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The role of nitrogenous nutrients in the occurrence of the harmful dinoflagellate blooms caused by *Cochlodinium polykrikoides* in Long Island estuaries (NY, USA)

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Abstract of the Thesis

The role of nitrogenous nutrients in the occurrence of the harmful dinoflagellate blooms caused by *Cochlodinium polykrikoides* in Long Island estuaries (NY, USA)

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The harmful dinoflagellate *Cochlodinium polykrikoides* is well known for forming ichthyotoxic blooms in coastal regions of Asia and North America, but the nutritional factors supporting and promoting these blooms have not been well studied. To better understand the nutritional ecology of the harmful dinoflagellate blooms caused by *Cochlodinium polykrikoides* in Long Island estuaries (NY, USA), laboratory and field studies of this species were conducted. I documented the spatial and temporal dynamics of nutrients, *C. polykrikoides* cells, and co-occurring phytoplankton within two New York estuaries from 2006 - 2008. I quantified the growth response of *C. polykrikoides* and co-occurring phytoplankton during experimental enrichment of different nitrogen sources. Furthermore, I quantified growth rates of *C. polykrikoides* clonal isolates on a variety of nitrogen sources (urea, ammonium, glutamic acid, nitrate) and over a range of concentrations (2-200 μM). Finally, I quantified the uptake rates of various N compounds in both field and laboratory conditions using ^{15}N-enriched compounds. *C. polykrikoides* cultures grown on glutamic acid displayed significantly faster growth and N-uptake rates compared to cultures grown on urea, ammonium, and nitrate. From 2006 – 2008, blooms of *C. polykrikoides* occurred in regions with a variety of N concentrations, but were only monospecific (in the >20 μm size range) when concentrations of nitrate and ammonium were < 2 μM. During blooms, the addition of a variety of N compounds (urea, ammonium, glutamic acid, nitrate) significantly increased the growth of *C. polykrikoides* more frequently than other phytoplankton groups suggesting blooms were N-limited. Finally, the dominant N compounds assimilated by bloom communities differed among sites, with nitrate and nitrite being taken up fastest at the most eutrophic locations and urea and glutamic acid being assimilated quickest at mesotrophic sites. The sum of these observations suggests that *C. polykrikoides* is a nutritionally flexible species, capable of assimilating a variety of N compounds, with the compound yielding maximal growth or uptake depending on prevailing nutrient

conditions. Results further suggest monospecific blooms may be promoted by modest levels of labile N compounds (2 – 10 µM).

Dedication

This thesis is dedicated to my parents, Jim and Jane Burson, for their unfaltering belief in my dreams.

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INTRODUCTION:

Harmful algal blooms (HABs) are a significant threat to fisheries, public health, and economies around the world. HABs are classified harmful for a suite of reasons including the ability of many HAB-forming dinoflagellates to produce potent biotoxins which can sicken or kill humans. While many HABs do not directly harm marine life, others can be lethal to marine animals (Landsberg, 2002; Sunda et al. 2006). In Asian waters, the red tide forming dinoflagellate *Cochlodinium polykrikoides* is well known for its harmful effects on marine organisms (Yuki and Yoshimatsu, 1989; Yamatogi et al., 2002; Huang and Dong, 2000; Lee, 2006; Kim, 1998). *Cochlodinium polykrikoides* and other closely related species in the genus are catenating dinoflagellates with cells which are approximately 20 μm in size, athecate, and known to perform diel vertical migration (Kudela et al., 2008). The life history of *C. polykrikoides* has not been well studied but Kim et al. (2002) have found cells in culture can form resting cysts. Several studies have demonstrated the fish killing capabilities of *Cochlodinium sp.* (Onoue et al., 1985; Yuki and Yoshimatsu, 1989; Guzmán et al., 1990; Qi et al., 1993; Gárrate-Lizárraga. et al., 2004; Whyte et al., 2001; Kim et al., 1999; Gobler et al., 2008) and *C. polykrikoides* blooms have been responsible for hundreds of millions of USD in fisheries losses in Korea alone (Kim, 1998).

Cochlodinium polykrikoides blooms have also occurred in many locations across North America. This species was first identified in Phosphorescent Bay, Puerto Rico by Margalef (1961). At Vancouver Island, British Columbia fishery losses exceeding $3 million USD were attributed to a 1999 bloom of *C. polykrikoides* (Whyte et al., 2001). Blooms of *Cochlodinium sp.* have also been reported in the Gulf of California (Garate-Lizárraga et al., 2004). Within coastal waters of the United States, *Cochlodinium sp.* blooms have been reported in Rhode Island (Hargraves and Maranda, 2002; Tomas and Smayda, 2008), California (Kudela et al., 2008; Curtiss et al., 2008), New Jersey (Sousa e Silva, 1976), and Chesapeake Bay (Marshall, 1995; Mulholland et al 2009). *Cochlodinium polykrikoides* has formed dense blooms in the Peconic Estuary and Shinnecock Bay of Long Island, NY during late summer early fall months annually since 2004 (Gobler et al., 2008). Originally described as nuisance blooms (Nuzzi, 2004), experiments have shown blooms of *C. polykrikoides* in NY can be lethal to fish and shellfish (Gobler et al., 2008). Contact with bloom waters or clonal isolates having densities greater than 1×10^3 cells ml^{-1} resulted in rapid mortality in fish (i.e. hours) and shellfish (i.e. days; Gobler et al., 2008; Tang and Gobler 2009).

Globally, nutrients are generally considered a prime promoter of HABs (Anderson et al 2008;Heisler et al. 2008). However, the manner in which nutrients may promote blooms of *C. polykrikoides* is not well understood. Jeong et al. (2004, 2005) have reported that *C. polykrikoides* isolates from Southeast Asia can be mixotrophic, making its nutritional options diverse. Kim et al. (2001) reported that the Korean strain of *C. polykrikoides* showed a preference for ammonium over nitrate for growth. Kudela et al. (2008) studied *Cochlodinium fulvescens* (Iwataki et al., 2008) blooms on the west coast of the US and found that at elevated nutrient concentrations, ammonium and urea uptake rates exceeded those of nitrate. While *C. polykrikoides* blooms have become common along the US east coast (Marshall, 1995; Hargraves and Maranda, 2002; Gobler et al., 2008; Mulholland et al 2009), the nutrient sources promoting these blooms are

unknown. Understanding the nutrient regime which may control HABs is crucial to developing strategies for management and remediation. It is the intention of this study, therefore, to understand the nutrient ecology of *Cochlodinium polykrikoides* in New York estuaries.

To better understand the nutritional ecology of the harmful dinoflagellate blooms caused by *Cochlodinium polykrikoides* in Long Island estuaries (NY, USA), laboratory and field studies of this species were conducted. I documented the spatial and temporal dynamics of *C. polykrikoides* cells, nutrients, and co-occurring phytoplankton within two New York estuaries from 2006 - 2008. I quantified of the growth response of *C. polykrikoides* and co-occurring phytoplankton during enrichment of different nitrogen sources. Furthermore, I quantified growth rates of culture isolates of *C. polykrikoides* on a variety of nitrogen sources (urea, ammonium, glutamic acid, nitrate) and through a range of concentrations (2-200 μM). Finally, I quantified the uptake rates of various N compounds in both field and laboratory conditions using ^{15}N-enriched nitrate, nitrite, urea, ammonium, and glutamic acid.

METHODS:

Culture Based Experiments:

***Cochlodinium polykrikoides* growth on various sources and concentrations of N:**

Cochlodinium polykrikoides strain CP1 was isolated from a 2006 bloom in Flanders Bay, Long Island, New York, USA. These cultures were grown on GSe medium (Doblin et al., 1999) made from artificial salts and supplemented with an antibiotic-antimycotic solution (a mixture of 10,000 I.U. penicillin, 10,000 μg mL^{-1} streptomycin, and 25 μg mL^{-1} amphotericin B; Mediatech. Inc., Hemdon, VA) added into the medium immediately before inoculation at a final concentration of 1-2% to minimize contamination by bacteria and fungi. Periodic DAPI-staining of cultures has indicated the absence of bacteria. Cultures were maintained at 21° C on a 14:10 light:dark cycle, illuminated by a bank of fluorescent lights that provided ~100 μmol quanta m^{-2} sec^{-1}. These conditions approximated temperature and light exposures found in Long Island estuaries during late summer months when *Cochlodinium polykrikoides* blooms (Gobler et al., 2008).

The growth of *C. polykrikoides* on different species and concentrations of nitrogen was examined in simultaneous experiments with four types of nitrogen provided at six concentrations in GSe medium. Cultures were grown in triplicate Pyrex test tubes (50 ml) at N concentrations of 200, 100, 50, 25, 10, 5, and 2 μM-N of nitrate, ammonium, urea, and glutamic acid. To assure nutrient saturation, the highest nitrogen concentrations were those of standard phycological media, but exceeded ranges found in *Cochlodinium polykrikoides* bloom-prone embayments (Gobler & Boneillo 2003; this study). Initial sets of tubes received an inoculum from a single microalgal culture grown under the conditions described above. Accumulation of cell biomass through time was estimated by *in vivo* fluorescence, measured the same time each day (to avoid diel fluctuations in cell fluorescence) in a Turner Designs TD-700 fluorometer. Previous research has demonstrated that *in vivo* fluorescence is proportional to cell densities of a variety of cultured phytoplankton species (Fogg and Thake, 1987; Taylor et al. 2006),

and I found this to be the case for *Cochlodinium polykrikoides*. Upon entering late exponential phase growth, cultures were transferred into the identical media they had been grown in previously to a cell density of ~100 cells ml^{-1}. Cultures were transferred under each concentration and source of N more than six times, to ensure N concentrations and nutrient stores from the initial transfer were diluted and to ensure exponential-phase cellular growth rates were representative of cells adapted to the culture conditions.

Cellular growth rates were calculated for all cultures in two ways. *In vivo* fluorescence was used to generate biomass production rate constants (d^{-1}) during exponential-phase growth. As such, cellular chlorophyll *a* quotients would not influence calculations as long as they are relatively constant during early to mid-exponential growth phase. Growth rates based on cell biovolume (μm^3) were also determined on 100 µl aliquots of Lugol's iodine-preserved samples using a Beckman-Coulter© Multisizer 3.0. All growth rates were calculated daily during exponential phase growth using the formula $\mu = \ln (B_t/B_0)/t$, where B_o and B_t are the initial and final biovolume, and t is the incubation duration in days. Growth rates were averaged over exponential phase, which typically persisted for 3 – 6 days, depending on the concentration of N. Growth curves from changes in cell volume and *in vivo* fluorescence were nearly identical and not statistically different. The Michealis-Menton kinetic terms μ_{max} (maximum growth rate) and K_S (half saturation constant) were derived through Lineweaver-Burk transformation of the growth curves and the competition coefficient, α, was calculated as μ_{max} / K_S. Determining the competition coefficient emphasizes both μ_{max} and K_s and provides a more descriptive picture of nutrient affinity at sub-saturating concentrations ($<K_s$) and when interspecies competition is likely to occur (Harrison et al 1989). Differences in growth rates among treatments were examined by means of a two-way analysis of variance, where nitrogen concentrations and nitrogen species were the main effects. Multiple comparisons among treatments were also examined using Tukey tests.

Uptake rates of nitrogenous nutrients:

To quantify the rate of uptake of different N compounds by cultures grown under different conditions, ^{15}N tracer experiments were conducted. Nitrogen uptake was measured using tracer additions (20 ± 11%) of highly enriched (98%) ^{15}N (Mulholland et al., 2002). Cultures were grown through seven transfers on each N source at concentrations of 2 µM and 20 µM N, which are similar to mean and maximal levels of nitrate and ammonium present during blooms (Table 1). In late exponential phase growth, cultures growing on 2 µM and 20 µM glutamic acid, urea, nitrate and ammonia were amended with the tracer addition of ^{15}N-labeled glutamic acid, urea, nitrate or ammonia (10%) plus 2 µM or 20 µM addition of each ^{14}N compound. Incubations were performed under normal culture conditions for 60 minutes, after which cultures were filtered onto pre-combusted (2 h @ 450ºC) GF/F glass fiber filters. The natural abundance ^{15}N signature of particulate organic nitrogen (PON) of cultures prior to enrichment was also determined. Samples were analyzed for PON and the ratio of ^{14}N:^{15}N at the U.C. Davis Stable Isotope Facility. Uptake rates were calculated according to the mixing model of Montoya et al. (2002) and equations from Orcutt et al. (2001). Rates were considered net uptake as they were not corrected for the effects of

isotopic dilution (Glibert et al., 1982) although these are expected to be minimal due to the short incubation times and absence of zooplankton and bacteria in cultures. Relative preferences for nitrogen sources were determined by comparing the uptake rate of each compound. A two-way analysis of variance with post-hoc Tukey multiple comparison tests was performed with ^{15}N compound added and N source cells were grown on as the main treatment effects.

Field Experiments:

Field Sampling:

During this study, estuaries historically prone to *C. polykrikoides* blooms (Shinnecock Bay and the Peconic Estuary; Gobler et al., 2008) were accessed by small vessels from the Stony Brook-Southampton Marine Science Center. Specific sampling sites included: Old Fort Pond (a tributary connecting to Shinnecock Bay, 40.8621° N, 72.4396° W) and Shinnecock Bay proper (40.8621° N, 72.4734° W), Great Peconic Bay (40.9252°N, 72.5614°W) and Flanders Bay (40.9255°N, 72.5928°W) in the western extent of the Peconic Estuary, and Meetinghouse Creek (40.9210° N, 72.6245°W), a tributary connecting to the north shore of Flanders Bay. Weekly sampling of all sites was performed in late summer (July - August), prior to the development of blooms, and continued into the fall when blooms had ended (October). During blooms, both dense bloom patches (surface swarms) and non-patch areas were sampled (Gobler et al., 2008). Surface and bottom salinity, temperature, and dissolved oxygen were measured at each sampling site using a hand held YSI© 556 sonde. Surface water was collected in 20-L, acid-cleaned carboys. Whole water from each station sampled was filtered for nutrient analysis using pre-combusted (2 hrs @ 450°C) glass fiber filters and frozen. Samples were analyzed colorimetrically for ammonium, nitrate, nitrite, phosphate, urea, silicate, total dissolved nitrogen and total dissolved phosphorus using wet chemistry and a spectrophotometric microplate reader (Valderma, 1981,; Jones, 1984; Parsons et al., 1984; Price and Harrison, 1987). Selected samples were analyzed for individual dissolved free amino acids in duplicate by high performance liquid chromatrography (HPLC; Cowie and Hedges 1992). Chlorophyll *a* was measured in whole (0.7 μm GFF), >5 μm, and >20 μm (5 μm and 20 μm polycarbonate filters) fractions using standard fluorometric techniques (Welschmeyer, 1994). Whole seawater samples were preserved in Lugol's iodine solution and species identification and enumeration was performed using an inverted light microscope (Hasle, 1978). Differences in biological, chemical, and physical parameters among sites and years were assessed by means of one-way analyses of variance (ANOVA) with post-hoc Tukey multiple comparison tests or Student's T-tests. For comparative purposes, a threshold of 330 cells mL^{-1} was used to define 'bloom' conditions, as this is the minimal density of this species capable of killing fish (Tang and Gobler 2009). The degree to which individual variables were correlated was evaluated by a Spearman's Rank Order Correlation Matrix. In all cases, a significance level of 0.05 was applied to justify statistically significant differences or correlations.

Nutrient amendment experiments:

During the initiation, peak, and demise of *C. polykrikoides* bloom events, nutrient amendment experiments were conducted to determine how the enrichment of different nutrient sources affected the growth of this species and other members of the

phytoplankton community. Experiments were conducted at various sampling sites during the summers of 2005, 2006, 2007, and 2008. Surface seawater was collected using acid-cleaned 20-L carboys and within two hours was used to fill 1.1-L acid-cleaned polycarbonate bottles. Triplicate bottles were used for each treatment, which included an unamended control, sodium nitrate (10 μM), urea (5 μM = 10 μM N), glutamic acid (10 μM), and ammonium (10 μM). Nutrient stocks were filter-sterilized (0.2 μm) and stored frozen. Bottles were incubated for 48 hours in eastern Shinnecock Bay under ambient light and temperature conditions. Termination of experiments included filtration of water to determine concentrations of total and >5 μm chlorophyll *a* and preservation in Lugol's iodine solution for microscopic quantification of *C. polykrikoides* and co-occurring phytoplankton, which were broadly grouped as 'diatoms' and 'other dinoflagellates'. Net growth rates (d^{-1}) of each component of the algal community were calculated as $\mu = [\ln(B_t/B_o)]/t$ where B_o and B_t are the initial and final biomass (pigment or cell density) of each algal group, respectively, and t is the incubation duration in days. One-way analyses of variance with post-hoc Tukey multiple comparison tests were performed to determine significant differences in growth rates among treatments for each algal group: *C. polykrikoides*, diatoms, other dinoflagellates, and small phytoplankton (< 5 μm).

Uptake rates of nitrogenous nutrients during bloom events:

^{15}N tracer experiments were conducted using 'bloom patches' (surface water cell swarms) following the methods described for cultures to assess the source of N assimilated by *C. polykrikoides* bloom populations. Differences included the use of nitrite as an additional N-tracer, and shorter incubations (30 minutes) performed under ambient light and temperature conditions. Since bloom patches of *C. polykrikoides* contained few other phytoplankton (65-97% of cells > 20μm were *C. polykrikoides* during this study), ^{15}N-amended experimental water was filtered on pre-combusted (2 h @ 450ºC) GF/F glass fiber filters with and without pre-filtration with a 20μm mesh to remove *C. polykrikoides* cells. While bacteria are known to rapidly degrade reduced N compounds, their uptake and degradation rates of urea, ammonium and amino acids (0.06, 2.4 and 2.5 nM-N h^{-1} respectively; Cho et al., 1996; Coffin, 1989; Hoch and Kirchman, 1995) are small relative to the ambient pools. The difference in uptake observed in the total and < 20 μm size fraction was ascribed to cells > 20 μm, and microscopic quantification was used to assess the relative abundance of *C. polykrikoides* cells in this size fraction during each experiment. A one-way analysis of variance with post-hoc Tukey multiple comparison tests was performed to determine significant differences among uptake rates for each compound from each experiment.

RESULTS:

Growth rates of <u>C. polykrikoides</u> on differing sources and concentrations of N:

Cultures of *Cochlodinium polykrikoides* strain CP1 grown on nitrate, ammonium, urea, or glutamic acid displayed standard Monod growth kinetics over the

range of N concentrations used (2 – 200 µM; Fig 1). Growth rates were similar among N sources at the low levels of N, at ~0.15-0.2 d^{-1} with growth rates on glutamic acid being somewhat higher (Fig 1). Growth rates seemed to saturate above 25 µM for all N species (Fig 1). Maximal growth rates (μ_{max}) achieved by *C. polykrikoides* on glutamic acid (0.50 ± 0.10 d^{-1}) were statistically significantly (Tukey test, $p<0.05$) higher than those for nitrate, ammonium, and urea (0.41 ± 0.10, 0.41 ± 0.07 and 0.42 ± 0.10 d^{-1}, respectively; Table 2). Half-saturation constants (K_s) were lower for glutamic acid and urea (1.84 ± 0.60 and 2.18 ± 0.51 µM, respectively) compared to ammonium and nitrate (2.60 ± 0.49, and 2.94 ± 0.70 µM, respectively; Table 2). Competition coefficients indicated *C. polykrikoides* would compete best for glutamic acid ($\alpha = 0.27$ $d^{-1}\mu M^{-1}$; Table 2), followed by urea and ammonium (0.19, and 0.16 $d^{-1}\mu M^{-1}$, respectively; Table 2). *C. polykrikoides* would be least competitive for nitrate ($\alpha = 0.14$; Table 2). Both nitrogen source and concentration were significant treatment effects for *C. polykrikoides* growth rates ($p < 0.001$; Two-way analysis of variance). *C. polykrikoides* growth rates on glutamic acid were significantly greater than those on all other N sources (Tukey test test, $p<0.05$). There were also expected significant differences between high and low N concentraions (e.g. 200 µM significantly greater than 2- 25 µM; Tukey test test, $p<0.05$). For all sources of N, increasing concentrations of N predictably yielded longer periods of exponential growth and larger final cell densities (data not shown). N concentrations were generally < 1µM at the end of exponential phase growth (data not shown).

N assimilation rates of <u>C. polykrikoides</u> on differing sources and concentrations of N:

Uptake rates of N differed among *C. polykrikoides* cultures grown on different N sources and concentrations. At both low and high N concentrations, glutamic acid uptakes rates were significantly higher than the uptake of all other compounds ($p<0.001$; Tukey Test; Fig 2). Ammonium uptake rates were significantly higher than those of urea and nitrate ($p<0.001$; Tukey Test; Fig 2), which did not differ from each other. Glutamic acid was the only nutrient with significantly faster uptake rates at higher than lower concentrations (Tukey test, $p<0.05$).

For comparative purposes, N uptake rates measured with ^{15}N compounds in culture were compared to the N demand of this species estimated from its N quota, cell densities, and growth rates. Based on concentrations of PON measured in *C. polykrikoides* cultures of known cell densities, this species contains 2.3 ± 0.3 x 10^{-11} mol N $cell^{-1}$. This value is similar to an estimate of its N content based on cell biovolume and a Refield C:N ratio (1.9 x 10^{-11} mol N $cell^{-1}$; Stoecker et al. 1994; Jiang et al, in press). For laboratory cultures grown on low levels of N (2 µM N), the amount of N uptake was almost identical to the estimated demand for urea and nitrate, but exceeded the estimated demand by two- to three-fold in ammonium and glutamic acid (Table 3). At higher concentrations (20 µM N), the uptake of nitrate and urea was below estimates of the N demand (~44%), while the assimilation of glutamic acid and ammonium was again higher by two-fold than estimates of N demand based on cell densities, growth rates, and cellular N quotas (Table 3), suggesting there was luxury uptake of these two compounds.

Uptake of different N species was affected by the N source on which the cultures had been grown (Fig 3). Cultures grown on 20 µM glutamic acid had significantly

higher uptake of all N species when compared to cultures grown on other N sources (Fig 3; Tukey test, $p<0.05$). In low N cultures (2 µM), cells grown on glutamic acid had significantly higher glutamic acid uptake compared to all other N compounds and significantly higher urea than ammonium uptake (Fig 3; Tukey test, $p<0.05$). For cultures grown on ammonium, urea uptake was significantly greater than all other N compounds (Fig 3; Tukey test, $p<0.05$) and for cultures grown on urea, nitrate and urea uptake were significantly greater than glutamic acid uptake, and glutamic acid uptake was significantly greater than ammonium uptake (Fig 3; Tukey test test, $p<0.05$).

Dynamics of phytoplankton and nutrients during C. polykrikoides blooms in NY estuaries, 2006 - 2008

Blooms (defined as >330 cells mL^{-1}) of *C. polykrikoides* occurred in Long Island estuaries during late summer (August) through early fall (September) from 2006 - 2008. Widespread (all study sites) and extended (> 1 month) *C. polykrikoides* blooms occurred in 2006 and 2008, while blooms in 2007 were isolated (Old Fort Pond and Flanders Bay only) and short (1 week). The bloom in 2006 was generally denser than the bloom in 2008 (Table 4). Blooms peaked at 55,000 cells mL^{-1} (Great Peconic Bay, 8/30/06; Table 4) and Great Peconic Bay experienced the densest blooms averaged over all three years (Table 4). Mean N levels among sites were generally low with nitrate, ammonium and urea ranging from 0.63-3.79 µM, 0.63-2.22 µM, and 0.15-1.11 µM respectively (Table 5). Silicate and phosphorous ranged from 32.92-65.01 µM and 1.14-2.04 µM, respectively (Table 5).

Analysis of all bloom and non-bloom conditions from all sites over all years (Table 6) revealed that, as would be expected, blooms had significantly more *C. polykrikoides* cells, total chlorophyll *a* and > 5 µm chlorophyll *a*, than non-bloom water ($p<0.001$). Bloom conditions also had significantly higher silicate levels and significantly lower salinity ($p<0.01$; Table 6). There were five-times more diatoms present under non-bloom conditions, compared to blooms (Table 6). Moreover, *C. polykrikoides* abundances were inversely correlated with diatom densities ($p < 0.05$) and significantly correlated with silicate concentrations ($p < 0.01$; Table 6). In addition, *C. polykrikoides* abundances were also significantly correlated with concentrations of dissolved organic nitrogen and phosphorous for all years (DON and DOP; $p < 0.05$ for each). In contrast, other phytoplankton groups such as other dinoflagellates, diatoms and small phytoplankton (< 5 µm) were not significantly correlated with DON or DOP. Comparisons between the bloom years (2006 and 2008) and the minor-bloom year (2007) revealed significantly higher concentrations of ammonium and urea in 2007 compared to 2006 and 2008 (Table 6?, $p<0.05$).

During blooms in Great Peconic Bay and Shinnecock Bay, *C. polykrikoides* cells comprised over 95% of the cells >20 µm, while in Meetinghouse Creek and Old Fort Pond *C. polykrikoides* was a significantly lower percentage of phytoplankton cells >20 µm (50-69%; $p<0.05$; Table 6?). Comparisons of all field parameters between monospecific (for phytoplankton >20 µm) bloom sites (Great Peconic and Shinnecock Bays) and the mixed bloom sites (Meetinghouse Creek and Old Fort Pond) indicated there was a significantly greater abundance of non-*C. polykrikoides* dinoflagellates at the mixed bloom sites ($p<0.05$). Furthermore, nitrate concentrations were significantly

higher at the mixed bloom locations (t-test, $p<0.005$) while during monospecific blooms higher salinity and urea concentrations were present ($p<0.05$).

N assimilation rates by plankton communities during C. polykrikoides blooms

Ten N-uptake experiments were conducted during August and September of 2008 in four locations: Old Fort Pond, Shinnecock Bay, Flanders Bay, and Great Peconic Bay. During experiments, *C. polykrikoides* cell densities ranged from 480 to 5,484 cells ml^{-1} while total chlorophyll *a* levels ranged from 18.3 to 55.7 µg L^{-1} (Table 7). Of the cells > 20 µm enumerated during experiments, *C. polykrikoides* represented a large majority of the total (72-97%), averaging 89±11% and being greater than 96% on three occasions (Table 7). Total N uptake for all N species ranged from 0.30 to 3.9 µmol N L^{-1} hr^{-1}, and averaged 1.8 ± 1.0 µmol N L^{-1} hr^{-1} (Fig 4a). The > 20 µm size fraction accounted for, on average, 34 ± 12% of the total N uptake, ranging from 12 – 48% (Fig 4). The N compound displaying the greatest uptake in the > 20µm size fraction varied by site and date. Within Old Fort Pond and Flanders Bay, nitrate and nitrite dominated total N uptake (69%; Fig 4). Of the four experiments in Old Fort Pond and Flanders Bay, the >20 µm plankton group displayed significantly greater uptake of nitrate and nitrite compared to glutamic acid in three experiments, significantly greater uptake of nitrate and nitrite compared to urea in two experiments and significantly greater uptake of nitrate and nitrite compared to ammonium in one experiment (Tukey test, $p<0.05$). In contrast, within Shinnecock Bay and Great Peconic Bay, urea was the compound taken up at the highest rates by plankton > 20 µm, ranging from 41 – 83% of the total N-assimilation rate (Fig 4). Of six experiments in Shinnecock Bay and Great Peconic Bay, the >20 µm plankton group displayed significantly greater uptake of urea compared to glutamic acid and nitrite in all experiments, and significantly greater uptake of urea compared to nitrate and ammonium within four experiments (Tukey test, $p<0.05$). Notably, glutamic acid was assimilated at the greatest observed uptake rate, when compared to other N sources, by the larger plankton in Shinnecock Bay on September 16th (20% of total, Fig 4). The N uptake characteristics of the > 20µm size fraction contrasted with those of the smaller plankton (< 20µm), which acquired the majority of their N from ammonium and urea, regardless of location (Fig 4).

Measured N uptakes rates were compared to the theoretical N demand of bloom populations on the three occasions in 2008 when *C. polykrikoides* was > 94% of the >20 µm phytoplankton community . Cellular N quotas of cultures ($2.3 \pm 0.3 \times 10^{-11}$ mol N $cell^{-1}$; see culture work for details) were applied to bloom cell densities, as were cellular growth rates measured for cultures at the levels of N present during blooms (0.2 d^{-1} at 2 µM N; Fig 1) on these three dates. The ^{15}N assimilation rates summed for all measured compounds on these dates accounted for 43%, 70% and 111% of the estimated N demand on 27 August in Great Peconic Bay, 10 September in Flanders Bay, and 16 September in Shinnecock Bay (Table 3).

Growth rates of plankton communities in response to nutrient amendment during C. polykrikoides blooms

Twenty-one nutrient amendment experiments were performed from 2005-2008. Enrichment with at least one of the N species significantly increased *C. polykrikoides* growth rates in 62% of experiments performed (Tukey test, $p<0.05$; Tables 8 and 9). Enrichment with nitrate, ammonium, urea, or glutamic acid yielded significantly higher

growth rates relative to the control treatment in 57, 53, 39, and 27% of experiments (Tukey test, $p<0.05$; Tables 8 and 9). Other members of the plankton community responded less frequently to N enrichment. For example, growth rates of diatoms, other dinoflagellates and small phytoplankton (< 5 μm) increased significantly in response to at least one form of N in 43, 17, and 38% of experiments conducted (Tukey test, $p<0.05$; Tables 8 and 9). These groups benefited most from nitrate enrichment (significantly increased growth in 36, 11, and 25% of experiments; Tukey test, $p<0.05$; Tables 8 and 9), but responded less frequently to other forms of N. For example, while *C. polykrikoides* experienced significantly increased growth when enriched with glutamic acid in 27% of experiments, this compound elicited a similar response in diatoms and other dinoflagellates in only 18% and 7% , respectivelt, of experiments (Tukey test, $p<0.05$; Tables 8 and 9) and never significantly altered the growth of small phytoplankton (0% of experiments; Tables 8 and 9).

The growth rates of *C. polykrikoides* in unamended control treatments were almost always slower than those of diatoms, dinoflagellates, or small phytoplankton (18 of 21; 86% of experiments; Table 8 and 9, Fig 5). However, in nearly 32% of amended treatments, enrichment by one of the N compounds resulted in a growth rate for *C. polykrikoides* which exceeded the other phytoplankton groups. For example, during experiments conducted in Shinnecock Bay in 2008 and in Old Fort Pond in August and September of 2005, enrichment with nitrate resulted in *C. polykrikoides* growth rates exceeding all other phytoplankton groups (Table 8 and 9, Fig 5). In experiments in Great Peconic Bay (2008) and in Meetinghouse Creek (2007 and 2008), the addition of urea led to *C. polykrikoides* growth rates outpacing all other algal groups (Table 8, Fig 5). Finally, glutamic acid enrichment also yielded a growth rate for *C. polykrikoides* which exceeded its competitors during the 2008 Great Peconic Bay experiment (Table 8).

DISCUSSION

Harmful algal blooms are an increasingly common phenomenon in coastal waters around the world, and nutrient enrichment is commonly an important contributor to the occurrence of these events (Heisler et al., 2008; Anderson et al., 2008). While *C. polykrikoides* has emerged during the past two decades as an ichthyotoxic HAB species which has caused annual blooms throughout Southeast Asia (Kim, 1998; Kim et al., 1999) and both coasts of North America (Curtiss et al., 2008; Gobler et al., 2008; Kudela et al., 2008; Mulholland et al., 2009), the nutritional regime supporting these blooms has not been determined. By combining laboratory and field studies, the present study indicated that *C. polykrikoides* is a nutritionally flexible species, capable of growing well on a variety of organic and inorganic forms of N. In addition, these results suggest *C. polykrikoides* is mostly likely to dominate estuarine algal communities when N levels are moderate to low (2 - 10 μM).

Growth of <u>C. polykrikoides</u> on differing N sources

During culture experiments, *C. polykrikoides* grew at rates consistent with prior studies of this species (0.4 d^{-1}; Kim et al., 2001) on both organic and inorganic forms of N. While many phytoplankton grow well on urea, robust growth on amino acids is less common (Antia et al., 1975; Bronk et al., 2007). However, *C. polykrikoides* cultures grown on glutamic acid attained significantly higher growth rates ($\mu max = 0.50 \pm 0.10$ d^{-1}) and had substantially lower half saturation constants ($Ks = 1.84 \pm 0.60$ µM) compared to all other N sources ($\mu max = \sim 0.4$; Ks = 2.2 – 2.9 µM; Table 2). Additionally, *C. polykrikoides'* low competition coefficient (α) for organic N sources indicates it would be a better competitor for organic N than inorganic N. Consistent with this hypothesis, *C. polykrikoides* densities were significantly correlated with DON concentrations in the field ($p < 0.05$) while other phytoplankton groups were not. *C. polykrikoides'* robust growth on organic N is consistent with many other dinoflagellates, including species which form HAB such as *Karenia brevis*, *Prorocentrum minimum*, and *Lingulodinium polyedrum* (Taylor, 1987, Smayda, 1997, Anderson et al., 2008, Heisler et al., 2008). The half saturation constants for growth reported here are similar to those measured for N uptake during a bloom of *C. fulvescens* in Monterey Bay (1.0 – 1.6 µM; Kudela et al., 2008) and for cultures of this species isolated from Korea grown on nitrate and ammonium (1.0 and 2.1 µM, respectively; Kim et al., 2001) and support the tenet that this species is adapted to moderate-to-low levels of N.

Beyond the faster growth on glutamic acid by *C. polykrikoides*, cultures grown on glutamic acid also assimilated this compound at rates significantly higher than cutures grown on nitrate (or urea) assimilated nitrate (or urea) (2 µM; Fig 3). Since the growth rates among different N sources varied by smaller amounts (Fig 1), the high uptakes rates of glutamic acid by cultures grown on glutamic acid suggest something other than N nutrition may be responsible for the higher uptake rates of this compound. Consistent with this hypothesis, N uptake rates by cultures grown on glutamic acid exceeded the theoretical demand for N by more than two-fold at both high and low levels of N, suggesting the high uptake rates of this compound represented luxury uptake perhaps as a means to obtain extra organic carbon. Mulholland et al. (2009) recently demonstrated that bloom populations of *C. polykrikoides* are capable of obtaining C from amino acids. This could be used to supplement their photosynthesis (Droop, 1974; Lewitus and Kana, 1995) and could establish a mechanism for 24-h C acquisition. In an ecosystem setting, exploiting such biochemical pathways could give this species a competitive advantage over algae obtaining C exclusively by means of photosynthesis. Interestingly, glutamic acid was the most abundant amino acid in selected (n = 10) seawater samples analyzed during *C. polykrikoides* blooms, present at a concentration of 0.2 ± 0.1 µM. Finally, during blooms, diel vertical migration of may allow *C. polykrikoides* to access such amino acids and other DON sources from sediments (MacIntyre et al., 2004; Kudela et al., 2008).

<u>C. polykrikoides</u> bloom dynamics

During this study, large (present at all study sites) extended (> 1 month) *C. polykrikoides* blooms occurred in 2006 and 2008, while blooms in 2007 were isolated (Old Fort Pond and Flanders Bay only) and brief (1 week). Blooms of *C. polykrikoides* occurred over a fairly wide range of nutrient conditions, including tributaries with high

nutrient levels, such as Old Fort Pond and Meetinghouse Creek (mean DIN = 2.2 ± 1.1), and open water sites such as Great Peconic Bay and Shinnecock Bay (DIN = 0.8 ± 0.3; Table 5). Organic nutrients such as urea and amino acids were generally less available during blooms (< 2 µM) in most locales, although the total DON pool was large (mean DON = 21 ± 4.9; Table 5). Interestingly, blooms were generally denser and more monospecific in the open parts of estuaries where nitrate levels were significantly lower ($p<0.05$; Tables 4 and 5). Also, concentrations of ammonium were significantly higher during 2007 when large blooms were not present ($p<0.05$; Table 5?). Together, these two observations suggest blooms of *C. polykrikoides*, particularly those which are monospecific, are less likely to form when concentrations of ammonium and nitrate are elevated. Since all half-saturation constants for growth for this species were relatively low (~ 2 µM), it seems persistent, monospecific *C. polykrikoides* blooms are associated with moderate, but not high, levels of nutrient enrichment.

Comparisons of diatoms, *C. polykrikoides*, and other dinoflagellates provide some preliminary insight into interspecific competition during *C. polykrikoides* blooms. The five-fold lower concentrations of diatoms during *C. polykrikoides* blooms, particularly in Flanders Bay and Meetinghouse Creek ($p<0.001$; Table 1) suggest this species may compete with diatoms for dominance among the larger plankton. The success of *C. polykrikoides* at the expense of diatoms is the likely cause of the significantly higher concentrations of silicate during blooms (i.e. fewer diatoms = less Si-uptake = higher concentrations; Table 1, 4). The significantly greater abundance of non-*C. polykrikoides* dinoflagellates at mixed bloom sites ($p<0.05$; Table 1) indicates that other dinoflagellates are able to co-exist within more eutrophic tributary sites, but not within more open estuarine sites and suggests *C. polykrikoides* occupies a broader ecological niche than these other species.

Effects of nutrient enrichment on phytoplankton growth

During the months in which *C. polykrikoides* blooms occurred, phytoplankton growth in the Peconic and Shinnecock Bays was frequently stimulated by N enrichment. *C. polykrikoides*, diatoms, dinoflagellates and small (<5 µm) phytoplankton were stimulated by N enrichment in 62%, 43, 17, and 38% of experiments, indicating the growth of *C. polykrikoides* was more often limited by N supply during blooms than other algal populations. The low DIN:DIP ratios present during *C. polykrikoides* blooms (2.5 ± 0.4; Table 4) further support the hypothesis that phytoplankton populations are N limited during blooms. Half-saturation constants for individual nutrients are often utilized as proxies for nutrient limitation in marine ecosystems with N concentrations below half-saturation constants often considered limiting (Caperon and Meyer, 1972; Fisher et al., 1992). The concentrations of nitrate, ammonium, urea, and glutamic acid were generally near or below the half-saturation constants of *C. polykrikoides* cultures for these nutrients, further supporting the hypothesis that *C. polykrikoides* blooms are limited by N in NY estuaries. While *C. polykrikoides* grew slower than other phytoplankton in all but 14% of experimental control treatments, N enrichment led to *C. polykrikoides* displaying growth rates faster than other phytoplankton groups in at least one N treatment in 32% of experiments (Table 8), suggesting that N enrichment can, at times, promote *C. polykrikoides* dominance. Interestingly, this species has been shown to be allelopathic to other phytoplankton (Tang and Gobler, in prep) and is most toxic

when in exponential phase growth (Tang and Gobler, 2009). As such, the fastest relative growth rates in some N treatments could be due to both faster growth by *C. polykrikoides* and slower net growth rates by other phytoplankton due to allelopathic effects.

In a manner paralleling laboratory experiments, this species responded when enriched with both organic and inorganic forms of N during amendment experiments. However, *C. polykrikoides* was not always the best competitor for N during amendment experiments, as other groups such as diatoms and other dinoflagellates displayed higher growth rates in many treatments with enriched levels of N (Table 8). This finding is consistent with low half saturation constants for nutrients displayed by cultures (1.8 – 2.9 µM; Table 2), suggesting the 10 µM N used in experiments was sometimes more favorable for species with higher growth rates and presumably higher half saturation coefficients. The faster growth rate of other phytoplankton during 10 µM N enrichment is also consistent with field observation of monospecific blooms occurring when DIN levels were generally lower (Table 5). Therefore, I conclude that growth of *C. polykrikoides* monospecific blooms are associated with modest, but not heavy nutrient enrichment.

N uptake characteristics of C. polykrikoides blooms

Within near-monospecific microplankton (> 20 µm) blooms of *C. polykrikoides* (70 – 100% of cells), both organic and inorganic forms of N were assimilated although the dominant N source taken up varied by location. The former result is consistent with prior N-uptake experiments on bloom populations which were not size fractionated (Kudela et al., 2008; Mulholland et al., 2009) and with prior studies of HABs in general (Mulholland et al., 2002; Bronk et al., 2007). However, the N uptake by blooms was also partly dependent on location. Within the two most eutrophic and enclosed sites studied (Old Fort Pond and Flanders Bay), nitrate and nitrite were the primary forms of N assimilated. These two sites had the highest levels of nitrate during this study, and are known to be heavily loaded by nitrate-contaminated groundwater (Schubert, 1998; Motulcon and Sañudo-Wilhelmy, 2001). In contrast, within open water locations where levels of nitrate were lower and urea and DON were significantly more abundant, organic nitrogen compounds (urea and glutamic acid) were assimilated at higher rates (Figure 4?). *C. polykrikoides* densities were significantly correlated with DON concentrations during this study. As such, in open estuarine waters where this species was monospecific among the microphytoplankton (> 20 µm), the dominance of *C. polykrikoides* may be partly due to its ability to grow faster on DON sources such as glutamic acid, but also its ability to assimilate and grow rapidly on organic N when DIN is less available Although *C. polykrikoides* grew fastest on glutamic acid in culture, this compound never comprised more than 20% of its N assimilation in the field. This discrepancy was likely due to the lower glutamic acid concentrations during blooms (mean = 0.2 ± 0.1µM) which are below the half-saturation of *C. polykrikoides*, but likely within ideal concentrations for heterotrophic bacteria (Kirchman et al., 1994). Regardless, the overall pattern of differing nutrient sources being exploited by *C. polykrikoides* based on local environmental conditions is consistent with both my laboratory experiments and my field incubation experiments and suggests *C.*

polykrikoides employs flexible nutrient strategies to form blooms in both eutrophic and mesotrophic regions of estuaries.

The phagotrophic abilities of *C. polykrikoides* have been demonstrated within laboratory cultures of this species isolated from South Korea, and could circumvent the need to assimilate dissolved nutrients during blooms (Jeong et al., 2004). However, during this study, dissolved N uptake rates were high, being similar to those measured during non-phagotrophic algal blooms in NY waters (*A. anophagefferens*; Berg et al., 1997; Mulholland et al., 2002). As such, dissolved nutrient acquisition is likely an important pathway for NY bloom populations of *C. polykrikoides*. However, the shortfall of N uptake from dissolved compounds relative to the calculated N requirement on two dates during this study (8/27/08, GPB, and 9/10/08, Flanders Bay; Table 3) could be due to phagotrophic acquisition of N, the assimilation of dissolved N compounds not measured during this study (e.g. amino acids besides glutamic acid), an overestimate of N demand, or some combination of these factors.

In summary, *C. polykrikoides* was observed to grow rapidly on both organic and inorganic forms of N. During field experiments, growth of this species was frequently stimulated by the enrichment of either organic or inorganic forms of N. Densities of *C. polykrikoides* were significantly correlated with concentrations DON and concentrations of urea were significantly higher in locations where *C. polykrikoides* was monospecific among the microphytoplankton (> 20 µm). In contrast, nitrate levels were significantly higher in regions where *C. polykrikoides* was mixed with other dinoflagellates. Finally, the dominant source of N assimilated by bloom populations of *C. polykrikoides* changed with location, with inorganic forms such as nitrate and nitrite being the primary forms of N assimilated in the more eutrophic locations, while organic N was more commonly assimilated within mesotrophic locations where levels of DIN were lower. Overall, this species displays nutritional flexibility which may facilitate its ability to form large (> 50km) and extended (> 1 month) blooms on the US Atlantic coast. It is more likely to form monospecific blooms in regions with higher DON and lower DIN, but can also bloom along with other dinoflagellates in regions with higher DIN concentrations. Finally, the generally low growth rates displayed by this species in culture (μ_{max} = 0.4 – 0.5 d^{-1}) and in the field (0.1 – 1.0 d^{-1}; commonly 0.2 d^{-1}) compared other phytoplankton (Tables 8) suggest that other processes such as allelopathy and predator deterrence are likely to be important for bloom formation.

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	Total	Bloom	Non-Bloom
C. polykrikoides (mL^{-1})	4830 ± 1570	**6890 ± 2980**	**65.6 ± 89.1**
Diatoms (mL^{-1})	177 ± 203	82.3 ± 107	421 ± 605
Dinoflagellates (mL^{-1})	331 ± 320	470 ± 548	107 ± 21.6
Chlorophyll *a* (µg L-1) Total	53.3 ± 6.31	**69.5 ± 9.36**	**20.4 ± 33.7**
Chlorophyll *a* (µg L-1) <5µm	26.6 ± 8.83	26.4 ± 8.55	22.4 ± 30.0
Chlorophyll *a* (µg L-1) >5µm	33.0 ± 3.60	**41.3 ± 6.27**	**8.92 ± 5.71**
Salinity (psu)	27.9 ± 1.17	**27.5± 1.62**	**29.0 ± 0.480**
Temperature (°C)	24.2 ± 0.580	24.0 ± 0.720	24.6 ± 0.410
Dissolved Organic Nitrogen (µM)	21.1 ± 8.67	21.9 ± 8.20	19.5 ± 9.60
Dissolved Organic Phosphorous (µM)	1.46 ± 1.07	1.58 ± 1.16	1.04 ± 0.488
Nitrate (µM)	1.35 ± 0.890	1.55 ± 1.30	1.13 ± 1.02
Ammonium (µM)	1.30 ± 0.350	1.33 ± 0.570	1.20 ± 0.170
Urea (µM)	0.540 ± 0.350	0.54 ± 0.350	0.490 ± 0.330
Sillicate (µM)	40.7 ± 11.8	**44.9 ± 13.1**	**30.6 ± 14.1**
Phosphate (µM)	1.48 ± 0.260	1.61 ± 0.320	1.27 ± 0.230

Table 1. Mean ± standard deviation (SD) for biological and chemical parameters measured from all sites from all years for bloom, non-bloom and both (total) periods. Parameters with statistically significant differences (t-test, $p<0.008$) between bloom and on-bloom are bolded.

	Glutamic acid	**Ammonium**	**Urea**	**Nitrate**
μ_{max} (d^{-1})	0.50 ± 0.10	0.41 ± 0.07	0.42 ± 0.10	0.41 ± 0.10
K_s (µM)	1.84 ± 0.60	2.60 ± 0.49	2.18 ± 0.51	2.94 ± 0.70
α (d^{-1} μM^{-1})	0.27	0.16	0.19	0.14

Table 2. Maximal growth rates (μ_{max}), half-saturation constants (K_s) and competition coefficients (d^{-1} μM^{-1}) for growth curves of *C. polykrikoides* cultures grown on glutamic acid, ammonium, urea, and nitrate.

Treatment	Measured Cells L^{-1}	Measured Growth rate d^{-1}	Measured N content	Estimated $\mu M N L^{-1} d^{-1}$	Estimated $\mu M N L^{-1} h^{-1}$	Measured $\mu M N L^{-1} h^{-1}$	Measured / Estimated
Glutmic acid, 2µM	246,064	0.3	2.3E-11	1.70E-06	7.07E-08	2.00E-07	2.83
Ammonium, 2µM	232,751	0.2	2.3E-11	1.07E-06	4.46E-08	1.00E-07	2.24
Nitrate, 2µM	222,397	0.2	2.3E-11	1.02E-06	4.26E-08	5.00E-08	1.17
Urea, 2µM	251,980	0.2	2.3E-11	1.16E-06	4.83E-08	5.00E-08	1.04
Glutmic acid, 20µM	453,151	0.5	2.3E-11	5.21E-06	2.17E-07	5.00E-07	2.30
Ammonium, 20µM	288,960	0.4	2.3E-11	2.66E-06	1.11E-07	2.50E-07	2.26
Nitrate, 20µM	299,315	0.4	2.3E-11	2.75E-06	1.15E-07	5.00E-08	0.44
Urea, 20µM	302,273	0.4	2.3E-11	2.78E-06	1.16E-07	5.00E-08	0.43
GPB, 8/27/08	5,484,000	0.2	2.3E-11	2.52E-05	1.05E-06	4.55E-07	0.43
FB, 9/10/08	3,376,000	0.2	2.3E-11	1.55E-05	6.47E-07	4.48E-07	0.69
SB, 9/16/08	2,884,000	0.2	2.3E-11	1.33E-05	5.53E-07	6.11E-07	1.11

Table 3. A comparison of N demand of *C. polykrikoides* cultures and field populations estimated from its N quota, cell densities, and growth rates to N uptake rates measured with ^{15}N. Cell densities and growth rates from cultures were quantified in situ. Field growth rates were extrapolated from low N cultures. N content was determined from PON measurements of cultures with known cell densities. Measured, field uptake rates were summed for all compounds, while measured uptake rates for cultures were for a single compound.

Site	Year	Date	*C. polykrikoides* (mL^{-1})	*C. polyrikoides* as % of cells >20 µm	Diatoms (mL^{-1})	Other Dinoflagellates (mL^{-1})	Chlorophyll *a* (µg L^{-1}) Total	Chlorophyll *a* (µg L^{-1}) >5µm
Flanders Bay	2006	22-Aug	20700 ± 2790	----	----	----	58.8 ± 1.66	42.6 ± 8.49
		25-Aug	3650 ± 116	----	----	----	30.4 ± 2.22	23.7 ± 0.30
		30-Aug	30000 ± 9490	----	----	----	269 ± 105	182 ± 24.8
	2007	22-Aug	13.0 ± 1.50	1	1810 ± 23.8	176 ± 12.5	7.32 ± 0.28	6.13 ± 0.52
		28-Aug	10.0 ± 1.50	0	2330 ± 99.4	29.0 ± 2.31	3.66 ± 0.19	2.79 ± 0.13
		30-Aug	8.00 ± 0.660	9	75.8 ± 9.87	5.50 ± 2.10	14.6 ± 1.03	6.28 ± 0.39
		4-Sep	974 ± 17.4	91	40.0 ± 5.02	54.0 ± 7.58	93.7 ± 1.08	49.5 ± 6.25
	2008	27-Aug	1050 ± 322	57	16.0 ± 0.00	786 ± 33.9	22.7 ± 1.36	10.3 ± 1.45
		28-Aug	5930 ± 554	96	4.00 ± 1.66	248 ± 33.2	53.5 ± 1.27	18.2 ± 6.04
		29-Aug	5770 ± 1090	95	4.00 ± 1.67	280 ± 22.6	61.1 ± 3.29	26.5 ± 7.64
		4-Sep	608 ± 147	70	124 ± 50.9	140 ± 5.66	8.80 ± 0.81	3.40 ± 0.80
		10-Sep	4950 ± 56.6	95	132 ± 16.9	120 ± 56.6	68.8 ± 5.07	25.6 ± 2.79
		17-Sep	4120 ± 690	94	12.0 ± 5.66	256 ± 102	55.8 ± 3.68	12.5 ± 2.25
		24-Sep	480 ± 45.3	72	80.0 ± 22.6	104 ± 56.6	18.3 ± 0.25	18.0 ± 1.14
Great Peconic Bay	2006	22-Aug	13.7 ± 5.31	----	----	----	7.12 ± 0.18	2.09 ± 0.52
		30-Aug	5590 ± 9450	----	----	----	242 ± 1.13	204 ± 17.7
	2007	22-Aug	0.00 ± 0.00	0	14.0 ± 2.31	120 ± 40.0	4.75 ± 0.57	4.04 ± 0.23
		28-Aug	0.00 ± 0.00	0	0.00 ± 0.00	66.7 ± 23.1	1.50 ± 0.13	0.83 ± 0.05
		30-Aug	0.00 ± 0.00	0	0.00 ± 0.00	80.0 ± 17.3	5.41 ± 1.04	3.13 ± 0.20
		4-Sep	0.00 ± 0.00	0	0.00 ± 0.00	160 ± 26.5	6.38 ± 1.35	4.48 ± 0.19
	2008	27-Aug	9140 ± 2460	94	16.0 ± 2.63	548 ± 77.2	51.2 ± 1.66	37.8 ± 4.07
		28-Aug	3690 ± 475	97	0.00 ± 0.00	108 ± 16.9	56.4 ± 1.98	22.4 ± 2.16
		29-Aug	3960 ± 869	95	8.00 ± 0.00	216 ± 56.6	55.0 ± 4.41	24.5 ± 3.71
		4-Sep	6920 ± 492	93	0.00 ± 0.00	492 ± 50.9	66.7 ± 2.20	35.6 ± 2.55
		10-Sep	1860 ± 49.2	96	44.0 ± 5.66	36.0 ± 5.66	45.5 ± 1.66	17.7 ± 1.80
		11-Sep	2290 ± 90.5	99	24.0 ± 0.00	0.00 ± 0.00	49.7 ± 1.11	24.1 ± 1.88
Meeting House Creek	2006	21-Aug	159 ± 27.8	----	----	----	242 ± 1.13	204 ± 17.7
		22-Aug	910 ± 82.3	----	----	----	42.6 ± 0.53	20.5 ± 2.17
		30-Aug	20800 ± 1670	----	----	----	62.8 ± 1.50	37.4 ± 2.81
		5-Sep	1950 ± 213	----	----	----	48.9 ± 1.92	21.6 ± 3.73
		7-Sep	13700 ± 5700	----	----	----	102 ± 7.64	67.8 ± 1.78
	2007	22-Aug	26.6 ± 3.09	20	13.0 ± 2.31	93.3 ± 6.11	3.30 ± 0.50	2.28 ± 0.08
		28-Aug	10.0 ± 1.50	1	656 ± 39.1	96.0 ± 17.3	26.2 ± 2.62	12.9 ± 4.19
		30-Aug	23.0 ± 2.00	11	54.3 ± 4.32	124 ± 9.67	37.2 ± 0.51	27.2 ± 1.09
		4-Sep	133 ± 61.0	31	107 ± 42.2	187 ± 46.2	93.9 ± 2.21	10.0 ± 2.75
	2008	27-Aug	1120 ± 117	18	0.00 ± 0.00	5270 ± 361	75.2 ± 2.83	64.5 ± 12.8
		28-Aug	4160 ± 147	99	0.00 ± 0.00	58.5 ± 4.95	79.9 ± 3.02	65.7 ± 12.2
		29-Aug	1270 ± 93.9	46	40.0 ± 11.3	1460 ± 238	21.9 ± 0.76	9.85 ± 0.95
		4-Sep	1220 ± 31.7	55	604 ± 17.0	384 ± 11.3	21.9 ± 0.38	19.4 ± 1.10
		11-Sep	952 ± 102	51	572 ± 130	336 ± 67.9	41.5 ± 0.20	22.6 ± 1.18
		24-Sep	1120 ± 56.6	48	0.00 ± 0.00	1190 ± 106	55.2 ± 0.94	46.4 ± 1.56
Old Fort Pond	2006	24-Aug	868 ± 22.6	----	----	----	48.8 ± 14.39	25.7 ± 4.19
		31-Aug	33300 ± 9090	----	----	----	----	----
		1-Sep	12000 ± 1760	----	----	----	----	----
		7-Sep	984 ± 52.6	----	----	----	----	----
	2007	22-Aug	1210 ± 266	43	0.00 ± 0.00	1600 ± 1220	45.5 ± 2.36	41.9 ± 1.31
		28-Aug	1270 ± 55.6	46	0.00 ± 0.00	1470 ± 254	54.8 ± 4.83	52.9 ± 9.90
		30-Aug	430 ± 20.7	24	40.0 ± 2.62	1290 ± 244	35.3 ± 4.56	19.8 ± 1.25
		4-Sep	160 ± 14.4	12	0.00 ± 0.00	1160 ± 69.3	27.7 ± 4.27	5.92 ± 0.38
	2008	13-Aug	892 ± 16.9	24	1930 ± 136	896 ± 113	28.9 ± 0.98	18.1 ± 0.36
		22-Aug	276 ± 107	7	1220 ± 10.0	2430 ± 90.5	34.4 ± 2.94	20.0 ± 1.66
		3-Sep	476 ± 39.6	69	180 ± 73.5	36.0 ± 2.83	31.9 ± 0.87	24.4 ± 2.50
		5-Sep	9630 ± 741	100	0.00 ± 0.00	8.00 ± 1.13	172 ± 17.33	90.7 ± 11.7
		23-Sep	2772.00 ± 254.55	97	32.0 ± 2.26	56.0 ± 4.53	53.2 ± 3.67	36.9 ± 3.10
Shinnecock Bay	2006	5-Sep	5110 ± 97.9	----	----	----	173 ± 9.07	----
	2007	22-Aug	107 ± 23.1	50	13.3 ± 2.30	93.3 ± 6.11	3.49 ± 0.29	----
		28-Aug	0.00 ± 0.00	0	53.3 ± 3.09	160 ± 40.0	2.36 ± 0.09	----
		30-Aug	0.00 ± 0.00	0	26.7 ± 6.42	93.3 ± 14.6	5.30 ± 0.14	----
		4-Sep	0.00 ± 0.00	0	80.0 ± 10.5	120 ± 40.0	4.91 ± 0.81	----
	2008	22-Aug	2390 ± 668	80	44.0 ± 5.10	552 ± 0.00	25.3 ± 1.33	20.8 ± 1.25
		28-Aug	8540 ± 735	99	4.00 ± 2.66	116 ± 16.9	45.9 ± 2.72	38.3 ± 3.46
		29-Aug	8130 ± 328	98	16.0 ± 2.62	184 ± 47.1	59.7 ± 2.13	37.7 ± 1.79
		4-Sep	7580 ± 56.6	95	64.0 ± 9.05	372 ± 62.2	53.7 ± 5.52	56.4 ± 3.45
		5-Sep	5300 ± 1320	97	36.0 ± 16.9	120 ± 0.00	121 ±14.58	47.3 ± 0.83
		16-Sep	2880 ± 226	97	16.0 ± 4.31	60.0 ± 19.9	40.7 ± 2.07	35.8 ± 4.37
		23-Sep	2550 ± 192	95	72.0 ± 22.6	64.0 ± 11.3	54.0 ± 4.17	36.1 ± 5.20

Table 4. Cell abundances (mL^{-1}) and chlorophyll *a* biomass (µg L^{-1}) at all sampling sites from 2006-2008.

Site	Year	Date	Salinity (psu)	Temperature (°C)	Dissolved Organic Nitrogen (µM)	Dissolved Organic Phosphorous (µM)	Nitrate (µM)	Ammonia (µM)	Urea (µM)	Sillicate (µM)	Phosphate (µM)	DIN:DIP
Flanders Bay	2006	22-Aug	24.0	20.6	----	2.72 ± 0.04	0.41 ± 0.14	2.32 ± 0.24	----	80.6 ± 4.94	1.46 ± 0.11	1.87
		25-Aug	25.8	22.3	----	1.38 ± 0.30	0.20 ± 0.19	1.57 ± 0.11	----	82.9 ± 4.49	1.42 ± 0.06	1.25
		30-Aug	24.0	20.7	----	1.47 ± 1.14	4.50 ± 0.82	1.48 ± 0.10	----	45.5 ± 0.24	1.63 ± 0.81	3.67
	2007	22-Aug	28.9	23.6	21.9 ± 3.10	0.93 ± 0.50	5.44 ± 2.78	1.33 ± 0.43	0.37 ± 0.30	34.1 ± 6.49	1.16 ± 0.30	5.84
		28-Aug	28.1	24.5	10.9 ± 0.19	0.59 ± 0.02	1.38 ± 1.31	1.10 ± 0.30	0.08 ± 0.03	27.6 ± 1.30	1.07 ± 0.26	2.32
		30-Aug	29.1	24.0	8.48 ± 3.19	0.64 ± 0.00	1.15 ± 0.65	1.02 ± 0.20	0.37 ± 0.42	20.5 ± 6.70	0.86 ± 0.39	2.52
		4-Sep	28.5	24.2	25.3 ± 4.72	1.42 ± 0.10	0.511 ± 0.36	8.30 ± 0.21	0.81 ± 0.51	35.2 ± 7.42	1.38 ± 0.43	5.37
	2008	27-Aug	23.9	23.6	27.6 ± 4.03	1.87 ± 0.35	0.49 ± 0.23	1.23 ± 0.20	0.04 ± 0.01	54.0 ± 3.65	2.35 ± 0.16	0.73
		28-Aug	27.4	24.6	30.2 ± 6.80	3.37 ± 1.60	0.72 ± 0.26	1.26 ± 0.44	0.06 ± 0.02	50.7 ± 7.58	2.10 ± 0.40	0.94
		29-Aug	27.9	24.3	26.1 ± 2.86	2.17 ± 0.47	0.41 ± 0.12	0.88 ± 0.35	0.05 ± 0.02	40.8 ± 7.80	1.57 ± 0.22	0.82
		4-Sep	26.8	25.9	25.8 ± 1.44	1.37 ± 0.38	0.80 ± 0.61	0.68 ± 0.24	0.04 ± 0.02	50.2 ± 3.05	1.37 ± 0.11	1.08
		10-Sep	27.4	23.8	16.4 ± 2.52	1.75 ± 0.06	0.47 ± 0.13	1.06 ± 0.30	0.04 ± 0.03	30.0 ± 7.40	1.35 ± 0.45	1.13
		17-Sep	27.6	23.1	24.9 ± 7.20	0.00 ± 0.19	0.30 ± 0.09	1.12 ± 0.53	0.06 ± 0.03	25.1 ± 3.23	2.04 ± 0.21	0.70
		24-Sep	27.6	19.3	44.0 ±7.03	4.26 ± 0.15	0.32 ±0.11	0.22 ± 0.16	0.12 ±0.23	39.9 ± 1.90	1.22 ± 0.22	0.44
Great Peconic Bay	2006	22-Aug	28.0	24.9	----	----	----	----	----	----	----	----
		30-Aug	27.7	25.0	----	----	----	----	----	----	----	----
	2007	22-Aug	30.0	24.2	12.1 ± 7.18	0.52 ± 0.30	1.37 ± 1.52	1.80 ± 0.18	0.46 ± 0.16	9.11 ± 0.74	1.72 ± 0.14	1.84
		28-Aug	30.1	24.4	13.7 ± 1.75	0.78 ± 0.05	0.22 ± 0.16	1.27 ± 0.19	0.27 ± 0.17	16.4 ± 3.33	0.83 ± 0.09	1.80
		30-Aug	30.3	24.5	12.5 ±3.39	1.55 ± 0.30	0.53 ± 0.29	1.26 ± 0.07	0.17 ± 0.05	21.5 ± 0.64	0.78 ± 0.09	2.29
		4-Sep	30.6	23.7	7.92 ± 5.41	1.35 ± 0.60	0.17 ± 0.14	1.33 ± 0.40	0.18 ± .011	16.9 ± 5.29	1.20 ± 0.18	1.25
	2008	27-Aug	28.3	24.2	16.4 ± 3.91	2.06 ± 0.46	0.60 ± 0.32	0.27 ± 0.27	0.36 ± 0.17	47.3 ± 5.48	1.79 ± 0.25	0.49
		28-Aug	28.0	24.2	12.8 ± 2.71	1.37 ± 0.50	0.70 ± 0.20	1.12 ± 1.03	0.36 ±0.08	45.0 ± 8.81	2.14 ± 0.96	0.85
		29-Aug	28.4	24.7	17.0 ± 0.61	1.81 ± 0.69	0.90 ± 0.39	0.77 ± 0.28	0.46 ±0.12	39.3 ± 6.31	1.29 ± 0.25	1.29
		4-Sep	28.4	25.2	17.8 ± 3.96	2.22 ± 0.60	0.70 ± 0.10	0.35 ± 0.23	0.42 ± 0.12	53.8 ± 1.86	1.56 ± 0.23	0.67
		10-Sep	28.0	23.9	14.9 ± 3.95	1.36 ± 0.37	0.45 ± 0.10	0.99 ± 0.56	0.44 ± 0.08	33.6 ± 11.2	1.02 ± 0.38	1.41
		11-Sep	28.3	23.5	15.3 ± 2.34	0.79 ± 0.30	0.74 ± 0.09	0.28 ± 0.07	0.68 ± 0.36	45.8 ± 1.21	1.48 ± 0.17	0.69
Meeting House Creek	2006	21-Aug	25.4	26.1	12.2 ± 1.48	0.27 ± 0.01	1.45 ± 0.15	1.91 ± 0.09	----	110 ± 5.95	2.66 ± 0.09	1.26
		22-Aug	24.9	25.4	9.29 ± 2.74	0.85 ± 0.10	2.07 ± 0.46	2.91 ± 0.73	----	88.7 ± 6.80	3.60 ± 0.36	1.38
		30-Aug	21.8	20.8	22.9 ± 0.50	0.14 ± 0.01	1.84 ± 0.02	5.55 ± 1.95	----	51.5 ± 6.84	3.17 ± 0.63	2.33
		5-Sep	24.4	20.2	19.6 ± 5.12	3.51 ±0.80	2.02 ± 0.49	6.00 ± 0.40	----	55.3 ± 4.55	2.46 ± 0.87	3.26
		7-Sep	24.7	20.1	37.5 ± 17.7	1.93 ± 0.47	2.25 ± 0.26	3.34 ± 0.70	----	45.4 ± 3.93	0.72 ± 0.09	7.76
	2007	22-Aug	29.8	24.0	41.9 ± 17.8	1.18 ± 1.07	0.02 ± 0.006	1.38 ± 0.34	0.25 ± 0.03	31.1 ± 6.64	2.67 ± 0.15	0.52
		28-Aug	30.2	24.4	32.7 ± 17.6	2.26 ± 0.77	1.20 ± 0.85	0.91 ± 0.09	0.86 ± 0.19	47.3 ± 1.01	0.44 ± 0.16	4.80
		30-Aug	28.2	25.3	25.9 ± 5.03	1.46 ± 0.09	0.10 ± 0.005	0.59 ± 0.06	0.23 ± 0.14	22.8 ± 0.43	0.17 ± 0.03	4.06
		4-Sep	26.8	25.7	22.4 ± 5.35	0.81 ± 0.20	0.46 ± 0.19	1.08 ± 0.06	0.45 ± 0.15	37.1 ± 5.20	1.69 ± 0.88	0.91
	2008	27-Aug	26.3	24.7	31.1 ± 6.26	3.69 ± 0.36	1.37 ± 0.34	2.68 ± 1.28	0.20 ± 0.16	73.3 ± 7.44	2.29 ± 0.46	1.77
		28-Aug	26.4	25.6	32.1 ± 6.50	3.57 ± 1.17	0.96 ± 0.48	1.13 ± 0.53	0.97 ± 0.20	63.9 ± 13.4	2.05 ± 0.64	1.02
		29-Aug	26.5	24.6	26.7 ± 3.46	0.59 ± 0.20	0.90 ± 0.36	0.16 ± 0.08	0.26 ± 0.06	74.9 ± 5.91	1.67 ± 0.70	0.63
		4-Sep	26.3	25.7	16.2 ± 0.82	0.20 ± 0.05	0.65 ± 0.15	0.12 ± 0.10	0.36 ± 0.03	53.8 ± 9.57	1.37 ± 0.79	0.56
		11-Sep	25.4	23.5	15.5 ± 0.91	1.19 ± 0.03	0.97 ± 0.42	0.15 ± 0.07	0.34 ± 0.15	79.4 ± 2.25	1.69 ± 0.52	0.66
		24-Sep	26.2	20.9	14.2 ± 1.63	0.47 ± 0.03	1.08 ± 0.49	0.18 ± 0.09	0.39 ± 0.19	64.0 ± 6.68	1.40 ± 0.41	0.90
Old Fort Pond	2006	24-Aug	----	----	33.4 ± 2.29	0.3 ± 0.03	0.27 ± 0.13	1.62 ± 0.16	----	37.2 ± 1.18	2.02 ± 0.07	0.94
		31-Aug	----	----	36.8 ± 8.02	0.55 ± 0.15	0.27 ± 0.16	1.46 ± 0.03	----	45.4 ± 1.66	2.83 ± 0.33	0.61
		1-Sep	----	----	----	----	----	----	----	----	----	----
		7-Sep	----	----	19.9 ± 3.73	0.19 ± 0.17	0.10 ± 0.06	1.71 ± 0.16	----	72.4 ± 2.58	1.71 ± 0.03	1.06
	2007	22-Aug	30.2	25.0	29.7 ± 8.26	0.90 ± 0.59	7.92 ± 1.20	1.85 ± 0.69	0.36 ± 0.08	32.4 ± 5.33	2.95 ± 0.63	3.31
		28-Aug	29.8	25.1	13.5 ± 5.22	1.20 ± 0.40	9.61 ± 2.50	2.62 ± 0.03	1.13 ± 0.43	26.8 ± 14.1	2.96 ± 2.15	4.13
		30-Aug	30.0	25.1	8.27 ± 2.05	1.09 ± 0.00	8.82 ± 0.90	0.99 ± 0.03	0.28 ± 0.01	41.3 ± 4.37	0.54 ± 0.14	18.2
		4-Sep	30.6	24.6	16.9 ± 4.13	1.20 ± 0.19	0.21 ± 0.02	1.94 ± 0.03	1.78 ± 0.20	22.8 ± 0.72	2.69 ±1.80	0.80
	2008	13-Aug	28.6	25.0	16.5 ± 1.02	1.24 ± 0.14	1.91 ± 1.02	0.65 ± .31	0.46 ± 0.44	13.2 ± 3.53	0.29 ± 0.14	8.83
		22-Aug	26.7	25.5	18.2 ± 0.41	1.21 ± 0.21	1.10 ± 1.07	0.09 ± 0.06	0.15 ± 0.13	36.1 ± 1.58	0.07 ± 0.04	17.0
		3-Sep	30.2	23.1	24.9 ± 0.09	0.87 ± 0.18	1.27 ± 1.26	0.44 ± 0.27	0.31 ± 0.26	16.8 ± 0.83	0.55 ± 0.11	3.11
		5-Sep	22.8	26.2	20.9 ± 0.35	1.72 ± 0.12	1.42 ± 1.14	0.65 ± 0.31	0.63 ± 0.30	37.5 ± 13.6	0.90 ± 0.49	2.30
		23-Sep	30.3	19.1	15.3 ± 0.06	0.20 ± 0.09	0.54 ± 0.30	0.23 ± 0.17	0.08 ± 0.07	12.5 ± 4.50	0.90 ± 0.14	0.86
Shinnecock Bay	2006	5-Sep	----	----	----	----	----	----	----	----	----	----
	2007	22-Aug	----	----	----	----	----	----	----	----	----	----
		28-Aug	----	----	----	----	----	----	----	----	----	----
		30-Aug	----	----	----	----	----	----	----	----	----	----
		4-Sep	----	----	----	----	----	----	----	----	----	----
	2008	22-Aug	28.3	25.6	11.0 ± 1.12	0.71 ± 0.18	0.72 ± 0.25	0.82 ± 0.24	0.42 ± 0.14	33.7 ± 14.0	0.64 ± 0.16	2.41
		28-Aug	28.8	24.2	23.1 ± 2.04	2.37 ± 0.14	0.73 ± 0.21	1.26 ± 0.37	0.87 ± 0.32	32.1 ± 9.23	1.64 ± 0.39	1.21
		29-Aug	29.4	24.2	26.3 ± 6.71	2.18 ± 0.23	0.67 ± 0.16	1.85 ± 0.59	0.90 ± 0.38	21.3 ± 2.63	1.53 ± 0.23	1.65
		4-Sep	28.9	24.5	25.5 ± 2.95	0.38 ± 0.10	0.10 ± 0.09	0.84 ± 0.47	0.85 ± 0.23	39.1 ± 2.59	1.44 ± 0.28	0.65
		5-Sep	27.7	26.9	21.7 ± 1.45	4.87 ± 0.19	0.51 ± 0.12	0.96 ± 0.56	1.09 ± 0.42	55.4 ± 3.20	0.85 ± 0.17	1.73
		16-Sep	26.9	21.7	23.1 ± 3.92	2.18 ± 0.15	0.84 ± 0.18	0.52 ± 0.29	0.77 ± 0.58	31.1 ± 15.3	1.01 ± 0.15	1.35
		23-Sep	30.7	19.0	11.7 ± 0.38	1.24 ± 0.09	0.83 ± 0.17	1.96 ± 0.24	2.85 ± 0.51	23.5 ± 1.63	0.87 ± 0.14	3.21

Table 5. Salinity (psu), temperature (°C), and dissolved nutrient concentrations (µM) at all sampling sites from 2006-2008.

Site		*C. polyrikoides* as % of cells >20 µm	*C. polykrikoides* (mL^{-1})	Diatoms (mL^{-1})	Other Dinoflagellates (mL^{-1})	Chlorophyll *a* (µg L-1) Total	Chlorophyll *a* (µg L-1) >5µm	Salinity (psu)	Temperature (°C)	Dissolved Organic Nitrogen (µM)	Dissolved Organic Phosphorous (µM)	Nitrate (µM)	Ammonia (µM)	Urea (µM)	Sillicate (µM)	Phosphate (µM)	DIN:DIP
Flanders Bay	Total	----	5590 ± 8850	421 ± 825	200 ± 215	54.8 ± 67.5	30.5 ± 45.8	27.0 ± 1.83	23.3 ± 1.72	23.8 ± 9.68	1.84 ± 1.07	1.22 ± 1.63	1.68 ±1.96	0.185 ± 0.242	44.1 ± 18.8	1.49 ± 0.42	2.12 ± 1.91
	Bloom	96	7120 ± 9480	51.5 ± 53.5	248 ± 232	67.4 ± 71.4	37.5 ± 49.8	26.5 ± 1.78	22.9 ± 1.92	27.5 ± 7.74	1.98 ± 1.14	0.462 ± 0.175	1.83 ± 2.21	0.153 ± 0.267	48.6 ± 18.6	1.63 ± 0.369	1.50 ± 1.66
	Non-Bloom	<1	10.3 ± 2.53	1410 ± 1180	70.2 ± 02.4	8.54 ± 5.59	5.07 ± 1.97	28.7 ± 0.541	24.2 ± 0.517	13.7 ± 7.12	0.720 ± 0.184	2.66 ± 2.41	1.15 ± 0.161	0.273 ± 0.167	27.4 ± 6.83	1.03 ± 0.154	3.56 ± 1.97
Great Peconic Bay	Total	----	10500 ± 18600	21.2 ± 13.9	201 ± 200	49.4 ± 65.7	31.8 ± 55.9	28.8 ± 1.04	24.4 ± 0.515	14. 1 ± 2.91	1.38 ± 0.561	0.638 ± 0.344	0.944 ± 0.516	0.380 ± 0.151	32.9 ± 15.7	1.38 ± 0.439	1.26 ± 0.592
	Bloom	97	11900 ± 19600	23.0 ± 15.4	296 ± 261	81.0 ± 71.5	52.4 ± 67.5	28.2 ± 0.32	24.4 ± 0.59	15.7 ± 1.78	1.60 ± 0.53	0.682 ± 0.149	0.630 ± 0.379	0.453 ± 0.118	44.1 ± 6.95	1.55 ± 0.389	0.901 ± 0.371
	Non-Bloom	10	13.7 ± 0.103	14.0 ± 0.321	107 ± 42.2	5.03 ± 2.17	2.91 ± 1.48	29.7 ± 1.13	24.3 ± 0.453	11.6 ± 2.51	1.05 ± 0.481	0.573 ± 0.555	1.42 ± 0.259	0.270 ± 0.134	15.9 ± 5.13	1.13 ± 0.434	1.79 ± 0.428
Meeting House Creek	Total	----	3170 ± 5970	292 ± 300	919 ± 160	63.7 ± 56.8	42.2 ± 49.7	26.2 ± 2.45	24.1 ± 1.97	24.0 ± 9.69	1.47 ± 1.25	1.16 ± 0.690	1.87 ± 1.89	0.431 ± 0.268	59.9 ± 23.0	1.87 ± 0.977	2.12 ± 2.05
	Bloom	72	4730 ± 6890	405 ± 316	1450 ± 1950	55.3 ± 25.6	37.6 ± 22.0	25.1 ± 1.65	23.7 ± 2.32	22.5 ± 9.12	1.61 ± 1.46	1.41 ± 0.58	2.22 ± 2.25	0.42 ± 0.28	65.0 ± 13.8	2.04 ± 0.869	2.03 ± 2.19
	Non-Bloom	17	70.3 ± 69.9	207 ± 301	125 ± 43.4	80.6 ± 96.4	51.4 ± 86.1	28.9 ± 1.83	24.9 ± 0.787	27.0 ± 11.2	1.19 ± 0.743	0.646 ± 0.648	1.17 ± 0.501	0.448 ±0.292	49.7 ± 35.1	1.53 ± 1.19	2.31 ± 1.97
Old Fort Pond	Total	----	4950 ± 9330	679 ± 855	994 ± 833	53.3 ± 43.1	33.6 ± 24.1	28.6 ± 2.87	24.9 ± 1.06	21.2 ± 8.48	0.889 ± 0.484	2.79 ± 3.68	1.19 ± 0.791	0.576 ± 0.548	32.9 ± 16.6	1.53 ± 1.12	5.09 ± 6.27
	Bloom	87	5810 ± 9950	111 ± 86.8	795 ± 718	58.9 ± 47.0	38.8 ± 24.1	28.6 ± 3.24	24.8 ± 1.31	21.9 ± 9.18	0.826 ± 0.509	3.56 ± 3.98	1.22 ± 0.753	0.464 ± 0.338	33.5 ± 18.0	1.57 ± 1.07	4.32 ± 5.45
	Non-Bloom	8	218 ± 82.0	608 ± 859	1790 ± 899	31.0 ± 4.77	12.9 ± 9.96	28.7 ± 2.72	25.0 ±0.636	17.6 ± 0.849	1.21 ± 0.007	0.655 ±0.629	1.02 ± 1.31	0.965 ± 1.15	29.5 ± 9.46	1.39 ± 1.85	8.89 ± 11.5
Shinnecock Bay	Total	----	4730 ± 2950	38.7 ± 25.9	175 ± 152	49.2 ± 51.9	38.9 ± 10.9	28.7 ± 1.21	24.1 ± 2.71	20.3 ± 6.33	1.99 ± 1.49	0.629 ± 0.258	1.17 ± 0.547	1.11 ± 0.795	33.7 ± 11.3	1.14 ± 0.391	1.74 ± 0.839
	Bloom	96	5310 ± 2550	36.0 ± 25.7	209 ± 185	71.8 ± 49.9	38.9 ± 10.9	28.7 ± 1.21	24.1 ±2.71	20.3 ± 6.33	1.99 ± 1.49	0.629 ±0.258	1.17 ± 0.547	1.11 ± 0.795	33.7 ± 11.3	1.14 ± 0.391	1.74 ± 0.839
	Non-Bloom	14	26.7 ± 53.3	43.3 ± 29.6	117 ± 31.5	4.02 ± 1.35	----	----	----	----	----	----	----	----	----	----	----

Table 6. Mean ± standard deviation (SD) of cell abundances, chlorophyll *a* levels, salinity, temperature and ambient dissolved nutrients during bloom, non-bloom and both (total) periods at all sites during 2006-2008. A bloom is defined as > 330 cells mL^{-1}.

Site	Date	Salinity (psu)	Temperature (°C)	Total chlorophyll *a* (µg L^{-1})	*C. polykrikoides* (mL^{-1})	*C. polykrikoides* as % of total cells >20 µm	PON (µg N) of matter >20 µM	Ambient Concentrations (µM)				
								DFAA	Ammoinium	Urea	Nitrate	Nitrite
Old Fort Pond	9/3/2008	30.2	23.1	32.0 ± 0.87	476 ± 40	68	13.1 ± 3.26	0.423 ± 0.017	0.442 ± 0.27	0.308 ± 0.26	1.27 ± 1.2	0.602 ± 0.24
Shinnecock Bay	8/22/2008	28.3	25.6	25.3 ± 1.3	2390 ± 670	80	13.2 ± 6.78	0.151 ± 0.006	0.816 ± 0.24	0.418 ± 0.14	0.400 ± 0.16	0.680 ± 0.16
	8/25/2008	29.4	23.7	27.4 ± 3.0	1200 ± 280	78	7.18 ± 2.23	0.519 ± 0.021	0.446 ± 0.15	0.807 ± 0.33	0.216 ± 0.16	0.445 ± 0.20
	9/5/2008	27.7	26.9	48.2 ± 5.9	2020 ± 240	76	42.5 ± 5.41	0.824 ± 0.033	0.958 ± 0.56	1.08 ± 0.42	0.070 ± 0.06	0.569 ± 0.19
	9/16/2008	26.9	21.7	40.7 ± 2.1	2880 ± 51	97	36.5 ± 9.67	0.147 ± 0.006	0.522 ± 0.29	0.768 ± 0.58	0.268 ± 0.18	0.469 ± 0.13
Great Peconic Bay	8/27/2008	28.3	24.2	55.4 ± 4.0	5480 ± 85	96	19.9 ± 3.58	0.569 ± 0.023	0.431 ± 0.27	0.358 ± 0.17	0.598 ± 0.32	0.608 ± 0.15
	9/4/2008	28.4	25.2	55.7 ± 2.8	2710 ± 110	81	35.5 ± 2.15	0.572 ± 0.023	0.350 ± 0.23	0.422 ± 0.12	0.690 ± 0.20	0.336 ± 0.10
Flanders Bay	8/29/2008	27.9	24.3	33.1 ± 1.0	1040 ± 23	83	12.8 ± 2.96	0.516 ± 0.021	0.883 ± 0.35	0.100 ± 0.02	0.721 ± 0.26	0.636 ± 0.26
	9/10/2008	27.4	23.8	71.1 ± 1.5	3380 ± 290	94	28.7 ± 0.823	1.50 ± 0.060	1.06 ± 0.30	0.100 ± 0.03	0.474 ± 0.13	0.445 ± 0.16
	9/24/2008	27.6	19.3	18.3 ± 0.25	480 ± 45	78	13.3 ± 2.03	0.280 ± 0.011	0.222 ± 0.16	0.214 ± 0.14	0.323 ± 0.11	0.326 ± 0.04

Table 7. Salinity (psu), temperature (°C), total chlorophyll *a* (µg L^{-1}), cell density of *C. polykrikoides* (mL^{-1}), particulate organic nitrogen (PON, ug N L^{-1}), ambient concentrations (µM) of dissolved free amino-acids (DFAA) and other N sources, and percentage of total cells >20 µm that were *C. polykrikoides* from field ^{15}N experiments.

Site and Date	*C. polykrikoides* (µ)					Other dinoflagellates (µ)					Diatoms (µ)					Chlorophyll *a* <5 µm (µ)				
Old Fort Pond	Control	Nitrate	Urea	Glutamic acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia
9/3/2008	0.37 ± 0.22	**0.75 ± 0.10**	**0.64 ± 0.13**	0.32 ± 0.13	**0.64 ± 0.13**	0.69 ± 0.11	0.73 ± 0.32	0.70 ± 0.16	0.11 ± 0.35	0.45 ± 0.21	-0.93 ± 0.49	-0.54 ± 0.19	-0.61 ± 0.54	-0.33 ± 0.07	-0.33 ± 0.47	-0.51 ± 0.25	-0.36 ± 0.32	-0.44 ± 0.73	-1.06 ± 0.00	**0.14 ± 0.01**
9/8/2008	0.15 ± 0.20	0.23 ± 0.24	0.36 ± 0.33	0.60 ± 0.14	0.05 ± 0.25	-0.46 ± 0.31	-0.18 ± 0.25	-0.18 ± 0.50	-0.13 ± 0.35	-0.54 ± 0.00	0.35 ± 0.15	0.51 ± 0.10	0.47 ± 0.37	-0.28 ± 0.00	-0.63 ± 0.00	0.00 ± 0.00	0.13 ± 0.06	0.02 ± 0.20	0.03 ± 0.14	-0.45 ± 1.01
10/2/2008	0.39 ± 0.39	-0.03 ± 0.20	0.07 ± 0.08	0.42 ± 0.37	0.47 ± 0.15	0.38 ± 0.12	0.43 ± 0.15	-0.14 ± 0.23	0.57 ± 0.16	0.41 ± 0.39	0.75 ± 0.45	1.47 ± 0.15	10.03 ± 0.30	`.46 ± 0.04	1.58 ± 0.51	0.03 ± 0.12	**0.54 ± 0.06**	**0.52 ± 0.06**	0.25 ± 0.10	0.14 ± 0.18
8/30/2007	-0.08 ± 0.02	**0.02 ± 0.01**	**0.03 ± 0.01**	-0.09 ± 0.04	**0.19 ± 0.04**	0.53 ± 0.04	**0.74 ± 0.01**	0.43 ± 0.03	**0.67 ± 0.01**	0.51 ± 0.05	1.20 ± 0.05	0.63 ± 0.17	1.11 ± 0.04	1.01 ± 0.05	1.18 ± 0.06	-0.29 ± 0.07	0.03 ± 0.13	-0.05 ± 0.03	-1.15 ± 0.36	0.20 ± 0.05
8/30/2005	-0.09 ± 0.35	**0.63 ± 0.21**	NA	NA	NA	-0.001 ± 0.03	0.06 ± 0.09	NA	NA	NA	-0.005 ± 0.09	0.01 ± 0.04	NA	NA	NA	NA	NA	NA	NA	NA
9/13/2005	-0.001 ± 0.04	0.06 ± 0.04	NA	NA	NA	0.00 ± 0.04	-0.03 ± 0.04	NA	NA	NA	-0.004 ± 0.08	**0.85 ± 0.05**	NA	NA	NA	NA	NA	NA	NA	NA
9/27/2005	0.08 ± 0.34	**0.29 ± 0.09**	NA	NA	NA	-0.002 ± 0.05	-0.004 ± 0.04	NA	NA	NA	-0.0004 ± 0.03	**0.29 ± 0.01**	NA	NA	NA	NA	NA	NA	NA	NA
	C. polykrikoides (µ)					Other dinoflagellates (µ)					Diatoms (µ)					Chlorophyll *a* <5 µm (µ)				
Great Peconic Bay	Control	Nitrate	Urea	Glutamic acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia
9/4/2008	-0.06 ± 0.07	0.14 ± 0.12	0.10 ± 0.02	**0.11 ± 0.05**	**0.21 ± 0.07**	-0.07 ± 0.04	-0.18 ± 0.10	-0.14 ± 0.07	-0.23 ± 0.03	-0.26 ± 0.07	NA	NA	NA	NA	NA	-0.19 ± 0.33	-0.28 ± 0.13	-0.20 ± 0.01	-0.28 ± 0.15	-0.13 ± 0.08
9/11/2008	-0.07 ± 0.04	-0.18 ± 0.10	-0.14 ± 0.07	-0.04 ± 0.13	-0.23 ± 0.03	0.64 ± 0.14	0.42 ± 0.48	0.58 ± 0.08	0.45 ± 0.14	0.52 ± 0.09	NA	NA	NA	NA	NA	-1.36 ± 0.24	**-0.27 ± 0.33**	-0.82 ± 9.42	-0.28 ± 0.16	-1.24 ± 1.19
	C. polykrikoides (µ)					Other dinoflagellates (µ)					Diatoms (µ)					Chlorophyll *a* <5 µm (µ)				
Meeting House Creek	Control	Nitrate	Urea	Glutamic acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia
10/3/2008	0.59 ± 0.35	**0.81 ± 0.21**	**0.85 ± 0.19**	**0.75 ± 0.38**	**0.78 ± 0.18**	0.64 ± 0.24	0.73 ± 0.08	0.70 ± 0.04	0.66 ± 0.01	0.70 ± 0.08	0.87 ±0.57	0.89 ± 0.15	0.83 ± 0.32	1.05 ± 0.23	1.07 ± 0.06	0.57 ± 0.07	0.61 ± 0.02	0.64 ± 0.02	0.66 ± 0.01	0.62 ± 0.02
8/30/2007	0.13 ± 0.03	**0.93 ± 0.11**	**1.15 ± 0.05**	**0.43 ± 0.05**	**1.08 ± 0.03**	-1.35 ± 0.07	-1.86 ± 0.19	-1.25 ± 0.07	-1.50 ± 0.10	-1.50 ±0.12	0.58 ± 0.05	**1.10 ± 0.14**	**1.34 ± 0.01**	0.50 ± 0.04	0.65 ± 0.04	NA	NA	NA	NA	NA
9/5/2006	0.13 ± 0.05	**0.25 ± 0.03**	**0.34 ± 0.07**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-0.08 ± 0.06	**0.20 ± 0.08**	**0.10 ± 0.03**	NA	NA
	C. polykrikoides (µ)					Other dinoflagellates (µ)					Diatoms (µ)					Chlorophyll *a* <5 µm (µ)				
Shinnecock Bay	Control	Nitrate	Urea	Glutamic acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia
8/25/2008	0.06 ± 0.04	**0.15 ± 0.14**	0.04 ± 0.09	0.01 ± 0.03	**0.12 ± 0.02**	0.53 ± 0.02	0.53 ± 0.12	0.33 ± 0.10	0.32 ± 0.26	0.49 ± 0.21	-0.37 ± 0.64	0.00 ± 0.00	-0.56 ± 0.96	-1.11 ± 0.96	-1.00 ± 0.88	1.61 ± 0.00	1.60 ± 0.32	1.54 ± 0.17	1.37 ± 0.48	1.63 ± 0.07
9/5/2008	0.05 ± 0.08	**0.18 ± 0.10**	0.02 ± 0.06	0.03 ± 0.12	0.02 ± 0.04	-0.06 ± 0.11	-0.04 ± 0.12	-0.14 ± 0.13	-0.28 ± 0.17	-0.22 ± 0.11	NA	NA	NA	NA	NA	-0.74 ± 0.13	-0.97 ± 0.42	-1.23 ± 0.34	-0.30 ± 0.00	-0.83 ± 0.18
9/23/2008	-0.35 ± 0.08	-0.59 ± 0.27	-0.42 ± 0.16	-0.39 ± 0.47	-0.50 ± 0.20	-0.20 ± 0.18	-0.10 ± 0.45	**0.97 ± 0.32**	-0.34 ± 0.66	-0.92 ± 9.40	-0.78 ± 0.56	-0.10 ± 0.18	-0.21 ±0.09	-0.55 ±0.38	-0.11 ±0.53	-0.86 ± 0.00	-1.25 ± 0.52	-0.61 ± 0.34	-0.83 ± 1.00	-0.75 ± 0.15
	C. polykrikoides (µ)					Other dinoflagellates (µ)					Diatoms (µ)					Chlorophyll *a* <5 µm (µ)				
Flanders Bay	Control	Nitrate	Urea	Glutamic acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia	Control	Nitrate	Urea	Glutamic Acid	Ammonia
8/29/2008	0.14 ± 0.02	**0.33 ± 0.10**	**0.29 ±0.08**	0.14 ± 0.20	**0.42 ± 0.11**	0.65 ± 0.08	0.56 ± 0.08	0.46 ± 0.07	0.71 ± 0.13	0.49 ± 0.16	NA	NA	NA	NA	NA	-0.89 ± 0.81	-1.23 ± 1.05	-0.58± 0.13	-0.59 ± 0.41	-0.44 ± 0.05
9/24/2008	0.20 ± 0.08	0.25 ± 0.14	0.18 ± 0.07	-0.05 ± 0.31	0.15 ± 0.09	1.08 ± 0.12	1.07 ± 0.47	1.03 ± 0.21	0.75 ± 0.44	0.99 ± 0.34	0.26 ± 0.45	1.52 ± 0.31	0.39 ± 0.68	1.20 ± 0.29	0.64 ± 0.20	-0.10 ± 0.03	**0.23 ± 0.08**	-0.11 ± 0.02	0.09 ± 0.03	0.06 ± 0.12
8/22/2007	-0.09 ± 0.03	**0.14 ± 0.01**	0.06 ± 0.07	**0.24 ± .0.1**	**0.13 ± 0.05**	0.05 ± 0.08	**0.29 ± 0.01**	**0.29 ± 0.07**	0.04 ± 0.03	0.19 ± 0.09	-0.11 ± 0.11	**0.82 ± 0.05**	0.05 ± 0.10	**0.41 ± 0.15**	**0.65 ± 0.02**	0.15 ± 0.14	0.20 ± 0.10	0.13 ± 0.15	0.36 ± 0.14	0.22 ± 0.06
9/4/2007	0.14 ± 0.07	-0.16 ± 0.31	0.07 ± 0.04	-0.18 ± 0.15	-0.33 ± 0.37	0.79 ± 0.02	0.64 ± 0.09	0.65 ± 0.07	0.60 ± 0.05	0.70 ± 0.05	-1.30 ± 0.08	**-0.11 ± 0.12**	**-0.54 ± 0.22**	**-0.41 ± 0.18**	**-0.67 0.22**	NA	NA	NA	NA	NA
8/23/2006	-0.08 ± 0.01	**0.13 ± 0.05**	**0.14 ± 0.05**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-0.07 ± 0.10	-0.63 ± 0.19	**0.27 ± 0.06**	NA	NA
9/11/2006	-0.27 ± 0.13	0.17 ± 0.10	0.03 ± 0.15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-0.02 ± 0.93	0.04 ± 0.18	0.26 ± 0.17	NA	NA

Table 8. Net growth rates of all nutrient amendment experiments from 2008, 2007, 2006 and 2005 from all sites under all treatments. Significantly increased net growth rates (Tukey test, $p<0.05$) when compared to control are highlighted red.

	C. polykrikoides	Other dinoflagellates	Diatoms	Small phytoplankton
Any N compound	62% (13/21)	17% (3/18)	43% (6/14)	38% (6/16)
Nitrate	57% (12/21)	11% (2/18)	36% (5/14)	25% (4/16)
Ammonium	53% (8/15)	0% (0/15)	18% (2/11)	8% (1/13)
Urea	39% (7/18)	13% (2/15)	18% (2/11)	19% (3/16)
Glutamic acid	27% (4/15)	7% (1/15)	18% (2/11)	0% (0/13)

Table 9. The percentage of experiments in which N compounds significantly increased the net growth rate of four phytoplankton groups relative to control treatments ($p<0.05$) during nutrient amendment experiments. Percentages and number of significant treatments out of total number of experiments (in parentheses) shown.

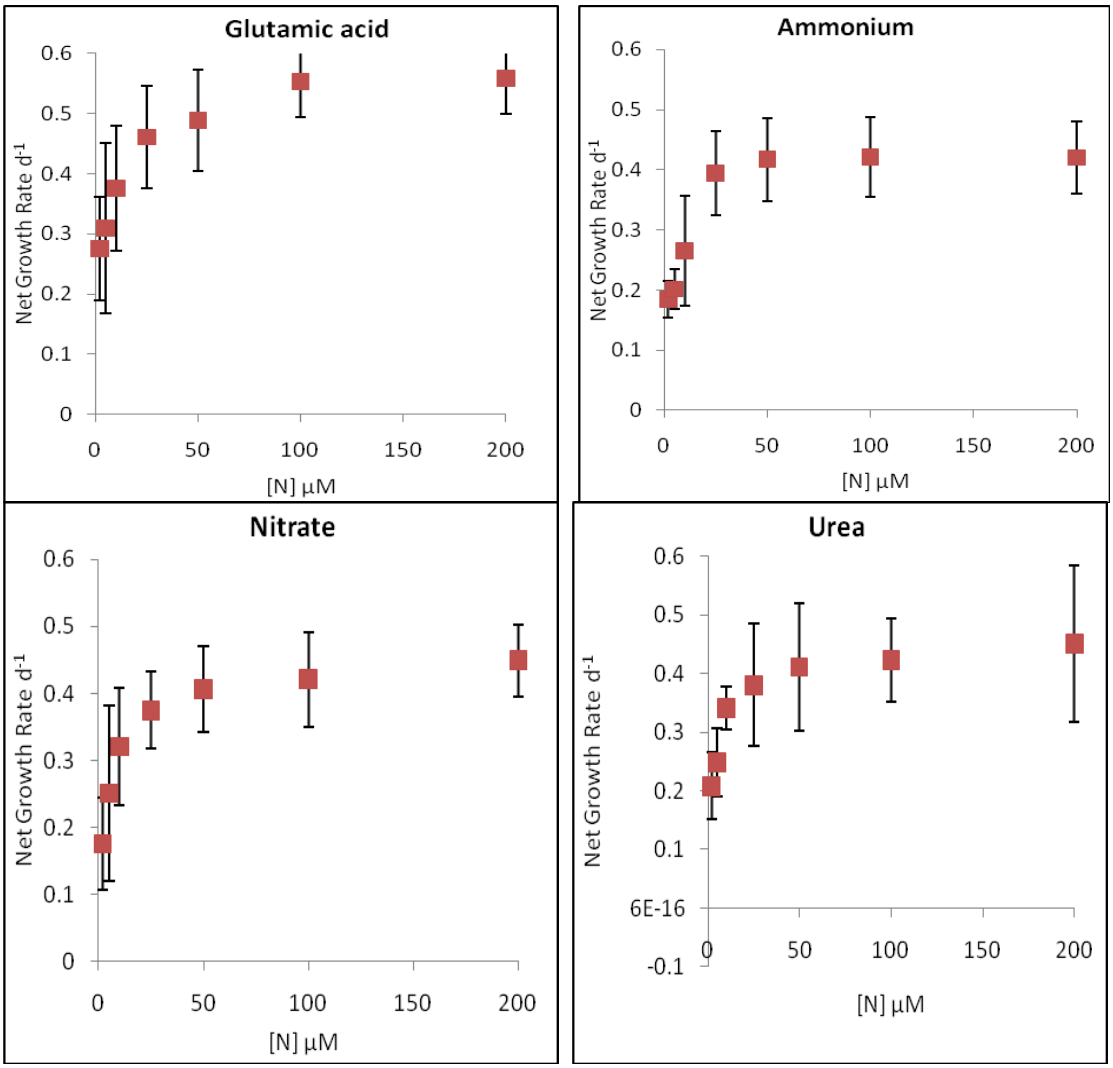

Figure 1. Growth rates (±SD) of *C. polykrikoides* cultures grown on multiple concentrations (2, 5, 10, 25, 50, 100, 200 µM) of glutamic acid, ammonium, urea and nitrate.

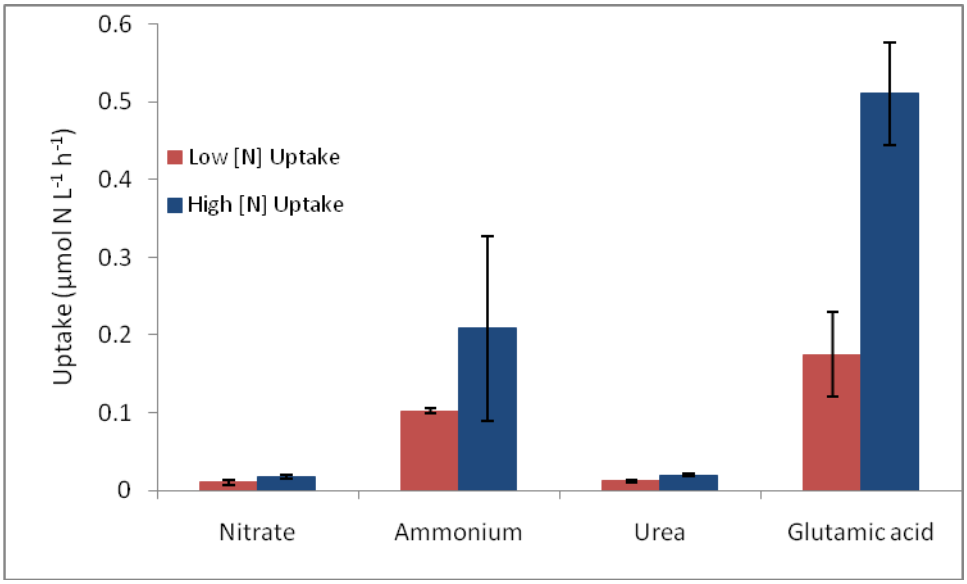

Figure 2. Nitrogen uptake of cultures grown on nitrate, urea, glutamic acid, and ammonium at high concentrations (20µM) and low concentrations (2µM) of N. Labeled nitrogen added was the same as the nitrogen species in which each culture was grown.

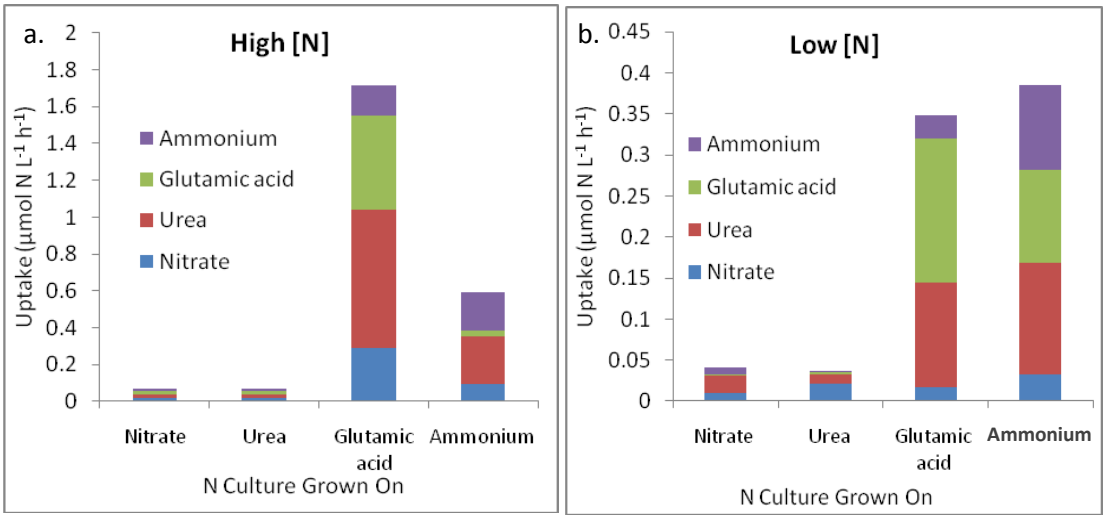

Figure 3 a-b. Uptake of high concentrations (20µM) and low concentrations (2µM) of various N species by cultures grown on different N sources. Mean relative standard deviation (RSD) for all rates was 0.3.

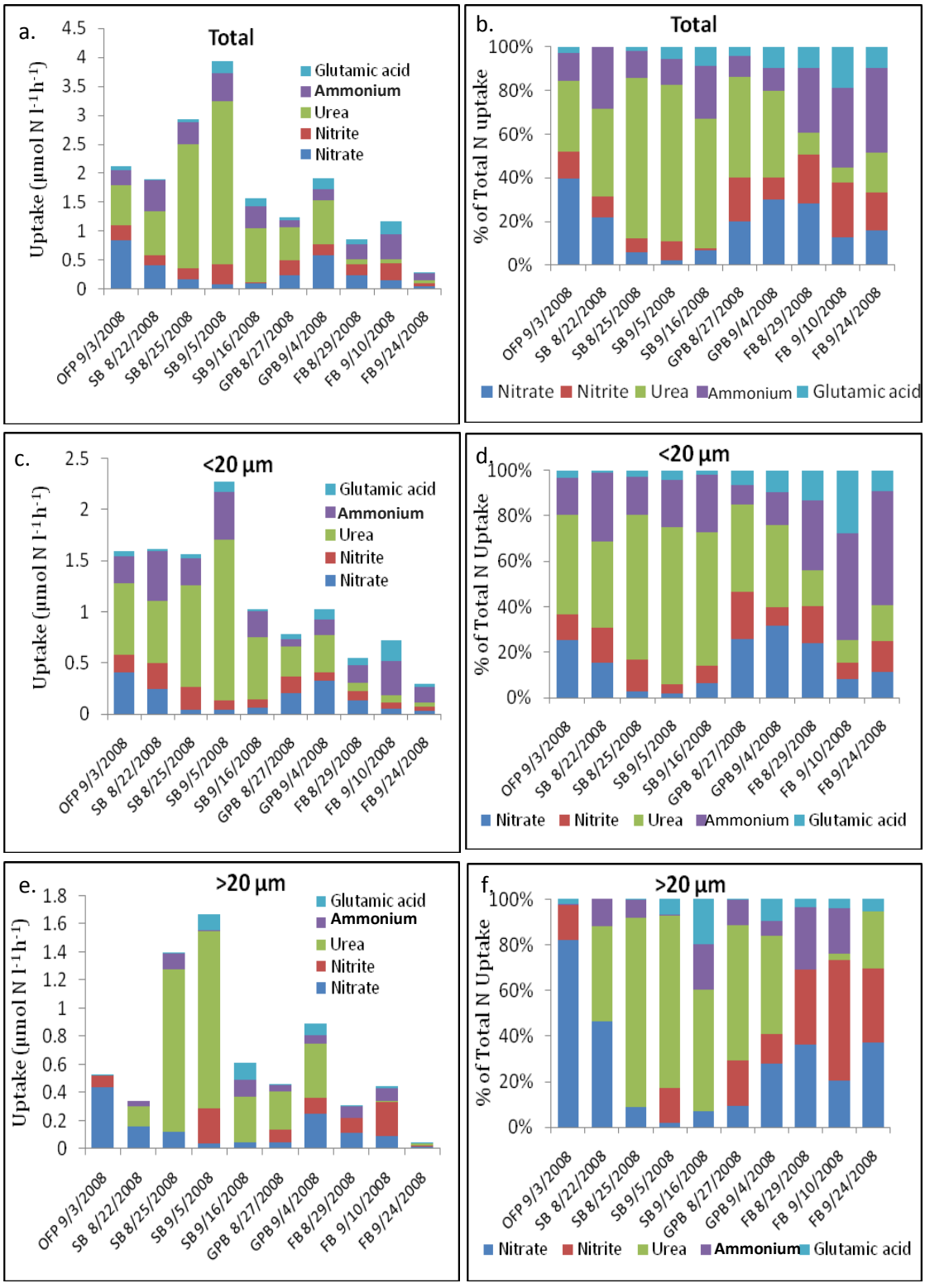
a.
Total
Uptake (µmol N l-1h-1)
Glutamic acid
Ammonium
Urea
Nitrite
Nitrate
b.
Total
% of Total N uptake
Nitrate Nitrite Urea Ammonium Glutamic acid
c.
<20 µm
Uptake (µmol N l-1h-1)
d.
<20 µm
% of Total N Uptake
e.
>20 µm
Uptake (µmol N l-1h-1)
f.
>20 µm
% of Total N Uptake
OFP 9/3/2008
SB 8/22/2008
SB 8/25/2008
SB 9/5/2008
SB 9/16/2008
GPB 8/27/2008
GPB 9/4/2008
FB 8/29/2008
FB 9/10/2008
FB 9/24/2008

Figure 4 a-f. Uptake (µmol N l^{-1} h^{-1}) and % of total uptake of ^{15}N-labeled N compounds by three plankton size fractions (total, <20 µm, and >20 µm) in *C. polykrikoides* bloom water. Water was obtained from Old Fort Pond (OFP), Shinnecock Bay (SB), Great Peconic Bay (GPB) and Flanders Bay (FB). Mean RSD of uptake rates for all experiments was 0.27.

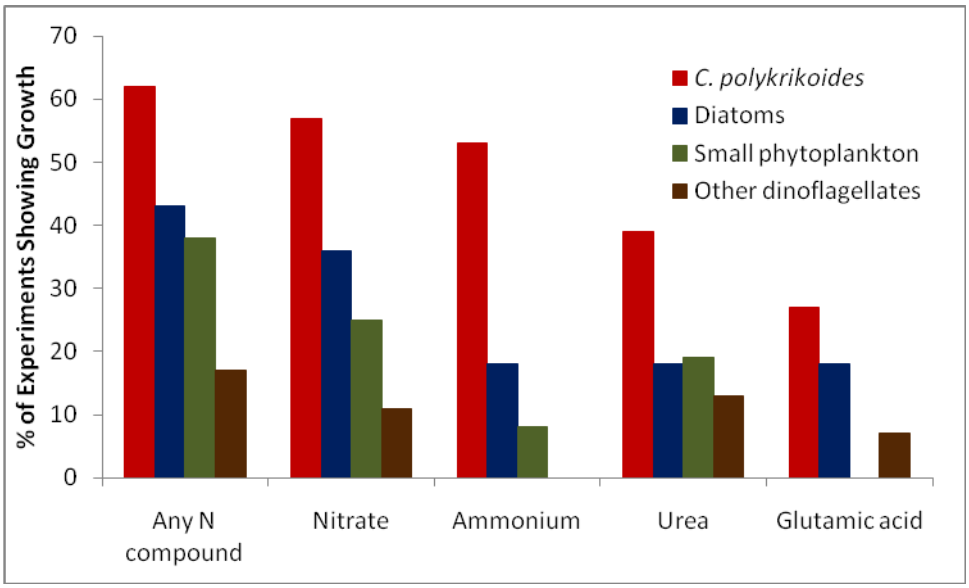

Figure 5. Percentage of growth experiments where each phytoplankton group (*C. polykrikoides*, other dinoflagellates, diatoms and small phytoplankton) showed faster growth than the control after addition of an N source (any N compound, nitrate, ammonium, urea, and glutamic acid).