ABSTRACT

Title of Document: THE EFFECT OF AMBIENT N:P RATIO AND LIGHT ON THE NITROGEN UPTAKE AND GROWTH OF SELECT ESTUARINE AND OCEANIC DINOFLAGELLATES

Ji Li, Doctor of Philosophy, 2011

Directed By: Patricia M. Glibert, Professor
Marine Estuarine Environmental Science

Increasing frequency of harmful algal blooms (HABs) have been observed in eutrophic coastal ecosystems. The relationship between environmental factors (nutrients and light) and bloom-forming dinoflagellates were explored in this dissertation by both historical data analysis and laboratory experiments. The growth and nitrogen (N) acquisition of the HAB dinoflagellates *Prorocentrum minimum*, *P. donghaiense*, *Karlodinium veneficum* and *Karenia brevis*, were studied. It is hypothesized that estuarine species *Prorocentrum* spp. develops blooms in relative high N:P ratio water, while *K. veneficum* blooms near or lower than the Redfield ratio; these species will grow faster in the N:P ratio in which they develop blooms, even when these nutrients are not at limiting levels; *Prorocentrum* spp. preferentially take up more DIN in high DIN:DIP ratio water, while *Karlodinium* can better use

other source of N in the low DIN water; low-light-adapted nitrogen acquisition by *Prorocentrum* spp. serves as an adaptive advantage to grow in low light waters.

Historical data analysis showed that *P. minimum* generally develops blooms in high DIN, high N:P ratio, but turbid water in Chesapeake Bay, while *K. veneficum* blooms near or lower than the Redfield ratio, when DIN was depleted, but organic N sources were still available. Following these results, the effects of ambient N:P ratio and light on the growth and N acquisition of *P. minimum* and *P. donghaiense* were studied in both batch and continuous culture (turbidistat). *Prorocentrum* spp. were grown in a wide range of N:P ratios, and across a wide range of light intensities in turbidistat. Experiments to determine rates of N acquisition of different N sources were conducted using ^{15}N tracer techniques at each N:P ratio and light treatment.

However, in culture, the growth of the *Prorocentrum* species was not regulated by the ambient N:P ratio. When nutrients were sufficient, light, instead of ambient N:P ratio, regulated the algal ability to acquire N. The adaptive strategies of the two types of dinoflagellates, *Prorocentrum* spp. and *Karlodinium/Karenia* spp., are different. *Prorocentrum minimum* was shown to take up N in the dark. This light independent N uptake allows it to be more competitive in the relative low light near-shore water. *Karlodinium/Karenia* spp. apparently only takes up N in the light phase, but it can be mixotrophic and directly use organic sources, and thus may be more competitive after DIN was depleted.

The Droop model, which describes the growth rate regulated by the cell quota, was used to interpret the relationship between N acquisition and the growth rate over the diel cycle of growth. *Prorocentrum* spp. continuously take up nitrogen at night to

supplement the cell quota, and reaches the maximum cell quota at the beginning of light phase, when they reach the higher growth rate in a diel cycle.

In eutrophic coastal systems (e.g., Chesapeake Bay), the ambient N:P ratio, as well as light may be critical factors for HAB growth. The dinoflagellates studied here have different adaptive strategies to grow in low light and to take advantage of high nutrients in the eutrophic waters. *Prorocentrum* spp. may dominant the high DIN water, while *Karlodinium/Karenia* spp. prefers organic nutrients.

THE EFFECT OF AMBIENT N:P RATIO AND LIGHT ON THE NITROGEN UPTAKE AND GROWTH OF SELECT ESTUARINE AND OCEANIC DINOFLAGELLATES

By

Ji Li

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2011

Advisory Committee:
Professor Patricia M. Glibert, Chair
Research Associate Professor Jeffrey C. Cornwell
Professor Ming Li
Dr. Peter Tango
Professor Daniel Terlizzi, Dean's Representative

Dedication

I dedicate this dissertation to my loving and supportive family.

Acknowledgements

I would like to thank all people who have helped and inspired me during my study.

I especially want to thank my advisor, Dr. Patricia M. Glibert, for her hard work and guidance throughout this entire study and for believing in my abilities. Her perpetual energy and enthusiasm in research had greatly motivated me. In addition, she was always accessible and willing to help my research.

I would like to express my gratitude to Dr. Jeffrey Cornwell, Dr. Ming Li, Dr. Peter Tango and Dr. Daniel Terlizzi as my academic committee members and advisors. Their suggestions and encouragement helped me throughout the research and writing of this thesis.

I like to express my thanks to Jeff Alexander, Cindy Shoemaker and Caroline Solomon. Without their support in both lab and life, this dissertation would not have been written.

I am appreciative for the support from Horn Point Laboratory fellowship and student small grant.

Especially, I would like to give my special thanks to my wife Yonghui whose patient love enabled me to complete this work.

Table of Contents

Dedication ii
Acknowledgements iii
Table of Contents iv
List of Tables vii
List of Figures viii
Chapter 1: **Introduction** 1
Eutrophication and Harmful Algal Blooms 1
N:P Ratio and Nutrient Limitation 2
N:P Ratio Regulating Dinoflagellate Blooms 4
Light Dependence of Photosynthesis and Nutrient Uptake 6
Perspective of N:P ratio and Light - the Matrix 8
Hypothesis 10
Research Objectives 10
References 12
Figures 19
Chapter 2: An Exploratory Analysis of Nutrients and Other Related Variables Related to Select Harmful Algal Bloom Events, in the Chesapeake Bay 1991-2008 21
Abstract 21
Introduction 22
Methods 25
Results 25
Eutrophic Level and Bloom Events 25
Spatial Distribution 27
Salinity 28
Seasonal Records and Temperature 28
Secchi Depths and TSS 29
Dissolved Oxygen 30
Dissolved Carbon and Nutrients 31
Components of Particulate Matter 33
Discussion 34
A Comparison with Ecosystems with Similar Nutrient Dynamics 34
A Comparison with Spatial Historical Data 36
The Spatial Variation of Nutrients and HAB Events 39
The Environmental Factors and HAB Species 41
References 45
Figures 54
Chapter 3: Effects of Ambient DIN : DIP Ratio on the Nitrogen Uptake of Harmful Dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* in Turbidistat* 90
Abstract 90
Introduction 91

Materials and Methods 93
Algae Culture 93
Continuous Culture Design 94
Experimental Design and Algal Growth 95
Nitrogen Uptake and Assimilation Experiments 96
Analytical Methods 97
Data Analysis 98
Results 98
Ambient Parameters and Cell Counts 98
Culture Growth in the Turbidistat 99
Nitrogen Uptake Experiments 100
Application of Droops Model 103
Discussion 104
Value of Tubidostat Approach 105
Value of Droop's Model Approach 108
The N:P Ratios in the Coastal Ecosystems 109
References 112
Tables 120
Figures 122
Chapter 4: Effects of Light on Growth and Nitrogen Uptake of *Prorocentrum minimum* and *Prorocentrum donghaiense* 133
Abstract 133
Introduction 134
Materials and Methods 136
Algal Culture 136
Continuous Culture Design 137
Experimental Design and Algal Growth 138
Nitrogen Uptake and Assimilation Experiments 139
Analytical Methods 140
Data Analysis 141
Results 142
Ambient Parameters 142
Growth Rate in the Turbidistat 142
Nitrogen Uptake Experiments 143
Discussion 145
Growth Regulation in Turbidistat 145
N Uptake versus Irradiance 149
Light Condition and Prorocentrum Blooms 151
References 153
Tables 161
Figures 163
Chapter 5: Growth and Competition of Several Harmful Dinoflagellates under Different Nutrient and Light Condition 173
Abstract 173
Introduction 174
Materials and Methods 177

Algae Cultures and Methods of Growth 177
Experimental Design 178
Analytical Protocols 180
Data Analysis and Simulation 181
Results 181
Experiment 1 181
Experiment 2 183
Experiment 3 183
Experiment 4 184
Experiment 5 185
Experiment 6 185
Discussion 186
Growth Rate 186
Nutrient Strategy – Autotrophy and Mixotrophy 188
References 196
Tables 205
Figures 208
Chapter 6: Research Conclusions 222
Dinoflagellate Blooms and the Matrix of Nutrients and Light 222
The Application of Cell Quota Model 226
The Improvement of Cell Quota Model 229
References 230
Figure 232
Complete References 233

List of Tables

Table 3-1 Ambient nutrient concentrations in 4 different tubidistat systems supplied by medium at different DIN:DIP ratios. 120
Table 3-2 Calculated parameters of uptake kinetics of nitrogen sources (NH_4^+, urea, and glycine) by *Prorocentrum minimum* at different ambient DIN:DIP ratio in turbidistat based on the Michaelis-Menten equation. 121
Table 4-1 Ambient nutrient concentrations in 4 different tubidistat systems supplied by medium at different DIN:DIP ratios. 120
Table 4-2 Calculated parameters of uptake kinetics of nitrogen sources (NH_4^+, urea, and glycine) by *Prorocentrum minimum* at different ambient DIN:DIP ratio in turbidistat based on the Michaelis-Menten equation. 121
Table 5-1 Monoculture exponential growth rates of *Prorocentrum minimum* and *Karlodinium veneficum* 198
Table 5-2 Exponential growth rates (μ day^{-1}) of *Prorocentrum minimum* and *Karlodinium veneficum* in mixed culture experiments. 206
Table 5-3 Exponential growth rates (μ day^{-1}) of *Prorocentrum minimum* and *Karlodinium veneficum* with *Rhodomonas* in mixed culture experiments. 207
Table 5-4 Monoculture exponential growth rates of *Prorocentrum donghaiense* and *Karlodinium brevis* 208
Table 5-5 Exponential growth rates of *Prorocentrum donghaiense* and *Karlodinium brevis* in mixed culture experiments 209
Table 5-6 Exponential growth rates of *Prorocentrum donghaiense* and *Karlodinium brevis* with *Synechococcus* in mixed culture experiments 210
Table 5-7 Summary of the growth rates of 4 dinoflagellates reported in laboratory experiments 211

List of Figures

Figure 1-1 Comparison of the range in ambient nitrogen: phosphorus ratios during different phases of the blooms in the East China Sea and in different phytoplankton assemblages among the southwest Florida shelf during May 2005.. 19
Figure 1-2 Predominant dinoflagellate life-form Types associated with the turbulence-nutrient matrix along an onshore–offshore continuum characterizing pelagic habitats 20
Figure 2-1 The routinely sampled stations in the Chesapeake Bay and its tributaries by Maryland Departments of Natural Resources 54
Figure 2-2 a. samples of different type of eutrophic level chlorophyll *a* recorded at Chesapeake Bay area (1991-2008); b, samples of different type of *Prorocentrum minimum* cell density level recorded; c, samples of different type of *Karlodinium veneficum* cell density level recorded; d, the number of samples of different type of cyanobacteria cell density level recorded..... 55
Figure 2-3 The spatial distribution of Chl *a* samples in the Chesapeake Bay and its tributaries 1991-2008..... 56
Figure 2-4 The spatial distribution of *Prorocentrum minimum* in the Chesapeake Bay and its tributaries 1991-2008. 57
Figure 2-5 The spatial distribution of *Karlodinium veneficum* in the Chesapeake Bay and its tributaries 2003-2008. 58
Figure 2-6 The spatial distribution of cyanobacteria in the Chesapeake Bay and its tributaries 1991-2008..... 59
Figure 2-7 The salinity of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay..... 60
Figure 2-8 The ambient parameters of *Prorocentrum minimum* (PM), *Karlodinium veneficum* (KV) and cyanobacteria (Cyn) in blooms in the Chesapeake Bay 61
Figure 2-9 The month number of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay..... 62
Figure 2-10 The water temperature of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 63
Figure 2-0-11 The secchi depth of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 64
Figure 2-12 The total suspended solids (TSS) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay..... 65
Figure 2-13 The dissolved oxygen (DO) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 66

Figure 2-14 The dissolved organic carbon (DOC) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 67
Figure 2-15 The dissolved organic nitrogen (DIN) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 68
Figure 2-16 The phosphate (PO_4^{3-}) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 69
Figure 2-17 The dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 70
Figure 2-18 The ambient carbon, nitrogen and phosphorus rates of *Prorocentrum minimum* (PM), *Karlodinium veneficum* (KV) and cyanobacteria (Cyn) in blooms in the Chesapeake Bay 71
Figure 2-19 The particle carbon (PC) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 72
Figure 2-20 The particle nitrogen (PN) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 73
Figure 2-21 The particle phosphorus (PP) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 74
Figure 2-22 The particle carbon (PC) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 75
Figure 2-23The particle carbon (PC) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 76
Figure 2-24 The particle nitrogen (PN) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay 77
Figure 2-25 Comparison of the range in ambient N:P ratios during different phase of the blooms in the East China Sea (ECS) during May 2005, in different phytoplankton assemblages along the southwest Florida Shelf (SFS) during May 2003 and different phase of blooms in the Chesapeake Bay from 1991 to 2008. 78

Figure 2-26 The spatial distribution of salinities in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987..... 79
Figure 2-27 The spatial distribution of dissolved inorganic nitrogen (DIN) in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 80
Figure 2-28 The spatial distribution of phosphate (PO_4^{3-}) in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 81
Figure 2-29 The spatial distribution of dissolved organic nitrate: dissolved organic phosphate (N:P) ratio in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987. 82
Figure 2-30 The spatial distribution of chlorophyll *a* in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987. 83
Figure 2-31. The summer surface water salinity in the Chesapeake Bay 84
Figure 2-32 The seasonal variation of monthly average dissolved inorganic nitrogen (DIN) concentrations in 3 zones of Chesapeake Bay. 85
Figure 2-33 The seasonal variation of monthly average phosphate (PO_4^{3-}) concentrations in 3 zones of Chesapeake Bay. 86
Figure 2-34 The seasonal variation of ambient dissolved inorganic nitrogen (N): phosphate (P) ratios in 3 zones of Chesapeake Bay. 87
Figure 2-35 The seasonal variation of monthly average chlorophyll *a* concentrations in 3 zones of Chesapeake Bay. 88
Figure 2-36 The seasonal variation of monthly maximum cell densities of cyanobacteria in Zone I and *Prorocentrum minimum* and *Karlodinium veneficum* in Zone II of Chesapeake Bay. 89
Figure 3-1 The setup of a turbidistat system..... 122
Figure 3-2 Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system for 4 constant 12 h: 12 h light dark cycles (96 hrs). 123
Figure 3-3 a. Diurnal growth rates of *Prorocentrum minimum* in turbidistat system at 14: 10 h light dark cycle. b. Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system at 12: 12 h light dark cycle..... 124
Figure 3-4 a. Average growth rates of *Prorocentrum minimum* in a 14h: 10h light dark cycle at different ambient N:P ratios. b. Average growth rates of *P. minimum* in the light phase of 14h: 10h light dark cycle at different ambient N:P ratios. c. Average growth rates of *P. donghaiense* in a 12h: 12h light dark cycle at different ambient N:P ratios. d. Average growth rates of *P. donghaiense* in the light phase of 12h: 12h light dark cycle at different ambient N:P ratios 125
Figure 3-5 Kinetic relationships between nitrogen specific uptake rates *V* (h^{-1}, upper panels) and assimilation rates *A* (h^{-1}, lower panels) of NH_4^+, urea, and glycine with ^{15}N substrate concentration (μM-N) by *Prorocentrum minimum* at different DIN:DIP ratio cultures. 126

Figure 3-6 Nitrate specific uptake rates V (h^{-1}) and assimilation rates A (h^{-1}) of NH_4^+, urea, and glycine with ^{15}N substrate concentration (μM-N) by *Prorocentrum minimum* grown at different DIN:DIP ratios. 127
Figure 3-7 a. The NO_3^- specific uptake rates (V, h^{-1}) of *Prorocentrum donghaiense* grown at different ambient N:P ratios; b. The NO_3^- specific assimilation rates (A, h^{-1}) of *P. donghaiense* grown at different ambient N:P ratios. c. The NH_4^+ uptake rates of *P. donghaiense* grown at different ambient N:P ratios; d. The NH_4^+ assimilation rates of *P. donghaiense* grown at different ambient N:P ratios. 128
Figure 3-8 The assimilation efficiencies % (assimilation rate/ uptake rate) of NO_3^- by *Prorocentrum donghaiense* grown at different ambient N:P ratios; b. The assimilation efficiencies % (assimilation rate/ uptake rate) of NH_4^+ by *P. donghaiense* grown at different ambient N:P ratios 129
Figure 3-9 The ratio of specific N assimilation rate/ growth rate (A/μ) of *Prorocentrum minimum* for major N sources at different DIN:DIP ratios 130
Figure 3-10 The ratio of specific N uptake rate/ growth rate (V/μ) of *Prorocentrum donghaiense* for NO_3^- (a) and NH_4^+ (b) at different ambient N:P ratios; the ratio of specific N assimilation rate/ growth rate (A/μ) of *P. donghaiense* for NO_3^- (c) and NH_4^+ (d) at different ambient N:P ratios 131
Figure 3-11 The conceptual model of the diel cell nutrient quota variation in a light-dark cycle..... 132
Figure 4-1 Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system for 4 constant 12 h: 12 h light dark cycles (96 hrs).. 163
Figure 4-2 a. Diurnal growth rates based on changes in optical signals of *Prorocentrum minimum* in turbidistat system at 14 h: 10 h light dark cycle. b. Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system at 14 h: 10 h light dark cycle. 164
Figure 4-3 a. Average growth rates of *Prorocentrum minimum* in a 14 h: 10 h light dark cycle at different ambient N:P ratios. b. Average growth rates of *P. minimum* in the light phase of 14 h: 10 h light dark cycle at different ambient N:P ratios. 165
Figure 4-4 Diurnal growth rates based on changes in optical signals of *Prorocentrum donghaiense* in turbidistat systems in 12 h: 12 h light dark cycles at different light intensities..... 166
Figure 4-5 Average diurnal growth rates (●) and average growth rates based on changes in optical signals of *Prorocentrum donghaiense* in the light phase (○) in a 12 h: 12 h light dark cycle at different ambient light irradiance.. 167
Figure 4-6 The relationship of N (Nitrate (NO_3^-), ammonium (NH_4^+), urea and glycine) uptake rates of *Prorocentrum minimum* at different irradiances.. 168
Figure 4-7 Average specific uptake rates V (h^{-1}) of 4 nitrogen substrates (Nitrate (NO_3^-), ammonium (NH_4^+), urea and glycine) near ambient concentration by *Prorocentrum donghaiense* in high light and low light incubation at different ambient N:P ratios. 169
Figure 4-8 Kinetic relationships between nitrogen specific uptake rates V (h^{-1}) of NO_3^- and NH_4^+ with ^{15}N substrate concentration (μM-N) by *Prorocentrum donghaiense* in different light irradiance..... 170

Figure 4-9 Average specific uptake rates *V* (h^{-1}) and assimilation rates *A* (h^{-1}) of nitrogen substrates nitrate (NO_3^-) and ammonium (NH_4^+) near ambient concentration by *Prorocentrum donghaiense* growing in high light and low light treatments 171
Figure 4-10 Ratio of assimilation rates *A* : uptake rates *V* of nitrogen substrates nitrate (NO_3^-) and ammonium (NH_4^+) by *Prorocentrum donghaiense* growing in high light and low light treatments. 172
Figure 5-1 Time course of growth of *Prorocentrum minimum* (a) and *Karlodinium veneficum* (b) in monoculture with 2 nitrogen sources NO_3^- (a, c) and urea (b, d) at high and low N:P ratios (HNP, LNP) and high and low light intensities (HL, LL) 212
Figure 5-2 Time course of growth of *Prorocentrum minimum* and *Karlodinium veneficum* in mixed culture with 2 nitrogen sources at 2 N:P ratios and 2 light intensities..... 214
Figure 5-3 Time course of growth of *Prorocentrum minimum* and *Karlodinium veneficum* with *Rhodomonas* in mixed culture with NO_3^- at 2 N:P ratios and 2 light intensities..... 215
Figure 5-4 Time course of growth of *Prorocentrum donghaiense* (a., b.) and *Karenia brevis* (c., d.) in monoculture with 2 nitrogen sources NO_3^- (a., c.) and urea (b., d.) at high and low N:P ratios (HNP, LNP) and high and low light intensities (HL, LL) 216
Figure 5-5 Time course of growth of *Prorocentrum donghaiense* and *Karenia brevis* in mixed culture with 2 nitrogen sources at 2 N:P ratios and 2 light intensities 218
Figure 5-6 Time course of growth of *Prorocentrum donghaiense*, *Karenia brevis* and *Synechococcus* in mixed culture at 2 N:P ratios and 2 light intensities 219
Figure 5-7 The exponential grow rates of 4 harmful dinoflagellates *Prorocentrum minimum* (PM), *P. donghaiense* (PD), *Karlodinium veneficum* (KV) and *Karenia brevis* (KB) in 3 sets of culture setting with treatments of high light and low light; low N:P ratio and high N:P ratio 221
Figure 6-1 The conceptual model of the bloom development in the Chesapeake Bay: *Prorocentrum* minimum developed blooms on DIN in the spring; *Karlodinium veneficum* developed blooms on DON or directly feeding on other microalgae as food sources. 232

Chapter 1: Introduction

Eutrophication and Harmful Algal Blooms

There has been a global increase in nutrient input to the coastal marine ecosystem as a result of increasing population in coastal areas, more urbanization, and increasing use of fertilizer for agriculture (Smith 2003, Smith et al. 2006, Andersen et al. 2006, Glibert et al. 2008). Eutrophication, defined as "the enrichment of water by nutrients, especially nitrogen (N) and/or phosphorus (P), causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of water concerned" by European Commission Nitrates Directive (http://www.cearac-project.org/2ndCEAWS/presen_PDF /Ulrich_CLAUSSEN.pdf), has been considered as one of the major threats to health of marine ecosystems (Smith et al. 1999, Smith 2003, Andersen et al. 2006, Smith et al. 2006). Using this definition, eutrophication is mostly concerned with the macronutrients (N and P) and the undesirable increases of phytoplankton biomass. Human activities have been one of the most important contributors to the increase in N and P loading to the coastal system. In the United States, N fertilizer consumption has a four-fold increase from the 1960s and phosphate (PO_4^{3-}) fertilizer consumption also doubled (USDA 2008). As a result, N and P enrichment to the coastal ecosystem by human activities has been increasing (Howarth et al. 2002). One prominent example is that N fluxes have increased 10 to 15 fold or more in coastal areas in past few decades (Jaworski et al. 1997, Howarth 2008).

In the past several decades, there have also been global increases in harmful algal blooms (HABs) in frequency, intensity and geographic distribution. HABs have caused many ecological, economic and human health problems in coastal areas (Hallegraeff 1993, Smayda 1997, Anderson et al. 2002, Glibert et al. 2005a, Glibert et al. 2008a, Heisler et al. 2008). At least in part, the increases of HABs have been strongly correlated to the global increase in eutrophication level (Paerl 1997, Anderson et al. 2002, Anderson et al. 2008, Glibert et al. 2008a, Heisler et al. 2008). Eutrophication caused by the high terrestrial nutrients input is one of the most important mechanisms of HABs events (Hallegraeff 1993, Anderson et al. 2002, Smayda 2002, Glibert and Burkholder 2006).

N:P Ratio and Nutrient Limitation

Even though phytoplankton biomass has shown strong positive relationships with N and P enrichment all over the world (Anderson et al. 2002, Smith 2006, Anderson et al. 2008, Glibert et al. 2008a, Heisler et al. 2008), the linkages between bulk quantities of nutrients (N, P) and algal blooms are complex (Anderson et al. 2008, Glibert et al. 2008a). The ratios of these nutrients could be very critical to regulate phytoplankton communities (Smayda 1990, Hodgkiss and Ho 1997, Flynn 2002, Li et al. 2009b). There have been many studies on N:P ratios and their effect on the phytoplankton biomass (Radach et al. 1990, Smayda 1990, Riegman 1995, Burkholder and Glasgow 1997). N:P ratios have been used as an important determinant in the question of whether N or P is the more limiting nutrient. The Redfield ratio (N:P = 16:1, Redfield 1934, 1958) has been used successfully to define

the limiting nutrient in many ecosystems (Hecky et al. 1993, Doering et al. 1995, Justic et al. 1995, Koerselman and Meuleman 1996, Lee et al. 1996). However, N and P load in the coastal water is complicated and does not always keep a close stoichometric balance in most ecosystems. While the total N and P load to the coastal environments has increased in the past several decades, the increases of N and P to coastal ecosystems have not necessarily been proportionate. For example, while both N and P fertilizer consumption has increased in USA since the 1960s, N fertilizer consumption has increased over 4 fold , but P fertilizer consumption only increased 80% up to 2006 (USDA 2008). Actually, the consumption of P fertilizer reached its peak in 1979 and has slightly decreased after that year (USDA 2008). One of reasons is that the principal source of effluent P was from P in laundry detergent, and the use of P detergent has been gradually replaced by P-free detergent since the late 1970s (Doemel and Brooks 1975, Sharfstein et al. 1977, Hartig and Horvath 1982, Berthouex et al. 1983, Maki et al. 1984, Hoffman and Bishop 1994, Lee and Joneslee 1995). This unbalanced increase of N and P nutrients loading could change the N: P ratio in the coastal water and subsequently change the ecosystem structure. This shift could be from seasonal to decades. A shift in the N:P ratios in coastal waters could result in an alteration in dominant species in the phytoplankton community (Phillips and Tanabe 1989, Bulgakov and Levich 1999, Stelzer and Lamberti 2001, Lagus et al. 2004, Paerl et al. 2004, Vrede et al. 2009). For example, the phytoplankton community of downstream Neuse River, USA, shifted from an N-limited cyanobacteria dominated freshwater phytoplankton community to a P-limited community after the P detergent ban in 1988 in this area (Paerl et al. 2004). As a

result of P reduction, N substrates taken up by phytoplankton in the downstream decreased and more N was delivered to the lower estuary and stimulated HABs comprised of dinoflagellates, cryptomonads, and diatoms there (Paerl et al. 2004).

N:P Ratio Regulating Dinoflagellate Blooms

Changes in the N:P ratio in inorganic and organic forms were suggested to be important in the bloom succession in the Chanjiang River estuary and East China Sea (ECS, Li et al. 2009). The Changjiang River is the third longest river of the world. Annual freshwater input is 9.32×10^{11} m^3, and annual input of N is 6.3×10^6 tonne (t) and P is 0.13×10^6 t (Shen et al. 2003), representing roughly a doubling of nutrient input in the past 20 years (Shen et al. 2003). While both N and P input by human usage have increased, the recent increases have been far greater in N. In 2005, China nitrogenous fertilizer production reached 36×10^6 t, including 20×10^6 t of urea fertilizer, a 5-fold increase since 1989 (Shen et al. 2003). The annual dissolved inorganic nitrogen (DIN) load to the coastal water from the Changjiang River has reached 1.4×10^6 t, which is higher than the Mississippi River and the Amazon River (Goolsby and Battaglin 2000, Duan et al. 2008). The total N off Changjiang River estuary has been shown to be about 70 to 110 μM-N, and is mostly in the form of NO_3^--N (Shen et al. 2003). The total P is about 2 to 25 μM-P, mostly as particulate P; PO_4^{3-}-P is only about 10% to 20% of the total P (Yan and Zhang 2003). As a result, the N:P ratio is typically > 100 near the river mouth (Chai et al. 2006, Zhang et al. 2007), which is much higher than the Redfield ratio and suggests P limitation.

Large scale dinoflagellate blooms in these areas in late spring and early summer have been recorded in the past decade (Zhou et al. 2003). These more

frequent and persistent algal blooms are thought to result from increasing nutrient inputs to the ECS (Anderson et al. 2002, Zhou et al. 2003, Glibert et al. 2006). The dinoflagellate *Prorocentrum donghaiense* has been found to be the dominant species in this region since large-scale field studies began in 2000. During late spring and early summer of 2005, large scale (> 15,000 km^2) mixed dinoflagellate blooms developed in the coastal ECS. *Karenia mikimotoi* was the dominant HAB species in the first stage of the bloom from late May and was succeeded by *P. donghaiense* approximately 2 weeks later. Samples were taken and experiments were conducted in the bloom region during 3 cruises of the Chinese Ecology and Oceanography of Harmful Algal Blooms (CEOHAB) Program during the bloom progression (Li et al. 2009). Experiment results supported that there was P limitation in these regions and suggested that the dominant bloom species and their temporal and spatial distribution were related to the ambient N:P ratios (Li et al. 2010). The uptake rates of N nutrients were also related to the ambient N:P ratios (Li et al. 2009). These results suggested ambient N:P ratio might be an important factor regulating the succession of dominant blooms species from *K. mikimotoi* to *P. donghaiense*. Ambient N:P ratio was also suggested to drive the phytoplankton community in the southwest Florida Shelf (Heil et al. 2007), where the spatial distribution of dinoflagellates (*Karenia brevis* and *Prorocentrum minimum*), cyanobacteria and diatom dominated communities reflected the variation of N:P ratios. Therefore, the tolerance and preference to certain N:P ratios might be an important regulator for the bloom expansion in these areas.

Furthermore, a direct comparison of the ambient N:P ratios during both the Florida and East China Sea blooms shows considerable correspondence (Fig. 1-1). In

both systems, the diatom dominant community was found associated with a high ambient DIN:DIP ratio (> 45), and the *Karenia* spp. dominant communities were found associated with DIN:DIP ratios that were below the Redfield ratio (< 16). The *P. donghaiense* community in the ECS and the mixed dinoflagellate assemblage off Florida were both observed to be associated with DIN: DIP ratios that more closely approximated the Redfield ratio. This comparison suggested there might be interesting similar ambient N:P preference for similar species: *P. donghaiense* and *P. minimum* or *K. mikimotoi* and *K. brevis*, and different N:P ratio preference between *Prorocentrum* and *Karenia* spp.

Light Dependence of Photosynthesis and Nutrient Uptake

Light is a very important environmental parameter for the phytoplankton community. Light is the essential source for the phytoplankton photosynthesis. Light energy is utilized by the algae cell during the photosynthesis process, distributed to ATP storage, and produced reductants for carbon fixation and nutrient reduction. Photosynthesis is a highly integrated metabolic process which could be regulated by both light and other environmental factors including nutrients.

There is always a periodic diel cycle in nature, and the photosynthesis variation on the diel cycle among different phytoplankton species could be wide (Behrenfeld et al. 2004). In coastal areas and adjacent oceans, the irradiance available to phytoplankton is varying over a wide range, due to possible high turbidity and/or high biomass in the water column. The vertical migration of many dinoflagellate species also allows cells to live in different depth where the light irradiance is different. In many cases, light is limiting factor of phytoplankton growth (Goldman et

al. 1979, Tett et al. 1985). Therefore, those bloom forming dinoflagellate species might not only dominate and bloom in their favorable nutrient condition (N:P ratio), but also have special strategy to dominate and bloom in their favorable light condition.

Nitrogen uptake and assimilation are energy consuming process, and can be regulated by light factors. Uptake rates varys over diel cycles, and the maximum uptake rates occurred at noon for some species (Anderson and Roels 1981). The light dependence of nutrient uptake has been studied in both laboratory cultures and in field communities, and the response to light is varied among different species. Numerous studies have shown that uptake of nutrient responding to light are different among taxonomic groups (Eppley et al. 1971, Wheeler et al. 1983). For example, the diatom *Skeletonema costatum* has lower dark uptake rates of NO_3^- and NH_4^+, while the coccolithophore *Emiliania huxleyi* was more uniform over a night range (Eppley et al. 1971). The light dependence of N uptake rates also varies for different nutrients. The ability of some cyanobacteria to uptake NH_4^+, a reduced N source, was relatively unaffected by darkness (Mulholland et al. 1999). The NO_3^- uptake rates appeared to be more limited at night than uptake of NH_4^+ (Cochlan et al. 1991). NO_3^- needs to be reduced before it can be assimilated by the algae, and it is an energy consuming process. NO_3^- uptake generally shows stronger light dependence than reduced N sources (NH_4^+ etc.) (Cochlan et al. 1991). However, for dinoflagellates, the light dependence of NO_3^- uptake still varied. *P. minimum* can continuously take up NO_3^- in the dark; while *K. mikimotoi* has been found to show almost no NO_3^- uptake in the

dark (Paasche et al. 1984). The light dependence might be weakened with increasing nutrient limitation (Healey 1977).

Irradiance-induced variation in nutrient uptake can alter the competitive abilities of species: those capable of maintaining high uptake in the dark may have a competitive advantage in light limiting environment. The different nutrient uptake rates in the dark might be an ecological strategy. If a species can continuously take up and assimilate N at high rates in darkness, it may be advantageous in low ambient light.

The bloom regions of ECS, west Florida Shelf and Chesapeake Bay area are under strong interaction of relative turbid terrestrial runoff and clear sea water, during a HAB progression, the available light is limited by both turbidity and biomass, therefore, the strategy of bloom forming dinoflagellates in different light condition is also very important to be considered for bloom development.

Perspective of N:P ratio and Light - the Matrix

Smayda has suggested dinoflagellates have diverse habitat preferences and adaptive strategies and characterized the dinoflagellate bloom species in coastal areas as 5 types which fit to a nutrient-turbulence matrix (Smayda and Reynolds 2001, Smayda 2002). Each dinoflagellate species has its specific niche for the bloom expansion, and these niches are described by available nutrients and turbulence/mixing intensity (Fig. 1-2). Based on Smayda's model, *Prorocentrum* spp. (length 15~22 μm width 9~14 μm) are described as Type II species which are small, rapidly growing competitors blooming in nutrient rich near-shore regions. Conversely, *Karenia* (length 18-37 μm, width 14-35 μm) are relatively slower

growing, larger, motile Type IV, V, VI species, which are generally considered to be off-shore species. These 2 groups of algae have different adaptive strategies to the ambient environment and have their own niches, described by different nutrient and turbulence preferences. The ambient N:P ratio has been suggested to be an important nutrient parameter regulating the *Prorocentrum* and *Karenia* spp. bloom progression (Li et al. 2009, Li et al. 2010). Ambient N:P ratio could the key factor which make the niches of these two type of algae different, as distinct N:P ratio variation distributed between the near-shore and off-shore water in the high frequent bloom area of these species (Heil et al. 2007, Li et al. 2009, Li et al. 2010). Moreover, near-shore regions are generally considered higher turbidity and less light available for the phytoplankton than clearer off-shore water. The light strategy of these 2 types of algae might also be different. The physiology differences of these 2 types of algae might determine their success in water column with different ambient N:P ratios and light condition.

Among these 2 types of algae, *K. mikimotoi* bloomed with *P. donghaiense* in the ECS (Li et al. 2009), *K. brevis* bloomed with *P. minimum* in the southwest Florida coast (Heil et al. 2007), and *Karlodinium veneficum* bloomed with *P. minimum* in the Chesapeake Bay (Zhang et al. 2008). Either temporal or spatial gradient existed when two bloom species developed the blooms. The range of N:P ratios for bloom expansion was different between *Prorocentrum* spp. and other species. The dependence of light might also be different among these species.

Therefore, the range of N:P ratio and light irradiance preferred by each dinoflagellate group could be critical for bloom development. In this dissertation, the

research is focused on, but not limited to, *P. minimum* and *K. veneficum* in Chesapeake Bay area. Historical observational data were analyzed to obtain the linkage between bloom-forming dinoflagellates and their ambient N:P ratios and light condition. Laboratory experiments were conducted to test physiological characteristics at specific ambient N:P ratios and light condition.

Hypothesis

The following hypotheses were tested.

- In Chesapeake Bay, *P. minimum* develops blooms in relative high DIN:DIP ratio water; while *K. veneficum* blooms near or lower than the Redfield ratio.
- These species will grow faster in the N:P ratio in which they develop blooms, even when these nutrients are not at limiting levels.
- *Prorocentrum* spp. preferentially take up more DIN in high DIN:DIP ratio water, while *Karlodinium* can better use other source of N in the low DIN water (e.g. DON, particulate N).
- Low-light-adapted nitrogen acquisition by *Prorocentrum* spp. serves as an adaptive advantage to grow in low light waters

Research Objectives

Historical observational data were analyzed and laboratory experiments were conducted to test the hypotheses and accomplish the following objectives:

1. Undertake a comprehensive analysis on decadal observational data of major dinoflagellate bloom species and cyanobacteria with the changes of ambient nutrient parameters (i.e., ambient N:P ratio) and related physical variables (i.e., light) in the Chesapeake Bay. (Chapter II)

2. Identify the quantitative relationship between growth and physiological characteristics of selected dinoflagellate, *Prorocentrum minimum* and *P. donghaiense*, with ambient N:P ratio in laboratory experiments; identify related parameters (growth rate, N uptake rate, etc.), and compare the results between these two species. (Chapter III)

3. Evaluate the importance of light on the nitrogen uptake and growth of *Prorocentrum minimum* and *P. donghaiense* and compare the difference. (Chapter IV)

4. Simulate the interspecies competition between three near-shore harmful dinoflagellates, *Prorocentrum minimum*, *P. donghaiense* and *Karlodinium veneficum*, and one off-shore dinoflagellate, *Karenia brevis*, at different ambient N:P ratio and light condition. (Chapter V)

5. Synthesize the findings in an overall conceptual framework, leading to suggestions to further study. (Chapter VI)

The experimental results were combined with the long term observational environmental data with statistical and mathematical approaches to understand the ambient N:P ratio and light regulation on these harmful algal bloom dynamics.

References

Andersen JH, Schluter L, Ærtebjerg G (2006) Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. Journal of Plankton Research 28:621-628

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Anderson SM, Roels OA (1981) Effects of light-intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Marine Biology 62:257-261

Behrenfeld MJ, Prasil O, Babin M, Bruyant F (2004) In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. Journal of Phycology 40:4-25

Berthouex PM, Pallesen L, Booman K, Sedlack R (1983) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 55:323-325

Bulgakov NG, Levich AP (1999) The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv Fur Hydrobiologie 146:3-22

Burkholder JM, Glasgow HB (1997) *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnology and Oceanography 42:1052-1075

Chai C, Yu ZM, Song XX, Cao XH (2006) The status and characteristics of eutrophication in the Yangtze River (Changjiang) estuary and the adjacent East China Sea, China. Hydrobiologia 563:313-328

Cochlan WP, Harrison PJ, Denman KL (1991) Diel periodicity of nitrogen uptake by marine-phytoplankton in nitrate-rich environments. Limnology and Oceanography 36:1689-1700

Doemel WN, Brooks AE (1975) Detergent phosphorus and algal growth. Water Research 9:713-719

Doering PH, Oviatt CA, Nowicki BL, Klos EG, Reed LW (1995) Phosphorus and nitrogen limitation of primary production in a simulated estuarine gradient. Marine Ecology-Progress Series 124:271-287

Duan SW, Liang T, Zhang S, Wang LJ, Zhang XM, Chen XB (2008) Seasonal changes in nitrogen and phosphorus transport in the lower Changjiang River before the construction of the Three Gorges Dam. Estuarine Coastal and Shelf Science 79:239-250

Eppley RW, Rogers JN, Mccarthy JJ, Sournia A (1971) Light/dark periodicity in nitrogen assimilation of marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. Journal of Phycology 7:150-154

Flynn KJ (2002) How critical is the critical N : P ratio? Journal of Phycology 38:961-970

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Granéli E, Anderson DM (2008) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Goldman JC, Mccarthy JJ, Peavey DG (1979) Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210-215

Goolsby DA, Battaglin WA (2000) Nitrogen in the Mississippi basin-estimating sources and predicting flux to the Gulf of Mexico. USGS Fact Sheet 135-00

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99

Hartig JH, Horvath FJ (1982) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 54:193-197

Healey FP (1977) Ammonium and urea uptake by some freshwater algae. Canadian Journal of Botany-Revue Canadienne De Botanique 55:61-69

Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography 38:709-724

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8:3-13

Hodgkiss IJ, Ho KC (1997) Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147

Hoffman FA, Bishop JW (1994) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia. Water Research 28:1239-1240

Howarth RW (2008) Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae 8:14-20

Howarth RW, Boyer EW, Pabich WJ, Galloway JN (2002) Nitrogen use in the United States from 1961–2000 and potential future trends. AMBIO 32:88-96

Jaworski NA, Howarth RW, Hetling LI (1997) Atmospheric deposition of nitrogen oxides onto the landscape contributes to coastal eutrophication in the northeast United States. Environmental Science & Technology 31:1995-2004

Justic D, Rabalais NN, Turner RE (1995) Stoichiometric nutrient balance and origin of coastal eutrophication. Marine Pollution Bulletin 30:41-46

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33:1441-1450

Lagus A, Suomela J, Weithoff G, Heikkila K, Helminen H, Sipura J (2004) Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. Journal of Plankton Research 26:779-798

Lee GF, Joneslee A (1995) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia - comment. Water Research 29:1425-1426

Lee YS, Seiki T, Mukai T, Takimoto K, Okada M (1996) Limiting nutrients of phytoplankton community in Hiroshima Bay, Japan. Water Research 30:1490-1494

Li J, Glibert PM, Zhou MJ (2010) Temporal and spatial variability in nitrogen uptake kinetics during harmful dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou MJ, Lu SH, Lu DD (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology-Progress Series 383:11-26

Maki AW, Porcella DB, Wendt RH (1984) The impact of detergent phosphorus bans on receiving water-quality. Water Research 18:893-903

Mulholland MR, Ohki K, Capone DG (1999) Nitrogen utilization and metabolism relative to patterns of N_2 fixation in cultures of *Trichodesmium* NIBB1067. Journal of Phycology 35:977-988

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42:1154-1165

Paerl HW, Valdes LM, Joyner AR, Piehler MF (2004) Solving problems resulting from solutions: Evolution of a dual nutrient management strategy for the eutrophying Neuse river estuary, North Carolina. Environmental Science & Technology 38:3068-3073

Phillips DJH, Tanabe S (1989) Aquatic pollution in the Far-East. Marine Pollution Bulletin 20:297-303

Radach G, Berg J, Hagmeier E (1990) Long-term changes of the annual cycles of meteorological, hydrographic, nutrient and phytoplankton time-series at Helgoland and at Lv Elbe 1 in the German Bight. Continental Shelf Research 10:305-328

Redfield A.C., On the proportions of organic derivations in sea water and their relation to the composition of plankton. In James Johnstone Memorial Volume. (ed. R.J. Daniel). University Press of Liverpool, pp. 177-192, 1934

Redfield, A.C., The biological control of chemical factors in the environment, American Scientist, 1958

Riegman R (1995) Nutrient-related selection mechanisms in marine phytoplankton communities and the impact of eutrophication on the planktonic food web. Water Science and Technology 32:63-75

Sharfstein B, Roels OA, Harris V, Lee V (1977) Effect of detergent legislation on phosphorus in effluent and receiving waters. Journal Water Pollution Control Federation 49:2017-2021

Shen ZL, Liu Q, Zhang SM, Miao H, Zhang P (2003) A nitrogen budget of the Changjiang River catchment. Ambio 32:65-69

Smayda T (1990) Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, New York

Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42:1137-1153

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems - A global problem. Environmental Science and Pollution Research 10:126-139

Smith VH (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnology and Oceanography 51:377-384

Smith VH, Joye SB, Howarth RW (2006) Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51:351-355

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179-196

Stelzer RS, Lamberti GA (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography 46:356-367

Tett P, Heaney SI, Droop MR (1985) The Redfield ratio and phytoplankton growth-rate. Journal of the Marine Biological Association of the United Kingdom 65:487-504

USDA (2008) Fertilizer consumption and use - By year. United States Department of Agriculture: http://www.ers.usda.gov/Data/FertilizerUse/

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2009) Effects of N:P loading ratios on phytoplankton

community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54:331-344

Wheeler PA, Olson RJ, Chisholm SW (1983) Effects of photocycles and periodic ammonium supply on 3 marine-phytoplankton species .2. ammonium uptake and assimilation. Journal of Phycology 19:528-533

Yan WJ, Zhang S (2003) The composition and bioavailability of phosphorus transport through the Changjiang (Yangtze) River during the 1998 flood. Biogeochemistry 65:179-194

Zhang H, Litaker W, Vandersea MW, Tester P, Lin SJ (2008) Geographic distribution of *Karlodinium veneficum* in the US east coast as detected by ITS-ferredoxin real-time PCR assay. Journal of Plankton Research 30:905-922

Zhang J, Liu SM, Ren JL, Wu Y, Zhang GL (2007) Nutrient gradients from the eutrophic Changjiang (Yangtze River) Estuary to the oligotrophic Kuroshio waters and re-evaluation of budgets for the East China Sea Shelf. Progress in Oceanography 74:449-478

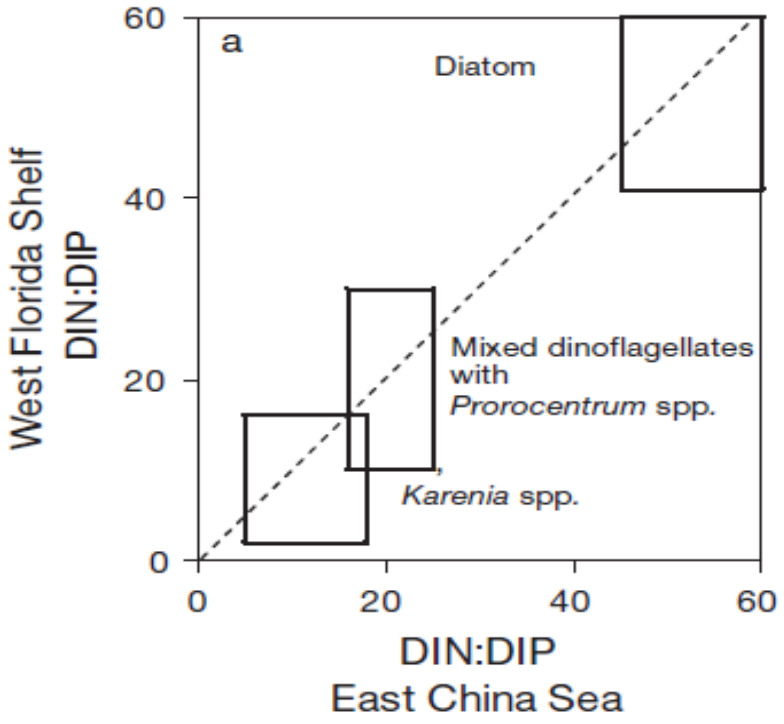


Figure 1-1 Comparison of the range in ambient nitrogen: phosphorus ratios during different phases of the blooms in the East China Sea and in different phytoplankton assemblages among the southwest Florida shelf during May 2005. The southwest Florida Shelf data are derived from Heil et al. (2007).

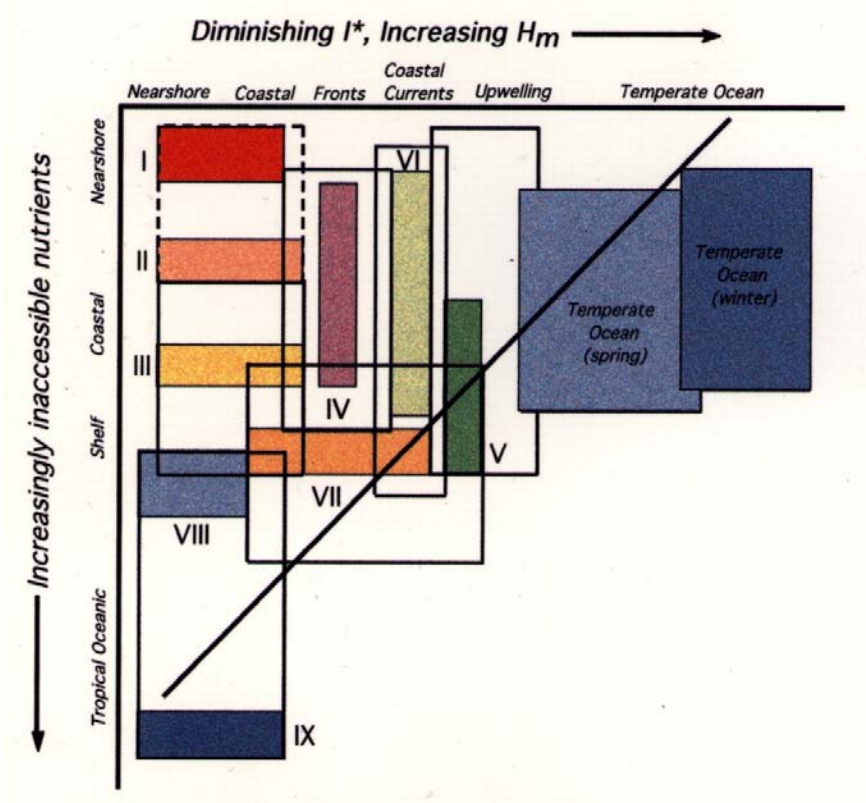


Figure 1-2 Predominant dinoflagellate life-form Types associated with the turbulence-nutrient matrix along an onshore–offshore continuum characterizing pelagic habitats (From Smayda 2001).

Type I = gymnodinioids; Type II = peridinioids and prorocentroids; Type III = ceratians; Type IV = frontal zone species; Type V = upwelling relaxation taxa; Type VI = coastal current entrained taxa; Type VII = dinophysoids; Type VIII = tropical oceanic flora; Type IV = tropical shade flora. Consult text for Type species.

Chapter 2: An Exploratory Analysis of Nutrients and Other Related Variables Related to Select Harmful Algal Bloom Events in the Chesapeake Bay 1991-2008

Abstract

An exploratory analysis was conducted relating Chesapeake Bay nutrient concentrations and other related water quality variables to the harmful algal bloom events of select species: the dinoflagellates *Prorocentrum minimum*, *Karlodinium veneficum,* and a composite density of freshwater cyanobacteria species. Chesapeake Bay, especially the upper bay and its tributaries, is characterized as a turbid, low light available, highly eutrophic system. *Prorocentrum minimum*, *K. veneficum* and cyanobacteria blooms can further decreased the light availability, enrich particle organic matter to the water column and carry the potential to be toxic. *Prorocentrum minimum* and *K. veneficum* generally develop blooms in the mesohaline religions, while freshwater cyanobacteria mostly bloomed in the tidal freshwater or oligohaline endmembers of tributaries. In contrast to *P. minimum* concentrations that correlates with high nitrogen (N) water, *K. veneficum* is associated with high phosphate (P) concentrations and low DIN:DIP ratio water in the summer. *K. veneficum* may be less limited by P in the low DIN:DIP ratio water after *P. minimum* blooms. The nutrients ratios during dinoflagellates events were comparable to those previous reported for analogous blooms in the East China Sea and southwest Florida Shelf.

Introduction

Chesapeake Bay is the largest estuarine system of the USA. Chesapeake Bay extends over 300 km from the Susquehanna River at its north head, to the Atlantic Ocean at its mouth, with an average depth of 8 m. The majority of freshwater flow (64%) and nutrient-loading of Chesapeake Bay is from the Susquehanna River (Boynton et al. 1995, Kemp et al. 2005), while there are several major tributaries on both sides of the Chesapeake Bay, including the Patuxent River, Potomac River, Rappahannock River and Choptank River. Extensive studies of nutrient loading and its processing by primary producers have been conducted on the Chesapeake Bay ecosystem (Fisher et al. 1990, Fisher et al. 1992, Boynton et al. 1995, Glibert et al. 1995, Cornwell et al. 1996, Malone et al. 1996, Harding and Perry 1997, Glibert et al. 2001, Glibert and Magnien 2004, Glibert et al. 2005b, Fisher et al. 2006). Massive terrestrial loading results in excessive phytoplankton production in the Chesapeake Bay (Malone et al. 1988, Malone et al. 1996, Glibert and Magnien 2004, Adolf et al. 2006c, Fisher et al. 2006). Generally, the peak biomass of Chesapeake Bay occurs during the spring diatom blooms, while peak production occurs in the summer when summer temperature reaches its maximum (Malone et al. 1988, Malone et al. 1996, Harding et al. 2002). Even though there is a large amount of nutrient loading into the Chesapeake Bay, phosphorus (P) and silica still tend to be limiting nutrients in spring, while nitrogen (N) is the primary limiting nutrient in summer (Fisher et al. 1992, Glibert et al. 1995, Malone et al. 1996, Fisher et al. 1999).

The Chesapeake Bay region has been subject to eutrophication which is linked to the pressures of increasing human population, urbanization (e.g. Washington DC,

Baltimore area), development of animal and plant agriculture and non-point nutrient pollution in its watershed (Glibert and Magnien 2004, Glibert et al. 2005b, Hagy et al. 2004, Kemp et al. 2005, Fisher et al. 2006). As a consequence of this eutrophication, Chesapeake Bay has suffered from major harmful algal bloom problems for decades (Glibert et al. 2001, Goshorn et al. 2004, Marshall et al. 2004, Tango et al. 2004, Tango et al. 2005, Tango and Butler 2008). In the Chesapeake Bay, harmful algal blooms have caused environmental damage including hypoxia, mortalities of fish and shellfish, decline of submerged aquatic vegetation, illness and death of various invertebrates, seabirds, and marine mammals (Deeds et al. 2002, Gallegos and Bergstrom 2005, Heil et al. 2005, Tango et al. 2005, Adolf et al. 2006a, Tango and Butler 2008).

Prorocentrum minimum is one of the major bloom forming harmful dinoflagellates, both globally distributed in coastal waters (Heil et al. 2005, Glibert et al. 2008b) and common in Chesapeake Bay (Glibert et al. 2001, Tango et al. 2005). *Karlodinium veneficum*, formerly recorded as *Gyrodinium galatheanum, Gymnodinium galatheanum* and *Karlodinium micrum* (Li et al. 2000, Adolf et al. 2006b, Deeds 2009), is also a widespread mixotrophic dinoflagellate which has caused HABs since 1950 in coastal waters of Southwest Africa, Europe, United States, Western Australia, and other temperate coastal environments, including Chesapeake Bay (Li et al. 2000, Zhang et al. 2008, Deeds 2009). *Karlodinium veneficum* is a toxigenic dinoflagellate, which can produce Karlotoxin and has been implicated to cause fish-kill events in the Chesapeake Bay area (Deeds et al. 2006,

Adolf et al. 2008). High biomass blooms of *K. veneficum* have been recorded in middle and upper Chesapeake Bay between May and September (Li et al. 2000).

Some toxic species of cyanobacteria, including *Microcystis* spp. and *Anabaena* spp., have also been reported to cause cyanobacterial HAB events in the Chesapeake Bay area (Marshall et al. 2005). Diverse toxic activity of cyanobacteria has been recorded in the tidal waters of Chesapeake Bay and has caused environmental problems in recent years (Tango and Butler 2008).

In this study, the occurrences of these two major bloom developing algae (dinoflagellates *P. minimum*, *K. veneficum*) and a composite density of freshwater cyanobacteria in Chesapeake Bay regions are described in terms of their relationship with eutrophication indicators. This study provided interpretations of the relationships between these HAB species and water quality and enabled consideration of questions such as:

- did the scale and frequency of HAB species in the Chesapeake Bay estuary change over time?
- how were biological changes related to water quality?
- what water quality conditions best explain the success of HAB species that developed blooms?

By addressing these questions, this study is helpful in gaining a better understanding of the complex mechanisms that influence the development of HABs in eutrophic ecosystem.

Methods

Long-term observational data on the surface dinoflagellate and cyanobacteria abundance and water quality were acquired from the Chesapeake Bay Program (http://www.chesapeakebay. net). These data were derived from routinely sampling in the Chesapeake Bay and its tributaries for water chemistry and phytoplankton community composition since the mid-1980s (http://www.chesapeakebay.net/data_waterquality.aspx, Fig. 2-1). The dinoflagellate data were derived from phytoplankton samples which were preserved with Lugol's, and subsequently counted by conventional light microscopy techniques. The cyanobacteria data were also derived from phytoplankton samples which were preserved with Lugol's, and subsequently counted by conventional light microscopy techniques. Picocyanobacteria (e.g. *Synechococcus*), which are generally smaller than the resolution limit of light microscopy, were not identified or counted, and are not involved in this anaylsis. Water quality and chemical analysis included ambient parameters (salinity, temperature, secchi depth, particulate and dissolved nitrogen/ phosphorus and carbon) and biological variables (chlorophyll). Data are available from samples which were conducted from biweekly to monthly.

Results

Eutrophic Level and Bloom Events

From 1991 to 2008, there were ~ 2830 records of *P. minimum* and ~ 4755 records of cyanobacteria sampled in the Chesapeake Bay and its tributaries. However,

the records of *K. veneficum* (~ 4755 records) were only available from 2003 in the available Chesapeake Bay Program data sets. Each species was either documented as mixed with other species or as population dominant in bloom conditions. Cell densities varied across wide range. According to United States National Estuarine Eutrophication Assessment (Bricker et al. 1999), Chlorophyll (Chl) *a* can be used as an indicator of algal blooms to define estuarine eutrophic condition. The thresholds and ranges are defined as: hypereutrophic: > 60 μg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 μg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 μg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 μg Chl *a* L^{-1}. Of the Chesapeake Bay records, over half of the samples (3350) were medium eutrophic when *P. minimum*, *K. veneficum* and cyanobacteria were observed. Over 1000 samples were in hyper and high eutrophic condition (Fig. 2-2 a).

For this analysis, the bloom status of the two dinoflagellate species in the water column was defined according to Tango et al (2005) and Maryland Eco-check (http://www.eco-check.org/), and the thresholds are set as: Type I: bloom condition, cell density > 5,000,000 cell L^{-1}; Type II, sub-bloom condition, cell density > 1,000,000 but ≤ 5,000,000 cell L^{-1}, dinoflagellates could be dominant species to develop bloom, but have not reached bloom condition; Type III, cell density >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV, cell density > 0 but ≤ 1,000 cell L^{-1}; Type V, cell density = 0, dinoflagellates were not observed at this station. Similar types were defined on cyanobacteria, however, the thresholds of each type are 10 times of dinoflagellates, as cyanobacteria have much smaller cell size (e.g. *Microcystis aeruginosa* cell size 2-3 μm; *P. minimum* 14-22 μm).

A total of 48 *P. minimum* Type I samples were recorded, with a maximum cell density of 60,915,000 cell L^{-1}, in the lower Potomac River database (Fig. 2-2b). There were 133 Type II samples. Most of the records of *P. minimum* (2157) were Type III level. Cell abundance data of *K. veneficum* were only available from 2003 in the available Chesapeake Bay Program data sets. Since 2003, there were 10 Type I records of *K. veneficum* and 38 Type II records, with a maximum cell density of ~16,000,000 cell L^{-1} in the lower Patuxent River (Fig. 2-2c). There were 196 Type III samples recorded. For the composite density of freshwater cyanobacteria, there were 329 Type I samples recorded, with a maximum cell density of 632,000,000 cell L^{-1} in the upper Sassafras River (Fig. 2-2d). There were 717 Type II records. Over 70% of the cyanobacteria records (3521) were Type III level.

Spatial Distribution

Hyper and high eutrophic level Chl *a* samples were documented all over the Chesapeake Bay (Fig. 2-3). However, most of these observations were from in the upper part of the Chesapeake Bay and major tributaries. The Type I and II levels of *P. minimum* have been also recorded throughout the Chesapeake Bay (Fig. 2-4), but especially in the lower Potomac River, Patuxent River, Choptank River and the upper Bay. *Karlodinium veneficum* were only recorded to have reached Type I and II level in upper Chesapeake Bay and lower Potomac River and Patuxent River (Fig. 2-5). High biomass of the cyanobacteria considered here were mostly found in the upper river of major tributaries (Fig. 2-6).

Salinity

Approximately 80% of hyper and high eutrophic samples had salinities less than 10, which represents the tidal freshwater and oligohaline regions of Chesapeake Bay (Fig. 2-7). Over 50% of the Type I and II *P. minimum* samples were collected in a narrow range of salinity of 6 - 10. *Prorocentrum minimum* cell densities decreased to Type IV as salinity increased to 20. Over 50% of the Type I and II *K. veneficum* samples were collected in a narrow range of salinity of 7.5 - 11. Of the *K. veneficum* samples, 80% were observed in the salinity range of 5 - 13. 90% Type I and 75% Type II levels of the cyanobacteria were recorded in salinity less than 10.

There was significant difference between the salinity where the dinoflagellate and the cyanobacteria blooms formed ($p < 0.001$) (Fig. 2-8). More than 80% of the *P. minimum* blooms (Type I) were developed in salinities of 5.5 and 11, while 80% of the *K. veneficum* blooms developed in salinities between 7 and 12.7, and 75% of the cyanobacterial blooms developed at stations where salinities were lower than 5.5. Dinoflagellate blooms developed in the tidal mixing area, generally in the lower part of the tributaries (Fig. 2-4 and 5), while cyanobacteria blooms developed in the non-tidal and tidal freshwater area, generally in the upper part of the tributaries (Fig. 2-6).

Seasonal Records and Temperature

More than 50% of the hyper, high and medium eutrophic samples were collected between May to August, and about 80% of these samples were collected between April and October (Fig. 2-9). More than 50% of the hyper, high and medium eutrophic conditions occurred when water temperature was in the range of 14.2 ˚C to 26.6 ˚C with a median over 21 ˚C (Fig. 2-10). Levels of Chl *a* dropped to Type IV

conditions when median temperatures were lower than 15 ˚C. Most of the Type I and II *P. minimum* samples were recorded in April and May, when water temperatures were in the range of 13.7 ˚C to 19.1 ˚C. *Karlodinium veneficum* reached Type II levels mostly in May, June and July, when temperatures were higher than 17.1˚C. However, 50% of the *K. veneficum* blooms developed in July, August and September. Blooms developed when temperature were between 21 ˚C and 29.5 ˚C. Cyanobacteria also bloomed mostly in July, August and September when water temperatures were higher than 20 ˚C. Cyanobacteria reached Type II level from May, when water temperatures were higher than 15 ˚C.

More than 80% of the *P. minimum* blooms developed when temperatures were between 12 ˚C and 21.4 ˚C. 80% of the *K. veneficum* blooms developed in the temperature range between 20.7 ˚C and 30 ˚C. The water temperature ranges of the two dinoflagellates blooms were significantly different ($p < 0.001$). Therefore, even though these two dinoflagellates typically bloom in similar area in the Chesapeake Bay (Fig. 2-4 and 5), these two species bloomed at different time of the year. *Prorocentrum minimum* blooms started from late spring, while *K. veneficum* bloomed in summer and autumn.

Secchi Depths and TSS

The median secchi depths in the hyper and high eutrophic were less than 0.5 m (Fig. 2-11), which is lower than the thresholds (0.65 m) for underwater sea grass growing in oligohaline and tidal freshwater of Chesapeake Bay area (Carter et al. 1994). Even though freshwater carries massive sediments and particles to the bay and its tributaries, secchi depths in low eutrophic stations were still less than 1.5 m. For *P.*

minimum, *K. veneficum* and cyanobacteria, the median secchi depths decreased from over 1 m to less than 0.5 m as the cell densities increased to bloom level (Fig. 2-11).

The median total suspended solids (TSS) increased from 10 mg L^{-1} in low and medium eutrophic stations to about 26 mg L^{-1} in high and hyper eutrophic stations (Fig. 2-12). TSS values were mostly around than 10 mg L^{-1}, when the two dinoflagellates were observed, except during *P. minimum* blooms, when the median TSS was about 18 mg L^{-1}. The median TSS also increased from 12 mg L^{-1} in stations with Type IV cyanobacteria level to 22 mg L^{-1} in stations with Type I cyanobacteria level.

Dissolved Oxygen

Dissolved oxygen in surface water was replete in most of the reported data. Over 80% of the observations had DO higher than 6 mg L^{-1}, and the median was over 8 mg L^{-1} (Fig. 2-13). This is likely due to the fact that all the samples were taken from less than 0.5 m and wind influenced air-water exchange and the photosynthesis activities kept the surface DO at a high level. All observations were also made during the midday. The hyper eutrophic samples had even higher DO, as the results of high photosynthesis activities of the blooming algae. When *P. minimum* was present, the median DO was higher than 8 mg L^{-1}. However, the median DO decreased to 5.5 mg L^{-1}, when *K. veneficum* bloomed. The median DO in Type I and II cyanobacteria-dominated stations was about 7.5 mg L^{-1}.

Dissolved Carbon and Nutrients

The median of dissolved organic carbon (DOC) in hyper and high eutrophic samples was about 400 μM-C (Fig. 2-14), and 50% of the samples were in the range of 310 - 450 μM-C, while the median of the low eutrophic samples decreased to 250 μM-C. The DOC concentration decreased from 400 μM-C to 230 μM-C as cell densities of *P. minimum* and cyanobacteria species decreased from Type I to Type IV level, but this decrease was not significant ($p>0.05$). The DOC concentrations of *K. veneficum* were steady in a narrow range around 320 μM-C.

Dissolved inorganic nitrogen (DIN, sum of NO_2^-+ NO_3^- and NH_4^+) concentrations were higher in low eutrophic stations. The median was about 36 μM-N in low eutrophic water, and 50% of the samples were in the range of 5 - 70 μM-N (Fig. 2-15). DIN concentrations were lower in the samples characterized as more eutrophic; more DIN was incorporated into biomass. The median decreased to 10-16 μM-N in medium and high eutrophic water, and 75% of the samples were less than 40 μM-N. When *P. minimum* was observed, the median DIN concentrations decreased from ~ 20 μM-N in Type I and II level to 10 μM-N in Type III level, then further decreased to less than 5 μM-N in Type IV level samples, which was significantly lower than the Type VI condition ($p<0.001$). However, an opposite trend was observed as *K. veneficum* cell densities increased, NO_3^- concentrations also increased. The median DIN concentration increased from less than 5 μM in Type I and II level samples to 10 μM-N in Type III, and continuously increased to over 40 μM in Type IV level, which was significantly higher than the Type V condition

($p<0.001$). There was no significant difference in change of DIN when cyanobacteria cell densities changed.

Dissolved inorganic phosphorus (DIP, $\sim PO_4^{3-}$) concentrations did not show a significant trend among stations characterized as different eutrophic levels. About 70% of the samples had DIP concentrations less than 0.5 μM-P (Fig. 2-16). The median concentration of hyper eutrophic stations was 0.4 μM-P, similar to the median of low eutrophic stations. DIP concentrations were around 0.1 μM-P in Type I and II *P. minimum* samples. In the *K. veneficum* samples, the median DIP concentrations were also in the range of 0.1 - 0.2 μM-P. The DIP concentrations were higher when cyanobacterial bloom developed. The median values were about 0.3 - 0.4 μM-P in the Type I, II and III samples, which were significantly higher than the values of Type V samples (0.2 μM-P, $p<0.05$).

Both *P. minimum* and cyanobacteria had significantly higher ambient DIN concentrations than *K. veneficum* when blooms occurred ($p<0.05$). However, both *K. veneficum* and cyanobacteria had significantly higher ambient DIP concentrations than *P. minimum* when blooms occurred ($p<0.05$).

The DIN:DIP ratios of all eutrophic level samples were mostly higher than the Redfield ratio (16), and the median values were in the range 40 to 60. The DIN:DIP ratios varied in a much larger range for the dinoflagellate-dominated samples (Fig. 2-17). The median values of Type I and Type II *P. minimum* samples were ~170, and 50% of DIN:DIP ratios were in the range of 10 - 370. The median value of Type III samples decreased to ~50, and 50% of DIN:DIP ratios were in the range of 10 - 180. The median value of Type IV samples decreased to around 16. An opposite trend was

observed in *K. veneficum*, as *K. veneficum* cell densities increased, DIN:DIP ratios decreased to the Redfield ratio instead. The DIN:DIP ratios at which *P. minimum* blooms were documented were significantly higher than those at which *K. veneficum* and cyanobacteria blooms were documented ($p<0.001$), which had median DIN:DIP values around the Redfield ratio. The median DIN:DIP ratio was about 16 and all the values were under 110 in bloom condition. The median DIN:DIP ratios slightly increased to 20-30 in Type II and III sample, and the upper range of 50% values increased to 200. The median DIN:DIP ratio increased to 360 in Type IV sample, and 50% of the values were in the range of 160 - 500. When cyanobacteria were observed, the DIN:DIP ratios were relatively stable with median value around 16 and 75% of the ratios were less than 70 in all types of samples.

Components of Particulate Matter

Particulate carbon (PC) in the water samples increased with the eutrophic level. The median PC concentrations significantly increased from 65 μM-C under low eutrophic conditions to 380 μM-C ($P<0.001$) under hyper eutrophic conditions (Fig. 2-19). The values varied in a narrow range at each defined eutrophic level. Similar trends were observed for all 3 target species or species groups. Particulate nitrogen (PN) also increased with the eutrophic level. The median PN concentrations increased from 12 μM-N under low eutrophic conations to 24 μM-N under hyper eutrophic conditions (Fig. 2-20). There were no significantly different changes of PN for the HABs, considered here. Particulate phosphorus (PP) also increased with the eutrophic level. The median PP concentrations increased from 1.4 μM-P to 2.7 μM-P (Fig. 2-

21). However, the increase of PP in relation to cell density for each species or species groups was not significant.

The PC:PN ratios of the particulate matter increased from near Redfield ratio (6.6) in middle and low eutrophic observations to a median value of 15 in hyper eutrophic observations (Fig. 2-22). The PC:PP values increased to over 200 in hyper eutrophic samples (Fig. 2-23). A similar trend was observed for *P. minimum*. When this species bloomed, the median of PC:PN increased to 20. Median PC:PP ratios also increased to over 200, when *P. minimum* and *K. veneficum* bloomed. The PC:PP ratios of the particulate matter were generally around 50 in low eutrophic water which was lower than Redfield ratio (106). The PN:PP ratios were in similar range in all eutrophic level samples. Most of the samples had ratios lower than Redfield ratio (16) (Fig. 2-24). The median number of PN:PP were only higher than 16 in Type I, II and III *K. veneficum* samples.

Discussion

A Comparison with Ecosystems with Similar Nutrient Dynamics

In a healthy Chesapeake Bay ecosystem, the most important event for the primary production of the bay is the spring diatom bloom, when the N nutrients accumulate during the winter and spring are consumed to support the production (Malone et al. 1988, Glibert et al. 1995, Malone et al. 1996, Harding et al. 2002). Due to the extensive terrestrial nutrient input in the past several decades (Glibert and Magnien 2004, Hagy et al. 2004, Kemp et al. 2005), the upper bay and its tributaries has been in high eutrophic condition indicated by Chl *a* level. Not only nutrient

concentrations, but also the nutrients ratios could be important for the succession of phytoplankton assemblages (Smayda 1997, Bulgakov and Levich 1999, Stelzer and Lamberti 2001, Vrede et al. 2009). Surveys conducted on the nutrients and phytoplankton in the East China Sea (Li et al. 2009) and southwest Florida Shelf (Heil et al. 2007) have suggested that ambient nutrients ratios may regulate the dominant species of the phytoplankton community. In both systems, the diatom dominated community was found associated the highest ambient DIN:DIP ratios, and the *Prorocentrum* spp. (*P. donghaiense* in the East China Sea and *P. minimum* in southwest Florida Shelf) dominant communities were associated with DIN:DIP ratios approximated higher than, but still close to, Redfield ratio. The *Karenia* spp. (*K. mikimotoi* in the East China Sea and *K. minimum* in southwest Florida Shelf) dominant communities were found in association with DIN:DIP ratio less than Redfield ratio. Remarkable correspondence of community succession was also observed in the Chesapeake Bay (Fig. 2-25). Diatoms bloomed in the spring when the ambient DIN:DIP ratios were the highest, followed by the *P. minimum* blooms when the ambient DIN:DIP ratios began to decrease. *K. veneficum* bloomed in the late summer when ambient DIN:DIP ratios decreased to the Redfield ratio. All three areas appeared to display similar patterns in the species dominance relative to DIN:DIP ratios, except the DIN:DIP ratios during the diatom and *P. minimum* blooms were higher in the Chesapeake Bay, which was due to the relatively higher DIN concentrations (compared to the southwest Florida Shelf) and lower PO_4^{3-} concentrations (compared to the East China Sea) in the Chesapeake Bay.

A Comparison with Spatial Historical Data

Fisher et al. (1992) presented the evidence of seasonal shifts from P to N as the limiting nutrient of phytoplankton biomass based on the data collected in the major axis of the Chesapeake Bay from 1982 to1987. Although the major axis is not a high frequent HAB area compared to the near-shore and tributary areas, there were still data collected along the stations in the same month along the major axis which were associated with the HAB species in the 1990s and 2000s. The decadal variation of salinity, nutrient and chlorophyll data from the 1980s (Fisher et al. 1992) were directly compared with the data in the 1990s and 2000s, along the major axis of the Chesapeake Bay.

The seasonal variation of salinity is significant. The fresh water river flow in the Chesapeake Bay is dominated by the Susquehanna River. A salinity gradient developed from the Susquehanna River mouth to the lower Chesapeake Bay. The river flow in the late spring (May) is higher than late summer (August), and as a result, the surface salinities in May are 3-5 lower than August. The salinity distribution was almost the same over the past 3 decades, except the samples from the 2000s (Fig. 2-26), when the salinities of 2000-2008 in May and August dropped about 10 at stations near the Potomac River mouth. The samples were likely taken after large scale of rainfalls in the nearby watershed, which caused the dilution. River flow from the major tributaries on both sides of the Chesapeake Bay also had significantly dilution effects on the major axis. The dilution impact on the water column was not only on salinity, but also on the water quality which is discussed below.

The seasonal variation of DIN concentrations along the major axis of the Chesapeake Bay is also significant ($p < 0.05$) and well documented (Fisher 1992). In May, the DIN is highest near the head of the Chesapeake Bay (60-100 μM-N) and gradually decreases to 10 μM-N at the mouth of the bay (Fig. 2-27). In August, DIN decreases to 10 μM-N from 80 km away from the head of the bay, and is depleted throughout the bay. However, decadal comparisons suggest that the DIN concentrations in the upper river have increased, as previous study suggested (Hagy et al. 2004, Kemp et al. 2005), which increased almost 20 μM-N every decade in May (Fig. 2-27). In August, the DIN concentrations were 30-50 μM-N higher in recent decades than 1980s. DIN concentration peaks match with the low salinity stations in the 2000s data. The N input from the major tributaries on both sides of the Chesapeake Bay could be important complement for the main channel. The PO_4^{3-} concentrations were slightly higher in the August than in the May. Near the head of the Chesapeake Bay, the PO_4^{3-} concentrations reached 0.7 μM-P in August, but only 0.4 μM in May. In the lower part of the Chesapeake Bay, the PO_4^{3-} concentrations were around 0.2 μM-P in August, but only slightly higher than 0.1 μM-P in May (Fig. 2-28). There was no significant difference among PO_4^{3-} concentrations in three decades ($p > 0.05$), except near the head of the Chesapeake Bay, where the peak value of PO_4^{3-} concentrations increased since 1980s.

Due to the different pattern of the DIN and PO_4^{3-} concentrations, the seasonal ambient DIN:DIP ratio variation is significant (Fig. 2-29). The DIN:DIP ratios are mostly higher than 100 in May, but lower than 100 in August. The ambient DIN:DIP ratios from the 1990s were significantly higher than those of 1980s ($p < 0.01$) in

August. In May, the DIN:DIP ratios only decreased to near Redfield ratio in the lower bay in the 1980s. In August, most of the DIN:DIP ratios were low all three decades, except the first 50 km near the head of the Chesapeake Bay. The DIN:DIP ratios decreased to less than 10 and even less than 1 in the lower bay in the 1980s. However, the DIN:DIP ratios in the past 2 decades only decreased to near Redfield ratio, not as low as the 1980s. In May, the Chl *a* concentrations were highest in the 1980s, when Chl *a* peaks (~40 μg L^{-1}) were observed in the stations about 40 km to the head of the bay and near the mouth of the bay (Fig. 2-30). However, the Chl *a* concentrations were generally lower than 40 μg L^{-1} in the recent two decades. In August, high Chl *a* concentrations were only observed in the upper bay, and there was no significant difference among the three decades.

As shown here and elsewhere (Hagy et al. 2004, Kemp et al. 2005), the DIN concentrations have increased since 1980s, especially in the upper bay. As a result, the overall ambient DIN:DIP ratios increased, which suggests that Chesapeake Bay might be more P limited in the later spring and early summer than the 1980s. The spring blooms depleted DIN (<1 μM-N) in summer in the 1980s, however, in recent decades, DIN in the upper and middle bay remains high (20-50 μM-N). High PO_4^{3-} concentrations were also observed in the upper bay. These PO_4^{3-} could be supplied by P released from the hypoxia zone which has been increased in recent decades (Kemp et al. 2005). Therefore, historical data comparison suggested the Chesapeake Bay is more eutrophic now than the 1980s in the upper and middle bay (Hagy et al. 2004, Kemp et al. 2005), which could provide more nutrients sources available for dinoflagellate species to develop blooms in the summer and early autumn.

The Spatial Variation of Nutrients and HAB Events

Based on the ambient parameters (i.e., salinity and eutrophic level) and spatial distributions of 3 types of harmful algal blooms, the Chesapeake Bay and its tributaries can be divided to 3 zones (Fig. 2-31). Zone I: The tidal fresh water area of the Chesapeake Bay, where surface salinity is generally less than 5, very eutrophic. Zone II: the lower part of the tributaries, upper and middle Chesapeake Bay, where surface salinity is generally between 5 and 15, and eutrophic. Zone III: the middle-lower bay, where surface salinity is higher than 15, but low eutrophic.

Zone I is the area where most of the cyanobacteria blooms developed. This area is characterized by significantly higher nutrient levels than the other 2 zones, due to the terrestrial nutrients input. In winter and early spring, the DIN concentrations accumulates to over 100 μM-N (Fig. 2-32), and the DIP concentration accumulates to over 0.5 μM-P (Fig. 2-33). The ambient DIN:DIP ratios are over 200, suggesting P limitation (Fig. 2-34). When the phytoplankton biomass begins to develop from March (Fig. 2-35, 36), the ambient DIN is consumed and starts to decease, while DIP concentrations are stable, and even slightly increase. As a result, the ambient DIN:DIP ratios start to decrease. When cyanobacterial blooms develop in the summer (Fig. 2-36), the ambient DIN:DIP ratios decrease to median value less than 40, which is a factor of 2 of the Redfield ratio. Cyanobacterial blooms generally last until October.

Zone II is the area where most of the *P. minimum* and *K. veneficum* blooms were documented. This area has lower nutrients compared to Zone I. In winter and early spring, the DIN concentrations are generally less than 50 μM-N (Fig. 2-32), and

the DIP concentrations accumulate to around 0.2 μM-P (Fig. 2-33). The ambient DIN:DIP ratios can reach over 500, suggesting P limitation (Fig. 2-34). The peak of *P. minimum* blooms occurred in April and May in both frequency and biomass, when the ambient DIN:DIP ratios dropped to 200. When *P. minimum* biomass accumulates in these high N low P environments, the ambient DIN further drops to ~ 20 μM-N in June, but PO_4^{3-} increases. When *K. veneficum* blooms develop in summer and autumn (June to September), the ambient DIN:DIP ratios drop to near the Redfield ratio. These changes are not only caused by the decrease of DIN, but also by the significantly increased DIP concentrations, which increases from ~ 0.2 μM-P to ~ 1 μM-P.

Zone III is the area mostly under the influence of oceanic water of all 3 zones. It is characterized as low DIN (mostly less than 10 μM-N), and low DIP (~ 0.1 μM-P), even in the early spring (Fig. 2-32, 33). The Chl *a* concentrations are less than 20 μg L^{-1} most times of the year and there are generally no significant blooms of the species groups considered here. The ambient DIN:DIP ratios drop from over 100 in the spring to near the Redfield ratio in July. The low DIN:DIP ratios last until November, even though there is no significant decrease in DIN concentrations.

The decrease of NO_3^- concentrations from spring to late summer was caused by the consumption by phytoplankton communities. However, the maxima of DIP were observed in the late summer. It has been suggested that the DIP is from the remineralization of organic matter (Taft and Taylor 1976, Boynton and Kemp 1985, Jordan et al. 2008). Spring and summer blooms and terrestrial input accumulate massive organic matter in the bottom layer of the Chesapeake Bay and its tributaries.

DIP released from the remineralization may still not available to the primary producer due to absorption of ferric (Fe^{3+}) oxides (Jordan et al. 2008). Decomposition of organic matter consumes the oxygen in the bottom layer after stratification develops in the water column in summer, and hypoxia develops in the bottom layer. DIP escapes precipitation when ferric oxides were reduced in the anoxic condition (Boynton and Kemp 1985). This DIP can be vertically transported to the euphotic layer and be available for the phytoplankton, or can be accessed by dinoflagellates which can vertically immigrate to the deeper layer of the water column.

The Environmental Factors and HAB Species

In a eutrophic estuary like Chesapeake Bay, the potential relationship between the frequency of HABs and increased anthropogenic nutrient inputs to coastal waters is a particular concern (Glibert et al. 2005a), as the phytoplankton assemblages will respond to excessive nutrient inputs with increased biomass and decreased diversity, and nutrient enrichment generally promotes a shift in dominance from diatoms to dinoflagellates and cyanobacteria (Pinckney et al. 2001, Anderson et al. 2008). The variations in nutrient loadings and supply ratios can regulate algal growth rates and community composition (Rudek et al. 1991, Smayda 1997, Heil et al. 2007).

Herein, species specific correspondences were found between selected HAB species and nutrients and other related environmental variables. These correspondences are consistent with the physiological characters of each species, which are reported in previous field and laboratory studies.

Prorocentrum minimum blooms have been often linked to eutrophication and occur in waters influenced by freshwater inputs (coastal waters and estuaries) and/or

anthropogenic loads (Heil et al. 2007, Glibert et al. 2001). In the lower part of the tributaries and the upper Chesapeake Bay, nutrients accumulate in the winter and early spring and DIN concentration can reach over 50 μM-N (Fig. 2-32). *Prorocentrum minimum* may have competitive advantages over other phytoplankton populations in such nutrient-enriched, but very turbid environments. *Prorocentrum minimum* can survive in dark environment for months (Harding and Coats 1988), and grow well under low light conditions (Grzebyk and Berland 1996). *Prorocentrum minimum* also shows light independent N uptake ability (Fan and Glibert 2005). *Prorocentrum minimum* appears to be a good competitor in such high turbidity waters. Although the DIP concentrations are generally very low (~ 0.1 μM-P, Fig. 2-16) during the *P. minimum* blooms, *P. minimum* is capable to induce alkaline phosphatases and utilize organic phosphorus (Dyhrman 2005).

In contrast to *P. minimum* which bloomed in high DIN water, *Karlodinium veneficum* were generally associated with high DIP concentrations and low DIN:DIP ratio (lower than Redfield ratio) water in the summer. *Karlodinium veneficum* developed blooms, even when ambient DIN has been depleted. *Karlodinium veneficum*, known as a mixotrophic dinoflagellate, may have competitive advantages in such waters. It can take up organic nitrogen to support growth, and ingest bacteria and cryptophytes (e.g. *Rhodomonas salina*) (Nygaard and Tobiesen 1993, Li et al. 1996, Li et al. 1999, Li et al. 2000, Adolf et al. 2003, Adolf et al. 2006a, Adolf et al. 2007, Adolf et al. 2008). Growth rates of mixotrophic *K. veneficum* (0.52 - 0.75 day^{-1}) have been found to be comparable to or greater than maximum growth rate in phototrophic mode (0.55 day^{-1}) (Adolf et al. 2006b). Phagotrophy of *K. veneficum* has

been intensively studied in Chesapeake Bay (Li et al. 1996, Li et al. 1999, Li et al. 2000, Adolf et al. 2003, Adolf et al. 2006a, Adolf et al. 2007, Adolf et al. 2008). From field and laboratory data, phagotrophy of *K. veneficum* was positively correlated with prey density (Li et al. 2000). N and/or P deficiency, or N:P ratios that were substantially higher or lower than the optimum N:P ratio (~10), also increased phagotrophic activity, suggesting feeding as a supplement for major nutrients (N, P) (Li et al., 2000). Phagotrophic activity might also supplement organic carbon sources or assist in acquiring trace growth factors (Li et al., 2000). High cryptophyte abundance in the summer has been suggested to trigger *K. veneficum* blooms as additional nutrient source in the Chesapeake Bay area (Adolf et al. 2008).

Cyanobacterial blooms (not including the picocyanobacteria species) are most common in the tidal freshwater of the Chesapeake Bay (Marshall et al. 2006), which is spatial difference from the regions of maximum abundance of the two dinoflagellates, which generally did not bloom in such fresh water. High frequent cyanobacterial blooms are generally observed in the summer and associated with high DIP, low DIN:DIP ratio water. Some cyanobacteria (e.g. *Anabaena* spp.) have been reported to be capable of fixing N_2 as a new N source in relative low N waters in the Chesapeake Bay (Burns et al. 2002, Moisander et al. 2007). This N_2-fixation ability may an important competitive advantage for these cyanobacteria to develop blooms.

Cyanobacterial blooms are also associated with very turbid water (secchi dept ~ 0.5 m). Cyanobacteria can possess gas vesicles in the cell, or/and form filaments to keep high buoyancy and stay in the surface water (Sellner 1997), where they can

reach light and N_2. These characters make cyanobacteria excellent competitors in the low N, high turbidity freshwater.

In summary, *P. minimum* blooms generally develop in high DIN water in the spring and early summer in mesohaline water of Chesapeake Bay, while *K. veneficum* blooms develop after DIN was depleted in similar areas. These trends were consistent with East China Sea and west Florida shelf for analogous species. Cyanobacterial blooms (not including the picocyanobacteria species) generally develop in the tidal freshwater of the Chesapeake Bay in the summer. Each species has corresponding physiological strategies that may provide completive advantages to the environments. The eutrophic areas in the Chesapeake Bay are the high frequent bloom areas. *Prorocentrum minimum*, *K. veneficum* and cyanobacterial blooms also further decreased the light availability in the water column.

References

Adolf J, Bachvaroff T, Place AR (2007) Cryptophytes drive blooms of mixotrophic harmful algae: A testable hypothesis based on *Karlodinium veneficum* in Chesapeake Bay. Journal of Phycology 43:31-31

Adolf JE, Bachvaroff T, Place AR (2008) Can cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? Harmful Algae 8:119-128

Adolf JE, Bachvaroff TR, Krupatkina DN, Nonogaki H, Brown PJP, Lewitus AJ, Harvey HR, Place AR (2006a) Species specificity and potential roles of *Karlodinium micrum* toxin. African Journal of Marine Science 28:415-419

Adolf JE, Stoecker DK, Harding LW (2003) Autotrophic growth and photoacclimation in *Karlodinium micrum* (Dinophyceae) and *Storeatula major* (Cryptophyceae). Journal of Phycology 39:1101-1108

Adolf JE, Stoecker DK, Harding LW (2006b) The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). Journal of Plankton Research 28:737-751

Adolf JE, Yeager CL, Miller WD, Mallonee ME, Harding LW (2006c) Environmental forcing of phytoplankton floral composition, biomass, and primary productivity in Chesapeake Bay, USA. Estuarine Coastal and Shelf Science 67:108-122

Andersen JH, Schluter L, Ærtebjerg G (2006) Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. Journal of Plankton Research 28:621-628

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Anderson SM, Roels OA (1981) Effects of light-intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Marine Biology 62:257-261

Behrenfeld MJ, Prasil O, Babin M, Bruyant F (2004) In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. Journal of Phycology 40:4-25

Berthouex PM, Pallesen L, Booman K, Sedlack R (1983) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 55:323-325

Bricker, S.B., C.G. Clement, D.E. Pirhalla, S.P. Orlando, and D.R.G. Farrow. 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. Silver Spring, MD: National Oceanic and Atmospheric Administration (NOAA), National Ocean Service. 71

Bulgakov NG, Levich AP (1999) The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv Fur Hydrobiologie 146:3-22

Burkholder JM, Glasgow HB (1997) *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnology and Oceanography 42:1052-1075

Burns JA, Zehr JP, Capone DG (2002) Nitrogen-fixing phylotypes of Chesapeake Bay and Neuse River estuary sediments. Microbial Ecology 44:336-343

Chai C, Yu ZM, Song XX, Cao XH (2006) The status and characteristics of eutrophication in the Yangtze River (Changjiang) estuary and the adjacent East China Sea, China. Hydrobiologia 563:313-328

Cochlan WP, Harrison PJ, Denman KL (1991) Diel periodicity of nitrogen uptake by marine-phytoplankton in nitrate-rich environments. Limnology and Oceanography 36:1689-1700

Doemel WN, Brooks AE (1975) Detergent phosphorus and algal growth. Water Research 9:713-719

Doering PH, Oviatt CA, Nowicki BL, Klos EG, Reed LW (1995) Phosphorus and nitrogen limitation of primary production in a simulated estuarine gradient. Marine Ecology-Progress Series 124:271-287

Duan SW, Liang T, Zhang S, Wang LJ, Zhang XM, Chen XB (2008) Seasonal changes in nitrogen and phosphorus transport in the lower Changjiang River before the construction of the Three Gorges Dam. Estuarine Coastal and Shelf Science 79:239-250

Dyhrman, S., 2005. Ectoenzymes in *Prorocentrum minimum*: alkaline phosphatase and leucine aminopeptidase. Harmful Algae 4, 619–627

Eppley RW, Rogers JN, Mccarthy JJ, Sournia A (1971) Light/dark periodicity in nitrogen assimilation of marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. Journal of Phycology 7:150-154

Flynn KJ (2002) How critical is the critical N : P ratio? Journal of Phycology 38:961-970

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Granéli E, Anderson DM (2008) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Glibert PM, Magnien R, Lomas MW, Alexander J, Fan CL, Haramoto E, Trice M, Kana TM (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. Estuaries 24:875-883

Goldman JC, Mccarthy JJ, Peavey DG (1979) Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210-215

Goolsby DA, Battaglin WA (2000) Nitrogen in the Mississippi basin-estimating sources and predicting flux to the Gulf of Mexico. USGS Fact Sheet 135-00

Hagy JD, Boynton WR, Keefe CW, Wood KV (2004) Hypoxia in Chesapeake Bay, 1950-2001: Long-term change in relation to nutrient loading and river flow. Estuaries 27:634-658Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99

Hartig JH, Horvath FJ (1982) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 54:193-197

Healey FP (1977) Ammonium and urea uptake by some freshwater algae. Canadian Journal of Botany-Revue Canadienne De Botanique 55:61-69

Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography 38:709-724

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8:3-13

Hodgkiss IJ, Ho KC (1997) Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147

Hoffman FA, Bishop JW (1994) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia. Water Research 28:1239-1240

Howarth RW (2008) Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae 8:14-20

Howarth RW, Boyer EW, Pabich WJ, Galloway JN (2002) Nitrogen use in the United States from 1961–2000 and potential future trends. AMBIO 32:88-96

Jaworski NA, Howarth RW, Hetling LI (1997) Atmospheric deposition of nitrogen oxides onto the landscape contributes to coastal eutrophication in the northeast United States. Environmental Science & Technology 31:1995-2004

Jordan TE, Cornwell JC, Boynton WR, Anderson JT (2008) Changes in phosphorus biogeochemistry along an estuarine salinity gradient: The iron conveyer belt. Limnology and Oceanography 53:172-184

Justic D, Rabalais NN, Turner RE (1995) Stoichiometric nutrient balance and origin of coastal eutrophication. Marine Pollution Bulletin 30:41-46

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33:1441-1450

Lagus A, Suomela J, Weithoff G, Heikkila K, Helminen H, Sipura J (2004) Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. Journal of Plankton Research 26:779-798

Lee GF, Joneslee A (1995) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia - comment. Water Research 29:1425-1426

Lee YS, Seiki T, Mukai T, Takimoto K, Okada M (1996) Limiting nutrients of phytoplankton community in Hiroshima Bay, Japan. Water Research 30:1490-1494

Li AS, Stoecker DK, Adolf JE (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquatic Microbial Ecology 19:163-176

Li AS, Stoecker DK, Coats DW (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. Journal of Plankton Research 22:2105-2124

Li AS, Stoecker DK, Coats DW, Adam EJ (1996) Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquatic Microbial Ecology 10:139-147

Li J, Glibert PM, Zhou MJ (2010) Temporal and spatial variability in nitrogen uptake kinetics during harmful dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou MJ, Lu SH, Lu DD (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology-Progress Series 383:11-26

Maki AW, Porcella DB, Wendt RH (1984) The impact of detergent phosphorus bans on receiving water-quality. Water Research 18:893-903

Moisander PH, Morrison AE, Ward BB, Jenkins BD, Zehr JP (2007) Spatial-temporal variability in diazotroph assemblages in Chesapeake Bay using an oligonucleotide nifH microarray. Environmental Microbiology 9:1823-1835

Mulholland MR, Ohki K, Capone DG (1999) Nitrogen utilization and metabolism relative to patterns of N_2 fixation in cultures of *Trichodesmium* NIBB1067. Journal of Phycology 35:977-988

Nygaard K, Tobiesen A (1993) Bacterivory in algae - a survival strategy during nutrient limitation. Limnology and Oceanography 38:273-279

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42:1154-1165

Paerl HW, Valdes LM, Joyner AR, Piehler MF (2004) Solving problems resulting from solutions: Evolution of a dual nutrient management strategy for the eutrophying Neuse river estuary, North Carolina. Environmental Science & Technology 38:3068-3073

Phillips DJH, Tanabe S (1989) Aquatic pollution in the Far-East. Marine Pollution Bulletin 20:297-303

Pinckney JL, Paerl HW, Tester P, Richardson TL (2001) The role of nutrient loading and eutrophication in estuarine ecology. Environmental Health Perspectives 109:699-706

Radach G, Berg J, Hagmeier E (1990) Long-term changes of the annual cycles of meteorological, hydrographic, nutrient and phytoplankton time-series at Helgoland and at Lv Elbe 1 in the German Bight. Continental Shelf Research 10:305-328

Riegman R (1995) Nutrient-related selection mechanisms in marine phytoplankton communities and the impact of eutrophication on the planktonic food web. Water Science and Technology 32:63-75

Sharfstein B, Roels OA, Harris V, Lee V (1977) Effect of detergent legislation on phosphorus in effluent and receiving waters. Journal Water Pollution Control Federation 49:2017-2021

Shen ZL, Liu Q, Zhang SM, Miao H, Zhang P (2003) A nitrogen budget of the Changjiang River catchment. Ambio 32:65-69

Smayda T (1990) Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, New York

Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42:1137-1153

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems - A global problem. Environmental Science and Pollution Research 10:126-139

Smith VH (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnology and Oceanography 51:377-384

Smith VH, Joye SB, Howarth RW (2006) Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51:351-355

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179-196

Stelzer RS, Lamberti GA (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography 46:356-367

Tango P, Butler W, Lacouture R, Goshorn D, Magnien R, Michael B, Hall H, Browhawn K, Wittman R, Betty W (2004) An unprecedented bloom of *Dinophysis acuminata* in Chesapeake Bay. In: Steidinger KA, Landsberg JA, Tomas CR, Vargo GA (eds) Proceedings of the Xth International Conference on Harmful Algae, St. Petersburg, Florida, p 358–363

Tango PJ, Butler W (2008) Cyanotoxins in tidal waters of Chesapeake Bay. Northeastern Naturalist 15:403-416

Tango PJ, Magnien R, Butler W, Luckett C, Luckenbach M, Lacouture R, Poukish C (2005) Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. Harmful Algae 4:525-531

Tett P, Heaney SI, Droop MR (1985) The Redfield ratio and phytoplankton growth-rate. Journal of the Marine Biological Association of the United Kingdom 65:487-504

USDA (2008) Fertilizer consumption and use. United States Department of Agriculture. http://www.ers.usda.gov/Data/FertilizerUse/

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2009) Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54:331-344

Wheeler PA, Olson RJ, Chisholm SW (1983) Effects of photocycles and periodic ammonium supply on 3 marine-phytoplankton species .2. ammonium uptake and assimilation. Journal of Phycology 19:528-533

Yan WJ, Zhang S (2003) The composition and bioavailability of phosphorus transport through the Changjiang (Yangtze) River during the 1998 flood. Biogeochemistry 65:179-194

Zhang H, Litaker W, Vandersea MW, Tester P, Lin SJ (2008) Geographic distribution of *Karlodinium veneficum* in the US east coast as detected by ITS-ferredoxin real-time PCR assay. Journal of Plankton Research 30:905-922

Zhang J, Liu SM, Ren JL, Wu Y, Zhang GL (2007) Nutrient gradients from the eutrophic Changjiang (Yangtze River) Estuary to the oligotrophic Kuroshio waters and re-evaluation of budgets for the East China Sea Shelf. Progress in Oceanography 74:449-478

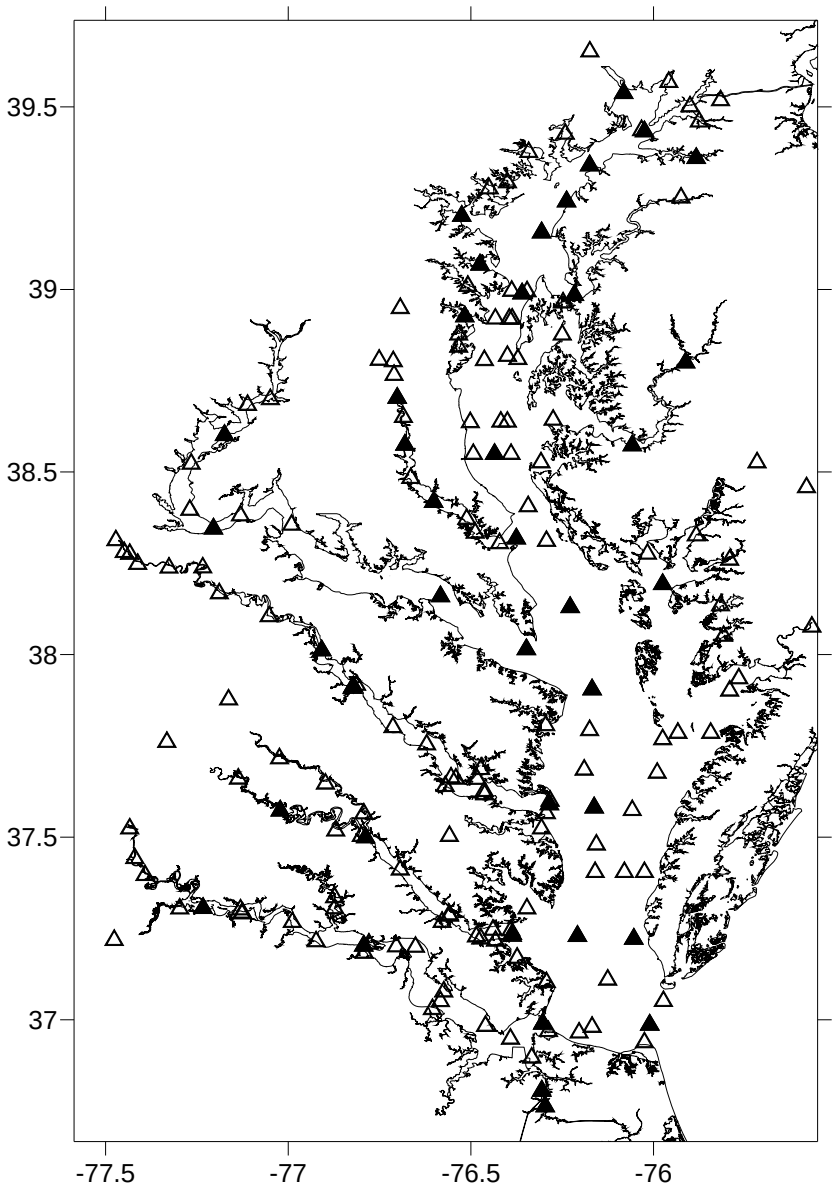


Figure 2-1 The routinely sampled stations (all the triangles) in the Chesapeake Bay and its tributaries by Maryland Departments of Natural Resources. Black triangles: the stations where *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria were recorded (cyanobacteria only include the species which could be counted in the resolution limit of light microscopy).

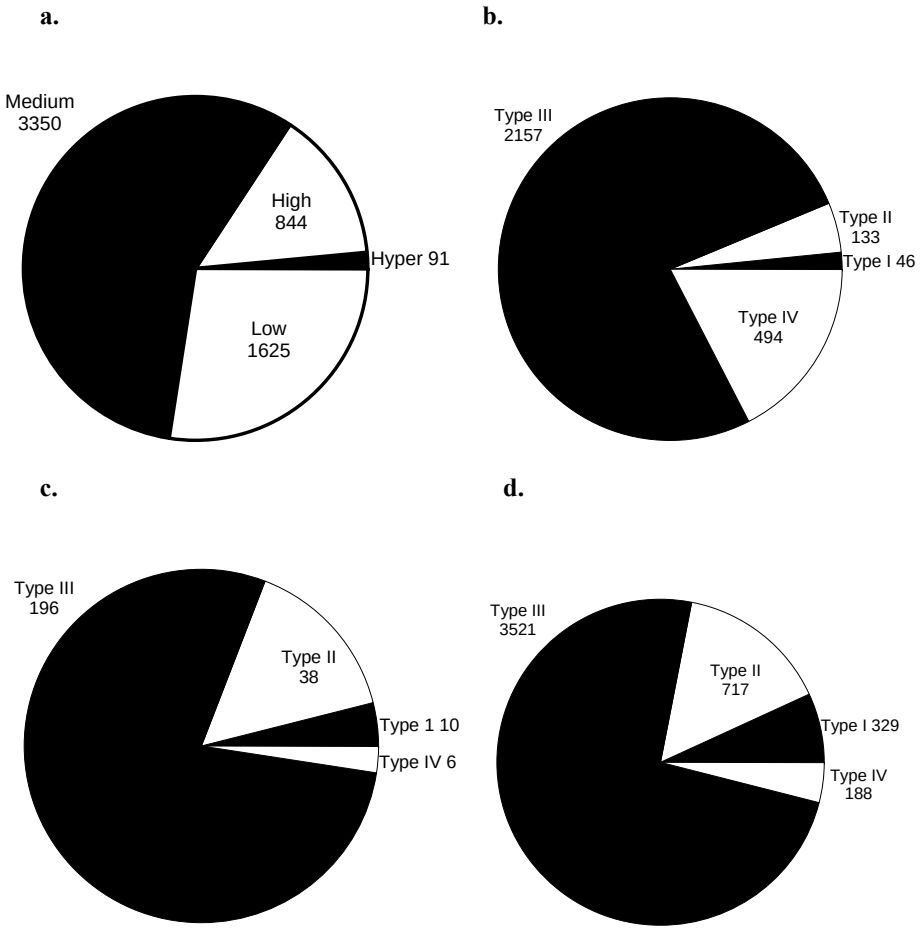


Figure 2-2 a. the number of samples of different type of eutrophic level chlorophyll *a* recorded at Chesapeake Bay area (1991-2008); b, the number of samples of different type of *Prorocentrum minimum* cell density level recorded; c, the number of samples of different type of *Karlodinium veneficum* cell density level recorded; d, the number of samples of different type of cyanobacteria cell density level recorded. Type I: hypereutrophic; type II: high eutrophic; Type III: medium eutrophic; Type IV: low eutrophic.

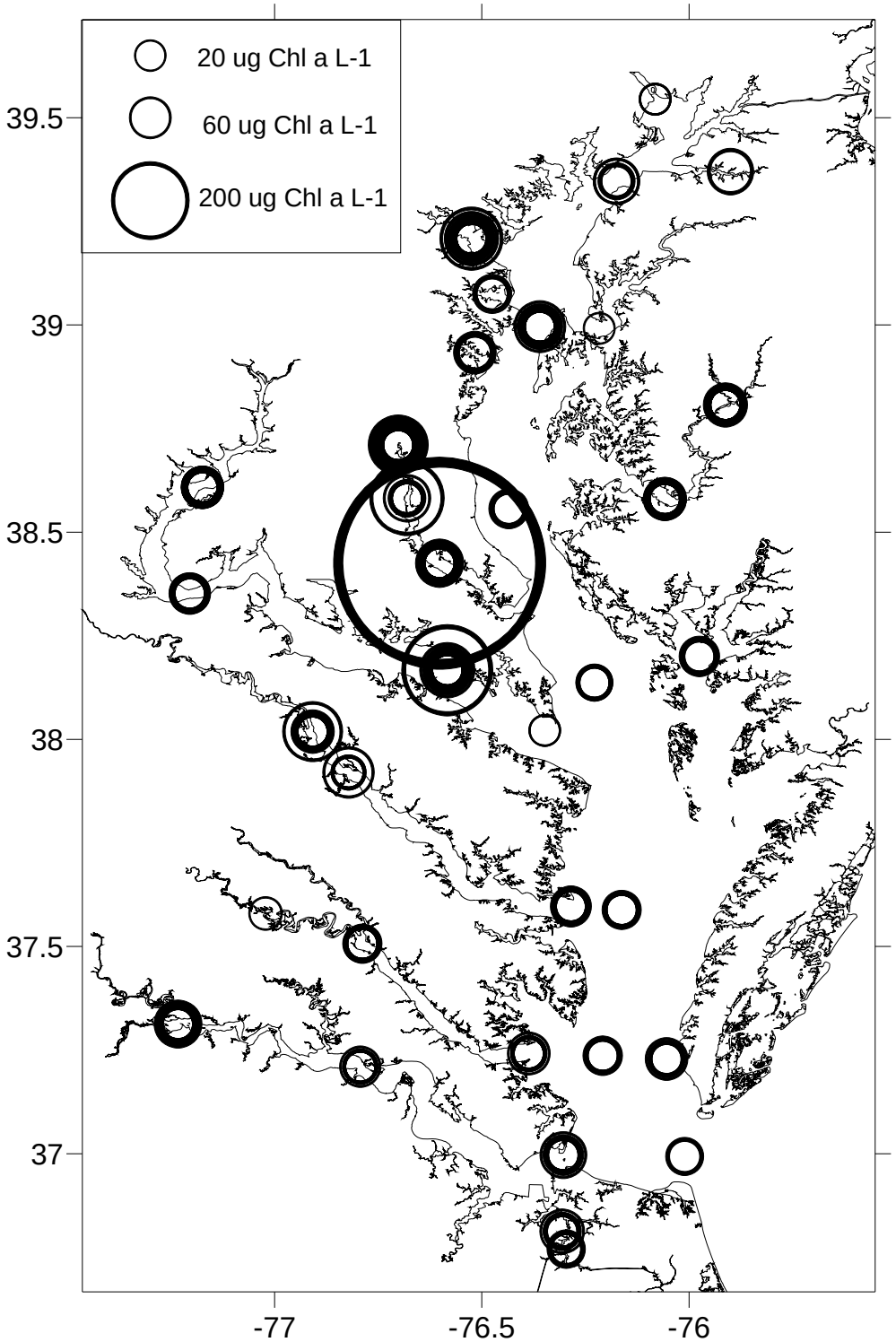


Figure 2-3 The spatial distribution of Chlorophyll *a* samples (> 10 mg L^{-1}) in the Chesapeake Bay and its tributaries 1991-2008. Data were derived from routinely sampling by Maryland Department of Natural Resources. Each circle stands for one sample, and the diameters of the circles are propositional to the Chlorophyll *a* concentrations according to the scale in the inset. The circles overlap at stations where high Chlorophyll *a* concentrations were frequently recorded.

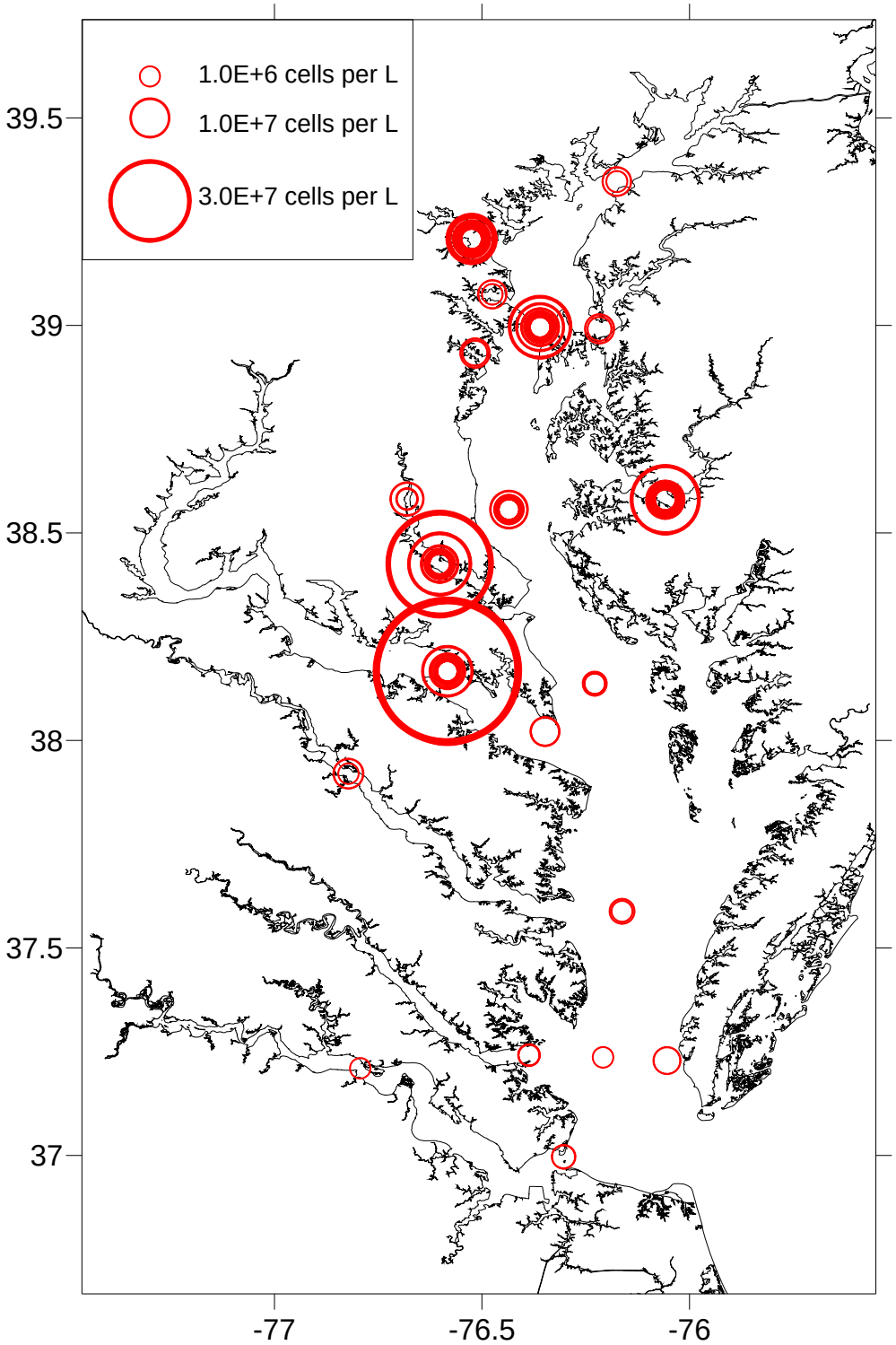


Figure 2-4 The spatial distribution of *Prorocentrum minimum* (cell density > 1,000,000 cells L^{-1}) in the Chesapeake Bay and its tributaries 1991-2008. Data were derived from routinely sampling by Maryland Department of Natural Resources. Each circle stands for one sample, and the diameters of the circles are propositional to the cell densities according to the scale in the inset. The circles overlap at stations where cells were frequently recorded.

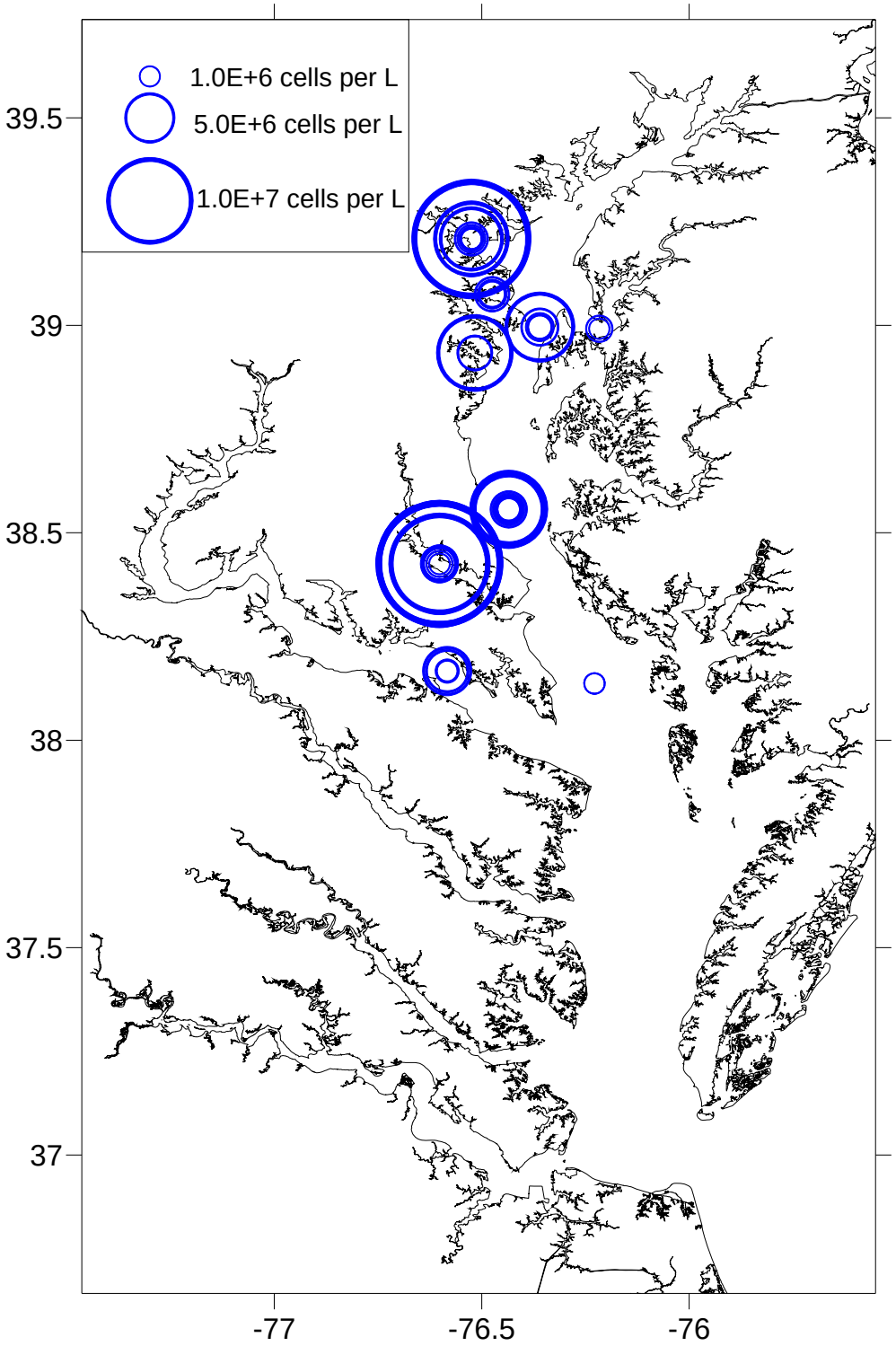


Figure 2-5 The spatial distribution of *Karlodinium veneficum* (cell density > 1,000,000 cells L^{-1}) in the Chesapeake Bay and its tributaries 2003-2008. Data were derived from routinely sampling by Maryland Department of Natural Resources. Each circle stands for one sample, and the diameters of the circles are propositional to the cell densities according to the scale in the inset. The circles overlap at stations where cells were frequently recorded.

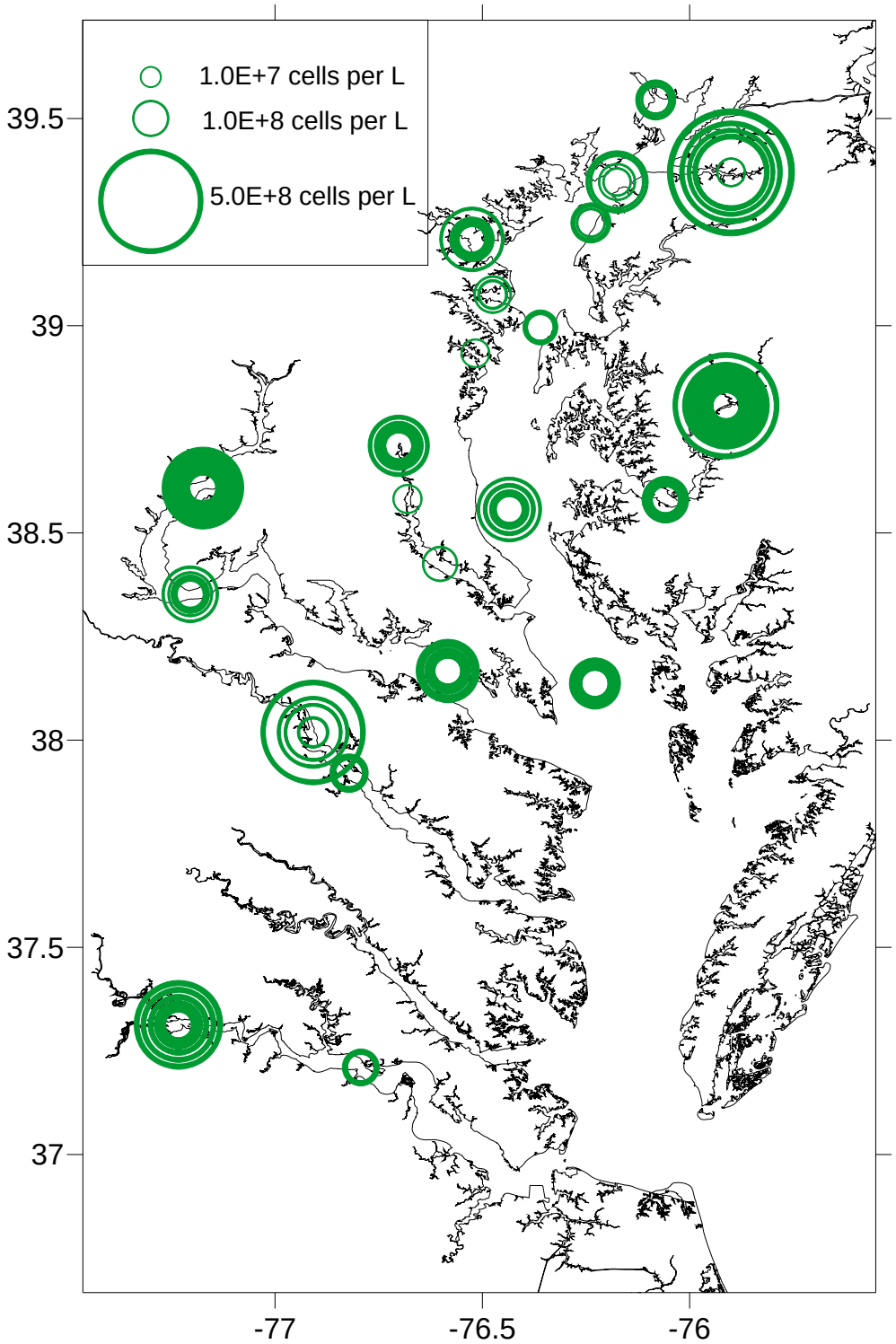


Figure 2-6 The spatial distribution of cyanobacteria (cell density > 10,000,000 cells L^{-1}) in the Chesapeake Bay and its tributaries 1991-2008. Data were derived from routinely sampling by Maryland Department of Natural Resources. Each circle stands for one sample, and the diameters of the circles are propositional to the cell densities according to the scale in the inset. The circles overlap at stations where cells were frequently recorded.

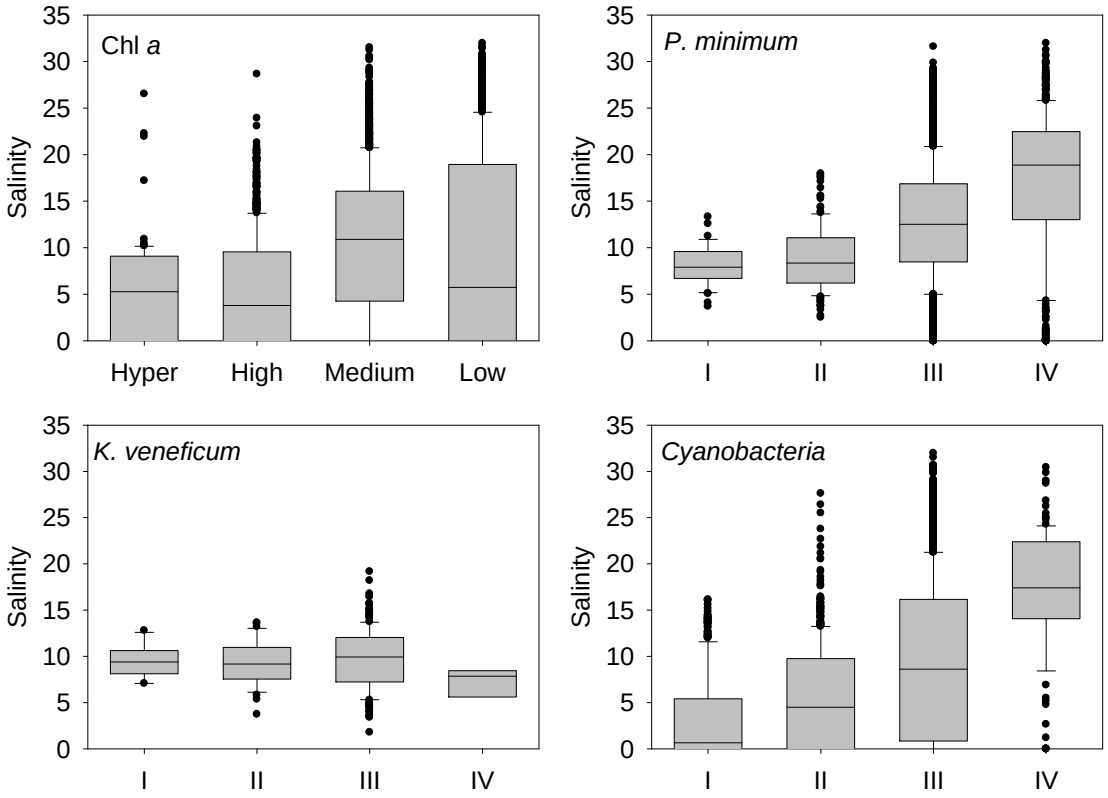


Figure 2-7 The salinity of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

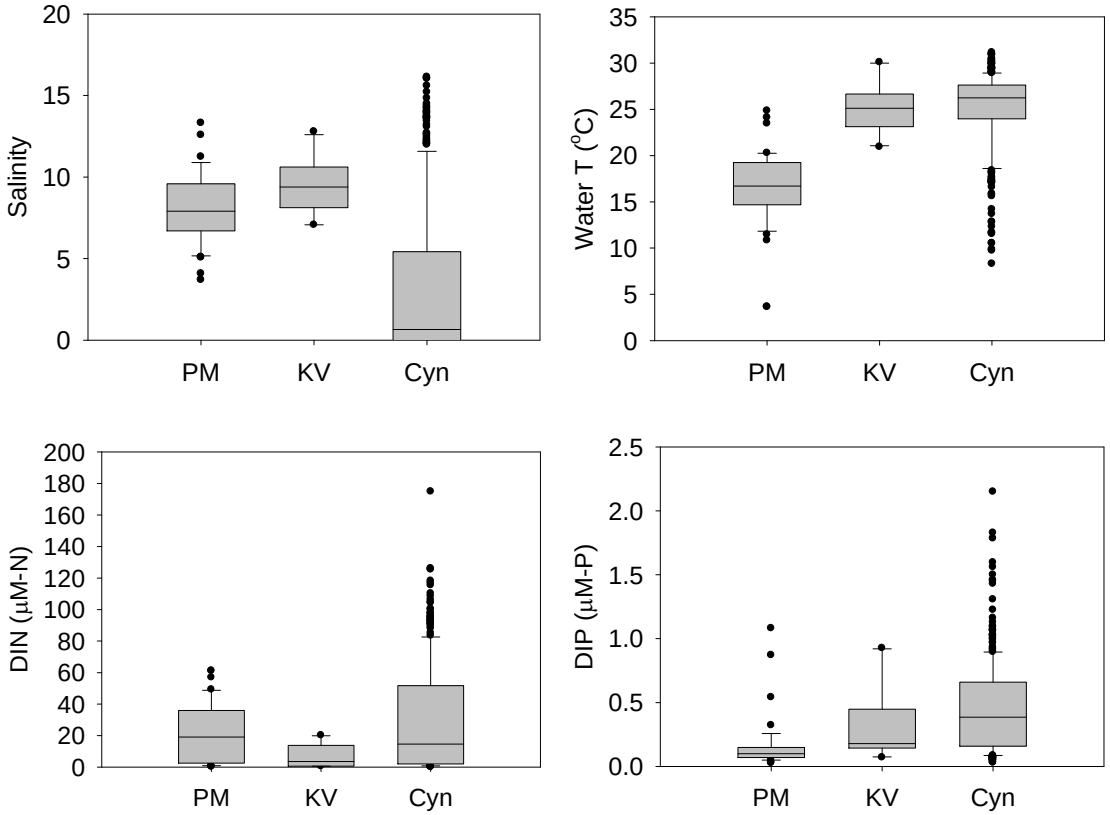


Figure 2-8 The ambient parameters (salinity, water temperature, dissolved inorganic nitrogen and phosphorus) of *Prorocentrum minimum* (PM), *Karlodinium veneficum* (KV) and cyanobacteria (Cyn) in blooms in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

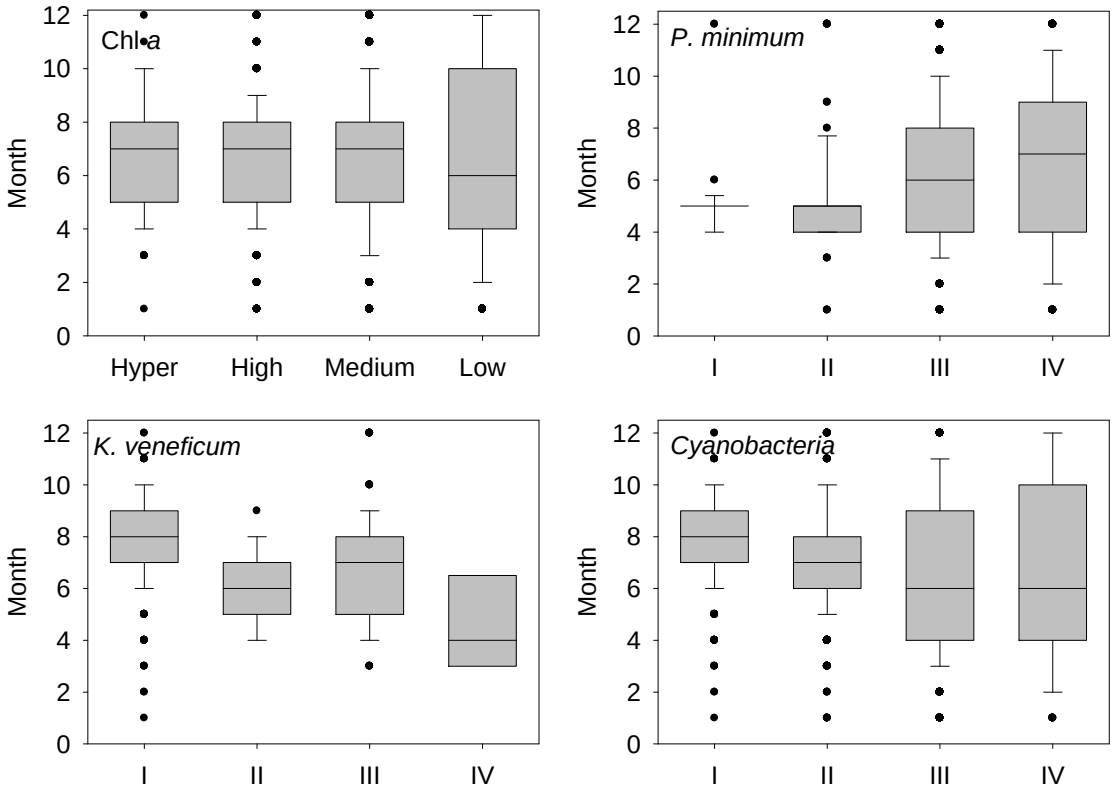


Figure 2-9 The month number of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

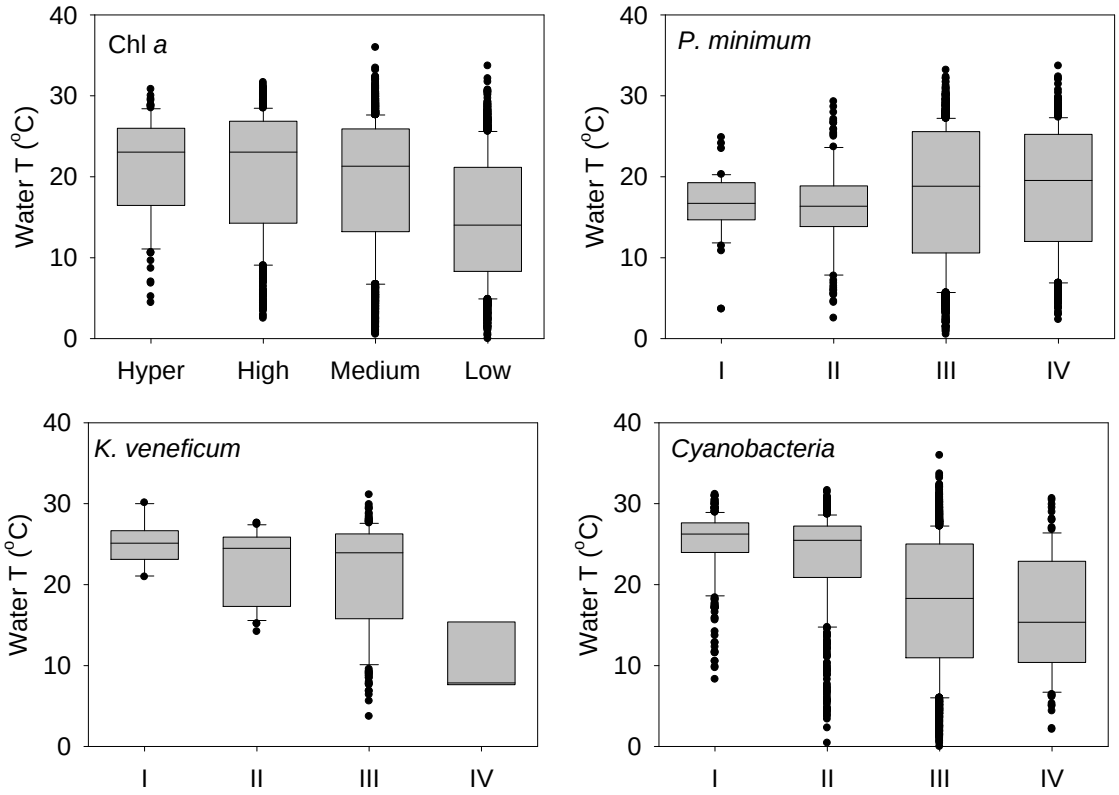


Figure 2-10 The water temperature of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

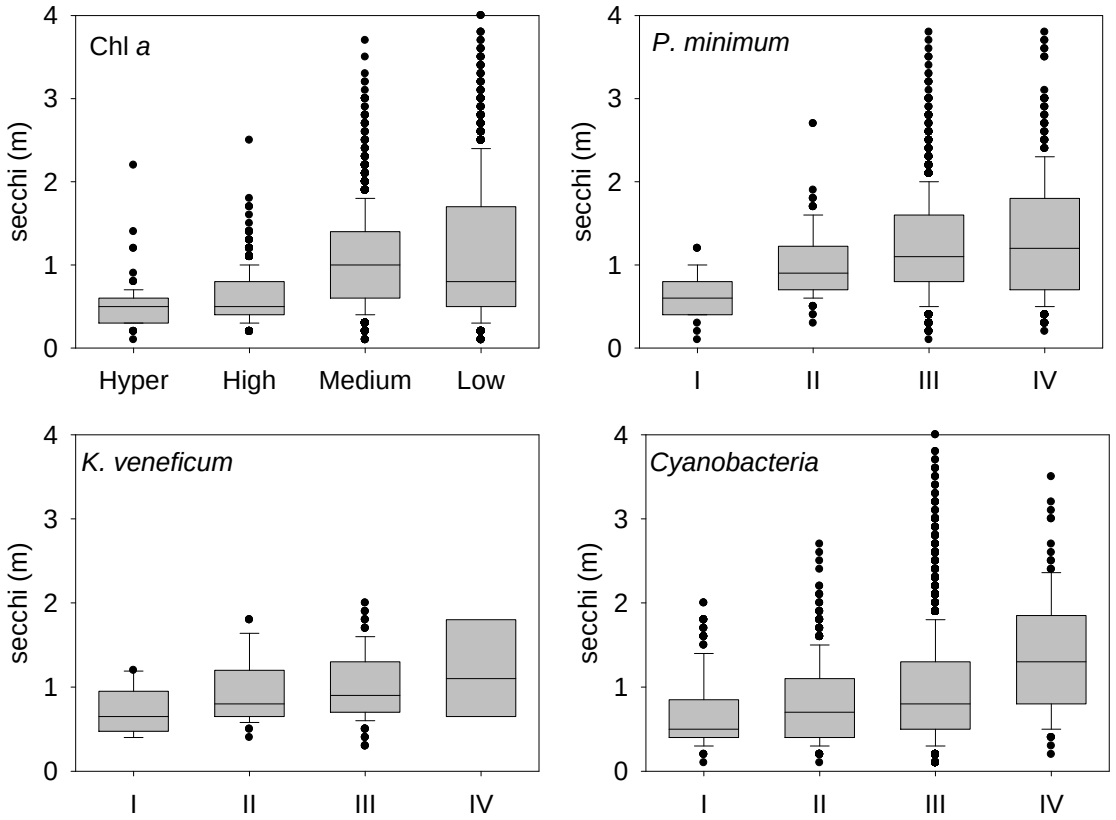


Figure 2-11 The secchi depth of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

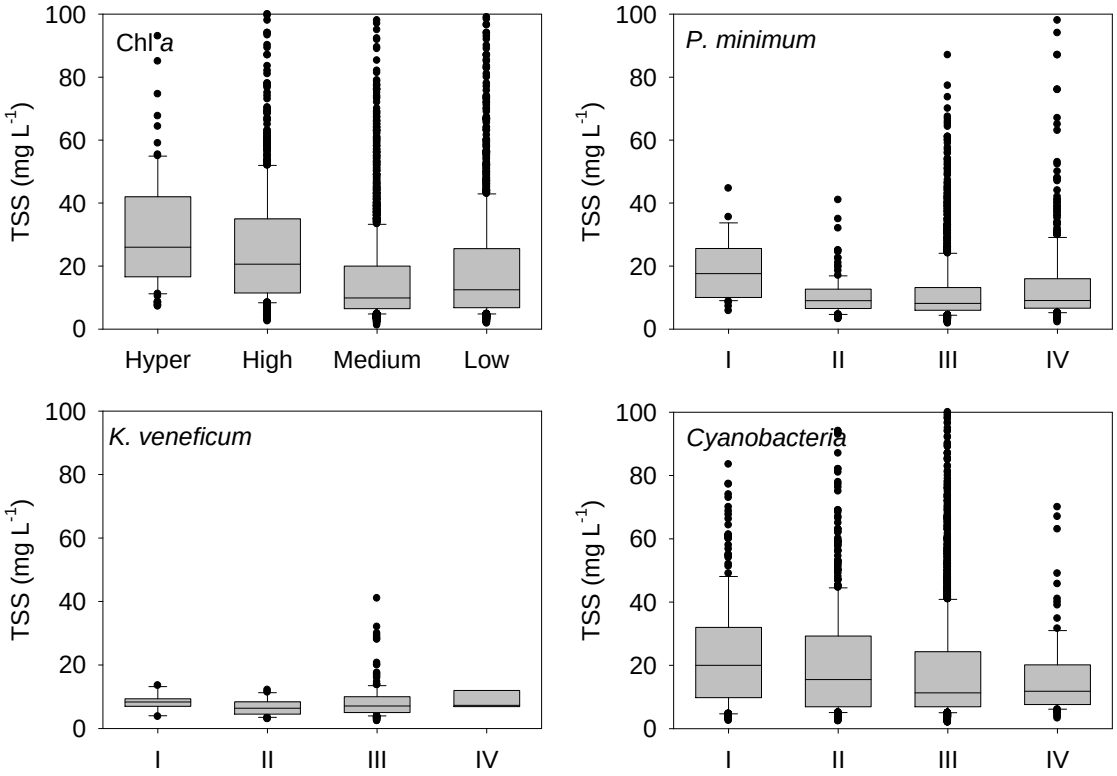


Figure 2-12 The total suspended solids (TSS) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

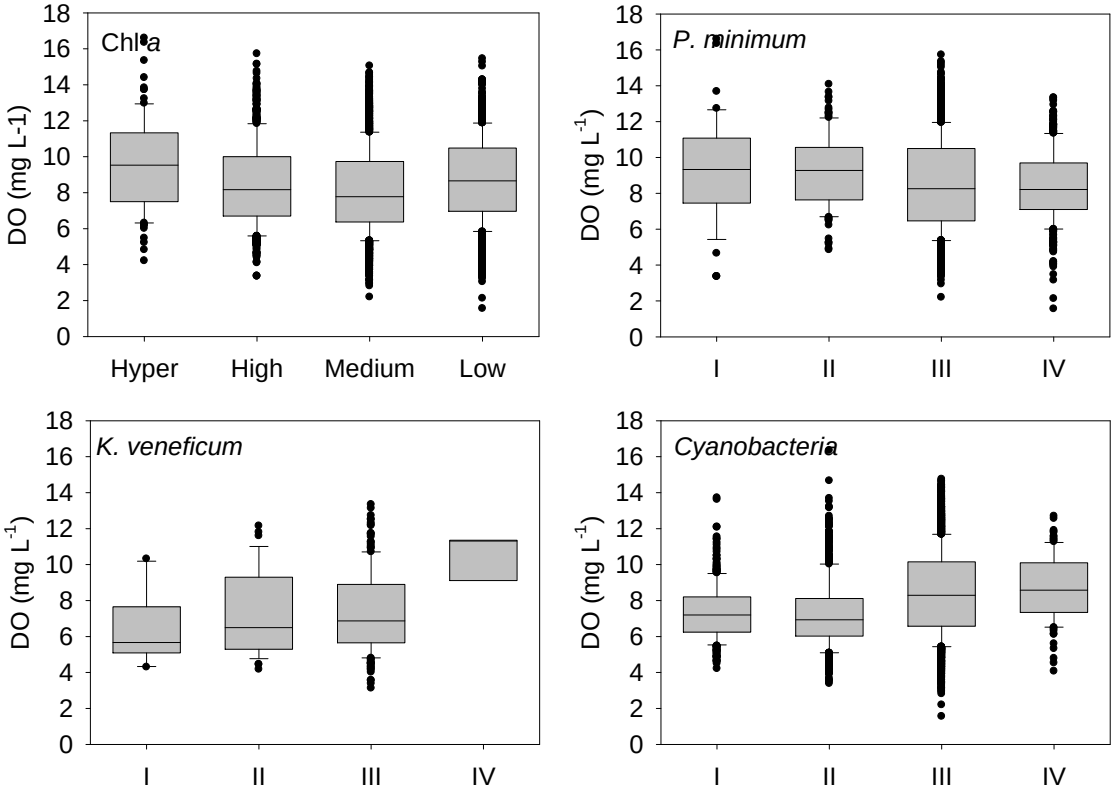


Figure 2-13 The dissolved oxygen (DO) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

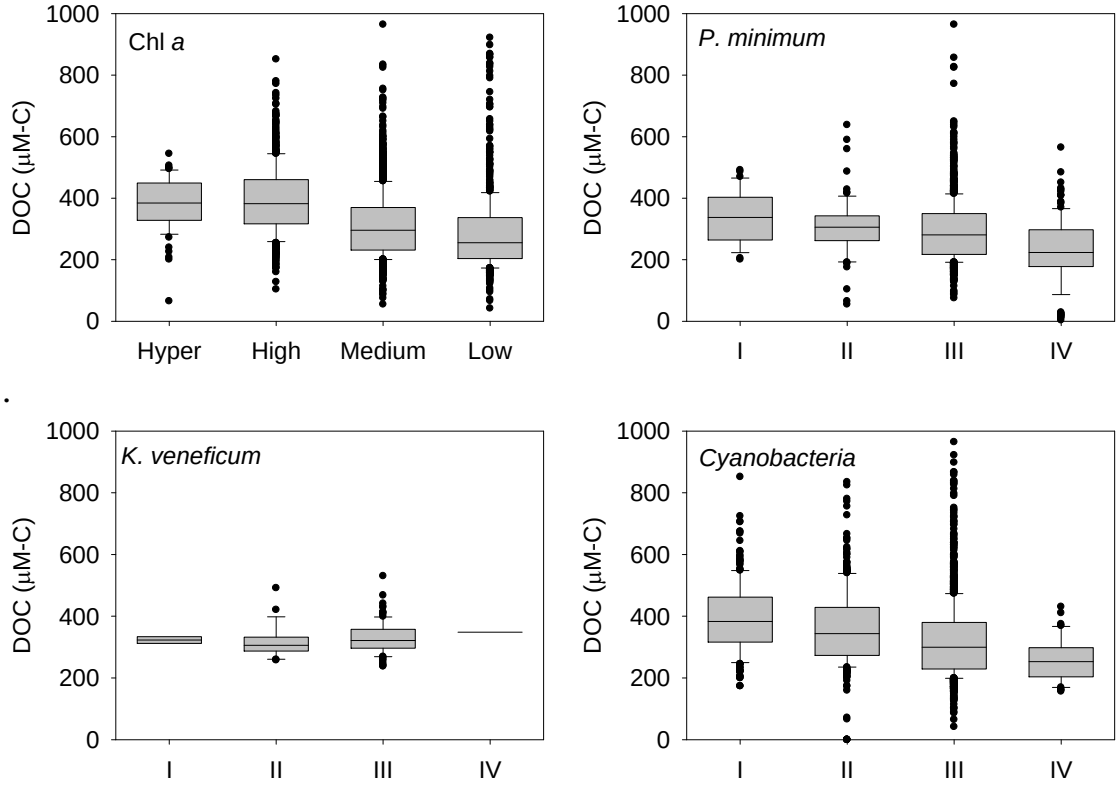


Figure 2-14 The dissolved organic carbon (DOC) of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

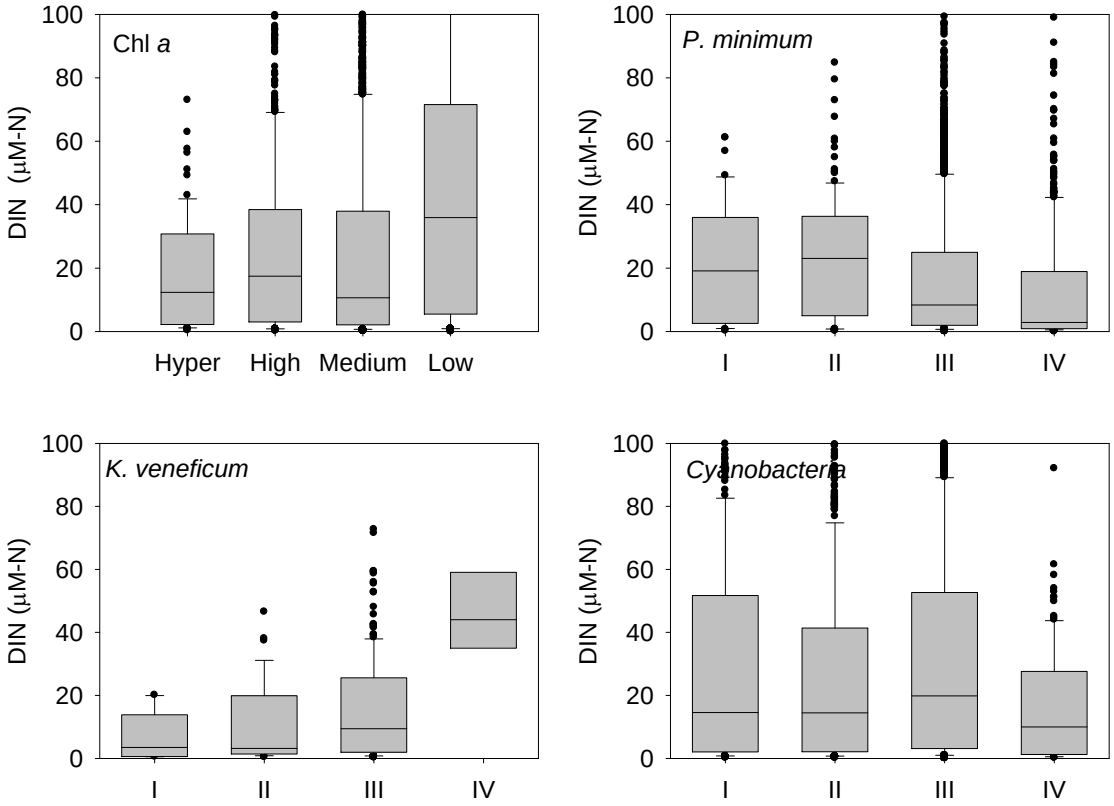


Figure 2-15 The dissolved inorganic nitrogen (DIN) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

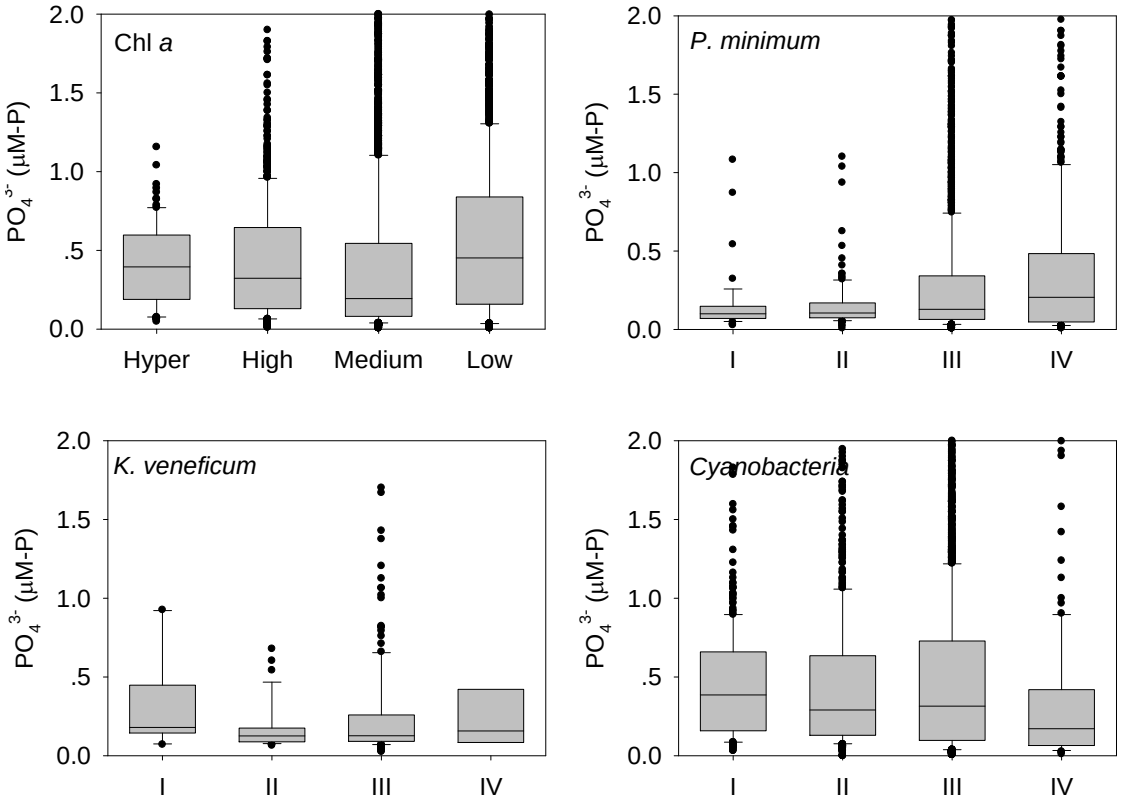


Figure 2-16 The phosphate (PO_4^{3-}) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

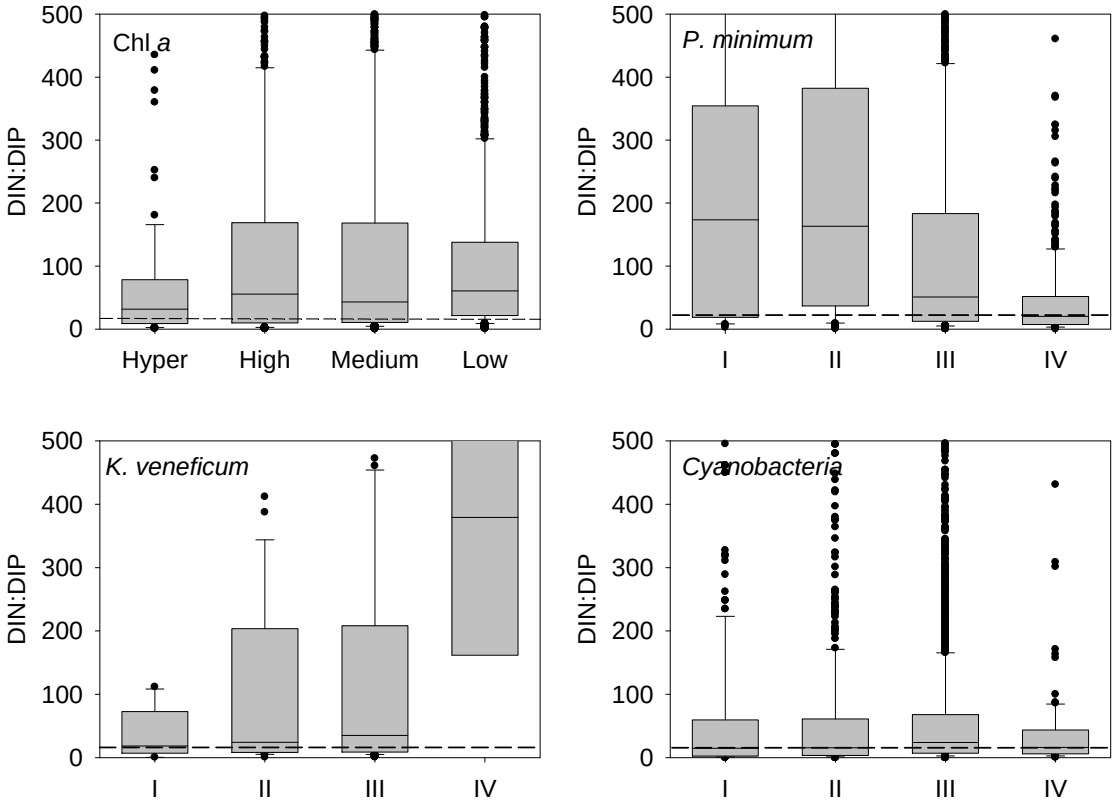


Figure 2-17 The dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

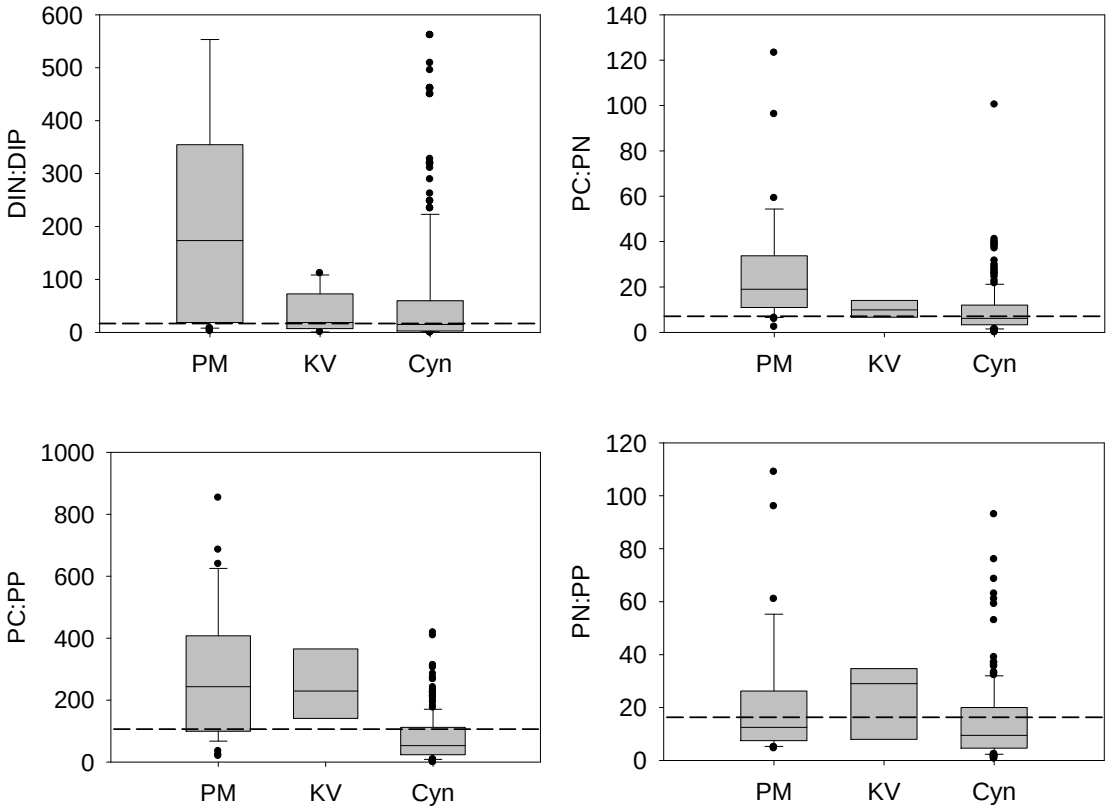


Figure 2-18 The ambient carbon, nitrogen and phosphorus rates of *Prorocentrum minimum* (PM), *Karlodinium veneficum* (KV) and cyanobacteria (Cyn) in blooms in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

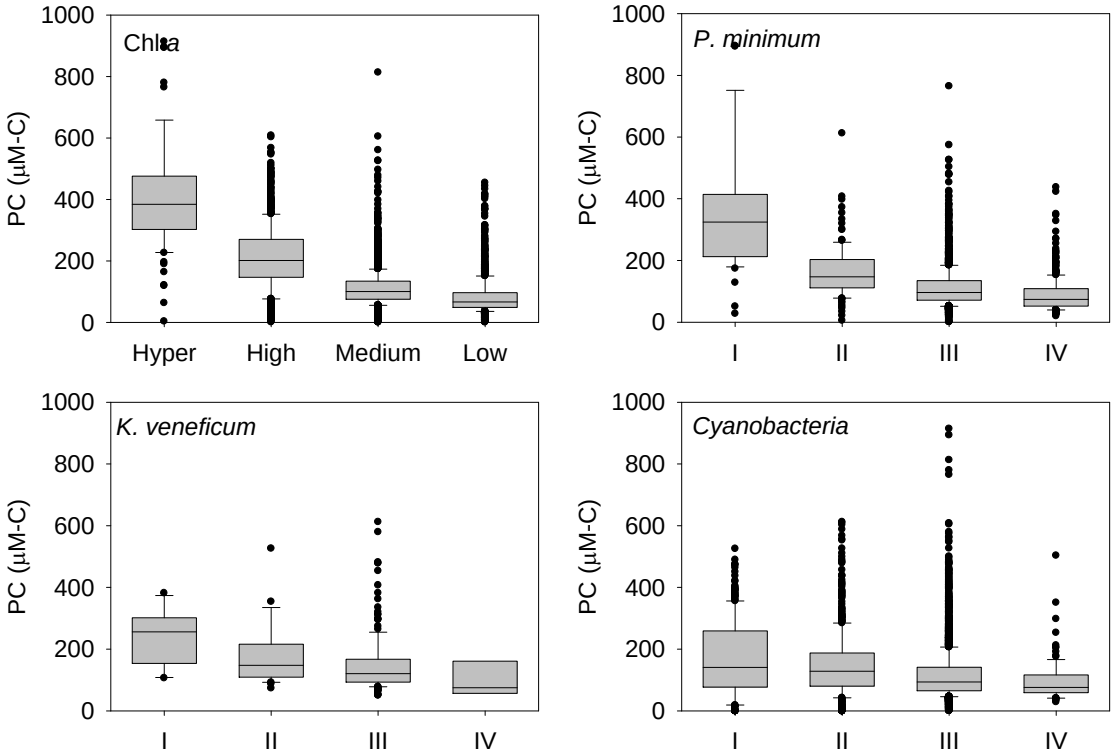


Figure 2-19 The particle carbon (PC) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

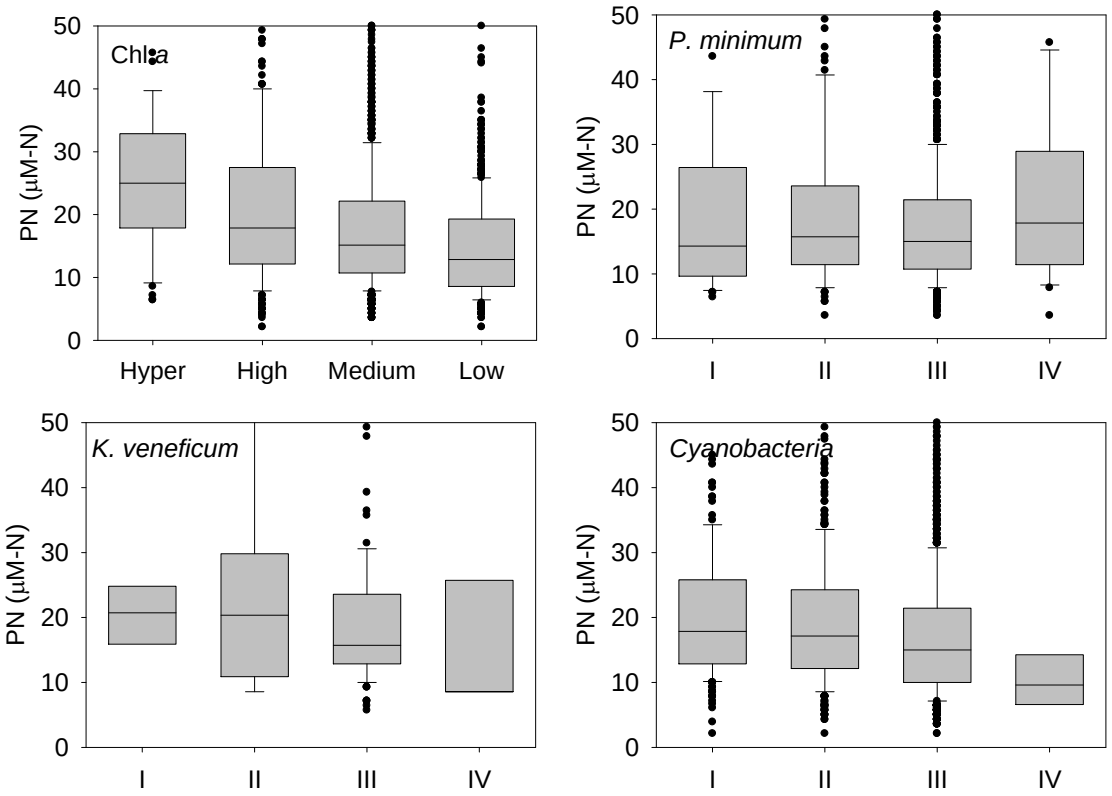


Figure 2-20 The particle nitrogen (PN) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

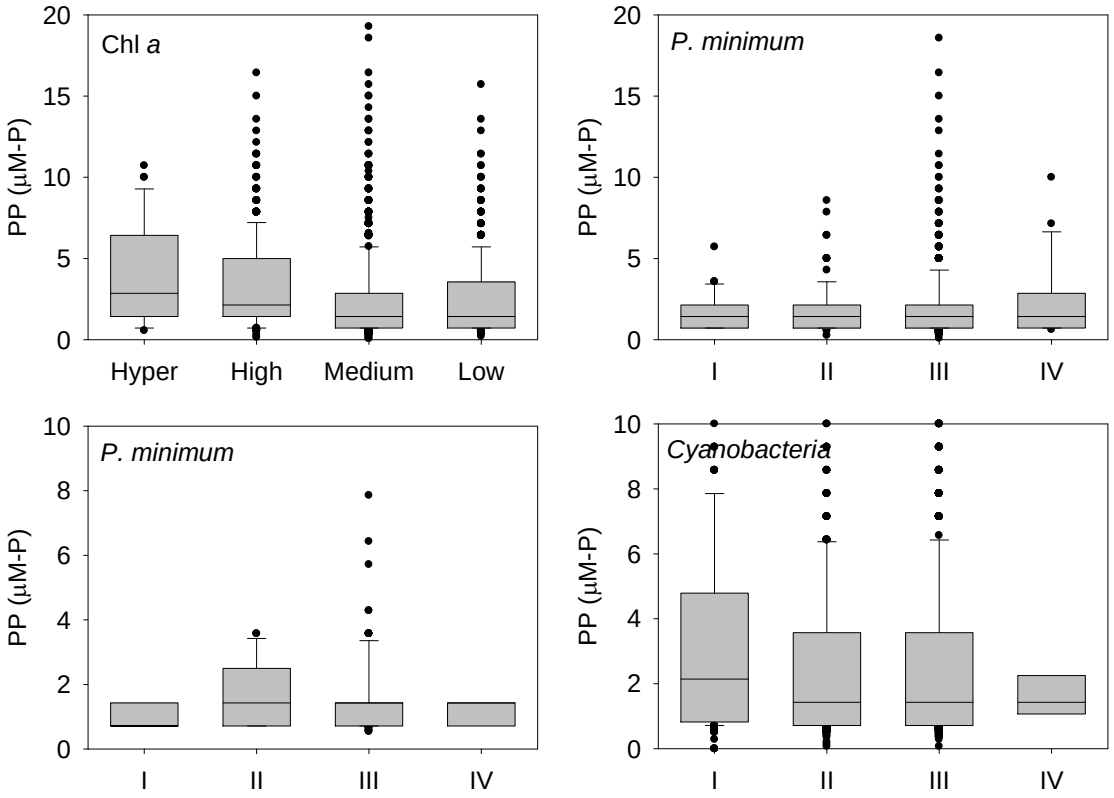


Figure 2-21 The particle phosphorus (PP) concentration of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

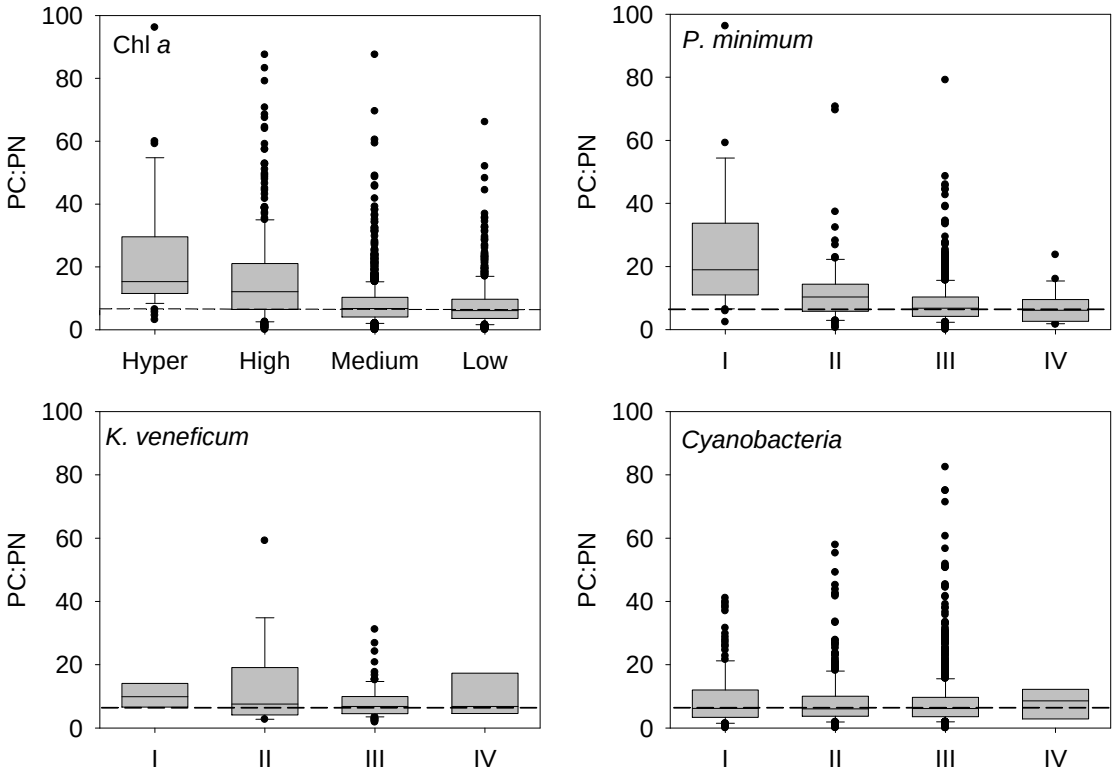


Figure 2-22 The particle carbon (PC) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

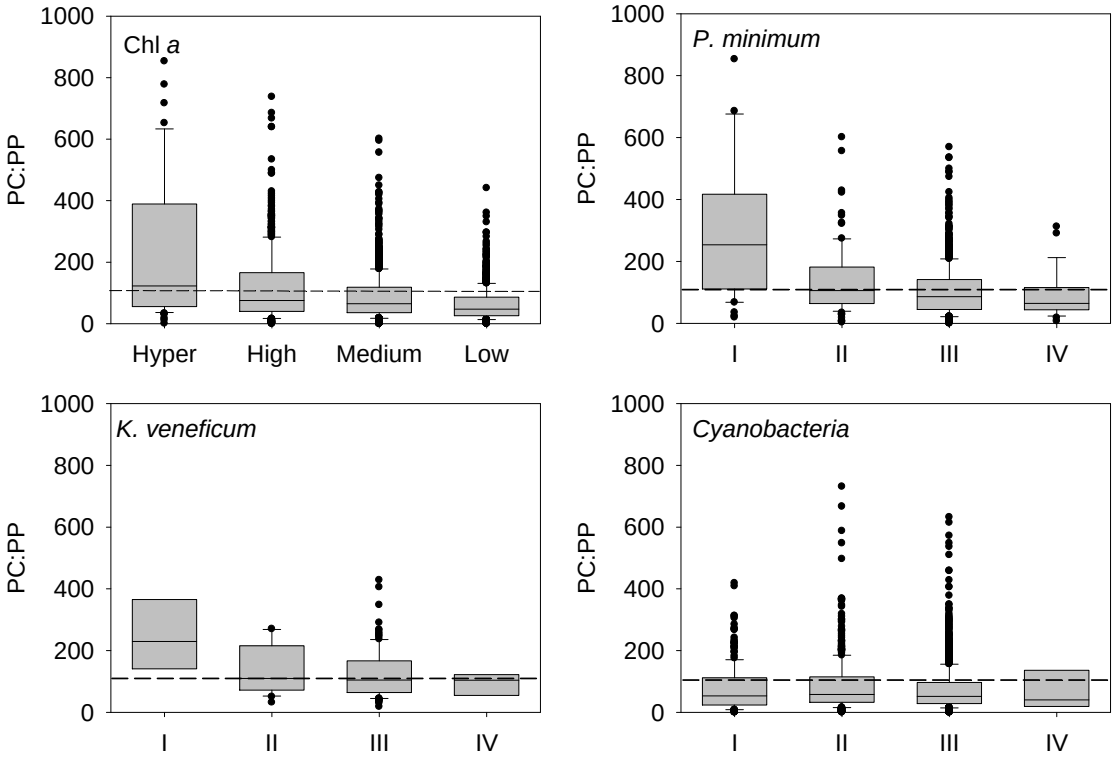


Figure 2-23 The particle carbon (PC) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Dash line: PC:PP = 106. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

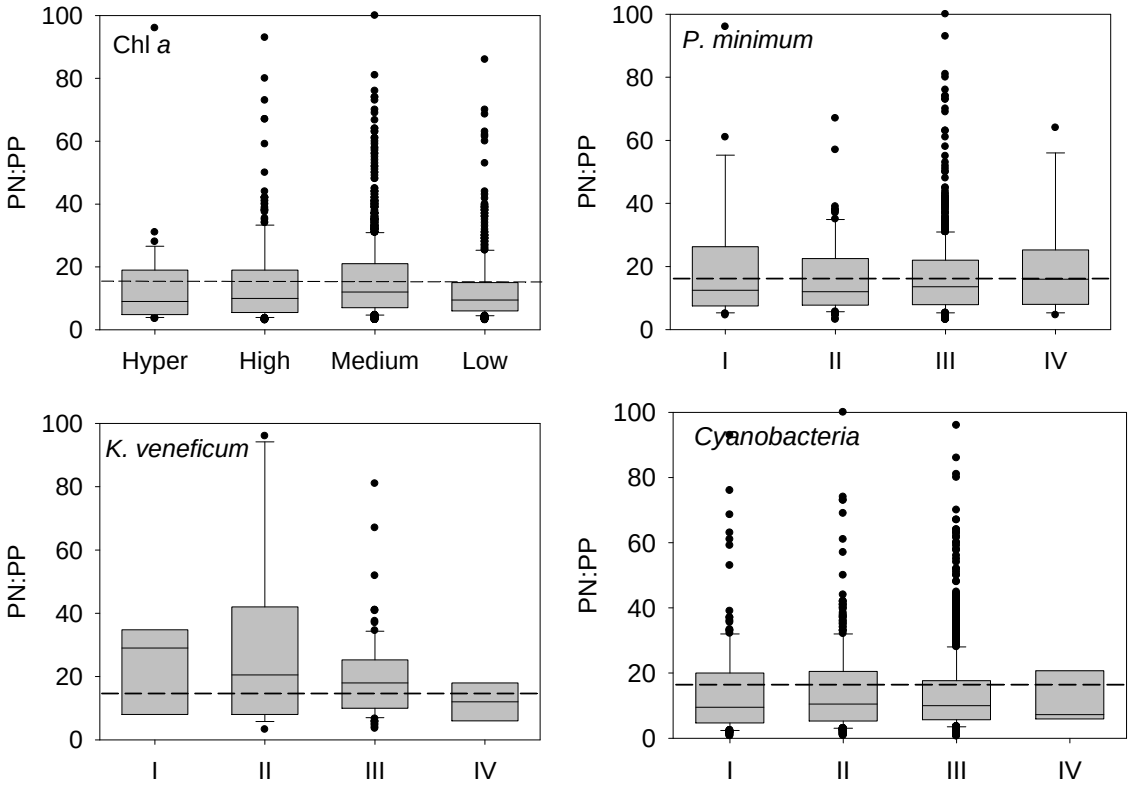


Figure 2-24 The particle nitrogen (PN) to particle phosphorus (PP) ratios of samples with different eutrophic levels of Chl *a* and harmful algae densities: *Prorocentrum minimum*, *Karlodinium veneficum* and cyanobacteria in the Chesapeake Bay. Dash line: the Redfield ratio. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers. Hypereutrophic: > 60 µg Chl *a* L^{-1}; high eutrophic: > 20 but ≤ 60 µg Chl *a* L^{-1}; medium eutrophic: > 5 but ≤ 20 µg Chl *a* L^{-1}; low eutrophic: > 0 but ≤ 5 µg Chl *a* L^{-1}. For *P. minimum* and *K. veneficum*,Type I: > 5,000,000 cell L^{-1}; Type II: > 1,000,000 but ≤ 5,000,000 cell L^{-1}; Type III: >1,000 but ≤ 1,000,000 cell L^{-1}; Type IV: > 0 but ≤ 1,000 cell L^{-1}. For cyanobacteria, Type I: > 50,000,000 cell L^{-1}; Type II: > 10,000,000 but ≤ 50,000,000 cell L^{-1}; Type III: >10,000 but ≤ 10,000,000 cell L^{-1}; Type IV: > 0 but ≤ 10,000 cell L^{-1}

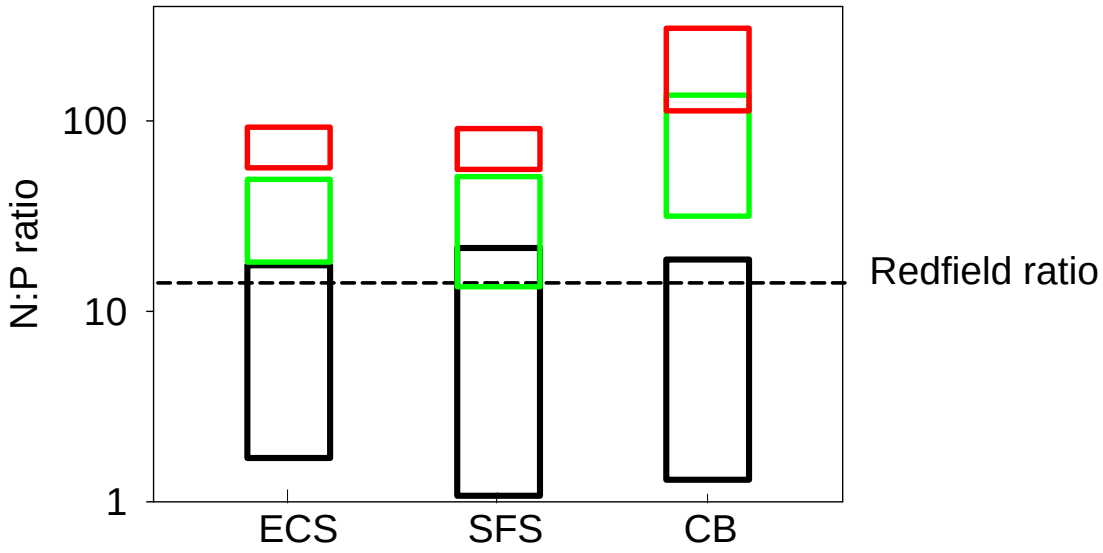


Figure 2-25 Comparison of the range in ambient N:P ratios during different phase of the blooms in the East China Sea (ECS) during May 2005, in different phytoplankton assemblages along the southwest Florida Shelf (SFS) during May 2003 and different phase of blooms in the Chesapeake Bay from 1991 to 2008. Black: *Karenica* spp. in ECS and SFS, and *Karlodinium veneficum* in Chesapeake Bay; Green: *Prorocentrum* spp.; Red: Diatom. The ECS data were derived from Li et al. (2009). The SFS data were derived from Heil et al. (2007)

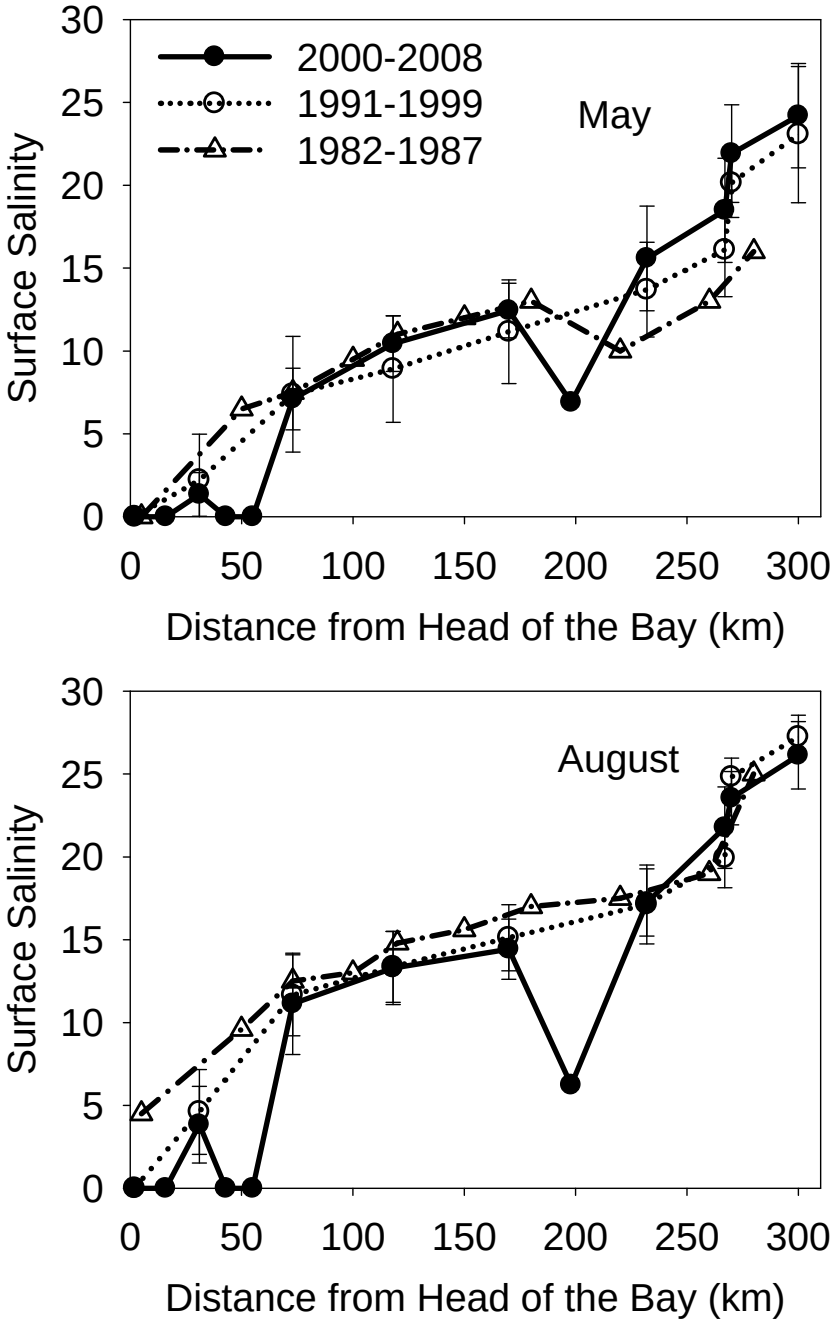


Figure 2-26 The spatial distribution of salinities in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 (data from Fisher et al. 1992).

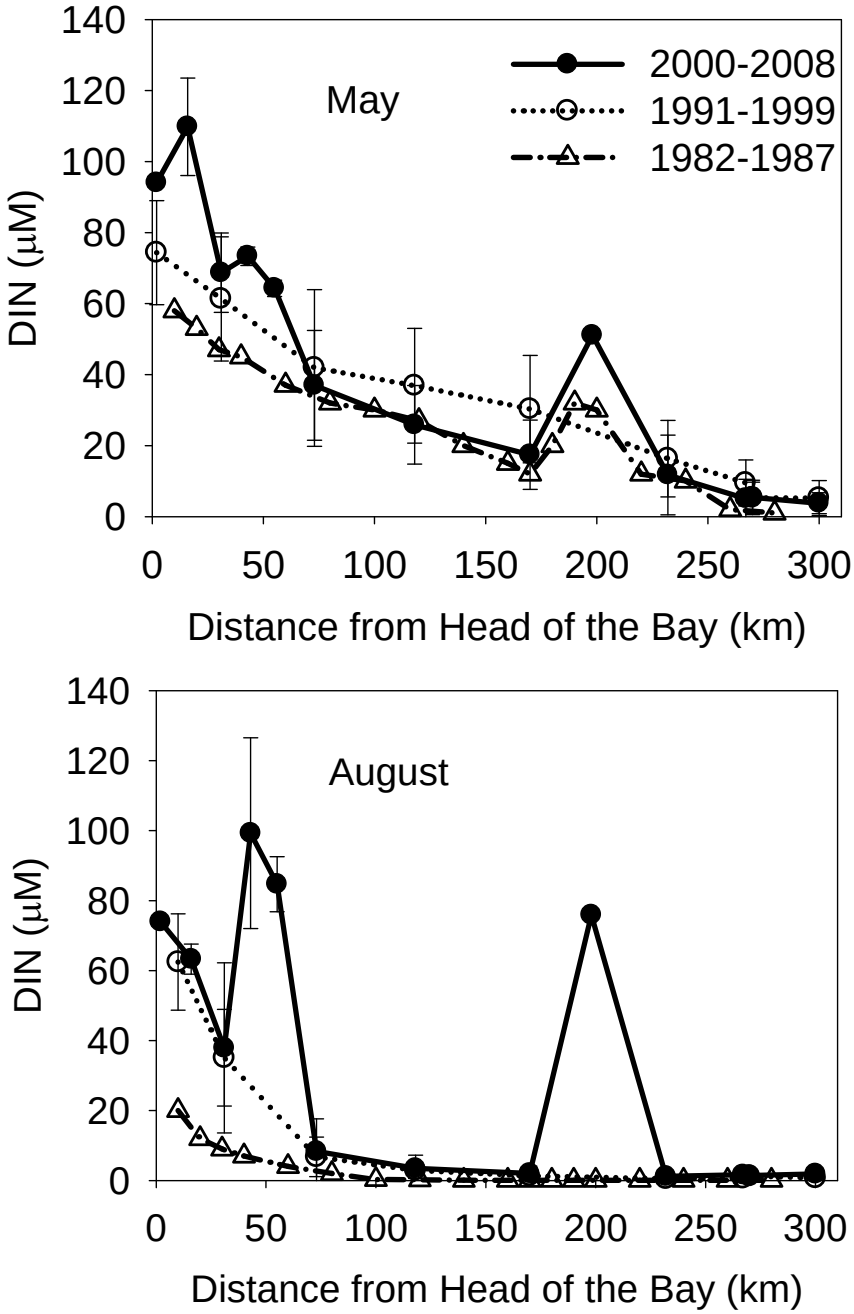


Figure 2-27 The spatial distribution of dissolved inorganic nitrogen (DIN) in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 (data from Fisher et al. 1992).

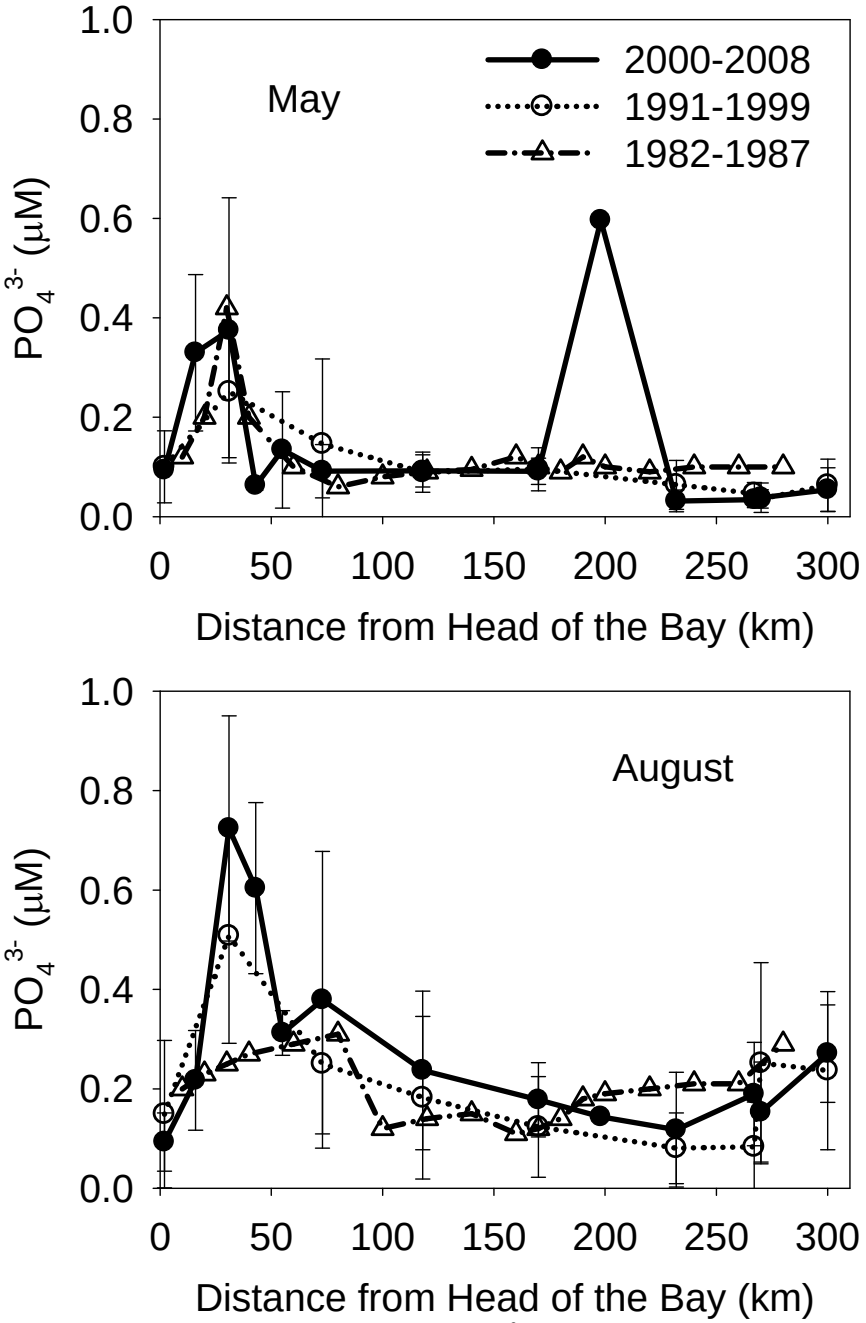


Figure 2-28 The spatial distribution of phosphate (PO_4^{3-}) in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 (data from Fisher et al. 1992).

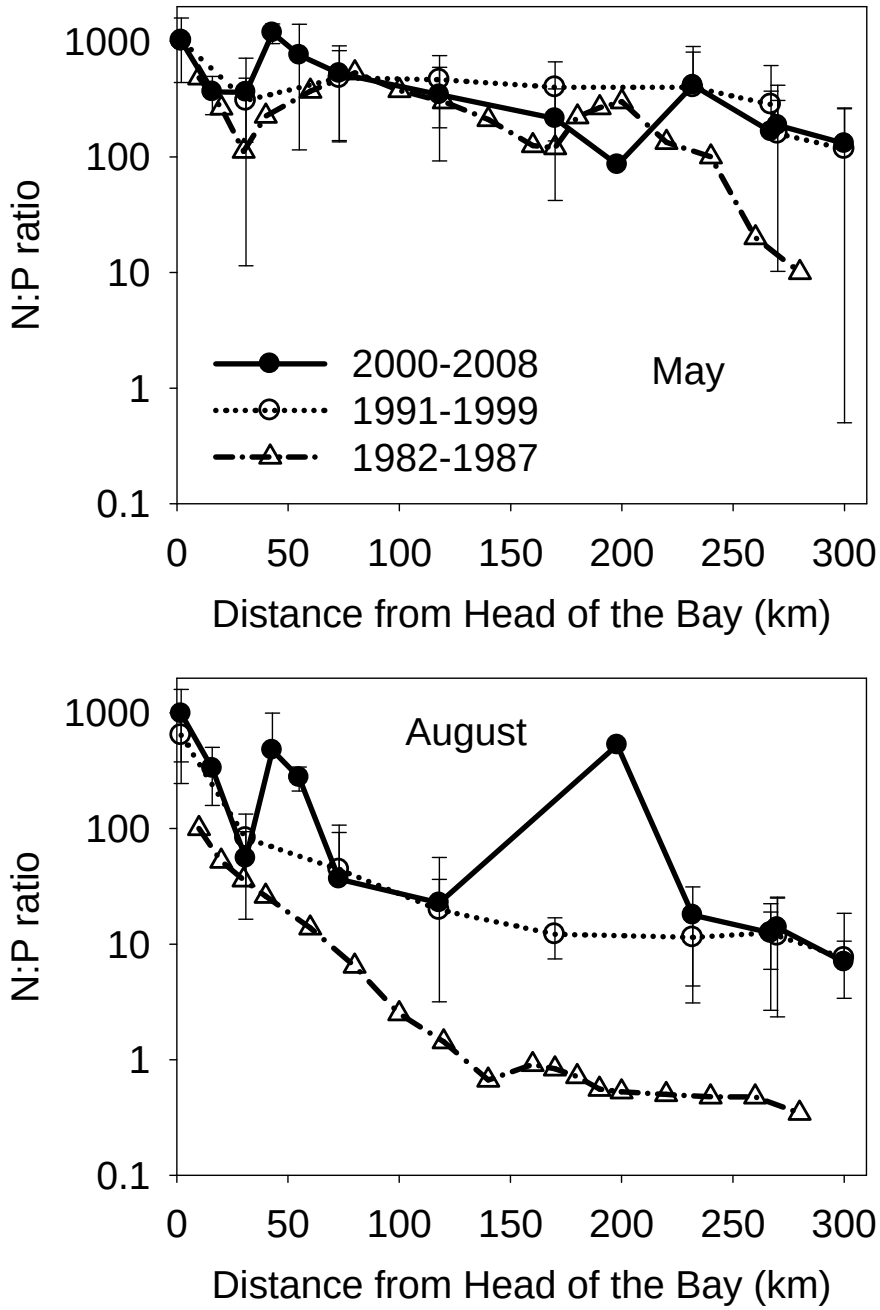


Figure 2-29 The spatial distribution of dissolved inorganic nitrate: dissolved inorganic phosphate (N:P) ratio in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 (data from Fisher et al. 1992).

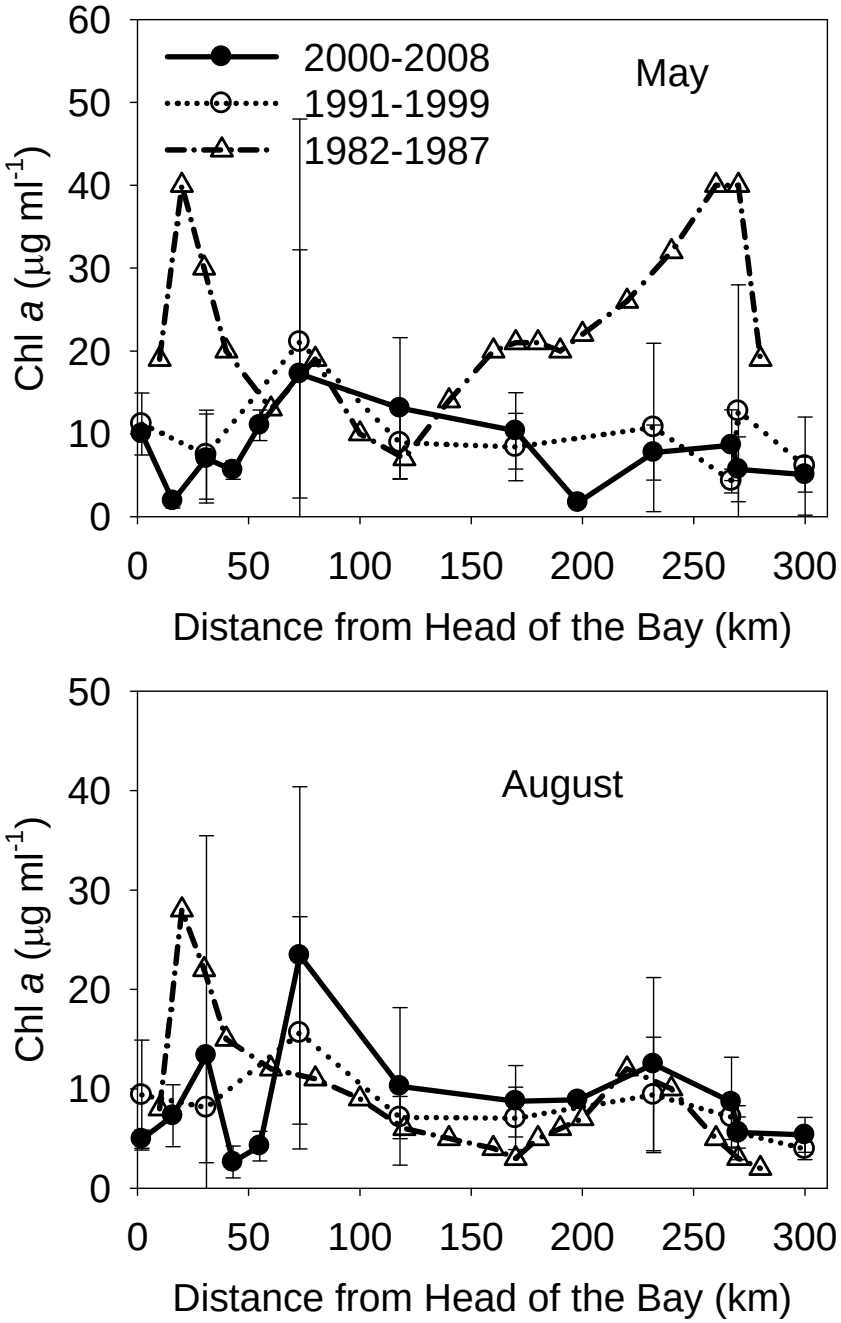


Figure 2-30 The spatial distribution of chlorophyll *a* in surface waters along the length of Chesapeake Bay major axis in May and August in the time range of 2000-2008, 1991-1999, and 1982-1987 (data from Fisher et al. 1992).

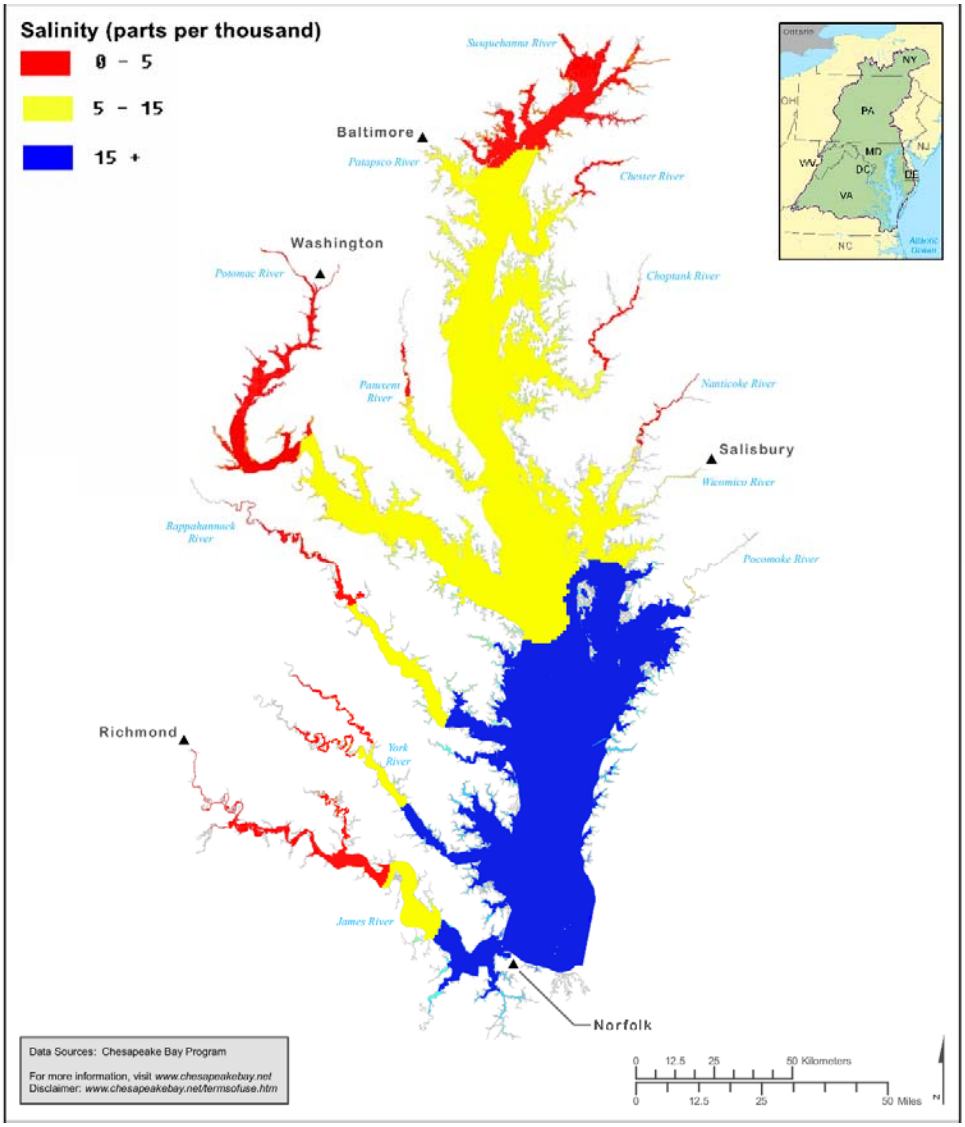


Figure 2-31. The summer surface water salinity in the Chesapeake Bay (modified from http://www.ncnr.nist.gov/programs/CHRNS/pdf/salinity_fall.png)

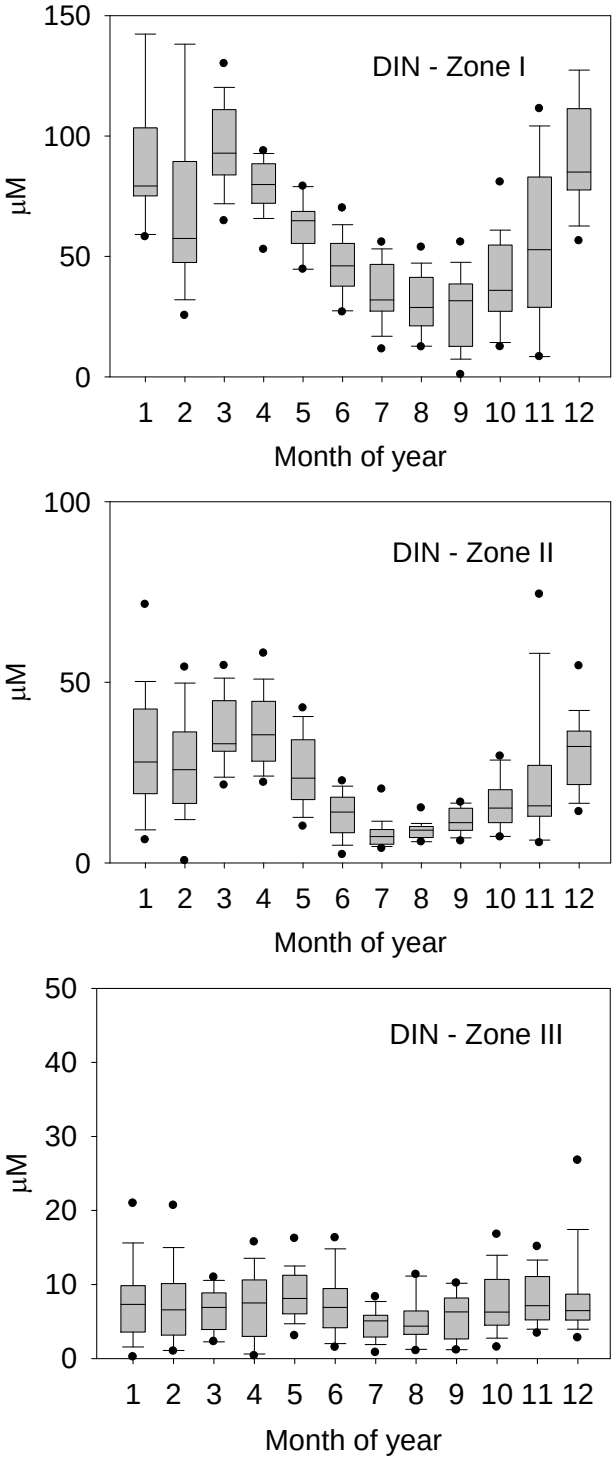


Figure 2-32 The seasonal variation of monthly average dissolved inorganic nitrogen (DIN) concentrations in 3 zones of Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

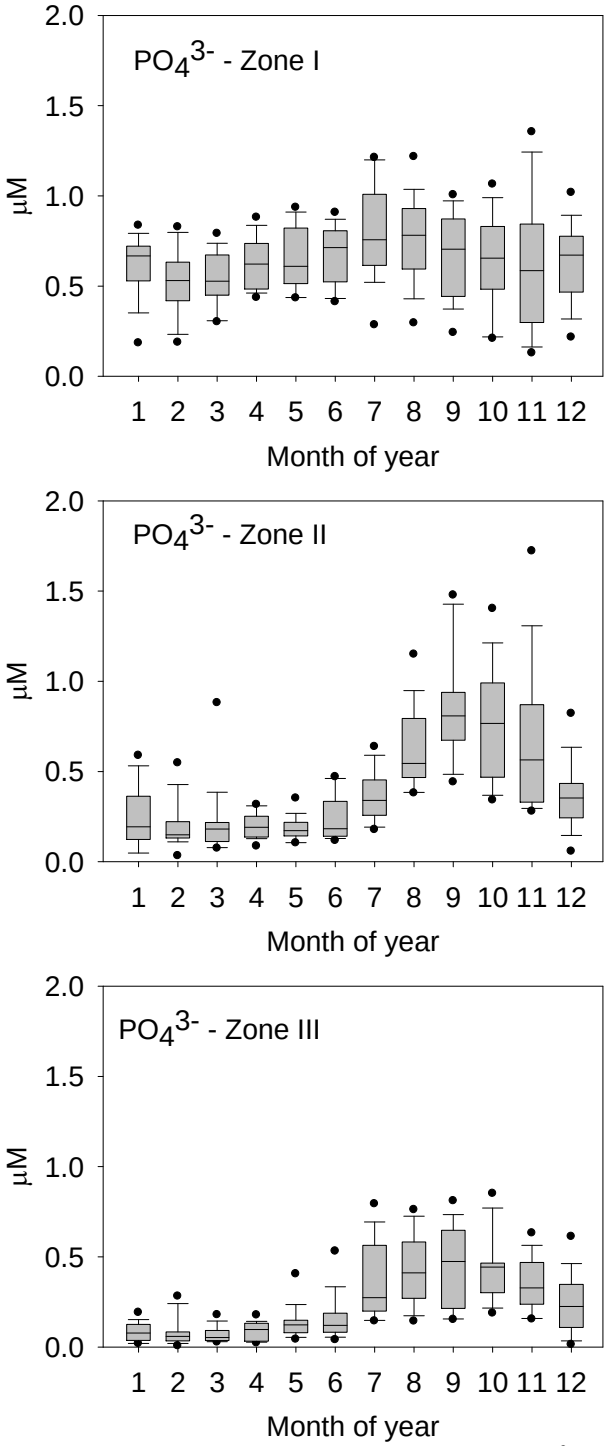


Figure 2-33 The seasonal variation of monthly average phosphate (PO_4^{3-}) concentrations in 3 zones of Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

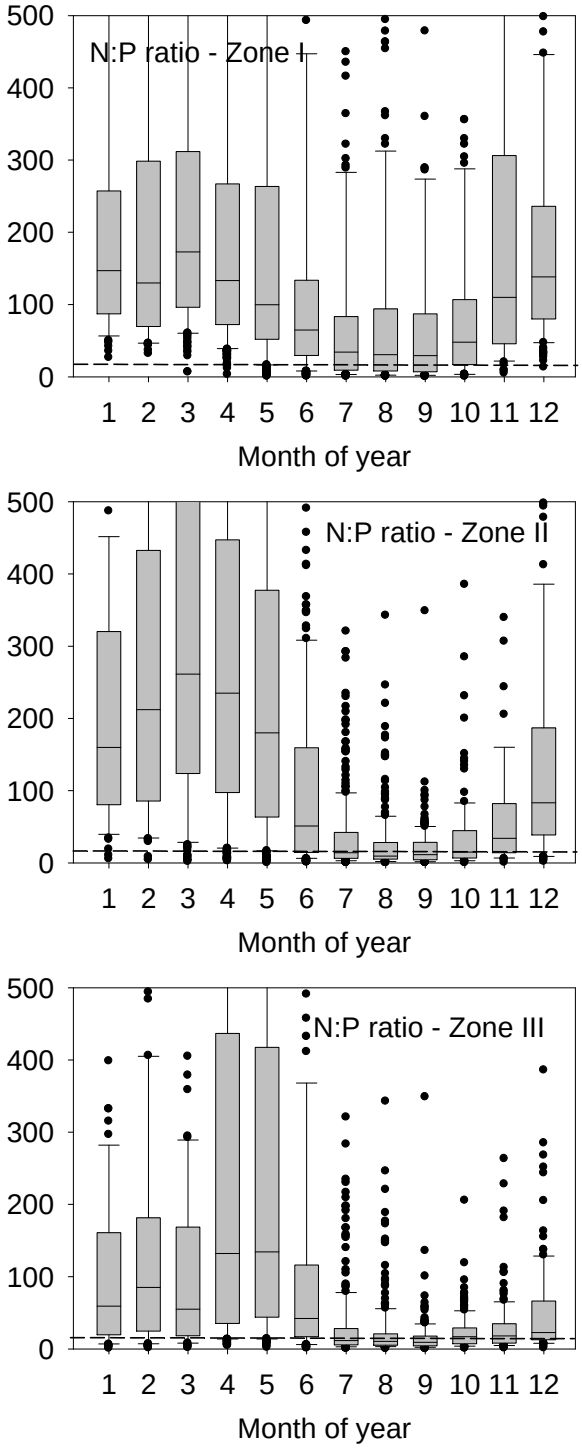


Figure 2-34 The seasonal variation of ambient dissolved inorganic nitrogen (N): phosphate (P) ratios in 3 zones of Chesapeake Bay. Dash line: the Redfield ratio. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

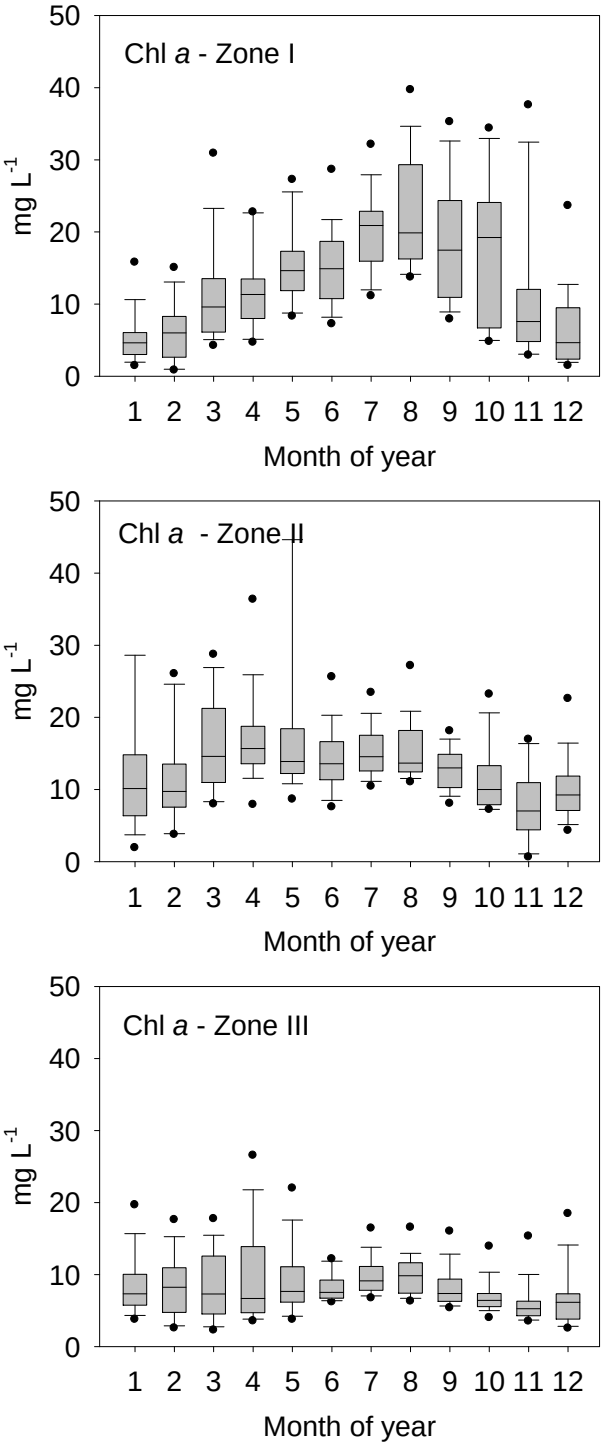


Figure 2-35 The seasonal variation of monthly average chlorophyll *a* concentrations in 3 zones of Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

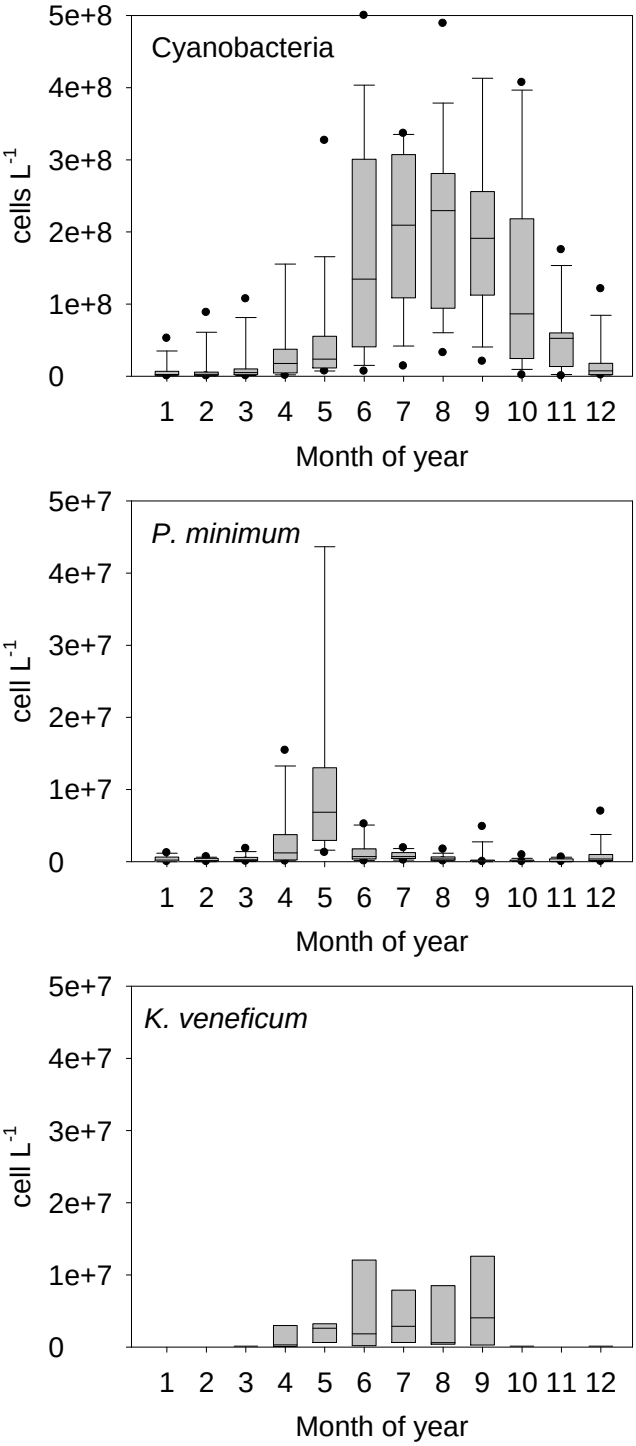


Figure 2-36 The seasonal variation of monthly maximum cell densities of cyanobacteria in Zone I and *Prorocentrum minimum* and *Karlodinium veneficum* in Zone II of Chesapeake Bay. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

Chapter 3: Effects of Ambient DIN : DIP Ratio on the Nitrogen Uptake of Harmful Dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* in Turbidistat*

Abstract

The effects of varying nitrogen (N): phosphorus (P) ratios on the growth and N-uptake /assimilation of the harmful dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* were examined in turbidistat culture experiments. Algal cultures were supplied with media containing PO_4^{3-} in various concentrations to obtain a wide range of N:P ratios. Experiments to determine rates of N uptake and assimilation of different N sources (NO_3^-, NH_4^+, urea and glycine by *P. minimum* and NO_3^-, NH_4^+ by *P. donghaiense*) were conducted using ^{15}N tracer techniques at each N:P ratio. The growth rates suggested nutrient limitation at both high and low N:P ratios relative to the Redfield ratio. On a diel basis, the growth of both species was regulated by the light-dark cycle, which may be a result of regulation of both light-dependent growth and light-independent nutrient uptake. Maximum growth rates of both species always occurred at the beginning of the light phase. In P-rich medium (low N:P ratio), both species had higher N assimilation rates, suggesting N limitation. Low assimilation coefficients at high N:P ratios suggested P limitation of N uptake and assimilation. NO_3^- and NH_4^+ contributed more than 90% of the total N uptake of *P. minimum*. Reduced N sources were more quickly assimilated than NO_3^-. Highest average daily growth rates were recorded near N:P ratio of 12 for both species. The N uptake rates of cultures at N:P ratio near Redfield ratio were more balanced with the

* Li J, Glibert PM, Alexander JA Chinese Journal of Oceanology and Limnology (in press)

growth rates. The linkage between growth rates and N uptake/assimilation rates were conceptually described by the variation of cell N quota. The N:P ratios affect the N uptake and growth of *Prorocentrum* spp., and may regulate their bloom progression in eutrophic ecosystems.

Introduction

Prorocentrum minimum, a bloom forming dinoflagellate, has been shown to be widely distributed globally (Heil et al. 2005, Glibert et al. 2008b). Its expanding geographical distribution is indicative of a strong relationship between both dissolved inorganic nitrogen (DIN) export and dissolved organic nitrogen (DON) export into coastal waters globally (Heil et al. 2005, Glibert and Burkholder 2006, Glibert et al. 2008a). A related pelagic *Prorocentrum* species, *Prorocentrum donghaiense*, has also been suggested to be an eutrophication-related species, becoming one of the dominant bloom species observed along coast of China in recent years (Lu and Goebel 2001, Zhou et al. 2003, Li et al. 2009).

The nitrogen nutritional strategies of *P. minimum* have been well studied, and the understanding of those of *P. donghaiense* are advancing. Both species can take up a range of nitrogen substrates, including NO_3^-, NH_4^+, and urea (Lomas and Glibert 1999a, b, Burns et al. 2000, Fan et al. 2003a, Li et al. 2010). The affinity for different nitrogen substrates varies with growth condition (Fan et al. 2003b) and with the rate of resupply (Sciandra 1991). Organic forms of nitrogen have also been found to be taken up via a range of mechanisms. The protease leucine aminopeptidase (LAP), one of the enzymes that liberates amino acids, has been found to be active in this species

in both laboratory and field studies (Stoecker and Gustafson 2003, Salerno and Stoecker 2009). Moreover, *P. minimum* is mixotrophic and has been shown to graze upon a wide range of organisms, from organic particles (Li et al. 1996) to other microalgae (Stoecker et al. 1997, Heil et al. 2005, Jeong et al. 2005b). The nutrient strategies of *P. donghaiense* are less well documented, but from what is currently known, they appear to be similar to those of *P. minimum*. *P. donghaiense* has also been shown to graze on cyanobacteria (*Synechococcus* etc.) and cryptophytes (Jeong et al. 2005a, Jeong et al. 2005b).

The uptake of NO_3^- by *P. minimum* under varying light-dark regimes was contrasted with that of several other dinoflagellates in studies by Paasche et al. (1984). They found that *P. minimum* continuously took up NO_3^-, while many other dinoflagellates stopped NO_3^- uptake at night. *P. minimum* also has been shown to display considerable plasticity in its photosynthetic parameters and can adapt to low light and be sustained for long periods of darkness (Tyler and Seliger 1978, Harding et al. 1983, Harding and Coats 1988). However, mixotrophy, as an adaptive strategy, is more likely to be a response to nutrient limitation than to carbon limitation (Stoecker et al. 1997). Less is known about *P. donghaiense* either in terms of photosynthetic adaption or its mixotrophic tendencies.

As with many other flagellate species, both *P. minimum* and *P. donghaiense* are more commonly observed when inorganic N:P ratios are at the Redfield ratio or below due to their relatively high requirement for P (Fan and Glibert 2005, Heil et al. 2007, Li et al. 2009). A key question that has not been addressed is whether N

nutritional strategies vary across a spectrum of N:P ratios. In order to assess this question, a series of laboratory experiments were conducted on both species.

The conceptual framework for these experiments is the cell quota equation of Droop (Droop 1968, 1973), who described the growth of phytoplankton growing under a nutrient limited environment using the equation:

$$\mu = \mu_{max}(1 - Q_{min}/Q)$$

where the cell quota (Q) is the intracellular content of the limiting chemical element (N, P etc.), μ is the growth rate, and μ_{max} is the maximum growth rate for a given set of environmental conditions. In this model framework, cells can not grow if the cell quota is lower than the minimum quota (Q_{min}), which defines the minimum requirement of the cells. Nutrients are taken up, then subsequently assimilated into the nitrogenous components of the cell. *P. minimum* has previously been shown to have a large, but varying cell quota (Sciandra 1991).

In this study, cells of *P. minimum* and *P. donghaiense* were grown across an N:P spectrum in order to achieve a range of growth rates and a range of physiological states. The rates of uptake of different forms of nitrogen were compared, as were the comparative rates of uptake and assimilation.

Materials and Methods

Algae Culture

Cultures of *Prorocentrum minimum* were isolated from Choptank River, a tributary of Chesapeake Bay in spring 1995 and were maintained in f/2 medium

(Guillard and Ryther 1962) in the Horn Point Laboratory culture collection. For the experiments, *P. minimum* cells were first transferred to f/20 batch cultures for 2 weeks, then grown in turbidistat, an automatic continuous culture system, as described below.

Cultures of *Prorocentrum donghaiense* were obtained from University of Connecticut Department of Marine Sciences, USA. These cultures were originally isolated from the East China Sea. They, too, were transferred to f/2 batch cultures for several weeks, then grown in turbidistat.

Continuous Culture Design

A continuous turbidistat culturing device (Fig. 3-1) was used to grow the *Prorocentrum* cultures. A modulated infrared (880 mm) LED beam was directed though the culture vessel (a 2 L glass carboy) to a photosensor, the analog signal output of which was related to optical transmission (i.e., turbidity). Real time turbidity signals were sent to a computer that controlled a peristaltic pump which diluted the culture based on attaining a threshold value. The system was programmed to dilute the culture by 5% in terms of the optical signal. This corresponds to a volume dilution of approx. 10%, based on calibrations using a volumetric dilution. The threshold was set to a turbidity that represented a *P. minimum* cell density of ~ 16,000 cell ml^{-1} and a *P. donghaiense* cell density of ~ 30,000 cell ml^{-1}. The culture was stirred at a speed of ~ 60 rpm and generally bubbled with air to keep the culture well mixed and aerated. In this condition, the cells grew at a maximum rate of growth as set by the environmental conditions of the culture. The specific growth rate over a 20 minute period was calculated from the optical signal and recorded every minute.

The use of growth rate, in this study, refers to the rate calculated based on the change in the optical signals.

Experimental Design and Algal Growth

Unialgal, but not axenic, *P. minimum* were grown in the turbidistats in filtered Choptank River water, at a salinity of 15, a temperature of 22 °C and a 14: 10 h light dark cycle at 430 µmol photons m^{-2} sec^{-1}. Modified f/2-medium without silicate was used, modified by reducing nitrate (NO_3^-) to 40 µM-N, but by adding PO_4^{3-} in varying concentration from 0.2 to 10 µM-P in different turbidistats, therefore providing N:P ratios of 5, 16, 40 and 200. The cells were thus grown in a N:P gradient from relatively low P to low N conditions.

Unialgal, but not axenic, cultures of *P. donghaiense* cells were grown in the turbidistats, in filtered Choptank River water, at a salinity of 30, a temperature of 22 °C and a 12: 12 h light dark cycle at 430 µmol photons m^{-2} sec^{-1}. Modified f/2-medium without silicate was used. The turbidistats were supplied with media NO_3^- at 80 µM and PO_4^{3-} at 8 µM. Then, the media N:P ratios were gradually changed following day 20. In one treatment, media NO_3^- concentrations were enriched to ~ 220 µM-N and PO_4^{3-} concentrations were gradually decreased to 2 µM-P. In the second treatment, media NO_3^- concentrations were enriched to 200 µM-N and PO_4^{3-} concentrations were gradually enriched to 140 µM-P by adding additional PO_4^{3-} every 3 days.

The volume of each turbidistat culture was kept about 1.6 L in the 2 L glass carboys. Before each day of experiments, 100 ml water samples were collected from each tubidostat culture carboy and filtered though pre-combusted (2 h at 400 °C)

Whatman GF/F filters for ambient nutrients analysis. The filtrates were stored frozen at -20°C for later analysis as described below. Subsamples (10 ml) were taken each day of experiments and preserved in 4% glutaraldehyde for algae cell counts. Cells were manually counted by optical microscopy.

Nitrogen Uptake and Assimilation Experiments

N Uptake Kinetics and Rates of P. minimum

P. minimum cells were grown in turbidistat at each N:P ratio for at least 3 days to get a steady growth state before the N uptake experiments were initiated. ^{15}N isotopically-labeled dissolved N substrates were used to measure the culture uptake and assimilation rates (Glibert and Capone 1993) at each N:P ratio. Experiments were conducted around 10 am. Culture samples of 40 ml were dispensed into a series of acid-clean 50 ml incubation cuvettes to which ^{15}N substrates (Cambridge Isotope Laboratories, Inc.) were added respectively. Different experiments involved different substrates and/or concentrations of ^{15}N. Due to high background NO_3^- concentration in the media, ^{15}N labelled NO_3^- was only added at a concentration of 5 μM-N, representing ~ 8% of NO_3^- media concentration. For the kinetic experiments, ^{15}N labelled NH_4^+, urea and glycine were added in a gradient of 0.2, 0.5, 1, 5, 20 μM-N. All the cuvettes were incubated for 0.5 h at 430 μmol photons m^{-2} sec^{-1}, and then the incubations were terminated by filtration onto pre-combusted GF/F filters. A 20 ml aliquot for each treatment was filtered directly onto one filter, which was treated with 10% ice-cold Tri-Chloroacetic Acid (TCA) for 10 seconds after filtration. For samples with incoming media at N:P ratio 16 and 200, an extra 20 ml aliquot was filtered to a second filter without further treatment. The TCA-treated filter represents

the N substrate assimilated into proteinaceous material, while the untreated filter represents the total N substrate which was taken into the cell. The filters were stored frozen until dried at 50 ˚C for 48 h, then analyzed as described below.

N Uptake Kinetics and Rates of P. donghaiense

Similar to the *P. minimum* experiments, for *P. donghaiense* at each N:P ratio, culture samples of 40 ml were dispensed into acid-clean 50 ml incubation cuvettes to which ^{15}N substrates were added. These experiments only involved ^{15}N labelled NO_3^- and NH_4^+ added at a concentration of 20 µM. All the cuvettes were incubated for 0.5 h at 430 µmol photons m^{-2} sec^{-1}, and then the incubations were terminated as described above, with one filter untreated and one treated with 10% ice-cold TCA for 10 seconds after filtration. The filters were stored frozen until dried at 50 ˚C for 48 h, then subsequently analyzed as described below.

Analytical Methods

Concentrations of ambient inorganic nutrients (NO_3^-+NO_2^--N, NH_4^+-N, PO_4^{3-}-P) were determined using a Technicon Auto-Analyzer (Lane et al. 2000). Concentrations of dissolved free amino acid (DFAA) were determined by fluorometric analysis according to Lindroth and Mopper (1979) and urea was analyzed using the method of Revilla et al. (2005). DFAA concentrations were used to approximate the glycine concentrations in the N uptake rate calculations. Glycine is generally considered to be a dominant amino acid (Degens and Mopper 1976), but their rates of uptake should be considered potential rates based on the uncertainty of their contribution to the DFAA pool. All the ^{15}N sample filters were analyzed for

isotope enrichment using a Sercon Mass Spectrometer. Rates of ^{15}N uptake and assimilation were calculated according to Glibert and Capone (1993).

Data Analysis

The nitrogen uptake parameters were plotted and fitted to the Michaelis-Menten formulation:

$$A = A_{\max}\frac{S}{K_s + S} \quad \text{or} \quad V = V_{\max}\frac{S}{K_s + S}$$

where A is the N specific assimilation rate (h^{-1}), A_{max} is the maximal N specific uptake rate, V is the N specific uptake rate (h^{-1}), V_{max} is the maximal N specific uptake rate (h^{-1}), S is the substrate concentration (μM-N) and K_s is the half-saturation constant (μM-N) for the substrate. A_{max}, V_{max} and K_s for each of 4 N sources during the blooms were calculated by the regression of uptake dynamic by Sigmaplot software (Systat Software, Inc.).

One way ANOVA analyses were conducted to compare the uptake and assimilation data in the different N:P ratio and light intensity treatments.

Results

Ambient Parameters and Cell Counts

In the *P. minimum* culture, the ambient N:P ratios in the culture vessels at the time of the experiments were 3.5, 12, 21, 300 (Table 3-1), which were slightly different from the ratios of incoming media (5, 16, 40 and 200). NO_3^- became depleted (< 2 μM-N) in the 2 relatively high P treatments (N:P ratio 3.5 and 12), but

high concentrations (>20 μM-N) remained in the two relatively low P treatments (N:P ratio 21 and 300). PO_4^{3-} was depleted in the treatment where N:P was 13. *P. minimum* cell densities on the day of experiments were in the range of 14,000 to 18,000 cells ml^{-1}.

In the *P. donghaiense* culture, the ambient NO_3^- concentration varied from 32.8 μM to 197 μM. NO_3^- concentration decreased with time when supplied with relatively P rich media, and NO_3^- concentration increased with time when supplied with relatively N rich media. Ambient PO_4^{3-} concentration varied from 0.33 μM to 127 μM. PO_4^{3-} concentration decreased with time when supplied with relatively N rich media, and PO_4^{3-} concentration increased with time when supplied with relatively P rich media. The imbalance of N and P resulted in ambient N:P ratios from 0.9 to 600. *P. donghaiense* cell densities on the day of ^{15}N experiments were relatively constant in the range of 28, 000 to 33, 000 cells ml^{-1}.

Culture Growth in the Turbidistat

When supplied with a constant media supply, the growth rates of both *P. minimum* and *P. donghaiense* in turbidistat showed highly repeatable-diel cycles. Figure 2 is an example of the high frequency, optical-signal growth rates of *P. donghaiense* during 4 continuous light-dark cycles, supplied with a steady ambient media in which the N:P ratio was 12. Both species showed a clear diel cycle (Fig. 3-3). Growth rapidly increased at the beginning of the light period and the rates increased to the highest value of the day in less than an hour. Growth rates decreased rapidly when the light was off and the calculated rates were negative in the dark. Similar diel growth cycles were observed for all treatments of both species.

In the *P. minimum* culture, at ambient N:P ratios of 3.5, 12, 21 and 300, the average hourly growth rates averaged over the full day (r_{day}) were 0.0085, 0.024, 0.013 and 0.012 h^{-1}, respectively (Fig.4 a), while the average growth rates for only the light phase (r_L) were 0.016, 0.047, 0.027 and 0.026 h^{-1}, respectively (Fig. 3-4 b). In the *P. donghaiense* culture, the r_{day} varied from ~ 0.004 h^{-1} to 0.021 h^{-1} (Fig. 3-4 c), while the r_L varied from ~ 0.017 h^{-1} to 0.052 h^{-1} (Fig. 3-4 d), when the ambient N:P ratios varied from 0.9 to 600. The maximum r_{day} was recorded at an N:P ratio of 12.0 (157 μM NO_3^-, 13.1 μM PO_4^{3-}). The maximum r_L was recorded at an N:P ratio of 5.8 (51 μM NO_3^-, 9.0 μM PO_4^{3-}). Both the minimum r_{day} and minimum r_L were recorded at an N:P ratio of 1.5 (135 μM NO_3^-, 94.0 μM PO_4^{3-}), followed by the values at N:P ratios of 264.5 (114 μM NO_3^-, 0.44 μM PO_4^{3-}) and 288.4 (143 μM NO_3^-, 0.5 μM PO_4^{3-}).

For both species, relative high maximum growth rate (0.021 h^{-1}) were recorded near an N:P ratio of 12, and the maximum growth rates decreased to less than 0.01 h^{-1} at both very low and very high N:P ratios (Fig. 3-4 c, d). Both r_{day} and r_L of the high N:P ratio cultures (N:P ratio > 50) were significantly lower than the lower N:P ratio culture (P = 0.008).

Nitrogen Uptake Experiments

In the *P. minimum* experiments, both N uptake and assimilation rates of NH_4^+, urea, and glycine at the N:P ratios of 12.5 and 300 (the only ratios at which full kinetics were tested) displayed saturating-type kinetics in the concentration range of the experiments (Fig. 3-5). Samples from the low P cultures (i.e., N:P ratio = 300) had higher V_{max} and higher K_s than samples from the culture where N:P ratio was 12.5

(Table 3-2). Samples from P-rich cultures (i.e., N:P ratio = 3.5) had highest rates of A_{max} for all 3 N nutrients, but have the lowest K_s, except for glycine (Table 3-2). Samples from the low P cultures had the lowest NH_4^+ A_{max}. There were no significance differences between the rates of the 2 relatively N:P balanced cultures (N:P ratio 12.5 and 20).

To further our understanding of the N uptake and assimilation rates at ambient concentrations, the uptake and assimilation rates at the lowest ^{15}N enrichment concentration (NO_3^- 5 μM-N; other 3 N sources 0.2 μM-N) were compared (Fig. 3-6). The NO_3^- uptake rate (V) at N:P ratio 300 (0.030 h^{-1}) was higher than that at an N:P ratio of 12.5 (0.021 h^{-1}). Assimilation rates (A) of NO_3^- (0.021 h^{-1}) in the relatively P-rich cultures (i.e., N:P ratio = 3.5) were 3 - 4 times higher than the rates at the rest of the N:P treatments. The ambient assimilation rates of the reduced N forms (NH_4^+, urea and glycine) were also highest in the N:P ratio treatments of 3.5 in all 4 treatments.

When N uptake rates were measured at approximate ambient concentrations, NO_3^- contributed more than 60% percent of the total of the 4 N substrates. In contrast, NH_4^+ contributed more than 30% of total N uptake rate when N:P ratios were 12 and 300. However, when comparing the assimilation rates with uptake rates, relatively less NO_3^- (50%), but more NH_4^+ (over 40%), was assimilated of the 4 N sources. The assimilation efficiencies (assimilation rate/ uptake rate) of all N substrates were higher in P-rich cultures (N:P ratio = 12) relative to P-poor cultures (N:P ratio 300). Also, the assimilation efficiencies of the reduced forms of N (NH_4^+, urea and glycine) were higher than those of NO_3^-. The assimilation efficiencies of NO_3^- were less than

40%. The assimilation efficiencies also showed evidence of P limitation, which were lower in high N:P ratio cultures. The assimilation efficiencies of NH_4^+ in P-poor cultures (21%) were less than half of those in culture with N:P ratio near Redfield ratio.

All 4 N:P ratio treatments were supplied with the same concentration of NO_3^- in the media, and were kept at similar biomass and all grew at their maximum growth rates for the condition of the culture, but the ambient nutrient concentrations varied in the 4 treatments at the time of experiments (Table 3-1). NO_3^- was the primary N source in the media; however, NO_3^- must be reduced to NH_4^+ before being assimilated by the algae. This process requires NO_3^- reductase as well as ATP and NADPH for energy supply (Lomas and Glibert 1999b, 2000). Phosphorus can be a critical limiting nutrient for the assimilation of NO_3^-. Therefore, the assimilation of NO_3^- in the high N:P ratio cultures (i.e., N:P ratio = 300) showed more P limitation, and as a result, there was high NO_3^- concentration remaining in this culture (Table 3-1). However, in the P-rich cultures (i.e., N:P ratio 12.5 and 3.5), *P. minimum* took up the NO_3^- rapidly, resulting in the depletion of ambient NO_3^- to ~ 1 μM-N, even though there was a supply of new media during the daytime to dilute the culture as the cells grew. Cells in the P-rich cultures (i.e., N:P ratio 3.5) had significantly higher ($p < 0.05$) N assimilation rates at all concentrations, indicating that N limitation resulted in high P residuals in the media (Fig. 3-6). Cells in the low N:P ratio cultures also had relative higher assimilation efficiencies (A/V), especially for NO_3^- and NH_4^+, indicating that the N substrates were more quickly used by the cells in the P-rich media (Fig. 3-7).

There were no significant differences in the relative contributions of the 4 N substrates to total N uptake and assimilation at different N:P ratios.

In the *P. donghaiense* experiments, the N-specific uptake rates (V, h^{-1}) and assimilation rates (A, h^{-1}) varied over different ambient N:P ratios (Fig. 3-7). The uptake rates of NO_3^- not only decreased from an average of 0.021 h^{-1} to 0.01 h^{-1} when the ambient N:P ratios decreased from ~ 7 to ~ 1, but they also significantly decreased to 0.006 h^{-1} ($p < 0.05$) when ambient N:P ratio increased from ~ 7 to over 100 (Fig. 3-7 a). The assimilation rates of NO_3^- were close to 0.01 h^{-1} where ambient N:P ratio was <10, but they decreased to 0.003 h^{-1} when ambient N:P ratio were between 10 and 50 (Fig. 3-7 b). They further decreased significantly to ~ 0.0007 h^{-1} at higher N:P ratios ($p < 0.05$). The uptake rates of NH_4^+ varied from 0.046 h^{-1} to 0.080 h^{-1}, and the assimilation rates of NH_4^+ varied from 0.028 h^{-1} to 0.058 h^{-1} (Fig. 3-7 c, d). The assimilation efficiencies (assimilation rate/ uptake rate) of NO_3^- significantly decreased from 70 % at low N:P ratios to ~ 6% at high N:P ratios ($p < 0.05$), while the assimilation efficiencies varied from ~ 60 % to 88 % (Fig. 3-8).

Similar to the results in the *P. minimum* experiments, the uptake and assimilation of NO_3^- in the high N:P ratio cultures were significantly lower than those in lower N:P ratio cultures ($p < 0.05$), suggesting strong P control on the uptake and assimilation of NO_3^-. However, the uptake and assimilation of NH_4^+, which are less energy costly processes, did not show evidence of N:P ratio regulation.

Application of Droops Model

The nutrient uptake and assimilation rates represent the processes whereby nutrients are taken up from ambient water, which was measured as V in this

experiment, and then assimilated to build the cell, which measured as *A*. The balance of nutrient uptake and assimilation further affects the growth rate. The ratio of these processes to growth rate allows comparison across treatments. In the *P. minimum* experiments, although growth rates were lower at highest and lowest N:P ratios compared to near Redfield ratios, the patterns of how A/μ values for the 4 N substrates varied at different N:P ratios was opposite (Fig. 3-9). A/μ values were therefore higher at the highest and lowest N:P ratios. Under low N concentrations, *P. minimum* quickly responded to the N pulse and assimilated more than their growth needs in the N:P treatment of 3.5, where *P. minimum* had lowest growth rate, but highest A/μ value. The growth of *P. minimum* at N:P ratio 12.5 were most balanced of all N:P treatments with the highest average growth rates and lowest A/μ value. The A/μ values increased when P was reduced.

In the *P. donghaiense* experiments, the N-poor treatments (low N:P ratios) also had a significantly enhanced ($p < 0.05$) N uptake and assimilation relative to growth rates (Fig. 3-10), and this enhancement decreased as the N:P ratios increased to near-Redfield proportion. The V/μ values increased again at high N:P ratios (Fig. 3-10 a, b). However, the A/μ values did not increase at the high N:P values, since N assimilation was under a greater degree of P regulation (Fig.10 c, d).

Discussion

Herein we took an approach to examine the effect of different ambient N:P ratios on the growth and N uptake kinetics of *P. minimum* and *P. donghaiense* in turbidistat. For *P. minimum*, two relatively N-P balanced treatments (N:P ratio 12.5 and 21) and

two relatively N:P unbalanced treatments (N:P ratio 3.5 and 300) were tested. For *P. donghaiense*, over 20 N:P ratio treatments were tested. For both species, the highest growth rates were recorded near the Redfield ratio (16), and growth rate decreased when ambient N:P ratios were more unbalanced, which suggested the regulation of growth rate by the ambient N:P ratio. The N uptake and assimilation rates of the 2 species also showed a response to the different N:P ratio treatments.

Value of Tubidostat Approach

Batch culture and continuous culture are widely used in laboratory experiments for microalgae culture. The growth of algae in a batch culture typically includes 4 phases: lag phase, exponential, stationary phase, and death phase. The physiology in the exponential phase is very valuable to help understand algal growth. However, the growth of batch cultures are regulated by the limiting nutrient, and batch cultures can not provide a relatively long and steady exponential phase of growth without causing significant change in irradiance caused by self-shading and nutrient depletion. In continuous culture, cells can keep growing in exponential phase and the growth rate can be calculated (Monod 1950). There are two common continuous culture devices, chemostat and turbidistat. Both systems include a medium reservoir and a culture incubator. New media can be pumped into the incubator to support the continuous growth of the cells, and extra culture is removed from the incubator to keep a steady culture volume. In chemostat, steady-state can be established at a fixed rate of limiting nutrient inflow (Yoshida et al. 1979, Rhee and Gotham 1981). In another words, the cell growth rate is equal to the culture dilution rate and nutrient uptake rate is equal to the growth rate when steady state is established (Hirsbrunner 1981,

Goldman and Glibert 1982). The dilution rate must be set at a value less than the maximum growth rate to avoid washout. The culture can reach steady state, and the nutrient uptake and cell quota of limited nutrient are assumed to be invariant with time (Rhee 1973, Rhee 1978, Gotham and Rhee 1981). Higher biomass can be achieved by increasing the nutrient concentration of the media. Furthermore, chemostat cultures are typically operated in continuous light (Falkowski et al. 1985), which does not exist in natural condition. A cyclostat is a modified chemostat system in which culture grows under light-dark cycles (Gotham and Frisch 1981, Gotham and Rhee 1982). In cyclostats, nutrient uptake rate, growth rate, and cell quota are not invariant, but have been demonstrated to have periodicities relating to light-dark cycles (Eppley and Renger 1974, Chisholm et al. 1975, Malone et al. 1975, Frisch and Gotham 1977). The culture can reach rhythmic steady state with a period of light-dark cycle. Alternatively, turbidistats are run under turbidity control, and new medium inflow only occurs in response to an increase in the culture biomass to a pre-set value. Biomass is set by the optical threshold of dilution. Cell growth is limited by light and temperature; nutrients are usually added in excess of demand (Watson 1972, Skipnes et al. 1980, Fenaux et al. 1985, Hill et al. 1985, Kalyuzhin 1998). Light in turbidistat can run in a light-dark cycle which is more close to real situations (Falkowski et al. 1985).

The independent and dependent variables are opposite in chemostat and turbidistat. For example, cell density is dependent and growth rate is independent in chemostat, but cell density is independent and growth rate is dependent in turbidistat (Bennett and Boraas 1989). Turbidistats are better than chemostats for the

experiments conducted which study cells growing near their maximum specific growth rate. In the experiments conducted herein, both species achieved continuous growth and repeatable diel cycle in turbidistat. The cells grew at their maximum growth, which was regulated by the ambient nutrient condition (N:P ratios).

However, due to the dilution process, the mass balance in the turbidistats changed due to the difference between N and P mass in the culture removed and the new media pumped in. For example, a *P. minimum* cell in exponential phase has ~ 2.1 $\times 10^{-6}$ μM-N per cell (Li unpublished data), which represents ~ 34 μM-N and ~ 2.1 μM-P mass of cells at a cell density of 16, 000 cell ml^{-1}, with the assumption that the N:P ratio of cells was near the Redfield ratio. The fastest growing culture (ambient N:P ratio 12) took up ~1.6 μM-N h^{-1} in the light phase, based on an average growth rate of 0.047 h^{-1}. While the total ambient N (sum of 4 N sources) was only 3.2 μM-N, which could only support the growth for 2 hrs, there was very limited residual N in the culture, and the growth relied on the new media pumped into the culture. The total N and P mass pumped from new media (enriched with 40 μM-N and 2.5 μM-P) was approximately equal to the N removed from the culture in cells and media. The growth of culture near the Redfield ratio was balanced. However, in the low N:P ratio culture (N:P ratio 3.5), there were also very limited residual N and P in the culture. The P in the new media was much higher than that in the culture and in the cells, as a result, the culture became more N limited. In the culture growing at ambient N:P ratio of 300, the residual N was high (over 40 μM-DIN), but the P was low (0.15 μM-DIP), which supported the growth for less than 3 hrs. Furthermore, the P in the new media was very low (0.2 μM-N), which was much less than the P in the cells. When the

dilution started, the P pumped in the culture was less than the P removed in the cells, and the culture became more P limited.

Value of Droop's Model Approach

Based on the growth rates recorded by the turbidistat, the growth of both species showed strong light-regulated diel cycles. Cell growth, measured as the change of turbidity in the culture vessel, started at the beginning of light cycle. Dinoflagellate species have been reported to have a maximum growth limit of 1 division per day (McDuff and Chisholm 1982), as a result of dark: light regulated diel synchrony. The growth rates in the tubidistat system were consistent with these reports. The maxima growth rates in the light phase were around 1 day^{-1}. Maximum growth rate generally occurred in the first hour at the beginning of the light phase, and growth rate decreased during the day time.

The growth rate, in this study, refers to the rate of changing of the optical signals, which attenuated with the turbidity of the culture. The turbidity was caused by the total mass concentration in the culture. Therefore, the growth rate is interpreted as the variation rate of total mass density of the culture, instead of cell density, which was calibrated by diluted the culture by volume. During the dark phase, there were negative growth rates, suggesting decreases in mass concentration in the culture. When the photosynthesis activities stopped in the dark phase, the production rates of these species were less than the respiration rate, causing the negative growth rate. This state lasted until the start of the light cycle, when production rates increased quickly over respiration and the turbidity of the culture increased again.

Based on Droop's model, these *Prorocentrum* species had their maximum cell quotas when they reached the highest growth rates at the beginning of light phase. The decreasing growth rates during the day are indicative of decreasing cell quotas. However, the low growth rates in the dark cycle were not necessary indicative of a small cell quota, since the cells were light regulated instead of nutrient regulated. *P. minimum* has been reported to continuously uptake the NO_3^- in the dark (Paasche et al. 1984). Cells stopped growth at night due to light limitation, but nutrients were likely continuously taken up into and stored in the cell, causing an increasing cell nutrient quota (Fig. 3-11). The cell quota reached its maximum value of the day at the beginning of light phase, as did growth rates. Since the experiments conducted here were not focused on the diel cycle of *Prorocentrum* growth and nutrient uptake, the conceptual model of diel cell quota variation (Fig. 3-11) needs to be tested by further studies.

The N:P Ratios in the Coastal Ecosystems

There has been evidence of a global increase of harmful algal blooms (HABs), which have increased not only in frequency, but also in intensity and geographic distribution in the coastal area, and have caused ecological, economical and human health problems (Smayda 1997, Anderson et al. 2002). In many cases, the increase in HABs has been strongly correlated to the global increase of eutrophication (Hallegraeff 1993, Glibert and Terlizzi 1999, Anderson et al. 2002, Smayda 2002, Glibert et al. 2005a, Glibert and Burkholder 2006, Glibert et al. 2006, Anderson et al. 2008, Glibert et al. 2008a, Heisler et al. 2008). Growth of human population, agriculture fertilization and urbanization has caused remarkable increase in N and P

loading to the coastal water. The progress toward controlling N loading has been relatively slow, and N load to the coastal environments has been increasing in the past several decades (NRC 2000). In the U.S., N fertilizer consumption has increased over 4 fold since 1960s, however, P fertilizer consumption only increased 80% up to 2006 (USDA 2008). Actually, the consumption of P fertilizer reached its peak in 1979 and has slightly decreased after that year (USDA 2008). There were also downward trends of P concentrations in many riverine sources (Litke 1999). One of reasons is that the principal source of effluent P was from P in laundry detergent, which has been gradually replaced by P-free detergent since the late 1970s (Doemel and Brooks 1975, Sharfstein et al. 1977, Hartig and Horvath 1982, Maki et al. 1984). Therefore, the increases of N and P loading to coastal ecosystems have not been proportionate. As the P-detergent banned expanded globally, export of dissolved inorganic P (DIP, mostly in form of phosphate), the most important form of bio-available P source, did not increase as much as N. This unbalanced increase of N and P nutrients loading has contributed to the change of the N:P ratios in the coastal water.

Changes of ambient N:P ratios could regulate the phytoplankton community composition and HAB developments (Hodgkiss and Ho 1997). In freshwater, N-fixing cyanobacteria could dominate in a low N:P ratio water column by fixing its own N source (Vrede et al. 2009). Good N competitors, including diatoms, may dominate the low N:P ratio community, but good P competitors (e.g. *Synechococcus*) may dominate the high N:P ratio community (Suttle and Harrison 1988). In seawater, a shift of dominant species in the phytoplankton community caused by the alternation of ambient N:P ratios could happen in time ranges of seasons to decades (Phillips and

Tanabe 1989, Bulgakov and Levich 1999, Lagus et al. 2004, Paerl et al. 2004). For example, the phytoplankton community of downstream Neuse River, USA, shifted from an N-limited cyanobacteria dominant freshwater phytoplankton community to a P-limited community after the P-detergent ban in 1988 in this area (Paerl et al. 2004). As a result of P loading reduction, N substrates taken up by phytoplankton decreased and more N was delivered to the lower estuary which stimulated HABs comprised of dinoflagellates, cryptomonads, and diatoms (Paerl et al. 2004). Spatially, high N:P ratio runoff mixed with relatively low N:P ratio seawater can develop a gradient of N:P ratio, which may regulate not only the HAB distribution and biomass, but also dominant species and its nutrient uptake rates and preference (Fisher et al. 1992, Li et al. 2009, Li et al. 2010).

In summary, the study of the growth and N uptake of *P. minimum* (a global HAB species) and *P. donghaiense* (a regional HAB species) on different N:P ratios showed evidence that N:P ratios could not only affect the growth, but also N uptake and assimilation. Relatively N:P balanced cultures reached the highest growth rates. N-poor cultures showed an enhanced N uptake in N enrichment experiments. Highest average daily growth rates of both species were recorded near N:P ratios of 12. Highest N assimilation rates for 4 N sources by *P. minimum* were recorded at N:P ratio of 3.5. *P. donghaiense* also has highest N assimilation coefficient at low N:P ratio. These results were consistent with the report that the optimal N:P ratio for *P. minimum* was 4 - 13 (Hodgkiss and Ho 1997) and the field report of *P. donghaiense* dominance under N:P ratios of 16 - 24 (Li et al. 2009).

References

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Bennett WN, Boraas ME (1989) Comparison of population-dynamics between slow-growing and fast-growing strains of the rotifer *Brachionus calyciflorus* pallas in continuous Culture. Oecologia 81:494-500

Bulgakov NG, Levich AP (1999) The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv Fur Hydrobiologie 146:3-22

Burns CL, Pennock JR, Lores EM, R.M. G (2000) The effect of nitrogen source on the growth and toxicity of three potentially harmful dinoflagellates. Journal of Phycology 36

Chisholm SW, Nobbs PA, Stross RG (1975) Simulation of algal growth and competition in a phosphate-limited cyclostat. Abstracts of Papers of the American Chemical Society:42-42

Degens ET, Mopper K (1976) Factors controlling distribution and early diagenesis of organic marine sediments. In: Riley JP, Chester R (eds) Chemical oceanography, Vol 6. Academic London, New York, San Francisco, p 60-114

Doemel WN, Brooks AE (1975) Detergent phosphorus and algal growth. Water Research 9:713-719

Droop MR (1968) Vitamin B12 and marine ecology 4. kinetics of uptake growth and inhibition in *Monochrysis lutheri*. Journal of the Marine Biological Association of the United Kingdom 48:689-733

Droop MR (1973) Some thoughts on nutrient limitation in algae. Journal of Phycology 9:264-272

Eppley RW, Renger EH (1974) Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. Journal of Phycology 10:15-23

Falkowski PG, Dubinsky Z, Wyman K (1985) Growth-irradiance relationships in phytoplankton. Limnology and Oceanography 30:311-321

Fan C, Glibert PM, Alexander J, Lomas MW (2003a) Characterization of urease activity in three marine phytoplankton species, *Aureococcus anophagefferens*, *Prorocentrum minimum*, and *Thalassiosira weissflogii*. Marine Biology 142:949-958

Fan CL, Glibert PM (2005) Effects of light on nitrogen and carbon uptake during a *Prorocentrum minimum* bloom. Harmful Algae 4:629-641

Fan CL, Glibert PM, Burkholder JM (2003b) Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. Harmful Algae 2:283-299

Fenaux R, Malara G, Claustre H (1985) A turbidostat driven and controlled by microcomputer. Aquaculture 48:91-95

Fisher TR, Peele ER, Ammerman JW, Harding LW (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Marine Ecology-Progress Series 82:51-63

Frisch HL, Gotham IJ (1977) Periodic algal cyclostat populations. Journal of Theoretical Biology 66:665-678

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Granéli E, Anderson DM (2008a) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Glibert PM, Capone DG (1993) Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques, Vol 243-272. Academic Press

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea - a global change contributing to coastal eutrophication. Biogeochemistry 77:441-463

Glibert PM, Mayorga E, Seitzinger S (2008b) *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: Application of spatially explicit nutrient export models. Harmful Algae 8:33-38

Glibert PM, Terlizzi DE (1999) Cooccurrence of elevated urea levels and dinoflagellate blooms in temperate estuarine aquaculture ponds. Applied and Environmental Microbiology 65:5594-5596

Goldman JC, Glibert PM (1982) Comparative rapid ammonium uptake by 4 species of marine-phytoplankton. Limnology and Oceanography 27:814-827

Gotham IJ, Frisch HL (1981) A simple-model for cell-volume and developmental compartments in nutrient limited cyclostat cultures of algae. Journal of Theoretical Biology 92:435-467

Gotham IJ, Rhee GY (1981) Comparative kinetic-studies of phosphate-limited growth and phosphate-uptake in phytoplankton in continuous culture. Journal of Phycology 17:257-265

Gotham IJ, Rhee GY (1982) Effects of nitrate and phosphate limitation on cyclostat growth of 2 fresh-water diatoms. Journal of General Microbiology 128:199-205

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99

Harding LW, Coats DW (1988) Photosynthetic physiology of *Prorocentrum mariae-lebouriae* (Dinophyceae) during Its subpycnocline transport in Chesapeake Bay. Journal of Phycology 24:77-89

Harding LW, Meeson BW, Tyler MA (1983) Photoadaptation and diel periodicity of photosynthesis in the dinoflagellate *Prorocentrum mariae-lebouriae*. Marine Ecology-Progress Series 13:73-85

Hartig JH, Horvath FJ (1982) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 54:193-197

Heil CA, Glibert PM, Fan CL (2005) *Prorocentrum minimum* (Pavillard) Schiller - a review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449-470

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8:3-13

Hill SH, Abbott MR, Denman KL (1985) A computer-controlled turbidostat for the culture of planktonic algae. Canadian Journal of Fisheries and Aquatic Sciences 42:744-753

Hirsbrunner M (1981) A chemostat-apparatus for continuous culture of agae. Schweizerische Zeitschrift Fur Hydrologie-Swiss Journal of Hydrology 43:370-376

Hodgkiss IJ, Ho KC (1997) Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147

Jeong HJ, Du Yoo Y, Park JY, Song JY, Kim ST, Lee SH, Kim KY, Yih WH (2005a) Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquatic Microbial Ecology 40:133-150

Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Jeng C, Seong CN, Lee KY, Yih WH (2005b) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquatic Microbial Ecology 41:131-143

Kalyuzhin VA (1998) The growth of a turbidostat yeast culture in the presence of high concentrations of various compounds in a steady-state regime and under osmotic shock. Microbiology 67:499-503

Lagus A, Suomela J, Weithoff G, Heikkila K, Helminen H, Sipura J (2004) Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. Journal of Plankton Research 26:779-798

Lane L, Rhoades S, Thomas C, Van Heukelem L (2000) Analytical services laboratory standard operating procedures, University of Maryland Center for Environmental Science, Cambridge, Maryland.

Li AS, Stoecker DK, Coats DW, Adam EJ (1996) Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquatic Microbial Ecology 10:139-147

Li J, Glibert PM, Zhou M (2010) Temporal and spatial variability in nitrogen uptake kinetics during dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou M, Lu S, Lu D (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology Progress Series 383:11-26

Lindroth P, Mopper K (1979) High-performance liquid-chromatographic determination of subpicomole amounts of amino-acids by precolumn fluorescence derivatization with ortho-phthaldialdehyde. Analytical Chemistry 51:1667-1674

Litke DW (1999) Review of phosphorus control measures in the United States and their effects on water quality, U.S. Geological Survey

Lomas MW, Glibert PM (1999a) Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Marine Biology 133:541-551

Lomas MW, Glibert PM (1999b) Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnology and Oceanography 44:556-572

Lomas MW, Glibert PM (2000) Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. Journal of Phycology 36:903-913

Lu DD, Goebel J (2001) Five red tide species in genus *Prorocentrum* including the description of *Prorocentrum donghaiense* Lu Sp. Nov. from the East China Sea. Chinese Journal of Oceanology and Limnology 4:337-344

Maki AW, Porcella DB, Wendt RH (1984) The impact of detergent phosphorus bans on receiving water-quality. Water Research 18:893-903

Malone TC, Garside C, Haines KC, Roels OA (1975) Nitrate Uptake and Growth of Chaetoceros Sp in Large Outdoor Continuous Cultures. Limnology and Oceanography 20:9-19

McDuff RE, Chisholm SW (1982) The calculation of in situ growth-rates of phytoplankton populations from fractions of cells undergoing mitosis - a clarification. Limnology and Oceanography 27:783-788

Monod J (1950) La technique de culture continue theorie et applications. Annales De L Institut Pasteur 79:390-410

NRC (2000) Clean coastal waters: Understanding and reducing the effects of nutrient pollution. National Academies Press.

Paasche E, Bryceson I, Tangen K (1984) Interspecific variation in dark nitrogen uptake by dinoflagellates. Journal of Phycology 20:394-401

Paerl HW, Valdes LM, Joyner AR, Piehler MF (2004) Solving problems resulting from solutions: Evolution of a dual nutrient management strategy for the eutrophying Neuse river estuary, North Carolina. Environmental Science & Technology 38:3068-3073

Phillips DJH, Tanabe S (1989) Aquatic pollution in the Far-East. Marine Pollution Bulletin 20:297-303

Revilla M, Alexander J, Glibert PM (2005) Urea analysis in coastal waters: comparison of enzymatic and direct methods. Limnology and Oceanography-Methods 3:290-299

Rhee G-Y (1978) The continuous culture in phytoplankton ecology. In: Droop MR, Jannasch HW (eds) Advances in Aquatic Microbiology

Rhee GY (1973) Continuous culture study of phosphate uptake, growth-rate and polyphosphate in *Scenedesmus* sp. Journal of Phycology 9:495-506

Rhee GY, Gotham IJ (1981) The effect of environmental-factors on phytoplankton growth - light and the interactions of light with nitrate limitation. Limnology and Oceanography 26:649-659

Salerno M, Stoecker DK (2009) Ectocellular glucosidase and peptidase activity of the mixotrophic dinoflagellate *Prorocentrum minimum* (Dinophyceae). Journal of Phycology 45:34-45

Sciandra A (1991) Coupling and uncoupling between nitrate uptake and growth rate in *Prorocentrum minimum* (Dinophyceae) under different frequencies of pulsed nitrate supply. Marine Ecology-Progress Series 72:261-269

Sharfstein B, Roels OA, Harris V, Lee V (1977) Effect of detergent legislation on phosphorus in effluent and receiving waters. Journal Water Pollution Control Federation 49:2017-2021

Skipnes O, Eide I, Jensen A (1980) Cage culture turbidostat - a device for rapid-determination of algal growth-rate. Applied and Environmental Microbiology 40:318-325

Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42:1137-1153

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Stoecker DK, Gustafson DE (2003) Cell-surface proteolytic activity of photosynthetic dinoflagellates. Aquatic Microbial Ecology 30:175-183

Stoecker DK, Li AS, Coats DW, Gustafson DE, Nannen MK (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Marine Ecology-Progress Series 152:1-12

Suttle CA, Harrison PJ (1988) Ammonium and phosphate-uptake rates, N-P supply ratios, and evidence for N-limitation and P-limitation in some oligotrophic lakes. Limnology and Oceanography 33:186-202

Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area - water circulation patterns and organism distributions in Chesapeake Bay. Limnology and Oceanography 23:227-246

USDA (2008) Fertilizer consumption and use. United States Department of Agriculture. http://www.ers.usda.gov/Data/FertilizerUse/

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2009) Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54:331-344

Watson TG (1972) Present status and future prospects of turbidostat. Journal of Applied Chemistry and Biotechnology 22:229

Yoshida T, Rao BSM, Ohasa S, Taguchi H (1979) Dynamic analysis of a mixed culture in chemostat. Journal of Fermentation Technology 57:546-553

Zhou M, Yan T, Zou J (2003) Preliminary analysis of the characteristics of red tide areas in Changjiang River estuary and its adjacent sea. Chinese Journal of Applied Ecology 14:1031-1038

Table 3-1 Ambient nutrient concentrations in 4 different turbidistat systems supplied by media at different DIN:DIP ratios.

Medium Enriched N:P Ratio	NO_3^- µM-N	NH_4^+ µM-N	Urea µM-N	DFAA µM-N	DIP µM-P	Culture DIN:DIP Ratio
5	1.95	0.05	1.18	0.063	0.57	3.5
16	0.37	1.05	3.14	0.011	0.11	12.5
40	21.98	3.59	2.09	0.038	1.17	21
200	42.4	2.52	2.05	1.123	0.15	300

Table 3-2 Calculated parameters of uptake kinetics of nitrogen sources (NH_4^+, urea, and glycine) by *Prorocentrum minimum* at different ambient DIN:DIP ratio in turbidistat based on the Michaelis-Menten equation. V_{max} and A_{max} are the specific nitrogen uptake and assimilation rates. K_s is the Michaelis constant where uptake rates equal to ½ of maximum uptake rates.

Culture DIN:DIP	NH_4^+		Urea		Glycine		NH_4^+		Urea		Glycine	
Ratio	V_{max}	K_s	V_{max}	K_s	V_{max}	K_s	A_{max}	K_s	A_{max}	K_s	A_{max}	K_s
3.5							0.031	0.20	5E-04	0.43	0.004	1.27
12.5	0.046	1.248	3E-04	0.052	0.004	0.48	0.024	2.32	7E-04	16.50	0.001	0.04
21							0.02	1.43	4E-04	2.63	0.001	0.10
300	0.065	4.646	8E-04	5.43	0.007	3.54	0.016	2.70	4E-04	1.56	0.003	3.39

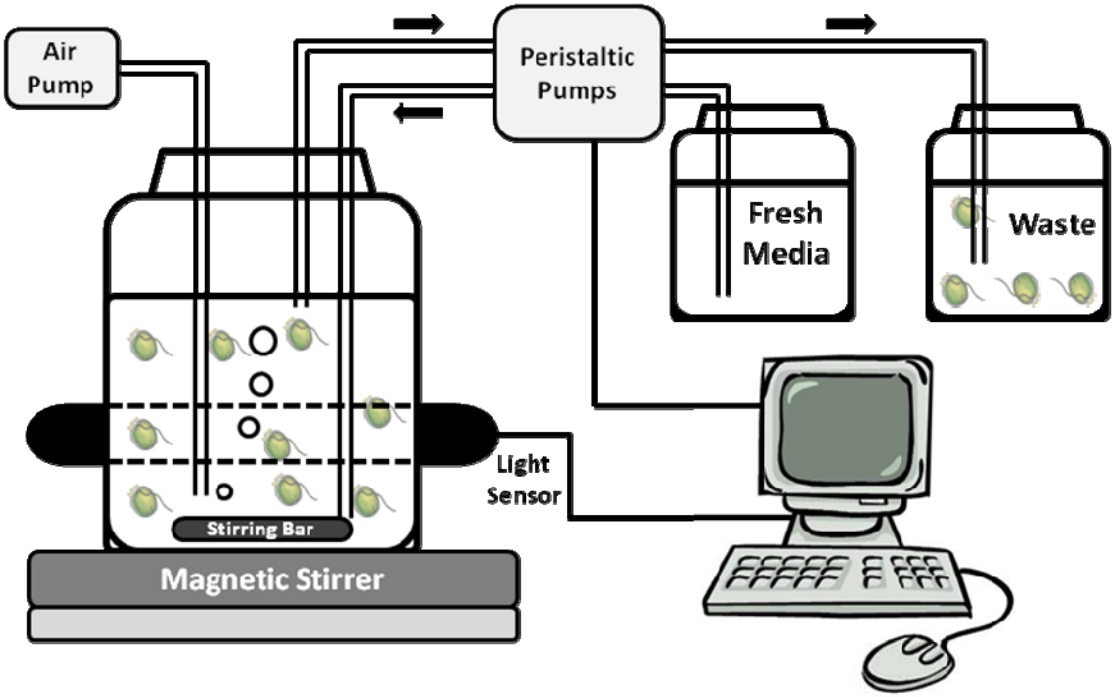


Figure 3-1 The setup of a turbidistat system

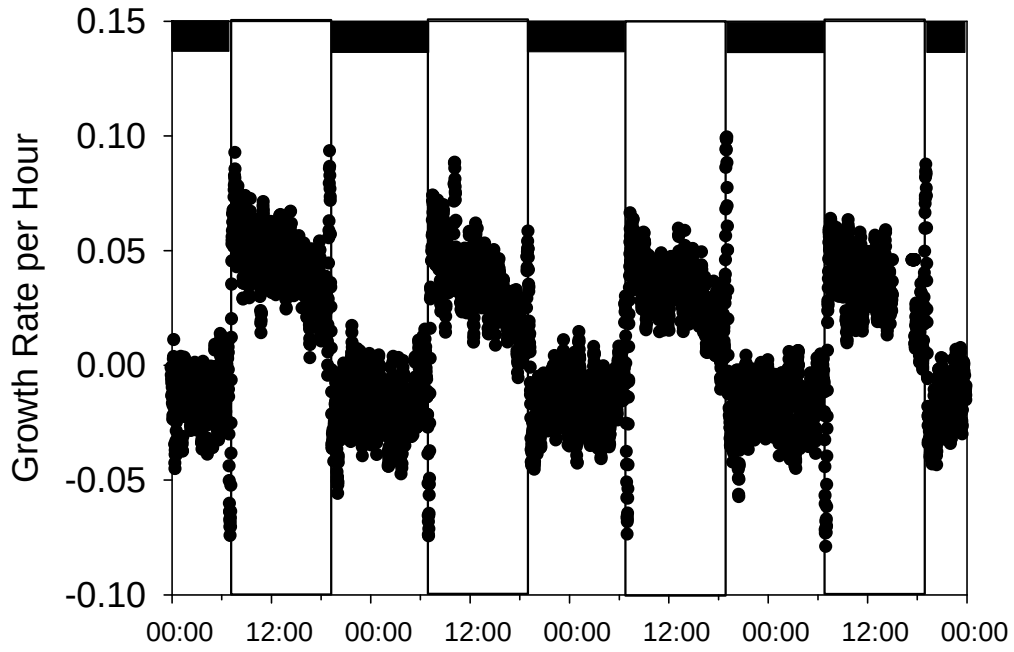


Figure 3-2 Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system for 4 constant 12 h: 12 h light dark cycles (96 hrs). Each point stands for the growth rate calculated based on the change in the optical signals over a 20-minute time period.

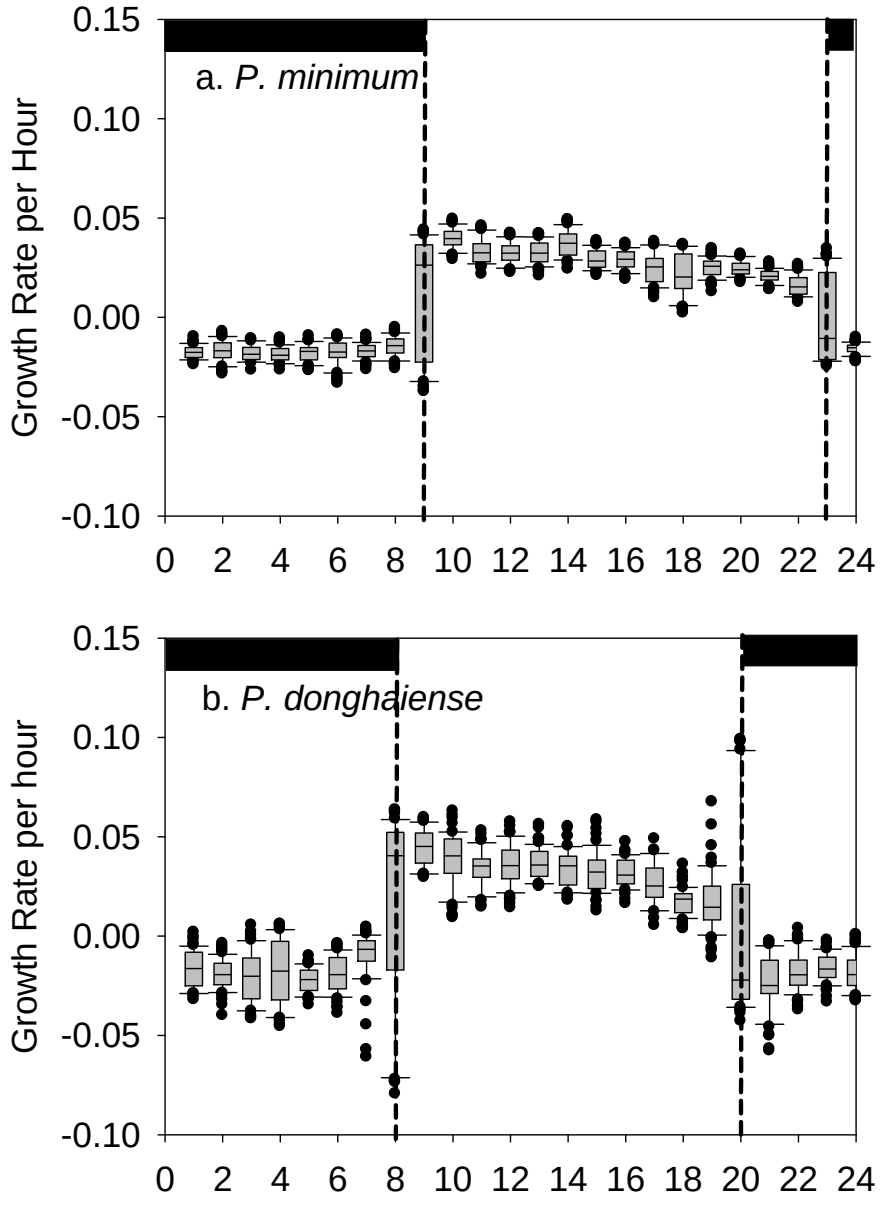


Figure 3-3 a. Diurnal growth rates of *Prorocentrum minimum* in turbidistat system at 14: 10 h light dark cycle. b. Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system at 12: 12 h light dark cycle. Box plots — line: median; box: 25th and 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

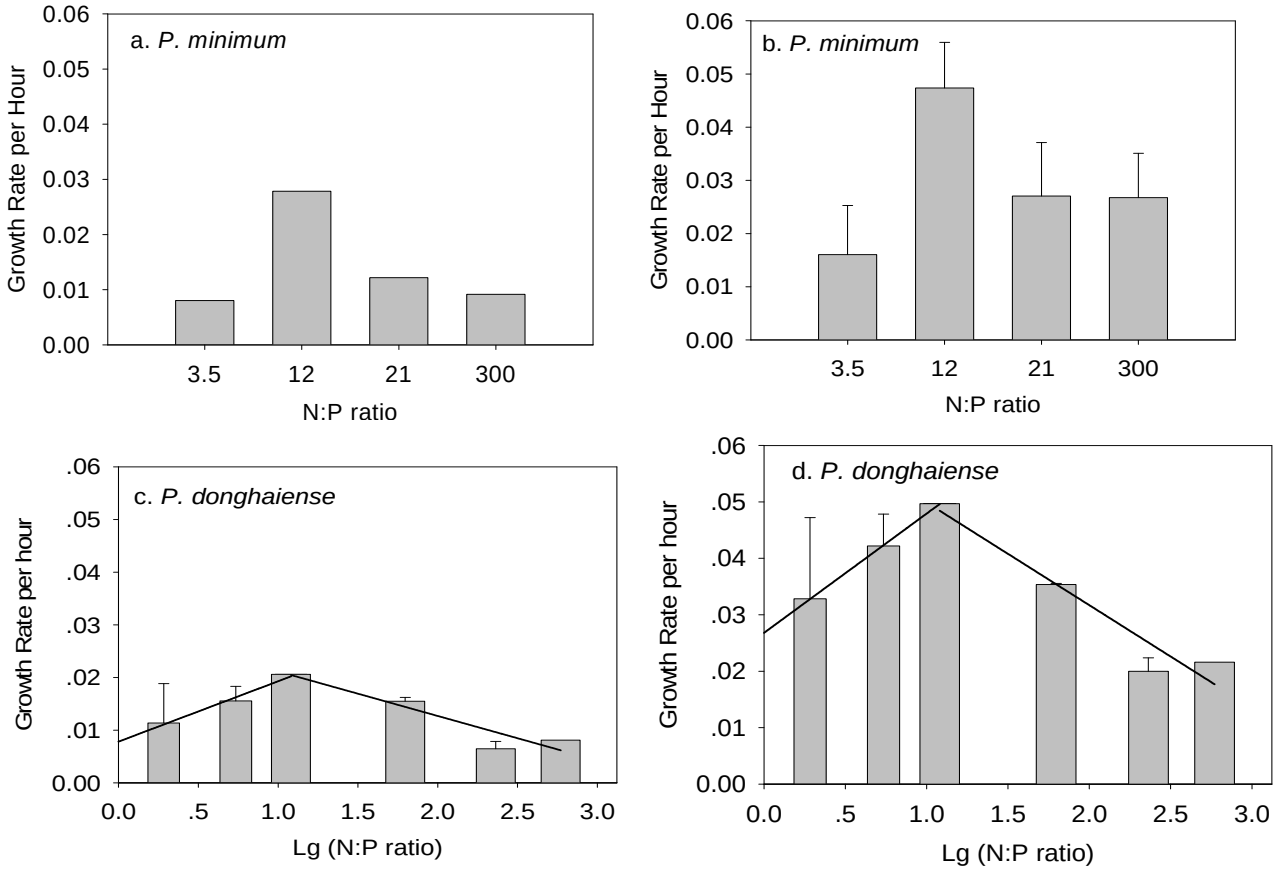


Figure 3-4 a. Average growth rates of *Prorocentrum minimum* in a 14h: 10h light dark cycle at different ambient N:P ratios. b. Average growth rates of *P. minimum* in the light phase of 14h: 10h light dark cycle at different ambient N:P ratios. c. Average growth rates of *P. donghaiense* in a 12h: 12h light dark cycle at different ambient N:P ratios. d. Average growth rates of *P. donghaiense* in the light phase of 12h: 12h light dark cycle at different ambient N:P ratios

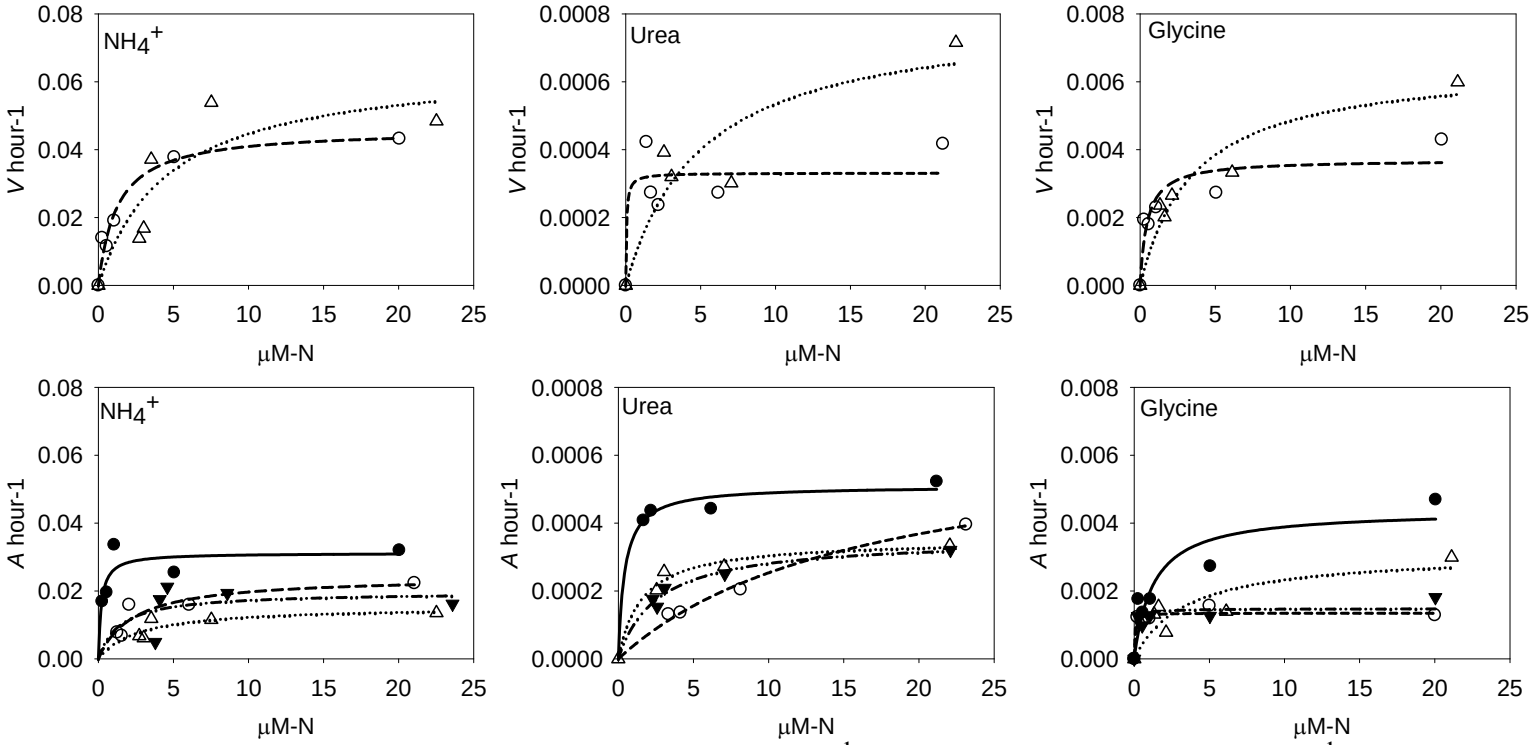


Figure 3-5 Kinetic relationships between nitrogen specific uptake rates *V* (h^{-1}, upper panels) and assimilation rates *A* (h^{-1}, lower panels) of NH_4^+, urea, and glycine with ^{15}N substrate concentration (μM-N) by *Prorocentrum minimum* at different DIN:DIP ratio cultures. All curves $R^2 > 0.6$.

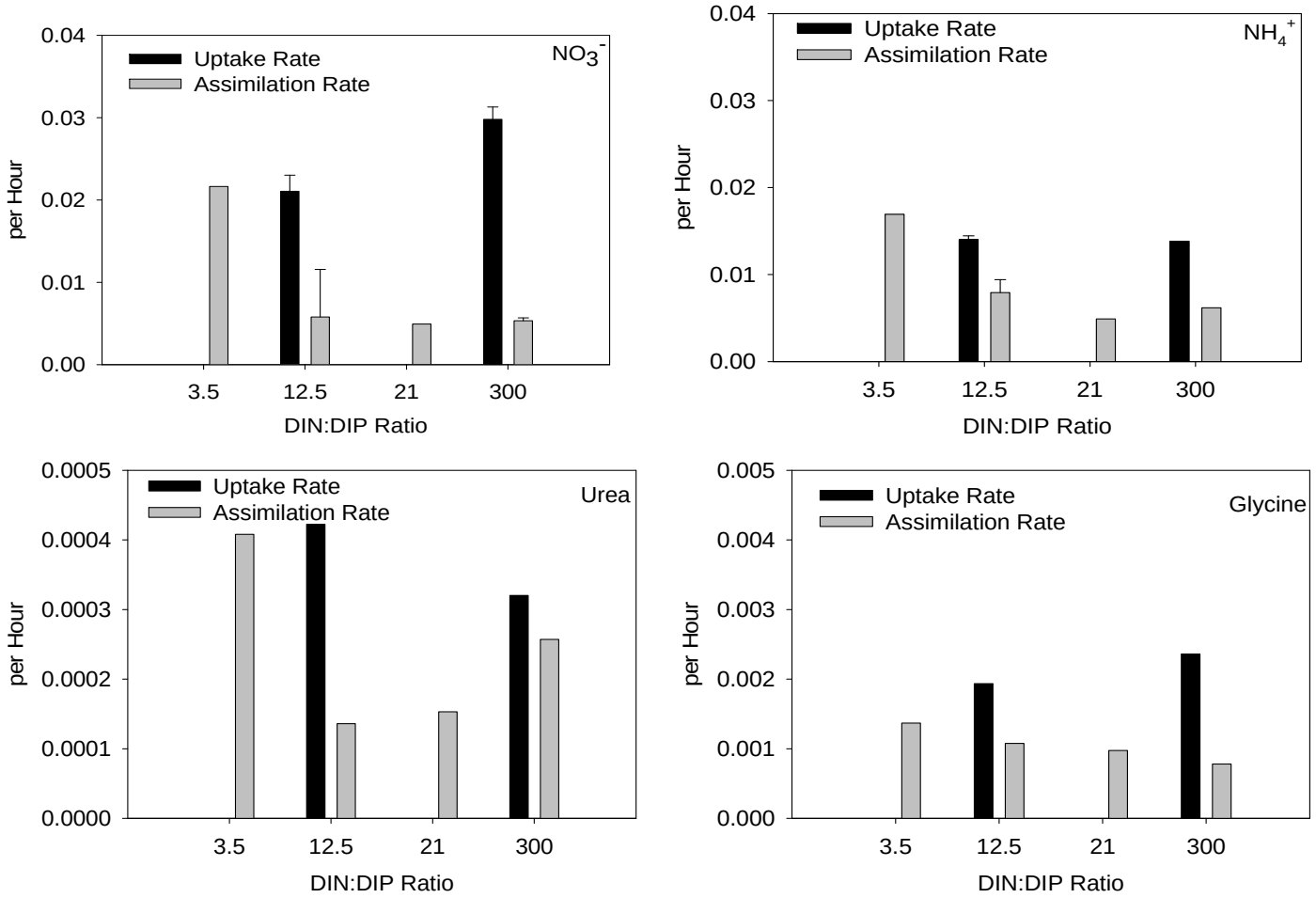


Figure 3-6 Nitrate specific uptake rates *V* (h^{-1}) and assimilation rates *A* (h^{-1}) of NH_4^+, urea, and glycine with ^{15}N substrate concentration (µM-N) by *Prorocentrum minimum* grown at different DIN:DIP ratios.

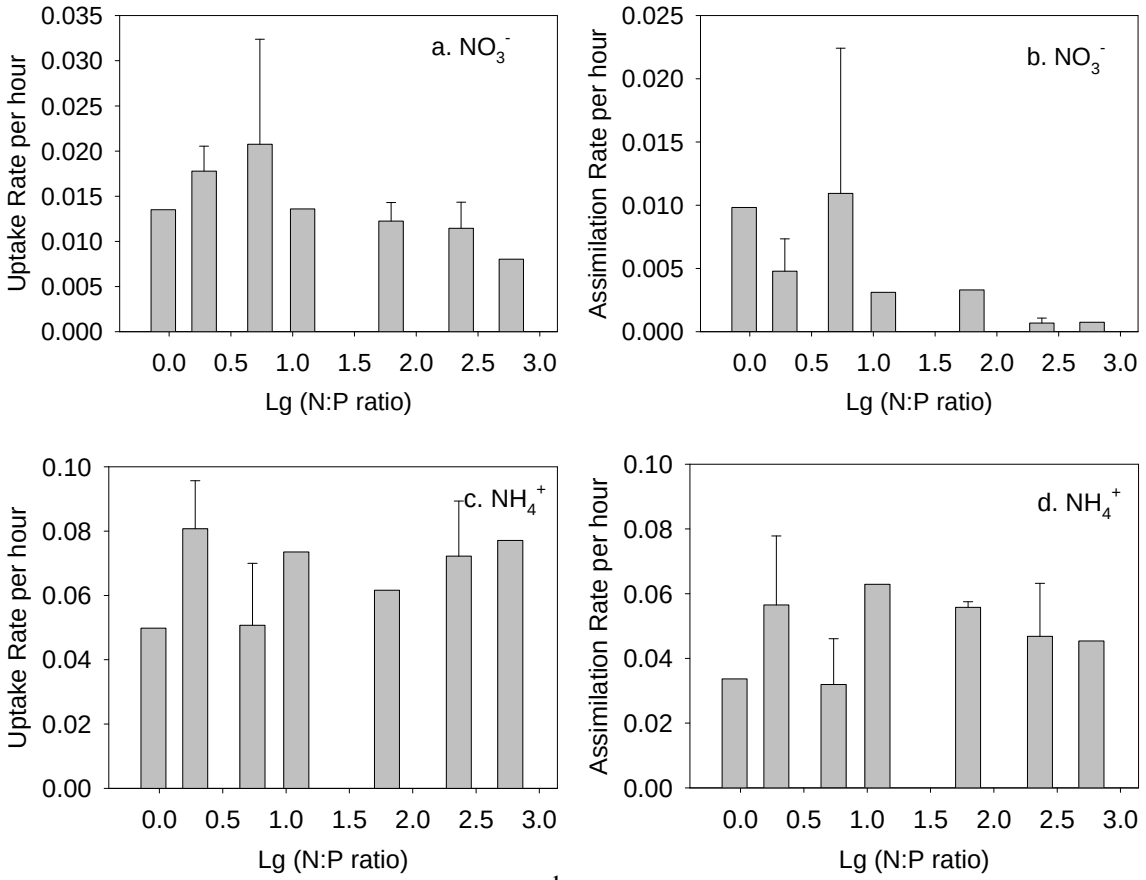


Figure 3-7 a. The NO_3^- specific uptake rates (V, h^{-1}) of *Prorocentrum donghaiense* grown at different ambient N:P ratios; b. The NO_3^- specific assimilation rates (A, h^{-1}) of *P. donghaiense* grown at different ambient N:P ratios. c. The NH_4^+ uptake rates of *P. donghaiense* grown at different ambient N:P ratios; d. The NH_4^+ assimilation rates of *P. donghaiense* grown at different ambient N:P ratios.

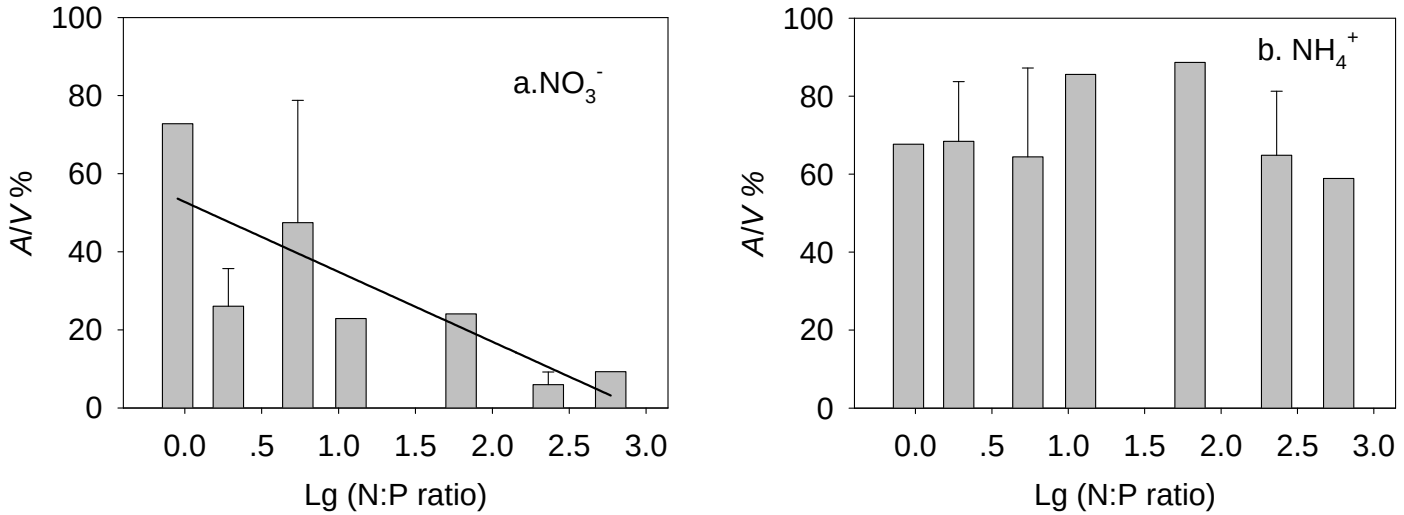


Figure 3-8 The assimilation efficiencies % (assimilation rate/ uptake rate) of NO_3^- by *Prorocentrum donghaiense* grown at different ambient N:P ratios; b. The assimilation efficiencies % (assimilation rate/ uptake rate) of NH_4^+ by *P. donghaiense* grown at different ambient N:P ratios

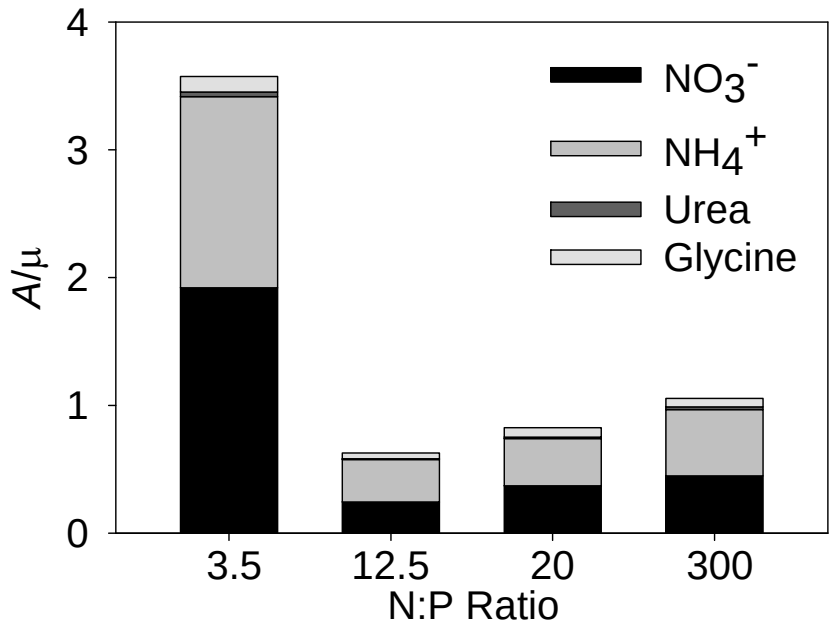


Figure 3-9 The ratio of specific N assimilation rate/ growth rate (*A*/μ) of *Prorocentrum minimum* for major N sources at different DIN:DIP ratios

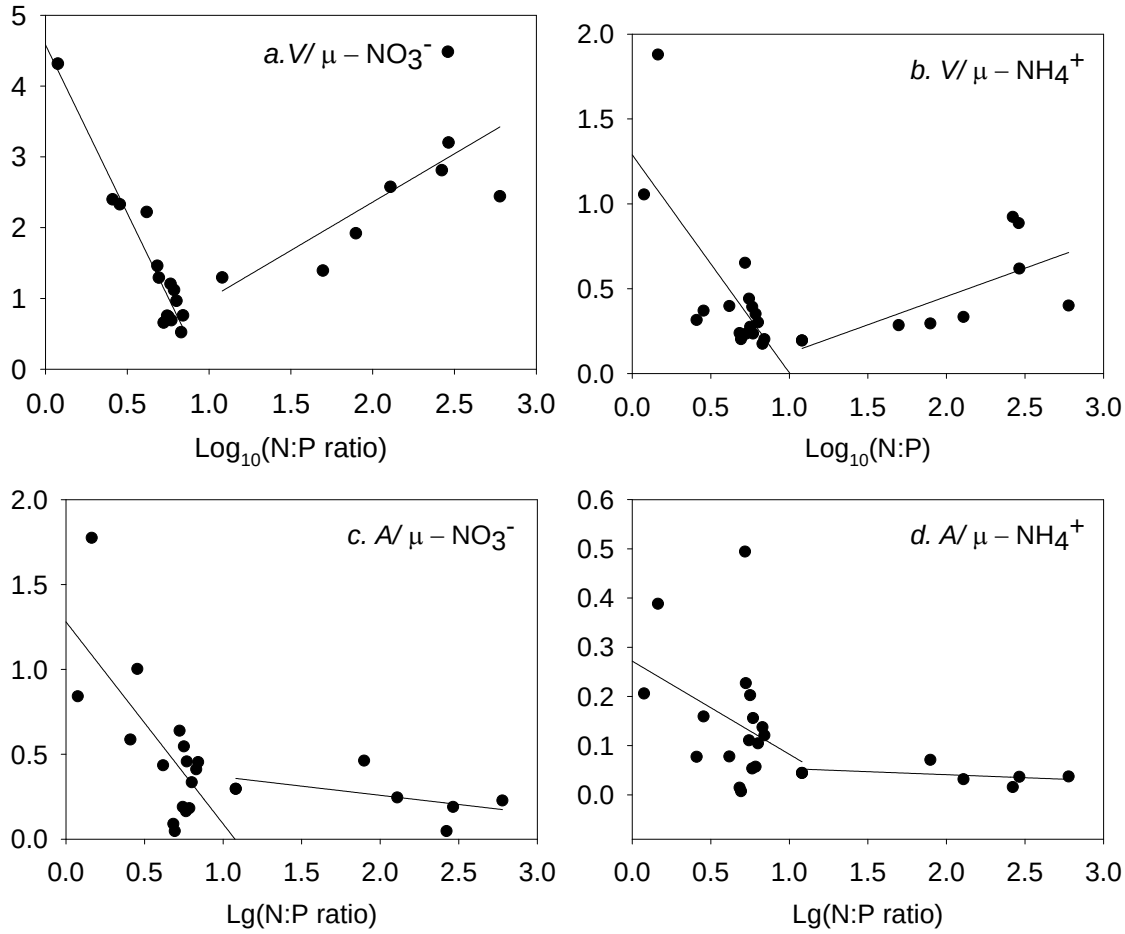


Figure 3-10 The ratio of specific N uptake rate/ growth rate (*V*/μ) of *Prorocentrum donghaiense* for NO_3^- (a) and NH_4^+ (b) at different ambient N:P ratios; the ratio of specific N assimilation rate/ growth rate (*A*/μ) of *P. donghaiense* for NO_3^- (c) and NH_4^+ (d) at different ambient N:P ratios

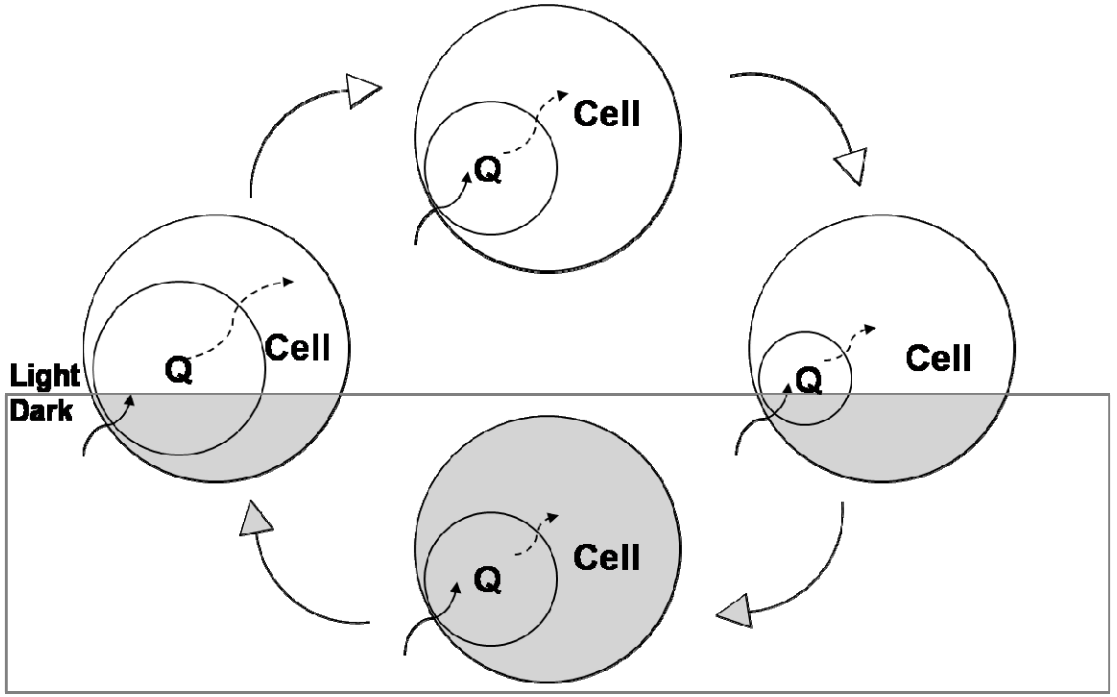


Figure 3-11 The conceptual model of the diel cell nutrient quota variation in a light-dark cycle. Q is the cell nutrient quota; the shading area stands for dark phase in a diel cycle; the filled arrows stand for the nutrients uptake from ambient water into the cell quota; the dash arrows stand for the process that nutrient in the cell quota is assimilated to build the cell.

Chapter 4: Effects of Light on Growth and Nitrogen Uptake of *Prorocentrum minimum* and *Prorocentrum donghaiense*

Abstract

The effects of varying light irradiance on the growth, nitrogen (N) uptake and assimilation of the harmful dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* were examined in turbidistat culture experiments. Algal cultures were grown in a wide range of ambient light intensities from high light to dark, when their growth rates and N acquisition were studied. Experiments to determine rates of N uptake and assimilation of different N sources (NO_3^-, NH_4^+, urea and glycine by *P. minimum* and NO_3^-, NH_4^+ by *P. donghaiense*) were conducted using ^{15}N tracer technique at each light irradiance. The growth rates suggested strong light regulation on the growth of *P. donghaiense*. On a diel basis, the growth of both species was regulated by the light-dark cycle, which may be a result of regulation of both light-dependent growth and light-independent N nutrient uptake. Maximum growth rates of both species always occurred at the beginning of light phase. N uptake by *P. minimum* for all tested substrates showed no obvious light limitation on a short-term basis. However, NH_4^+ uptake of *P. donghaiense* showed significant light limitation on long-term basis. The light independent N uptake by *P. minimum* and *P. donghaiense* allow them to be competitive in the relative low light available near-shore water. However, the capability of light independent N uptake between the two *Prorocentrum* species might be different.

Introduction

An increasing frequency and intensity of harmful algae blooms (HABs) has been observed in coastal waters all over the world and these events threaten the structure and function of coastal ecosystems (Smayda 1990, Hallegraeff 1993, Anderson 1997, Moncheva et al. 2001, Heil et al. 2005, Wang and Wu 2009). It has been suggested that many HABs are correlated with the acceleration of eutrophication in coastal ecosystems (Hallegraeff 1993, Anderson et al. 2002, Glibert et al. 2005, Glibert and Burkholder 2006, Glibert et al. 2006, Glibert et al. 2008a, Heisler et al. 2008). Smayda (2001) suggested that dinoflagellates, a major group of bloom-forming algae, have diverse habitat preferences and adaptive strategies and characterized dinoflagellate bloom species in coastal areas in a nutrient-turbulence matrix (Smayda and Reynolds 2001, Smayda 2002). Each dinoflagellate species has its specific niche for bloom expansion, and the niches are described by available nutrients and turbulence/mixing intensity. In this matrix, *Prorocentrum* spp. (length 15~22 μm width 9~14 μm) are Type II species which are small, rapidly growing competitors blooming in nutrient-rich near-shore regions.

Prorocentrum minimum has caused HAB problems in both tropical and temperate coastal waters (Heil et al. 2005, Glibert et al. 2008b). It is a common HAB species in coastal and estuarine systems in USA, and frequently develops blooms in the Chesapeake Bay and southwest Florida shelf (Glibert et al. 2001, Tango et al. 2005, Heil et al. 2007). *Prorocentrum donghaiense*, is a major bloom species near the Changjiang Estuary and in the coastal area of East China Sea. Large scale *P. donghaiense* blooms have been observed in the past two decades (Zhou et al. 2006, Li

et al. 2009, Li et al. 2010). *Prorocentrum minimum* and *P. donghaiense* are very similar species and can be classified in the same category in the Smayda's model, and their expanding geographical distribution is indicative of a strong relationship to terrestrial nutrients discharge into coastal waters (Heil et al. 2005, Glibert and Burkholder 2006, Glibert et al. 2008a). However, there are still differences between the habitats of these two species. *Prorocentrum donghaiense* can only grow well in high salinity (25 - 35) water (Xu et al. 2010). The high frequent bloom areas of *P. donghaiense* have been documented mostly in the adjacent area of the Changjiang River estuary, which is the largest river of the China (Zhou et al. 2003, Zhou et al. 2006). The Changjiang River water develops huge plumes from the river mouth, with characteristically high nutrients, high turbidity, but low salinity water (Lie et al. 2003). The distribution of *P. donghaiense* is usually observed relatively off-shore near the edge of the river plume, where these cells could access the riverine nutrients, but still remain in the water column with high clarity (Li et al. 2009). On the other hand, *P. minimum* can grow over a broad salinity range, and develops blooms in the coastal water with salinity as low as 5 (Heil et al. 2005, Tango et al. 2005). In the Chesapeake Bay, high frequent *P. minimum* were documented in the muddy tributaries, where the secchi depth was only ~ 1m (Tango et al. 2005).

Considering these field observation, it is reasonable to suggest these two species can be exposed to different light in these habitats, when they develop blooms. Studying the effects of light on the growth and nutrient uptake of these two species will be helpful to understand the mechanisms of bloom development. Previous studies suggested that *P. minimum* grew well under low light conditions (Grzebyk and

Berland 1996), and show light-independent N uptake ability (Fan and Glibert 2005). However, according to recent literature review, there is no information on the influence of light on the N uptake of *P. donghaiense.*

Herein, the utilization of N nutrients by the phytoplankton are described as two steps. Nitrogen uptake is the step in which N nutrients are transported across the cell membrane into the nutrient pool inside the cell. When taken up into the cell, NO_3^- and NO_2^- are reduced to $NH_4^+$with the aid of NO_3^-reductase or NO_2^-reductase, or both (Lomas and Glibert 2000). Reduced forms of N nutrients can be utilized in the synthesis of organic compounds, which is the step of assimilation (Conway 1977, Glibert and McCarthy 1984).

In this study, cultures of *P. minimum* and *P. donghaiense* were grown across a light irradiance spectrum in order to achieve a range of growth rates and a range of physiological states. Ambient N:P ratio, which has been reported to be related to the regulating growth and physiology of these two species (Li et al. 2009, Li et al. in press), was also considered as a variable in the experiments of *P. minimum*. The effect of light on these two species was tested on growth rate and the rates of uptake of different forms of N, as were the comparative rates of uptake and assimilation.

Materials and Methods

Algal Culture

Cultures of *Prorocentrum minimum* were isolated from Choptank River, a tributary of Chesapeake Bay in spring 1995 and were maintained in f/2 medium in the

Horn Point Laboratory culture collection. For the present experiments, *P. minimum* cells were first transferred to f/2 batch cultures for 2 weeks, then grown in turbidistat, an automatic continuous culture system, as described in Li et al. (in press), at a cell density ~ 16,000 cell ml^{-1}.

Cultures of *Prorocentrum donghaiense* were obtained from University of Connecticut Department of Marine Sciences, USA. These cultures were originally isolated from the East China Sea. They, too, were transferred to f/2 batch cultures for several weeks, then grown in turbidistat at a cell density ~ 30,000 cell ml^{-1}.

Continuous Culture Design

A continuous turbidistat culturing device was used to grow the *Prorocentrum* cultures. A modulated infrared (880 mm) LED beam was directed though the culture vessel (a 2 L glass carboy) to a photosensor, the analog signal output of which was related to optical transmission (i.e., turbidity). Real time turbidity signals were sent to a computer that controlled a peristaltic pump which diluted the culture based on attaining a threshold value. The system was programmed to dilute the culture by 5% in terms of the optical signal. This corresponds to a volume dilution of approx. 10%, based on calibrations using a volumetric dilution. The threshold was set to a turbidity that represented a *P. minimum* cell density of ~ 16,000 cell ml^{-1} and a *P. donghaiense* cell density of ~ 30,000 cell ml^{-1}. The culture was stirred at a speed of ~ 60 rpm and generally bubbled with air to keep the culture well mixed and aerated. In this condition, the cells grew at a maximum rate of growth as set by the environmental conditions of the culture. The specific growth rate over each 20 minute period was

calculated from the optical signal and recorded every minute. The use of growth rate, in this study, refers to the rate calculated based on the change in the optical signals.

Experimental Design and Algal Growth

Unialgal, but not axenic, *P. minimum* were grown in the turbidistats in filtered Choptank River water, at a salinity of 15, a temperature of 22 °C and a 14 h: 10 h light dark cycle at 430 µmol photons m^{-2} sec^{-1}. Modified f/2-medium without silicate was used, modified by reducing nitrate (NO_3^-) to 40 µM-N, but by adding PO_4^{3-} in varying concentrations from 0.2 to 10 µM-P in different turbidistats, therefore providing N:P ratios of 5, 16, 40 and 200. The cells were thus grown in a N:P gradient from relatively low P to low N conditions.

Unialgal, but not axenic, cultures of *P. donghaiense* cells were grown in the turbidistats, in filtered Choptank River water, at a salinity of 30, a temperature of 22 °C and a 12 h: 12 h light dark cycle at 500 µmol photons m^{-2} sec^{-1}. Modified f/2-medium without silicate was used. The turbidistat were supplied with media NO_3^- at 80 µM and PO_4^{3-} at 5 µM.

The volume of each turbidistat culture was kept about 1.6 L in the 2 L glass carboys. Before the initiation of experiments, 100 ml water samples were collected from each turbidistat culture and filtered though pre-combusted (2 h at 400 °C) Whatman GF/F filters for ambient nutrient analysis as described below.

Nitrogen Uptake and Assimilation Experiments

N Uptake of P. minimum

Prorocentrum minimum cells were grown in turbidistat at each N:P ratio for at least 3 days to obtain a steady growth state before the N uptake experiments were initiated. ^{15}N isotopically labeled dissolved N substrates were used to measure the culture uptake rates (Glibert and Capone 1993) across a light irradiance spectrum at each N:P ratio. Experiments were conducted around 12 pm. Culture samples of 40 ml were dispensed into a series of acid-clean 50 ml incubation cuvettes to which ^{15}N substrates (Cambridge Isotope Laboratories, Inc.) were added respectively. Considering NO_3^- was the primary N nutrient in the media, ^{15}N labelled NO_3^- was added at a concentration of 20 μM-N. ^{15}N labelled NH_4^+, urea and glycine were added at 2 μM-N. Each group of cuvettes, after adding the 4 substrates, were incubated for 0.5 h in a light irradiance gradient of 9, 35, 200, 430 μmol photons m^{-2} sec^{-1}. Irradiance was obtained by covering the cuvettes with different number layer of black mesh screens. The incubations were terminated by filtration onto pre-combusted GF/F filters. A 20 ml aliquot for each treatment was filtered directly onto one filter. The filters were stored frozen until dried at 50 ˚C for 48 h, then analyzed as described below.

N Uptake and Assimilation of P. donghaiense

Prorocentrum donghaiense cells were grown in turbidistats at a light irradiance gradient of 500, 200, 66, 16, 5 μmol photons m^{-2} sec^{-1}. Cells were grown at each light irradiance for at least 3 days. Experiments were conducted around 10 am. Culture samples of 40 ml were dispensed into acid-clean 50 ml incubation cuvettes to

which ^{15}N substrates were added. These experiments only involved ^{15}N labelled NO_3^- and NH_4^+. NO_3^- was added in a gradient of 1, 2, 5, 10, 25, 37.5, 50 µM-N. NH_4^+ was added in a gradient of 0.1, 0.5, 2, 5, 10, 20 µM-N. All the cuvettes were incubated for 0.5 h at the same light irradiance which the cultures were grown at the time of experiments. The incubations were terminated by filtration onto pre-combusted GF/F filters. A 20 ml aliquot for each treatment was filtered directly onto one filter, which was treated with 10% ice-cold Tri-Chloroacetic Acid (TCA) for 10 seconds after filtration. An extra 20 ml aliquot was filtered to a second filter without further treatment. The TCA-treated filter represents the N substrate assimilated into proteinaceous material, while the untreated filter represents the total N substrate which was taken into the cell. The filters were stored frozen until dried at 50 ˚C for 48 h, then analyzed as described below.

Analytical Methods

Concentrations of ambient inorganic nutrients (NO_3^-+NO_2^--N, NH_4^+-N, PO_4^{3-}-P) were determined using a Technicon Auto-Analyzer (Lane et al. 2000). Concentrations of dissolved free amino acid (DFAA) were determined by fluorometric analysis according to Lindroth and Mopper (1979) and urea was analyzed using the method of Revilla et al. (2005). DFAA concentrations were used to approximate the glycine concentrations in the N uptake rate calculations. Glycine is generally considered to be a dominant amino acid (Degens and Mopper 1976), but their rates of uptake should be considered potential rates based on the uncertainty of their contribution to the DFAA pool. All the ^{15}N sample filters were analyzed for

isotope enrichment using a Sercon Mass Spectrometer. Rates of ^{15}N uptake and assimilation were calculated according to Glibert and Capone (1993).

Data Analysis

The nitrogen uptake parameters relating to ambient N concentrations were plotted and fitted to the Michaelis-Menten formulation:

$$V = V_{\max} \frac{S}{K_s + S}$$

where V is the N specific uptake rate (h^{-1}), V_{max} is the maximal N specific uptake rate (h^{-1}), S is the substrate concentration (μM-N) and K_s is the half-saturation constant (μM-N) for the substrate. V_{max} and K_s for each N source were calculated by using Sigmaplot software (Systat Software, Inc.).

The growth rate parameters relating to ambient light irradiances were also plotted and fitted to a modified formulation (Baly 1935, Aalderink and Jovin 1997):

$$r = r_{\max} \frac{I}{K_I + I} - D$$

where r is the specific growth rate (h^{-1}), r_{max} is the maximal growth rate, I is the ambient light irradiance (μmol photons m^{-2} sec^{-1}), K_I is the half-saturation light irradiance (μmol photons m^{-2} sec^{-1}), and D is the specific dark respiration rate (h^{-1}), which is generally considered opposite to the growth process. r_{max}, K_I and D for *P. donghaiense* were also calculated by the regression of dynamic by Sigmaplot software (Systat Software, Inc.).

Two way ANOVA analyses were conducted to compare the growth and uptake and assimilation data in the different N:P ratio and light intensity treatments.

Results

Ambient Parameters

In the *P. minimum* culture, the ambient N:P ratios in the culture vessels at the time of the experiments were 3.5, 12, 21, 300 (Table 4-1), which were slightly different from the ratios of incoming media (5, 16, 40 and 200). NO_3^- became depleted (< 2 μM-N) in the two relatively high P treatments (N:P ratios 3.5 and 12), but high concentrations (>20 μM-N) remained in the two relatively low P treatments (N:P ratios 21 and 300). PO_4^{3-} was depleted in the treatment where N:P ratio was 13. In the *P. donghaiense* culture, the ambient NO_3^- concentration varied from 1.9 μM-N to 17.8 μM-N, and ambient NH_4^+ concentration varied from 0.72 μM-N to 2.57 μM-N.

Growth Rate in the Turbidistat

When supplied with a constant media supply, the growth rates of both *P. minimum* and *P. donghaiense* in turbidistats showed highly repeatable diel cycles, and cells reached high frequency optical-signal continuous growth in light-dark cycles (e.g. Fig. 4-1). Both species showed a clear diel cycle (Fig. 4-2). Growth rapidly increased at the beginning of the light period and the rates increased to the highest value of the day in less than an hour. Growth rates decreased rapidly when the light was off and the calculated rates were negative in the dark, which indicated the decrease of turbidity in the dark. Similar diel growth cycles were observed for all treatments of both species.

In the *P. minimum* cultures, at ambient N:P ratios of 3.5, 12, 21 and 300, the hourly growth rates averaged over the full day (r_{day}) were 0.0085, 0.024, 0.013 and 0.012 h^{-1}, respectively (Fig. 4-3 a), while the average growth rates for only the light phase (r_L) were 0.016, 0.047, 0.027 and 0.026 h^{-1}, respectively (Fig. 4-3 b). The maximum r_{day} and r_L was recorded at a N:P ratio of 12.0 (157 μM NO_3^-, 13.1 μM PO_4^{3-}). In the *P. donghaiense* cultures, the growth rates decreased as the ambient irradiance decreased (Fig. 4-4). The r_{day} decreased from ~ 0.022 h^{-1} at 500 μmol photons m^{-2} sec^{-1} to ~ 0.011 h^{-1} at 5 μmol photons m^{-2} sec^{-1} (Fig. 4-5), while the r_L decreased from ~ 0.071 h^{-1} at 500 μmol photons m^{-2} sec^{-1} to -0.014 h^{-1} at 5 μmol photons m^{-2} sec^{-1}. There was no evidence of high light growth inhibition in the light range of experiments. For *P. donghaiense*, the average r_{max} over one light-dark cycle was 0.042 h^{-1}, with half saturation coefficient (K_I) of 34 μmol photons m^{-2} sec^{-1} and respiration rate (D) of 0.017 h^{-1}. The r_{max} averaged in the light phase was 0.097 h^{-1}, with K_I of 56 μmol photons m^{-2} sec^{-1} and D of 0.021 h^{-1}.

Nitrogen Uptake Experiments

In the *P. minimum* experiments, the N uptake rates did not show significant light limitation in the irradiance range of experiments (Fig. 4-6). The uptake rates of NO_3^-, NH_4^+, urea and glycine in the relatively high-P culture (N:P ratio 3.5) were significantly higher than in the low-P cultures ($p < 0.05$). There were no significant differences among the NO_3^-, NH_4^+ uptake rates in these higher N:P ratio cultures. A significant light inhibition of N uptake rates was only observed when the average uptake rates at relatively low light (9 and 35μmol photons m^{-2} sec^{-1}) and relatively high light (200 at 430 μmol photons m^{-2} sec^{-1}) were compared for the culture growing

at ambient N:P ratio of 3.5 (Fig. 4-7). Although NO_3^- was the major N nutrient in the total of the 4 N substrates in the culture, NH_4^+ contributed about 70% of total N uptake rate (sum of uptake rate of 4 N nutrients), while NO_3^- contributed about 18% of total N uptake rate. Glycine contributed about 11% of total N uptake rate. The uptake rates of urea were very low.

In the *P. donghaiense* experiments, the $V_{NO_3^-}$ was about 0.002 h^{-1} near ambient concentration, and increased to 0.005 - 0.01 h^{-1}, when enriched with 50 μM-NO_3^-. Although $V_{NO_3^-}$ did not saturate over the concentration range tested, V_{max} values can be estimated mathematically (Fig. 4-8). The highest calculated $V_{max NO_3^-}$ was 0.032 h^{-1}, measured in lowest light. However, the second highest calculated $V_{max NO_3^-}$ was 0.022 h^{-1}, measured in highest light. The differences among NO_3^- uptake rates under different light treatments were not significant. $V_{NH_4^+}$ reached saturation over the concentration range tested. The $V_{NH_4^+}$ in the 2 highest light treatments were significantly higher than the 2 lowest light treatments. $V_{max\ NH_4^+}$ varied from 0.02 to 0.07 h^{-1}.

The half-saturation constant K_s can be considered an index of competitive ability of bloom species at low nutrient concentration. Since the measured rates of NO_3^- uptake at some irradiance levels did not saturate over the concentration range tested, the K_s values were also estimated mathematically to be very high. Calculated K_s values of NO_3^- uptake were in range of 13.5 to 156.6 μM-N. The uptake rate curve was almost linear with a high slope with high K_s (Table 4-2). The $K_{s NH_4^+}$ values were negatively correlated to the ambient light, and increased from ~1 to over 20 μM-N as the ambient light irradiance decreased.

Not all the N substrate taken up into the cells was assimilated into proteinaceous material during the incubation time. Here, the two highest light irradiance, 500 and 200 μmol photons m^{-2} sec^{-1}, were averaged, and the two lowest light irradiance, 5 and 16 μmol photons m^{-2} sec^{-1}, were also averaged to compare high and low light rates. Near the ambient concentration level, ***P. donghaiense*** had higher $V_{NO_3^-}$ (~ 0.003 h^{-1}) and $A_{NO_3^-}$ (~ 0.0007 h^{-1}) in the low light than high light, however, it had higher $V_{NH_4^+}$ (~ 0.017 h^{-1}) and $A_{NH_4^+}$ (~ 0.07 h^{-1}) in the high light than low light (Fig. 4-9). The $V_{NH_4^+}$ and $A_{NH_4^+}$ is an order of magnitude higher than $V_{NO_3^-}$ and $A_{NO_3^-}$ respectively. The assimilation coefficients (assimilation rate : uptake rate) of NH_4^+ were also much higher than those of NO_3^-. *Prorocentrum donghaiense* growing in high light could assimilate average 35% of the NH_4^+ taken up during the incubation, but those in low light only assimilate less than 20%. *Prorocentrum donghaiense* could only assimilate on average less than 10% of the NO_3^- taken up.

Discussion

Growth Regulation in Turbidistat

Turbidistat is run under turbidity control, and new medium inflow only occurs in response to an increase in the culture biomass to a pre-set value. Biomass is set by the optical threshold of dilution. Cell growth is limited by light and temperature; nutrients are usually added in excess of demand (Watson 1972, Skipnes et al. 1980, Fenaux et al. 1985, Hill et al. 1985, Kalyuzhin 1998). Light in turbidistat can run on a light-dark cycle which is more close to real situations (Falkowski et al. 1985). Herein, turbidistats provided a relatively long and steady exponential phase of growth without

causing significant change in irradiance caused by self-shading and nutrient depletion, and also provided detailed records of real-time growth rates.

The growth rate, in this study, refers to the rate of changing of the optical signals, which attenuate with the turbidity of the culture. The turbidity is caused by the total mass concentration in the culture. Therefore, the growth rate is interpreted as the variation rate of total mass density of the culture, instead of cell density, which is calibrated by diluted the culture by volume. Based on the growth rates recorded by the turbidistat, the growth of both species showed strong light-regulated diel cycles. Cell growth, measured as the change of turbidity in the culture vessel, started at the beginning of light cycle.

Dinoflagellate species have been reported to have a maximum growth limit of 1 division per day (McDuff and Chisholm 1982), as a result of dark-light regulated diel synchrony. Here, the growth rates recorded in the high light treatments were around 0.7 division per day. Maximum growth rate generally occurred in the first hour at the beginning of the light phase, and growth rate decreased during the day time. During the dark phase, the cultures were still mixed and gently bubbled and to keep them well mixed. Therefore, the negative growth rates suggested decreases in mass concentration in the culture. Negative growth rate, therefore, were not to due to setting of cells. When the photosynthesis stopped in the dark phase, the production rates of these species were less than the respiration rate, causing the negative growth rate. This state lasted until the start of the light cycle, when production rates increased quickly over respiration and the turbidity of the culture increased again.

Diel phasing of the cell cycle is common among photoautotrophic dinoflagellates, although the precise temporal phasing varies in a species-specific or environment-specific manner (Van Dolah et al. 1995, Van Dolah and Leighfield 1999, Leighfield and Van Dolah 2001). In one cell cycle *Prorocentrum micans* and *P. minimum* divide (the M phase) during a narrow window of time in the early hours of the light phase (Pan and Cembella 1998). The dark phase of the diel cycle of *Prorocentrum* spp. may include the S and G2 phase of the cycle which is light independent in dinoflagellates (Olson and Chisholm 1986). The entry of M phase is suggested to be dictated by the time of S phase entry instead of light. However, the G1 phase is suggested to be light dependent (Olson and Chisholm 1986). Incubation in low light for multiple diel cycle could arrest cells in the G1 phase, instead of entering the S phase and finishing a division cycle. As the result, the cell growth was inhibited. Light limitation impacts ATP and reductant production, which is derived from photosynthesis, and therefore affects N uptake, reduction and assimilation. In a previous study, in contrast to tested a diatom (*Thalassiosira weissflogii*), in which the N uptake and cell division were regulated by the new N pulses, the cell division of a dinoflagellate (*Amphidinium carteri*) was strictly regulated by the light-dark cycle, regardless the time of new N pulses (Wheeler et al. 1983). However, light-dark cycle had a relatively weak effect on N uptake and assimilation. N was metabolized rapidly, under conditions of N limitation. This light regulated dinoflagellate cell cycle led to a large degree of uncoupling between N assimilation and cell division (Wheeler et al. 1983). In the present study, the culture was diluted by new media from 0 to 6 times a day, depending on the growth rates. Each dilution could be considered as a N pulse.

However, there were no significant variations on the growth rate pattern after each pulse, and the growth rates were still strictly regulated by the dark-light cycle, which was measured as the change of turbidity.

Wheeler et al. (1989) also reported maximal N uptake during the daytime and release (regeneration) of NH_4^+ exclusively at night, suggesting the capacity for large internal N pools for the N. Cells were able to take up and assimilate N, but could not proceed through the cell cycle until the correct portion of the dark-light cycle. At the beginning of the photoperiod, this pool of intracellular N may allow the assimilation process to proceed at an enhanced rate to support the fast growth, which was recorded by the turbidistats. This uncoupled N uptake and cell growth can also be explained by Droop's model (Droop 1968, 1973), which describes the growth rate regulated by the cell quota (intracellular content of the limiting chemical element). These *Prorocentrum* species had their maximum cell quotas (internal N pool), and reached the highest growth rates at the beginning of light phase. Decreasing growth rates during the day are indicative of decreasing cell quotas. However, low growth rates in the dark cycle were not necessary indicative of a small cell quota, since the cells were light regulated instead of nutrient regulated. *Prorocentrum minimum* has been reported to continuously uptake the NO_3^- in the dark (Paasche et al. 1984). Cell stopped growth at night due to light limitation, but nutrients were likely continuously taken up into and stored in the cell, causing an increasing cell nutrient quota. The cell quota reached its maximum value of the day at the beginning of light phase, as did growth rates.

The regulation of light on the growth of *P. donghaiense* was significant. Based on the kinetics simulation, the half saturation coefficient (K_I) was low (30 ~ 50 μmol photons m^{-2} sec^{-1}) in the range of light tested growth rates. The growth rates dropped rapidly when the ambient light was lower than the K_I. The negative growth rate in the dark phased indicated very low production, which could not compensate the respiration lost, as a result, negative growth rates were recorded. In the low light treatments, the growth activities of *P. donghaiense* were light limited.

N Uptake versus Irradiance

Nitrogen uptake by phytoplankton is an energy consuming process. Light dependent nutrient uptake of phytoplankton has been studied in both laboratory culture and field communities, and the responses to light are varied among different species (Anderson and Roels 1981, Megard et al. 1985, Lomas et al. 1996, Kudela et al. 1997, Sinclair et al. 2009). High light may enhance the rate of N uptake, as the energy for N uptake and NO_3^- reduction is derived primarily from photosynthesis in the light (Kanda et al. 1989). The uptake rates might be cyclic over diel cycles (Anderson and Roels 1981). Numerous studies have shown that uptake of nutrient responding to light were different among taxonomic groups (Eppley et al. 1971, Wheeler et al. 1983). For example, the diatom *Skeletonema costatum* had lower dark uptake rates of NO_3^- and NH_4^+, and coccolithophore *Emiliania huxleyi* was more consistent in both day and night (Eppley et al. 1971). Light dependent N uptake rates have also been shown to be different for different nutrients. NO_3^- needs to be reduced before it can be assimilated by the algae, and this process needs light, as an energy consuming process. NO_3^- uptake rates have been shown to decrease more than uptake

of NH_4^+ in diatom dominant assemblages at night, suggesting higher light dependence of NO_3^- uptake than reduced N sources (Cochlan et al. 1991). For dinoflagellates, the light dependence of N uptake also varied with species. *Prorocentrum minimum* could continuous uptake the NO_3^- in the dark; while *Karenia mikimotoi* showed almost no NO_3^- uptake in the dark (Paasche et al. 1984). The light dependence might be weaken with increasing nutrient limitation (Healey 1977).

In this experiment, the N uptake rates rate of *P. minimum* showed similar results to those reported by as Fan and Glibert (2005), which suggested little or no effect on N uptake during short term (~ half hour) incubations. Compared to the growth rates measured in the turbidistat, which had quick response to light and which decreased to near zero in 0.5 hour after the lights turned offs, the light independence of N uptake of *P. minimum* suggested that N uptake was not affected by low light. In this case, the growth stopped and but the cell N quota still increased.

However, after days of incubation in different irradiance levels, the regulation of light on the N uptake of *P. donghaiense* was significant. Although the uptake and assimilation rates of NO_3^- in the low light were even slightly higher than those in high light treatments, the uptake and assimilation rates of NO_3^- were an order of magnitude lower than those of NH_4^+. The contribution of NO_3^- to the total N uptake and assimilation was very limited. Both the uptake and uptake assimilation rates of NH_4^+ suggested significant light limitation when comparing the low and high light treatments. This light limitation of N uptake was consistent with light limitation on the growth rate (Fig. 4-9). The NH_4^+ uptake rates were less than the growth rate,

suggesting the contribution of other N nutrients to support growth (i.e., organic N, Li et al. 2009).

Light Condition and Prorocentrum Blooms

The annual recirculation of cell transportation in the Chesapeake Bay has been suggested as an important mechanism for the periodic outbreak of *P. minimum* blooms (Tyler and Seliger 1978). *P. minimum* cells are transported to upper Chesapeake Bay and its tributaries by the northward current from the bottom layer from the mouth of the Chesapeake Bay in the winter and early spring. It could take over a month before the *P. minimum* is transported to the tributaries, and more time to reach the surface water. During this period, the cells are in near or total dark environment. When *P. minimum* rises to the surface, the cells grow and develop blooms. Dinoflagellates have multiple strategies to survive extended periods of darkness. For example, both *P. minimum* and *P. donghaiense* have been reported to be mixotrophic dinoflagellates, and may directly feed on a wide range of food sources, including other algae, bacteria, and organic particles (Li et al. 1999, Stoecker 1999, Jeong et al. 2004, Burkholder et al. 2008, Jeong et al. 2010). Light independent N uptake is another strategy for the cells to keep taking up N nutrients supply during the dark phase in the bottom layer, which would allow them to be competitive in turbid coastal waters where light is low. When cells are transported to the deeper layer, although the growth may be light limited, cells can still take up N and keep a large cell N quota. When they reach surface water, cells with large cell nutrient quota can quickly reach high growth rate and develop blooms. When N is depleted in the surface layer, cells can also obtain N sources in the dark deep layer by taking the

advantage of vertical migration (Holmes et al. 1967, Eppley et al. 1968, Haraguchi et al. 2010, Rines et al. 2010). Dinoflagellates can access both the deep nutrient pool and near-surface light required for photosynthesis, at different times of the day. Bloom-forming dinoflagellates are capable of maintaining a relatively high nutrient uptake rate in a low-light or even completely dark environment (Heaney and Eppley 1981, Paasche et al. 1984, Cullen et al. 1985, Olsson and Granéli 1991, Cloern 2001, Fan and Glibert 2005), thus allowing them to store extra nutrients (especially regenerated nutrient PO_4^{3-} and NH_4^+) by luxury uptake in the deep layer before ascending to the surface for photosynthesis.

However, the regulation of light on N uptake between the two *Prorocentrum* species might be different. Although the growth rate of *P. donghaiense* only decreased about 40%, when ambient light decreased from 500 to 66 μmol photons m^{-2} sec^{-1}, there was significantly greater light limitation on NH_4^+ uptake rate. *Prorocentrum donghaiense* also had significantly lower K_s for NH_4^+ in high light treatments. These results suggested *P. donghaiense* might not adapt to low light as well as *P. minimum*, which is consistent with the field observation that *P. minimum* can bloom in very turbid near-shore estuarine waters, while *P. donghaiense* blooms are relatively off-shore.

References

Aalderink RH, Jovin R (1997) Estimation of the photosynthesis/irradiance (P/I) curve parameters from light and dark bottle experiments. Journal of Plankton Research 19:1713-1742

Anderson DM (1997) Turning back the harmful red tide - Commentary. Nature 388:513-514

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Anderson SM, Roels OA (1981) Effects of light-intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Marine Biology 62:257-261

Baly E (1935) The kinetics of photosynthesis. Proceedings of the Royal Society B 117:218-239

Burkholder JM, Glibert PM, Skelton HM (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8:77-93

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology-Progress Series 210:223-253

Cochlan WP, Harrison PJ, Denman KL (1991) Diel periodicity of nitrogen uptake by marine-phytoplankton in nitrate-rich environments. Limnology and Oceanography 36:1689-1700

Conway HL (1977) Interactions of inorganic nitrogen in uptake and assimilation by marine phytoplankton. Marine Biology 39:221-232

Cullen J, Zhu M, Davis R, Pierson D (1985) Vertical migration, carbohydrate synthesis, and nocturnal nitrate uptake during growth of *Heterocapsa niei* in a laboratory water column. In: Anderson D, White A, Baden D (eds) Toxic dinoflagellates. Elsevier, Amsterdam, p 189-194

Degens ET, Mopper K (1976) Factors controlling distribution and early diagenesis of organic marine sediments. In: Riley JP, Chester R (eds) Chemical oceanography, Vol 6. Academic London, New York, San Francisco, p 60-114

Droop MR (1968) Vitamin B12 and marine ecology 4. kinetics of uptake growth and inhibition in *Monochrysis lutheri*. Journal of the Marine Biological Association of the United Kingdom 48:689-733

Droop MR (1973) Some thoughts on nutrient limitation in algae. Journal of Phycology 9:264-272

Eppley RW, Holm-Hansen O, Strickland JD (1968) Some observations on vertical migration of dinoflagellates. Journal of Phycology 4:333-340

Eppley RW, Rogers JN, McCarthy JJ, Sournia A (1971) Light/dark periodicity in nitrogen assimilation of marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. Journal of Phycology 7:150-154

Falkowski PG, Dubinsky Z, Wyman K (1985) Growth-irradiance relationships in phytoplankton. Limnology and Oceanography 30:311-321

Fan CL, Glibert PM (2005) Effects of light on nitrogen and carbon uptake during a *Prorocentrum minimum* bloom. Harmful Algae 4:629-641

Fenaux R, Malara G, Claustre H (1985) A turbidostat driven and controlled by microcomputer. Aquaculture 48:91-95

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Granéli E, Anderson DM (2008a) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Glibert PM, Capone DG (1993) Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques, Vol 243-272. Academic Press

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea - a global change contributing to coastal eutrophication. Biogeochemistry 77:441-463

Glibert PM, Magnien R, Lomas MW, Alexander J, Fan CL, Haramoto E, Trice M, Kana TM (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. Estuaries 24:875-883

Glibert PM, Mayorga E, Seitzinger S (2008b) *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: Application of spatially explicit nutrient export models. Harmful Algae 8:33-38

Glibert PM, McCarthy JJ (1984) Uptake and assimilation of ammonium and nitrate by phytoplankton - Indexes of nutritional status for natural assemblages. Journal of Plankton Research 6:677-697

Grzebyk D, Berland B (1996) Influences of temperature, salinity and irradiance on growth of *Prorocentrum minimum* (Dinophyceae) from the Mediterranean Sea. Journal of Plankton Research 18:1837-1849

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99

Haraguchi K, Yamamoto T, Chiba S, Shimizu Y, Nagao M (2010) Effects of phytoplankton vertical migration on the formation of oxygen depleted water in a shallow coastal sea. Estuarine Coastal and Shelf Science 86:441-449

Healey FP (1977) Ammonium and urea uptake by some freshwater algae. Canadian Journal of Botany-Revue Canadienne De Botanique 55:61-69

Heaney S, Eppley R (1981) Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. Journal of Plankton Research 3:331-344

Heil CA, Glibert PM, Fan CL (2005) *Prorocentrum minimum* (Pavillard) Schiller - a review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449-470

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8:3-13

Hill SH, Abbott MR, Denman KL (1985) A computer-controlled turbidostat for the culture of planktonic algae. Canadian Journal of Fisheries and Aquatic Sciences 42:744-753

Holmes RW, Williams PM, Eppley RW (1967) Red water in La Jolla Bay 1964-1966. Limnology and Oceanography 12:503-512

Jeong H, Yoo Y, Kim J, Seong K, Kang N, Kim T (2010) Growth, feeding, and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Science Journal 45:65-91

Jeong HJ, Song JY, Lee CH, Kim ST (2004) Feeding by larvae of the mussel *Mytilus galloprovincialis* on red-tide dinoflagellates. Journal of Shellfish Research 23:185-195

Kalyuzhin VA (1998) The growth of a turbidostat yeast culture in the presence of high concentrations of various compounds in a steady-state regime and under osmotic shock. Microbiology 67:499-503

Kanda J, Ziemann DA, Conquest LD, Bienfang PK (1989) Light-dependency of nitrate uptake by phytoplankton over the spring bloom in Auke Bay, Alaska. Marine Biology 103:563-569

Kudela RM, Cochlan WP, Dugdale RC (1997) Carbon and nitrogen uptake response to light by phytoplankton during an upwelling event. Journal of Plankton Research 19:609-630

Lane L, Rhoades S, Thomas C, Van Heukelem L (2000) Analytical services laboratory standard operating procedures, University of Maryland Center for Environmental Science, Cambridge, Maryland.

Leighfield TA, Van Dolah FM (2001) Cell cycle regulation in a dinoflagellate *Amphidinium operculatum*: identification of the diel entraining cue and a possible role for cyclic AMP. Journal of Experimental Marine Biology and Ecology 262:177-197

Li AS, Stoecker DK, Adolf JE (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquatic Microbial Ecology 19:163-176

Li J, Glibert PM, Alexander JA (in press) Effects of ambient N:P ratio on the growth and nitrogen uptake of harmful dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* in turbidistat. Chinese Journal of Oceanology and Limnology

Li J, Glibert PM, Zhou M (2010) Temporal and spatial variability in nitrogen uptake kinetics during dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou M, Lu S, Lu D (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology Progress Series 383:11-26

Lie HJ, Cho CH, Lee JH, Lee S (2003) Structure and eastward extension of the Changjiang River plume in the East China Sea. Journal of Geophysical Research-Oceans 108:-

Lindroth P, Mopper K (1979) High-performance liquid-chromatographic determination of subpicomole amounts of amino-acids by precolumn fluorescence derivatization with ortho-phthaldialdehyde. Analytical Chemistry 51:1667-1674

Lomas MW, Glibert PM (2000) Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. Journal of Phycology 36:903-913

Lomas MW, Glibert PM, Berg GM, Burford M (1996) Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens*

(Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. Journal of Phycology 32:907-916

McDuff RE, Chisholm SW (1982) The calculation of in situ growth-rates of phytoplankton populations from fractions of cells undergoing mitosis - a clarification. Limnology and Oceanography 27:783-788

Megard RO, Berman T, Curtis PJ, Vaughan PW (1985) Dependence of phytoplankton assimilation quotients on light and nitrogen-source - implications for oceanic primary productivity. Journal of Plankton Research 7:691-702

Moncheva S, Gotsis-Skretas O, Pagou K, Krastev A (2001) Phytoplankton blooms in Black Sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: Similarities and differences. Estuarine Coastal and Shelf Science 53:281-295

Olson RJ, Chisholm SW (1986) Effects of light and nitrogen limitation on the cell cycle of the dinoflagellate *Amphidinium carteri*. Journal of Plankton Research 8:785-793

Olsson P, Granéli E (1991) Observations on diurnal vertical migration and phased cell-division for 3 coexisting marine dinoflagellates. Journal of Plankton Research 13:1313-1324

Paasche E, Bryceson I, Tangen K (1984) Interspecific variation in dark nitrogen uptake by dinoflagellates. Journal of Phycology 20:394-401

Pan Y, Cembella AD (1998) Flow cytometric determination of cell cycles and growth rates in *Prorocentrum* spp. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful Algae, Xunta de Galicia and Intergovernmental Oceanic Commission of UNESCO, p 173-176

Revilla M, Alexander J, Glibert PM (2005) Urea analysis in coastal waters: comparison of enzymatic and direct methods. Limnology and Oceanography-Methods 3:290-299

Rines JEB, McFarland MN, Donaghay PL, Sullivan JM (2010) Thin layers and species-specific characterization of the phytoplankton community in Monterey Bay, California, USA. Continental Shelf Research 30:66-80

Sinclair G, Kamykowski D, Glibert PM (2009) Growth, uptake, and assimilation of ammonium, nitrate, and urea, by three strains of *Karenia brevis* grown under low light. Harmful Algae 8:770-780

Skipnes O, Eide I, Jensen A (1980) Cage culture turbidostat - a device for rapid-determination of algal growth-rate. Applied and Environmental Microbiology 40:318-325

Smayda TJ (1990) Novel and nuisance phytoplankton blooms in the Sea: Evidence for a global epidemic. In: Toxic Marine Phytoplankton, 4th International Conference. Elsevier, Amsterdam, p 29-40

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

Stoecker DK (1999) Mixotrophy among dinoflagellates. Journal of Eukaryotic Microbiology 46:397-401

Tango PJ, Magnien R, Butler W, Luckett C, Luckenbach M, Lacouture R, Poukish C (2005) Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. Harmful Algae 4:525-531

Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area - water circulation patterns and organism distributions in Chesapeake Bay. Limnology and Oceanography 23:227-246

Van Dolah FM, Leighfield TA (1999) Diel phasing of the cell-cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*. Journal of Phycology 35:1404-1411

Van Dolah FM, Leighfield TA, Sandel HD, Hsu CK (1995) Cell division in the dinoflagellate *Gambierdiscus toxicus* is phased to the diurnal cycle and accompanied by activation of the cell cycle regulatory protein, Cdc2 kinase. Journal of Phycology 31:395-400

Wang JH, Wu JY (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of the Total Environment 407:4012-4021

Watson TG (1972) Present status and future prospects of turbidostat. Journal of Applied Chemistry and Biotechnology 22:229

Wheeler PA, Kirchman DL, Landry MR, Kokkinakis SA (1989) Diel periodicity in ammonium uptake and regeneration in the oceanic subarctic Pacific - implications for interactions in microbial food webs. Limnology and Oceanography 34:1025-1033

Wheeler PA, Olson RJ, Chisholm SW (1983) Effects of photocycles and periodic ammonium supply on 3 marine-phytoplankton species .2. ammonium uptake and assimilation. Journal of Phycology 19:528-533

Xu N, Duan SS, Li AF, Zhang CW, Cai ZP, Hu ZX (2010) Effects of temperature, salinity and irradiance on the growth of the harmful dinoflagellate *Prorocentrum donghaiense* Lu. Harmful Algae 9:13-17

Zhou M, Yan T, Zou J (2003) Preliminary analysis of the characteristics of red tide areas in Changjiang River estuary and its adjacent sea. Chinese Journal of Applied Ecology 14:1031-1038

Zhou W, Yin K, Zhu D (2006) Phytoplankton biomass and high frequency of *Prorocentrum donghaiense* harmful algal bloom in Zhoushan sea area in spring. Chinese Journal of Applied Ecology (In Chinese) 17:887-893

Table 4-1 Ambient nutrient concentrations in 4 different turbidistat systems supplied by medium at different DIN:DIP ratios.

Medium Enriched N:P Ratio	NO_3^- μM-N	NH_4^+ μM-N	Urea μM-N	DFAA μM-N	DIP μM-P	Culture DIN:DIP Ratio
5	1.95	0.05	1.18	0.063	0.57	3.5
16	0.37	1.05	3.14	0.011	0.11	12.5
40	21.98	3.59	2.09	0.038	1.17	21
200	42.4	2.52	2.05	1.123	0.15	300

Table 4-2 Calculated parameters of uptake kinetics of nitrogen sources (NH_4^+, urea, and glycine) by *Prorocentrum minimum* at different ambient DIN:DIP ratio in turbidistat based on the Michaelis-Menten equation. V_{max} and A_{max} are the specific nitrogen uptake and assimilation rates. K_s is the Michaelis constant where uptake rates equal to ½ of maximum uptake rates.

Culture DIN:DIP	NH_4^+		Urea		Glycine		NH_4^+		Urea		Glycine	
Ratio	V_{max}	K_s	V_{max}	K_s	V_{max}	K_s	A_{max}	K_s	A_{max}	K_s	A_{max}	K_s
3.5							0.031	0.20	5E-04	0.43	0.004	1.27
12.5	0.046	1.248	3E-04	0.052	0.004	0.48	0.024	2.32	7E-04	16.50	0.001	0.04
21							0.02	1.43	4E-04	2.63	0.001	0.10
300	0.065	4.646	8E-04	5.43	0.007	3.54	0.016	2.70	4E-04	1.56	0.003	3.39

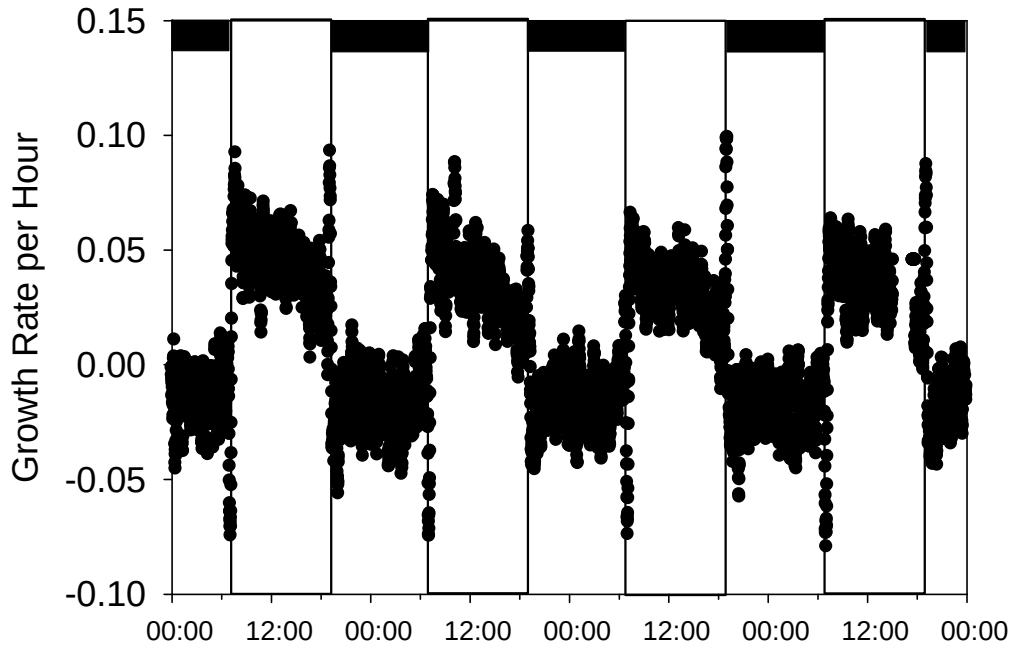


Figure 4-1 Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system for 4 constant 12 h: 12 h light dark cycles (96 hrs). Each point stands for the growth rate calculated based on the change in the optical signals over a 20-minute time period.

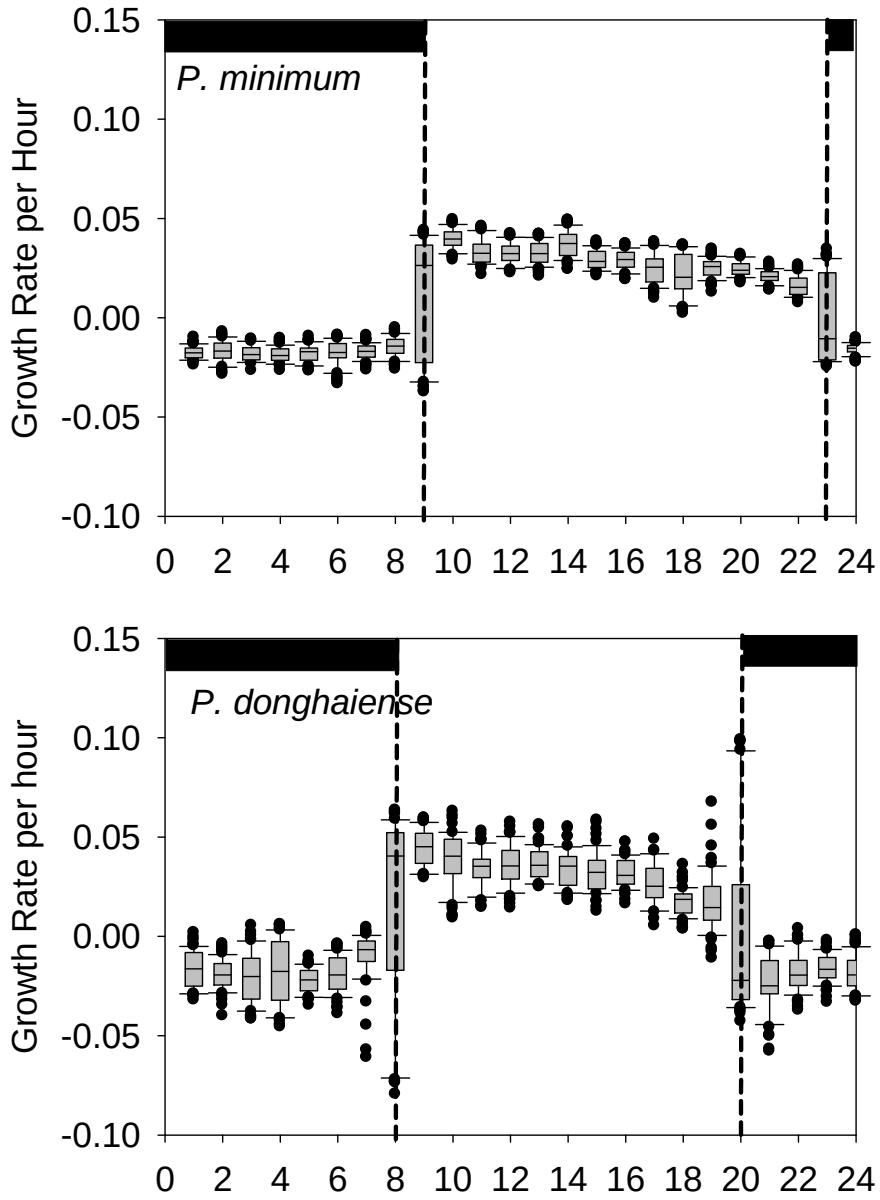


Figure 4-2 a. Diurnal growth rates based on changes in optical signals of *Prorocentrum minimum* in turbidistat system at 14 h: 10 h light dark cycle. b. Diurnal growth rates of *Prorocentrum donghaiense* in turbidistat system at 14 h: 10 h light dark cycle. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

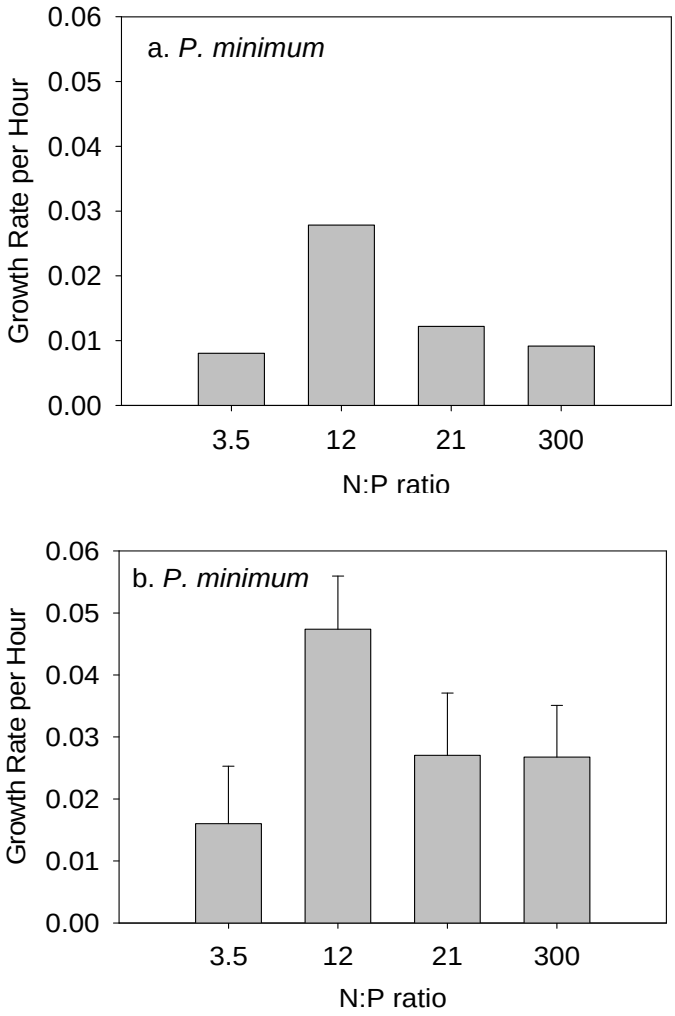


Figure 4-3 a. Average growth rates of *Prorocentrum minimum* in a 14 h: 10 h light dark cycle at different ambient N:P ratios. b. Average growth rates of *P. minimum* in the light phase of 14h: 10h light dark cycle at different ambient N:P ratios.

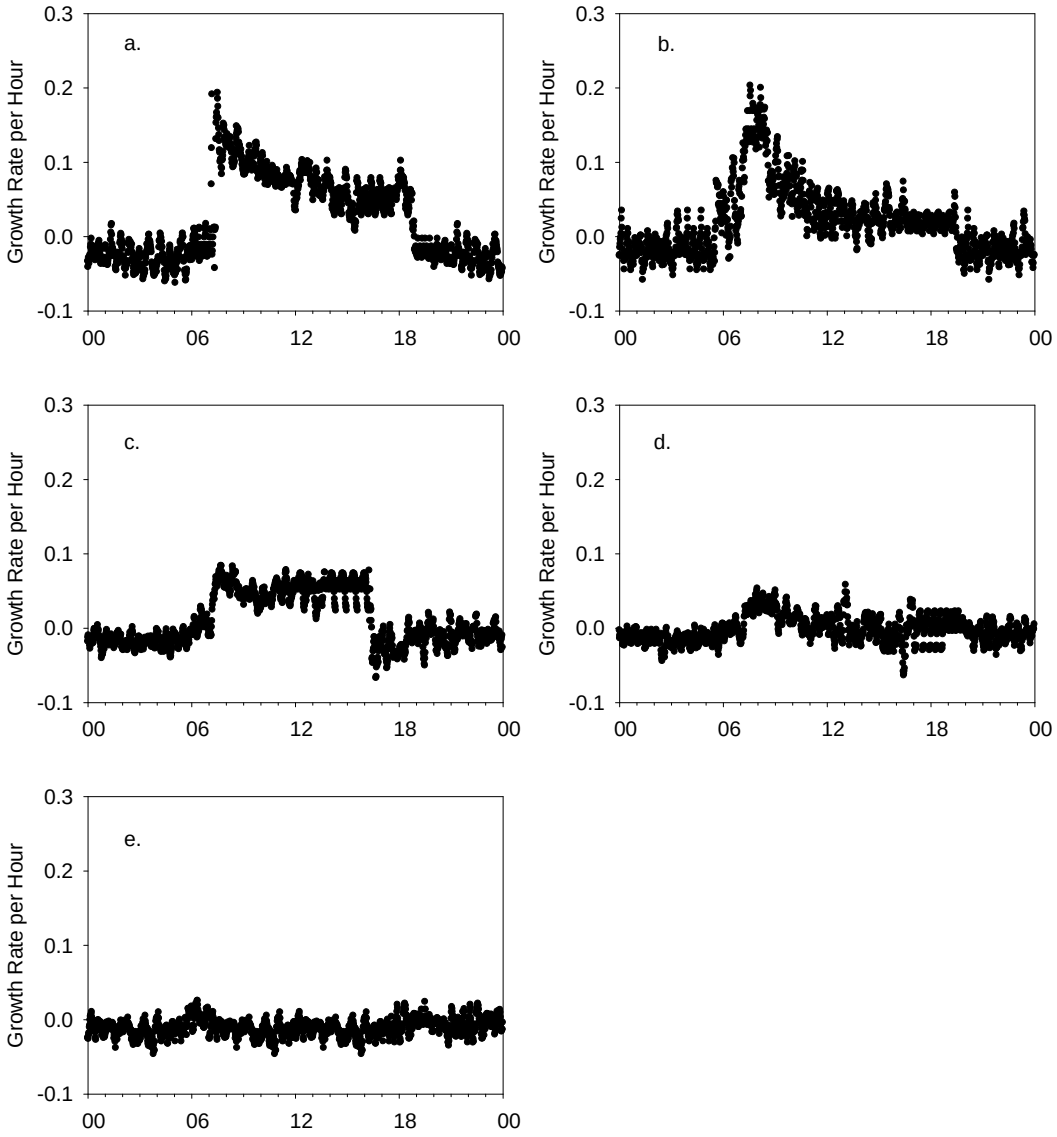


Figure 4-4 Diurnal (12 h: 12 h light dark cycle) growth rates based on changes in optical signals of *Prorocentrum donghaiense* in turbidistat systems at different light intensities. From 00 h to 00 h the next day, lights were on at 08 h and off at 20 h (X-axis). a. 500; b. 200; c. 66; d. 16; e. 5 (µmol photons m^{-2} sec^{-1}). Each point stands for the growth rate calculated based on the change in the optical signals over a 20-minute time period.

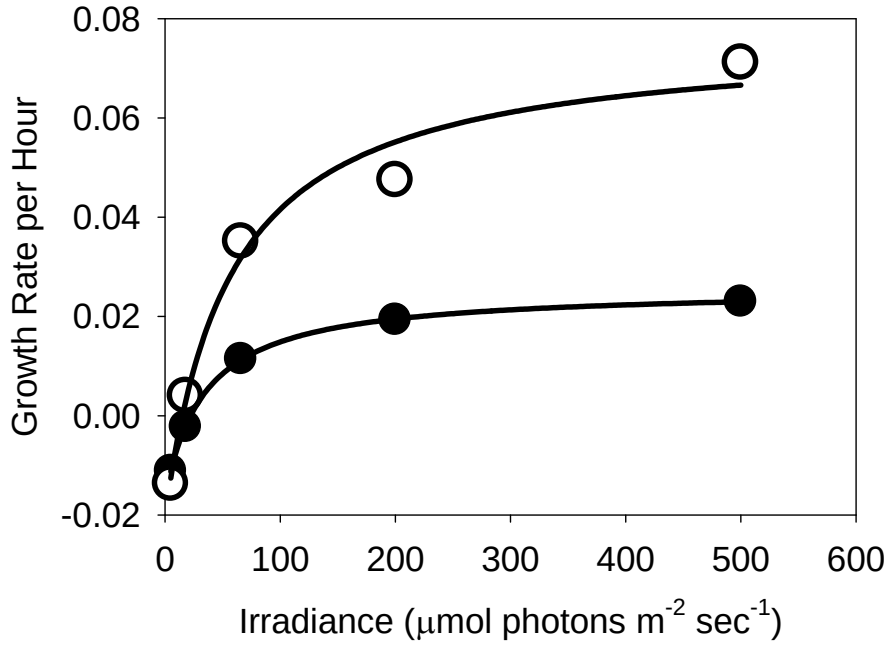


Figure 4-5 Average diurnal growth rates (●) and average growth rates based on changes in optical signals of *Prorocentrum donghaiense* in the light phase (○) in a 12 h: 12 h light dark cycle at different ambient light irradiance.

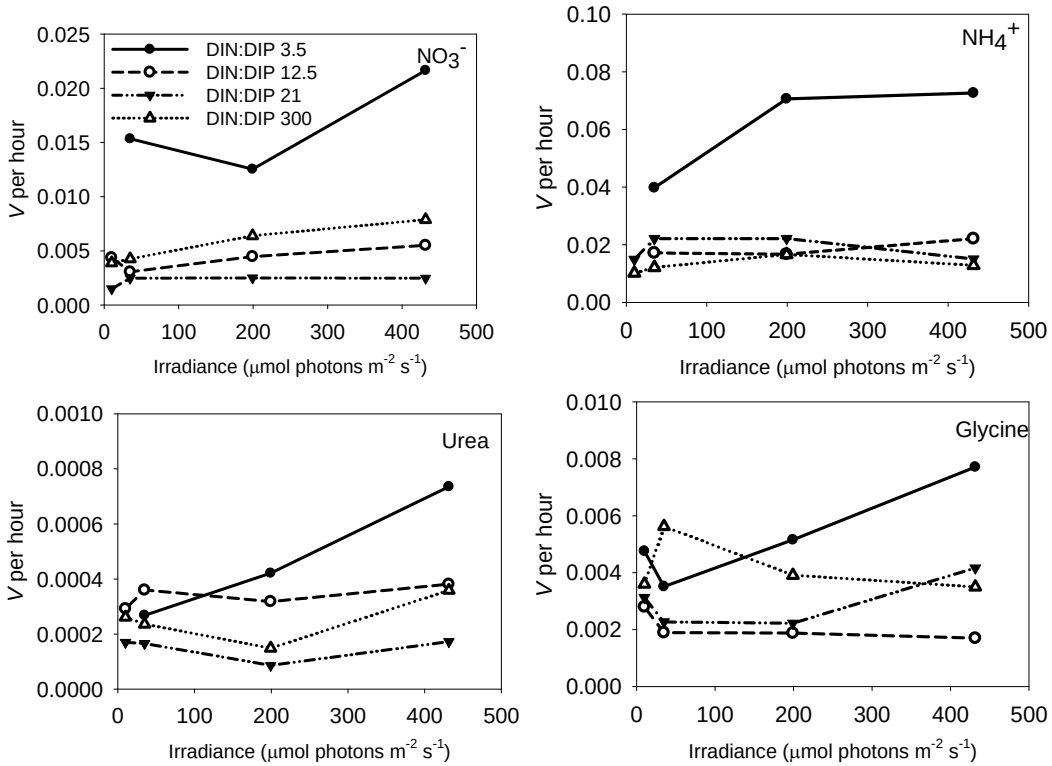


Figure 4-6 The relationship of N (Nitrate (NO_3^-), ammonium (NH_4^+), urea and glycine) uptake rates *V* (h^{-1}) of *Prorocentrum minimum* at different irradiance levels (μmol photons m^{-2} sec^{-1}) with different ambient DIN:DIP ratio treatments.

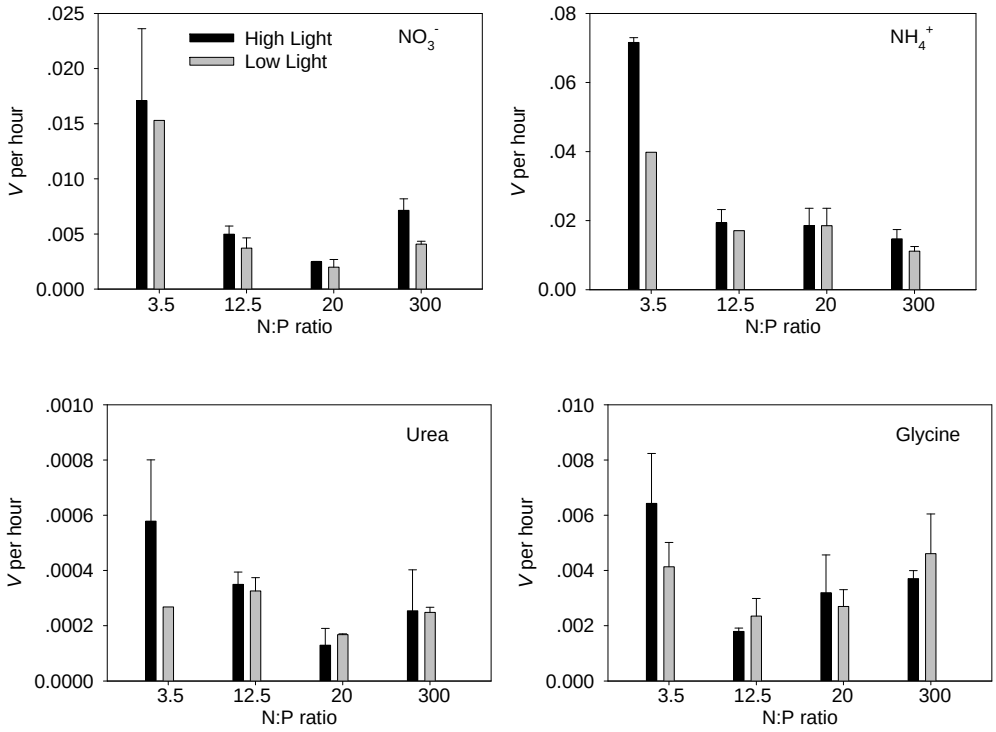


Figure 4-7 Average specific uptake rates *V* (h^{-1}) of 4 nitrogen substrates (Nitrate (NO_3^-), ammonium (NH_4^+), urea and glycine) near ambient concentration by *Prorocentrum minimum* in high light (average of treatments 9 and 35 µmol photons m^{-2} sec^{-1}) and low light (average of treatments 200 and 430 µmol photons m^{-2} sec^{-1}) incubation at different ambient N:P ratios.

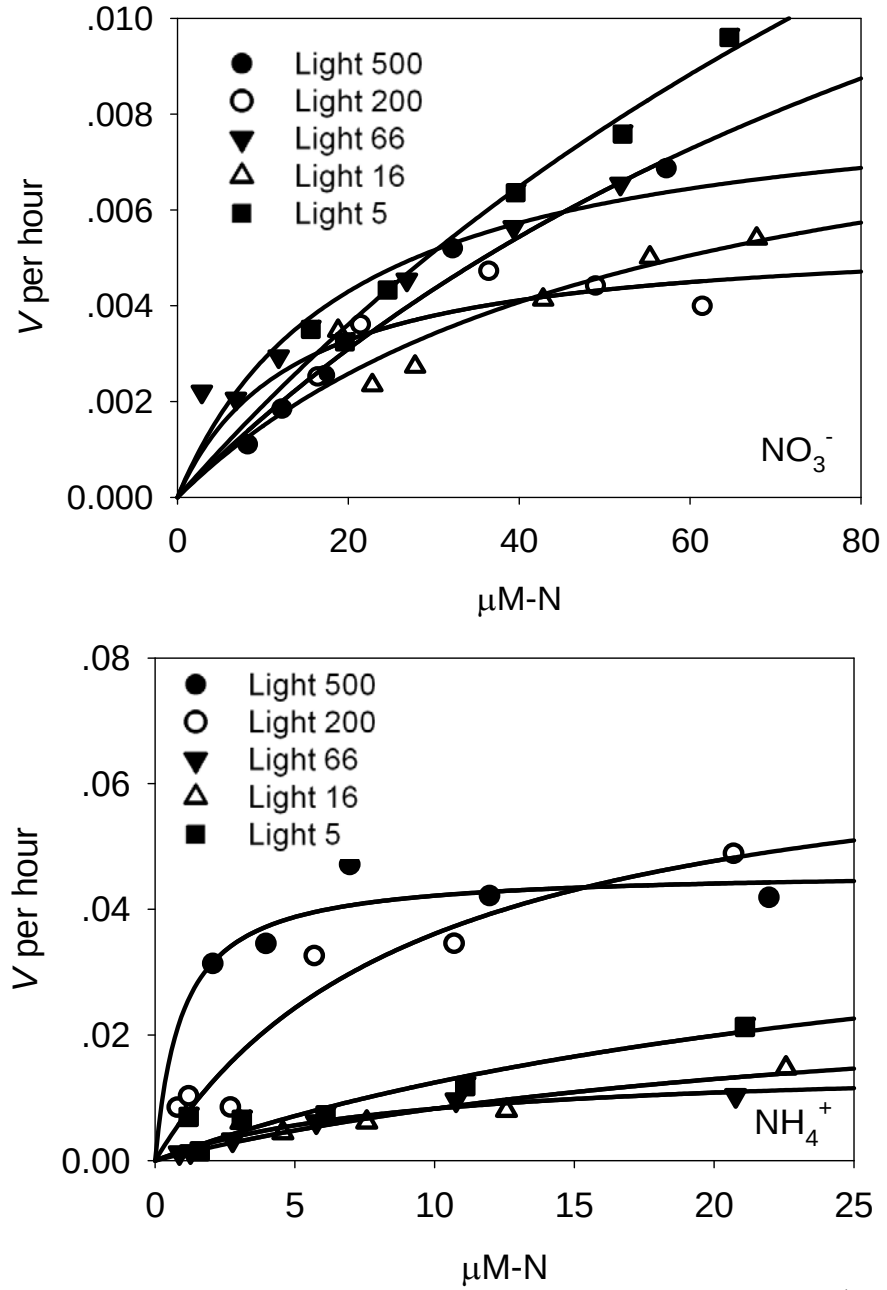


Figure 4-8 Kinetic relationships between nitrogen specific uptake rates *V* (h^{-1}) of NO_3^- and NH_4^+ with ^{15}N substrate concentration (μM-N) by *Prorocentrum donghaiense* in different irradiance levels (μmol photons m^{-2} sec^{-1}).

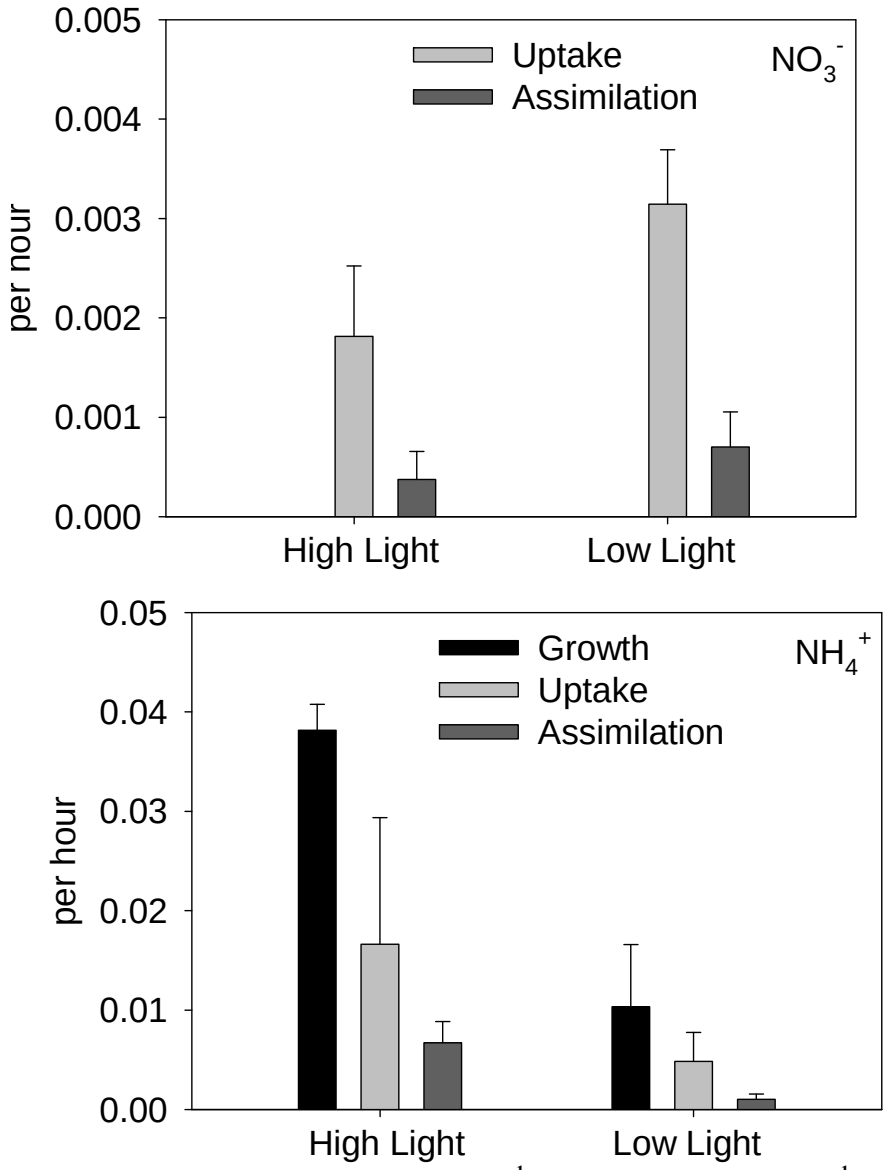


Figure 4-9 Average specific uptake rates *V* (h^{-1}) and assimilation rates *A* (h^{-1}) of nitrogen substrates nitrate (NO_3^-) and ammonium (NH_4^+) near ambient concentration by *Prorocentrum donghaiense* growing in high light (average of treatments 9 and 35 µmol photons m^{-2} sec^{-1}) and low light (average of treatments 200 and 430 µmol photons m^{-2} sec^{-1}) treatments. The average growth rate *r* (h^{-1}) of that light-dark cycle was also compared with *V* and *A* of NH_4^+.

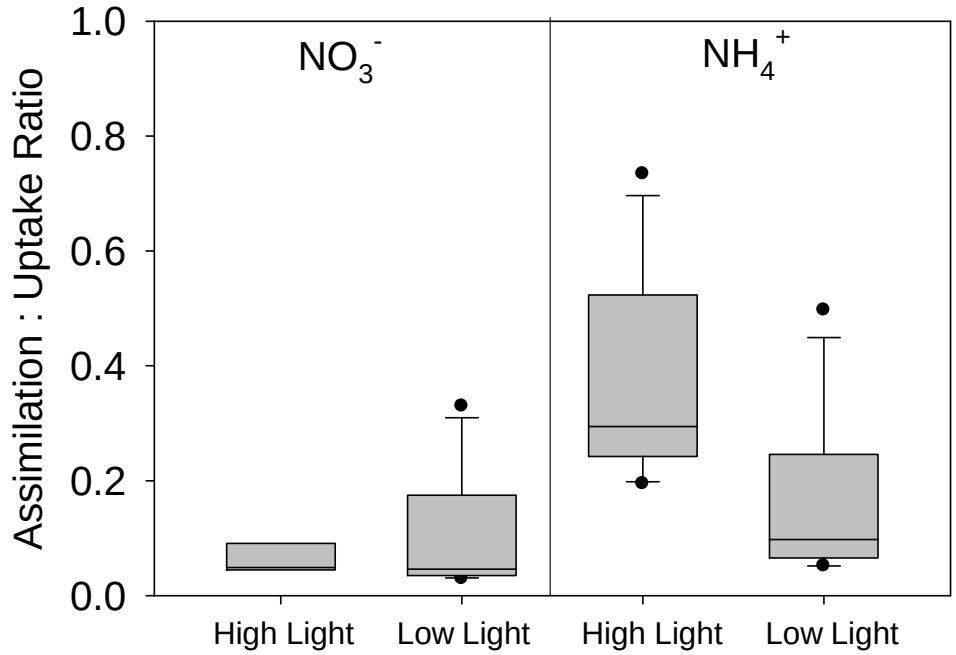


Figure 4-10 Ratio of assimilation rates *A* : uptake rates *V* of nitrogen substrates nitrate (NO_3^-) and ammonium (NH_4^+) by *Prorocentrum donghaiense* growing in high light and low light treatments. Box plots - line: median; box: 25th to 75th percentiles; whiskers: 10th and 90th percentiles; dots: outliers.

Chapter 5: Growth and Competition of Several Harmful Dinoflagellates under Different Nutrient and Light Condition

Abstract

Three near-shore type harmful dinoflagellates, *Prorocentrum minimum*, *P. donghaiense* and *Karlodinium veneficum*, and one off-shore dinoflagellate, *Karenia brevis*, were grown in laboratory monoculture and mixed batch cultures. The dinoflagellate cultures were grown on treatments of two ambient nitrogen (N) : phosphorus (P) ratios; two N substrates (nitrate and urea) and two light intensities. The microalgae *Rhodomonas* and *Synechococcus* were also added in separate treatments to the mixed culture treatments as potential food sources. All tested species grew well on both N substrates. In mixed culture, *P. minimum* outgrew *K. veneficum*, and *P. donghaiense* outgrew *K. brevis* in most treatments reaching higher growth rates and higher biomass. However, when a third algal, *Rhodomonas*, was added, the growth of *P. minimum* was inhibited relative to that of *K. veneficum*. In contrast, when grown with *K. brevis*, the growth rate of *P. donghaiense* was not significantly affected. *K. brevis* had a longer growth phase, and kept growing after *P. donghaiense* reached stationary phase, suggesting better adaptation of *K. brevis* to low inorganic nutrient conditions. The growth of *K. brevis* was also significantly limited in the low light treatment. *Karlodinium veneficum* overgrew *P. minimum* with the presence of *Rhodomonas*, a potential nutrient source. The growth of both *K.*

brevis and *P. donghaiense* were reduced with the presence of *Synechococcus*. Other mechanisms (e.g. allelopathy), in addition to nutrient competition, should be considered and may also play a role in determining the dominant species.

Introduction

Prorocentrum spp. are one of the major groups of bloom forming harmful dinoflagellates that have been reported to develop harmful algal blooms (HABs) in coastal waters worldwide, and to cause ecological damage by high biomass and to have related effects (Heil et al. 2005, Glibert et al. 2008b). *Prorocentrum minimum* is a common HAB species in coastal and estuarine systems in USA, and it is well documented in the Chesapeake Bay and southwest Florida shelf among many other parts of the world (Glibert et al. 2001, Tango et al. 2005, Heil et al. 2007, Glibert et al. 2008b). In the past two decades, over 800 events of *P. minimum* with cell density over 100, 000 cell L^{-1} were recorded in the Chesapeake Bay by the State of Maryland Department of Natural Resources (MD DNR) monitoring program. Another *Prorocentrum* species, *P. donghaiense*, is a major bloom species near the Changjiang River estuary and in the coastal area of East China Sea. Large scale *P. donghaiense* blooms have been observed in those waters in the past two decades (Zhou et al. 2006, Li et al. 2009, Li et al. 2010).

Another HAB species, which is increasing in both frequency and abundance in Chesapeake Bay, is *Karlodinium veneficum*. *Karlodinium veneficum* is a potentially toxic dinoflagellate and has been implicated to cause fish kill events (Heil et al. 2001, Deeds et al. 2002, Adolf et al. 2006a, Deeds et al. 2006). Both *P. minimum* and *K. veneficum* are recognized as dominant late spring and summer

bloom species in the Chesapeake Bay area, and often co-occur (Glibert et al. 2001, Tango et al. 2005). In the southwest Florida shelf, *P. minimum* has also been recorded to bloom with *Karenia brevis*, a prevalent, potentially toxic dinoflagellate (Heil et al. 2007, Vargo et al. 2008, Vargo 2009). In the East China Sea, the dinoflagellate *Karenia mikimotoi* was reported to bloom just before or contemporaneously with *P. donghaiense* blooms (Li et al. 2009). Thus, there are numerous questions with regard to relative success of *Prorocentrum* species in the presence of the competing dinoflagellates, *Karlodinium* or *Karenia.*

In all these cases, bloom areas were enriched by large terrestrial nutrient inputs. The relationships between nutrient loading and bloom development are very complex (Glibert et al. 2005a, Glibert and Burkholder 2006, Glibert et al. 2006, Anderson et al. 2008, Glibert et al. 2008a). While dissolved inorganic nitrogen (DIN) still is the major N sources enriched to the coastal systems, urea and other dissolved organic N (DON) have became important N sources for many HAB species (Glibert et al. 2004, Glibert et al. 2006, Heil et al. 2007, Li et al. 2009). The nutrient composition might regulate the community composition of the blooms.

In addition to nutrient composition, the relative ambient N:P ratio may also regulate the phytoplankton community composition and HAB development (Phillips and Tanabe 1989, Hodgkiss and Ho 1997, Bulgakov and Levich 1999, Lagus et al. 2004). For *P. minimum*, *P. donghaiense* and *K. brevis*, the N:P ratio may not only relate to the bloom distribution and biomass, but also may regulate dominant species due to their differential nutrient uptake rates and preference (Fisher et al. 1992, Heil et al. 2007, Li et al. 2009, Li et al. 2010). In both East China Sea and southwest

Florida shelf, the *Prorocentrum* spp. communities were observed to be associated with DIN:DIP ratios that were slightly higher than the Redfield ratio (16), while the *Karenia* spp. dominated communities were found in association with DIN:DIP ratios that were below the Redfield ratio, but high DON:DOP ratio (>20), suggesting that *Karenia* spp. in both environments may have been consuming the organic N source (Heil et al. 2007, Li et al. 2009).

As an alternative nutrient strategy, mixotrophy, a combination of phototrophic and phagotrophic nutrition, has been reported for *P. minimum*, *P. donghaiense*, *K. veneficum* and *K. brevis* (Li et al. 1999, Stoecker 1999, Jeong et al. 2004, Jeong et al. 2005a, Adolf et al. 2007, Burkholder et al. 2008, Glibert et al. 2009, Jeong et al. 2010). Phagotrophy may provide mixotrophic dinoflagellates a source of nutrients and/or carbon from other organisms that supplement that which they assimilate from the water. Some dinoflagellates (e. g. *K. veneficum* and *K. brevis*) approach a higher maximum growth rate when growing mixotrophically (Adolf et al. 2006b, Glibert et al. 2009). Therefore, phagotrophy of dinoflagellates could serve as a competition strategy during nutrient limitation and could be a means of sustaining the growth of mixotrophic dinoflagellates. Mixotrophic activity may play a substantial role in sustaining blooms (Glibert et al. 2009). Dinoflagellates feed on a wide range of food sources, including other algae, bacteria, and organic particles (Burkholder et al. 2008). In estuaries with high terrestrial input, organic particles and their primary decomposers, heterotrophic bacteria, could be important food sources for mixotrophic dinoflagellates.

The effects of various nutrient sources and growth conditions on the growth competition of *P. minimum*, *P. donghaiense*, *K. veneficum* and *K. brevis* singly and in combination were tested in the present study. The factors tested included different type of N sources (nitrate and urea), different ambient N:P ratios (12 and 145), different light availability (50 and 300 µmol photons m^{-2} s^{-2}) and the potential of mixotrophic food sources. The euryhaline cryptophyte, *Rhodomonas*, and cyanobacteria, *Synechococcus*, were grown with the dinoflagellates as potential food sources to test their contribution as a nutrient source in sustaining the growth of the dinoflagellates.

Materials and Methods

Algae Cultures and Methods of Growth

Cultures of *Prorocentrum minimum* were isolated from Choptank River, a tributary of Chesapeake Bay in spring 1995 and were maintained in f/2 medium in the Horn Point Laboratory culture collection. Cultures of *Prorocentrum donghaiense* were obtained from University of Connecticut Department of Marine Sciences, USA. These cultures were originally isolated from the East China Sea. Cultures of *Karlodinium veneficum*, *Karenia brevis* (CCMP 2229), *Rhodomonas* and *Synechococcus* (CCMP 1768) were obtained from the Provasoli-Guillard National Culture Collection, Bigelow Laboratory for Ocean Sciences, Maine, USA. The *K. brevis* strain was originally isolated from Manasota Key, Florida. *Synechococcus* strain CCMP1768 was originally isolated from the Gulf of Mexico. All the cultures in the present study were unialgal, but not axenic.

The *P. minimum* and *K. veneficum* cultures were grown in modified f/2 media at a salinity of 12; *P. donghaiense* and *K. brevis* cultures were grown in modified f/2 media at a salinity of 30. The N and P source concentration varied with the experiments, as described below. Maintenance cultures were grown at 22 ˚C and on a 12 h light: 12 h dark cycle in a walk-in temperature-controlled incubator. The seawater used in media preparation was artificial seawater. Fluorescent lights were set to provide a range of light intensities, which varied by experiments. Cultures were not axenic, but aseptic techniques were used to minimize additional bacterial contamination during the growth periods.

Experimental Design

Experiment 1: Phototrophic growth of P. minimum and K. veneficum monoculture with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum minimum and *K. veneficum* were diluted by fresh media at salinity of 12 to a final concentration of ~1.0×10^4 cells ml^{-1} for each species. Monocultures were grown in triplicate 50 ml glass cuvettes with the N substrate as NO_3^- or urea and P substrate as PO_4^{3-} at two N:P ratios (12 and 145) and two light intensities (50 and 300 μmol photons m^{-2} s^{-2}). One set of cultures was enriched to a final nutrient concentrations of 42.8 μM-N and 3.56 μM-P to reach an N:P ratio of 12, and the other set was enriched to 224.9 μM-N and 1.55 μM-P to reach the N:P ratio of 145.

Experiment 2: Phototrophic growth competition between P. minimum and K. veneficum with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum minimum and *K. veneficum* were mixed and diluted by fresh media at a salinity of 12 to a final concentration of ~1.0×10^4 cells ml^{-1} for each species. Cells were grown in duplicate 1.5 L glass bottles with two N substrates (NO_3^- and urea) at two N:P ratios (12 and 145) and two light intensities (50 and 300 µmol photons m^{-2} s^{-2}). Nutrients were added as in experiment 1. All bottles were gently bubbled with air to keep the culture well mixed and aerated.

Experiment 3: Phototrophic/ mixotrophic growth competition between P. minimum and K. veneficum with Rhodomonas as potential prey, and with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum minimum and *K. veneficum* were mixed and diluted by fresh media at a salinity of 12 to a final concentration of ~2.5×10^4 cells ml^{-1} for each species. *Rhodomonas* were also added at a final concentration of ~7.5×10^4 cells ml^{-1}. Cells were grown in duplicate 1.5 L glass bottles with NO_3^- at two N:P ratios (12 and 145) and two light intensities (50 and 300 µmol photons m^{-2} s^{-2}). Nutrients were added as in experiment 1. All bottles were gently bubbled with air to keep the culture well mixed and aerated.

Experiment 4: Phototrophic growth of P. donghaiense and K. brevis with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum donghaiense and *K. brevis* were diluted by fresh media at salinity of 30 to a final concentration of ~1.0×10^3 cells ml^{-1} for each species. Monocultures were grown in triplicate 50 ml glass cuvettes with two substrates (NO_3^- and urea) at two N:P ratios (12 and 145) and two light intensities (50 and 300 µmol photons m^{-2} s^{-2}). Nutrients were added as in experiment 1.

Experiment 5: Phototrophic growth competition between P. donghaiense and K. brevis with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum donghaiense and *K. brevis* were mixed and diluted by fresh media at salinity of 30 to a final concentration of ~1.0×10^3 cells ml^{-1}. Cells were grown in duplicate 1.5 L glass bottles with 2 N substrates (NO_3^- and urea), at two N:P ratios (15.5 and 145) and two light intensities (50 and 300 μmol photons m^{-2} s^{-2}). Nutrients were added as in experiment 1. All bottles were gently bubbled with air to keep the culture well mixed and aerated.

Experiment 6: Phototrophic growth competition between P. donghaiense and K. brevis with Synechococcus as potential prey, and with NO_3^- or urea at high or low light levels and 2 N:P ratios.

Prorocentrum donghaiense and *K. brevis* were mixed and diluted by fresh media at salinity of 30 to a final concentration of ~1.0×10^3 cells ml^{-1}. *Synechococcus* were also added at a final concentration of ~7.5×10^4 cells L^{-1}. Cells were grown in duplicate 1.5 L glass bottles with two forms of N sources (NO_3^- and urea), at two N:P ratios (15.5 and 145) and two light intensities (50 and 300 μmol photons m^{-2} s^{-2}). Nutrients were added as in experiment 1. All bottles were gently bubbled with air to keep the culture well mixed and aerated.

Analytical Protocols

The growth of each dinoflagellate monoculture was tracked by the change of its fluorescence using a TD-700 Fluorometer. The growth of mixed dinoflagellates and *Rhodomonas* in the mixed culture was tracked by optical microscopy. On an approximate daily basis, 7 ml of culture were sampled from each bottle and fixed

with Lugol's solution for cell counting. The cell densities of *Synechococcus* were also measured on a daily basis. To do so, fresh culture was added to a quartzose cuvette and the fluorescence emission of 560 nm was recorded on a Jobin Yvon Fluoro Max-3 spectrofluorometer according to Glibert et al. (2009). The results were calibrated by microscopy cell counting.

Data Analysis and Simulation

Statistical comparisons between groups and treatments were compared and analyzed using ANOVA and *t*-tests. All comparisons were made at a significance level of 0.05.

The exponential growth rates of each culture in the early stage of the growth phase were determined with Sigmaplot software (Systat Software, Inc.) by the equation:

$$N(t) = N_0 e^{\mu t} \qquad (1)$$

where μ is the specific growth rate (d^{-1}); N_0 is the initial cell density (cells ml^{-1}); N(t) is the cell density at time t. Herein, only the cell densities of the early exponential phase were used for the growth rate regression to estimate the exponential growth rate.

Results

Experiment 1

Prorocentrum minimum monocultures in NO_3^- media grew for about 3 weeks before reaching the stationary phase, except for the treatment with low light and high

N:P ratio, which started to decrease in 2 weeks (Fig. 5-1 a). The growth rates were in the range of 0.44 day^{-1} to 0.49 day^{-1} (Table 5-1) in high light, which were significantly higher than the rates (~0.25 day^{-1}) in low light ($p<0.001$). There was no significant difference between growth rates with low and high N:P ratio treatments. The *P. minimum* monocultures in urea media had a long lag phase, and rapid growth started after one week of incubation and only lasted for a week, with a growth rate about 0.39 day^{-1} in the high light treatment. In low light, exponential growth lasted about 2 weeks; however, the growth rates (~0.24 day^{-1}) were significantly lower than those at high light (Fig. 5-1 b). There was no significant difference between growth rates with low and high N:P ratio treatments.

Karlodinium veneficum monocultures in NO_3^- media also grew exponentially for more than 3 weeks, except for the treatments with low light and high N:P ratio, which started to decrease at day 18 (Fig. 5-1 c.). The growth rates were about 0.32 day^{-1} in high light, significantly higher than those in low light (0.19 day^{-1}, $p=0.004$). There was no significant difference in growth rates between the low and high N:P ratio treatments. In NO_3^- media, the growth rates of *P. minimum* were significantly higher than *K. veneficum* for all treatments ($p<0.05$). The *K. veneficum* monoculture in urea media also had a long lag phase, and growth started after over one week of incubation and only lasted for a week at a growth rate of ~ 0.24 day^{-1} in high light treatment, and 0.16 day^{-1} in low light (Fig. 5-1 d.). There was no significant difference between growth rates with low and high N:P ratio treatments.

Experiment 2

Prorocentrum minimum biomass in the mixed culture increased in the low N:P ratio treatment for about 10 days (Fig. 5-2), while exponential growth lasted more than 20 days in the high N:P ratio in low light. The growth rates in the low N:P ratio treatment were in the range of 0.17 day^{-1} to 0.27 day^{-1} (Table 5-2). The growth rates in the high N:P ratio treatment were in the range of 0.23 day^{-1} to 0.33 day^{-1}, which were significantly higher than those of the low N:P ratio treatment ($p<0.001$). There was no significant difference between the *P. minimum* growth rates at high light and low light treatments. *Prorocentrum minimum* grown in urea media in low light had significantly higher growth rates than the comparable cultures growing on NO_3^- ($p<0.05$). The exponential growth phase of *K. veneficum* in the mixed culture lasted from a week to 10 days. The growth rates of *K. veneficum* were in the range of 0.06 day^{-1} to 0.28 day^{-1}. However, the difference among growth rates of *K. veneficum* was not significant among all treatments. The growth rates of *K. veneficum* were significantly lower than those of *P. minimum* ($p<0.001$). As a result of a longer period of exponential growth and faster growth rates, *P. minimum* reached significantly higher cell densities than *K. veneficum* in most treatments, except for the culture in urea at a N:P ratio of 12 in high light. Neither species grew well in the urea treatment in high light.

Experiment 3

With the addition of *Rhodomonas*, the growth rate of *P. minimum* was very low, only ~ 0.04 day^{-1}, in high light (Table 5-3), and no significant growth was observed in low light. The growth rate of *K. veneficum* was only ~ 0.085 day^{-1}, and no

significant difference in growth rates was observed between light or N:P ratio treatments. The increase in biomass for both species only lasted 4-5 days. However, *K. veneficum* outgrew *P. minimum*. The cell densities of *Rhodomonas* decreased over 50% during first 2-3 days and were subsequently held at < 5% of the initial densities (Fig. 5-3). There were no significant differences of growth rates among different treatments for each species.

Experiment 4

Prorocentrum donghaiense in monoculture grew exponentially for about 20 days before reaching the stationary phase, except for the treatments in high N:P ratio, which started to decrease in 10 days (Fig. 5-4). The highest growth rates were 0.37 day^{-1} in NO_3^- media at low N:P ratio and high light (Table 5-4). The lowest growth rates were 0.16 day^{-1} in NO_3^- media at low N:P ratio and low light, but the high and low light growth rates were not significantly different. The growth rates in the low N:P ratio media were significantly higher than those in high N:P ratio media in high light ($p<0.001$). The growth rates on urea were significantly higher than those in NO_3^- media in low light ($p<0.001$). *Karenia brevis* biomass in monoculture increased for about 10 days in high light, then started to decrease. However, *K. brevis* in low light kept increasing during the entire study (Fig. 5-4). The growth rates in high light were in the range of 0.22 day^{-1} to 0.28 day^{-1}. The growth rates in low light were in the range of 0.08 day^{-1} to 0.14 day^{-1}, and were significantly lower than those in high light ($p<0.001$). There were no significant differences in growth rates between the low and high N:P ratio treatments. In monoculture, the growth rates of *P. donghaiense* were significantly higher than *K. brevis* for all treatments ($p=0.015$).

Experiment 5

In high light, *Prorocentrum donghaiense* growth rates were in the range of 0.30 day^{-1} to 0.38 day^{-1} (Table 5-5). *P. donghaiense* biomass in the mixed culture kept increasing at high light for about 7-10 days, and reached cell densities of ~ 40, 000 cell ml^{-1} (Fig. 5-5). *Karenia brevis* growth rates were in the range of 0.17 day^{-1} to 0.25 day^{-1}in high light, and reached cell densities of ~ 20, 000 cell ml^{-1}. However, at low light, *P. donghaiense* grew in the range of 0.12 day^{-1} to 0.23 day^{-1}, and the growth lasted longer and reached maximal cell densities of ~ 80, 000 cell ml^{-1}. *Karenia brevis* only grew at 0.025 day^{-1} or lower, and only reached cell densities of ~ 4, 000 cell ml^{-1}. Both species grew significantly faster in high light than low light ($p<0.01$). There were no significant differences between NO_3^- and urea treatments. The growth rates of *P. donghaiense* were significantly higher than *K. brevis* ($p=0.004$).

Experiment 6

In experiments 6 with addition of *Synechococcus*, the growth rates of *P. donghaiense* in high light were in the range of 0.36 day^{-1} to 0.39 day^{-1}, which were significant higher than rates in low light (0.14 day^{-1}, $p=0.001$). The growth rates of *K. brevis* at low light were in the range of 0.019 day^{-1} to 0.055 day^{-1}. The growth rates in high light were about 0.17 day^{-1}, which were significantly higher than those at low light ($p<0.001$). The growth rates of *P. donghaiense* were significantly higher than *K. brevis* ($p<0.05$). The *Synechococcus* growth rates were about 0.32 day^{-1} in high light and 0.06 day^{-1} in low light. *Synechococcus* grew exponentially for a week before it decreased at high light, but it continued growing at low light (Fig. 5-6).

Discussion

Growth Rate

Numerous studies have been conducted on the four dinoflagellate species studied herein. The growth rates in the present study were compared with previous work (Table 5-7).The growth rates of *P. minimum* monocultures (0.23-0.46 day^{-1}) in this study were within the range of the previous work, which mostly were in the range of 0.13 to 1 day^{-1} except for one report of rates reaching 3.54 day^{-1} (Smayda 1996). The growth rates of *P. minimum* in mixed culture with *K. veneficum* (0.17-0.32 day^{-1}) fell in the lower range of previous work, and the growth rates of *P. minimum* in cultures mixed with *K. veneficum* and *Rhodomonas* (<0.05 day^{-1}) were lower than previous results. The growth rates of *K. veneficum* monoculture (0.19-0.33 day^{-1}) in this study were within the range of the previous work (< 0.75 day^{-1}, Table 5-7). The growth rates of *K. veneficum* in mixed culture with *P. minimum* (0.06-0.28 day^{-1}) were also in the in the range of previous work, and the growth rates of *K. veneficum* with *P. minimum* and *Rhodomonas* (<0.09 day^{-1}) were in the lower range of previous results. The growth rates of *P. donghaiense* were in the range of 0.12-0.39 day^{-1}, which were within the range of the previous work (0.1-0.78 day^{-1}). There was only one report of growth rates reaching 1.40 day^{-1} (Wang and Tang 2008). The growth rates of *K. brevis* monoculture (0.08-0.28 day^{-1}) in this study were within the range of the previous work (0-0.36 day^{-1}, Table 5-7). The growth rates of *K. brevis* in mixed culture with *P. donghaiense* (0.0-0.26 day^{-1}) were in also in the range of previous work, and the growth rates of *K. brevis* with *P. donghaiense* and *Synechococcus* (0.02-0.15day^{-1}) were in the lower range of previous results.

Dinoflagellates are one of the major groups of algae causing HABs in coastal waters. Smayda suggested that dinoflagellates have diverse habitat preferences and adaptive strategies. He characterized the dinoflagellate bloom species in coastal areas as 5 types, which fit to a nutrient-turbulence matrix (Smayda and Reynolds 2001, Smayda 2002). Each dinoflagellate species had its specific niche for bloom expansion, and the niches were described by available nutrients and turbulence/mixing intensities. In Smayda's model, small, rapidly growing species (e.g. *Prorocentrum* spp.) are better competitors in near-shore regions. Conversely, relative slower growing, larger, motile species (e.g. *Karenia* spp.) are better suited to off-shore regions. The near-shore regions are generally considered nutrient rich but with high turbidity, while light might be the limiting factor for the phytoplankton. The off-shore regions are generally considered to have relatively low nutrients but with higher light availability. These two groups of algae have different adaptive strategies to the environment. Therefore, not only are the nutrient preferences but also light adaptation strategies of these two general genera of algae potentially differ.

In this study, the growth and competition between the near-shore dinoflagellates (*P. minimum* and *K. veneficum*) and between near-shore and off-shore species (*P. donghaiense* and *K. brevis*) were tested under different ambient conditions, including variable nutrient conditions and light intensities. The two near-shore dinoflagellate species, *P. minimum* and *P. donghaiense*, both had higher growth rates in monoculture than their competitors in this study. When grown with *K. veneficum*, the average growth rates of *P. minimum* were inhibited to about 40% of the rates observed in monocultures. After adding *Rhodomonas*, *K. veneficum* outgrew

P. minimum, and reached higher cell densities than *P. minimum*. However, when grown with a different type (off-shore) of dinoflagellate, *K. brevis*, the growth rate of *P. donghaiense* was not significantly affected. Although *K. brevis* grew slower than *P. donghaiense*, they still reached similar cell densities in mixed culture in high light treatment. *K. brevis* had a longer growth phase, and kept growing after *P. donghaiense* reached stationary phase, suggesting better adaptation of *K. brevis* to low inorganic nutrient conditions. The growth of *K. brevis* was mostly limited by the low light treatment of the four species. This off-shore species only had an advantage at low ambient inorganic nutrient condition, and appeared to be more limited by light.

Nutrient Strategy – Autotrophy and Mixotrophy

All four tested dinoflagellates have developed HABs in eutrophic estuarine and marine coastal waters. The increase of many HABs has been suggested to correspond with high nutrient loading (Glibert et al. 2001, Anderson et al. 2002, Glibert et al. 2005a). Dissolved inorganic nitrogen forms (DIN, i.e., NO_3^-, NH_4^+) are generally considered the principal N sources for phytoplankton. Diatoms are often found to use NO_3^- at low temperatures and to develop blooms in the early spring (Lomas and Glibert 1999a, b). Spring blooms also deplete the NO_3^- in the ambient water in coastal systems, as often seen in the Changjiang River estuary (Li et al. 2009, Li et al. 2010), and the Chesapeake Bay (Jordan et al. 1991, Fisher et al. 1992, Glibert et al. 1995). Although the NO_3^-concentration decreases after the spring blooms, it is still an important N source, and it has been widely used as N source in the media in the culture experiments (Guillard and Ryther 1962).

The succession of dominant phytoplankton assemblages in eutrophic waters has been associated with shifts in nutrient supply ratios, especially decreased DIN:DIP ratios that reflect regional disproportionate P loading relative to N, which relates to anthropogenic and regenerated inorganic sources (Suttle and Harrison 1988, Jordan et al. 1991, Koerselman and Meuleman 1996, Bulgakov and Levich 1999, Stelzer and Lamberti 2001, Heil et al. 2007, Vrede et al. 2009). All four tested dinoflagellates have been documented to bloom in relatively lower DIN:DIP ratio waters (close to the Redfield ratio) than diatom blooms (Jordan et al. 1991, Heil et al. 2007, Li et al. 2009).

For dinoflagellates, another important N source is dissolved organic nitrogen (DON), which may represent 14 - 90% of the total N in lower river estuaries from natural and anthropogenic sources (Glibert et al. 1991, Seitzinger et al. 2002, Glibert et al. 2004). DON may also represent an important N source for phytoplankton in eutrophic coastal waters (Bronk et al. 2004, Glibert et al. 2004, Wiegner et al. 2006, Heil et al. 2007, Li et al. 2010). Urea has become a very important DON source because of its accelerated global use, which has increased more than 100-fold in the past 4 decades and contributes > 50% of global nitrogenous fertilizer usage (Glibert et al. 2006). Urea is increasingly found to be an important N nutrient source for HAB species and is positively correlated to some blooms (Glibert et al. 2004, Glibert et al. 2005b, Glibert et al. 2006). In Chesapeake Bay tributaries, high concentrations of urea (> 10 μM-N) have been observed frequently, generally in late spring when urea or poultry manure is applied to winter wheat and corn; this is also the time when high frequent *P. minimum* blooms have been recorded (Glibert et al. 2005b). *P. minimum*

have been observed to follow heavy rainfall and fertilizer application which result in short term increases of urea in the water column (Glibert et al. 2001). Urea can be directly taken up by the dinoflagellates. A high affinity of *P. minimum* for urea has previously been found (Fan et al. 2003). In the Western Florida Shelf, urea concentrations were also significantly greater over the entire region adjacent to the mouths of both the Shark and Caloosahatchee Rivers after hurricane flooding (Heil et al. 2007). In the East China Sea, urea contributed to over 20% of reduced N (NH_4^+, urea and glycine) uptake during the *K. brevis* and *P. donghaiense* bloom progression. Urea has also been reported to increased toxicity in some harmful algae. Toxigenic *Pseudo-nitzschia australis* assemblages have also been shown to double toxin production when grown on urea compared to growth on NO_3^- and NH_4^+ (Howard et al. 2007). *K. brevis* in culture was observed to increase in toxin content up to 6-fold after enrichment with urea compared to treatments without urea enrichment (Shimizu et al. 1995).

All four tested dinoflagellates have been reported to have mixotrophic abilities as a mechanism to supplement nutrient supplies (Jeong et al. 2005a, Stoecker et al. 2006, Burkholder et al. 2008, Jeong et al. 2010). This mechanism is suggested to be important in nutrient acquisition and growth of these species for the development and maintenance of their blooms in eutrophic waters (Burkholder et al. 2008). Although the high frequent bloom areas of these tested dinoflagellates have variable nutrient loads and concentrations, dissolved forms of nutrients still can be limiting factors in bloom progression, due to high biomass of the algae and the disproportionate nature of nutrient supply (e.g. very high DIN:DIP ratio). Light deficiency is also considered

common in eutrophic systems, especially in shallow water and estuarine systems, where algal blooms, sediment from river input, precipitation, wind/river mixing process and other disturbance can cause limiting light availability for the phytoplankton (Hecky and Kilham 1988, Cloern and Jassby 2010). These low light and/or nutrient deficiency habitats may promote the mixotrophy of algae. Mixotrophy might be triggered by nutrient limitation (Granéli et al. 1999), light limitation (Caron et al. 1993, Jones et al. 1995), or high prey densities (Sanders et al. 1990).

Stoecker (1998) proposed three physiological types of protistan mixotrophs: Type I - ‘‘ideal’’ mixotrophs that can use phototrophy and phagotrophy equally well; Type II - predominantly phototrophic algae, which included many of the harmful algae in eutrophic estuarine and coastal waters; and Type III - predominantly heterotrophic algae. The four tested dinoflagellates are generally considered type II algae. Many Type II species become mixotrophic when DIN and/or DIP become limiting, and their feeding rates can be directly related to available light (Stoecker 1998, 1999, Li et al. 2000).

Prorocentrum minimum can not only consume DON sources via osmotrophy (Fan et al. 2003, Heil et al. 2005, Solomon and Glibert 2008), but also has been reported to ingest cyanobacteria and cryptophytes, including *Rhodomonas salina* (Stoecker et al. 1997, Jeong et al. 2005a, Jeong et al. 2005b, Jeong et al. 2010). Mixotrophy by *P. minimum* was found to be higher in spring than the summer in the Chesapeake Bay, when ingestion of cryptophytes was observed in over 50% of the cells in natural assemblages (Stoecker et al. 1997). But, ingestion was low when the cryptophyte densities were high. Ingestion was also inhibited after nutrient

enrichment (DIN or DIP), suggesting it a mechanism for obtaining organic nutrients rather than organic carbon (Stoecker et al. 1997).

Karlodinium veneficum also takes up urea to support growth, and can ingest eubacteria and cryptophytes *Rhodomonas salina* (Nygaard and Tobiesen 1993, Li et al. 1996, Li et al. 1999, Li et al. 2000, Adolf et al. 2003, Adolf et al. 2006a, Adolf et al. 2007, Adolf et al. 2008). Growth rates of mixotrophic *K. veneficum* (0.52 - 0.75 day^{-1}) have been found to be comparable to or greater than maximum growth rate in phototrophic mode (0.55 day^{-1}) (Adolf et al. 2006b). Phagotrophy of *K. veneficum* has been intensively studied in Chesapeake Bay (Li et al. 1996, Li et al. 1999, Li et al. 2000, Adolf et al. 2003, Adolf et al. 2006a, Adolf et al. 2007, Adolf et al. 2008). From field and laboratory data, phagotrophy of *K. veneficum* was positively correlated with prey density, and negatively correlated with depth, salinity, and PO_4^{3-} concentration (Li et al. 2000). N and/or P deficiency, or N:P ratios that were substantially higher or lower than the optimum N:P ratio (~10), increased phagotrophic activity, suggesting feeding as a supplement for major nutrients (N, P) (Li et al., 2000). Phagotrophic activity might also supplement organic carbon sources or assist in acquiring trace growth factors (Li et al., 2000).

Prorocentrum donghaiense also has been shown to ingest cyanobacteria, cryptophytes and other dinoflagellates (*P. minimum*) (Jeong et al. 2005a, Jeong et al. 2010). In low ambient light (20 μmol photons m^{-2} sec^{-1}), mixotrophic *P. donghaiense* reached higher maximum growth rate (0.051 day^{-1}) by feeding on cryptophytes than in autotrophic mode (0.038 day^{-1}) (Jeong et al. 2004, Jeong et al. 2005b).

Karenia brevis has been shown to take up DON from *Trichodesmium* blooms (Mulholland et al. 2006), and also urea to support growth (Glibert et al. 2009). *K. brevis* can ingest cyanobacteria *Synechococcus* (Jeong et al. 2005b, Glibert et al. 2009). *K. brevis* was observed grazing on *Synechococcus* at higher rate in low light (43 μmol photons m^{-2} sec^{-1}) when N-depleted (Glibert et al. 2009).

In the present study, the four tested dinoflagellates were grown on treatments which promoted different growth strategies, from autotrophic growth on NO_3^- at high light, to osmotrophic growth on urea and mixotrophic growth on eukaryote prey, the co-cultured dinoflagellate, or phagotrophic growth on the prey, *Rhodomonas* or *Synechococcus*. The initial ambient nutrients were replete. There were no significant differences of the growth rate among the monocultures growing on NO_3^- and urea. A significant light limitation on the growth rate was observed. All four species could use these dissolved N sources well. When two dinoflagellates were grown together, the growth of *P. minimum* and *K. veneficum* were both inhibited in high light, while only the growth of *K. brevis* was inhibited when *P. donghaiense* was co-cultured. After adding the *Rhodomonas* or *Synechococcus*, the cell densities of these potential food sources either decreased at the beginning of the incubation or decreased when the culture became more nutrient limited. The tested dinoflagellates could feed on the prey, however, the competition between two dinoflagellates is more complicated and needs further experimental study.

Both *P. minimum* and *K. veneficum* have been documented to bloom in the Chesapeake Bay (Deeds et al. 2002, Glibert and Magnien 2004, Goshorn et al. 2004). *Prorocentrum minimum* generally blooms in the late spring and early summer, when

ambient water N:P ratios are higher than the Redfield ratio, suggesting P limitation (Fisher et al. 1992, Tango et al. 2005, Ptacnik et al. 2010). *Prorocentrum minimum* outgrew *K. veneficum* in relative N-rich natural water, which was consistent with the present study. In contrast, *K. veneficum* generally develops more blooms in the later summer and autumn (Li et al. 2000, Glibert and Magnien 2004), when ambient inorganic N is depleted, and ambient N:P ratios are lower than the Redfield ratio, suggesting N limitation (Fisher et al. 1992, Ptacnik et al. 2010). High cryptophyte abundance has been suggested to trigger *K. veneficum* blooms (Adolf et al. 2008). Mixotrophic *K. veneficum* may feed on the cryptophyte as an N source. Herein, in the experiments simulating this condition, *K. veneficum* outgrew *P. minimum* after enrichment with *Rhodomonas.*

In the East China Sea, *P. donghaiense* has been the dominant bloom forming dinoflagellate in recent years, even when the off-shore type species *Karenia mikimotoi* and *Alexandrium tamarense* have been documented to mix with the *P. donghaiense* (Zhou et al. 2006, Li et al. 2009). The only recent exception to this pattern was in 2005, when *K. mikimotoi* developed blooms before the *P. donghaiense* in the East China Sea. However, *P. donghaiense* still outgrew *K. mikimotoi* in approximately two weeks (Li et al. 2009). Herein, *Prorocentrum donghaiense* also outgrew the off-shore type *K. brevis* in present study.

In summary, dinoflagellates have multiple nutrient strategies to grow in coastal waters, which depend on the nutrients and light availabilities. The dinoflagellate species in present study have slightly different nutrient strategies, which may drive the results of the growth competition. The effectiveness of utilizing

dissolved organic nutrients and prey appears to play important roles in determining the competition between *P. minimum* and *K. veneficum.* It may be advantageous for *K. brevis* to feed on *Rhodomonas*, but this needs further direct study. The growth of off-shore type species *K. brevis* was mostly limited by the low light treatments. However, *K. brevis* had longer growth phase, and kept growing after *P. donghaiense* reached stationary phase, suggesting better adaptation of *K. brevis* to low nutrient (or nutrient depleted) condition, because of its greater mixotrophic tendencies. The laboratory experiment results were consistent with the field observation in waters where the blooms of these species were documented. It must be noted, however, that these experiments were still conducted in relatively nutrient-replete media, and the N and P nutrients concentrations may not be limiting factors in the early growth phase. Because of these high nutrient levels, the effect of N:P ratios on the growth of these species were not significant.

References

Adolf J, Bachvaroff T, Place AR (2007) Cryptophytes drive blooms of mixotrophic harmful algae: A testable hypothesis based on *Karlodinium veneficum* in Chesapeake Bay. Journal of Phycology 43:31-31

Adolf JE, Bachvaroff T, Place AR (2008) Can cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? Harmful Algae 8:119-128

Adolf JE, Bachvaroff TR, Krupatkina DN, Nonogaki H, Brown PJP, Lewitus AJ, Harvey HR, Place AR (2006a) Species specificity and potential roles of *Karlodinium micrum* toxin. African Journal of Marine Science 28:415-419

Adolf JE, Stoecker DK, Harding LW (2003) Autotrophic growth and photoacclimation in *Karlodinium micrum* (Dinophyceae) and *Storeatula major* (Cryptophyceae). Journal of Phycology 39:1101-1108

Adolf JE, Stoecker DK, Harding LW (2006b) The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). Journal of Plankton Research 28:737-751

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Bronk DA, Sanderson MP, Mulholland MR (2004) Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA (eds) Harmful Algae 2002, Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, St. Petersberg, Florida, USA, p 80-82

Bulgakov NG, Levich AP (1999) The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv Fur Hydrobiologie 146:3-22

Burkholder JM, Glibert PM, Skelton HM (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8:77-93

Caron DA, Sanders RW, Lim EL, Marrase C, Amaral LA, Whitney S, Aoki RB, Porter KG (1993) Light dependent phagotrophy in the fresh water mixotrophic chrysophyte *Dinobryon cylindricum*. Microbial Ecology 25:93-111

Cloern JE, Jassby AD (2010) Patterns and scales of phytoplankton variability in estuarine-voastal ecosystems. Estuaries and Coasts 33:230-241

Deeds JR, Reimschuessel R, Place AR (2006) Histopathological effects in fish exposed to the toxins from *Karlodinium micrum*. Journal of Aquatic Animal Health 18:136-148

Deeds JR, Terlizzi DE, Adolf JE, Stoecker DK, Place AR (2002) Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae) – a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae 1:169-189

Fan CL, Glibert PM, Burkholder JM (2003) Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. Harmful Algae 2:283-299

Fisher TR, Peele ER, Ammerman JW, Harding LW (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Marine Ecology-Progress Series 82:51-63

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005a) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Granéli E, Anderson DM (2008a) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Glibert PM, Burkholder JM, Kana TM, Alexander J, Skelton H, Shilling C (2009) Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquatic Microbial Ecology 55:17-30

Glibert PM, Conley DJ, Fisher TR, Harding LW, Malone TC (1995) Dynamics of the 1990 winter spring bloom in Chesapeake Bay. Marine Ecology-Progress Series 122:27-43

Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay Estuary and its regulation by large heterotrophs. Limnology and Oceanography 36:895-909

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea - a global change contributing to coastal eutrophication. Biogeochemistry 77:441-463

Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Marine Ecology-Progress Series 280:73-83

Glibert PM, Magnien R, Lomas MW, Alexander J, Fan CL, Haramoto E, Trice M, Kana TM (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. Estuaries 24:875-883

Glibert PM, Magnien RE (2004) Harmful algal blooms in the Chesapeake Bay, USA: Common species, relationships to nutrient loading, management approaches, successes, and challenges. In: Hall S, Anderson D, Kleindinst J, Zhu M, Zou Y (eds) Harmful Algae Management and Mitigation. Asia-Pacific Economic Cooperation, Singapore

Glibert PM, Mayorga E, Seitzinger S (2008b) *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: Application of spatially explicit nutrient export models. Harmful Algae 8:33-38

Glibert PM, Trice TM, Michael B, Lane L (2005b) Urea in the tributaries of the Chesapeake and Coastal Bays of Maryland. Water Air and Soil Pollution 160:229-243

Goshorn D, Deeds J, Tango P, Poukish C, Place AR, McGinty M, Butler W, Luckett C, Magnien R (2004) Occurrence of *Karlodinium micrum* and its association with fish kills in Maryland estuaries. In: Steidinger KA, Landsberg JA, Tomas CR, Vargo GA (eds) Proceedings of Harmful Algae 2002. IOC-UNESCO, St. Petersberg, p 361–363

Granéli E, Carlsson P, Legrand C (1999) The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. Aquatic Ecology 33:17-27

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology 8:229

Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in fresh-water and marine environments - a review of recent evidence on the effects of enrichment. Limnology and Oceanography 33:796-822

Heil CA, Glibert PM, Al-Sarawl MA, Faraj M, Behbehani M, Husain M (2001) First record of a fish-killing *Gymnodinium* sp bloom in Kuwait Bay, Arabian Sea: chronology and potential causes. Marine Ecology-Progress Series 214:15-23

Heil CA, Glibert PM, Fan CL (2005) *Prorocentrum minimum* (Pavillard) Schiller - a review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449-470

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Hodgkiss IJ, Ho KC (1997) Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147

Howard MDA, Cochlan WP, Ladizinsky N, Kudela RM (2007) Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (*Bacillariophyceae*) from field and laboratory experiments. Harmful Algae 6:206-217

Jeong H, Yoo Y, Kim J, Seong K, Kang N, Kim T (2010) Growth, feeding, and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Science Journal 45:65-91

Jeong HJ, Du Yoo Y, Park JY, Song JY, Kim ST, Lee SH, Kim KY, Yih WH (2005a) Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquatic Microbial Ecology 40:133-150

Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Jeng C, Seong CN, Lee KY, Yih WH (2005b) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquatic Microbial Ecology 41:131-143

Jeong HJ, Song JY, Lee CH, Kim ST (2004) Feeding by larvae of the mussel *Mytilus galloprovincialis* on red-tide dinoflagellates. Journal of Shellfish Research 23:185-195

Jones HLJ, Durjun P, Leadbeater BSC, Green JC (1995) The relationship between photoacclimation and phagotrophy with Respect to Chlorophyll *a*, carbon and nitrogen content, and cell size of *Chrysochromulina brevifilum* (*Prymnesiophyceae*). Phycologia 34:128-134

Jordan TE, Correll DL, Miklas J, Weller DE (1991) Nutrients and chlorophyll at the interface of a watershed and an estuary. Limnology and Oceanography 36:251-267

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33:1441-1450

Lagus A, Suomela J, Weithoff G, Heikkila K, Helminen H, Sipura J (2004) Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. Journal of Plankton Research 26:779-798

Li AS, Stoecker DK, Adolf JE (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquatic Microbial Ecology 19:163-176

Li AS, Stoecker DK, Coats DW (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. Journal of Plankton Research 22:2105-2124

Li AS, Stoecker DK, Coats DW, Adam EJ (1996) Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquatic Microbial Ecology 10:139-147

Li J, Glibert PM, Zhou M (2010) Temporal and spatial variability in nitrogen uptake kinetics during dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou M, Lu S, Lu D (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology Progress Series 383:11-26

Lomas MW, Glibert PM (1999a) Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Marine Biology 133:541-551

Lomas MW, Glibert PM (1999b) Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnology and Oceanography 44:556-572

Mulholland MR, Bernhardt PW, Heil CA, Bronk DA, O'Neil JM (2006) Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. Limnology and Oceanography 51:1762-1776

Nygaard K, Tobiesen A (1993) Bacterivory in algae - a survival strategy during nutrient limitation. Limnology and Oceanography 38:273-279

Phillips DJH, Tanabe S (1989) Aquatic pollution in the Far-East. Marine Pollution Bulletin 20:297-303

Ptacnik R, Andersen T, Tamminen T (2010) Performance of the Redfield Ratio and a family of nutrient limitation indicators as thresholds for phytoplankton N vs. P limitation. Ecosystems 13:1201-1214

Sanders RW, Porter KG, Caron DA (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. Microbial Ecology 19:97-109

Seitzinger SP, Kroeze C, Bouwman AF, Caraco N, Dentener F, Styles RV (2002) Global patterns of dissolved inorganic and particulate nitrogen inputs to coastal systems: Recent conditions and future projections. Estuaries 25:640-655

Shimizu Y, Watanabe N, Wrensford G (1995) Biosynthesis of brevetoxins and heterotrophic metabolism in *Gymnodinium breve*. In: Watanabe N, Wrensford G, Lassus P, Arzul G, Erard-Le Denn E, Gentien P, Marcaillou C (eds) Harmful Marine Algal Blooms. Lavoisier Publishing Inc., New York, p 351-357

Smayda TJ (1996) Dinoflagellate bloom cycles: what is the role of cellular growth rate and bacteria? In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

Solomon CM, Glibert PM (2008) Urease activity in five phytoplankton species. Aquatic Microbial Ecology 52:149-157

Stelzer RS, Lamberti GA (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography 46:356-367

Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. European Journal of Protistology 34:281-290

Stoecker DK (1999) Mixotrophy among dinoflagellates. Journal of Eukaryotic Microbiology 46:397-401

Stoecker DK, Li AS, Coats DW, Gustafson DE, Nannen MK (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Marine Ecology-Progress Series 152:1-12

Stoecker DK, Tillmann U, Granéli E (2006) Phagotrophy in harmful algae. In: Granéli E, Turner JT (eds) Ecology of Harmful Algae. Springer-Verlag, Berlin, p 177-187

Suttle CA, Harrison PJ (1988) Ammonium and phosphate-uptake rates, N-P supply ratios, and evidence for N-limitation and P-limitation in some oligotrophic lakes. Limnology and Oceanography 33:186-202

Tango PJ, Magnien R, Butler W, Luckett C, Luckenbach M, Lacouture R, Poukish C (2005) Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. Harmful Algae 4:525-531

Vargo GA (2009) A brief summary of the physiology and ecology of *Karenia brevis* Davis (G. Hansen and Moestrup comb. nov.) red tides on the West Florida Shelf and of hypotheses posed for their initiation, growth, maintenance, and termination. Harmful Algae 8:573-584

Vargo GA, Heila CA, Fanning KA, Dixon LK, Neely MB, Lester K, Ault D, Murasko S, Havens J, Walsh J, Bell S (2008) Nutrient availability in support of *Karenia brevis* blooms on the central West Florida Shelf: What keeps Karenia blooming? Continental Shelf Research 28:73-98

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2009) Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54:331-344

Wang Y, Tang XX (2008) Interactions between *Prorocentrum donghaiense* Lu and *Scrippsiella trochoidea* (Stein) Loeblich III under laboratory culture. Harmful Algae 7:65-75

Wiegner TN, Seitzinger SP, Glibert PM, Bronk DA (2006) Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquatic Microbial Ecology 43:277-287

Zhou W, Yin K, Zhu D (2006) Phytoplankton biomass and high frequency of *Prorocentrum donghaiense* harmful algal bloom in Zhoushan sea area in spring. Chinese Journal of Applied Ecology (In Chinese) 17:887-893

Table 5-1 Monoculture exponential growth rates (μ day^{-1}) of *Prorocentrum minimum* and *Karlodinium veneficum*. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum minimum*			*Karlodinium veneficum*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.485	0.0012	0.949	0.321	<0.0001	0.986
NO_3^-	high	145	0.443	0.0003	0.969	0.312	<0.0001	0.990
Urea	high	12	0.377	<0.0001	0.829	0.249	<0.0001	0.940
Urea	high	145	0.405	<0.0001	0.898	0.237	<0.0001	0.960
NO_3^-	low	12	0.277	<0.0001	0.999	0.187	<0.0001	0.984
NO_3^-	low	145	0.232	<0.0001	0.993	0.185	<0.0001	0.982
Urea	low	12	0.257	<0.0001	0.989	0.175	<0.0001	0.918
Urea	low	145	0.235	<0.0001	0.958	0.150	<0.0001	0.844

Table 5-2 Exponential growth rates (μ day^{-1}) of *Prorocentrum minimum* and *Karlodinium veneficum* in mixed culture experiments. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum minimum*			*Karlodinium veneficum*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.173	0.0189	0.675	0.06	0.0399	0.507
NO_3^-	high	145	0.328	0.0029	0.862	0.145	0.0369	0.568
Urea	high	12	0.182	0.0287	0.593	0.135	0.0391	0.492
Urea	high	145	0.245	0.0056	0.729	0.283	0.0033	0.796
NO_3^-	low	12	0.187	0.0006	0.897	0.079	0.0122	0.646
NO_3^-	low	145	0.225	<0.0001	0.939	0.102	0.0057	0.701
Urea	low	12	0.276	0.0022	0.861	0.124	0.002	0.799
Urea	low	145	0.276	<0.0001	0.935	0.104	0.0024	0.728

Table 5-3 Exponential growth rates (μ day^{-1}) of *Prorocentrum minimum* and *Karlodinium veneficum* with *Rhodomonas* in mixed culture experiments. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum minimum*			*Karlodinium veneficum*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.048	0.3521	0.204	0.086	0.0041	0.843
NO_3^-	high	145	0.038	0.1054	0.434	0.09	0.1181	0.461
NO_3^-	low	12	~ 0			0.081	0.0712	0.532
NO_3^-	low	145	~ 0			~0		

Table 5-4 Monoculture exponential growth rates (μ day^{-1}) of *Prorocentrum donghaiense* and *Karlodinium brevis*. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum donghaiense*			*Karlodinium brevis*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.374	<0.0001	0.999	0.267	<0.0001	0.948
NO_3^-	high	145	0.206	0.0002	0.911	0.219	<0.0001	0.942
Urea	high	12	0.292	0.0001	0.939	0.278	<0.0001	0.971
Urea	high	145	0.18	0.0016	0.913	0.278	<0.0001	0.989
NO_3^-	low	12	0.158	<0.0001	0.992	0.136	<0.0001	0.979
NO_3^-	low	145	0.216	<0.0001	0.988	0.124	<0.0001	0.971
Urea	low	12	0.269	<0.0001	0.996	0.116	<0.0001	0.968
Urea	low	145	0.282	<0.0001	0.986	0.078	<0.0001	0.943

Table 5-5 Exponential growth rates (μ day^{-1}) of *Prorocentrum donghaiense* and *Karlodinium brevis* in mixed culture experiments. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum donghaiense*			*Karlodinium brevis*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.380	0.0004	0.871	0.173	<0.0001	0.974
NO_3^-	high	145	0.301	0.002	0.79	0.249	<0.0001	0.987
Urea	high	12	0.334	0.0014	0.818	0.235	<0.0001	0.97
Urea	high	145	0.344	0.0069	0.709	0.224	<0.0001	0.987
NO_3^-	low	12	0.127	0.0164	0.566	~0		
NO_3^-	low	145	0.153	0.0001	0.88	0.024	0.0131	0.552
Urea	low	12	0.116	<0.0001	0.92	~0		
Urea	low	145	0.233	0.166	0.926	0.026	0.1391	0.266

Table 5-6. Exponential growth rates (μ day^{-1}) of *Prorocentrum donghaiense* and *Karlodinium brevis* with *Synechococcus* in mixed culture experiments. R^2 is the coefficient of determination of growth rate from the regression of daily cell counts.

N source	Light	N:P ratio	*Prorocentrum donghaiense*			*Karlodinium brevis*		
			μ day^{-1}	*p*	R^2	μ day^{-1}	*p*	R^2
NO_3^-	high	12	0.385	<0.0001	0.951	0.172	0.0563	0.587
NO_3^-	high	145	0.355	0.0061	0.866	0.16	0.0086	0.802
NO_3^-	low	12	0.137	0.0003	0.894	0.019	0.2738	0.166
NO_3^-	low	145	0.141	<0.0001	0.922	0.055	0.0011	0.762

Table 5-7 Summary of the growth rates of 4 dinoflagellates reported in laboratory experiments.

μ day^{-1}	μ_{max}	Growth Condition	Clone ID and Origin	Reference
Prorocentrum minimum				
0.26-0.46		F/2, variable light, 15 °C	Chesapeake Bay isolate	(Harding et al. 1983)
0.24-0.62		Enriched seawater, 150 μE m^{-2} s^{-1}	N.D.	(Granéli et al. 1985)
0.12-0.36		F/2, 258 μE m^{-2} s^{-1}, 15 °C	N.D.	(Coats & Harding 1988)
0.25-0.74		F/2, 187 μE m^{-2} s^{-1}, 20 °C	Clone 1PM	(Antia et al. 1991)
0.38		F/2, 19 W m^{-2}, 19 °C	LAC6KA83 (Kattegat clone)	(Nielsen & Bjorn 1995)
0.37		F/2, 19 W m^{-2}, 19 °C	LAC4LI (Atlantic clone)	(Nielsen & Bjorn 1995)
2.84	3.54(± 0.21)	F/2-Si, in situ light and temp	Clone EX	(Smayda 1996)
1.13		33 psu, 475 W m^{-2}, 26.5 °C	Mediterranean Sea isolate	(Grzebyk & Berland 1996)
0.24-1.03		F/2-Si, in situ light and temp	Clone S1-25-6, Rhode Island	(Heil 1996)
0.24-0.48		F/2-Si, 100 μE m^{-2} s^{-1}, 15 °C	Clone S1-25-6, Rhode Island	(Heil 2005)
0.23-0.43		F/2, 80 μE m^{-2} s^{-1}	Gulf of Trieste, Italy	(Micheli et al. 1996)
0.26-0.81		L1, 175 μmol quanta m^{-2} s^{-1}, 18 °C	Clone LAC5 ME66	(Pan & Cembella 1998)
0.25-0.98		F/2, 180 μE m^{-2} s^{-1}, 4-20 °C	Chesapeake Bay isolate	(Lomas & Glibert 1999a, b)
0.47-0.98		ASW, F/2, 100 μM photons m^{-2} s^{-1}	Chesapeake Bay isolate	(Fan et al. 2003a, Fan et al. 2003b)
0.23-0.76		Modified F/2-Si, 430 μmol photons m^{-2} s^{-1} , 22 °C, in turbidistat	Chesapeake Bay isolate	Li, unpublished data
0.23-0.46		Modified F/2-Si, variable light, nitrogen source 22 °C	Chesapeake Bay isolate	This study
Prorocentrum donghaiense				
0.1-0.78		F/2, variable temperature, salinity and irradiance	East China Sea isolate	(Xu et al. 2010)
0.60-1.40		F/2, 20°C	East China Sea Isolate	(Wang & Tang 2008)
	0.21-0.71	Modified F/2-Si, 430 μmol photons m^{-2} s^{-1} , 22 °C, in turbidistat	East China Sea Isolate	Li, unpublished data
0.12-0.39		Modified F/2-Si, variable light, 22 °C,	East China Sea Isolate	This study
Karlodinium veneficum				
0.14		L1, 21°C, 110 μmol photons m^{-2} s^{-1}		(Fuentes-Grunewald et al. 2009)
0.52-0.75		Modified F/2-Si, 250 μmol photons m^{-2} s^{-1}, 15 °C	CCMP 1974	(Adolf et al. 2006)
0.15-0.52	0.52	F/2-Si, variable light	CCMP 1974	(Adolf et al. 2003)
0-0.66	0.23-0.66	F/2, 15%, variable light, 20 °C	Chesapeake Bay isolate	(Li et al. 1999)
0.19-0.32		Modified F/2-Si, variable light, nitrogen sources 22 °C	Chesapeake Bay isolate	This study
Karenia brevis				
0-0.36	0.36	L1-si, variable light temperature and salinity	CCMP sp3	(Magana & Villareal 2006)
0.12-0.18		L20, variable nitrogen sources, 30 μmol photons m^{-2} s^{-1}, 22 °C	CCMP 2229, C3, C6	(Sinclair et al. 2009)
0.2-0.5		Enriched seawater	Florida isolate	(Wilson 1966)
0.2-0.5			Florida isolate	(Steidinger 1983)
0.16-0.19			Florida isolate	(Doig III 1973)
0.2-1				(Shanley & Vargo 1993)

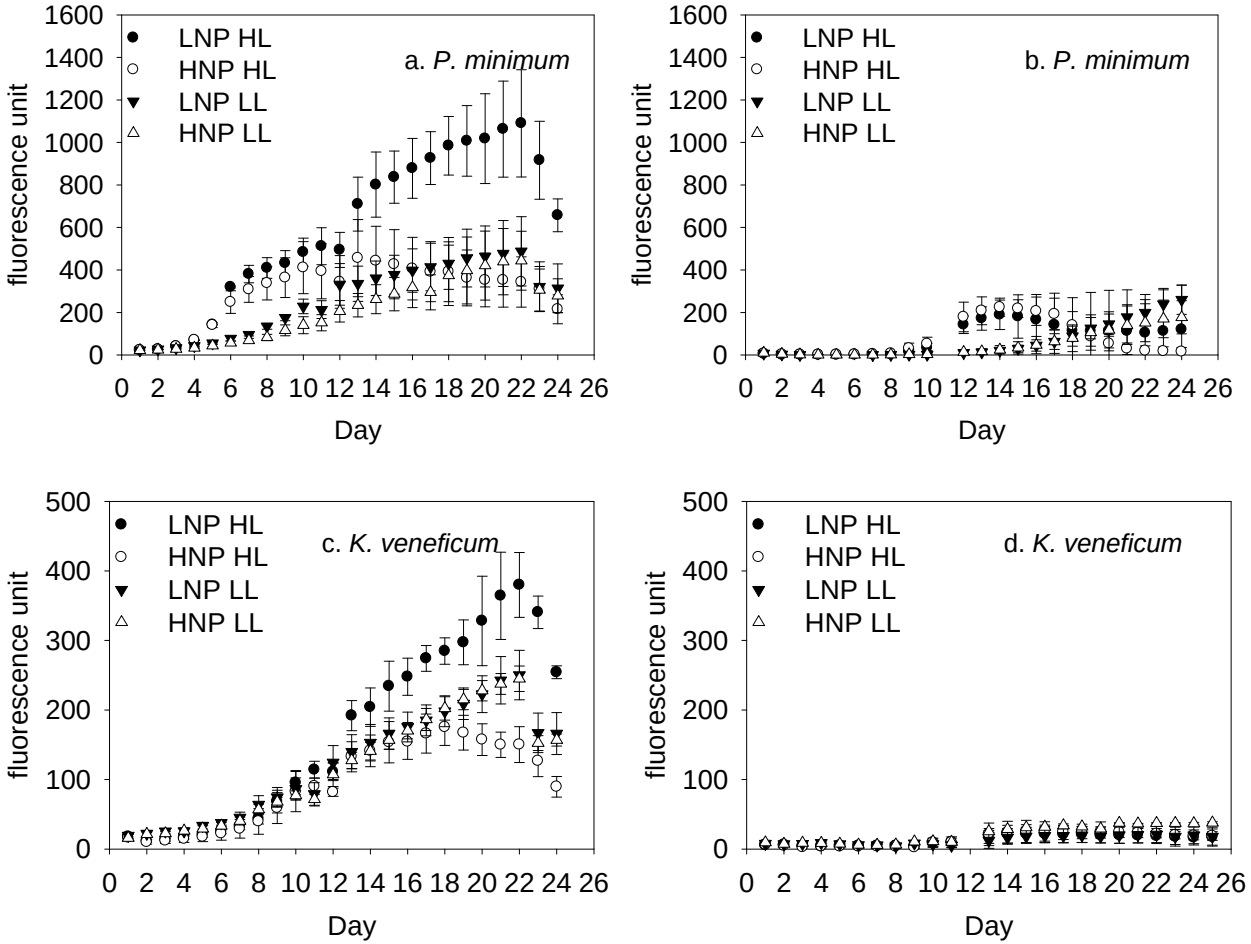
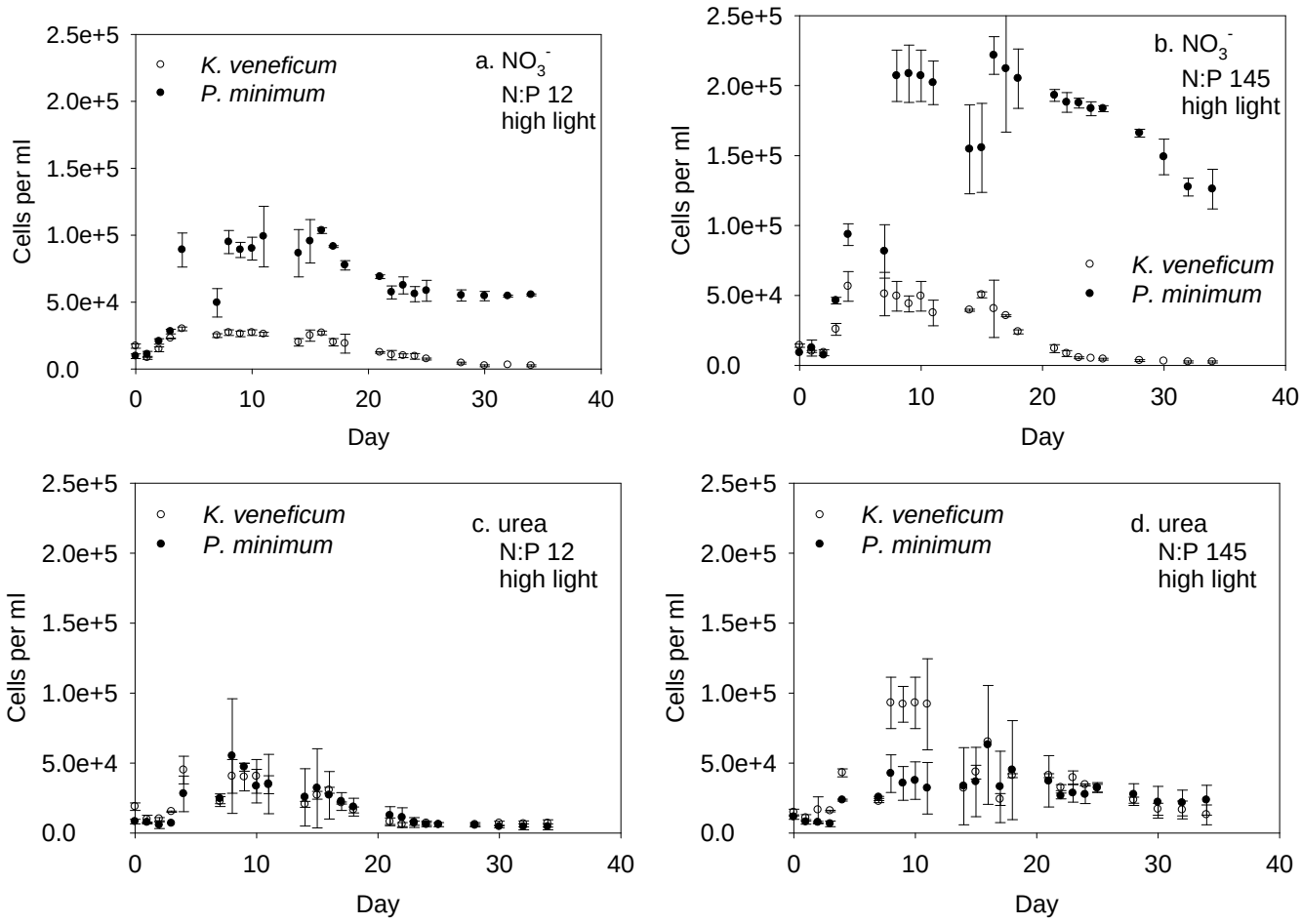


Figure 5-1 Time course of growth of *Prorocentrum minimum* (a) and *Karlodinium veneficum* (b) in monoculture with 2 nitrogen sources NO_3^- (a, c) and urea (b, d) at high and low N:P ratios (HNP, LNP) and high and low light intensities (HL, LL). The error bar is the standard deviation of triplicate treatments.


a. NO_3^-
N:P 12
high light
K. veneficum
P. minimum
Cells per ml
Day
b. NO_3^-
N:P 145
high light
c. urea
N:P 12
high light
d. urea
N:P 145
high light
2.5e+5
2.0e+5
1.5e+5
1.0e+5
5.0e+4
0.0
0
10
20
30
40

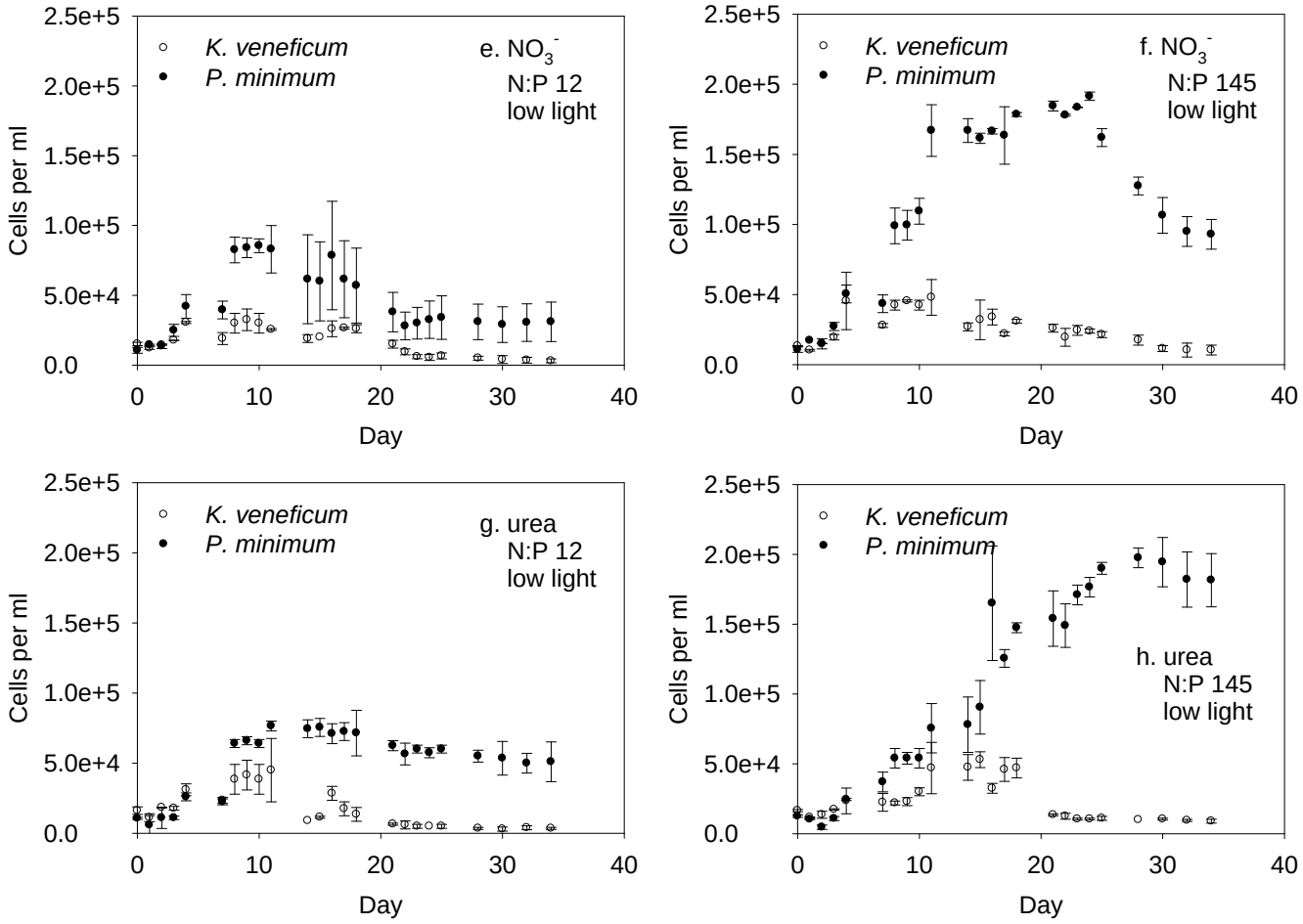


Figure 5-2 Time course of growth of *Prorocentrum minimum* and *Karlodinium veneficum* in mixed culture with 2 nitrogen sources at 2 N:P ratios and 2 light intensities. The error bar is the standard deviation of duplicate treatments.

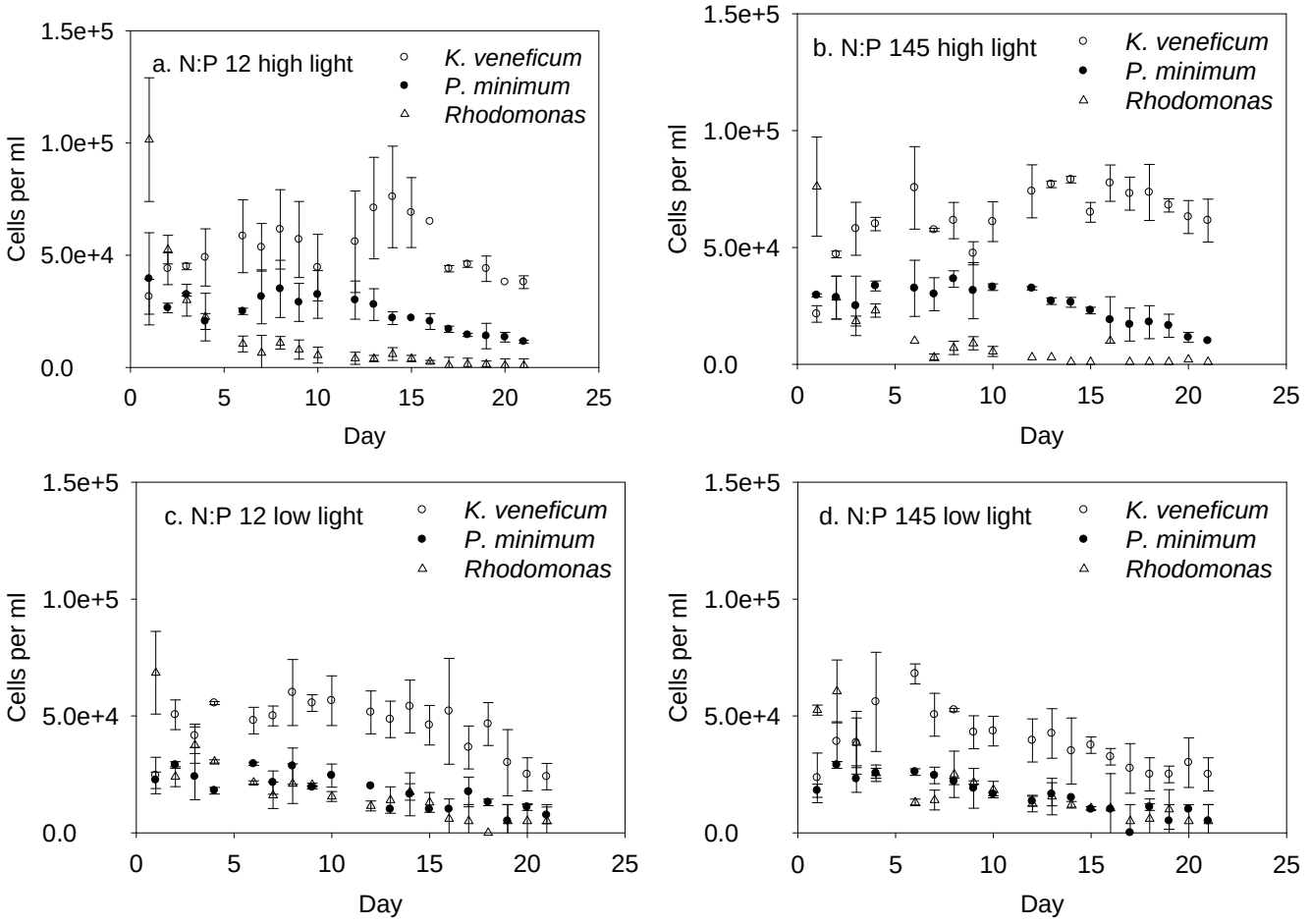


Figure 5-3 Time course of growth of *Prorocentrum minimum* and *Karlodinium veneficum* with *Rhodomonas* in mixed culture with NO_3^- at 2 N:P ratios and 2 light intensities. The error bar is the standard deviation of duplicate treatments.

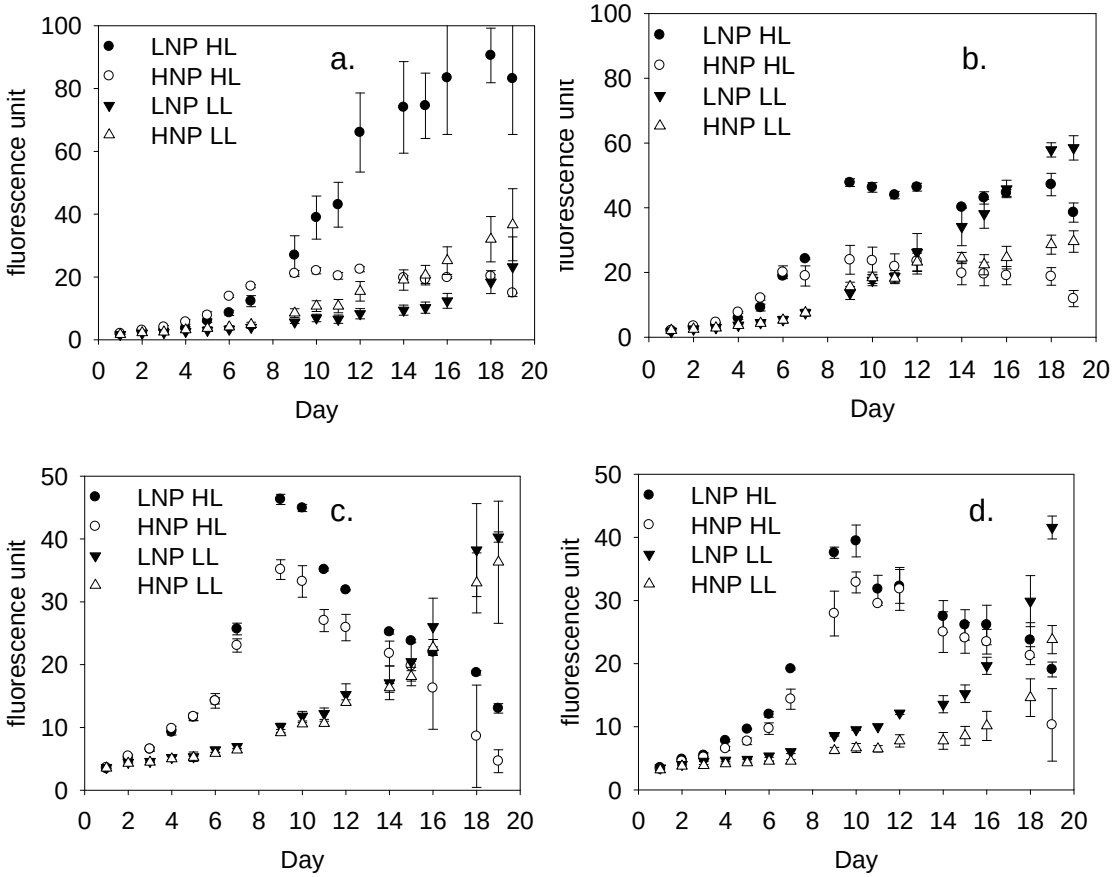
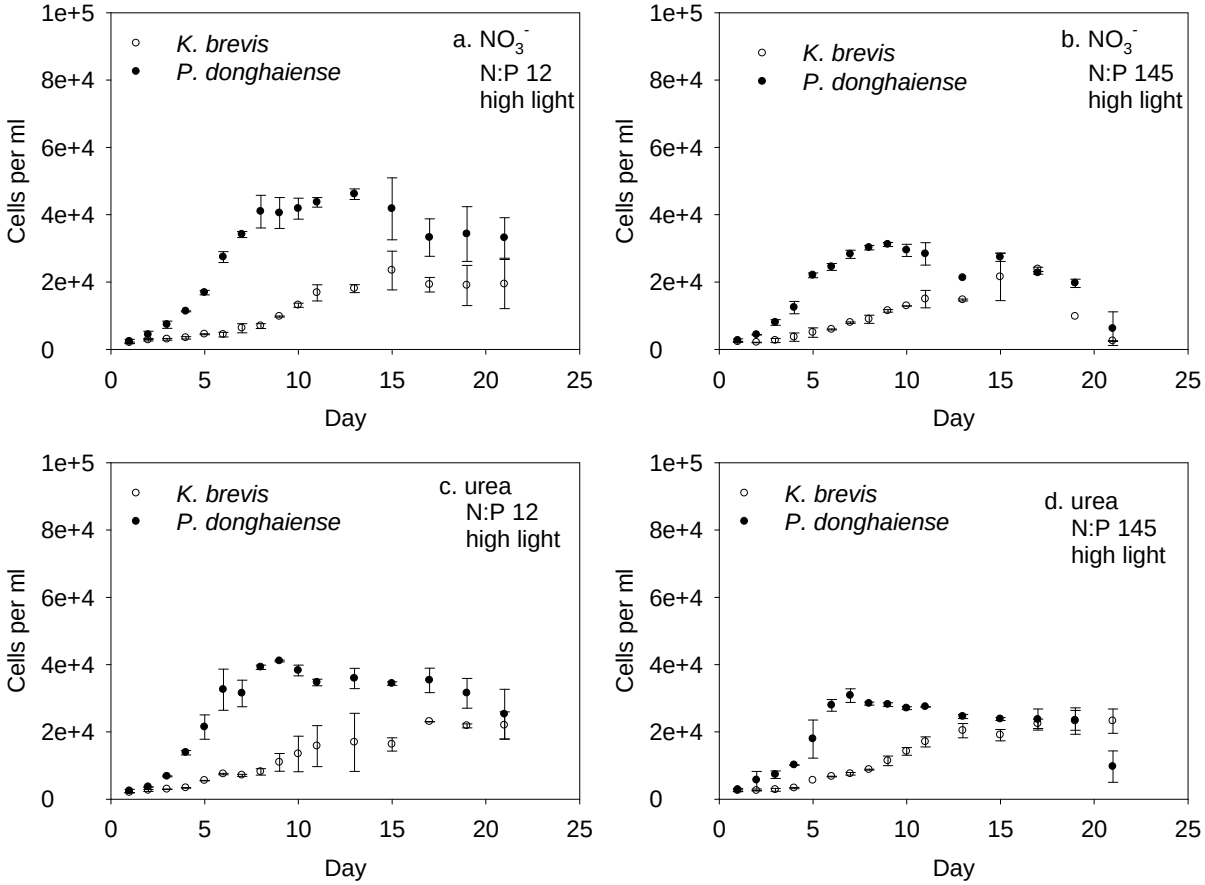


Figure 5-4 Time course of growth of *Prorocentrum donghaiense* (a., b.) and *Karenia brevis* (c., d.) in monoculture with 2 nitrogen sources NO_3^- (a., c.) and urea (b., d.) at high and low N:P ratios (HNP, LNP) and high and low light intensities (HL, LL). The error bar is the standard deviation of triplicate treatments.


a. NO_3^-
N:P 12
high light
b. NO_3^-
N:P 145
high light
c. urea
N:P 12
high light
d. urea
N:P 145
high light
K. brevis
P. donghaiense
Cells per ml
Day
1e+5
8e+4
6e+4
4e+4
2e+4
0
0
5
10
15
20
25

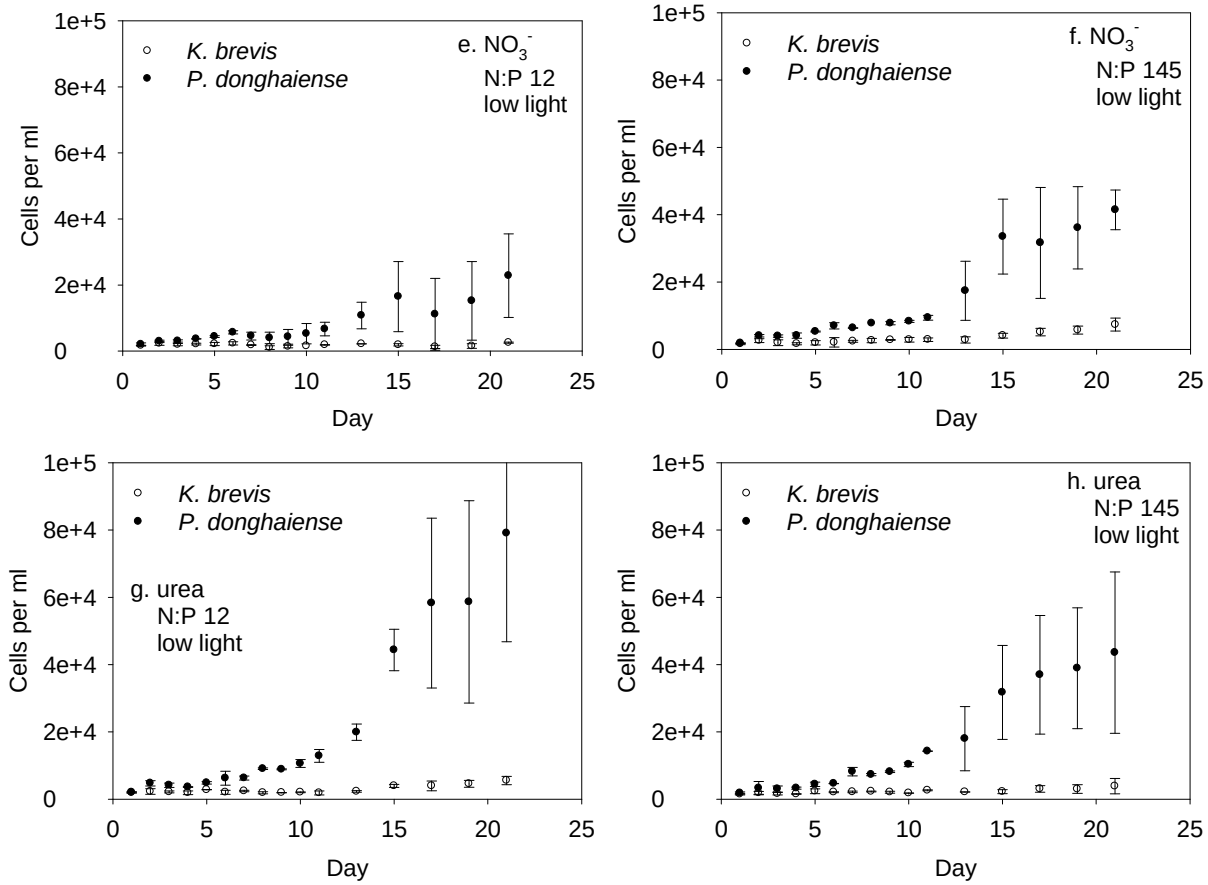


Figure 5-5 Time course of growth of *Prorocentrum donghaiense* and *Karenia brevis* in mixed culture with 2 nitrogen sources at 2 N:P ratios and 2 light intensities. The error bar is the standard deviation of duplicate treatments.

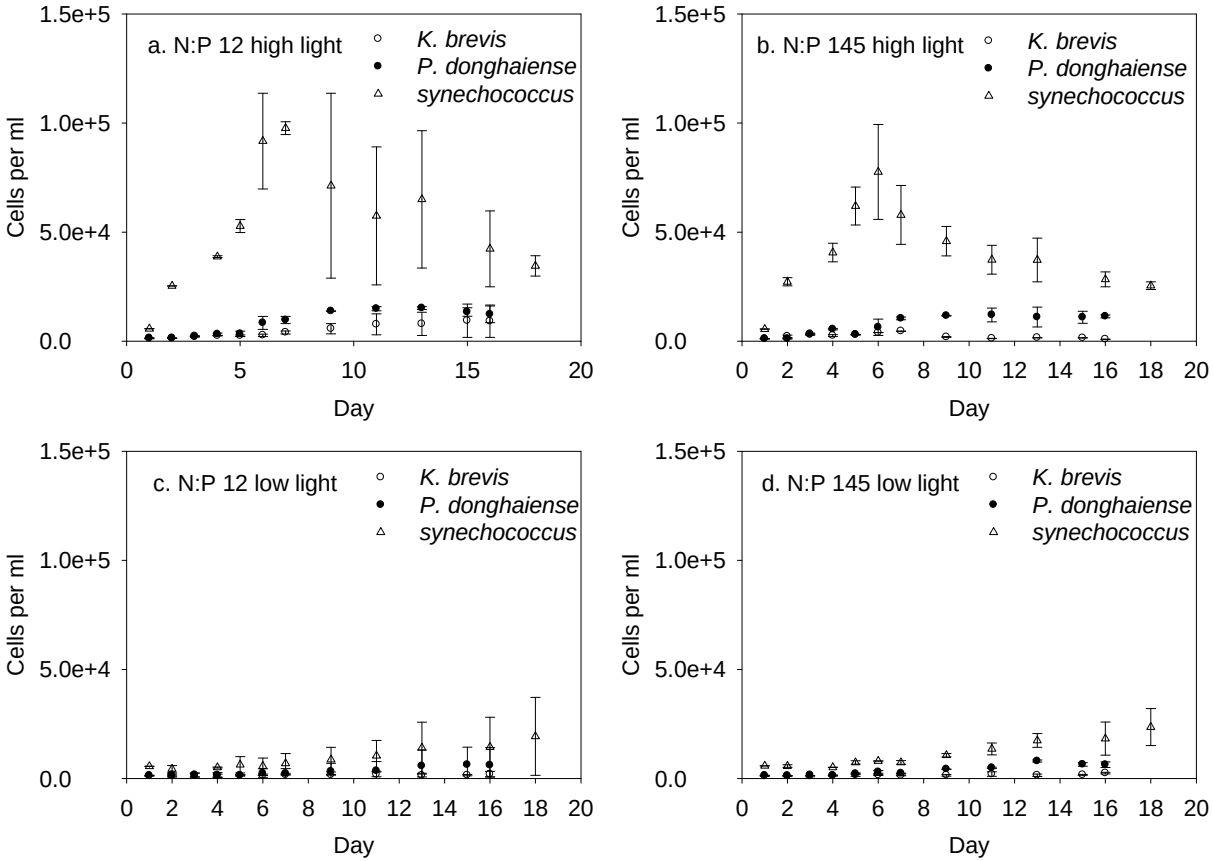
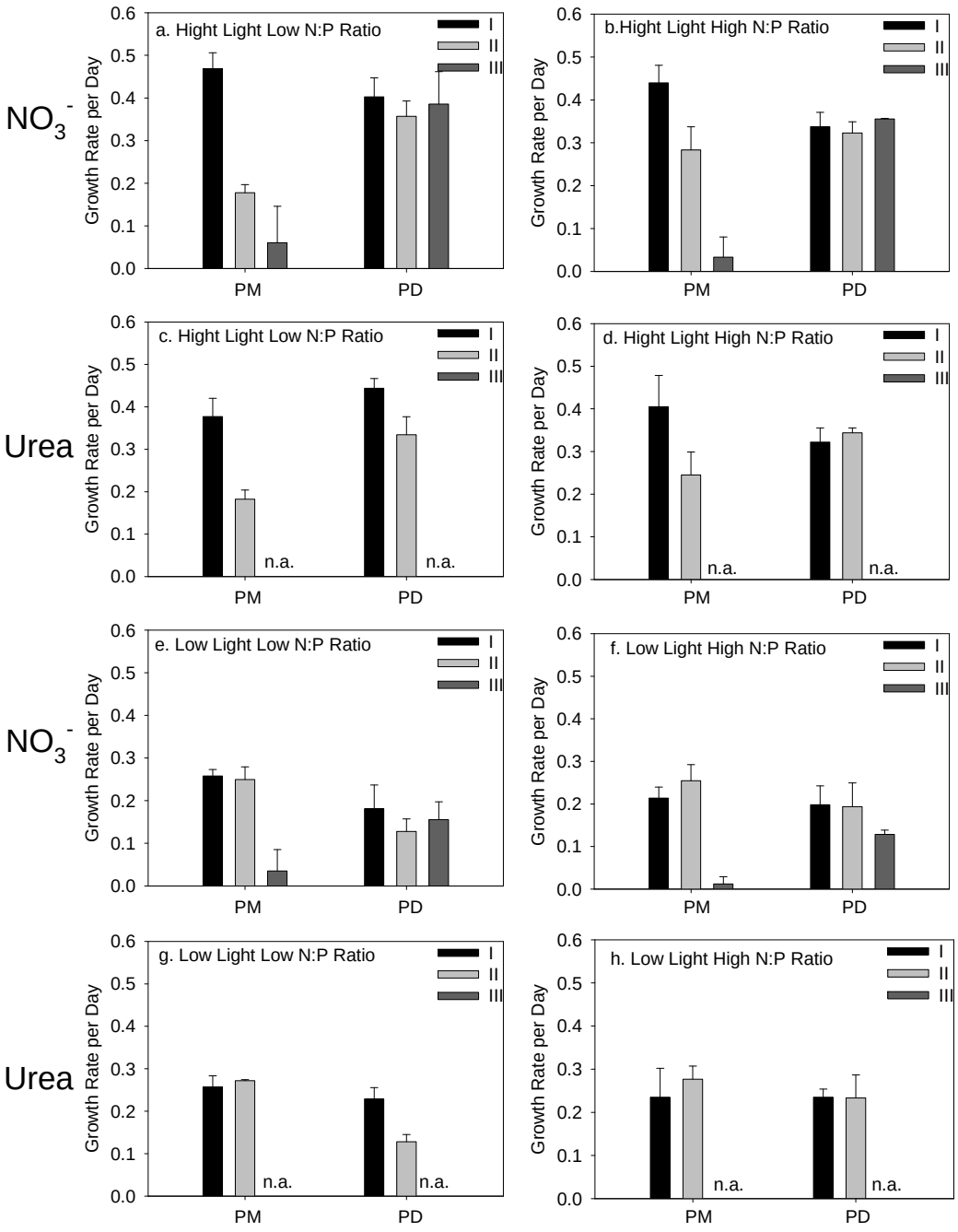


Figure 5-6 Time course of growth of *Prorocentrum donghaiense*, *Karenia brevis* and *Synechococcus* in mixed culture at 2 N:P ratios and 2 light intensities. The error bar is the standard deviation of duplicate treatments.


NO3-
Urea
NO3-
Urea
a. Hight Light Low N:P Ratio
b.Hight Light High N:P Ratio
c. Hight Light Low N:P Ratio
d. Hight Light High N:P Ratio
e. Low Light Low N:P Ratio
f. Low Light High N:P Ratio
g. Low Light Low N:P Ratio
h. Low Light High N:P Ratio
Growth Rate per Day
0.0
0.1
0.2
0.3
0.4
0.5
0.6
PM
PD
I
II
III
n.a.

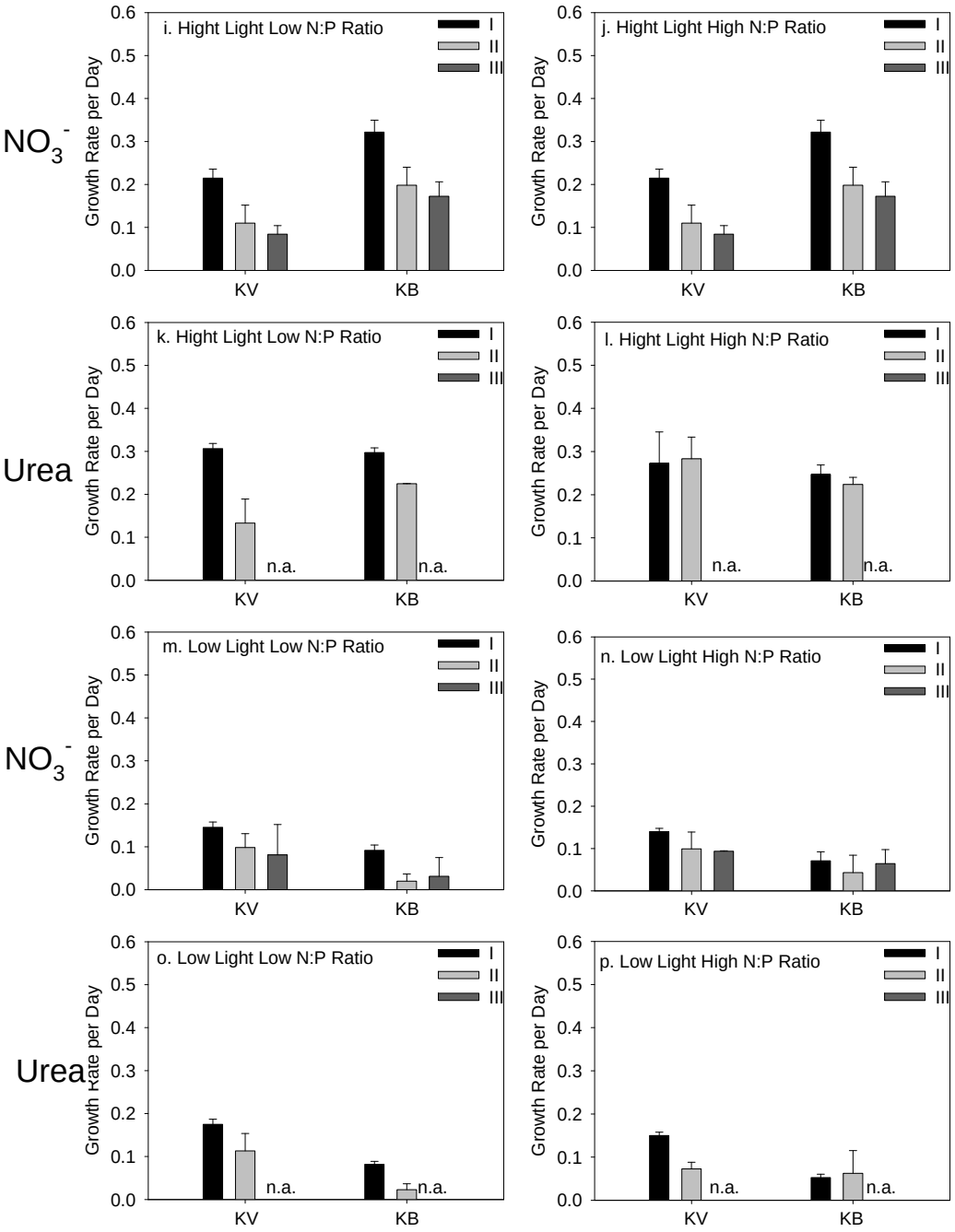


Figure 5-7 The exponential grow rates of 4 harmful dinoflagellates *Prorocentrum minimum* (PM), *P. donghaiense* (PD), *Karlodinium veneficum* (KV) and *Karenia brevis* (KB) in 3 sets of culture setting with treatments of high light (a, b, c, d, i, j, k, l) and low light (e, f, g, h, m, n, o, p); low N:P ratio (a, c, e, j, i, k, m, o) and high N:P ratio (b, d, f, h, j, l, n, p). Culture setting I: Monoculture; II: mixed culture of *P. minimum* and *K. veneficum*; mixing culture of *P. donghaiense* and *K. brevis*; III: mixed culture of *Rhodomonas*, *P. minimum* and *K. veneficum*; mixed culture of *Synechococcus*, *P. donghaiense* and *K. brevis*

Chapter 6: Research Conclusions

Dinoflagellate Blooms and the Matrix of Nutrients and Light

Smayda (Smayda and Reynolds 2001, Smayda 2002) has suggested that the increase in dinoflagellate blooms can be categorized as responses to a changing environment (e.g. eutrophication). Each dinoflagellate requires a common set of ecological characteristics (niche) that support it to develop blooms (Smayda 1990, Smayda 1997, 2002). Smayda (2002) refined this environmental adaptation of dinoflagellates by classifying them into different functional groups in a matrix, based on ecophysiological preferences organized by a near-shore / off-shore gradient in decreasing nutrients, reduced mixing, and increasing light. In this matrix, estuarine species are defined as the dinoflagellates which are better adapted to low-light high-nutrient waters, but oceanic species are better adapted to high-light low-nutrient waters.

Estuarine dinoflagellate *Prorocentrum minimum* and *P. donghaiense* have been reported to coexist with *Karenia brevis* and *K. mikimotoi* in the southwest Florida shelf and the East China Sea respectively (Heil et al. 2007, Li et al. 2009). Each pair of algae developed blooms in either spatial or temporal succession in these coastal waters, and a remarkable similarity was found between the dominant species and their ambient N:P ratios (Li et al. 2009). This dissertation study was conducted to answer the research question whether this relationship between the ambient N:P ratio and the dominance species can be expanded to other regions, where estuarine species

P. minimum and *Karlodinium veneficum* develop blooms, and to address the physiological basis for such a relationship. The research conducted for this dissertation set out to study the growth and physiological mechanisms of nitrogen acquisition with respect to nutrient and light, which are two factors used to characterize the niches of dinoflagellates by Smayda's matrix.

The following hypotheses were tested:

1) In Chesapeake Bay, *P. minimum* develops blooms in relative high DIN:DIP ratio water; while *K. veneficum* blooms near or lower than the Redfield ratio.

2) These species will grow faster in the N:P ratio in which they develop blooms, even when these nutrients are not at limiting levels.

3) *Prorocentrum* spp. preferentially take up more DIN in high DIN:DIP ratio water, while *Karlodinium* can better use other source of N in the low DIN water (e.g. DON, particulate N).

4) Low-light-adapted nitrogen acquisition by *Prorocentrum* spp. serves as an adaptive advantage to grow in low light waters.

From this dissertation study, the following results were found, corresponding to the hypotheses:

In the Chesapeake Bay, the estuarine species *P. minimum* developed blooms in high DIN, high N:P ratio, turbid water; while *K. veneficum* bloomed after DIN was depleted (Chapter 2) and ambient N:P ratio was near the Redfield ratio. These trends are consistent with East China Sea and west Florida shelf for similar species (Chapter

2). *K. veneficum* needs additional N sources to bloom, which may be the relatively high concentrations of DON in the water column.

However, in culture, the estuarine species *P. minimum* and *P. donghaiense* did not reach the highest maximum growth rates in N-rich high N:P ratio water, instead, they reached the highest maximum growth rates at more N:P balanced ratio (near the Redfield ratio) in turbidistat (Chapter 3). When ambient N:P ratio varied, but nutrients were sufficient, there was also no significant variation ($p > 0.05$) of the growth rates for both *P. minimum* and *K. veneficum* in batch culture (Chapter 5). Instead, *P. minimum* grew faster on NO_3^-, a DIN source, while, *K. veneficum* grew faster on urea, a DON source.

Light is another important factor in the Smayda's matrix. All four tested species showed light limitation in the low light treatments in batch culture (Chapter 5) and turbidistat (Chapter 4). However, light affected the growth rate differently: low-light adapted estuarine species *Prorocentrum* spp. reached about 60% of their high-light growth rate; while, oceanic species *K. brevis* only reached about 30% of its high-light growth rate. Similar results were recorded in both batch culture and turbidistat.

The diel growth of *P. minimum* and *P. donghaiense* is strictly light regulated. Positive growth was only recorded during the light phase by the turbidistat (Chapter 4). The highest growth rates were recorded at the beginning of the light phase, and the growth rates decreased during the light phase. This diel cycle of growth rate can be interpreted by Droop's model as the light-dark regulation of the N acquisition by *Prorocentrum* species. The growth rate appears to be more driven by the variation of

cell quota, than by ambient nutrient concentrations. *Prorocentrum* spp. continuously take up nitrogen at night to supplement the cell quota, when the growth stopped. The cells have the maximum cell N quota and reach highest growth rate at the beginning of light phase (Chapter 3). In contrast, oceanic species *K. brevis* and *K. mikimotoi* take up N in the light phase only, even when nutrient is saturated (Paasche et al. 1984, Sinclair et al. 2009). Therefore, the physiological mechanisms of nitrogen acquisition are regulated by light differentially between these two types of algae. In turbid estuarine water, *Prorocentrum* spp. can take up N at high rates and may be better competitors than *Karenia* and *Karlodinium* spp., which have a greater light requirement for dissolved N uptake.

When two types of algae were grown in mixed culture, the estuarine species *Prorocentrum* spp. overgrew the opponents in most of the treatments (Chapter 5). However, *Karlodinium veneficum* overgrew *P. minimum* with the presence of *Rhodomonas*, a potential nutrient source. The growth of both *K. brevis* and *P. donghaiense* were reduced with the presence of *Synechococcus*. Other mechanisms (e.g. allelopathy), in addition to nutrient competition, should be considered and may also play a role in determining the dominant species. Therefore, in the mixed culture, growth rates do not need to be at the physiological maximum for the given set of conditions for species to become dominant. There might be other competition mechanisms (e.g. allelopathy), other than nutrient competition, to determine the dominant species.

In summary, there are high DIN (mostly NO_3^-) sources accumulated in the upper Chesapeake Bay and its tributaries during the winter and spring (Chapter 2).

When the water temperature increased over 10-15 °C, estuarine species *P. minimum* can take the advantage of high DIN uptake rate in turbid water, and overgrow other dinoflagellates or diatoms (Fig. 6-1). However, DIN is depleted in the summer, but there are still relatively high organic forms of nutrients available in the water column. *Karlodinium veneficum* may be mixotrophic and feed on the organic sources, or directly feed on other micro-algae in the water column to develop blooms.

In eutrophic coastal systems (e.g. Chesapeake Bay), there are high residual nutrient sources or/and nutrient inputs. The exponential growth phase of batch culture and continuous culture are used to simulate the growth of dinoflagellates in such environment. From this study, the availability of different nutrient sources (not limited to inorganic nutrients), but not the ratio of those nutrients, are critical for the nutrient acquisition and bloom development. P-limitation may not develop in high N:P ratio eutrophic water, due to the development of light-limitation (Flynn 2010b). Light, instead of ambient N:P ratio, significantly affected the growth of all species in this study. In Chesapeake Bay, the eutrophic areas are very turbid, and the two types of bloom-forming dinoflagellates have different nutrient acquisition strategies: *Prorocentrum minimum* keeps taking N in the dark, while *K. veneficum* can be mixotrophic and may directly use organic sources. Availability of nutrient sources and light are two key environmental factors necessary to understand the bloom mechanisms in eutrophic waters.

The Application of Droop Model

Nutrient limitation of phytoplankton grow can either be described by the Monod model (Monod 1950) or by the Droop model (Droop 1968, 1973). Although

the Monod model originated as a description of steady-state bacterial growth, it has been used as the default description of nutrient acquisition in dynamic systems in many ecosystem models (Flynn 2010a). It is widely used, because it is the simplest mathematical description of nutrient limited growth, and only considers external nutrients. The Redfield ratio (Redfield 1958), originated as an empirical observation, has been used as a key index to identify the limited nutrient in the application of Monod model, in which the model simulation will follow the fate of the limiting nutrient and the consumption of other nutrients follow in proportion of the Redfield ratio (Flynn 2010a). However, the ambient N:P ratio is the sum of both abiotic and biotic processes. Not only nutrient consumption, but also nutrient availability affects the ratios. For example, in Chesapeake Bay (Chapter 2), when the water column is dominated by the abiotic processes (i.e., terrestrial input) in the winter, the ambient N:P ratio converge to the ratios of input nutrients, which is much higher than the Redfield ratio. However, when the water column is dominated by the biotic processes (i.e., algal blooms), the ambient N:P ratio converge to Redfield ratio, the stoichiometric ratio of phytoplankton cells.

In this dissertation study, the effects of ambient N:P ratio on the growth and N acquisition *Prorocentrum* species were studied. The N uptake rates of *Prorocentrum* spp. were more balanced with the growth rates at N:P ratios near Redfield ratio. However, when nutrients were sufficient, there was no significant variation of the growth rates at different N:P ratios. There was no direct evidence that growth was regulated by the ambient N:P ratio, instead, the growth is regulated by the nutrient acquisition ability of the cells.

When studying N acquisition, both the N uptake and assimilation process can be measured and linked to the growth rates by the Droop cell quota model (Droop 1968, 1973). An intracellular nutrient pool (cell quota) is regulated by nutrient taken into the cell and assimilated to support the growth. The growth rate is regulated by the size of cell quota, instead of the external nutrient pool (Droop 1968, 1973). The quota concept is considered more realistic than the Monod model, because growth can now continue at the expense of previously accumulated nutrient (Flynn 2008). It is easier to configure a relationship between growth rate and the internal nutrient concentration (quota) than between growth and the external nutrient concentration (Monod) (Goldman 1977, Flynn 2010a). Thus, growth rate is solely a function of the quota equation with no reference to external nutrient concentrations in the application of Droop model. In this dissertation study, the physiological mechanisms of cell quota regulated by nutrient uptake are supported by the turbidistat study, and can be described using individual cell quota based model. *Prorocentrum* spp. can take up N at night, and supplement the cell quota, which is consistent with the observation of maximum growth rate of *Prorocentrum* spp. at the beginning of the light phase in a diel cycle. Therefore, the Droop model may better describe the physiological activities and growth of phytoplankton cells, and can be further applied to ecosystem modeling work. The Monod model can not biologically capture this fundamental dynamic. The growth and physiology of phytoplankton should be directly linked to the cell quota, which is regulated by their ability to acquire nutrients (Flynn 2010b), instead of the ambient nutrient concentrations or ratios.

The Improvement of Droop Model

The cell quota model initially described in term of cell (e.g. N per cell), but cell size varies not only with cell cycle, but also with various environmental and physiological factors, including nutrient stress (Lehman 1976, Gotham and Frisch 1981). Therefore, the changes of cell quota may not directly respond to the nutrient content on a cell, but due to the change of cell size. To overcome these issues, the quota relationship has been strongly suggested to be normalized to biomass and described in terms of carbon (Flynn 2008). However, in this dissertation study, both the uptake and assimilation rates were measured as specific rates (h^{-1}). The cell N quota can be described as a dynamic system of N acquisition and consumption, and the growth rate can be mathematically described as variation rate of cell quota.

Compared to the Monod model, the application of Droop model needs more parameters. In the future research, experiments should be designed to get complete data sets for the Droop model. For example, in a growth experiment, not only the nutrients and cell number variation during the growth phase should be measured, the variation of particulate C, N, and P in the culture also should be measured, if possible. Also, when using C or N isotope tracer techniques to study the C-fixation or N-uptake, the variation of C and N in the cell should be linked with the growth rate during the incubation. The Droop model will be not only more appropriate to be applied to simulate the phytoplankton growth and nutrient dynamics in steady state culture, but also may be applied in ecosystem modeling.

References

Droop MR (1968) Vitamin B12 and marine ecology 4. kinetics of uptake growth and inhibition in *Monochrysis lutheri*. Journal of the Marine Biological Association of the United Kingdom 48:689-733

Droop MR (1973) Some thoughts on nutrient limitation in algae. Journal of Phycology 9:264-272

Flynn KJ (2008) Use, abuse, misconceptions and insights from quota models - The Droop cell quota model 40 years on. Oceanography and Marine Biology: An Annual Review, Vol 46 46:1-23

Flynn KJ (2010a) Ecological modelling in a sea of variable stoichiometry: Dysfunctionality and the legacy of Redfield and Monod. Progress in Oceanography 84:52-65

Flynn KJ (2010b) Do external resource ratios matter? - Implications for modelling eutrophication events and controlling harmful algal blooms. Journal of Marine Systems 83; 170-180

Goldman JC (1977) Steady-state growth of phytoplankton in continuous culture - comparison of internal and external nutrient equations. Journal of Phycology 13:251-258

Gotham IJ, Frisch HL (1981) A simple-model for cell-volume and developmental compartments in nutrient limited cyclostat cultures of algae. Journal of Theoretical Biology 92:435-467

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Lehman JT (1976) Photosynthetic capacity and luxury uptake of carbon during phosphate limitation in *Pediastrum duplex* (Chlorophyceae). Journal of Phycology 12:190-193

Li J, Glibert PM, Zhou M, Lu S, Lu D (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology Progress Series 383:11-26

Monod J (1950) La technique de culture continue theorie et applications. Annales De L Institut Pasteur 79:390-410

Paasche E, Bryceson I, Tangen K (1984) Interspecific variation in dark nitrogen uptake by dinoflagellates. Journal of Phycology 20:394-401

Redfield, A.C., The biological control of chemical factors in the environment, American Scientist, 1958

Sinclair G, Kamykowski D, Glibert PM (2009) Growth, uptake, and assimilation of ammonium, nitrate, and urea, by three strains of *Karenia brevis* grown under low light. Harmful Algae 8:770-780

Smayda T (1990) Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, New York

Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42:1137-1153

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

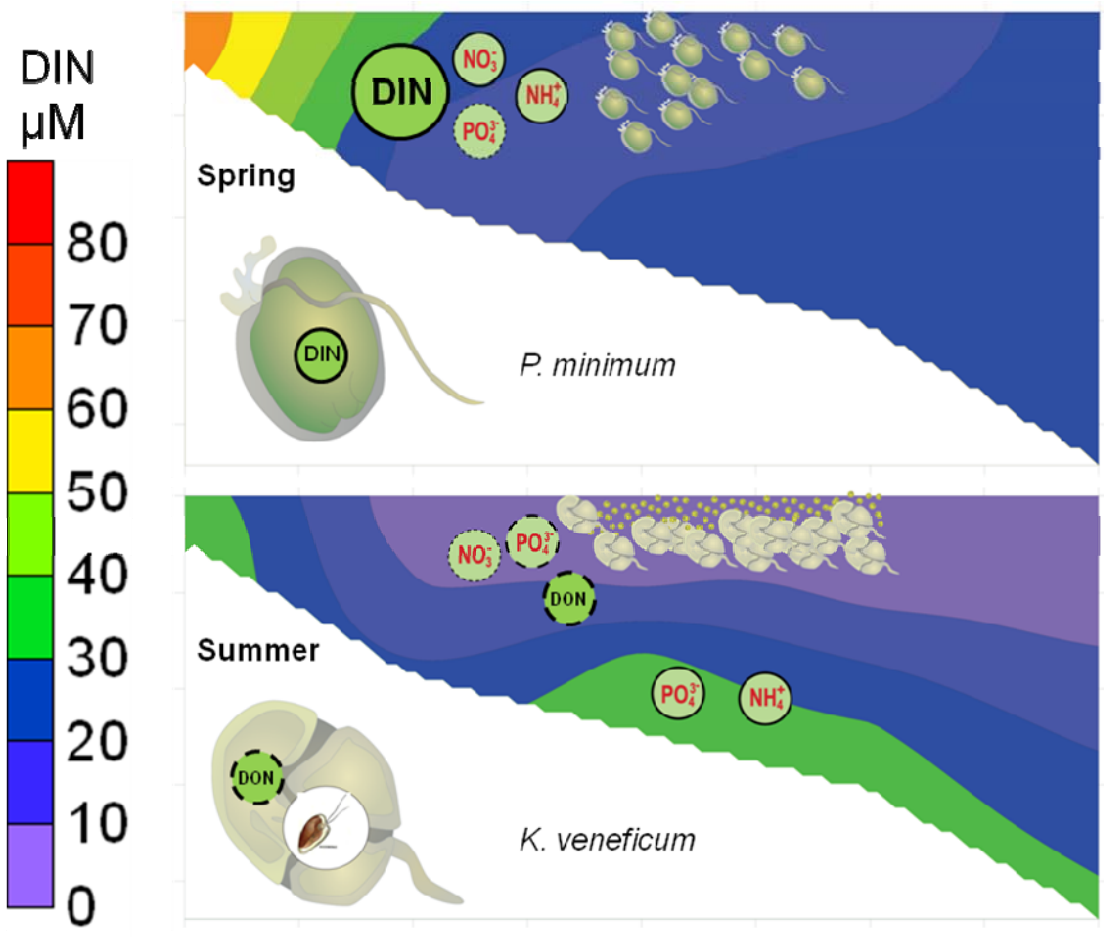


Figure 6-1 The conceptual model of the bloom development in the Chesapeake Bay: *Prorocentrum* minimum developed blooms on DIN in the spring; *Karlodinium veneficum* developed blooms on DON or directly feeding on other microalgae as food sources.

Complete References

Aalderink RH, Jovin R (1997) Estimation of the photosynthesis/irradiance (P/I) curve parameters from light and dark bottle experiments. Journal of Plankton Research 19:1713-1742

Adolf J, Bachvaroff T, Place AR (2007) Cryptophytes drive blooms of mixotrophic harmful algae: A testable hypothesis based on *Karlodinium veneficum* in Chesapeake Bay. Journal of Phycology 43:31-31

Adolf JE, Bachvaroff T, Place AR (2008) Can cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? Harmful Algae 8:119-128

Adolf JE, Bachvaroff TR, Krupatkina DN, Nonogaki H, Brown PJP, Lewitus AJ, Harvey HR, Place AR (2006a) Species specificity and potential roles of *Karlodinium micrum* toxin. African Journal of Marine Science 28:415-419

Adolf JE, Stoecker DK, Harding LW (2003) Autotrophic growth and photoacclimation in *Karlodinium micrum* (Dinophyceae) and *Storeatula major* (Cryptophyceae). Journal of Phycology 39:1101-1108

Adolf JE, Stoecker DK, Harding LW (2006b) The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). Journal of Plankton Research 28:737-751

Adolf JE, Yeager CL, Miller WD, Mallonee ME, Harding LW (2006c) Environmental forcing of phytoplankton floral composition, biomass, and primary productivity in Chesapeake Bay, USA. Estuarine Coastal and Shelf Science 67:108-122

Andersen JH, Schluter L, Ærtebjerg G (2006) Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. Journal of Plankton Research 28:621-628

Anderson DM (1997) Turning back the harmful red tide - Commentary. Nature 388:513-514

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39-53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726

Anderson SM, Roels OA (1981) Effects of light-intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Marine Biology 62:257-261

Baly E (1935) The kinetics of photosynthesis. Proceedings of the Royal Society B 117:218-239

Behrenfeld MJ, Prasil O, Babin M, Bruyant F (2004) In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. Journal of Phycology 40:4-25

Bennett WN, Boraas ME (1989) Comparison of population-dynamics between slow-growing and fast-growing strains of the rotifer *Brachionus calyciflorus* pallas in continuous Culture. Oecologia 81:494-500

Berthouex PM, Pallesen L, Booman K, Sedlack R (1983) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 55:323-325

Boynton WR, Garber JH, Summers R, Kemp WM (1995) Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries 18:285-314

Boynton WR, Kemp WM (1985) Nutrient regeneration and oxygen-consumption by sediments along an estuarine salinity gradient. Marine Ecology-Progress Series 23:45-55

Bricker, S.B., C.G. Clement, D.E. Pirhalla, S.P. Orlando, and D.R.G. Farrow. 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. Silver Spring, MD: National Oceanic and Atmospheric Administration (NOAA), National Ocean Service. 71

Bronk DA, Sanderson MP, Mulholland MR (2004) Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA (eds) Harmful Algae 2002, Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, St. Petersberg, Florida, USA, p 80-82

Bulgakov NG, Levich AP (1999) The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv Fur Hydrobiologie 146:3-22

Burkholder JM, Glasgow HB (1997) *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnology and Oceanography 42:1052-1075

Burkholder JM, Glibert PM, Skelton HM (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8:77-93

Burns CL, Pennock JR, Lores EM, R.M. G (2000) The effect of nitrogen source on the growth and toxicity of three potentially harmful dinoflagellates. Journal of Phycology 36

Burns JA, Zehr JP, Capone DG (2002) Nitrogen-fixing phylotypes of Chesapeake Bay and Neuse River estuary sediments. Microbial Ecology 44:336-343

Caron DA, Sanders RW, Lim EL, Marrase C, Amaral LA, Whitney S, Aoki RB, Porter KG (1993) Light dependent phagotrophy in the fresh water mixotrophic chrysophyte *Dinobryon cylindricum*. Microbial Ecology 25:93-111

Carter V, Rybicki NB, Landwehr JM, Turtora M (1994) Role of weather and water quality in population dynamics of submersed macrophytes in the tidal Potomac River. Estuaries 17:417-426

Chai C, Yu ZM, Song XX, Cao XH (2006) The status and characteristics of eutrophication in the Yangtze River (Changjiang) estuary and the adjacent East China Sea, China. Hydrobiologia 563:313-328

Chisholm SW, Nobbs PA, Stross RG (1975) Simulation of algal growth and competition in a phosphate-limited cyclostat. Abstracts of Papers of the American Chemical Society:42-42

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology-Progress Series 210:223-253

Cloern JE, Jassby AD (2010) Patterns and scales of phytoplankton variability in estuarine-voastal ecosystems. Estuaries and Coasts 33:230-241

Cochlan WP, Harrison PJ, Denman KL (1991) Diel periodicity of nitrogen uptake by marine-phytoplankton in nitrate-rich environments. Limnology and Oceanography 36:1689-1700

Conway HL (1977) Interactions of inorganic nitrogen in uptake and assimilation by marine phytoplankton. Marine Biology 39:221-232

Cornwell JC, Conley DJ, Owens M, Stevenson JC (1996) A sediment chronology of the eutrophication of Chesapeake Bay. Estuaries 19:488-499

Cullen J, Zhu M, Davis R, Pierson D (1985) Vertical migration, carbohydrate synthesis, and nocturnal nitrate uptake during growth of *Heterocapsa niei* in a laboratory water column. In: Anderson D, White A, Baden D (eds) Toxic dinoflagellates. Elsevier, Amsterdam, p 189-194

Deeds J (2009) The evolving story of *Gymnodinium galatheanum* = *Karlodinium micrum* = *Karlodinium veneficum*. A ten year perspective. Journal of Phycology 45:1-2

Deeds JR, Reimschuessel R, Place AR (2006) Histopathological effects in fish exposed to the toxins from *Karlodinium micrum*. Journal of Aquatic Animal Health 18:136-148

Deeds JR, Terlizzi DE, Adolf JE, Stoecker DK, Place AR (2002) Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae) – a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae 1:169-189

Degens ET, Mopper K (1976) Factors controlling distribution and early diagenesis of organic marine sediments. In: Riley JP, Chester R (eds) Chemical oceanography, Vol 6. Academic London, New York, San Francisco, p 60-114

Doemel WN, Brooks AE (1975) Detergent phosphorus and algal growth. Water Research 9:713-719

Doering PH, Oviatt CA, Nowicki BL, Klos EG, Reed LW (1995) Phosphorus and nitrogen limitation of primary production in a simulated estuarine gradient. Marine Ecology-Progress Series 124:271-287

Droop MR (1968) Vitamin B12 and marine ecology 4. kinetics of uptake growth and inhibition in *Monochrysis lutheri*. Journal of the Marine Biological Association of the United Kingdom 48:689-733

Droop MR (1973) Some thoughts on nutrient limitation in algae. Journal of Phycology 9:264-272

Duan SW, Liang T, Zhang S, Wang LJ, Zhang XM, Chen XB (2008) Seasonal changes in nitrogen and phosphorus transport in the lower Changjiang River before the construction of the Three Gorges Dam. Estuarine Coastal and Shelf Science 79:239-250

Dyhrman, S., 2005. Ectoenzymes in *Prorocentrum minimum*: alkaline phosphatase and leucine aminopeptidase. Harmful Algae 4, 619–627

Eppley RW, Holm-Hansen O, Strickland JD (1968) Some observations on vertical migration of dinoflagellates. Journal of Phycology 4:333-340

Eppley RW, Renger EH (1974) Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. Journal of Phycology 10:15-23

Eppley RW, Rogers JN, McCarthy JJ, Sournia A (1971) Light/dark periodicity in nitrogen assimilation of marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. Journal of Phycology 7:150-154

Falkowski PG, Dubinsky Z, Wyman K (1985) Growth-irradiance relationships in phytoplankton. Limnology and Oceanography 30:311-321

Fan C, Glibert PM, Alexander J, Lomas MW (2003a) Characterization of urease activity in three marine phytoplankton species, *Aureococcus anophagefferens*,

Prorocentrum minimum, and *Thalassiosira weissflogii*. Marine Biology 142:949-958

Fan CL, Glibert PM (2005) Effects of light on nitrogen and carbon uptake during a *Prorocentrum minimum* bloom. Harmful Algae 4:629-641

Fan CL, Glibert PM, Burkholder JM (2003b) Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. Harmful Algae 2:283-299

Fenaux R, Malara G, Claustre H (1985) A turbidostat driven and controlled by microcomputer. Aquaculture 48:91-95

Fisher T, Boicourt W, Boynton W, Capone D, Chao SY, Conley D, Cornwell J, Costanza R, Delia C, Glibert P, Kemp M, Malone T, Peele E, Sanford L, Ulanowicz R (1990) Responses of Chesapeake Bay to nutrient Inputs - recycling, production, and export. Abstracts of Papers of the American Chemical Society 199:35-Geoc

Fisher TR, Gustafson AB, Sellner K, Lacouture R, Haas LW, Wetzel RL, Magnien R, Everitt D, Michaels B, Karrh R (1999) Spatial and temporal variation of resource limitation in Chesapeake Bay. Marine Biology 133:763-778

Fisher TR, Hagy JD, Boynton WR, Williams MR (2006) Cultural eutrophication in the Choptank and Patuxent estuaries of Chesapeake Bay. Limnology and Oceanography 51:435-447

Fisher TR, Peele ER, Ammerman JW, Harding LW (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Marine Ecology-Progress Series 82:51-63

Flynn KJ (2002) How critical is the critical N : P ratio? Journal of Phycology 38:961-970

Flynn KJ (2008) Use, abuse, misconceptions and insights from quota models - The Droop cell quota model 40 years on. Oceanography and Marine Biology: An Annual Review, Vol 46 46:1-23

Flynn KJ (2010a) Ecological modelling in a sea of variable stoichiometry: Dysfunctionality and the legacy of Redfield and Monod. Progress in Oceanography 84:52-65

Flynn KJ (2010b) Do external resource ratios matter? - Implications for modelling eutrophication events and controlling harmful algal blooms. Journal of Marine Systems 83; 170-180

Frisch HL, Gotham IJ (1977) Periodic algal cyclostat populations. Journal of Theoretical Biology 66:665-678

Gallegos CL, Bergstrom PW (2005) Effects of a *Prorocentrum minimum* bloom on light availability for and potential impacts on submersed aquatic vegetation in upper Chesapeake Bay. Harmful Algae 4:553-574

Glibert PM, Anderson DM, Gentien P, Granéli E, Sellner KG (2005a) The global, complex phenomena of harmful algal blooms. Oceanography 18 (2):136-147

Glibert PM, Burkholder JM (2006) The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J (eds) Ecology of Harmful Algae. Springer, p 341-354

Glibert PM, Burkholder JM, Graneli E, Anderson DM (2008a) Advances and insights in the complex relationships between eutrophication and HABs: Preface to the special issue. Harmful Algae 8:1-2

Glibert PM, Burkholder JM, Kana TM, Alexander J, Skelton H, Shilling C (2009) Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquatic Microbial Ecology 55:17-30

Glibert PM, Capone DG (1993) Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles R, Blackburn TH (eds) Nitrogen isotope techniques, Vol 243-272. Academic Press

Glibert PM, Conley DJ, Fisher TR, Harding LW, Malone TC (1995) Dynamics of the 1990 winter spring bloom in Chesapeake Bay. Marine Ecology-Progress Series 122:27-43

Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay Estuary and its regulation by large heterotrophs. Limnology and Oceanography 36:895-909

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea - a global change contributing to coastal eutrophication. Biogeochemistry 77:441-463

Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Marine Ecology-Progress Series 280:73-83

Glibert PM, Magnien R, Lomas MW, Alexander J, Fan CL, Haramoto E, Trice M, Kana TM (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. Estuaries 24:875-883

Glibert PM, Magnien RE (2004) Harmful algal blooms in the Chesapeake Bay, USA: Common species, relationships to nutrient loading, management approaches, successes, and challenges. In: Hall S, Anderson D, Kleindinst J, Zhu M, Zou Y (eds) Harmful Algae Management and Mitigation. Asia-Pacific Economic Cooperation, Singapore

Glibert PM, Mayorga E, Seitzinger S (2008b) *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: Application of spatially explicit nutrient export models. Harmful Algae 8:33-38

Glibert PM, McCarthy JJ (1984) Uptake and assimilation of ammonium and nitrate by phytoplankton - Indexes of nutritional status for natural assemblages. Journal of Plankton Research 6:677-697

Glibert PM, Terlizzi DE (1999) Cooccurrence of elevated urea levels and dinoflagellate blooms in temperate estuarine aquaculture ponds. Applied and Environmental Microbiology 65:5594-5596

Glibert PM, Trice TM, Michael B, Lane L (2005b) Urea in the tributaries of the Chesapeake and Coastal Bays of Maryland. Water Air and Soil Pollution 160:229-243

Goldman JC (1977) Steady-state growth of phytoplankton in continuous culture - comparison of internal and external nutrient equations. Journal of Phycology 13:251-258

Goldman JC, Glibert PM (1982) Comparative rapid ammonium uptake by 4 species of marine-phytoplankton. Limnology and Oceanography 27:814-827

Goldman JC, Mccarthy JJ, Peavey DG (1979) Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210-215

Goolsby DA, Battaglin WA (2000) Nitrogen in the Mississippi basin-estimating sources and predicting flux to the Gulf of Mexico. USGS Fact Sheet 135-00

Goshorn D, Deeds J, Tango P, Poukish C, Place AR, McGinty M, Butler W, Luckett C, Magnien R (2004) Occurrence of *Karlodinium micrum* and its association with fish kills in Maryland estuaries. In: Steidinger KA, Landsberg JA, Tomas CR, Vargo GA (eds) Proceedings of Harmful Algae 2002. IOC-UNESCO, St. Petersberg, p 361–363

Gotham IJ, Frisch HL (1981) A simple-model for cell-volume and developmental compartments in nutrient limited cyclostat cultures of algae. Journal of Theoretical Biology 92:435-467

Gotham IJ, Rhee GY (1981) Comparative kinetic-studies of phosphate-limited growth and phosphate-uptake in phytoplankton in continuous culture. Journal of Phycology 17:257-265

Gotham IJ, Rhee GY (1982) Effects of nitrate and phosphate limitation on cyclostat growth of 2 fresh-water diatoms. Journal of General Microbiology 128:199-205

Granéli E, Carlsson P, Legrand C (1999) The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. Aquatic Ecology 33:17-27

Grzebyk D, Berland B (1996) Influences of temperature, salinity and irradiance on growth of *Prorocentrum minimum* (Dinophyceae) from the Mediterranean Sea. Journal of Plankton Research 18:1837-1849

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology 8:229

Hagy JD, Boynton WR, Keefe CW, Wood KV (2004) Hypoxia in Chesapeake Bay, 1950-2001: Long-term change in relation to nutrient loading and river flow. Estuaries 27:634-658

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99

Haraguchi K, Yamamoto T, Chiba S, Shimizu Y, Nagao M (2010) Effects of phytoplankton vertical migration on the formation of oxygen depleted water in a shallow coastal sea. Estuarine Coastal and Shelf Science 86:441-449

Harding LW, Coats DW (1988) Photosynthetic physiology of *Prorocentrum mariae-lebouriae* (Dinophyceae) during Its subpycnocline transport in Chesapeake Bay. Journal of Phycology 24:77-89

Harding LW, Mallonee ME, Perry ES (2002) Toward a predictive understanding of primary productivity in a temperate, partially stratified estuary. Estuarine Coastal and Shelf Science 55:437-463

Harding LW, Meeson BW, Tyler MA (1983) Photoadaptation and diel periodicity of photosynthesis in the dinoflagellate *Prorocentrum mariae-lebouriae*. Marine Ecology-Progress Series 13:73-85

Harding LW, Perry ES (1997) Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. Marine Ecology-Progress Series 157:39-52

Hartig JH, Horvath FJ (1982) A preliminary assessment of Michigan's phosphorus detergent ban. Journal Water Pollution Control Federation 54:193-197

Healey FP (1977) Ammonium and urea uptake by some freshwater algae. Canadian Journal of Botany-Revue Canadienne De Botanique 55:61-69

Heaney S, Eppley R (1981) Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. Journal of Plankton Research 3:331-344

Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography 38:709-724

Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in fresh-water and marine environments - a review of recent evidence on the effects of enrichment. Limnology and Oceanography 33:796-822

Heil CA, Glibert PM, Al-Sarawl MA, Faraj M, Behbehani M, Husain M (2001) First record of a fish-killing *Gymnodinium* sp bloom in Kuwait Bay, Arabian Sea: chronology and potential causes. Marine Ecology-Progress Series 214:15-23

Heil CA, Glibert PM, Fan CL (2005) *Prorocentrum minimum* (Pavillard) Schiller - a review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449-470

Heil CA, Revilla M, Glibert PM, Murasko S (2007) Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnology and Oceanography 52:1067-1078

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8:3-13

Hill SH, Abbott MR, Denman KL (1985) A computer-controlled turbidostat for the culture of planktonic algae. Canadian Journal of Fisheries and Aquatic Sciences 42:744-753

Hirsbrunner M (1981) A chemostat-apparatus for continuous culture of agae. Schweizerische Zeitschrift Fur Hydrologie-Swiss Journal of Hydrology 43:370-376

Hodgkiss IJ, Ho KC (1997) Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147

Hoffman FA, Bishop JW (1994) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia. Water Research 28:1239-1240

Holmes RW, Williams PM, Eppley RW (1967) Red water in La Jolla Bay 1964-1966. Limnology and Oceanography 12:503-512

Howard MDA, Cochlan WP, Ladizinsky N, Kudela RM (2007) Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (*Bacillariophyceae*) from field and laboratory experiments. Harmful Algae 6:206-217

Howarth RW (2008) Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae 8:14-20

Howarth RW, Boyer EW, Pabich WJ, Galloway JN (2002) Nitrogen use in the United States from 1961–2000 and potential future trends. AMBIO 32:88-96

Jaworski NA, Howarth RW, Hetling LI (1997) Atmospheric deposition of nitrogen oxides onto the landscape contributes to coastal eutrophication in the northeast United States. Environmental Science & Technology 31:1995-2004

Jeong H, Yoo Y, Kim J, Seong K, Kang N, Kim T (2010) Growth, feeding, and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Science Journal 45:65-91

Jeong HJ, Du Yoo Y, Park JY, Song JY, Kim ST, Lee SH, Kim KY, Yih WH (2005a) Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquatic Microbial Ecology 40:133-150

Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Jeng C, Seong CN, Lee KY, Yih WH (2005b) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquatic Microbial Ecology 41:131-143

Jeong HJ, Song JY, Lee CH, Kim ST (2004) Feeding by larvae of the mussel *Mytilus galloprovincialis* on red-tide dinoflagellates. Journal of Shellfish Research 23:185-195

Jones HLJ, Durjun P, Leadbeater BSC, Green JC (1995) The relationship between photoacclimation and phagotrophy with Respect to Chlorophyll *a*, carbon and nitrogen content, and cell size of *Chrysochromulina brevifilum* (*Prymnesiophyceae*). Phycologia 34:128-134

Jordan TE, Cornwell JC, Boynton WR, Anderson JT (2008) Changes in phosphorus biogeochemistry along an estuarine salinity gradient: The iron conveyer belt. Limnology and Oceanography 53:172-184

Jordan TE, Correll DL, Miklas J, Weller DE (1991) Nutrients and chlorophyll at the interface of a watershed and an estuary. Limnology and Oceanography 36:251-267

Justic D, Rabalais NN, Turner RE (1995) Stoichiometric nutrient balance and origin of coastal eutrophication. Marine Pollution Bulletin 30:41-46

Kalyuzhin VA (1998) The growth of a turbidostat yeast culture in the presence of high concentrations of various compounds in a steady-state regime and under osmotic shock. Microbiology 67:499-503

Kanda J, Ziemann DA, Conquest LD, Bienfang PK (1989) Light-dependency of nitrate uptake by phytoplankton over the spring bloom in Auke Bay, Alaska. Marine Biology 103:563-569

Kemp WM, Boynton WR, Adolf JE, Boesch DF, Boicourt WC, Brush G, Cornwell JC, Fisher TR, Glibert PM, Hagy JD, Harding LW, Houde ED, Kimmel DG, Miller WD, Newell RIE, Roman MR, Smith EM, Stevenson JC (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology-Progress Series 303:1-29

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33:1441-1450

Kudela RM, Cochlan WP, Dugdale RC (1997) Carbon and nitrogen uptake response to light by phytoplankton during an upwelling event. Journal of Plankton Research 19:609-630

Lagus A, Suomela J, Weithoff G, Heikkila K, Helminen H, Sipura J (2004) Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. Journal of Plankton Research 26:779-798

Lane L, Rhoades S, Thomas C, Van Heukelem L (2000) Analytical services laboratory standard operating procedures, University of Maryland Center for Environmental Science, Cambridge, Maryland.

Lee GF, Joneslee A (1995) Impacts of a phosphate detergent ban on concentrations of phosphorus in the James River, Virginia - comment. Water Research 29:1425-1426

Lee YS, Seiki T, Mukai T, Takimoto K, Okada M (1996) Limiting nutrients of phytoplankton community in Hiroshima Bay, Japan. Water Research 30:1490-1494

Lehman JT (1976) Photosynthetic capacity and luxury uptake of carbon during phosphate limitation in *Pediastrum duplex* (Chlorophyceae). Journal of Phycology 12:190-193

Leighfield TA, Van Dolah FM (2001) Cell cycle regulation in a dinoflagellate *Amphidinium operculatum*: identification of the diel entraining cue and a possible role for cyclic AMP. Journal of Experimental Marine Biology and Ecology 262:177-197

Li AS, Stoecker DK, Adolf JE (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. Aquatic Microbial Ecology 19:163-176

Li AS, Stoecker DK, Coats DW (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. Journal of Plankton Research 22:2105-2124

Li AS, Stoecker DK, Coats DW, Adam EJ (1996) Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquatic Microbial Ecology 10:139-147

Li J, Glibert PM, Alexander JA (in press) Effects of ambient N:P ratio on the growth and nitrogen uptake of harmful dinoflagellate *Prorocentrum minimum* and *Prorocentrum donghaiense* in turbidistat. Chinese Journal of Oceanology and Limnology

Li J, Glibert PM, Zhou MJ (2010) Temporal and spatial variability in nitrogen uptake kinetics during harmful dinoflagellate blooms in the East China Sea. Harmful Algae 9:531-539

Li J, Glibert PM, Zhou MJ, Lu SH, Lu DD (2009) Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. Marine Ecology-Progress Series 383:11-26

Lie HJ, Cho CH, Lee JH, Lee S (2003) Structure and eastward extension of the Changjiang River plume in the East China Sea. Journal of Geophysical Research-Oceans 108:-

Lindroth P, Mopper K (1979) High-performance liquid-chromatographic determination of subpicomole amounts of amino-acids by precolumn fluorescence derivatization with ortho-phthaldialdehyde. Analytical Chemistry 51:1667-1674

Litke DW (1999) Review of phosphorus control measures in the United States and their effects on water quality, U.S. Geological Survey

Lomas MW, Glibert PM (1999a) Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Marine Biology 133:541-551

Lomas MW, Glibert PM (1999b) Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnology and Oceanography 44:556-572

Lomas MW, Glibert PM (2000) Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. Journal of Phycology 36:903-913

Lomas MW, Glibert PM, Berg GM, Burford M (1996) Characterization of nitrogen uptake by natural populations of Aureococcus anophagefferens (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. Journal of Phycology 32:907-916

Lu DD, Goebel J (2001) Five red tide species in genus *Prorocentrum* including the description of *Prorocentrum donghaiense* Lu Sp. Nov. from the East China Sea. Chinese Journal of Oceanology and Limnology 4:337-344

Maki AW, Porcella DB, Wendt RH (1984) The impact of detergent phosphorus bans on receiving water-quality. Water Research 18:893-903

Malone TC, Conley DJ, Fisher TR, Glibert PM, Harding LW, Sellner KG (1996) Scales of nutrient-limited phytoplankton productivity in Chesapeake Bay. Estuaries 19:371-385

Malone TC, Crocker LH, Pike SE, Wendler BW (1988) Influences of River Flow on the Dynamics of Phytoplankton Production in a Partially Stratified Estuary. Marine Ecology-Progress Series 48:235-249

Malone TC, Garside C, Haines KC, Roels OA (1975) Nitrate Uptake and Growth of Chaetoceros Sp in Large Outdoor Continuous Cultures. Limnology and Oceanography 20:9-19

Marshall HG, Burchardt L, Lacouture R (2005) A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. Journal of Plankton Research 27:1083-1102

Marshall HG, Egerton T, Stem T, Hicks J, Kokocinski M (2004) Extended bloom concentration of the toxic dinoflagellate *Dinophysis acuminata* in Virginia estuaries during late winter and early spring, 2002 In: Steidinger KA, Landsberg JA, Tomas CR, Vargo GA (eds) Proceedings of Harmful Algae 2002. IOC-UNESCO, St. Petersberg, p 364–366

Marshall HG, Lacouture RV, Buchanan C, Johnson JM (2006) Phytoplankton assemblages associated with water quality and salinity regions in Chesapeake Bay, USA. Estuarine Coastal and Shelf Science 69:10-18

McDuff RE, Chisholm SW (1982) The calculation of in situ growth-rates of phytoplankton populations from fractions of cells undergoing mitosis - a clarification. Limnology and Oceanography 27:783-788

Megard RO, Berman T, Curtis PJ, Vaughan PW (1985) Dependence of phytoplankton assimilation quotients on light and nitrogen-source - implications for oceanic primary productivity. Journal of Plankton Research 7:691-702

Moisander PH, Morrison AE, Ward BB, Jenkins BD, Zehr JP (2007) Spatial-temporal variability in diazotroph assemblages in Chesapeake Bay using an oligonucleotide nifH microarray. Environmental Microbiology 9:1823-1835

Moncheva S, Gotsis-Skretas O, Pagou K, Krastev A (2001) Phytoplankton blooms in Black Sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: Similarities and differences. Estuarine Coastal and Shelf Science 53:281-295

Monod J (1950) La technique de culture continue theorie et applications. Annales De L Institut Pasteur 79:390-410

Mulholland MR, Bernhardt PW, Heil CA, Bronk DA, O'Neil JM (2006) Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. Limnology and Oceanography 51:1762-1776

Mulholland MR, Ohki K, Capone DG (1999) Nitrogen utilization and metabolism relative to patterns of N_2 fixation in cultures of *Trichodesmium* NIBB1067. Journal of Phycology 35:977-988

NRC (2000) Clean coastal waters: Understanding and reducing the effects of nutrient pollution. National Academies Press.

Nygaard K, Tobiesen A (1993) Bacterivory in algae - a survival strategy during nutrient limitation. Limnology and Oceanography 38:273-279

Olson RJ, Chisholm SW (1986) Effects of light and nitrogen limitation on the cell cycle of the dinoflagellate *Amphidinium carteri*. Journal of Plankton Research 8:785-793

Olsson P, Granéli E (1991) Observations on diurnal vertical migration and phased cell-division for 3 coexisting marine dinoflagellates. Journal of Plankton Research 13:1313-1324

Paasche E, Bryceson I, Tangen K (1984) Interspecific variation in dark nitrogen uptake by dinoflagellates. Journal of Phycology 20:394-401

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42:1154-1165

Paerl HW, Valdes LM, Joyner AR, Piehler MF (2004) Solving problems resulting from solutions: Evolution of a dual nutrient management strategy for the

eutrophying Neuse river estuary, North Carolina. Environmental Science & Technology 38:3068-3073

Pan Y, Cembella AD (1998) Flow cytometric determination of cell cycles and growth rates in *Prorocentrum* spp. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful Algae, Xunta de Galicia and Intergovernmental Oceanic Commission of UNESCO, p 173-176

Phillips DJH, Tanabe S (1989) Aquatic pollution in the Far-East. Marine Pollution Bulletin 20:297-303

Pinckney JL, Paerl HW, Tester P, Richardson TL (2001) The role of nutrient loading and eutrophication in estuarine ecology. Environmental Health Perspectives 109:699-706

Ptacnik R, Andersen T, Tamminen T (2010) Performance of the Redfield Ratio and a family of nutrient limitation indicators as thresholds for phytoplankton N vs. P limitation. Ecosystems 13:1201-1214

Radach G, Berg J, Hagmeier E (1990) Long-term changes of the annual cycles of meteorological, hydrographic, nutrient and phytoplankton time-series at Helgoland and at Lv Elbe 1 in the German Bight. Continental Shelf Research 10:305-328

Redfield A.C. On the proportions of organic derivations in sea water and their relation to the composition of plankton. In James Johnstone Memorial Volume. (ed. R.J. Daniel). University Press of Liverpool, pp. 177-192, 1934

Redfield, A.C. The biological control of chemical factors in the environment, American Scientist, 1958

Revilla M, Alexander J, Glibert PM (2005) Urea analysis in coastal waters: comparison of enzymatic and direct methods. Limnology and Oceanography-Methods 3:290-299

Rhee G-Y (1978) The continuous culture in phytoplankton ecology. In: Droop MR, Jannasch HW (eds) Advances in Aquatic Microbiology

Rhee GY (1973) Continuous culture study of phosphate uptake, growth-rate and polyphosphate in *Scenedesmus* sp. Journal of Phycology 9:495-506

Rhee GY, Gotham IJ (1981) The effect of environmental-factors on phytoplankton growth - light and the interactions of light with nitrate limitation. Limnology and Oceanography 26:649-659

Riegman R (1995) Nutrient-related selection mechanisms in marine phytoplankton communities and the impact of eutrophication on the planktonic food web. Water Science and Technology 32:63-75

Rines JEB, McFarland MN, Donaghay PL, Sullivan JM (2010) Thin layers and species-specific characterization of the phytoplankton community in Monterey Bay, California, USA. Continental Shelf Research 30:66-80

Rudek J, Paerl HW, Mallin MA, Bates PW (1991) Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. Marine Ecology-Progress Series 75:133-142

Salerno M, Stoecker DK (2009) Ectocellular glucosidase and peptidase activity of the mixotrophic dinoflagellate *Prorocentrum minimum* (Dinophyceae). Journal of Phycology 45:34-45

Sanders RW, Porter KG, Caron DA (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. Microbial Ecology 19:97-109

Sciandra A (1991) Coupling and uncoupling between nitrate uptake and growth rate in *Prorocentrum minimum* (Dinophyceae) under different frequencies of pulsed nitrate supply. Marine Ecology-Progress Series 72:261-269

Seitzinger SP, Kroeze C, Bouwman AF, Caraco N, Dentener F, Styles RV (2002) Global patterns of dissolved inorganic and particulate nitrogen inputs to coastal systems: Recent conditions and future projections. Estuaries 25:640-655

Sellner KG (1997) Physiology, ecology, and toxic properties of marine cyanobacteria blooms. Limnology and Oceanography 42:1089-1104

Sharfstein B, Roels OA, Harris V, Lee V (1977) Effect of detergent legislation on phosphorus in effluent and receiving waters. Journal Water Pollution Control Federation 49:2017-2021

Shen ZL, Liu Q, Zhang SM, Miao H, Zhang P (2003) A nitrogen budget of the Changjiang River catchment. Ambio 32:65-69

Shimizu Y, Watanabe N, Wrensford G (1995) Biosynthesis of brevetoxins and heterotrophic metabolism in *Gymnodinium breve*. In: Watanabe N, Wrensford G, Lassus P, Arzul G, Erard-Le Denn E, Gentien P, Marcaillou C (eds) Harmful Marine Algal Blooms. Lavoisier Publishing Inc., New York, p 351-357

Sinclair G, Kamykowski D, Glibert PM (2009) Growth, uptake, and assimilation of ammonium, nitrate, and urea, by three strains of *Karenia brevis* grown under low light. Harmful Algae 8:770-780

Skipnes O, Eide I, Jensen A (1980) Cage culture turbidostat - a device for rapid-determination of algal growth-rate. Applied and Environmental Microbiology 40:318-325

Smayda T (1990a) Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, New York

Smayda TJ (1990b) Novel and nuisance phytoplankton blooms in the Sea: Evidence for a global epidemic. In: Toxic Marine Phytoplankton, 4th International Conference. Elsevier, Amsterdam, p 29-40

Smayda TJ (1996) Dinoflagellate bloom cycles: what is the role of cellular growth rate and bacteria? In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO

Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42:1137-1153

Smayda TJ (2002) Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. Journal of Oceanography 58:281-294

Smayda TJ, Reynolds CS (2001) Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. Journal of Plankton Research 23:447-461

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems - A global problem. Environmental Science and Pollution Research 10:126-139

Smith VH (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnology and Oceanography 51:377-384

Smith VH, Joye SB, Howarth RW (2006) Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51:351-355

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179-196

Solomon CM, Glibert PM (2008) Urease activity in five phytoplankton species. Aquatic Microbial Ecology 52:149-157

Stelzer RS, Lamberti GA (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography 46:356-367

Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. European Journal of Protistology 34:281-290

Stoecker DK (1999) Mixotrophy among dinoflagellates. Journal of Eukaryotic Microbiology 46:397-401

Stoecker DK, Gustafson DE (2003) Cell-surface proteolytic activity of photosynthetic dinoflagellates. Aquatic Microbial Ecology 30:175-183

Stoecker DK, Li AS, Coats DW, Gustafson DE, Nannen MK (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Marine Ecology-Progress Series 152:1-12

Stoecker DK, Tillmann U, Granéli E (2006) Phagotrophy in harmful algae. In: Granéli E, Turner JT (eds) Ecology of Harmful Algae. Springer-Verlag, Berlin, p 177-187

Suttle CA, Harrison PJ (1988) Ammonium and phosphate-uptake rates, N-P supply ratios, and evidence for N-limitation and P-limitation in some oligotrophic lakes. Limnology and Oceanography 33:186-202

Taft JL, Taylor WR (1976) Phosphorus distribution in the Chesapeake Bay Chesapeake Science 17:67-73

Tango P, Butler W, Lacouture R, Goshorn D, Magnien R, Michael B, Hall H, Browhawn K, Wittman R, Betty W (2004) An unprecedented bloom of *Dinophysis acuminata* in Chesapeake Bay. In: Steidinger KA, Landsberg JA, Tomas CR, Vargo GA (eds) Proceedings of the Xth International Conference on Harmful Algae, St. Petersburg, Florida, p 358–363

Tango PJ, Butler W (2008) Cyanotoxins in tidal waters of Chesapeake Bay. Northeastern Naturalist 15:403-416

Tango PJ, Magnien R, Butler W, Luckett C, Luckenbach M, Lacouture R, Poukish C (2005) Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. Harmful Algae 4:525-531

Tett P, Heaney SI, Droop MR (1985) The Redfield ratio and phytoplankton growth-rate. Journal of the Marine Biological Association of the United Kingdom 65:487-504

Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area - water circulation patterns and organism distributions in Chesapeake Bay. Limnology and Oceanography 23:227-246

USDA (2008) Fertilizer consumption and use. United States Department of Agriculture. http://www.ers.usda.gov/Data/FertilizerUse/

Van Dolah FM, Leighfield TA (1999) Diel phasing of the cell-cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*. Journal of Phycology 35:1404-1411

Van Dolah FM, Leighfield TA, Sandel HD, Hsu CK (1995) Cell division in the dinoflagellate *Gambierdiscus toxicus* is phased to the diurnal cycle and accompanied by activation of the cell cycle regulatory protein, Cdc2 kinase. Journal of Phycology 31:395-400

Vargo GA (2009) A brief summary of the physiology and ecology of *Karenia brevis* Davis (G. Hansen and Moestrup comb. nov.) red tides on the West Florida Shelf and of hypotheses posed for their initiation, growth, maintenance, and termination. Harmful Algae 8:573-584

Vargo GA, Heila CA, Fanning KA, Dixon LK, Neely MB, Lester K, Ault D, Murasko S, Havens J, Walsh J, Bell S (2008) Nutrient availability in support of *Karenia brevis* blooms on the central West Florida Shelf: What keeps Karenia blooming? Continental Shelf Research 28:73-98

Vrede T, Ballantyne A, Mille-Lindblom C, Algesten G, Gudasz C, Lindahl S, Brunberg AK (2009) Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54:331-344

Wang JH, Wu JY (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of the Total Environment 407:4012-4021

Wang Y, Tang XX (2008) Interactions between *Prorocentrum donghaiense* Lu and *Scrippsiella trochoidea* (Stein) Loeblich III under laboratory culture. Harmful Algae 7:65-75

Watson TG (1972) Present status and future prospects of turbidostat. Journal of Applied Chemistry and Biotechnology 22:229

Wheeler PA, Kirchman DL, Landry MR, Kokkinakis SA (1989) Diel periodicity in ammonium uptake and regeneration in the oceanic subarctic Pacific - implications for interactions in microbial food webs. Limnology and Oceanography 34:1025-1033

Wheeler PA, Olson RJ, Chisholm SW (1983) Effects of photocycles and periodic ammonium supply on 3 marine-phytoplankton species .2. ammonium uptake and assimilation. Journal of Phycology 19:528-533

Wiegner TN, Seitzinger SP, Glibert PM, Bronk DA (2006) Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquatic Microbial Ecology 43:277-287

Xu N, Duan SS, Li AF, Zhang CW, Cai ZP, Hu ZX (2010) Effects of temperature, salinity and irradiance on the growth of the harmful dinoflagellate *Prorocentrum donghaiense* Lu. Harmful Algae 9:13-17

Yan WJ, Zhang S (2003) The composition and bioavailability of phosphorus transport through the Changjiang (Yangtze) River during the 1998 flood. Biogeochemistry 65:179-194

Yoshida T, Rao BSM, Ohasa S, Taguchi H (1979) Dynamic analysis of a mixed culture in chemostat. Journal of Fermentation Technology 57:546-553

Zhang H, Litaker W, Vandersea MW, Tester P, Lin SJ (2008) Geographic distribution of *Karlodinium veneficum* in the US east coast as detected by ITS-ferredoxin real-time PCR assay. Journal of Plankton Research 30:905-922

Zhang J, Liu SM, Ren JL, Wu Y, Zhang GL (2007) Nutrient gradients from the eutrophic Changjiang (Yangtze River) Estuary to the oligotrophic Kuroshio waters and re-evaluation of budgets for the East China Sea Shelf. Progress in Oceanography 74:449-478

Zhou M, Yan T, Zou J (2003) Preliminary analysis of the characteristics of red tide areas in Changjiang River estuary and its adjacent sea. Chinese Journal of Applied Ecology 14:1031-1038

Zhou W, Yin K, Zhu D (2006) Phytoplankton biomass and high frequency of *Prorocentrum donghaiense* harmful algal bloom in Zhoushan sea area in spring. Chinese Journal of Applied Ecology (In Chinese) 17:887-893