

ABSTRACT

Title of Thesis: Nitrogen Uptake and Physiology of Dinoflagellate Blooms in the East China Sea

Ji Li, Master of Science, 2008

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Frequent and large scale harmful algal blooms have developed in the highly eutrophic water in the Changjiang River estuary and East China Sea coast in the past decade. Sampling and experiments were conducted during late spring and early summer of 2005 to study the dinoflagellate blooms in this region when large scale ($> 15,000 \text{ km}^2$) mixed dinoflagellate blooms developed. *Karenia mikimotoi* was the dominant harmful algal bloom (HAB) species in the first stage of the bloom and was succeeded by *Prorocentrum donghaiense* approximately 2 weeks later. Samples were collected from different stations along both north-south and west-east transects during 3 cruises. Nitrogen (N) uptake experiments were conducted during the time period that covered pre-bloom, bloom development and bloom decline. Kinetics of N uptake by phytoplankton assemblages were measured as a function of substrate concentration (4 N sources, NO_3^- , NH_4^+ , urea, glycine) with ^{15}N isotope techniques. The bloom progression was related to the change in available N and phosphorus (P) composition. Reduced N, especially NH_4^+ and urea, were preferentially taken up during the blooms. Both the biological availability of reduced N and the N:P ratio were suggested to be important factors for the bloom development. High inorganic nitrogen loading from the river and phosphorus from coastal water supported the bloom development. Relatively high organic nitrogen levels,

and low DIN:DIP led to a succession of dinoflagellates. The temporal and spatial variability in kinetics parameters were reported as N-specific maximum uptake rates (V_{\max} , h^{-1}), absolute maximum N uptake rates (ρ_{\max} , $\mu\text{M h}^{-1}$) and half-saturation constant (K_s , $\mu\text{mol L}^{-1}$) during the bloom progression. The results of comparison these indices with ambient nutrients and bloom progression confirmed the preferentially taken up of N by different species during the blooms. Remarkable correspondence was found about the nutrient ratios and specific uptake rates of urea between blooms in the East China Sea and in analogous blooms on the West Florida Shelf. Similar N uptake rates were also shown between the East China Sea and the Chesapeake Bay. These findings suggest that there may be general relationships between these blooms species and the availability of different nutrients and the N:P ratio. To more firmly estimate these relationships, additional laboratory experiments and more comparison among different ecosystem will be required. Such relationships will contribute to our understanding of, and ability to model, these bloom dynamics.

NITROGEN UPTAKE AND PHYSIOLOGY
OF DINOFLAGELLATE BLOOMS
IN THE EAST CHINA SEA

By

Ji Li

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DEDICATION

There are people without whom all my efforts would have been in vain. This thesis is dedicated to my wonderful parents, Changbo Li and Xiuchuan Zhang, who have raised me to be the person I am today. They have been with me every step of the way, through good times and bad. I would like to thank them for all the unconditional love, guidance, and support that they have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to.

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INTRODUCTION

Large scale dinoflagellate blooms in the Changjiang (Yangtze River) Estuary (CJRE) and adjacent area of East China Sea (ECS) in late spring and early summer have been recorded in the past decade (Zhou et al. 2003). The dinoflagellate *Prorocentrum donghaiense* has been recorded as the dominant species in this region since large scale field studies began in 2000, with blooms observed from late April to May almost every year (Tang et al. 2006). In 2005, this *P. donghaiense* bloom was preceded by a bloom of the dinoflagellate *Karenia mikimotoi* (Li et al. In preparation). The scale of these blooms has increased from thousands km² early this century to over 15,000 km² in 2005. Systematic research across fields such as biology, chemistry and physics has been carried out to elucidate the ecological and oceanographical mechanisms of these blooms. Yet, to date, virtually nothing is known about the nitrogen nutrition of these blooms. Consequently, this was the topic of this thesis.

Ecophysiology of *P. donghaiense*

Prorocentrum donghaiense in this region was first reported as *Prorocentrum dentatum* Stein, but then was renamed a new species – *Prorocentrum donghaiense* (Lu & Goebel 2001). Results of comparing both the morphology and molecular data of *P. donghaiense* with the original descriptions of *P. dentatum* by Stein (1883) and Dodge (1975) and culture of *P. dentatum* from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) suggested that distinct differences existed in respect to morphology and cell sizes with original descriptions (Lu et al. 2005). However, based on scanning electron-microscopic studies, the detailed morphology of these *P. donghaiense*

and *P. dentatum* samples was very similar and showed these two species might belong to one species. One of the explanations may be that there was a mistake in Stein's original description of cell size. The nucleotide divergence between *P. donghaiense* and *P. dentatum* was only 0.2% which suggested they should be the same species. The major difference was cell size (Lu et al. 2005). Despite of the similarity in these two species, the bloom forming species in the ECS was still suggested to be named a new species: *Prorocentrum donghaiense* Lu (Qi & Wang 2003).

Prorocentrum donghaiense blooms in ECS can develop in a temperature range of 14 °C to 22 °C and salinity of 14 to 32, but more commonly in a temperature and salinity range of 18 °C to 21 °C and 21 to 23 (Wang 2003). Typical meteorological conditions during these blooms are high solar and high sea surface pressure (1015 to 1017 h Pa) and no wind. Vertical migration of this species has been observed with *P. donghaiense* cells staying in the surface water in daylight hour and migrating to the deeper water at night during the period of the bloom (Wang 2003). Vertical migration activity of *P. donghaiense* could be important in order to access nutrients.

Some *Prorocentrum* spp. have been reported to be toxic and produce prorocontrolide, okadaic acid and derivatives, which are toxins that cause Diarrheic Shellfish Poisoning (DSP) (Hu et al. 1996, Bravo et al. 2001, Heil et al. 2005, Nascimento et al. 2005). However, there is no toxin reported yet for *P. donghaiense* in ECS. On the other hand, the toxic dinoflagellate *Alexandrium catenella* and *A. tamarense* have been found in

association with *P. donghaiense* blooms and paralytic shellfish poisoning (PSP) toxicity has been detected (Zhou, 2003).

Oceanography, Hydrology and Nutrient Budget of Bloom Region

These more frequent and persistent algal blooms in recent years have been related to the increasing nutrients input to the ECS (Anderson et al. 2002, Zhou et al. 2003, Glibert & Burkholder 2006a). The high frequent bloom-forming area, CJRE and ECS coast, is a very complex hydrological system (Fig i-1) and under the influence of terrestrial input and water current transportation. Several major rivers run into this region (e.g. Qiantangjiang River, Yongjiang River), and the most dominant river input is the Changjiang River Diluted Water (CRDW), which has a plume influencing hundreds of kilometers seaward at the river mouth. Several currents, including coastal currents from north and south and the Taiwan Warm Current (TWWC), a branch of the Kuroshio Current, also strongly influence this region. The seasonal variation of runoff and current strength makes the hydrological environment of this region more complex.

The major source of nitrogen (N) is thought to be terrestrial input from the watershed draining into Changjiang and other major rivers (Zhang et al. 2007). The Changjiang is the third longest river of the world. Annual freshwater input is $9.32 \times 10^{11} \text{ m}^3$, and annual input of N is 6.3×10^6 tonne and phosphorus (P) is 0.13×10^6 tonne (Shen et al. 2003).

While both N and P inputs of China have increased in the past 20 years (Shen et al. 2003), the recent increases have been far greater in N. In 2005, China nitrogenous fertilizer production reached 36 million tonnes (including 20 million tonnes of urea fertilizer, a 5-fold increase since 1989; IFIA 2007). N loading to the coastal water from the Changjiang

River watershed, which has a population of over 400 million including the most developed area, and the largest agriculture and fertilization zone of China, is thought to be important to the blooms in the ECS.

Changjiang River Diluted Water, the freshwater runoff, extends offshore and forms a huge plume, characteristically high in N, but relatively low in P. As a result, the coastal area of ECS has relatively low P concentration. The total N of CRDW has been shown to be about 70~110 $\mu\text{M-N}$, and is mostly in the form of NO_3^- -N (Shen et al. 2003). The total P is about 2~25 $\mu\text{M-P}$, mostly as particle P; PO_4^{3-} -P is only about 10%~20% of the total P (Yan & Zhang 2003). As a result, the dissolved inorganic N (DIN) to dissolved inorganic P (DIP) ratio is typically over 100 inside the river mouth (Chai et al. 2006, Zhang et al. 2007). Limitation by P has been reported along the China coast, from the Yellow River continental shelf in the north China (Turner et al. 1990) to Pearl River estuary (Yin et al. 2004), and coastal South China Sea (Harrison et al. 1990) in the south.

The discharge of Changjiang River is only a relatively small part of total P budget of the ECS region. Fluxes imported (mostly dissolved phase P) from the Kuroshio and the TWWC into the ECS are the primary P source of this region (Fang 2004). The TWWC originates from the Kuroshio northeastward across the continental shelf from northeast of Taiwan to the middle shelf of the ECS and provides warm P-rich water to the area off the Fujian and Zhejiang coasts and the Changjiang river mouth (Yuan et al. 2008). At one side, this TWWC could be blocked by the CRDW when runoff increases in early spring, and causes strong mixing and nutrients exchange. On the other side, the continental shelf

of ECS near the coast is very shallow, the TWWC is forced by landform and upwells from the bottom. Two strong upwelling zones are formed (Zhao 1993). One is around the 29°N, 12°30'E, and the other is around 31°-32°N, 122°20'-123°00'. Both the upwelling and groundwater inflow transport bring large nutrients input to the CJRE. Model calculations show that the upwelled water near CJRE comes from the deep water of Taiwan Strait north exit and takes 4 months to reach CJRE (Luo 1998). The mixing between freshwater and seawater also forms a halocline and front at 123°E. The halocline changes with season and can form a convergence zone (Chen et al. 2001). There is still no direct evidence showing that the current circulation may be the source of harmful algae; however, the fine grain sediments area, which might indicate a convergence zone (Fig. i-1), overlaps with bloom forming region. These hydrological factors could be very crucial for the development of the blooms.

The Annual Community Ecology

Early spring diatom blooms in the ECS have been recorded since the late 20th century (Chiang et al. 1999, Chiang et al. 2004, Ishikawa & Furuya 2004). The water column of coastal China Sea is well-mixed and re-suspended by the winter monsoon, tides and storms in the winter, and nutrient regeneration and remineralization are assumed to be enhanced during this period. Diatoms have been reported to be the major consumer of nutrients accumulated in these areas in the early spring as the temperature begin to rise (Ishikawa & Furuya 2004).

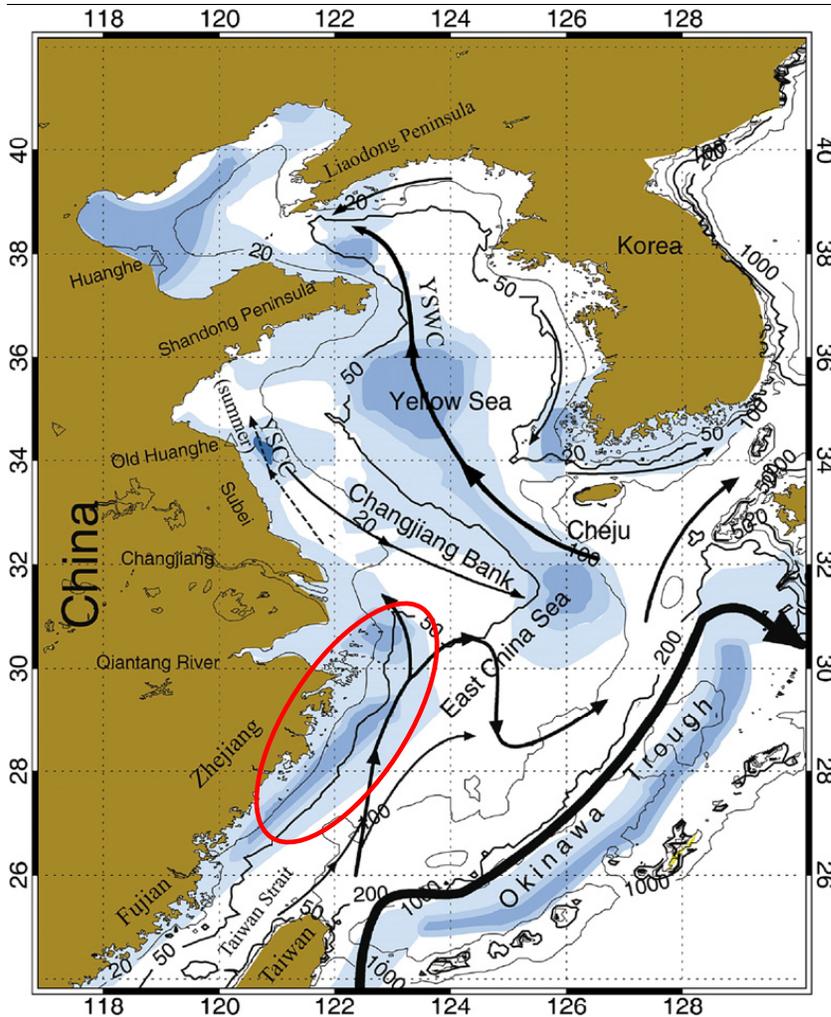


Figure i-1 Regional map and topography of the East China Sea and high frequent dinoflagellate bloom forming area (circled). The contour unit is meters. Shaded areas show distribution of mud patches at the ocean bottom, with darker colors indicating finer grain sediments. The arrows indicate the Yellow Sea Warm Current (YSWC), the Yellow Sea Coastal Current (YSCC), the Korea Coastal Current (KCC), the Tsushima Warm Current (TSWC), and the Taiwan Warm Current (TWC) in the map. The dash arrow along the Subei coast marks the YSCC in summer (After Yuan et al. 2008).

Diatoms have been reported to have higher NO_3^- uptake rates than dinoflagellates at low temperature (Lomas & Glibert 2000). The diatom *Skeletonema costatum* in nutrient-replete water, particularly those in which NO_3^- is the dominant nitrogenous nutrients, is favored and out competes *P. donghaiense* at temperatures $<15^\circ\text{C}$ (Li et al. 2003, Wang et al. 2006). *P. donghaiense* grows at maximum rates at $18^\circ\text{C}\sim 21^\circ\text{C}$. When the temperature rises over 20°C , *S. costatum* grows rapidly, but is replaced by *P. donghaiense* when the temperature rises over 23°C in late May (Wang, 2003). In June and July, when the salinity in CJRE is lowest, *S. costatum* can reach a density of 10^8 cell m^{-3} and can make up over 95% of the phytoplankton biomass (Wang, 1993).

Research Importance and Project

The delta zone of CJRE is the most populated and developed area of China and is home to the biggest city of China - Shanghai. This delta zone is just 1% of the total area, but it contains 5.8% of the population of China, and, it has over 18% of the GDP of China. The Zhoushang Archipelago is the largest fishery field of China, but it has suffered from overfishing and decay of fish stock for decades. Frequent and large scale blooms have caused both ecological and economic problems in the ECS coast. Therefore, the HABs in ECS, as one of the crucial environmental problems in China, have drawn attention from scientists and government. Protection of environment and ecosystem of CJRE and ECS coast is important for the sustainable development of China.

CEOHAB (Chinese Ecology and Oceanology of Harmful Algal Blooms), a national HAB project of China, was started in 2001. This project supported annual research cruises in the region of ECS and research across multiple subjects such as biology, chemistry and

physics in order to elucidate the ecological and oceanographical mechanisms of the blooms. Nitrogenous nutrient uptake and dynamics during the bloom progression is an important aspect of bloom dynamics, but were not studied in the CEOHAB program. This thesis is a report of a series of experiments on nitrogen uptake and physiology conducted in CEOHAB cruises in the 2005 HAB event. Ambient nutrients, N uptake rates and preference during the bloom period were measured and experiments were conducted to test the effect of P limitation on N uptake in this region. N uptake kinetics and related indices were calculated based on uptake rates and different ambient substrate. The following hypotheses were tested by these experiments:

- (1). P limitation develops in the CJRE from excess N loading, but P is made available from adjacent coastal water and its availability changes the N:P ratio and leads to blooms;
- (2). Dinoflagellates depend on reduced N sources (NH_4^+ , urea, amino acids) over NO_3^- as their N source.

CHAPTER I

Relationships between Nitrogen and Phosphorus Forms and Ratios and the Development of Dinoflagellates Blooms in the East China Sea, with a Note on Comparisons with Similar Algal Assemblages on the Southwest Florida Shelf*

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Li, J., P. M. Glibert, M. Zhou, S. Lu, D. Lu

Introduction

Large scale dinoflagellate blooms in the Changjiang (Yangtze) River estuary (CJRE) and adjacent area of East China Sea (ECS) in late spring and early summer have been recorded in the past decade (Zhou et al. 2003). The dinoflagellate *Prorocentrum donghaiense* has been recorded as the dominant species in this region since large scale field studies began in 2000, and large scale blooms have been observed from late April to May almost every year (Tang et al. 2006). The scale of the blooms has been increasing from thousands km² to over 15,000 km² (2005) in this century.

These more frequent and persistent algal blooms are thought to result from increasing nutrients input to the ECS (Anderson et al. 2002, Zhou et al. 2003, Glibert & Burkholder 2006a). The major source of nitrogen (N) and phosphorus (P) is terrestrial input from the watersheds of the Changjiang River and other major rivers (e.g. Qiantangjiang River, Yongjiang River). The Changjiang River is the third longest river of the world. Annual freshwater input is 9.32×10^{11} m³, 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 inputs 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 million tonnes, including 20 million tonnes of urea fertilizer, a 5-fold increase since 1989 (IFIA 2007). Nitrogen loading to the coastal water from the Changjiang River watershed, which has a population of over 400 million including the most developed area, and the largest agriculture and fertilization zone of China, is thought to be important to the blooms in the ECS.

The freshwater of Changjiang River extends offshore and forms a huge plume, which is called the Changjiang River Diluted Water (CRDW) and is characteristically high in N, but relatively low in P. As a result, the coastal area of ECS has a relatively low P concentration. The total N of CRDW has been shown to be about 70 to 110 $\mu\text{M-N}$, and is mostly in the form of NO_3^- -N (Shen et al. 2003). The total P is about 2 to 25 $\mu\text{M-P}$, mostly as particle P; PO_4^{3-} -P is only about 10% to 20% of the total P (Yan & Zhang 2003). As a result, the dissolved inorganic N (DIN) to dissolved inorganic P (DIP) ratio is typically > 100 inside the river mouth (Chai et al. 2006, Zhang et al. 2007). Limitation by P has also been reported along the China coast, from the Yellow River continental shelf in the north China (Turner et al. 1990) to Pearl River estuary (Yin et al. 2004), and coastal South China Sea (Harrison et al. 1990) in the south.

Compared to P-limited Changjiang River water, there is a branch of the Kuroshio current, the Taiwan Warm Current (TWWC), which is P-replete (Tang et al. 2000, Fang 2004). This bottom current passes the Taiwan Strait and flows northward along the coast, bringing similar levels of N as the East China Sea, but 8 - 17 times more DIP than the Changjiang with seasonal variation (Shen et al. 2003, Li et al. 2007). The TWWC is thought to be an important P source for the coastal ECS. The current strength of TWWC is weak in the winter but increases in the early spring and rises with the landform of the coast when it reached the CJRE area, and forms an upwelling zone (Chen et al. 1995). Mixing between the CRDW and the TWWC may result in P being carried to the surface water and becoming available to the phytoplankton community.

Although the general hydrography and nutrient loading rates to the ECS have been studied, the rates of nutrient uptake, and nutritional preferences of the phytoplankton during the blooms are not known. A comprehensive survey of the blooms area was conducted in 2005. Our objectives of study were to investigate the nutrient concentrations in the bloom area, assess the uptake rates of NO_3^- , NH_4^+ and dissolved organic N (DON), and the describe relationships between ambient N:P ratios and phytoplankton community composition. The bloom of 2005 was unusual in composition, with a progression of species beginning with *Karenia mikimotoi*, followed by *P. donghaiense*, and lastly, *Noctiluca scintillans*. This unusual character afforded the opportunity to explore the relationship between specific HAB species and nutrients dynamics. Comparisons in N:P ratios and N uptake rates are also drawn with similar measurements made in different assemblages dominated by *K. brevis*, mixed dinoflagellates, and diatoms on the southwest Florida shelf.

Materials and Methods

Sampling sites

Sampling was conducted during 3 cruises in 2005 in the coastal area of ECS, between 27.2° N and 31.5° N (Fig. 1-1). The first cruise was from April 23 to May 18, the second cruise was from May 25 to June 19, and the third cruise was from June 22 to July 1, 2005.

Transects were set between the 20 m and 60 m isobaths.

Sampling and Parameters Measurements

Water was collected using 30 L Niskin bottles from 10 m and near surface, or using a bucket for the surface sample at some stations. All samples were collected around noon.

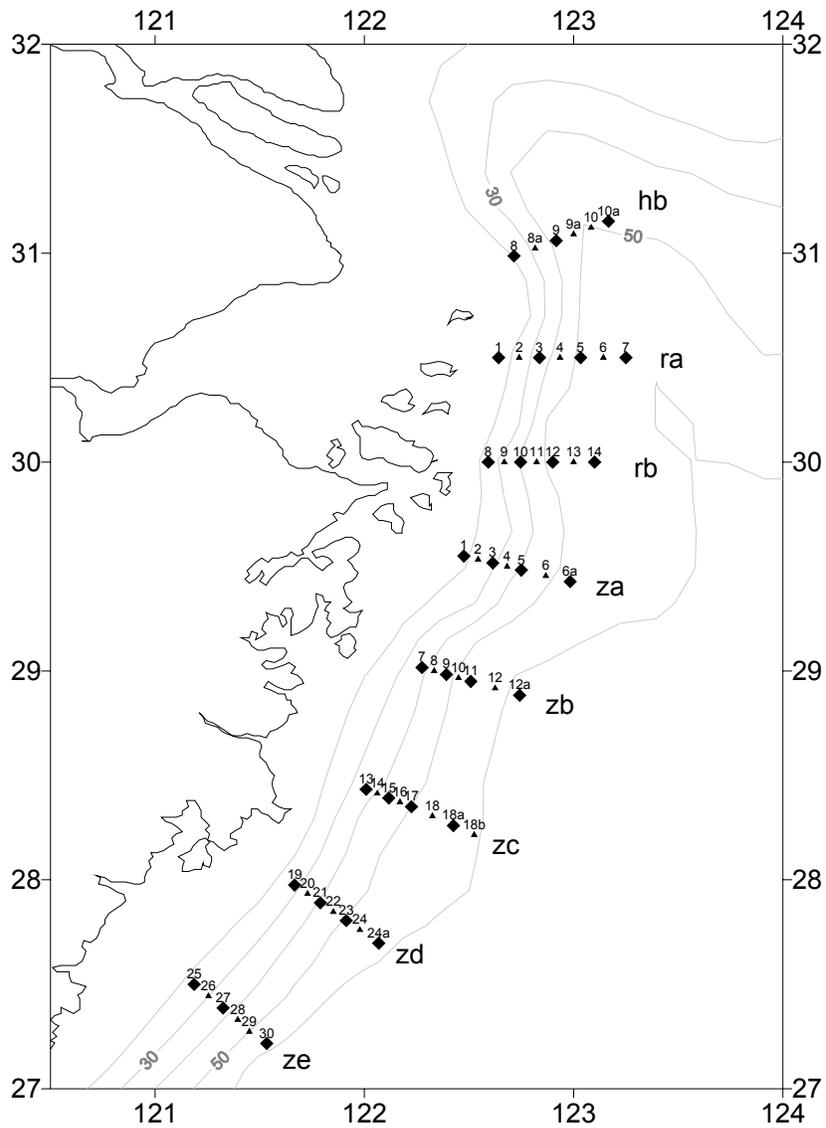


Figure 1-1 Locations of sampling station and transect setting of cruises on the dinoflagellate blooms in East China Sea, 2005.

Water samples were subsequently divided for different analysis and experimental purposes, as described below. Cell samples were identified microscopically during the cruises. Water samples were filtered through pre-combusted (2 h at 400 °C) Whatman GF/F filters and stored at -20°C for subsequent nutrient analysis. Filters were stored frozen for later pigment analysis and particle organic carbon C and N (POC and PON) analysis.

All the samples were transported frozen to Horn Point Laboratory and analyzed within 6 mo. Concentrations of ambient inorganic nutrients (NO_3^- -N, NH_4^+ -N, PO_4^{3-} -P, Si(OH)_4 -Si) were determined using a Technicon AutoAnalyzer (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). Filters for POC and PON were dried at 50°C for 24 hours. POC and PON concentrations were measured with a CHN elemental analyzer using acetanilide as standards.

Pigment analyses were performed by high performance liquid chromatography (HPLC) according to the method of Van Heukelem and Thomas (2001). The relative abundances of specific pigments were used to evaluate the abundance of specific algae. Fucoxanthin was used to indicate the present of diatoms (Boczar & Palmisano 1990, Yao et al. 2006), and peridinin was used as an indicators of photosynthetic dinoflagellates (e.g., *P. donghaiense*). The pigment gyroxanthin- diester was used as a specific indicator of *Karenia* spp. (Chang & Trench 1982, Le et al. 2001, Richardson & Pinckney 2004). The absolute values of specific pigments were used to indicate the abundance of the species groups in the water

and the ratios of specific pigments to chlorophyll *a* (Chl *a*) were used to indicate the relative abundance of the species in the phytoplankton community. Microscopic enumerations (not shown) verified the dominance of the particular algal groups and thus the appropriateness of the indicator pigments chosen.

Nitrogen uptake experiments

Experiments were conducted on May 7, 8, 9, 13, 28 and June 3, 9, 11, 16, 23 of 2005.

These periods bracketed the period before dinoflagellate dominance to the late phase of bloom progression. One experiment was conducted per day. Sample water was transferred into a clear 20L carboy immediately after collection and experiments were started immediately. Water samples were dispensed into acid- clean 250 ml bottles and inoculated with ¹⁵N labeled substrates (NO₃⁻, NH₄⁺, urea, and glycine). ¹⁵N labeled NO₃⁻ was added at 1 μM-N; ¹⁵N labeled NH₄⁺ and urea were added at 0.5 μM-N; and ¹⁵N labeled glycine was added at 0.1 μM-N. ¹⁵N labeled substrates represented ~ 10% to 50% of final ambient concentrations. Incubations were 0.5 hr on deck in a flowing seawater incubator around noon. Samples were then filtered onto pre-combusted GF/F filters. Due to the number of treatments, replication was not possible. However, routine analyses conducted with the same protocols and on the same instrumentation yield an average variation in ¹⁵N isotope enrichment of < 0.5% in replicated samples (Glibert et al. 2006). The uptake rates of NO₃⁻ were not measured after May 28 due to limited supply of this isotope.

To evaluate the possibility of P limitation, some experiments were also conducted on samples that were enriched with PO₄³⁻ to 3 μM-P and pre-incubated for either 2 hours or overnight before the start of the isotope ¹⁵N enrichment experiments.

All GF/ F filters for isotope analysis were dried for 24 h at 50 °C and were analyzed for isotope enrichment using a Sercon Mass Spectrometer. Rates of ^{15}N uptake were calculated according to Glibert and Capone (1993).

Results

Overview

The cruise time series covered a succession of phytoplankton species. No dinoflagellate blooms were observed during the first cruise (April 23 - May 18), although the presence of dinoflagellates were noted in the phytoplankton community which was dominated by diatoms during this period (maximum 10^7 cell L^{-1} ; Chiang et al. 1999). However, beginning with the first day of the second cruise (May 25), *K. mikimotoi* was observed to dominate the assemblages in all transects (maximum 8×10^6 cell L^{-1} at station za-1). The total area of the *K. mikimotoi* blooms was ultimately estimated to be over 15,000 km^2 . The *K. mikimotoi* blooms were also associated with a massive fish kill when the blooms were transported to the coastal aquaculture areas. High value aquaculture fish (*Sciaenops ocellatus*, *Miichthys miiuy*, *Epinephelus* spp., *Monotaxis grandoculis*) worth about \$2.5 million were lost due to this bloom (Zhou 2005). *K. mikimotoi* blooms were succeeded by *P. donghaiense* (maximum 5×10^6 cell L^{-1} at station ra-1) in our sample sites from the second week of June. The dinoflagellate blooms were observed to be in decay phase during the third cruise. *Noctiluca scintillans* was a common species observed throughout the cruises, but their density was highest after the blooms of *K. mikimotoi* and *P. donghaiense*.

Ambient Parameters Comparison

A total of 85 water samples from the 3 cruises were analyzed for nutrient concentrations. Total dissolved N (TN) in the surface water decreased from an average of 31 $\mu\text{M-N}$ to 16.3 $\mu\text{M-N}$ during the bloom progression (Table 1-1, Fig. 1-2 a). Similarly, during the study period, the average surface NO_3^- concentration decreased from 18.8 $\mu\text{M-N}$ to 7.2 $\mu\text{M-N}$ when *K. mikimotoi* were dominant, and further decreased to 3.0 $\mu\text{M-N}$ when *P. donghaiense* replaced *K. mikimotoi* as the dominant species (Table 1-1, Fig. 1-2 d).

In contrast to NO_3^- , reduced N (NH_4^- , urea and DFAA) did not decrease, but rather increased throughout the time series (Fig. 1-2 e, f, g). DON, however, remained relatively constant around 12 $\mu\text{M-N}$ (Fig. 1-2 c). Therefore, the percentage of DON in the TN pools in surface water increased from average 42% before the bloom to 62% when *K. mikimotoi* was dominant and 77% when *P. donghaiense* was dominant. During the period of bloom decay, the percentage of DON in the TN decreased to 66%.

The average DIP concentration decreased from about 0.35 $\mu\text{M-P}$ to 0.19 $\mu\text{M-P}$ during the *K. mikimotoi* blooms, but stayed relatively constantly around 0.2 $\mu\text{M-P}$ during the period of *P. donghaiense* (Fig. 1-3 a). DOP decreased from 0.43 $\mu\text{M-P}$ before the dinoflagellate blooms to about 0.3 $\mu\text{M-P}$ during the blooms, but increased in the bloom decay phase (Fig. 1-3 b). The percentage of DOP in the total dissolved phosphorus (TP) remained constant around 60%.

Average molar DIN (NO_3^- and NH_4^+): DIP (PO_4^{3-}) ratios were about 65 before the blooms, which was much higher than the Redfield ratio (16:1). DIN: DIP ratios decreased to an average of 40 in the surface water during the *K. mikimotoi* blooms and further declined to an average of 22 during the *P. donghaiense* blooms (Table 1-2). The DIN: DIP ratio rose to ~33 during the declining phase of the blooms when *N. scintillans* dominated (Fig. 1-3 d, Table 1-2). In contrast, the average molar DON: DOP ratio showed a different pattern, with ratios remaining <40 before and during early phase the dinoflagellate blooms, but were close to 50 during the peak of the bloom (Fig. 1-3 e, Table 1-2). TN: TP ratio also decreased from 44.5 before the bloom to 30 as the blooms declined.

Average Si(OH)_4 concentrations were around 20 $\mu\text{M-Si}$ throughout the time series, with slight increases in the surface, and decreases in the 10 m layer (Fig. 1-3 c). Average DIN: Si(OH)_4 ratios decreased from ~1.0 to ~0.4 during the period of *K. mikimotoi* dominance, and further decreased to ~0.17 during the *P. donghaiense* blooms and finally increased to 0.42 after the blooms (Fig. 1-3 f, Table 1-2).

In contrast to the variation in N, P and Si concentrations and their relative ratios that were associated with different phases of the bloom progression (Fig. 1-4), ratios of POC: PON ratios remained relatively constant for different nutrient conditions with an average of 7.6 and a range of 6.6 to 9.6, throughout the study period.

Table 1- 1 Ambient nutrients in the East China Sea during spring 2005. Values shown are average for the bloom period and depth indicated. Values for NO_3^- , NH_4^+ , urea, dissolved free amino acid (DFAA), total nitrogen (TN), particle organic nitrogen (PON) are given in $\mu\text{M-N}$; PO_4^{3-} and total phosphorus (TP) in $\mu\text{M-P}$; Si(OH)_4 in $\mu\text{M-Si}$ and particle organic carbon (POC) in $\mu\text{M-C}$.

CRUISE	I		II				III	
	April 23 - May 18 pre-blooms		May 25 - June 7 <i>K. mikimotoi</i> blooms		June 8 - Jun 19 <i>P. donghaiense</i> blooms		June 22 - June 25 late blooms	
Date	Surface	10 m	Surface	10 m	Surface	10 m	Surface	10m
NO_3^-	18.8	17.2	7.2	8.4	3.0	5.27	3.7	4.8
NH_4^+	0.95	0.85	1.0	1.51	1.3	1.13	1.62	3.08
Urea	0.38	0.41	0.71	0.52	1.45	0.85	1.31	1.03
DFAA	0.24	0.18	0.78	0.26	1.1151	0.40	0.75	0.48
PO_4^{3-}	0.35	0.31	0.20	0.18	0.21	0.15	0.68	0.25
Si(OH)_4	17.4	19.8	24.2	18.1	23.9	22.1	16.4	12.6
TN	30.5	27.4	20.4	20.9	17.8	16.9	33.0	17.3
TP	0.78	0.64	0.56	0.50	0.51	0.34	1.34	0.16
PON	4.3	4.0	20.4	7.6	13.8	12.7	9.4	6.4
POC	35.1	32.5	161.5	55.2	101.2	89.8	68.3	46.6

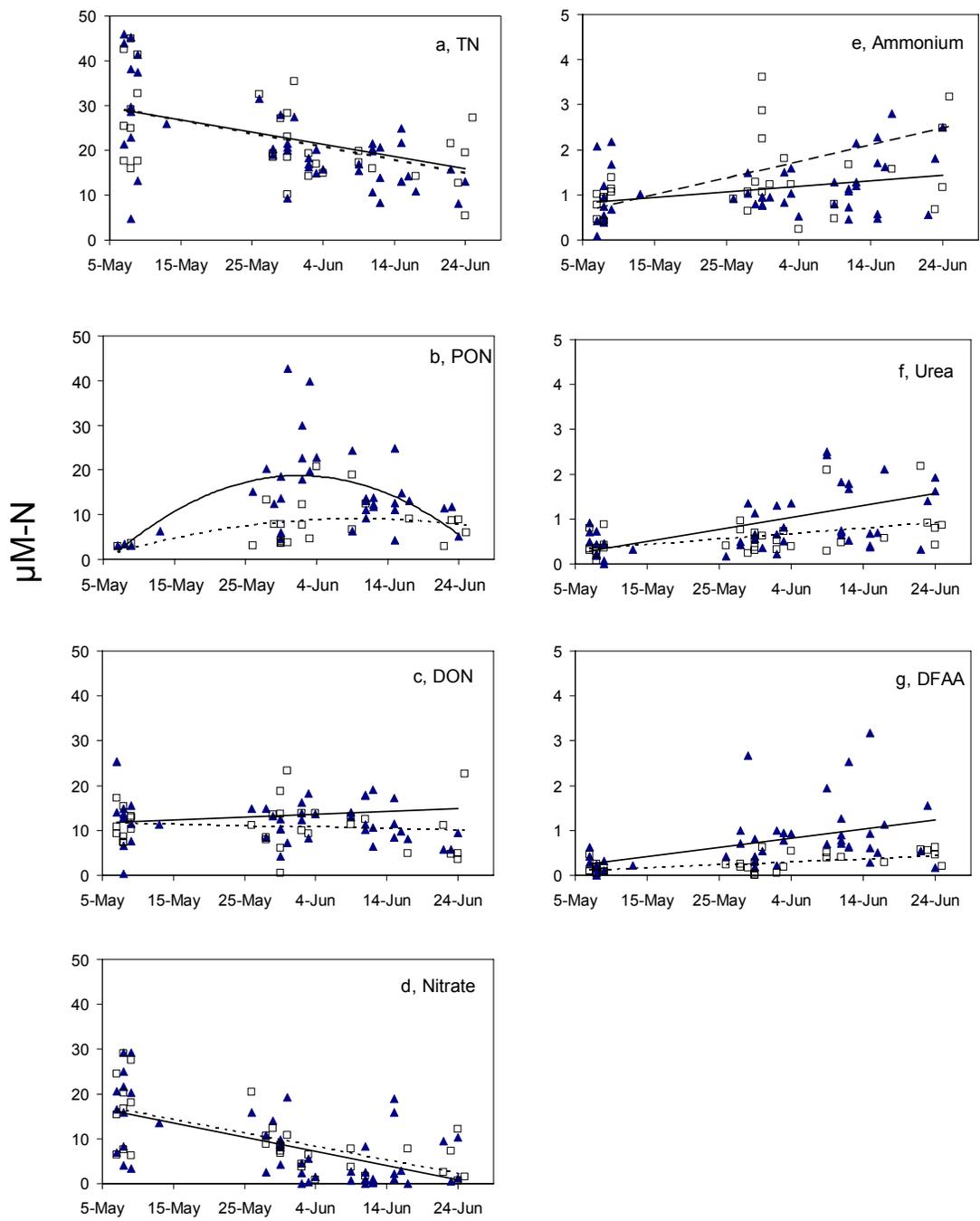


Figure 1-2 Major nitrogen components concentration ($\mu\text{M-N}$) in the water column in the bloom time series in surface water (triangle) and 10 m depth (square). TN: total dissolved nitrogen; PON: particle organic nitrogen; DON: dissolved organic nitrogen; DFAA: dissolved free amino acid

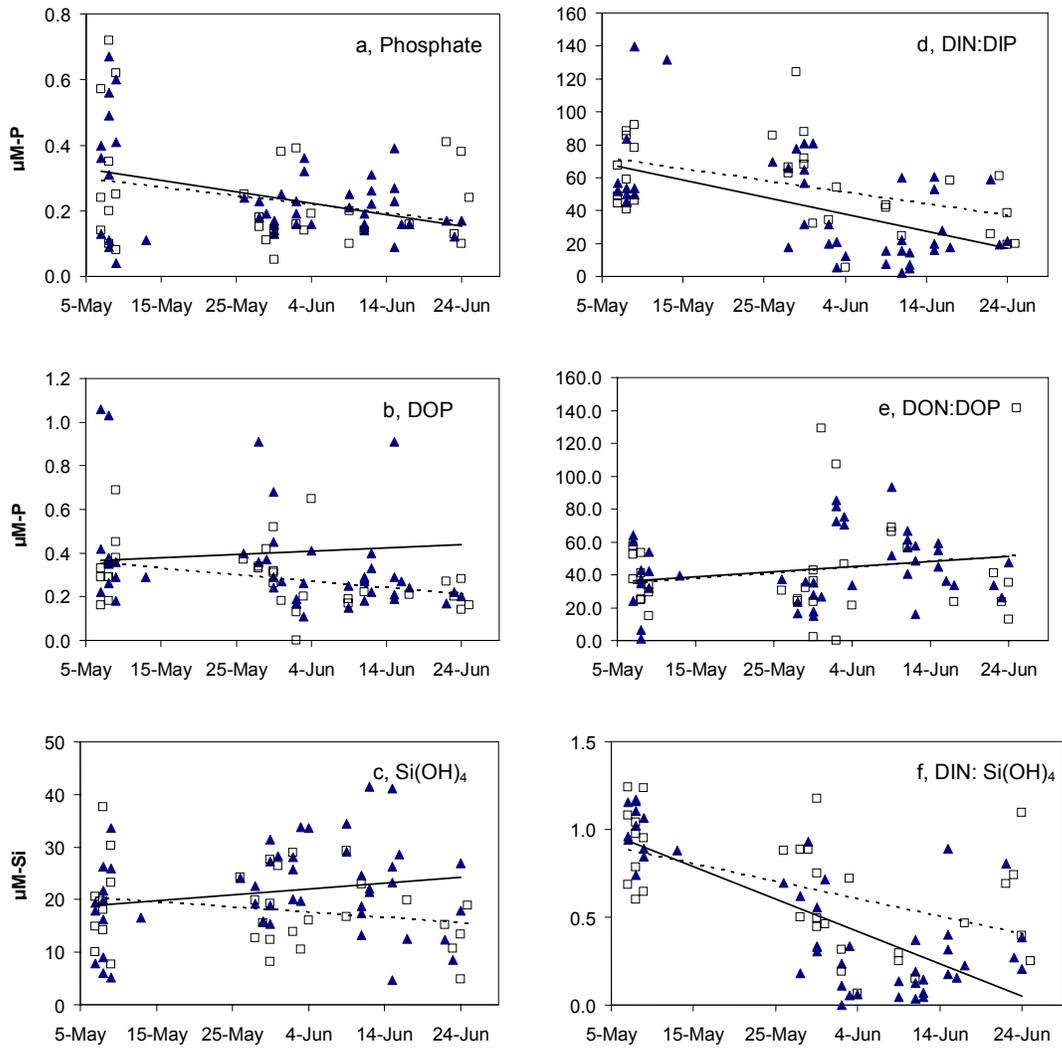


Figure 1-3 Dissolved phosphorus and silicate concentration ($\mu\text{mol L}^{-1}$) and the molar ratio of dissolved nitrogen to phosphorus and silicate in the water column in the bloom time series in surface water (triangle) and 10 m depth (square). DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus; DOP: dissolved organic phosphorus; DON: dissolved organic nitrogen; DSi: dissolved silicate

Table 1- 2 Dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen concentration ($\mu\text{M-N}$), DIN to dissolved inorganic phosphate (DIP) ratio, DON to dissolved organic phosphate (DOP) ratio and DIN to dissolved silicate (Si(OH)_4) ratio in different phases of the bloom series. I: Before blooms; II: Early *Karenia mikimotoi* blooms; III: Late *Karenia mikimotoi* blooms; IV: Early *Prorocentrum donghaiense* blooms; V: After dinoflagellate blooms

Phase	DIN $\mu\text{M-N}$		DON $\mu\text{M-N}$		DIN:DIP		DON:DOP		DIN: Si(OH)_4	
	Surface	10m	Surface	10m	Surface	10m	Surface	10m	Surface	10m
I	17.4 \pm 8.5	18.0 \pm 8.4	13.2 \pm 6.4	11.2 \pm 3.4	65.9 \pm 31.2	65.1 \pm 19.8	36.3 \pm 17.6	36.8 \pm 14.1	1.0 \pm 0.13	0.92 \pm 0.23
II	11.3 \pm 5.3	12.1 \pm 3.8	10.7 \pm 3.6	11.5 \pm 6.8	60.7 \pm 22.2	74.8 \pm 26.3	26.1 \pm 8.6	26.9 \pm 12.1	0.50 \pm 0.24	0.72 \pm 0.26
III	3.3 \pm 2.6	4.6 \pm 2.8	13.7 \pm 3.4	11.8 \pm 2.5	17.8 \pm 9.9	31.3 \pm 24.6	69.7 \pm 18.6	58.3 \pm 44.0	0.13 \pm 0.12	0.32 \pm 0.28
IV	3.0 \pm 2.5	5.4 \pm 2.8	13.4 \pm 4.2	12.28 \pm 0.9	11.1 \pm 6.77	36.5 \pm 10.6	54.7 \pm 20.6	64.0 \pm 6.4	0.12 \pm 0.10	0.23 \pm 0.08
V	7.7 \pm 6.5	8.2 \pm 4.4	9.5 \pm 3.6	8.6 \pm 7.2	32.7 \pm 18.9	37.5 \pm 18.9	42.1 \pm 11.4	27.2 \pm 11.3	0.39 \pm 0.24	0.61 \pm 0.30

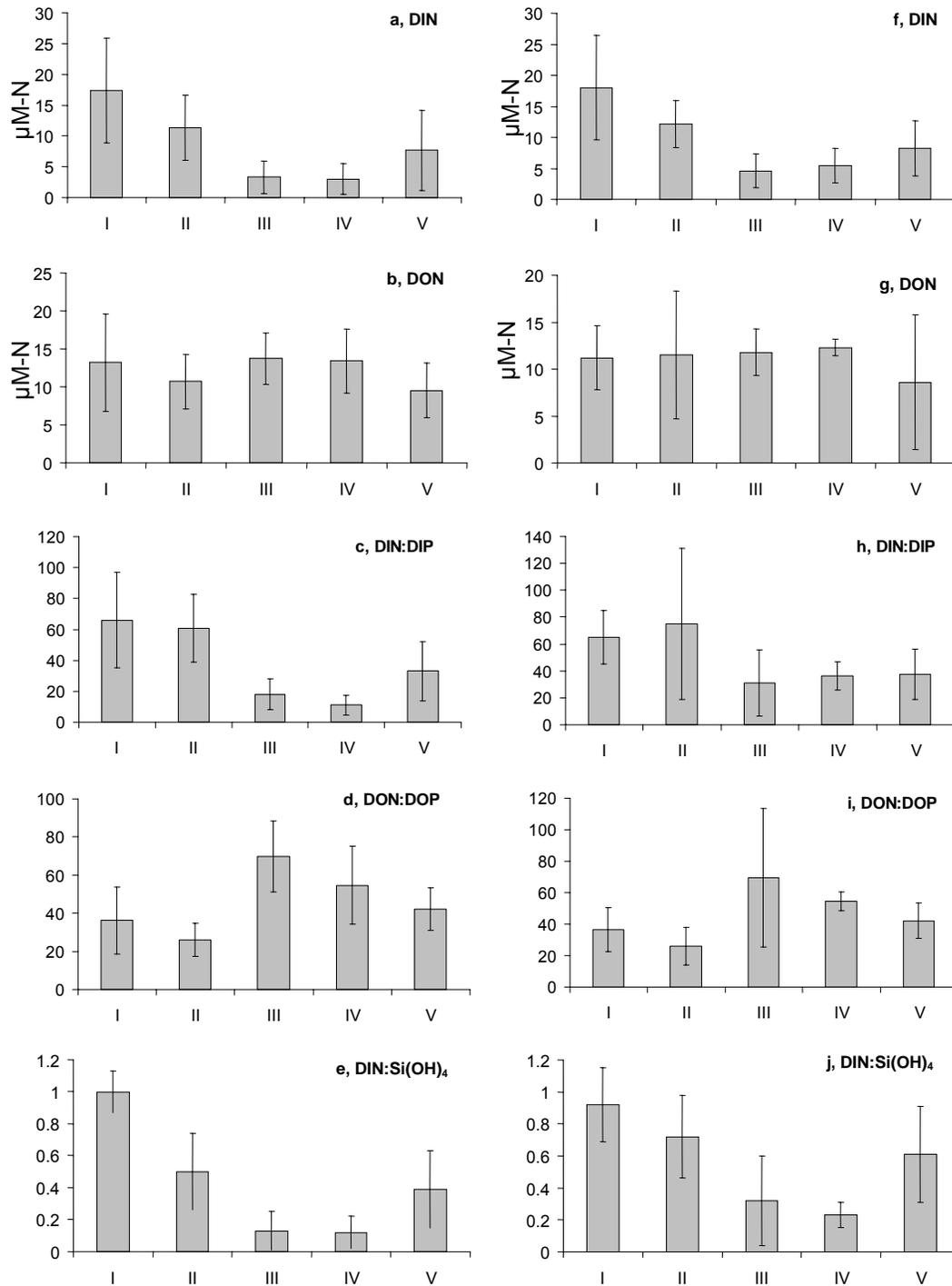


Figure 1-4 Dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen concentration ($\mu\text{M-N L}^{-1}$), DIN to dissolved inorganic phosphate (DIP) ratio, DON to dissolved organic phosphate (DOP) ratio and DIN to silicate (Si(OH)_4) ratios in different phases of the bloom series in surface water (a, b, c, d, e) and 10m water (f, g, h, i, j). I: Before blooms; II: Early *Karenia mikimotoi* blooms; III: Late *Karenia mikimotoi* blooms; IV: Early *Prorocentrum donghaiense* blooms; V: Late *Prorocentrum donghaiense* blooms.

Pigment concentrations and phytoplankton composition

In early May, total Chl *a* concentrations on average were low ($\sim 1.5 \mu\text{g L}^{-1}$) in both surface and 10 m samples. A higher Chl *a* concentration ($\sim 7.5 \mu\text{g L}^{-1}$) was observed on May 13 before the end of the first cruise. Significantly higher Chl *a* concentrations developed during the dinoflagellate blooms: $65 \mu\text{g L}^{-1}$ during the period of *K. mikimotoi* dominance and $80 \mu\text{g L}^{-1}$ during the phase of *P. donghaiense* dominance (Fig. 1-5 a). The PC: Chl *a* ratios decreased during the bloom succession (Fig. 1-5 e).

All 3 indicator pigments showed a trend line of increasing from a low value, reaching a peak and finally decay. Fucoxanthin and gyroxanthin-diester reached their maximum values during the phase of *K. mikimotoi* dominance, while peridinin reached its maximum value during the phase of *P. donghaiense* blooms (Fig. 1-5 b, c, d). The relative abundance of the indicator pigments also showed different trends during bloom succession. The relative abundance of fucoxanthin: Chl *a* decreased through the entire periods of study (Fig. 1-5f), while the relative abundance of gyroxanthin-diester: Chl *a* reached its peak during the midpoint of the time series when *K. mikimotoi* dominated (Fig. 1-5g), and peridinin: Chl *a* increased in the latter phase of the time series when *P. donghaiense* bloomed (Fig. 1-5h).

Nitrogen uptake experiments

The N-specific uptake rates ($V \text{ h}^{-1}$) of NO_3^- varied between 0.008 h^{-1} at an ambient NO_3^- of $2.6 \mu\text{M-N}$ and 0.013 h^{-1} at an ambient NO_3^- of $6.2 \mu\text{M-N}$ (Fig. 1-6 a). Due to the variation of biomass in the water column, absolute uptake rates of NO_3^- ($\mu\text{M-N h}^{-1}$) increased from $<0.05 \mu\text{M-N h}^{-1}$ in early May to $0.18 \mu\text{M-N h}^{-1}$ by late May (Fig. 1-6 e). NO_3^- contributed

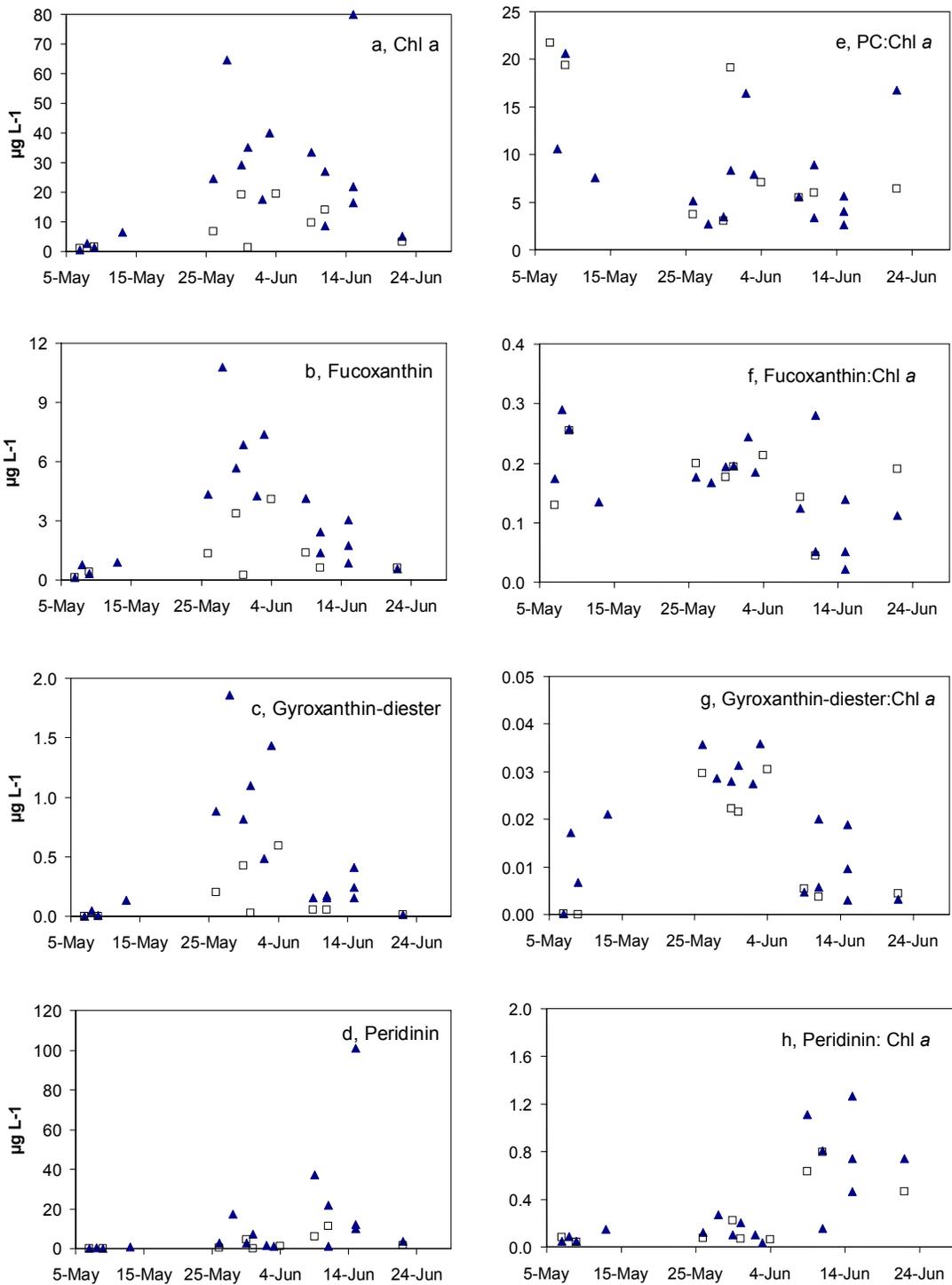


Figure 1-5 a, chlorophyll a (Chl a) concentration ($\mu\text{M-N}$); b, chlorophyll a to particle carbon ratio in the water column; and pigments fucoxanthin, gyroxanthin-diester and peridinin concentrations ($\mu\text{M-N}$) and their relative ratio to chlorophyll a in the water column in the bloom time series.

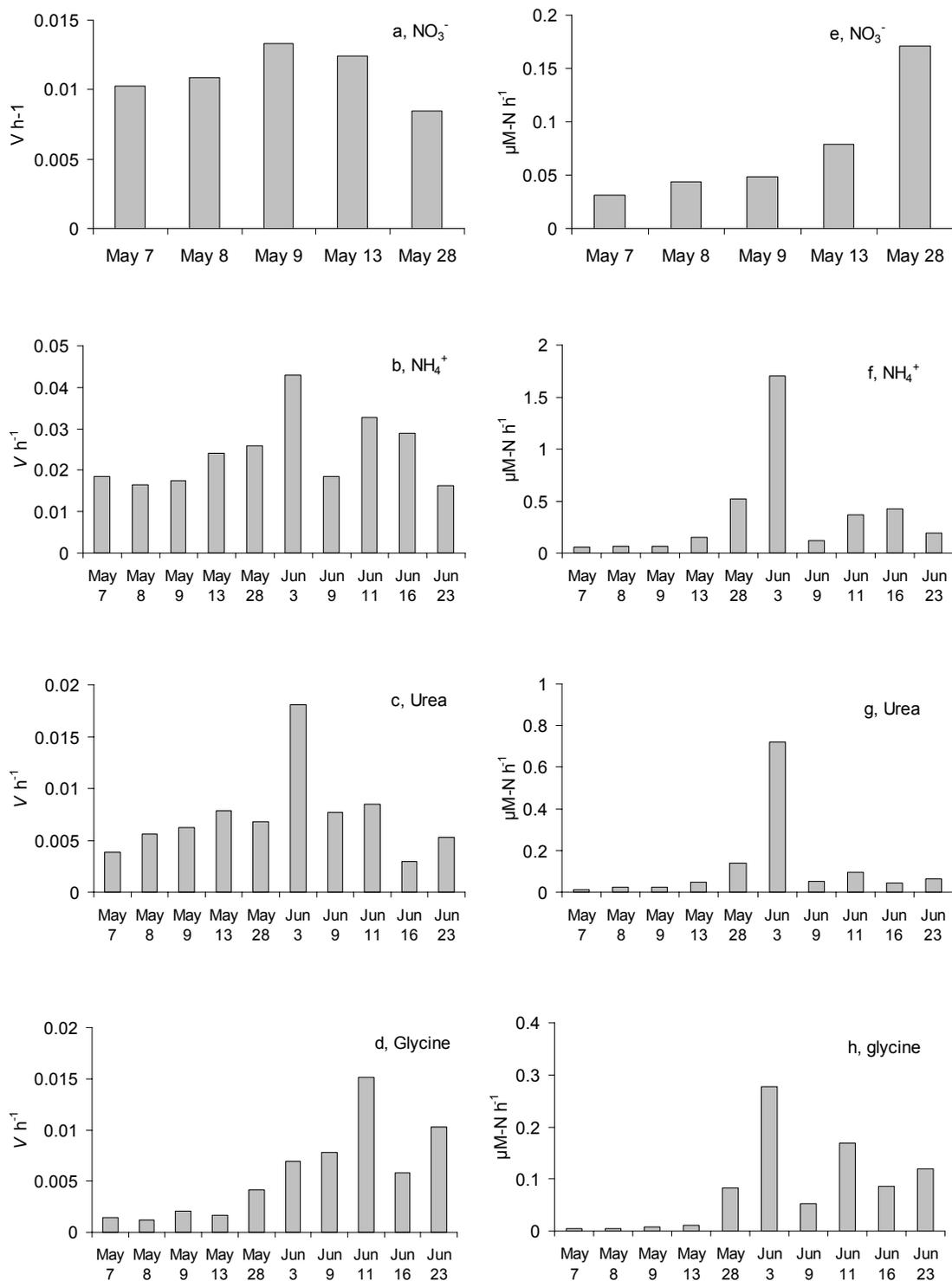


Figure 1-6 The nitrogen specific uptake rates V (h^{-1}) (a, b, c, d) and absolute nitrogen uptake rates ($\mu\text{M-N h}^{-1}$) (e, f, g, h) of nitrate (NO_3^-), ammonium (NH_4^+), urea and glycine in the bloom time series. No nitrate data after May 28.

about 30% of the total N uptake (sum of the uptake rates of NO_3^- , NH_4^+ , urea, and glycine) before the period of dinoflagellate dominance and decreased to < 20% of total N uptake after these blooms had become established (Fig. 1-7 a), after which rates of NO_3^- were not measured.

The rates of uptake of reduced N also varied with the bloom progression (Fig. 1-6 b, c, d, f, g, h). The N-specific uptake rates of NH_4^+ ($V h^{-1}$) were between $0.016 h^{-1}$ at an ambient NH_4^+ of $0.63 \mu\text{M-N}$ and $0.043 h^{-1}$ at an ambient NH_4^+ of $1.59 \mu\text{M-N}$. Absolute uptake rates of NH_4^+ increased from $0.06 \mu\text{M h}^{-1}$ before the *K. mikimotoi* blooms to a maximum rate of $1.71 \mu\text{M h}^{-1}$ and decreased to $0.18 \mu\text{M h}^{-1}$ when the *K. mikimotoi* blooms decayed. Overall, NH_4^+ contributed an average of 66% of the total reduced N uptake (sum of the uptake rates of NH_4^+ , urea, and glycine) throughout the time series.

Urea contributed on average 20% of the total reduced N uptake. Rates of urea uptake were highest on both an N-specific and absolute basis, when *K. mikimotoi* dominated (Figs. 1-6 c, g). Rates of glycine uptake increased during the bloom succession, as did the contribution of this N substrate to total N uptake. Glycine uptake was only about 5% of total reduced N before the dinoflagellate blooms, but its relative contribution increased to 11% during the *K. mikimotoi* blooms and 25% during the *P. donghaiense* blooms (Fig. 1-7 b). Higher reduced N uptake rates were geographically located in both south station and the station close to the Changjiang river mouth (Fig. 1-8).

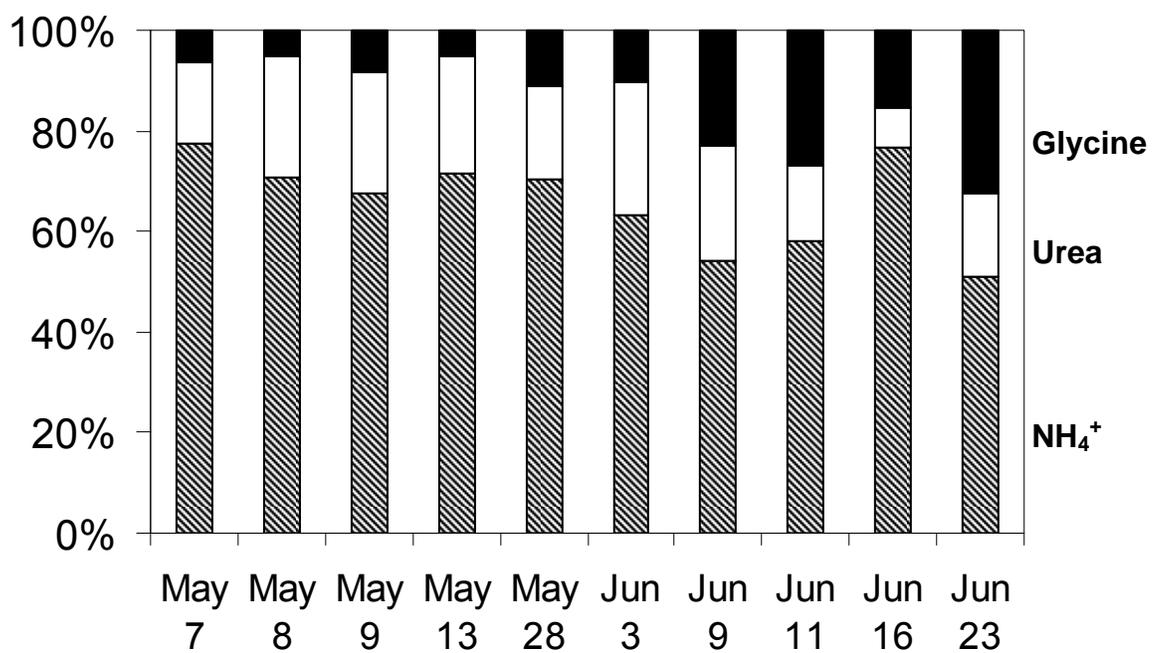


Figure 1-7 The relative contribution of reduced N (ammonium (NH₄⁺), urea and glycine) to total reduced N uptake rates in the bloom time series.

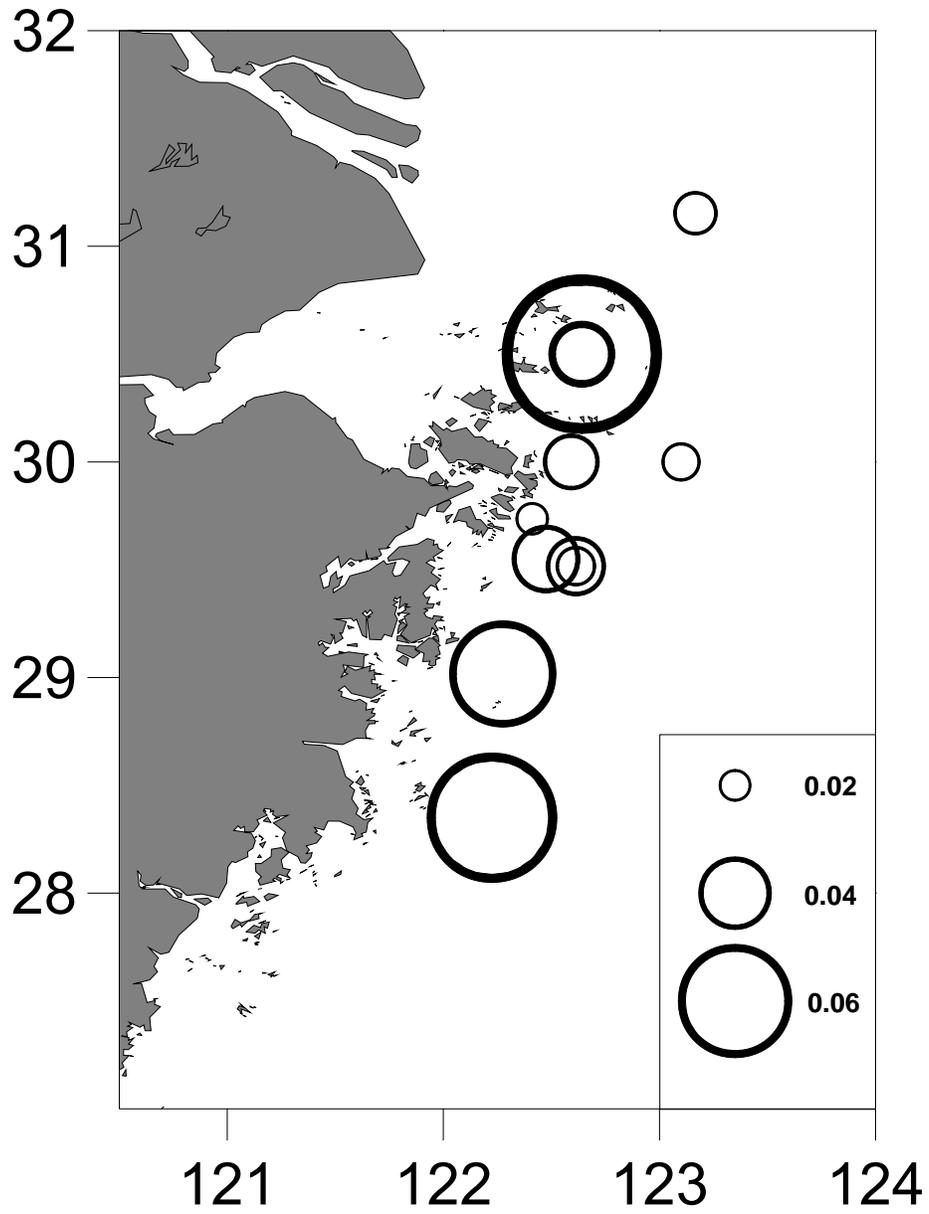


Figure 1-8 Geographical result of specific uptake rates of reduced nitrogen (V , h^{-1}) in the bloom area.

The relative preference index (*RPI*) defines the preference of a particular form of N relative to its relative availability. For example, for NH_4^+ :

$$RPI_{\text{NH}_4} = \frac{V_{\text{NH}_4}}{\sum V} / \frac{[\text{NH}_4]}{\sum [\text{N}]}$$

where V_{NH_4} and $\sum V$ are the N-specific uptake rates (h^{-1}) of NH_4^+ and total N uptake, and $[\text{NH}_4]$ and $\sum [\text{N}]$ are the concentration of NH_4^+ ($\mu\text{M-N}$) and the total concentration of N sources being compared. Values >1 represent preference for a particular N form.

Due to the fact that NO_3^- uptake rates were not measured after the first cruise, the calculated *RPI* was calculated 2 ways. For the first cruise, the 4 different N sources (NO_3^- , NH_4^+ , urea, and glycine) were compared. A separate calculation was done for the reduced N sources (NH_4^+ , urea, and glycine) only. Before the dinoflagellate blooms, including at the beginning of *K. mikimotoi* blooms, the *RPIs* for NO_3^- were all lower than 0.5. During this same period, the *RPIs* for NH_4^+ and urea were all higher than 1 (Fig. 1-9 a). The *RPIs* for reduced N only during the whole bloom time series generally converged on 1. However, *RPIs* for urea were higher than 1 during the *K. mikimotoi* blooms but lower than 1 during the *P. donghaiense* blooms. The *RPIs* of glycine showed the opposite trend (Fig. 1-9 b).

Effect of phosphate pre-enrichment

Pre-enrichment with P increased the rates of N uptake in virtually all experiments (Fig. 1-10). NO_3^- uptake rates increased an average 28% with a maximum increase of 60%. Rates of reduced N (NH_4^+ , urea) increased an average 10% with a maximum increase of 31%. Stations closed to the Zhoushan archipelago had the highest percentage increases in rates of reduced N uptake with P pre-enrichment. The increase was less in the south stations and

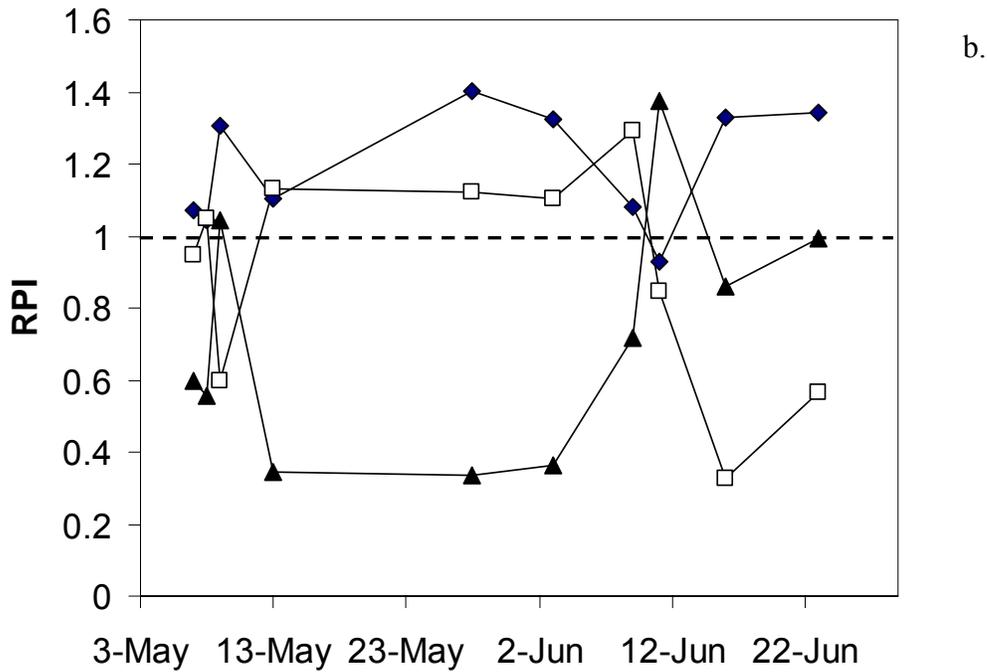
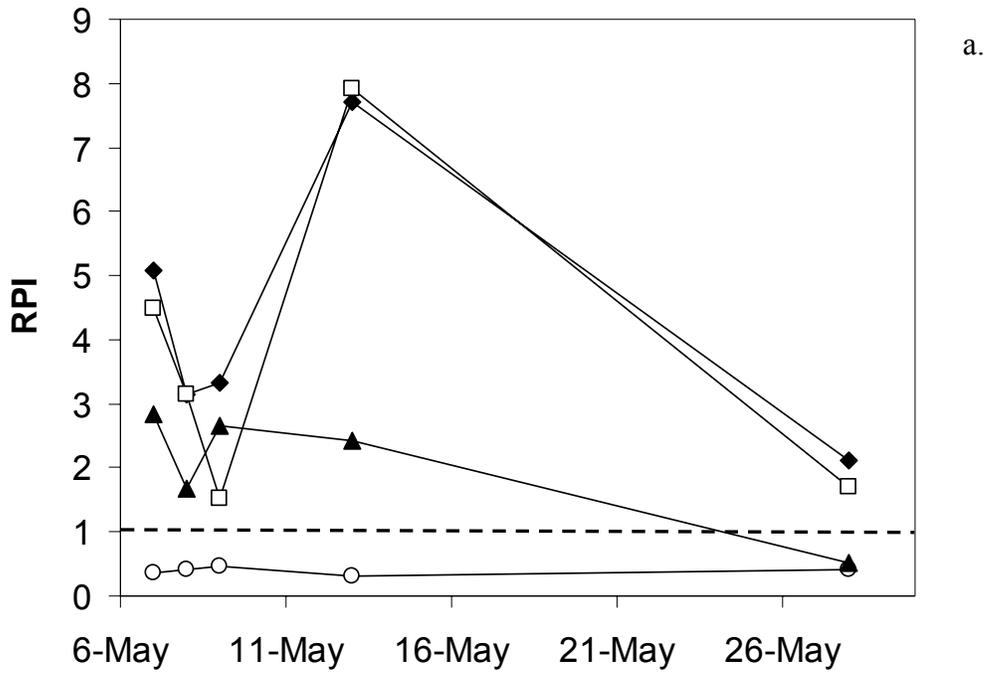


Figure 1-9 Relative preference index (RPI) of the different nitrogen sources uptake. Panel a: RPI values calculated using NO_3^- (○), NH_4^+ (◆), urea (□) and glycine (▲) for the period of the time series up to the beginning of the *Karenia mikimotoi* blooms. Panel b: RPI values calculated using only NH_4^+ (◆), urea (□) and glycine (▲) for the entire time series.

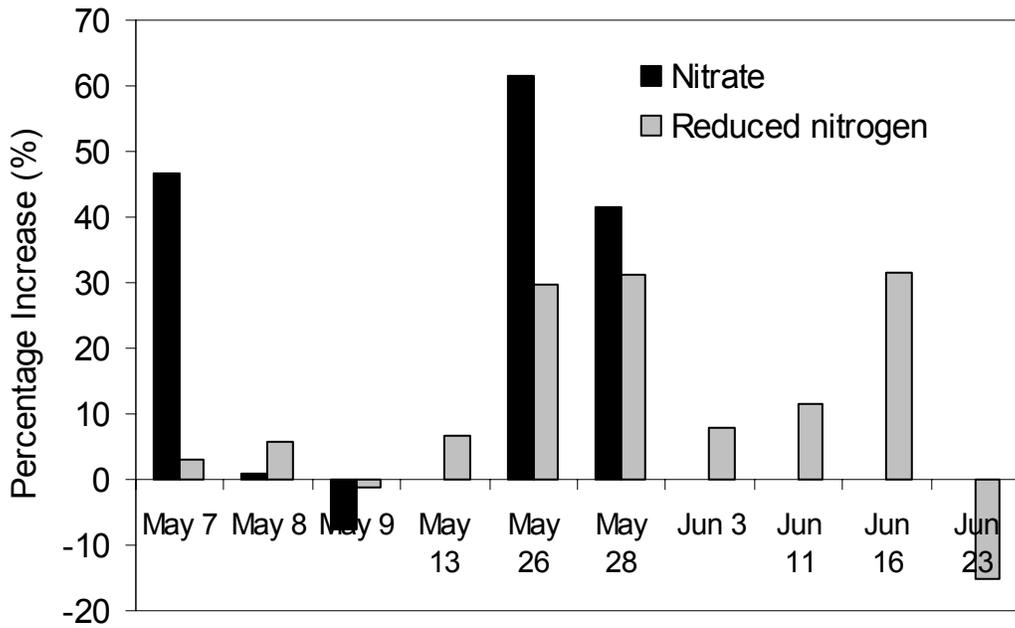


Figure 1-10 Percent increase in the rate of NO_3^- and reduced nitrogen forms (as the sum of NH_4^+ , urea and glycine) after phosphorus enrichment for 2 hours for the date shown.

off-shore (Fig. 1-11). On May 28, N uptake rates with PO_4^{3-} pre-enrichment were compared for samples incubated with P for 2 hours and overnight before N uptake rates were measured. The longer incubation time with P-enrichment yielded a significantly greater increase in the N uptake ($p= 0.044 < 0.05$, Fig. 1-12).

Discussion

Overview

The dinoflagellate blooms in the coast area of ECS in 2005 were large ($>15,000 \text{ km}^2$), and exhibited a unique succession of species compared to previous years (Tang et al. 2006). Here, the results on nutrient concentrations and N uptake rates are placed in the context of bloom succession. In addition, comparisons of N uptake rates and molar ratios are drawn with a comparative system that experiences similar diatom and dinoflagellate blooms, southwestern Florida Shelf, Gulf of Mexico (Heil et al. 2007).

Bloom Succession, nutrient availability and molar N:P ratios

In the late April in the ECS, before dinoflagellates became dominant, both DIN and PO_4^{3-} concentrations decreased gradually seaward from the CJRE, suggesting terrestrial sources of N and P at this time. During this period, although both N and P appeared to be land-derived, N was considerably higher than P, leading to a high ambient molar ratio of DIN:DIP (~ 65). *Skeletonema costatum* was the dominant species and cell densities up to 10^7 cells L^{-1} were recorded (Chiang et al. 1999). Spring diatom blooms in the early spring in the ECS have been recorded in this area since the late 20th century (Chiang et al. 1999, Chiang et al. 2004, Ishikawa & Furuya 2004). The water column of coastal China Sea is well mixed and re-suspended by the winter monsoon, tides and storms in the winter, and nutrient

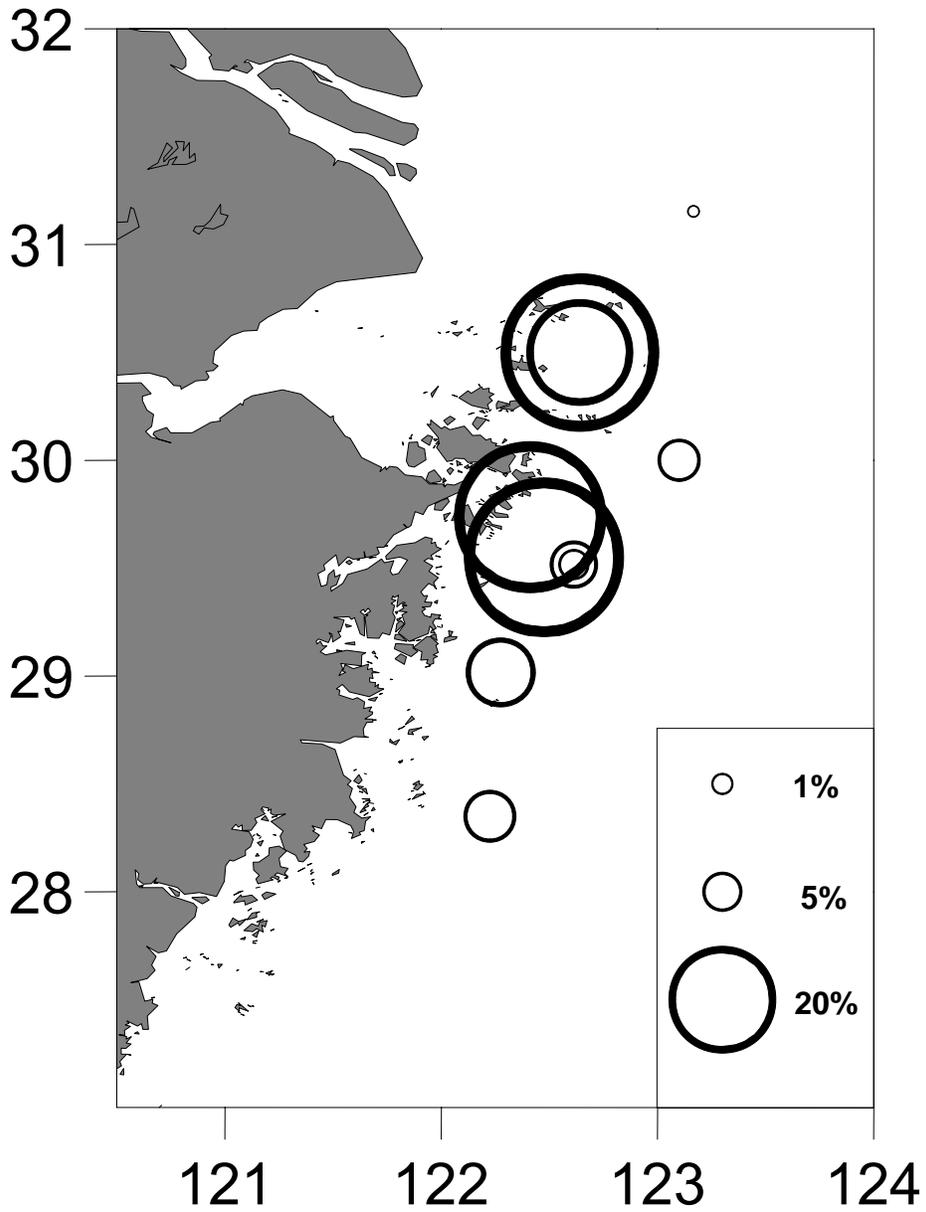


Figure 1-11 Spatial variation in the percent increase in the rate of reduced nitrogen uptake (as the sum of NH_4^+ , urea and glycine) after phosphorus enrichment for 2 hours in the bloom area from sample collected on different days as described in text

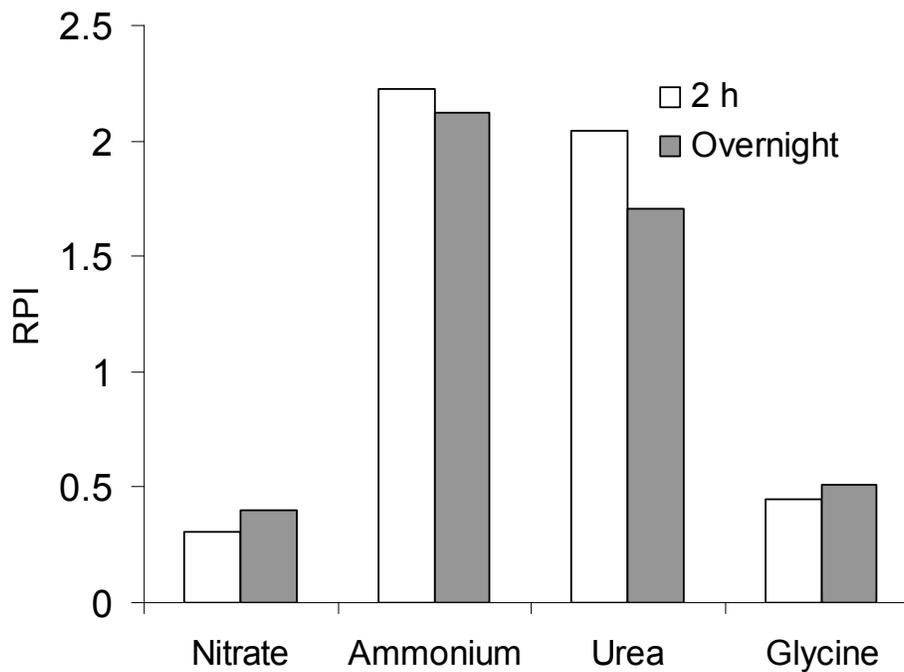
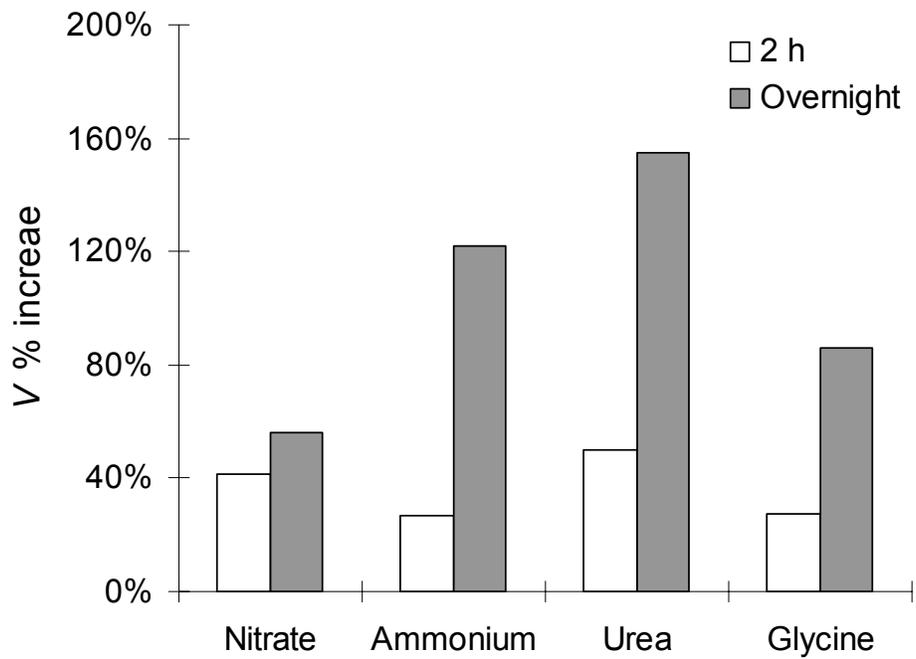


Figure 1-12 The percentage increasing of nitrogen specific uptake rates V (h^{-1}) when sample water was pre-incubated with $3 \mu\text{M PO}_4^{3-}$ for 2 hour and for 12 hour (overnight) on May 28, 2005 at station sra-1. DIN: DIP = 17.7

regeneration and demineralization are assumed to be enhanced during this period. Diatoms are thought to be the major consumer of nutrients accumulated in these areas in the early spring as the temperature begins to rise (Ishikawa & Furuya 2004). Diatoms are reported to have higher NO_3^- uptake rates than dinoflagellates at low temperature (Lomas & Glibert 2000). *S. costatum* in nutrient-replete water, particularly those in which NO_3^- is the dominant nitrogen form, is favoured and out competes *P. donghaiense* at temperatures $<15^\circ\text{C}$ (Li et al. 2003, Wang et al. 2006).

In Chesapeake Bay, USA, where diatoms typically comprise 80 - 90% of the total algae abundance during the early spring blooms, both the depletion of Si(OH)_4 and P have been suggested to be responsible for the decay of blooms (Conley & Malone 1992, Fisher et al. 1992). In Chesapeake Bay, P limitation lasts until more P-replete freshwater input in the summer is delivered, and this period is often associated with frequent blooms of *P. minimum* blooms in the upper and middle Chesapeake Bay (Tango et al. 2006). Our knowledge of relationship between spring diatom blooms with the later dinoflagellate blooms in ECS is still limited. However, similar to the nutrient dynamics in the Chesapeake Bay, after the spring diatom blooms, a P-limited (DIN: DIP ratio > 60 ; Table 1-2) condition forms. Increasing river input in late spring in the coastal ECS may result in the delivery of N in this area; however, due to the relative low P concentration in the runoff, additional P sources are important for dinoflagellate blooms to succeed. The TWWC is P-replete water current and its strength increases in the early spring and could reach the CJRE from the bottom, providing P from this off-shore source (Tang et al. 2000).

The role of this P source is supported by the spatial and temporal progression of N:P gradients, showing release of P limitation seaward (Fig. 1-13). The dinoflagellate species, *P. donghaiense*, *K. mikimotoi* and *A. tamananense* were observed to accumulate around the pycnocline in the deeper water toward the end of our first cruise and reached 10^3 cells L^{-1} .

Gyroxanthin-diester, the specific pigment indicated *K. mikimotoi* first bloomed when ambient molar DIN: DIP ratios were on the order of 60 ~ 80. Then as the blooms progressed, the DIN: DIP ratio dropped, and in fact, was ultimately lower than the Redfield ratio by the end of the *K. mikimotoi* dominance period (Table 1-2). During this progression, the corresponding molar DON: DOP range was 20 ~ 50. Peridinin, the specific pigment for *P. donghaiense* showed a different pattern of association with ambient N:P ratios. Blooms were dominant when ambient DIN: DIP ratios were around 20 and DON: DOP ratios were around 60 (Table 1-2). This progression suggests that *K. mikimotoi* proportionately depended on DIP and organic forms of N. *P. donghaiense* were more competitive than *K. mikimotoi* at DIN: DIP ratio of 16 and successfully became the dominant species. The DIN: DIP ratio remained close to the Redfield ratio during the *P. donghaiense* blooms.

Reduced N forms were preferentially taken up during the blooms. NH_4^+ was always used preferentially relative to its availability, as shown by the *RPI* calculation (Fig. 1-9). Urea was more preferentially taken up during the *K. mikimotoi* blooms, which suggests that urea was an important DON source for *K. mikimotoi*, but urea was not preferentially used during the *P. donghaiense* blooms. However, glycine was used preferentially during the *P. donghaiense* period but not during the *K. mikimotoi* period (Fig. 1-9).

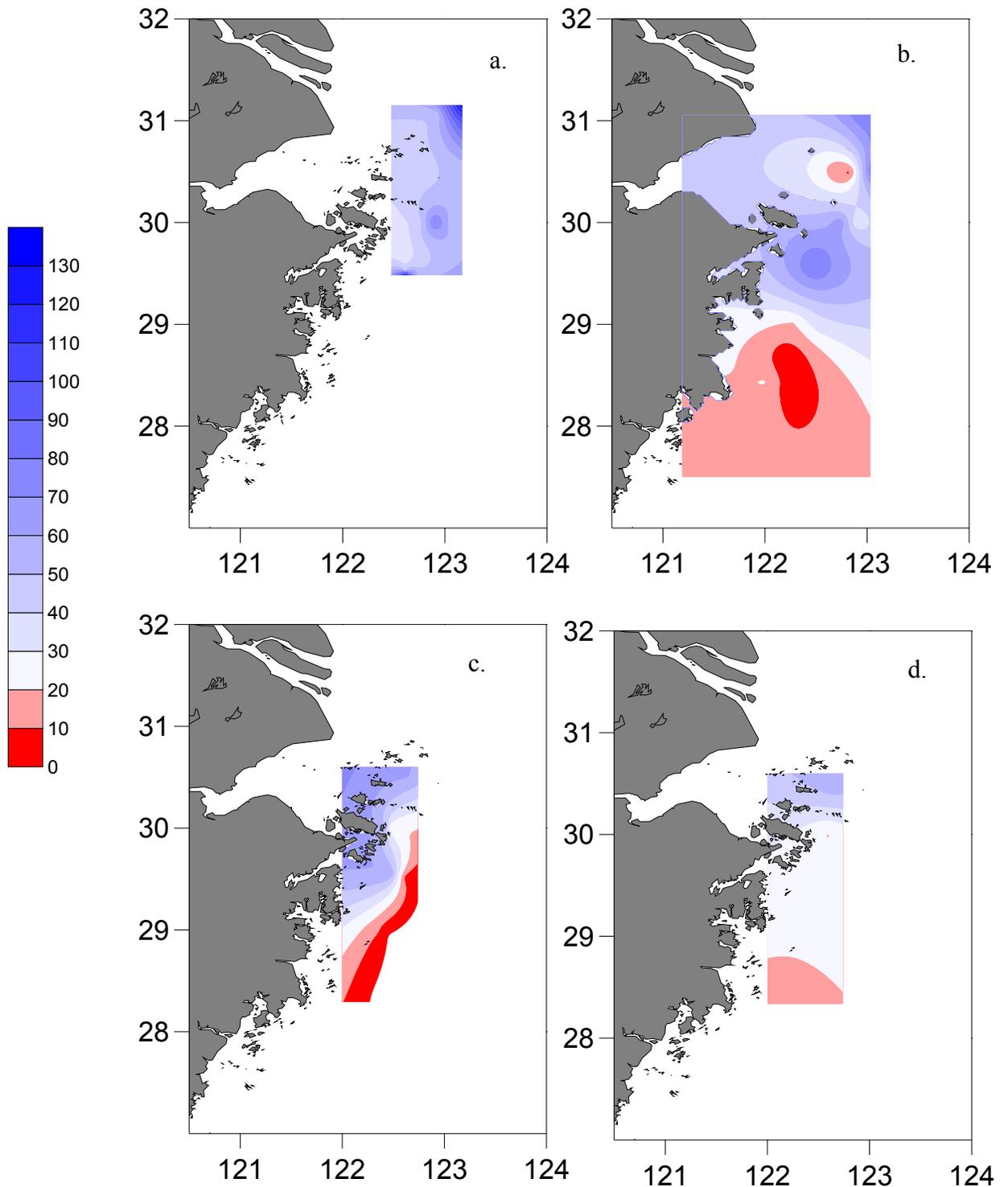


Figure 1-13 The mean ratio of DIN (NO_3^- and NH_4^+): DIP (PO_4^{3-}) in the surface water of the East China Sea during the spring 2005 time series. Panel a: Before the dinoflagellate blooms (May 7 - May 13). Panel b: during the period of *Karenia mikimotoi* dominance (May 26 - June 4). Panel c: during the period of *Prorocentrum donghaiense* dominance (June 9 - June 18). Panel d: during the bloom decline period (June 20 - June 24).

Uptake rates were strongly related to the DIN: DIP ratio in the water. With the progression of the blooms, not only did NO_3^- availability decrease, but that of NH_4^+ and urea actually increased, while DON remained roughly constant in availability (Fig. 1-2).

N-specific rates of NO_3^- uptake increased as DIN: DIP ratio increased (Fig. 1-14 a). The uptake of NO_3^- at elevated NO_3^- concentrations may be a function of both active transport and diffusion (Lomas & Glibert 2000). However, the uptake of reduced N shows a negative relationship with the DIN: DIP ratios when the ratio was larger than the Redfield ratio (Fig. 1-14 b, c, d), indicating P limitation of uptake of these substrates.

Pre-enrichment with PO_4^{3-} for 2-24 hr before measurement of N uptake rates confirmed P limitation. A longer P enrichment time resulted in even greater increases in the rates of N uptake. The percentage increase in reduced N uptake also increased during the latter stages of time series. The water column near Zhoushan archipelago were under more P stress than the off- shore area and the southern part of coastal Jejiang Province.

Relatively high abundance of *N. scintillans* developed after the *K. mikimotoi* and *P. donghaiense* blooms, although its presence was also detected following the period of diatom dominance. *N. scintillans* has been observed after diatom blooms in the Atlantic Ocean (Kiorboe et al. 1998, Tiselius & Kiorboe 1998), south coast of Australia (Dela-Cruz et al. 2002) and coast of South China sea (Huang & Qi 1997). *N. scintillans* as a eurythermal and euryhaline heterotrophic dinoflagellate could be categorized in the size range of micro- to meso- zooplankton and could play an important role in the bloom progression (Elbrächter & Qi, 1998).

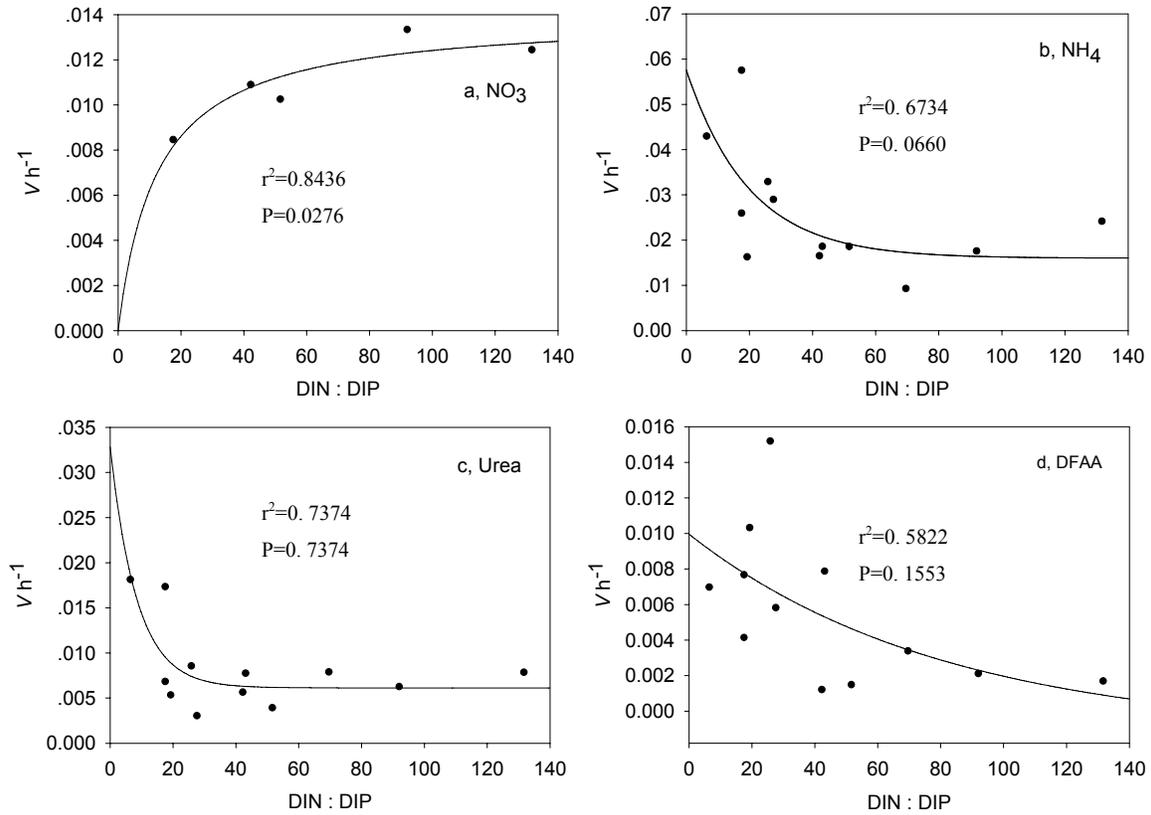


Figure 1-14 The rate of nitrogen-specific uptake rates V (h^{-1}) for each nitrogen form shown as a function of the dissolved inorganic nitrogen (DIN): dissolved inorganic phosphorus (DIP) ratio in the ambient surface water.

N. scintillans could prey on both diatoms (Tiselius & Kiorboe 1998) and dinoflagellates (Rodríguez 2005). On the other hand, *N. scintillans* can also affect zooplankton biomass by feeding on protozoa, copepods and eggs of copepod and fish (Nakamura 1998, Quevedo et al. 1999, Strom 2001). Ingestion rates of *N. scintillans* have been reported to be $0.008 \mu\text{g C cell}^{-1} \text{ day}^{-1}$ of phytoplankton, $0.008 \mu\text{g C cell}^{-1} \text{ day}^{-1}$ of zooplankton and $0.005 \mu\text{g C cell}^{-1} \text{ day}^{-1}$ of bacteria (Umani et al. 2004). These ingestion rates could be significant for the plankton community when *N. scintillans* abundance was high, since the cell densities were observed to vary from 10^4 to 10^7 cells L^{-1} , resulting in ingestion of 2 mg to 2 g $\text{C L}^{-1} \text{ day}^{-1}$ maximally.

High biomass of *N. scintillans* may also have been associated with the production of NH_4^+ in the latter stages of the time series. Their buoyancy is due to their large cell vacuole filling with NH_4^+ ions (Elbrächter & Qi, 1998). This high NH_4^+ in their biomass and its release back to the water could be important for the N budget of the bloom progression (Montani et al. 1998). Therefore, even though our knowledge of *N. scintillans* in the dinoflagellates bloom progression in coast of ECS is limited, their roles may be important in both the community construction and nutrient dynamics.

A comparison with southwestern Florida Shelf algal nutrient dynamics

During May 2005, a near-synoptic survey was conducted of the nutrients and phytoplankton on the southwestern Florida Shelf (Heil et al. 2007). During this period, phytoplankton communities were characterized as being dominated by *K. brevis*, a mixed non-*Karenia* dinoflagellates assemblage, and diatoms along different regions of the shelf. Both N:P ratios, and uptake rates of NO_3^- and urea were reported for these assemblages and

thus provide a spatial comparison to the ECS blooms which varied more temporally. Rates of NH_4^+ and glycine uptake are not available for the Florida blooms.

A direct comparison of the ambient DIN:DIP and DON:DOP ratios during both blooms shows considerable correspondence (Fig. 1-15a). In both systems, the diatom dominated community was found in association with a high ambient DIN:DIP ratio (>45), and the *Karenia* spp. dominated communities were found in association 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. In both systems also, *Karenia* spp. was observed in waters characterized by a high DON:DOP ratio (>20), but the diatom and other dinoflagellate assemblages both proliferated in even higher ambient DON:DOP regimes (Fig. 1-15b). This pattern is suggestive that *Karenia* spp. in both environments may have been consuming the organic N source.

The uptake of dissolved organic N was suggested to be important for the *K. brevis* blooms on the southwest Florida shelf (Heil et al. 2007), and previous studies have also shown that urea is used by *K. brevis* (Shimizu and Wrensford 1993, Bronk et al. 2004). In fact, the comparison of N-specific uptake rates for the *Karenia* dominated blooms in ECS and southwestern Florida shelf show nearly identical rates (Fig. 1-16). The comparison of urea uptake rates for the diatom-dominated communities and for the mixed dinoflagellate communities also reveal remarkable correspondence, but lower N-specific urea uptake rates than for *Karenia* spp. (Fig. 1-16). Thus, there appear to be similar general patterns in

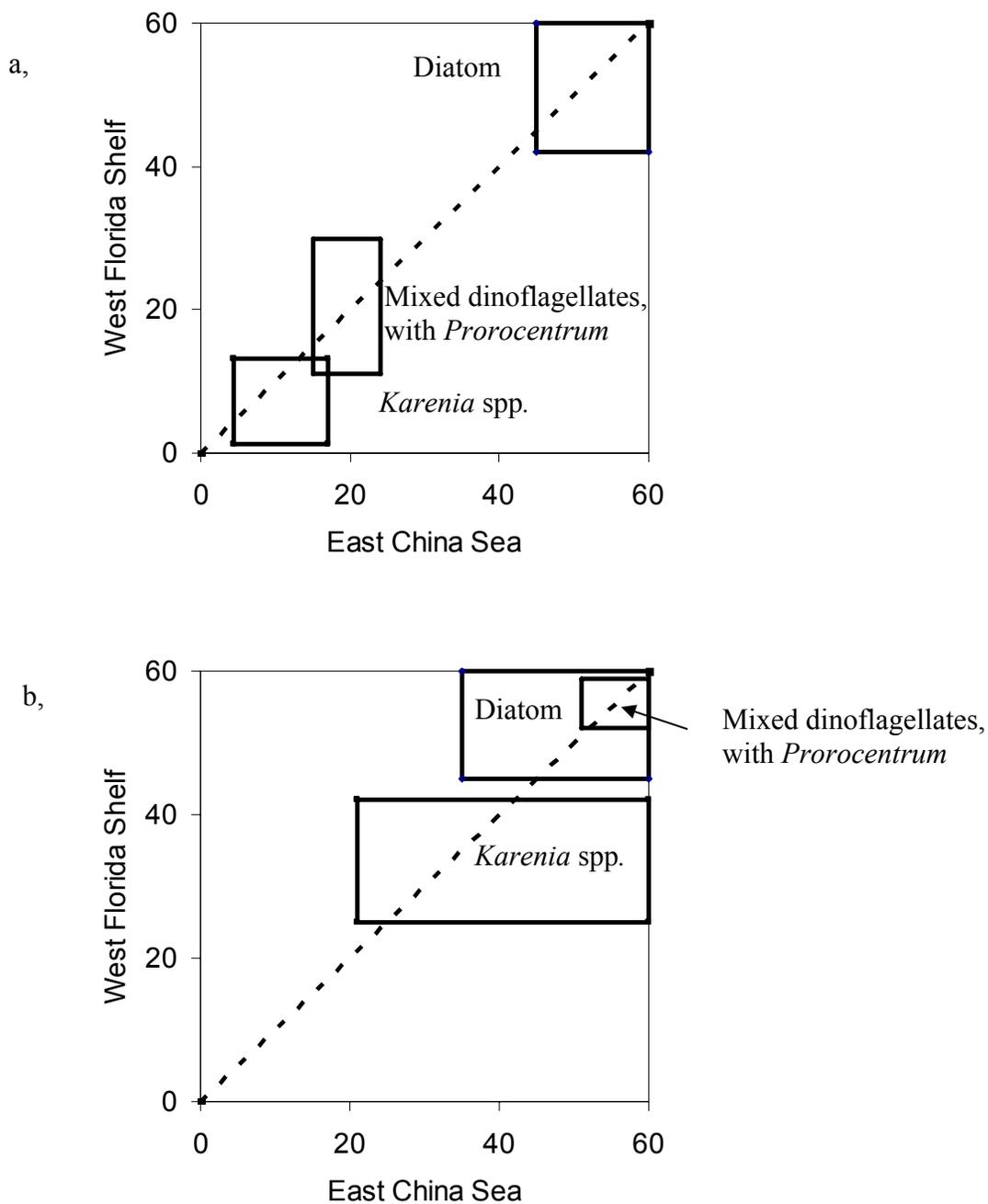


Figure 1-15 Comparison of the range in ambient nitrogen: phosphorus ratios during different phases of the blooms in the East China Sea and in different phytoplankton assemblages among the southwest Florida shelf during May 2005. Panel a: Dissolved inorganic nitrogen: Dissolved inorganic phosphorus (DIN:DIP); panel b: Dissolved organic nitrogen: Dissolved organic phosphorus (DON:DOP). The southwest Florida Shelf data are derived from Heil et al. (2007).

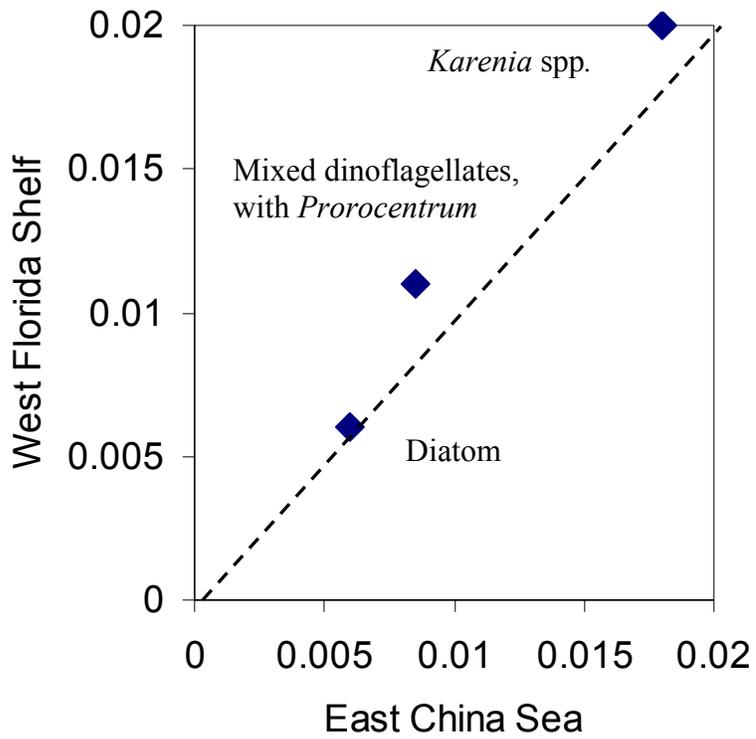


Figure 1-16 Comparison of nitrogen specific urea uptake rates ($V h^{-1}$) in different blooms in the East China Sea and different phytoplankton assemblages among southwest Florida shelf during May 2005. The southwest Florida Shelf data are derived from Heil et al. (2007).

species dominance relative to N:P ratios, and comparable N-specific uptake rates for these different systems, even though absolute biomass differed considerably.

Conclusions

In summary, the time series of the ECS showed that diatom blooms were dominant in N-replete spring conditions, but as Si and P limitation developed, *K. mikimotoi* blooms emerged. They further drew down the available DIN as well as DON. When the DIN: DIP ratio reached a level which was more close to the Redfield ratio, *P. donghaiense* became the dominant dinoflagellate. During the time series, surface water column concentrations of TDN declined, and could support less biomass. Organic nutrients (DON and DOP) were also important to the bloom progression, especially DOP in the P-limited water in the early blooms and DON in the potentially N-limited water in the late blooms. While all forms of reduced nitrogen (NH_4^+ , urea and glycine) were preferentially taken up throughout the study period, urea was more preferred during the *K. mikimotoi* blooms, while DFAA more preferred during the *P. donghaiense* blooms. N-specific rates of urea uptake in the ECS blooms were comparable to those previously reported for analogous blooms off the Florida coast (Heil et al. 2007). Nutrient regeneration was also apparently important, as increasing concentrations of NH_4^+ and urea occurred. Both stoichiometric ratios and enrichment experiments showed that P was the limiting nutrient early in the bloom progression and the N:P ratio was an important determinant for both the biomass and N uptake rates. These results also provide evidence of biological activities which tended to converge the N:P ratio to the Redfield ratio with time.

CHAPTER II

Temporal and Spatial Variability in Nitrogen Uptake Kinetics During Dinoflagellate Blooms in the East China Sea*

*To be submitted to Harmful Algae,

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Introduction

During the late spring and early summer of 2005, a series of large dinoflagellate blooms occurred off the coast of East China Sea (ECS) and adjacent area of Changjiang River (Yangtze River) Estuary (CJRE). Dominant species were the photosynthetic /mixotrophic dinoflagellates *Karenia mikimotoi*, and *Prorocentrum donghaiense*. Large scale dinoflagellate blooms, dominated by *P. donghaiense*, have been recorded in this region during the same season from the late 1990's (Zhou et al. 2003). However, 2005 was the first time that massive *K. mikimotoi* blooms (over 15,000 km²) were recorded. These blooms were also responsible for large scale fish kills (Zhou et al. 2006).

Eutrophication has been suggested to be one of the important trigger factors of algal blooms (Hallegraeff 1993, Glibert & Terlizzi 1999, Anderson et al. 2002, Glibert et al. 2005, Kemp et al. 2005, Glibert & Burkholder 2006). CJRE is the largest estuarine system of China. The terrestrial input from the river watershed is the major nitrogen source of this area, with an annual input of nitrogen of 6.3×10^6 tonne, which has roughly doubled in the past 20 years (Shen et al. 2003). As a result, the total nitrogen (mostly NO₃⁻-N) at the Changjiang River mouth is about 70 to 110 μM-N⁻¹, and this nitrogenous nutrient input has been related to the eutrophication of CJRE and the blooms forming in this area (Zhou et al. 2003, Chai et al. 2006, Glibert & Burkholder 2006).

While *P. donghaiense* and *K. mikimotoi* have been studied in both field and laboratory conditions in recent years, little is known about the nitrogenous nutrition and uptake kinetics of these species in this region. The aim of this study was to simultaneously

quantify the uptake of major nitrogen sources, as NO_3^- , NH_4^+ , urea, and glycine, and to assess the kinetics of uptake over the period of bloom progression. These results are from a series cruises during May, June and July, 2005 (Li et al. In preparation).

Materials and Methods

Sampling

This study was conducted during 3 cruises (April 23 to May 18, May 25 to June 19, June 22 to July 1) in 2005, along the coast area of Zhejiang province, China, between 21.2° N and 31.5° N (Fig. 2-1). Transects were set between the 20 m and 60 m isobaths. Sampling for this aspect of the study was conducted from May 7 to June 23, which encompassed all 3 research cruises in this area.

Water was collected around noon using 30 L Niskin bottles from 10 m and near surface, or using a clean plastic bucket for the surface sample at some stations. Water samples for nutrients and phytoplankton pigments were filtered through pre-combusted (2 h at 400 °C) Whatman GF/F filters. Both water and filters were frozen stored until analysis at Horn Point Laboratory as described below.

Nitrogen uptake experiments

The uptake of ^{15}N isotopically-labeled dissolved nitrogen substrates was used to measure the phytoplankton nitrogen uptake rates (Glibert & Capone 1993). On May 7, 8, 9, 13, 26, 28 and June 3, 9, 11, 16, 23, 2005, water was collected and transferred to a clear 20L carboy. Then, water was dispensed into acid-clean 250 ml polycarbonate incubation bottles to which ^{15}N was added.

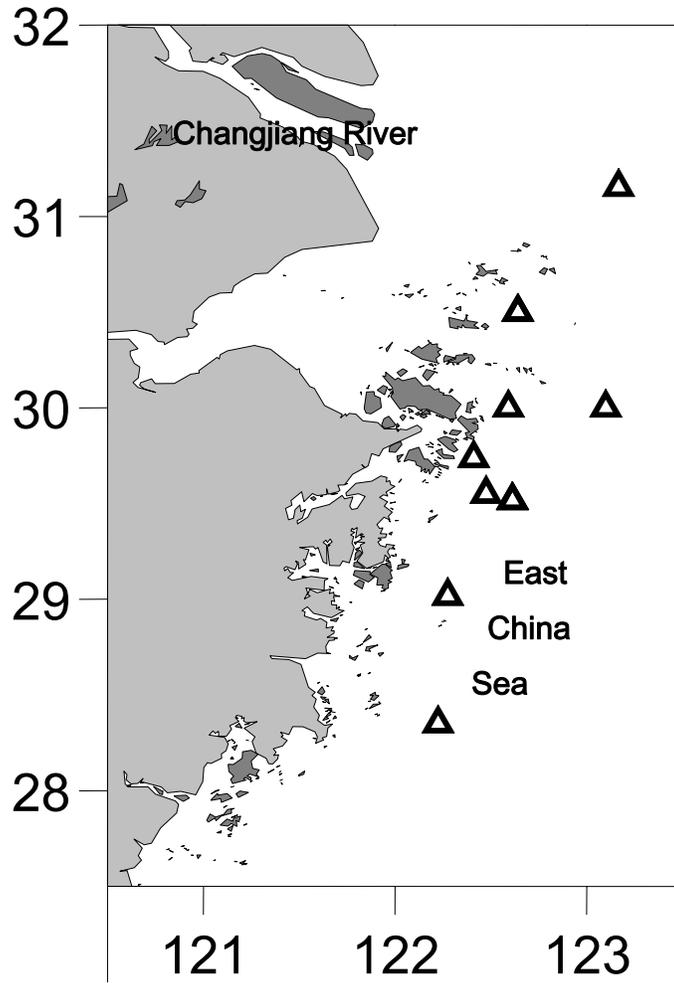


Figure 2-1 Sample sites of the nitrogen uptake experiments during the dinoflagellate bloom period in the East China Sea in 2005. The experiments were carried out from early May to late June.

^{15}N labelled NO_3^- was added in a gradient of 1, 10, 20, 50 $\mu\text{M-N}$; ^{15}N labelled NH_4^+ and urea were added in a gradient of 0.5, 1, 5, 15 $\mu\text{M-N}$; and ^{15}N labelled glycine was added in a gradient of 0.1, 0.5, 1, 5 $\mu\text{M-N}$. ^{15}N labelled substrates represented $\sim 10\%$ to 98% of final ambient concentrations. All the bottles were incubated for 0.5 hr on deck in a flowing seawater incubator, then terminated by filtration onto pre-combusted GF/F filters. The filters were stored frozen until dried at 50°C for 48 h. Due to the number of treatments, replication was not possible. However, routine analyses conducted with the same protocols and on the same instrumentation yield an average variation in ^{15}N isotope enrichment of $< 0.5\%$ in replicated samples (Glibert et al. 2006). The uptake rates of NO_3^- were not measured after May 28 due to limited supply of this isotope.

Analytical methods

All samples were transported frozen to Horn Point Laboratory and analyzed within 6 mo. Concentrations of ambient inorganic nutrients (NO_3^- -N, NH_4^+ -N, PO_4^{3-} -P, $\text{Si}(\text{OH})_4$ -Si) were determined using a Technicon AutoAnalyzer (Lane et al. 2000). Concentrations of dissolved free amino acid (DFAA) were determined by fluorometric analysis according to Lindroth and Mopper (1979) and urea concentrations were analyzed using the method of Revilla et al. (2005). DFAA concentrations were used to approximate for the glycine concentrations in uptake rate calculations, recognizing that an overestimate of substrate concentration will lead to an overestimated uptake rates. Filters for particulate carbon and nitrogen (POC and PON) were dried at 50°C for 24 hours. These filters were then measured with a CHN elemental analyzer using acetanilide as standards. Pigment analyses were performed analysis by high performance liquid chromatography (HPLC) according to the method of Van Heukelem and Thomas (2001).

All the ^{15}N sample filters were analyzed for isotope enrichment using a Sercon Mass Spectrometer. Rates of ^{15}N uptake were calculated according to Glibert and Capone (1993).

Data Analysis

For each nitrogen source, total concentrations were calculated based on the ambient N concentrations and ^{15}N addition. N-specific uptake rates were calculated according to Dugdale & Goering (1967). These nitrogen uptake parameters were plotted and fitted to the Michaelis-Menten formulation by Sigmaplot software (Systat Software, Inc.) and the curve indexes were simulated at the same time.

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

where V is the specific uptake rate (h^{-1}), V_{\max} is the maximal specific uptake rate (h^{-1}), S is the substrate concentration ($\mu\text{M-N}$) and K_s is the half-saturation constant ($\mu\text{M-N}$) for the substrate. V_{\max} and K_s of each of 4 nitrogen sources during the blooms were calculated. ρ_{\max} , the absolute uptake rate, was calculated base on the Michaelis-Menten formulation as well, and $\rho_{\max} = V_{\max} \times \text{PON}$.

Results

General Environmental Parameters

Massive dinoflagellate blooms dominated by *K. mikimotoi* were first observed in late May, 2005, near Zhoushan Archipelago ($\sim 30.7^\circ \text{N}$) outside of the CJE, and were further observed to the south along the coast to Jishan Archipelago ($\sim 28.5^\circ \text{N}$). The dominant species was gradually substituted by *P. donghaiense* from the second week of June and

blooms lasted until late June. The maximum cell density for *K. mikimotoi* was about 8×10^6 cell L^{-1} and for *P. donghaiense* was about 5×10^6 cell L^{-1} (Li et al. In preparation).

The ambient dissolved and particulate nutrient and chlorophyll *a* (Chl *a*) concentrations varied with time during the period of study (Table 2-1). Concentrations of NO_3^- were relatively high ($\sim 15 \mu M-N$) at the near-coast sites before the blooms, but became depleted as the blooms developed. On the other hand, regenerated forms of nitrogen (NH_4^+ , urea, DFAA) increased over the progression of the blooms (Table 2-1). Chl *a* concentrations were approximate $1 \mu g L^{-1}$ and increased to $65 \mu g L^{-1}$ on May 8. Both dissolved organic nitrogen (DON) and dissolved organic phosphate (DOP) concentration slightly decreased during the bloom progression.

Nitrogen Uptake Kinetics

The N-specific uptake rates (V , h^{-1}) of the four N sources as a function of substrate concentrations varied over different phases of the blooms (Fig. 2-2). The bloom phases have been grouped by time to be generally representative of the period before the dinoflagellate dominance (phase I), the period of *K. mikimotoi* dominance (phase II), the period of *P. donghaiense* dominance (phase III) and the period during bloom decline (phase IV). The relationship between V of NO_3^- and substrate concentration increased as the substrate concentration increased and did not show evidence of saturation, even at concentration of $50 \mu M-N$ (Fig. 2-2). However, the rates of N-specific uptake of NO_3^- as a function of substrate concentration were lower during phase II than during phase I. No measurements of NO_3^- uptake are available after phase II.

In contrast, all rates of N-specific uptake of the 3 forms of reduced N showed evidence of saturation even at relatively low substrate concentrations. The uptake rates at the highest concentration of the N addition gradient increased after the dinoflagellate blooms developed (Fig. 2-3). The highest N-specific uptake rates were observed for NH_4^+ and they were about twice the rates of V_{\max} for urea and glycine. All the N uptake curves were fit to the Michaelis-Menten kinetics equation. The specific and absolute maximum uptake rates (V_{\max} and ρ_{\max}) and half saturation constants (K_s) were calculated by date and bloom stage (Table 2-2).

Although the measured rates of NO_3^- uptake did not saturate over the concentration range tested, a V_{\max} can be estimated mathematically. The highest calculated V_{\max} of NO_3^- was 0.10 h^{-1} , measured before the dinoflagellate blooms. The calculated V_{\max} of NO_3^- decreased when the dinoflagellate blooms developed. The V_{\max} of NH_4^+ increased from 0.026 h^{-1} (May 7, phase I) to 0.059 h^{-1} (May 26, phase II) and then increased to 0.098 h^{-1} (June 16, phase III), and V_{\max} decreased only when the blooms decayed. A similar pattern was noticed on V_{\max} of glycine, which increased from 0.0046 h^{-1} (May 7, phase I) to 0.015 h^{-1} (May 26, phase II), and then to 0.033 h^{-1} (June 16, phase III). V_{\max} of urea ranged from 0.009 h^{-1} to 0.031 h^{-1} . Average V_{\max} of urea were higher in phase I and phase II than later, but the highest rates were recorded in phase III.

The maximum absolute nitrogen uptake rate (ρ_{\max} , $\mu\text{M-N h}^{-1}$) is a function of both V_{\max} and particulate N biomass. The highest calculated ρ_{\max} of NO_3^- before the blooms developed was $0.31 \mu\text{M-N h}^{-1}$ which was higher than the ρ_{\max} of any of 3 reduced-N sources.

Table 2- 1 Environmental data recorded at sampling stations during the 2005 blooms in the East China Sea, during the period before the dinoflagellate dominance (phase I), the period of *Karenia mikimotoi* dominance (phase II), the period of *Prorocentrum donghaiense* dominance(phase III) and the period during bloom decline (phase IV).

	DATE	Longitude	Latitude	NO ₃ ⁻ -N μM	NH ₄ ⁻ -N μM	Urea-N μM	DFAA-N μM	DON-N μM	PON-N μM	PO ₄ ³⁻ -P μM	DOP-P μM	Chl <i>a</i> μg/L
Phase I	7-May-07	122.6133	29.5167	16.56	2.08	0.49	0.30	25.26	3.07	0.36	0.42	0.59
	8-May-07	123.1	30	3.18	0.63	0.21	0.09	14.09	4.03	0.09	0.47	1.90
	9-May-07	123.1666	31.1533	6.24	1.13	0.88	0.17	10.23	3.63	0.08	0.69	1.52
	13-May-07	122.6133	29.5165	13.48	1.02	0.32	0.23	11.40	6.35	0.11	0.29	6.56
Phase II	26-May-07	122.4111	29.7363	15.82	0.92	0.16	0.42	14.86	15.08	0.24	0.40	24.63
	28-May-07	122.6408	30.4995	2.57	1.49	0.49	0.99	14.94	20.27	0.23	0.91	64.72
	3-Jun-07	122.225	28.35	0.78	1.59	0.81	0.94	18.25	39.80	0.36	0.26	39.97
Phase III	9-Jun-07	122.6417	30.5	7.85	0.80	0.28	0.51	11.25	6.64	0.20	0.17	9.68
	11-Jun-07	122.275	29.0167	1.14	2.50	0.71	0.78	17.72	11.14	0.14	0.29	8.66
	16-Jun-07	122.475	29.55	2.82	1.62	0.69	0.50	9.86	0.66	0.16	0.27	N/A
Phase IV	23-Jun-07	122.5917	30	0.53	1.80	1.39	1.55	5.78	11.66	0.12	0.22	N/A

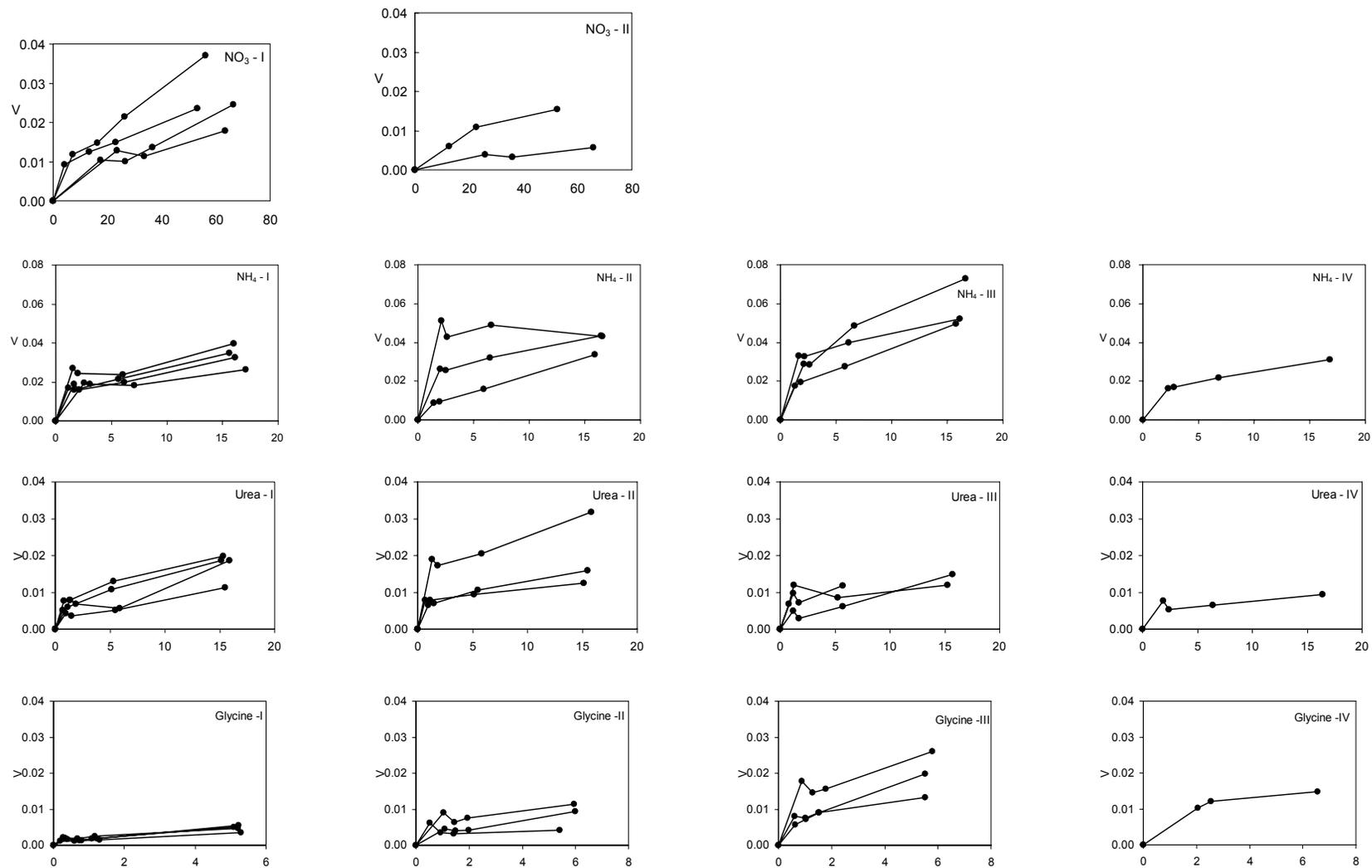


Figure 2-2 Kinetic relationships between nitrogen-specific uptake rates V (h⁻¹) of NO₃⁻, NH₄⁺, urea, and glycine and final substrate concentration (μM-N), before the bloom (I), during the *Karenia mikimotoi* bloom (II) and *Prorocentrum donghaiense* bloom (III), and decay phase of bloom (IV).

In phase II, ρ_{\max} of NO_3^- increased to $0.54 \mu\text{M-N h}^{-1}$ on May 28, which was lower than ρ_{\max} of NH_4^+ at that time. ρ_{\max} of the reduced N substrates were related to the phytoplankton biomass during the bloom progression. In phase I, the highest ρ_{\max} of NH_4^+ was $0.23 \mu\text{M-N h}^{-1}$, that for urea was $0.13 \mu\text{M-N h}^{-1}$, and that for glycine was $0.058 \mu\text{M-N h}^{-1}$. The highest ρ_{\max} of all 3 reduced N sources was observed on June 3 when the highest level of Chl *a* was recorded. At that time, ρ_{\max} of NH_4^+ was $1.83 \mu\text{M-N h}^{-1}$; ρ_{\max} of urea was $1.22 \mu\text{M-N L}^{-1} \text{ h}^{-1}$; and ρ_{\max} of glycine was $0.48 \mu\text{M-N h}^{-1}$. In phase III, the highest observed ρ_{\max} of NH_4^+ decreased to $0.59 \mu\text{M-N h}^{-1}$; that of urea decreased to $0.13 \mu\text{M-N h}^{-1}$; and that of glycine decreased to $0.32 \mu\text{M-N h}^{-1}$.

Since the measured rates of NO_3^- uptake did not saturate over the concentration range tested, the K_S was also estimated mathematically to be relatively high values. K_S values of NO_3^- uptake were in range of 30 to 60 $\mu\text{M-N}$ in most stations, with a maximum value over 200 in the first experiment of May 7th, when the uptake rates curve showed almost linear with a high slope. There were also high K_S values of NH_4^+ uptake (12.7, 6.1 $\mu\text{M-N}$) in the bloom phase, when uptake also did not saturate, but mostly K_S values of NH_4^+ were around 1, especially during the pre-bloom phase. K_S values of urea decreased from an average of around 5 $\mu\text{M-N}$ before the bloom to lower than 1 $\mu\text{M-N}$ after bloom developed. Urea uptake did not saturate on June 16th. K_S values of glycine were lower than 1 $\mu\text{M-N}$ at most stations with a maximum value of 4 $\mu\text{M-N}$.

During the bloom progression, there were higher rates of V_{\max} of NO_3^- at all stations before May 15, when the dinoflagellate blooms had not yet developed. When the blooms were

observed, after May 26, the rates of V_{\max} of NO_3^- had decreased and were relatively low compared to the rates of V_{\max} of reduced-N (Fig. 2-3). The pattern of V_{\max} of NH_4^+ and glycine increased until the blooms decayed. The pattern of V_{\max} with time of urea decreased but not significantly. All the rates of ρ_{\max} increased when the blooms developed but dropped in the period of bloom decay (Fig. 2-3). Spatially, V_{\max} values were higher at the station close to the Zhoushan archipelago, and lower at seaward stations, and ρ_{\max} values were higher in the southern sample stations (Fig. 2-4).

Discussion

Both *Karenia* spp. and *Prorocentrum* spp. are world-wide, bloom-forming harmful dinoflagellates (Leblond & Chapman 2002, Heil et al. 2005, Glibert et al. 2008, Redalje et al. 2008). Many ecological and physiological models have been developed to better understand bloom dynamics (Maier & Dandy 1997, Thompson 1998, Cloot & Pieterse 1999, Walsh et al. 2001, Hood et al. 2006). The soundness of these models depends on sufficient parameterization of both environmental and physiological variables. Nitrogen uptake rates are important parameters that have not been available for these species in this region to date.

The average V_{\max} values for different N sources were similar in scale even when the dominant species varied (Table 2-2 b). However, V_{\max} of NO_3^- decreased when blooms developed, and V_{\max} of NH_4^+ and glycine increased during the blooms progression, indicating strong demand for the regenerated nitrogen sources, especially when NO_3^- became depleted early in the development stage of the dinoflagellate blooms. V_{\max} of urea was relatively constant before and in the bloom period (0.014 – 0.031 h^{-1}).

Table 2- 2 a, Calculated parameters of uptake kinetics of four nitrogen sources (NO_3^- , NH_4^+ , urea, and glycine) by natural phytoplankton assemblages in the East China Sea coast, based on the Michaelis-Menten equation. V_{\max} and ρ_{\max} are the specific and absolute nitrogen uptake rates. K_s is the Michaelis constant where uptake rates equal to $\frac{1}{2}$ of maximum uptake rates. b, the V_{\max} in each stage of the bloom are given as mean (\pm S.E). Experiments were during the period before the dinoflagellate dominance (phase I), the period of *Karenia mikimotoi* dominance (phase II), the period of *Prorocentrum dinghaiense* dominance (phase III) and the period during bloom decline (phase IV).

a.

	DATE	NO_3^- -N				NH_4^+ -N				Urea-N				Glycine-N			
		V_{\max} h^{-1}	K_s μM	ρ_{\max} $\mu\text{M h}^{-1}$	Rsqr	V_{\max} h^{-1}	K_s μM	ρ_{\max} $\mu\text{M h}^{-1}$	Rsqr	V_{\max} h^{-1}	K_s μM	ρ_{\max} $\mu\text{M h}^{-1}$	Rsqr	V_{\max} h^{-1}	K_s μM	ρ_{\max} $\mu\text{M h}^{-1}$	Rsqr
Phase I	7-May-07	0.100	209.5	0.31	0.97	0.026	1.11	0.08	0.94	0.014	5.54	0.04	0.87	0.0046	1.67	0.014	0.87
	8-May-07	0.028	14.7	0.11	0.94	0.036	1.86	0.14	0.92	0.022	3.62	0.09	0.96	0.0078	2.98	0.032	0.97
	9-May-07	0.076	60.4	0.27	0.97	0.033	1.89	0.12	0.90	0.027	9.22	0.10	0.73	0.0054	0.90	0.019	0.88
	13-May-07	0.025	29.0	0.16	0.97	0.036	0.77	0.23	0.87	0.021	2.07	0.13	0.96	0.0091	3.45	0.058	0.90
Phase II	26-May-07	0.010	50.2	0.15	0.96	0.059	12.67	0.89	0.97	0.012	0.42	0.17	0.95	0.0044	0.30	0.066	0.98
	28-May-07	0.027	37.0	0.54	0.99	0.046	1.84	0.93	0.95	0.017	2.09	0.34	0.96	0.0153	3.95	0.310	0.95
	3-Jun-07					0.046	0.12	1.83	0.99	0.031	1.20	1.22	0.91	0.0122	0.78	0.484	0.88
Phase III	9-Jun-07					0.060	4.41	0.40	0.95	0.011	0.25	0.07	0.85	0.0144	0.70	0.096	0.96
	11-Jun-07					0.053	1.22	0.59	0.68	0.013	0.62	0.14	0.92	0.0286	0.94	0.319	0.92
	16-Jun-07					0.098	6.08	0.44	0.99	0.028	14.95	0.13	0.91	0.0327	3.62	0.145	0.99
Phase IV	23-Jun-07					0.035	3.15	0.41	0.98	0.009	0.81	0.10	0.87	0.0183	1.46	0.213	1.00

b.

V_{\max}	NO_3^- -N	NH_4^+ -N	Urea-N	Glycine-N
I	0.057 (0.037)	0.032 (0.05)	0.021 (0.005)	0.007 (0.002)
II	0.018 (0.012)	0.050 (0.08)	0.020 (0.010)	0.011 (0.006)
III	N/A	0.070 (0.024)	0.017 (0.009)	0.025 (0.010)
IV	N/A	0.035	0.009	0.018

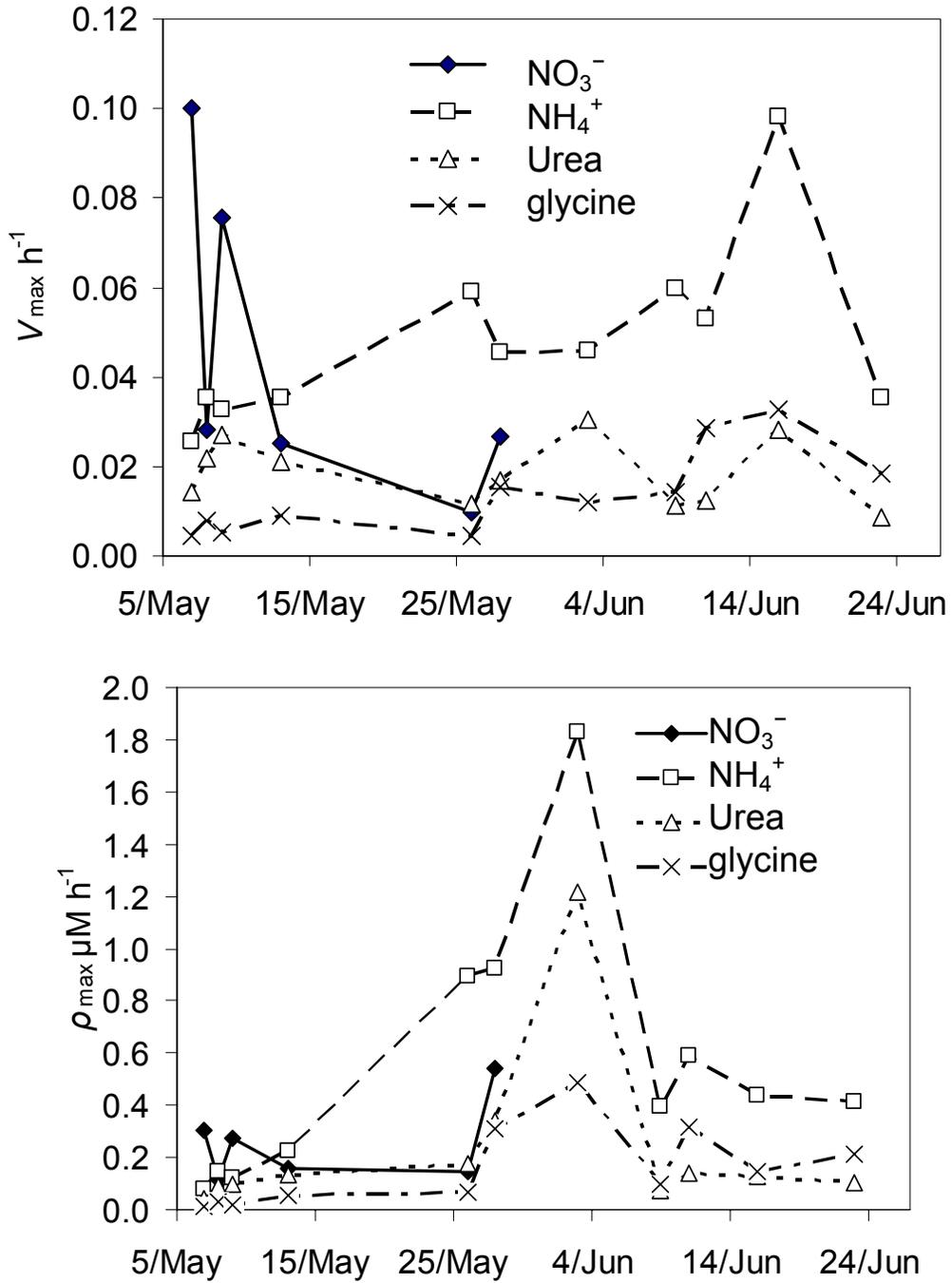


Figure 2-3 The maximum specific uptake rates of N (V_{\max} , h^{-1}) and absolute N uptake rates (ρ_{\max} , $\mu\text{M-N h}^{-1}$) of four nitrogen sources (NO_3^- , NH_4^+ , urea, and glycine) in the water column during the bloom progression in East China Sea, 2005.

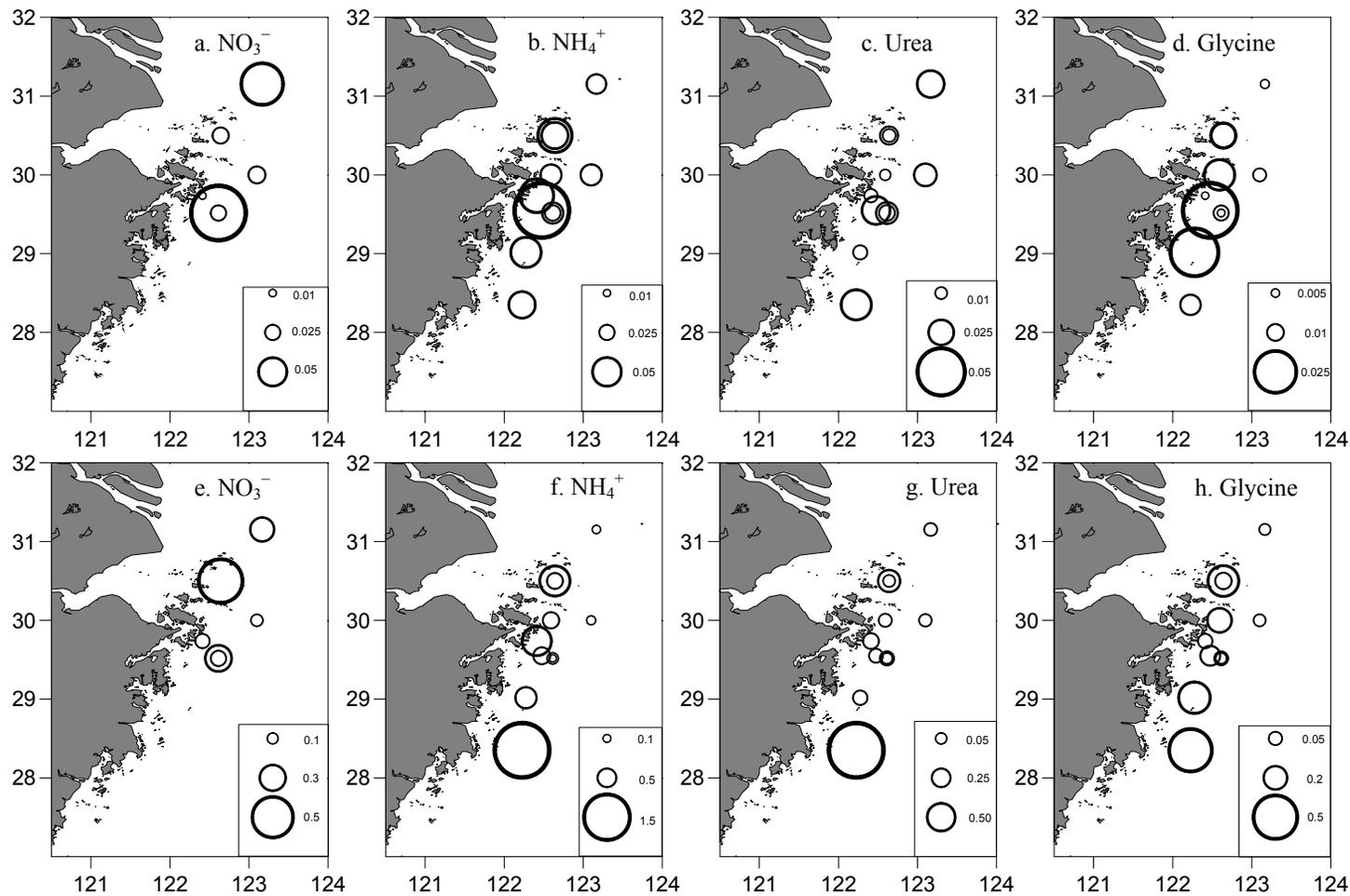


Figure 2-4 The spatial maximum specific uptake rates of N (V_{\max} , h^{-1} ; a, b, c, d) and absolute N uptake rates (ρ_{\max} , $\mu\text{M-N h}^{-1}$ e, f, g, h) of four nitrogen sources (NO_3^- , NH_4^+ , urea, and glycine) in the water column in bloom area from sample collected on different days as described in text.

Maximum uptake rates and half-saturation constants (K_s) of ECS coast were compared to other coastal regions (Table 2-3) as different phases of the bloom progression. Phase I was a period when phytoplankton assemblages switched from being diatom (*Skeletonema costatum*) dominated to dinoflagellate dominated (*K. mikimotoi* and *P. donghaiense*). Rates of V_{\max} of NO_3^- during this phase (0.029 - 0.10 h^{-1}) were similar to those measured for a *S. costatum* culture (0.10 h^{-1}) (Lomas & Glibert 2000), *Lingulodinium polyedrum* in southern California coast (0.022 h^{-1}) (Kudela & Cochlan 2000), *Karenia brevis* in the Gulf of Mexico (0.05 h^{-1}) (Bronk et al. 2004) and *Prorocentrum minimum* in the Choptank River, USA (0.012 h^{-1}) (Fan et al. 2003). Also, V_{\max} of urea (0.017 - 0.020 h^{-1}) and amino acid glycine (0.025 h^{-1}) were similar to *K. brevis* in the Gulf of Mexico (V_{\max} of urea 0.020 h^{-1} ; V_{\max} of amino acid glutamate 0.030 h^{-1}) (Bronk et al. 2004), and also in the lower range of Choptank River (V_{\max} of urea: 0.016- 0.090 h^{-1} ; V_{\max} of amino acid: 0.016- 0.36 h^{-1}) (Fan et al. 2003). However, V_{\max} of NH_4^+ (0.5 h^{-1}) in ECS was about 10 times higher than the Gulf of Mexico (0.055 h^{-1}) (Bronk et al. 2004) and southern California coast (0.04 h^{-1}) (Kudela & Cochlan 2000), but the similar to the values in Choptank River (0.20 h^{-1}) (Fan et al. 2003).

Rates of ρ_{\max} of NO_3^- , NH_4^+ , and urea (0.21, 0.14, 0.09 $\mu\text{M-N h}^{-1}$) in the diatom dominated phase in ECS were very similar to that of diatom sample in Chesapeake Bay (0.21, 0.12, 0.05 $\mu\text{M-N h}^{-1}$) (Lomas & Glibert 1999). ρ_{\max} and K_s of regenerated N during the bloom period in ECS, with the exception of the very high K_s for NH_4^+ found on May 26th, were also similar with that of a mixed dinoflagellates bloom with *P. minimum* in the

Table 2- 3 Summary of maximum nitrogen specific uptake rates (V_{\max} , h^{-1}), absolute nitrogen uptake rates (ρ_{\max} , $\mu\text{M h}^{-1}$) and half-saturation constants (K_s) of four nitrogen sources (NO_3^- , NH_4^+ , urea, and amino acid) determined for natural phytoplankton assemblages from coastal regions.

Area	Bloom species	NO_3^-			NH_4^+			Urea			Amino Acid			Source
		V_{\max}	ρ_{\max}	K_s	V_{\max}	ρ_{\max}	K_s	V_{\max}	ρ_{\max}	K_s	V_{\max}	ρ_{\max}	K_s	
East China Sea coast	diatom and dinoflagellate mixture, no bloom	0.057	0.21	78.4	0.32	0.14	1.41	0.021	0.091	5.12	0.007	0.031	2.25	This study
	<i>Karenia mikimotoi</i>	0.018	0.34	43.6	0.50	1.22	4.88	0.020	0.578	1.35	0.011	0.28	1.68	
	<i>Prorocentrum donghaiense</i>				0.70	0.47	3.90	0.017	0.113	5.273	0.025	0.19	1.76	
Culture	<i>Skeletonema costatum</i>	0.10	0.16	0.4										(Lomas & Glibert 2000)
Chesapeake Bay	Mixed diatoms with <i>Skeletonema costatum</i>		0.21			0.12			0.05					(Lomas & Glibert 1999b)
Gulf of Mexico	<i>Karenia brevis</i>	0.05			0.055			0.020			0.030			(Bronk et al. 2004)
Choptank River	<i>Prorocentrum minimum</i>	0.008-0.012	0.38-2.1	1.4-7.8	0.08-0.20	4.9-14.7	2.4-9.8	0.016-0.09	1.2-4.4	6.6-17.8	0.016-0.36	2.2-24.4	4.8-26.6	(Fan et al. 2003)
Neuse Estuary	dinoflagellates with <i>Prorocentrum minimum</i>		0.22	0.54		3	4.9		0.3	0.4		0.12	2.26	
Southern California coast	<i>Lingulodinium polyedrum</i>	0.022		0.47	0.04		0.59	0.062		0.99				(Kudela & Cochlan 2000)
Washington coast		0.0058		0.05	0.0068		0.71	0.0046		0.78				(Dortch & Postel 1989)

Neuse River, US in January (Fan et al. 2003). Fan et al. (2003) reported that ρ_{\max} of NH_4^+ were all about $1\sim 3 \mu\text{M-N h}^{-1}$; ρ_{\max} of urea were about $0.1 \sim 0.5 \mu\text{M-N h}^{-1}$; ρ_{\max} of glycine were about $0.12 \sim 0.28 \mu\text{M-N h}^{-1}$. Uptake rates were lower than that of a spring *P. minimum* bloom in the Choptank River (Fan et al. 2003). All three of these regions have very high regenerated N uptake rates compared to NO_3^- .

NO_3^- uptake rates did not show evidence of saturation in our range of experimental gradients, which resulted in very high saturation concentrations and high K_s values of NO_3^- . K_s of NO_3^- decreased in phase II. The non-saturation uptake of NO_3^- by diatom has been observed in both field studies (Lomas & Glibert 1999) and culture experiments (Lomas & Glibert 2000), and suggested to be the result of diffusion uptake at high substrate concentration, and the uptake might only fit Michaelis-Menten kinetics in a relatively low concentration range (Lomas & Glibert 2000). However, the slopes of increasing of NO_3^- uptake rates as function of substrate concentration were lower in dinoflagellate bloom period than diatom dominated pre-bloom phase. This decrease of slope could be temperature regulated as diatoms tend to have faster NO_3^- uptake rates at low temperature in early spring (Lomas & Glibert 1999). On the other hand, NH_4^+ , urea and glycine all approached uptake saturation (V_{\max}) and fit Michaelis-Menten kinetics as a result of carrier-mediated transfer uptake, except that uptake of NH_4^+ did not saturate over the concentration range tested on the first sampling day in bloom phase, when K_s value over 12 was estimated. In general, NH_4^+ , urea and glycine all have low K_s , which could be an important adaptation for the uptake of these substrates which were low in most of the water column.

When the ambient N concentration is close to, or higher than K_s , it indicates the phytoplankton assemblages may take up the nutrients sources at rates that approach the maximum rates. Such an ambient environment should be favorable for the uptake of nutrients and favorable for phytoplankton growth. The K_s of each study site was compared with the ambient nutrient concentration (S) and used to calculate the $S: K_s$ ratio (Fig. 2-5). NO_3^- concentration values were much lower than the K_s of NO_3^- uptake ($S: K_s < 0.1$) during all phases of the blooms, which suggested even though NO_3^- was the major N source in the water column, its uptake rate was lower than the calculated V_{\max} . An actual V_{\max} for NO_3^- was not attained due to incomplete saturation. On the other hand, the ambient NH_4^+ concentrations were closer in value to K_s or even higher than K_s ($S: K_s$ 1.8 to 4 in phase I and 0.8 to 1.4 in phase II), indicating that NH_4^+ uptake was likely operating close to V_{\max} . However, in the late phase of blooms, this ratio decreased slightly to 0.2 to 0.6, indicating lower rates of NH_4^+ uptake relative to V_{\max} . Urea and DFAA concentrations were also much lower than the corresponding K_s value before the bloom developed ($S: K_s < 0.2$). These ratios increased when blooms developed, especially in phase III when the ratios were larger than 1.

The diversity of $S: K_s$ ratios among different N sources and different phase of the blooms indicated a relationship among ambient nutrient concentrations, uptake rates and bloom species. NO_3^- was the major N nutrient in the water before the blooms, when diatoms dominated. Although NO_3^- concentration was not high enough to be taken up at V_{\max} by the dinoflagellates during the early phase of their development, it was still important and contributed significantly to total N uptake. Only NH_4^+ ambient concentration was

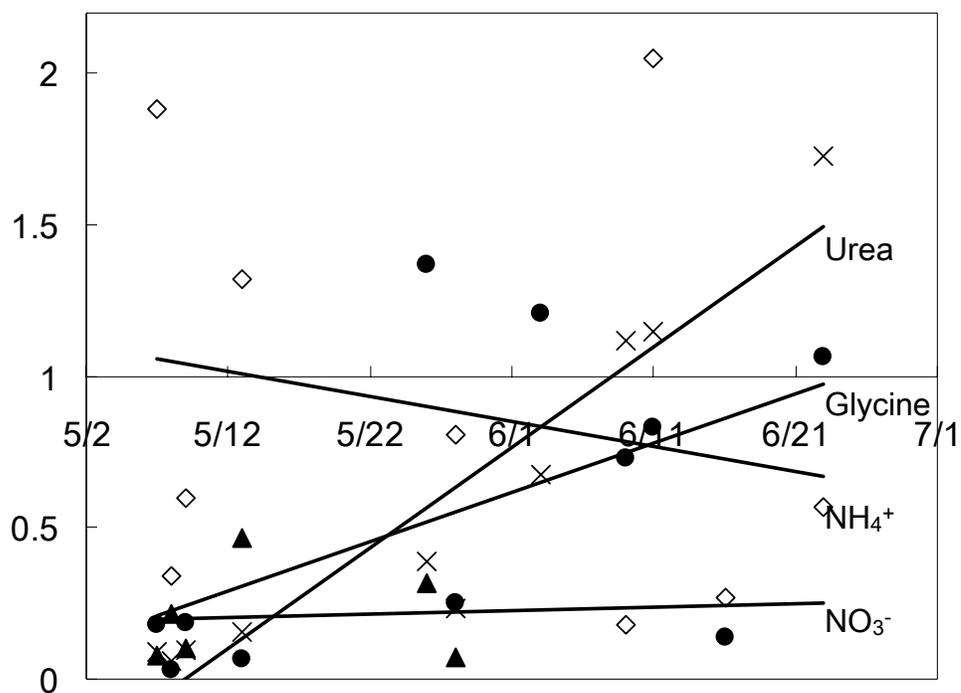


Figure 2-5 Ratio of 4 ambient nitrogen nutrients (NO_3^- (▲), NH_4^+ (◇), urea (x) and dissolved free amino acid (•)) to the correspond uptake Michaelis constant K_s , where uptake rates equal to $\frac{1}{2}$ of maximum uptake rates, and their trend lines during dinoflagellate bloom period in the East China Sea, 2005.

sufficiently high at some stations and close to K_s before the bloom developed. Species which had a low K_s took up NH_4^+ near V_{max} and contributed to the development of the blooms. During the bloom progression, a substantial quantity of regenerated N was released to the water, especially DON, which apparently contributed to the increasing concentrations of urea and DFSA. When concentrations of urea and glycine approached the K_s , the bloom algae took up urea and DFSA at high rates and these substrates became important for the persistence of the blooms. Therefore, $S:K_s$ ratios may be a better index to estimate of sufficiency of specific nutrients, than the absolute concentration of the nutrients in the water column in the bloom progression. The ambient NH_4^+ concentration was high enough to support a high uptake rate of in early phase of the blooms, however, the ambient DON concentration was high to enough support a high uptake rate DON after bloom developed. *K. mikimotoi* and *P. donghaiense* have been thought to have different nitrogen preferences (Li et al. In preparation). The variety of N sources and their variable availability in the ambient water in relation to the preferences of individual species under specific environmental conditions may contribute to the succession of dominant species.

CONCLUSIONS

The experiments were conducted to test the following hypotheses:

- (1). P limitation develops in the CJRE from excess N loading, but P is made available from adjacent coastal water and its availability changes the N:P ratio and leads to blooms;
- (2). Dinoflagellates depend on reduced N sources (NH_4^+ , urea, amino acids) over NO_3^- as their N source.

The results of this work confirmed the hypotheses:

High inorganic nitrogen, but low phosphorus loading from the Changjiang River estuary led to high DIN: DIP ratios in the sampling area and indicate the development of phosphorus limitation. The results of ^{15}N uptake experiments also suggested P limitation of the N uptake rates. This P limitation was at least partially released by high rates of NO_3^- uptake by spring diatom blooms by depleting the ambient NO_3^- . A DIN:DIP gradient was recorded in which the DIN:DIP ratio decreased from the Changjiang river mouth to the southern Zhejiang coast. This gradient was consistent with the pathway of Taiwan Warm Current (TWWC) which was suggested to be an important P source for this area. Dinoflagellate blooms occurred in a relative short time period (about one month) after the spring diatom bloom in 2005. The regenerated N and transported P from coastal water were important for the dinoflagellates blooms.

DIN: DIP ratio showed to be an important determinant for the dinoflagellate blooms in study area. Dinoflagellate biomass, dominant species and N uptake rates all showed strong

relationships with the DIN: DIP ratio. The adaptation of *K. mikimotoi* and *P. donghaiense* to N:P ratios was different. *K. mikimotoi* bloomed in high DIN:DIP ratio water and drew down DIN:DIP ratio by proportionately more DIN utilization, then *P. donghaiense* bloomed and over grew *K. mikimotoi* at DIN:DIP ratio close to the Redfield ratio and became the dominant species. Relatively high organic nitrogen levels, and lower DIN:DIP ratio led to a succession of dinoflagellates.

Nitrogen uptake rates were also related to DIN: DIP ratio. NO_3^- uptake rates increased with DIN: DIP ratios, however, uptake rates of reduced N decreased with increasing DIN: DIP ratios.

The result of ^{15}N uptake take experiments also showed the dinoflagellates preferred reduced forms of nitrogen, but in somewhat different proportions. *K. mikimotoi* had higher rates of urea uptake, while *P. donghaiense* had higher rates of glycine uptake. Both had high rates of ammonium uptake. These differences might be because of the availability of these two nutrients varied during the phases of the bloom.

These results suggest that there may be general relationships between these blooms species and the availability of different nutrients and the N:P ratio. To more firmly estimate these relationships, additional laboratory experiments and more comparisons among different ecosystems will be required. Future work should be focus on the nitrogen uptake rates and preference under different N: P ratios and the community competition in such environment. The role of autotrophic/ mixotrophic strategy played by dinoflagellates during the bloom

succession should be studied. Modeling work based on the N:P ratio and nutrient uptake rates indices could also be developed.

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