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The role of vitamin B_1 and B_{12} in controlling phytoplankton biomass, diversity, and dynamics

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Abstract of the Dissertation

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Most phytoplankton species have a requirement for one of the B-vitamins (B_1 and B_{12}). Prior studies have found low, picomolar, B-vitamin concentrations in the marine environment and have demonstrated that vitamin B_{12} can limit or co-limit phytoplankton growth in coastal and open ocean environments. The extent, to which vitamins influence plankton species succession, however has never been assessed. In addition no study has quantified vitamin uptake rates by marine plankton communities. My dissertation examined the role of vitamin B_1 and B_{12} in the ecology of phytoplankton communities from the disparate ecosystems of the Gulf of Alaska and coastal waters of Long Island, NY.

In the Gulf of Alaska, picoplankton (0.2-2 μm) were responsible for the majority of vitamin B_{12} uptake in both coastal and high nutrient low chlorophyll (HNLC) regions and B_{12} concentrations and B_{12} uptake rates were higher in HNLC regions compared to coastal regions with higher iron (Fe) concentrations. During vitamin amendment experiments, B_{12} alone and in

conjunction with other limiting nutrients (N or Fe) significantly enhanced algal biomass and stimulated the growth of multiple groups of larger (> 2 μm) phytoplankton.

Vitamin utilization in two contrasting NY estuaries was highest in the more eutrophic systems and positively correlated with primary production. Similar to the Gulf of Alaska, vitamin B_1 and B_{12} uptake were dominated by the picoplankton with multiple lines of evidence suggesting that heterotrophic bacteria were the main utilizers. Carbon-specific uptake vitamin uptake rates showed a much higher uptake by picoplankton compared to microplankton and were higher in the more eutrophic system. Combined with prior studies, these findings suggest that picoplankton are both the primary producers and users of B-vitamins in coastal ecosystems and that rapid microbial cycling of B-vitamins may sometimes deprive larger phytoplankton of these micronutrients and thus influence phytoplankton species succession. During a brown tide, the harmful algae *A. anophagefferens* and the associated microbial community rapidly utilized vitamins B_1 and B_{12}, drawing down ambient concentrations from >100 pM to <7 pM over the course of a bloom. At the peak of the bloom the majority of B_1 uptake occurred in the brown tide size class (1-5 µm) while the <1 µm and 1-5 µm community utilized equal parts of the B_{12} pool. Culture work evidenced the ability of *A. anophagefferens* to adapt to lower vitamin concentrations although vitamin amendment experiments demonstrated that vitamin B_{12} can limit the growth of this organism during the peak and demise of brown tides.

Similar to the brown tide, the presence of the red tide forming dinoflagellate, *Cochlodinium polykrikoides*, significantly increased nutrient utilization compared to non-bloom water, with ten-fold and five-fold higher uptake rates for nitrogen and vitamin B_{12}, respectively, during blooms while B_1 uptake was unchanged. In addition the heterotrophic bacterial community associated with blooms was more abundant and was comprised of unique species

with the lower carbon-specific vitamin B_{12} uptake rates. In a manner consistent with brown tides, the enrichment of bloom water with vitamin B_{12}, but not B_1, significantly enhanced the growth of *C. polykrikoides* in 60% of experiments performed.

In summary, this dissertation has revealed the strong effects of B-vitamins on planktonic species succession, the importance of picoplankton as vitamin consumers, and the role of vitamins in the occurrence of HABs while making the first measurements of vitamin uptake by pelagic plankton, These findings collectively demonstrate that, like more actively researched macronutrients (N and P) and micronutrients (Fe), B-vitamins can play a central role in ecology of the ocean plankton. As such, more research is required to clarify this role.

Dedication Page

I would like to dedicate this dissertation to my wife Kimberly and my children Lea, Rheana, Tristan and Sebastian. Even though I am the recipient of this degree it is really my entire family which has earned it. They have supported me through good and bad and have sacrificed much, mostly time spent with me. In addition I would like to thank my extended family for being there for us when we needed them most. I have had the privilege to be a member of an amazing lab full of people, all of which have become a second family to me, whose support in both my work and personal life are invaluable and to whom I owe much. I would also like to acknowledge the love and support of Grace Presbyterian Church and all of its members. Lastly I would like to thank my advisor, Dr. Christopher Gobler, who has inspired and guided me by his work ethic, his loyalty to his students/staff and his love for his family. Chris has poured much precious time into this document as well as my career and I am truly grateful and honored to have had the privilege of being part of his lab.

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Introduction

Background:

In recent decades, studies of the elements which influence primary production in marine ecosystems have focused primarily on nitrogen, phosphorus, silicon and more recently iron. During this time few studies have directly addressed the effects of organic micronutrients or coenzymes such as vitamins on phytoplankton population dynamics. This is despite the fact that earlier studies have shown that many phytoplankton have an absolute requirement for exogenous vitamins such as B_{12} (Carlucci and Silbernagel 1966; Guillard 1968; Swift and Guillard 1977). Provasoli and Carlucci (1974) and Swift (1980) described that many microalgal species are unable to synthesize vitamin B_{12} (cobalamin), vitamin B_1 (thiamine) and vitamin B_6 (biotin) *de novo* and thus are considered "auxotrophic", meaning they must obtain these vitamins from an external source. Each of these vitamins can play an important role in algal biochemistry. For example, cobalamin (B_{12}) is required for the biosynthesis of methionine, thiamine (B_1) facilitates the decarboxylation of pyruvic acid in the Calvin cycle and biotin (B_7) plays a critical role in fatty acid synthesis.

Vitamin B_{12} is a large organic molecule which serves as a cofactor for a variety of metabolic processes in many organisms, including phytoplankton and humans. Cobalamin is structurally similar to chlorophyll and heme and was first discovered in 1926 when Minot and Murphy noted that providing liver extracts to patients with pernicious anemia could reverse the disease. Soon after, the cofactor responsible was identified, isolated, and named cobalamin (Rickes et al. 1948; Smith 1948). There are many functions for this cofactor in organisms. Bacteria, for example, possess more than 20 cobalamin dependant enzymes and most bacteria synthesize B_{12} *de novo*. Some of these include methionine synthase, mutases (e.g., C-skeletal mutases) and enzymes important for ribonucleotide reduction (e.g., reductase; Ludwig and

Matthews 1997). The reactivity of these complex macromolecules is associated with the unique cobalt-carbon bond (Marsh 1999) and exists in two biologically active forms, methylcobalamin and adenosylcobalamin and their closely related cobamide forms. Methylcobalamin is required for the function of the folate-dependent enzyme, methionine synthase. This enzyme is required for the synthesis of the amino acid, methionine, from homocysteine. Methionine in turn is required for the synthesis of S-adenosylmethionine, a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA and RNA (Shane 2000). Adenosylcobalamin functions as a source of carbon-based free radicals that are released during the homolysis of B_{12}'s carbon-cobalt bond and is required by the enzyme that catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA. This biochemical reaction is important in generating energy from fats and proteins in the cells citric acid cycle. Succinyl CoA is also required for the synthesis of hemoglobin, the oxygen carrying pigment in red blood cells (Carmel 2006) In phytoplankton, B_{12} and its role in algal biochemistry has been the most studied of all the B-vitamins. Among the three known essential B-vitamins for algal growth, it is required by the largest number of phytoplankton species (see Table 1 in Croft et al. 2005). Of the 306 species surveyed, 155 required the presence of cobalamin in order to grow indicating that auxotrophy is the norm rather than the exception for phytoplankton despite the fact that they are photosynthetic organisms, and therefore autotrophs. Methionine synthesis has been implicated to be the primary metabolic pathway requiring $B_{12.}$ However the revelation by Croft (2006) that methionine additions to B_{12} starved algae did not completely alleviate B_{12} auxotrophy coupled with the fact that B_{12} dependent ribonucleotide reductase has been purified from *Euglena gracilis*, suggest that B_{12} might also play a major role in other metabolic pathways (i.e. DNA biosynthesis; Hamilton 1974). Cobalamin dependency among phytoplankton stretches over all

of the main phyla (Croft et al. 2005). Evolutionarily, if B_{12} dependency originated from a common eukaryotic ancestor, individual phyla of phytoplankton would either have a requirement or not need this coenzyme. Since B_{12} auxotrophs and non-auxotrophs can be found in all phyla, B_{12} dependency did not arise at one point in evolutionary time but has likely been selectively lost or arisen numerous times over evolutionary history. Regardless, vitamin B_{12} measurements made in both marine and freshwater systems during the 1960's and 1970's (Vishniac and Riley 1961; Menzel and Spaeth 1962; Swift 1972; Swift and Guillard 1978) and again in the past five years (Panzeca et al. 2006; Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007) have demonstrated that ambient concentrations of this vitamin are low (pM) and potentially growth limiting. In addition to date, only prokaryotic plankton (heterotrophic and cyanobacteria) have been found to possess the necessary genes to biosynthesis B_{12} (Raux et al. 2000; Bonnet et al. 2010).

Thiamine or vitamin B_1 is required by all higher animals and is supplied mostly by the food source or a microbial fauna (Provasoli 1971). Unlike cobalamin, some algal species synthesize thiamine *de novo*, whereas B_1 auxotrophs have an obligate need for this cofactor and do not possess the biochemical pathways required for thiamine synthesis (Croft et al. 2006). Thiamine is a cofactor in the decarboxylation of pyruvic and other α-keto acids. Decarboxylation of pyruvic acid to acetyl CoA is catalyzed in the mitochondria by the pyruvate dehydrogenase complex (Swift 1980). Vitamins, such as biotin, thiamine and cobalamin, as well as NAD and a lipooyl cofactor are part of this complex and also play an important role in the Calvin cycle in chloroplasts of higher plants (Hatch 1976). In addition, thiamine plays an essential role in the citric acid cycle, the catabolism of leucine, isoleucine, valine and threonine and the synthesis of cysteine. Croft et al. (2006) found that 67 (22%) of 306 phytoplankton species surveyed required B_1 to grow. The majority of euglenophyte (73%) and

prymnesiophytes (83%) species required exogenous B_1, while only a minority of species among other algal classes seem to require this B-vitamin (Croft et al. 2006). Like B_{12}, this vitamin has been found at pM levels in marine environments (Okbamichael and Sanudo-Wilhelmy 2005).

Of the two vitamins, B_{12} is required the most with approximately half of the phytoplankton surveyed having an absolute requirement for this micronutrient (see Table 1 in Croft et al. 2005) indicating that B_{12} auxotrophy is the norm rather than the exception for phytoplankton despite the fact that they are photosynthetic organisms, and therefore autotrophs. B_{12} auxotrophy is highest in dinophytes (89%), haptophytes (59%) and heterokontophytes (59%). The same study reported that 67 (22%) of all surveyed phytoplankton species required B_1 to grow. Even though the majority of euglenophyte (73%) and prymnesiophytes (83%) species required exogenous B_1, only a minority of species among other algal classes seem to require this B-vitamin (Croft et al. 2006). These numbers were recently updated by Tang et al. (2010) who found that HAB species, most of which belong to the dinophytes, are especially auxotrophic with respect to vitamins. In this study 97% and 79% of HAB species required an exogenous source of B_{12} and B_1, respectively. Biotin levels in the environment have not been measured directly but have been estimated via the bioassay technique (described below) and found to exist at pM levels in the ocean (Carlucci 1970a; Ohwada and Taga 1972; Carlucci and Cuhel 1975).

It has long been hypothesized that bacteria are the main source of vitamins to the water column and subsequently phytoplankton. Provasoli (1963) and Fogg (Fogg 1966) observed that B_{12} auxotrophic phytoplankton grew better in the presence of bacteria than in axenic, B_{12}-depleted media. Croft et al. (2005) observed that the B_{12} auxotrophic red algae *Porphyridium purpureum* could be grown in B_{12}-deficient media in the presence of the marine bacteria

Halomonas sp. The authors argued that the bacteria likely received organic carbon from the algae while in turn supplying it with required B vitamins. Taylor and Sullivan (2008) also argued that the bacterial communities in sea ice are likely a source of vitamins to its algal inhabitants since melt-water from the ice had 40 fold higher B_{12} concentrations than seawater. Besides being directly supplied by prokaryotes, other sources of vitamins to phytoplankton include viral lysis, zooplankton grazing, and cell lysis (Provasoli 1963; Croft et al. 2006; Taylor and Sullivan 2008). More recently, genomic evidence points to the presence of several genes, related to vitamin synthesis in prokaryotes (Raux et al. 2000; Bertrand et al. 2011) while cyanobacteria were found to produce vitamins in culture (Bonnet et al. 2010).

The rate at which vitamins are assimilated by phytoplankton in the field is poorly understood. Utilization of vitamins by the plankton community has largely been inferred by the disappearance of the ambient vitamin concentrations in the presence of known vitamin auxotrophs (Menzel and Spaeth 1962; Gobler et al. 2007). Direct measurements of vitamin utilization are scarce (B_{12}) or do not exist (B_1). Also, because the presence of bacteria and/or the death of phytoplankton in culture can cause vitamins to be released and then assimilated again, experiments assessing uptake from estimated total vitamin concentrations have been measurements of net, not total, uptake. The use of radioactive tracers to examine uptake of vitamin B_{12} in situ has previously been explored by Parker (1977), Hoffmann (1990) and more recently Bertrand et al. (2007) and Taylor and Sullivan (2008). In all instances ambient concentrations of vitamins were measured via the bioassay technique and with the exception of Taylor and Sullivan (2008) additions were not carried out at trace levels (~10%). B_1 uptake by plankton has never been studied before, nor do studies exist in which vitamin B_{12} and B_1 uptake has been investigated in tandem. Also the uptake of each vitamin has never been examined over

a complete annual cycle and the groups of plankton responsible for vitamin uptake in an ecosystem setting are unknown.

All studies of vitamins in aquatic ecosystems as well as culture-based experiments with plankton during the twentieth century, estimated ambient as well as intracellular concentrations of vitamins by means of bioassays. The first bioassay for cobalamin was developed in 1962 by Ryther and Guillard who used *T. pseudonana* strain 3H as the assay organisms. Cultures were grown in filtered sample water as well as in artificial seawater media with range of vitamin concentrations while all other growth regulating factors were optimal (light, temperature, and other nutrients). The vitamin concentrations within the unknown samples were estimated by comparing the final cell yields to those achieved in standards made with artificial media and known vitamin concentrations. Despite the plethora of reports of vitamin measurements made around the globe during the 20^{th} century, there are a series of reasons to approach this data with caution. For example, since vitamin B_{12} is known to rapidly photodegrade and become unstable at alkaline pH (Friedrich 1974) extended incubations under standard culture conditions could alter the available B_{12} concentrations. Furthermore bioassays required seawater to be passed through a 0.45 μm filter, which would allow passage of some bacteria and which in turn could change vitamin concentrations through bacterial synthesis during the one-week incubation (Roth et al. 1996; Croft et al. 2005). These problems may partly account for reports of different vitamin levels reported in the same water mass when bioassays were conducted with different phytoplankton species (Provasoli and Carlucci 1974; Sharma et al. 1979; Swift 1980). As such, surveys conducted in the Sargasso Sea (Menzel and Spaeth 1962), Long Island Sound (Vishniac and Riley 1961), the Gulf of Maine (Swift 1972; Swift and Guillard 1978), the Gulf of Alaska

(Natarajan and Dugdale 1966) and various other sites around the world should probably be viewed as estimates.

In 2004 , Okbamichael and Sanudo-Wilhelmy reported the first method for directly measuring vitamins in seawater using high performance liquid chromatography (HPLC). The availability of a method for directly quantifying vitamins in seawater and recent reports (Panzeca et al. 2006; Sanudo-Wilhelmy et al. 2006; Bertrand et al. 2007; Gobler et al. 2007) that phytoplankton growth can be altered by changes in the ambient vitamin pool has led to a renewed interest in vitamins and how they affect phytoplankton community composition and species succession.

Effects of vitamins on phytoplankton community composition and species succession

Coastal areas comprise 8% of the world's ocean surface but account for over 28% of the annual ocean primary production (Holligan and De Boois 1993). In addition, in a manner paralleling global trends, nearly 75% of the US population lives within 75 km of the coastline, making these regions subject to a suite of anthropogenic influences including intense nutrient loading (De Jonge et al. 2002; Valiela 2006) which in turn makes these regions vulnerable to algal blooms (Beman et al. 2005; Heisler et al. 2008). While the absolute amount of N and/or P entering coastal zones often controls the amount of phytoplankton biomass in that system, the availability, ratio, and/or type (e.g. inorganic vs. organic) of nutrients can also influence algal community composition (Smayda 1997; Heisler et al. 2008). For example, many studies have found that low levels of Si relative to N due to anthropogenic N loading and/or Si scavenging associated with water diversion (i.e. damming, irrigation) can shift phytoplankton communities from diatoms to flagellates (Smayda 1990; Gobler et al. 2006). Alternatively, dinoflagellates

have been shown to have an advantage over other phytoplankton species in low nitrate and/or high dissolved organic nitrogen (DON) environments since many species are able to utilize DON but are poor competitors for nitrate in an ecosystem setting (Margalef 1978; Anderson et al. 2002). For example, a recent study by Koch and Gobler (2009) showed that water from salt marshes, which is enriched in DON and depleted in nitrate, was capable of shifting phytoplankton community composition from diatom to dinoflagellate dominance. Since vitamins are essential for the growth of some phytoplankton species but not for others the availability of vitamins in conjunction with nutrient loading may therefore also influence species composition, species succession and algal growth rates in coastal zones.

Several studies have suggested that vitamin availability can shape phytoplankton communities. Culture studies by Droop (1955), Guillard and Ryther (1962) and Guillard (1968) showed that most centric diatoms which comprise the temperate spring bloom (i.e. *Skeletonema costatum, Thalassiosira pseudonana and Ditylum brightwelli*) require B_{12} whereas only half of pennate species seem to be B_{12} auxotrophs. Accordingly, many have argued that B_{12} concentrations might influence dynamics and composition of the spring bloom (Carlucci and Bowes 1970; Provasoli 1971; Swift 1980). The dinoflagellates (Dinophyceae) are another algal group which are common in coastal zones and have been shown to exhibit a large degree of B_{12} auxotrophy, with between nearly 87 % of species studied requiring this vitamin for growth (Loeblich 1967; Provasoli and Carlucci 1974; Croft et al. 2005). The auxotrophic tendencies of dinoflagellates are consistent with the observation that only half of the surveyed dinoflagellates are completely autotrophic with respect to carbon and nitrogen and thus obtain some or most of their nutrition from an external organic source (i.e. heterotrophy; Taylor 1987; Smayda 1997). There have been many examples of dinoflagellate bloom dynamics being linked with the

dynamics of vitamin B_{12} including blooms of *Lingulodinium polyedrum* (formerly known as *Gonyaulax polyedra*) off the coast of California (Carlucci 1970a) and blooms of *Karenia brevis* in the Gulf of Mexico (Aldrich 1962; Hunter and Provasoli 1964; Stewart et al. 1967; Collier et al. 1969). In contrast, the presence or absence of the B_{12} producing cyanobacteria *Oscillatoria sp.* was implicated in controlling the B_{12} concentrations of Lake Washington and so influencing the availability of this vitamin to the rest of the phytoplankton community (Parker 1977). Recent research in Long Island's coastal waters has shown that vitamin B_{12} and B_1 concentrations can enhance the biomass of large phytoplankton (> 5 µm; Sañudo-Wilhelmy et al 2006 ; Gobler et al 2007). All of these findings suggest vitamins can influence phytoplankton species composition in coastal ecosystems although most of the studies have focused on brief time periods, usually during the summer and fall. In addition, no study to date has examined phytoplankton species succession, vitamin assimilation rates, and actual vitamin concentrations over a full annual cycle.

The influence of vitamins on HNLC phytoplankton communities

In addition to having been implicated in affecting growth of phytoplankton in coastal zones, recent studies in in high latitude, high nutrient low chlorophyll (HNLC) regions suggest vitamins may limit or co-limit the growth of phytoplankton in these regions. The realization by John Martin in the1980s that iron, a micronutrient, was limiting the growth of phytoplankton in the Gulf of Alaska (Martin and Fitzwater 1988; Martin et al. 1989) led to a series of studies in many previously poorly studied regions of the world's oceans. During the last three decades a plethora of studies have investigated the role of iron within HNLC regions and found that the addition of iron stimulates diatom blooms, draw down macro nutrients and leads to increased export of organic matter (IronEx-1, Martin et al. 1994 ; IronEx-2, Coale et al 1996 ; SOIREE, Boyd et al. 2000 ; SEEDS, Tsuda et al. 2003 ; SERIES, Boyd et al. 2004). Like iron, vitamins

in HNLC areas are present at very low (pM) concentrations. Measurements using the bioassay technique yielded typical vitamin B_{12} concentrations in the North Pacific of < 0.5 pM and in some regions < 0.1 pM (Natarajan and Dugdale 1966; Carlucci 1970b). Vitamin surveys conducted in the Southern Ocean (Carlucci and Cuhel 1975) reported mean values of 0.14 pM. In light of the ample supply of inorganic nutrients (nitrate, phosphate) in near surface waters, Carlucci and Cuhel (1975) argued that vitamins must be limiting the growth of phytoplankton in this region. Despite the well-established iron limited nature of the Southern Ocean, the ability of vitamins to limit or co-limit phytoplankton was recently demonstrated by studies along the Antarctic Peninsula (Panzeca et al. 2006) and in the Ross Sea (Bertrand et al. 2007). Although neither study reported ambient vitamin concentrations, in both studies the experimental addition of vitamins (B_{12} and/or B_1) alone (Panzeca et al. 2006) or with iron (Bertrand et al. 2007) significantly enhanced phytoplankton biomass compared to unamended controls. Bertrand et al. (2007) also found an inverse relationship between bacterial densities and the phytoplankton community response to vitamin B_{12}, with algal growth being most stimulated in regions with the lowest densities of heterotrophic bacteria. This finding suggests that B_{12}-producing microbes are important for supplying vitamins to the phytoplankton in this region. Genomic data indicate that many prokaryotes (phototrophs and heterotrophs) could be significant sources of B_{12} to the oceans (Palenik et al. 2003; Rocap et al. 2003; Vitreschak et al. 2003) a finding recently confirmed by studies showing the ability of *Synchecoccus sp* and other prokaryotes to produce vitamins (Bonnet et al. 2010; Bertrand et al. 2011) The low rates of metabolic activity of prokaryotes in cold, high latitude regions (Church 2000; Kirchman et al. 2009) coupled with low cyanobacteria abundances in these regions (Boyd and Harrison 1999; Boyd et al. 2000) suggests that microbial B_{12} synthesis rates are likely to be relatively slow within high latitude oceans.

The relationship between B-vitamins and phytoplankton in the North Pacific Ocean has not been examined in more than 40 years (Carlucci 1970a) and direct measurements of vitamins, plankton assimilation rates of vitamins, and the response of phytoplankton to vitamins have never been investigated in this region.

The role of vitamins in harmful algal bloom dynamics

Beyond increasing phytoplankton community biomass, enhanced nutrient loading in coastal zones has recently been implicated in the increased frequency and intensity of harmful and ecosystem disruptive algal blooms (HABs and EDABs, Glibert et al. 2005, Sunda et al. 2006, Anderson et al. 2008, Heisler et al. 2008). In recent decades these blooms have caused widespread economic and ecological damage to coastal ecosystems worldwide (Cloern 2001). Mechanisms leading to formation and persistence of most HABs often include low rates of grazing mortality and a nutrient regime which the HAB species can exploit to the exclusion of competing algae (Sunda et al. 2006; Heisler et al. 2008). New York's coastal waters are subject to annual HABs caused by two contrasting phytoplankton species: *Aureococcus anophagefferens*, a pelagophyte also known as the brown tide, and *Cochlodinium polykrikoides*, an athecate, catenated dinoflagellate.

Aureococcus anophagefferens - The first occurrence of brown tide was documented on eastern Long Island in 1985 and resulted in a mass die-off of *Zostera marina*, a critical habitat for juvenile finfish as well as shellfish and other benthic fauna (Dennison 1988). Blooms devastated the local bay scallop population (*Argopecten irradians*) which resulted in the loss of one of Long Island's largest fisheries (Bricelj et al. 1989). Since 1985 the brown tide has recurred almost annually in the different bays on eastern and southern Long Island and has expanded southward to New Jersey, Delaware, Maryland and Virginia (Gobler et al. 2005; Sunda

et al. 2006). This small (2-3 µm diameter) organism seems to gain a competitive advantage over other phytoplankton by deterring grazing by micro- and mesozooplankton (Gobler et al. 2002; Caron et al. 2004; Deonarine et al. 2006) and bivalves (Bricelj et al. 1989; Bricelj et al. 2001). *A. anophagefferens* has also been shown to out-grow other phytoplankton in the presence of low nitrate and high organic nitrogen concentrations (Taylor et al. 2006). This finding is consistent with the observation that blooms in the Peconic Estuary have generally been observed either after periods of low groundwater discharge (high in nitrate, LaRoche et al. 1997) or elevated DON (Gobler and Sanudo-Wilhelmy 2001). Regarding vitamins, little is known about this species. All strains of *A. anophagefferens* examined (CCMP 1984, 1707, 1850) are auxotrophic for both vitamin B_{12} (cyanocobalamin) and vitamin B_1 (thiamine) but not for B_7 (biotin; Tang et al. 2010) and *A. anophagefferens* has been shown to possess the B_{12}-dependent methionine synthesis gene (metH; Gobler et al. 2011). Since vitamins have been found to limit the accumulation of phytoplankton biomass in estuaries where brown tides occur (e.g. Quantuck Bay, Great South Bay, NY; Sanudo-Wilhelmy et al. 2006), vitamins may play a role in the bloom dynamics of *A. anophagefferens.* This hypothesis is consistent with the ability of this species to grow on organic compounds (Gobler et al. 2005; Taylor et al. 2006). Furthermore summer vitamin concentrations in a Long Island south shore marine system have been shown to correlate positively with concentrations of dissolved organic nutrients (Gobler et al. 2007). *A. anophagefferens* has been shown to thrive in low dissolved inorganic nutrients/high dissolved organic nutrient conditions (Taylor et al. 2006), suggesting that vitamins may play a key role in these blooms.

Cochlodinium polykrikoides - Red tides caused by the dinoflagellate *C. polykrikoides* have plagued Asian coastal waters during the past two decades (Yuki and Yoshimatsu 1989; Kim

1998; Huang and Dong 2000), being responsible for over $100 million in fisheries losses during the 1990s in Korea alone (Kim 1998). This dinoflagellate has lethal effects on a variety of finfish and shellfish (Kim et al. 1999; Whyte et al. 2001; Tang and Gobler 2009) with high density exposures resulting in gill hyperplasia, hemorrhaging, squamation, and apoptosis in cells of gills and digestive tracts in fish and shellfish (Gobler et al. 2008). In addition *C. polykrikoides* has also shown to inhibit the growth of a variety of co-occuring phytoplankton species via allelopathic chemicals (Tang and Gobler 2010). *C. polykrikoides* and related *Cochlodinium* species are globally distributed (Kudela et al. 2008a) and have recently been observed in the coastal embayments of Long Island, NY(Nuzzi and Waters 2004; Gobler et al. 2008). Blooms have occurred annually in the Peconic Estuary and Shinnecock Bay from 2004 – 2008 during late summer and early fall reaching peak densities of $> 10^3$ cells ml^{-1} (Gobler et al. 2008; Kudela and Gobler 2012). These blooms manifest themselves as intense and sometimes ephemeral patches of red water which can appear or disappear in a matter of hours since they undergo intense vertical migration (Kudela et al. 2008a). Although the nutrient dynamics initiating and sustaining these blooms is still poorly understood, in a manner similar to other dinoflagellates, *C. polykrikoides* seems to display nutritional flexibility, being capable of assimilating and growing on a variety of organic and inorganic N sources (Kim et al. 2001; Kudela et al. 2008b; Gobler et al. 2012). Cultures of *C. polykrikoides* (clone CP-1) isolated from Long Island waters have shown an obligate requirement for extracellular B_{12} and B_1 but not B_7 (biotin; Tang et al. 2010). As described above, this finding is consistent with prior work showing that most dinoflagellates (>90%) are auxotrophs for vitamin B_{12} (Provasoli and Carlucci 1974; Croft et al. 2005) making them the phytoplankton class with the largest percentage of B_{12} auxotrophs outside of the geographically rarer rhodophytes of which 12 of 13 species (92%) require B_{12} (Croft et al. 2005).

In addition, members of this group also make up more than 50% of all surveyed algae requiring biotin (B_7). This includes species such as *Karenia brevis* and *Prorocentrum sp*., both implicated in the formation of HABs and EDABs.

Beyond documenting, for the first time, the annual cycle of vitamin concentrations, vitamin assimilation rates, and phytoplankton dynamics, the presence of two different harmful algal blooms in NY coastal waters caused by two vitamin auxotrophs presents an additional unique opportunity to make these measurements during both bloom and non-bloom conditions. During bloom events, both *A. anophagefferens* and *C. polykrikoides* occur at near monsospecific densities within their respective size grouping in the plankton (1 – 5 µm and > 20 µm respectively; Gobler et al. 2012, Gobler et al. 2005) which allows for species-specific size fractionation of vitamin uptake rates and primary production.

Factors affecting the uptake of vitamins by phytoplankton

The elemental composition and growth rate of phytoplankton is influenced by a number of external factors including light, temperature, and nutrient availability. The relationship between the elemental composition of algae and their growth rate has been extensively studied with nitrogen, phosphorus and silicon commonly emphasized (Droop 1983). Several studies suggest that nitrogen and carbon metabolic pathways in phytoplankton are linked, since diel variation in nitrogen metabolism is coordinated with photosynthesis and/or the products of photosynthesis (Turpin et al. 1988; Turpin 1991; Vergara et al. 1998). In contrast, silicate metabolism of diatoms has been shown to be coordinated with the cell cycle rather than photosynthesis (Brzezinski 1992; Brzezinski and Conley 1994) a finding which has been attributed to the fact that girdle formation must precede cell division (Claquin and Martin-Jezequel 2002). While these mechanisms for assimilating macronutrients have been extensively

studied, the exact mechanisms and process for vitamin assimilation are still unclear. It has been hypothesized that B_{12} uptake is comprised of an adsorption step followed by an energy-dependent uptake step (Droop 1968; Bradbeer 1971), suggesting a possible link with energy derived from photosynthesis. Ford (1958), Droop (1968) and others have described the presence of binding factors akin to siderophores which bind B_{12}, making it biounavailable. Recently this compound has been described in *E. coli* as "BtuF" periplasmic-binding protein (Karpowich et al. 2003). Other work with bacteria also ascribes the energy-dependent step of B_{12} uptake to ATP-binding cassette (ABC) transporters (Koster 2001; Borth et al. 2005). Taylor and Sullivan (2008) found that when sea ice microalgae were placed in the dark or photosynthesis was inhibited, uptake rates of B_{12} decreased significantly suggesting reliance on photosynthesis for the energy-dependent step of B_{12} uptake.

It has been well-established that increasing the ambient vitamin concentration in cultures of auxotrophic phytoplankton yields increased growth rates (e.g. Ford 1958 , Wood 1963 , Carlucci and Silbernagel 1969). In addition, Droop (1968) reported that when B_{12} starved cells of *M. lutheri* were exposed to increasing concentrations of B_{12}, the cellular uptake rates increased with increasing B_{12} concentrations an observation consistent with Carlucci and Silbernagel (1969) who reported that the cellular B_{12} quota of *Cyclotella nana* increased with increasing vitamin concentrations. The cellular B_{12} requirement of several algal species has previously been determined (Droop 1955; Droop 1968; Droop 1970) and Guillard (1963) reported there to be 3-24 molecules of B_{12} for every μm^3 of cell biovolume of several centric diatoms. Carlucci and Bowes (1972) reported internal B_{12}:C ratio of 12 (ngB_{12} : mgC) for the bloom forming centric diatom, *Skeletonema costatum.* To date, the effect of varying vitamin concentrations on the cellular vitamin : C ratios and cellular C and vitamin quotas has been

poorly studied. For example, cellular B_1:C, B_{12}: B_1 and B_7:C ratios have never been published. Moreover, determinations of vitamin uptake kinetics have focused almost entirely on B_{12} and have only been determined for six species of phytoplankton (Droop 2007). The determination of half-saturation constants (K_s), for ecologically significant phytoplankton species for all three B-vitamins will greatly enhance our understanding on how vitamin concentrations may contribute to species succession in aquatic environments.

Objectives:

More than half of the phytoplankton species in the world's oceans have an absolute requirement for exogenous vitamins, but a comprehensive understanding of the ability of vitamins to shape phytoplankton community composition and succession in aquatic ecosystems has not been attained. Taking advantage of newly available techniques, my dissertation aims to elucidate the intricate dynamics of B-vitamins, phytoplankton communities, carbon fixation, and B-vitamin utilization by phytoplankton communities in a variety of ecosystems from eutrophic coastal waters of Long Island to the HNLC regions of the North Pacific. Detailed examinations of high biomass, near-monospecific algal blooms caused by auxotrophic phytoplankton will offer an opportunity to examine the extent to which vitamins contribute to the initiation, sustenance, and demise of these events. Finally, culture experiments will provide an understanding of how key ecological factors such as ambient vitamin levels influence the growth, vitamin assimilation, and carbon fixation of auxotrophic phytoplankton. As such, this thesis will elucidate the extent to which B-vitamins influence phytoplankton community growth, composition, and succession in marine ecosystems.

This dissertation will investigate the following hypotheses:

H1: B-Vitamins (B_1, B_{12}) behave like a macronutrient, potentially limiting algal growth and influencing phytoplankton community composition as well as species succession in both coastal and open ocean environments.

H2: Vitamin uptake rates and vitamin limitation will be maximal when auxotrophic phytoplankton dominate high biomass, phytoplankton communities.

H3: Cellular B vitamin:carbon ratios, as well as V_{max}, μ_{max}, K_s for each vitamin will be affected by a variety of environmental conditions including vitamin concentrations and plankton community composition.

To test these hypotheses I have set the following research objectives:

1. Quantify an annual cycle of A) vitamin concentrations, B) size fractionated vitamin B_1 and B_{12} uptake rates, C) size fractionated primary productivity rates, D) phytoplankton community composition, and E) phytoplankton nutrient (N, B_1 and B_{12}) limitation within two contrasting coastal marine ecosystems (mesotrophic v hypereutrophic).

2. To quantify vitamin B_{12} concentrations, B_{12} uptake rates, primary productivity and the phytoplankton community composition and to establish whether vitamins limit or co-limit phytoplankton growth in both coastal and HNLC regions of the North Pacific Ocean.

3. To establish the importance of B-vitamins in the development, peak and demise of contrasting high biomass algal blooms caused by *A. anophagefferens* and *C. polykrikoides.*

4. To determine the effects of changing vitamin concentrations on cellular C : vitamin ratios, half saturation constants (K_s) as well as maximum growth rates (μ_{max}) and uptake rates (V_{max}) using cultures of auxotrophic phytoplankton, including *A. anophagefferens* and *C. polykrikoides.*

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Chapter Two

The effect of vitamin B_{12} on phytoplankton growth and community structure in the Gulf of Alaska

Abstract: A majority of eukaryotic phytoplankton species require an exogenous source of vitamin B_{12} for growth and recent field studies in some coastal and polar regions indicate that the addition of vitamin B_{12} alone, or with another limiting nutrient can influence the accumulation of phytoplankton biomass. We quantified the concentrations and uptake rates of vitamin B_{12}, characterized phytoplankton community composition, and examined the ability of vitamin B_{12} to alter the growth and composition of phytoplankton communities in the Gulf of Alaska. Picoplankton (0.2-2 μm) were responsible for the majority of vitamin B_{12} uptake in both coastal and high nutrient low chlorophyll (HNLC) regions and B_{12} concentrations and uptake rates were higher in HNLC regions compared to coastal regions with higher iron (Fe) concentrations. During vitamin amendment experiments, B_{12} alone and in conjunction with other limiting nutrients (N or Fe) significantly enhanced algal biomass and increased the growth rates of multiple groups of larger (> 2 μm) phytoplankton. This included ecologically significant, B_{12} auxotrophs, such as *Gymnodinium* sp. and *Alexandrium* sp. (in the coastal experiment) as well as *Chaetoceros* sp. and *Gymnodinium* sp. (in the HNLC experiment). The ability of vitamin B_{12} to shape algal community composition in coastal and HNLC areas of the Gulf of Alaska, even in cases where it does not limit total phytoplankton production, suggests that it may influence carbon export in this and other polar ecosystems.

Introduction

It has long been known that, in addition to macronutrients (e.g., NO_3^-, PO_4^{3-}, $Si(OH)_4$) and micronutrients (e.g., Fe, Zn, Cu, Co, Ni), many species of phytoplankton also have a requirement for cofactors such as cobalamin (vitamin B_{12}). Vitamin B_{12} is a cobalt containing organometallic compound which is only produced by some Archaea and Bacteria and eukaryotic phytoplankton must, therefore, obtain this cofactor (Guillard 1968; Swift and Guillard 1977). Phytoplankton possess several B_{12}-dependent enzymes, such as methyltransferases (e.g., methionine synthase), mutases (e.g., C-skeletal mutases), and reductases (e.g., ribonucleotide reduction; Ludwig and Matthews 1997) and approximately half of surveyed phytoplankton species have an absolute requirement for B_{12} (Croft et al. 2005).

During the second half of the twentieth century, significant efforts were made to better understand the role of vitamins in phytoplankton ecology. Following two decades of laboratory studies, Provasoli and Carlucci (1974) reviewed the B_{12} requirements of hundreds of species of phytoplankton. Field studies of the Atlantic, Pacific, and Southern Ocean reported low and potentially growth-limiting vitamin concentrations (Menzel and Spaeth 1962). All of these studies measured B_{12} concentrations using bioassays, an approach that did not yield direct measurements of vitamins but rather estimated concentrations based on the growth rate of assay organisms (Droop 1968). While prior studies estimated in situ concentrations of B_{12} via bioassays, direct measurements of B_{12} vitamin uptake by pelagic marine plankton assemblages has only been reported for one sample collected in the Ross Sea (Bertrand et al. 2007)..

Recently, the development of a method to directly measure vitamin B_{12} in seawater (Okbamichael and Sanudo-Wilhelmy 2004) as well as recent studies focusing on the role of B-vitamins on algal cell metabolism (Croft et al. 2006), the supply of B_{12} to autotrophs from

prokarytoes (Croft et al. 2005), the distribution of vitamin auxotrophy and requirements amongst algal classes (Croft et al. 2005), and field studies of coastal (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007) and open ocean (Panzeca et al. 2006; Bertrand et al. 2007) vitamin-phytoplankton dynamics have sparked renewed interest in this compound. Field studies have shown that vitamin B_{12} concentrations in the ocean (Okbamichael and Sanudo-Wilhelmy 2004) are similar to the range of half saturation constants for vitamin B_{12} for phytoplankton in culture (0.1 – 10 pmol L^{-1}; Droop 1968 ; Tang et al 2010). Incubation experiments have demonstrated that the amendment of seawater with vitamins in addition to nitrate (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007) or Fe (Panzeca et al. 2006; Bertrand et al. 2007) can significantly enhance phytoplankton biomass in multiple ecosystems demonstrating the potential for B-vitamins to influence primary production in the world's oceans.

Parts of the Gulf of Alaska have been characterized as one of the ocean's high-nutrient-low-chlorophyll (HNLC) regions where phytoplankton productivity is limited by the availability of Fe (Martin and Fitzwater 1988; Tsuda et al. 2003; Boyd 2004). Vitamin B_{12} concentrations in the Gulf of Alaska, previously measured via the bioassay technique, were found to be low (< 0.3 pmol L^{-1}; (Carlucci and Silbernagel 1966; Natarajan and Dugdale 1966). Experimental enrichment of seawater with vitamin B_{12} and Fe in other high latitude, HNLC regions has been shown to stimulate the growth of phytoplankton (Panzeca et al 2006; Bertrand et al. 2007). Since bacteria are a primary source of vitamin B_{12} (Croft et al 2005), vitamin limitation in cold, polar latitudes may be due to slow vitamin production rates, as the degree to which phytoplankton were limited by vitamins in the Southern Ocean was inversely proportional to in situ bacterial densities (Bertrand et al. 2007). Taken together, these findings suggest that the growth of some phytoplankton in high latitude, HNLC regions of the world's oceans may be

influenced by the bioavailability of B-vitamins in surface waters. Thus far, the effects of vitamins on the phytoplankton communities of the North Pacific have not been studied.

The goal of this study was to evaluate the influence of vitamin B_{12} on the phytoplankton communities within the Gulf of Alaska. Direct measurements of vitamin B_{12} concentrations and uptake rates by plankton along surface transects were assessed across the subarctic Gulf of Alaska. In addition, the effects of vitamin amendments alone and combined with the primary limiting nutrients (NO_3^- or Fe) on the phytoplankton community growth and composition were examined. By sampling across coastal, shelf, open water, and HNLC regions I was able to assess the importance of vitamin B_{12} across a diversity of environments within the Gulf of Alaska.

Methods

Field measurements

Samples were collected from 33 surface stations spanning across coastal areas, shelf, off-shelf waters, and HNLC regions of the Gulf of Alaska (Fig. 1) from 19 August – 18 September 2007 aboard the R/V *Thomas T. Thompson* (University of Washington). At each station, physical data (temperature, salinity, depth, latitude and longitude) was recorded from the ships underway data logging system equipped with a Sea-Bird Electronics-21 Seacat themosalinograph and a Bathy-2000 Ocean Data Equipment Corporation mapping depth sounder. Water was collected from near surface via a conductivity temperature depth (CTD) rosette or directly from the GeoFish clean underway surface sampling system. This system delivered water from 1 to 3 m depth into a trace metal clean room via a trace metal clean Teflon pumping system (Hutchins et al. 2002; Bruland et al. 2005). Triplicate chlorophyll *a* (Chl *a*) samples were collected on 0.2 and 2 μm polycarbonate filters and frozen and analyzed according to Welschmeyer (1994). To characterize the phytoplankton community, 250 mL of sample were preserved in 5% Lugols

iodine solution, settled in chambers, and quantified under an inverted microscope (Hasle 1978). Plankton larger than 5 μm were characterized to the genus level or higher and were generally grouped as diatoms, dinoflagellates, autotrophic nanoplankton, and ciliates. A minimum of 200 organisms or 100 fields of view were enumerated per sample (Omori and Ikeda 1984). For our coastal experiment, phytoplankton accessory pigments were quantified by high performance liquid chromatography (HPLC, (Ditullio and Geesey 2002). Five photopigments which are found exclusively in single classes of phytoplankton were examined as proxies for five major algal groups. Peridinin was used as an indicator of dinoflagellates, alloxanthin was analyzed as a proxy for cryptophytes, lutein indicated chlorophytes, zeaxanthin represented cyanobacteria, and fucoxanthin was used for diatoms (Ditullio and Geesey 2002). Whole water samples were preserved with 10% buffered formalin (final concentration of 1%), flash-frozen in liquid nitrogen and analyzed flow cytometrically to assess picoplankton densities (Olson et al. 1991). Abundance of heterotrophic bacteria (stained with SYBR™ Green I; Jochem 2001), phycoerythrin-containing picocyanobacteria, and photosynthetic picoeukaryotes were determined using a Fluorescence Activated Cell Scan (FACScan; Becton, Dickinson and Company) flow cytometer using fluorescence patterns and particle size from side angle light scatter (Olson et al. 1991). Samples for dissolved nitrogen (nitrate and nitrite, NO_x), phosphate (PO_4^{3-}), and silicic acid ($Si(OH)_4$) were measured on board on a Lachat QuickChem 8000 analyzer after filtration through 0.2 μm Millex filters as described in Parsons et al. (1984). Dissolved Fe samples were filtered through trace metal clean 0.2 μm filters and concentrations were determined onboard using a flow injection method with catalytic spectrophotometric detection (Lohan et al. 2006). Vitamin samples were collected by filtering 4 L of water through a 0.2 μm Millipore cartridge filter into 4 L amber high density polyethylene bottles

(Okbamichael and Sanudo-Wilhelmy 2004). Samples were acidified with 10% HCl to a pH between 6.1 and 6.7 and vitamins were then captured by slowly pumping (1 mL min^{-1}) samples through High Capacity Bondesil C18 (120 μm) beads (BioRad). The columns containing the bound vitamins were then stored in the freezer until subsequent methanol elution and reverse phase HPLC analysis (Okbamichael and Sanudo-Wilhelmy 2004).

B_{12} uptake rates and primary productivity measurements

A ^{57}Co-labeled vitamin B_{12} from Milan Panic (MP)-Biomedicals© (specific activity 212 μCi μg^{-1}) was used to measure planktonic uptake rates of this compound. A trace amount (0.5 pmol L^{-1} B_{12}, 0.04 μCi vs. mean ambient B_{12} concentrations ~2 pmol L^{-1}; Table 1) of ^{57}Co-cyanocobalamine was added to triplicate, 300 mL polycarbonate bottles, filled with near surface water collected using trace metal clean techniques. To assess non-specific binding and/or adsorption of the isotope a 1% gluteraldehyde 'killed-control' bottle was also be spiked with tracer and incubated along the 'live' bottles. To determine primary productivity rates, 10 μCi of ^{14}C-bicarbonate (MP-Biomedicals©, specific activity 55 mCi $mmol^{-1}$) was added to triplicate bottles according to Joint Global Ocean Flux Study (JGOFS) protocols (1994). Incubation bottles were then placed in on-deck, flow through incubators which maintained ambient temperatures under neutral density screening (~77% of ambient light). Incubations were terminated after 24 h by filtering up to 100 mL from both live and dead bottles onto 0.2 and 2 μm pore size polycarbonate filters, allowing for the determination of size fractionated uptake of the tracers. At the beginning and end of the incubation, a small aliquot of each bottle (250 μL and 1 mL for ^{14}C and ^{57}Co, respectively) was removed to determine total activity. The ^{57}Co and ^{14}C containing experimental filters were analyzed on a Beckman β-Mate II scintillation counter along with the total activities. ^{57}Co was counted using a scintillation counter following

the protocols of Gutcho et al. (1973). The background activity for detection of ^{57}Co was generally between 20 and 40 counts per minute while samples ranged from 80 to 1500 counts per minute. Uptake of vitamin B_{12} was calculated by using the equation: $((A_L - A_{KC} / A_{tot}) \times [vitamin])/ t$ where A_L is the activity on the live filters, A_{KC} is the activity on the gluteraldehyde – preserved killed control sample filters, A_{tot} is the total activity added, [vitamin] is the ambient B_{12} concentration and t equals the length of the incubation in days. Activity of filters from killed controls ranged from 5% to 25% of the live bottle's activity. Uptake of ^{14}C bicarbonate was determined according to the JGOFS (1994) protocol.

Nutrient amendment experiments

Water for experiments was collected from two stations (*see* Fig. 1): West of Glacier Bay at 59º 0.56'N, 139º 50.58'W (Coastal experiment) and southwest of Kodiak Island at 53º 44.82'N, 155º 18.7'W (HNLC experiment). Acid washed, trace metal clean 1.1 L polycarbonate bottles were filled with water from the GeoFish clean pumping system (Hutchins et al. 2002; Bruland et al. 2005) from an average depth of 1-3 m. To avoid trace metal contamination, all manipulating and processing of experimental bottles was performed in the trace metal clean room using trace metal clean techniques. For the coastal experiment, triplicate bottles were amended with NO_3^- (20 μmol L^{-1}), B_{12} (100 pmol L^{-1}), and both compounds. The second experiment was conducted using HNLC water and triplicate bottles were amended with 2 nmol L^{-1} of $FeSO_4$, B_{12} (100 pmol L^{-1}), and both compounds. The B_{12} and NO_3^- solutions were passed through columns containing Chelex-100 resin (BioRad Laboratories©) at a flow rate of < 1 mL min^{-1} prior to use to remove trace metals from these solutions and minimize contamination. Differential responses from phytoplankton communities to vitamin B_{12} and Fe within HNLC regions indicated that this procedure successfully removed Fe from the B_{12} solution and that

algal responses were not due to Fe contamination of vitamin B_{12} stocks. The bottles were placed in on-deck incubators, kept at ambient temperature by the ship's flow through system and covered with neutral density screening (~77% of ambient light). Incubations lasted for 4-6 days. Every two days the progress of the experiments was evaluated by removing a small aliquot from each bottle (100 mL) and filtering them onto pre-combusted glass fiber filter (GFF) for Chl *a*, saving the filtrate for nutrient (NO_3^-, PO_4^{3-}, $Si(OH)_4$) analysis. Experiments were terminated by processing the bottles for the analysis of size fractionated Chl *a* onto GF/F glass fiber, 0.2 μm and 2 μm polycarbonate filters. Samples were also preserved for nutrient analysis and for characterization of phytoplankton community composition using light microscopy, HPLC and flow cytometry as described above. Net growth rates were estimated for each plankton group characterized and the different size fractions based on changes in pigment concentrations using the formula: $\mu = \ln([\text{pigment}]_{\text{final}} / [\text{pigment}]_{\text{initial}}) /$ incubation time where $[\text{pigment}]_{\text{final}}$ was the concentration of a given photopigment in bottles at the end of experiments, $[\text{pigment}]_{\text{initial}}$ was the concentration of photopigment at the beginning of the experiment, and the incubation time was in days.

Data analysis

To examine spatial trends in the parameters, stations were characterized as on-shelf (<300 m depth), off-shelf (>300 m depth and < 5 μmol L^{-1} NO_x) and HNLC (>300 m and > 6 μmol L^{-1} NO_x). On-shelf stations were further divided by low (<31) and higher (>31) salinities to account for the influence of fluvial discharge on some near shore stations. The degree to which individual parameters were correlated with each other across all locations was evaluated by means of a Spearman rank order correlation matrix. *p*-values <0.05 were deemed to be significantly correlated and the correlation coefficient is reported as *r*. For nutrient amendment

experiments, differences in growth rates among treatments for each size class of plankton, pigments and plankton groups were statistically evaluated using one-way analyses of variance (ANOVA) followed by Tukey's multiple comparison tests. $p<0.05$ was used to establish significant differences among treatments and correlations among parameters.

Results

As expected, macronutrient concentrations across the Gulf of Alaska were highest amongst HNLC stations and inversely correlated to dissolved Fe concentrations which were substantially higher among coastal stations (Fig. 2, Table 1). Vitamin B_{12} concentrations were four-times higher among HNLC stations compared to off-shelf and coastal regions ($p<0.001$, 4.12 ± 1.66, 1.57 ± 0.60 and 1.13 ± 0.24 pmol L^{-1}, respectively). Plankton biomass was highest among on-shelf stations with no statistical differences between abundances of phytoplankton, heterotrophic-, and cyano-bacteria between low-, high salinity and off-shelf stations (Fig. 3, Table 2). HNLC stations exhibited the lowest abundance of plankton as well as primary production rates which were significantly lower than rates measured in other regions ($p<0.001$, Fig. 3). Large (> 2 μm) autotrophs were responsible for the majority of primary production within the low salinity, near-shore stations (64%), half of the primary production off-shelf, and a minor fraction within the HNLC zone (38%; Table 2). Uptake rates of vitamin B_{12} were similar between on- and off-shelf stations but were four-fold higher among HNLC stations ($p<0.01$, 0.602 ± 0.252 pmol L^{-1} d^{-1}; Fig. 3, Table 2). Unlike primary production, picoplankton were responsible for the majority of B_{12} utilization in all regions, accounting for 68% to 82% of the total B_{12} uptake (Table 2).

Nutrient-vitamin amendment experiments

Coastal experiment - The first experiment was conducted with water collected near Glacier Bay (Sta. 2, Fig. 1). Initial nutrient concentrations were indicative of a nitrogen limited coastal system: Low levels of NO_x (0.1 μmol L^{-1}) and a low N: P ratio (0.17; Table 1). Ambient Chl *a* and vitamin B_{12} concentrations were low (0.61 ± 0.02 µg L^{-1} and 0.1 pmol L^{-1}, respectively). The nano- and microplankton community was dominated by dinoflagellates (63% of cells; *Prorocentrum* sp., *Scrippsiella* sp., *Gymnodinium* sp. being most abundant), with diatoms (5%, *Nitzschia* sp.), ciliates (15%, *Strombidium* sp., *Myrionecta* sp., *Tintinnopsis* sp.), autotrophic nanoflagellates (10%) comprising the remaining dominant groups. Densities of heterotrophic bacteria, cyanobacteria, and eukaryotic phytoplankton were 2.5 ± 0.1 x 10^6, 2.7 ± 0.1 x 10^5 and 3.7 ± 0.7 x 10^4 cells mL^{-1}, respectively).

Although enrichment with vitamin B_{12} alone did not significantly change levels of bacterial, cyanobacterial, or algal biomass during these experiments (Fig. 4A-C), this vitamin did alter the composition of the plankton community. Enrichment with B_{12} caused a large decline in autotrophic nanoflagellates, as well as a significant (p<0.001) increase in the relative abundance of both diatoms and ciliates (Fig. 5). In contrast, enrichment with NO_3^- yielded a ten-fold increase in Chl *a*-specific net growth rates of large and small phytoplankton (> 2 μm, < 2 μm) compared to other treatments and the control (p<0.001, Fig. 4B). NO_3^- additions shifted the phytoplankton from a dinoflagellate-dominated (68% of micro- and nanoplankton cells) to a diatom-dominated (59%) community (Fig. 5) consisting primarily of *Nitzschia* spp. (98% of diatoms). Pigment analysis revealed that phytoplankton containing lutein (chlorophytes), zeaxanthin (cyanobacteria), and fucoxanthin (diatoms) experienced significantly increased net growth rates within the NO_3^- amendment (p<0.001 in all cases, Table 3A). Flow cytometric counts also documented these changes as net growth rates of cyanobacteria and eukaryotic

phytoplankton increased significantly (two- to three-fold increase, $p<0.05$, Fig. 4C). A unique response was observed in the combined NO_3^- and vitamin B_{12} amendment within which the >2 μm size class exhibited significantly higher net growth rates and the <2 μm size class exhibited significantly lower net growth rates compared to the NO_3^- only treatment and the control treatment ($p<0.001$, Fig. 4B). Dinoflagellates experienced significantly increased net growth rates when amended with both NO_3^- and B_{12} compared to the NO_3^- only treatment and the control treatment ($p<0.001$; Table 3A). Conversely, diatoms had a significantly lower net growth rate in the NO_3+B_{12} addition when compared to the NO_3 alone ($p<0.001$, Table 3A). The addition of NO_3^-+B_{12} resulted in a community co-dominated by dinoflagellates (48%; primarily *Prorocentrum* sp. and *Alexandrium* sp.) and ciliates (32%; primarily *Strombidium* sp., *Myrionecta* sp., *Tintinnopsis* sp.), while the relative abundance of diatoms (*Nitzschia* spp.) was similar to the control but significantly lower than the NO_3^- only treatment (Fig. 5). Flow cytometric analyses revealed that net growth rates of cyanobacteria within the NO_3+B_{12} treatment were double the NO_3^- only treatment (Fig. 4C). The addition of vitamin B_{12} in conjunction with NO_3^- also yielded significantly greater net growth rates of heterotrophic bacteria compared to all other treatments including the NO_3^- only treatment ($p<0.001$, Fig. 4C).

HNLC experiment - Water used for the HNLC experiment was collected southwest of Kodiak Island (Sta. 20, Fig. 1, Table 1). Levels of Fe (0.03 nmol L^{-1}) and Chl *a* in this regions were low (0.66 ± 0.02 μg L^{-1}), while NO_x, PO_4^-, and $Si(OH)_4$ levels were elevated (6.9, 0.8, and 13.8 μmol L^{-1}, respectively, Table 1). Initial vitamin B_{12} concentrations were 1.7 pmol L^{-1}, higher than most coastal stations but lower than what was found at other HNLC stations (Table 1). There were low abundances of nano- and microplankton cells (49 cells mL^{-1}) with the community being fairly equally represented by dinoflagellates (31%, *Prorocentrum* sp.,

Heterocapsa sp., *Scripsiella* sp., *Gymnodinium* sp.), autotrophic nanoflagellates (29%), ciliates (20%, *Strombidium* sp., *Myrionecta* sp.), and diatoms (17%, *Thalassiosira* sp., *Nitzschia* spp.). Water collected from this station contained heterotrophic bacteria, cyanobacteria, and small eukaryotic phytoplankton at densities of $1.4 \pm 0.1 \times 10^6$, $5.4 \pm 0.1 \times 10^4$, and $3.7 \pm 0.1 \times 10^4$ cells mL^{-1}, respectively.

As expected for this HNLC environment, Chl *a*-specific, eukaryotic phytoplankton, and heterotrophic bacterial net growth rates significantly increased within the Fe treatment ($p<0.001$; Fig. 4B). The addition of Fe resulted in a dramatic increase in the relative abundance of diatom cells among the nano- and microplankton (87% of total cells; $p<0.001$; Fig. 5) consisting mostly of *Nitzschia* sp. (84% of diatoms). Relative to unamended controls, the addition of vitamin B_{12} also resulted in a significant ($p<0.001$) increase in total Chl *a* accumulation and the net growth rates for each Chl *a* size fraction (Fig. 4A). This treatment also displayed a significant ($p<0.001$) increase in the growth rate of heterotrophic bacteria (Fig. 4C). The nano- and microplankton community response to vitamin B_{12} additions alone was nearly the opposite of the response observed for the Fe addition. Compared to the control treatment, B_{12} enrichment significantly decreased the relative abundance of diatoms ($p<0.001$) and significantly increased the relative abundance of autotrophic nanoflagellates, ciliates (*Strombidium* sp., *Myrionecta* sp.) and dinoflagellates (*Gyrodinium* sp. and *Prorocentrum* sp.; $p<0.001$ for all, Fig. 5) compared to the control treatment. Adding B_{12} in conjunction with Fe resulted in a significantly higher total Chl *a* accumulation than either individual compound ($p<0.05$; Fig. 4A). Growth in the Fe+B_{12} treatment was due to larger phytoplankton as the >2 μm Chl *a* net growth rates in this treatment were significantly higher than in the individual B_{12} or Fe treatments ($p<0.05$; Fig. 4B) while <2 μm growth rates were similar among the three treatments. The net growth rates of heterotrophic

bacteria, cyanobacteria, and eukaryotic phytoplankton in the Fe+B_{12} were all twice the rates displayed in the Fe alone treatment (p<0.05; Fig. 4C). Diatoms became less abundant in the Fe+B_{12} treatment compared to Fe alone (68% vs. 87% of microplankton) and their composition changed as the abundance of *Chaetoceros* sp. increased dramatically while *Rhizosolenia* sp. densities decreased more than four-fold. In contrast, there was a significant increase in autotrophic nanoflagellates observed in the Fe+B_{12} treatment compared to the Fe only treatment (p<0.05). Among dinoflagellates, *Prorocentrum* sp. abundances increased significantly (p<0.05) in the individual B_{12} and Fe treatments while *Gymnodinium* sp. became the dominant dinoflagellate (80% of dinoflagellates) in the Fe+B_{12} treatment. In a manner paralleling the B_{12} only treatment, the addition of Fe and B_{12} resulted in a significant increase (p<0.01) in ciliates (*Myrionecta* sp., *Strombindium* sp.) over the Fe only treatments (Fig. 5).

Discussion

This study presents the first direct measurements of vitamin B_{12} concentrations and vitamin B_{12} uptake rates in the sub-arctic North Pacific. Concentrations and uptake rates of vitamin B_{12} were highest within the HNLC region of the Gulf of Alaska where picoplankton dominated primary production and numerical plankton counts. Incubation experiments demonstrated that vitamin B_{12} alone or in conjunction with the primary limiting nutrients (Fe or N) was able to significantly alter plankton community structure and growth rates (Figs. 4, 5). Together, this data set provides a new understanding of the role of vitamin B_{12} in high latitude, ocean plankton communities.

The dynamics of vitamin B_{12} parallel macronutrients in the Gulf of Alaska

Concentrations of dissolved vitamin B_{12} were low (0.1 – 8 pmol L^{-1}) across the surface waters of the Gulf of Alaska likely reflecting rapid microbial cycling of this compound (Swift

1980; Croft et al. 2005). Vitamin B_{12} was distributed similarly to macronutrients with concentrations being low in the coastal zone, high in the HNLC region, and correlated with NO_3^- and PO_4^{3-} concentrations throughout the Gulf of Alaska (r = 0.38 and 0.39, respectively; p<0. 05 for both). In addition, NO_3^-, PO_4^-, and B_{12} concentrations were all inversely correlated with dissolved Fe concentrations (r = -0.75, -0.84 and -0.49, respectively; p<0.05 for all). Prior studies within the Strait of Georgia (British Columbia; Cattell 1973) and the Sargasso Sea (Menzel and Spaeth 1962) have found that concentrations of vitamin B_{12} paralleled those of NO_x and PO_4^-. The same study of the Strait of Georgia also found the highest B_{12} concentrations in off-shore waters, with decreasing concentrations towards the coast (Cattell 1973). Natarajan and Dugdale (1966) observed a similar spatial gradient for vitamin B_1 in the Gulf of Alaska; lower concentrations in the coastal regions and highest in offshore, HNLC regions. Both genomic data (Rocap et al. 2003; Rodionov et al. 2003; Vitreschak et al. 2003) and recent laboratory studies (Croft et al. 2005; Bonnet et al. 2010) indicate that many prokaryotes (phototrophs and heterotrophs) could be significant sources of B_{12} to the oceans. The low rates of metabolic activity of prokaryotes in cold, high latitude regions (Church 2000; Kirchman et al. 2009) coupled with lower densities of cyanobacteria in these regions (Boyd and Harrison 1999; Boyd et al. 2000) suggests that microbial B_{12} synthesis rates are likely to be relatively low within high latitude oceans. The low production of B_{12} in Gulf of Alaska coastal regions coupled with higher primary production rates (compared to off-shelf regions) and thus B_{12} uptake by larger, B_{12} auxotrophic phytoplankton during late summer likely contribute to the low B_{12} concentrations observed within coastal regions. The higher B_{12} concentrations found in the HNLC regions suggests Fe-replete prokaryotic picoplankton (>10^6 cells mL^{-1}; Fig. 3) grow and produce B_{12} (Parker 1977; Rodionov et al. 2003; Bonnet et al. 2010) which, like nitrate,

accumulates due to the inability of Fe-limited nano- and micropankton to grow (Martin and Fitzwater 1988; Martin et al. 1989; Boyd et al. 1996) and assimilate the vitamin. Furthermore, the small plankton in the HNLC Gulf of Alaska support an active microzooplankton community which rapidly cycles the standing crop of picoplankton and regenerates nutrients (Landry et al. 1997; Harrison et al. 1999; Strom et al. 2006) such as B_{12}.

Vitamin utilization by the plankton community

Throughout the Gulf of Alaska, primary productivity rates and Chl *a* concentrations were evenly distributed between the large and small plankton whereas most vitamin B_{12} uptake (68-83%) was by picoplankton (< 2 μm; Table 2). The higher proportion of uptake of B_{12} compared to bicarbonate (i.e., primary production) among picoplankton could indicate that heterotrophic bacteria, which would not assimilate bicarbonate, were responsible for the relatively higher < 2 μm B_{12} uptake. Although many prokaryotes have the capacity to synthesize B_{12} (Rodionov et al. 2003; Vitreschak et al. 2003; Bonnet et al. 2010), many are also capable of B_{12} uptake (i.e., contain the btu*B* gene; Medigue et al. 2005). Similarly, Parker (1977) found that cyanobacterial communities in Lake Washington were responsible for the bulk of vitamin production and utilization.

The small proportion of vitamin B_{12} uptake by larger phytoplankton in the Gulf of Alaska could be the outcome of competition for a limited resource. B_{12} auxotrophy is prevalent among larger phytoplankton such as diatoms (60% of species surveyed; Croft et al 2005) and dinoflagellates (91% of species surveyed; Tang et al. 2010). The picomolar vitamin concentrations found in the Gulf of Alaska (0.1 – 8 pmol L^{-1}, Table 1) were within the range of B_{12} half saturation constants (0.1 – 10 pmol L^{-1}) of these the larger phytoplankton (Droop 1968; Droop 2007; Tang et al. 2010) and thus may have limited their growth rates (Caperon and Meyer

1972). In addition, the high surface-to-volume ratios and thin surface diffusive boundary layers of small cells likely results in higher vitamin uptake rates per unit of biomass compared to their larger counterparts (Raven and Kubler 2002). Therefore, we hypothesize that large auxotrophic phytoplankton in the Gulf of Alaska are poor competitors for B_{12} under the low, ambient vitamin concentrations, and thus are more likely to be limited by B_{12} than picoplankton. Consistent with this hypothesis, during experiments performed in the Gulf of Alaska (Figs. 4, 5) and other ecosystems (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007), enriching seawater with vitamins stimulated the growth of large but not small, planktonic cells.

Effect of B_{12} vitamin amendments on community composition

Coastal experiment – The strong growth response from NO_3^- among phytoplankton in general and in diatoms particular was consistent with the low NO_3^- levels, low N:P and N:Si ratios present at the start of the experiment and with prior studies in the coastal Gulf of Alaska (Whitney et al. 2005; Strom et al. 2006), and other marine coastal zones (Nixon 1995). The addition of B_{12} alone did not alter total algal biomass but did significantly enhance the relative abundance of diatoms and ciliates. While the majority of diatoms are auxotrophic (Croft et al. 2005), the response of ciliates may have been due to direct use of the vitamin or consumption of more abundant prey such as diatoms. Interestingly, the addition of B_{12} combined with NO_3^- yielded an algal community dominated by auxotrophic dinoflagellates (*Gymnodinium* sp., and *Alexandrium* sp.; Tang et al. 2010) which were nearly absent in the diatom dominated NO_3^- only treatment. Verification of the precise auxotrophic nature of these phytoplankton would require the isolation and growth of these cells in culture since B_{12} auxotrophy can vary between species within a genera (Guillard 1968) and even among strains within a species (Tang et al. 2010). Regardless, the auxotrophy displayed by these and most other dinoflagellates (Tang et al. 2010)

is consistent with their tendency to derive nutrition from external and/or organic sources (i.e., heterotrophy; Taylor 1987 ; Smayda 1997; Heisler et al 2008). The specific increase in the growth of large phytoplankton in this experiment was consistent with experiments conducted within northwest Atlantic estuaries in which the addition of vitamin B_{12} and NO_3^- also promoted the growth of larger (>5 μm) phytoplankton (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007). Finally, the significant increase in growth rates of heterotrophic bacteria and ciliates in the NO_3^- + B_{12} could have been caused by a co-limitation by these compounds (Saito et al. 2008) or could have been in response to the enhanced growth of primary producers in this treatment (Strom et al. 2006; Kirchman et al. 2009).

HNLC experiment – In a manner consistent with experiments in the Southern Ocean (Panzeca et al. 2006; Bertrand et al. 2007) , the addition of B_{12} combined with Fe significantly enhanced phytoplankton growth rates in the Gulf of Alaska, indicating that B_{12} can be a secondary limiting element (Saito et al. 2008) in this regions. Our results also documented, for the first time, a strong algal response from vitamin B_{12} enrichment alone as well as an altered plankton community composition due to the presence of higher B_{12} concentrations, with or without Fe. Given the strong growth response to B_{12} among > 2 μm phytoplankton and the dominance of vitamin assimilation in < 2 μm plankton (> 80% of uptake), low Fe, HNLC regions may favor small plankton, in part, due to the rapid cycling of vitamin B_{12}. Additionally, microbially-derived organic compounds which have been shown to inhibit the bioavailability of dissolved vitamins to phytoplankton (Swift 1980) may be actively produced by microbial processes in the HNLC Gulf of Alaska and contribute toward B_{12} limitation in this region.

The influence of vitamin B_{12} in the HNLC Gulf of Alaska was evident within the response of individual taxa to each treatment. Consistent with prior HNLC experiments, the

addition of Fe enhanced the growth of the diatom *Nitzschia* spp. (Martin et al. 1989; De Baar et al. 2005). Unlike Fe, vitamin B_{12} shifted the algal community away from diatoms and towards autotrophic nanoflagellates and putatively auxotrophic dinoflagellates such as *Alexandrium, Prorocentrum,* and *Gyrodinium* (Fig. 5; Tang et al. 2010). These distinct differences in community composition between the B_{12} and the Fe only additions demonstrate that B_{12}, and not Fe contamination of the B_{12} stock, was responsible for the phytoplankton response in the B_{12} treatment. Within the combined Fe and B_{12} treatment favored the growth of the centric diatom, *Chaetoceros* sp., and the dinoflagellate, *Gymnodinium* sp., both genera which are comprised primarily of B_{12} auxotrophs (Provasoli and Carlucci 1974; Tang et al. 2010). Finally, patterns of net growth rates of heterotrophic bacteria paralleled eukaryotic phytoplankton, being significantly enhanced by Fe or B_{12} and being even higher in the dual Fe + B_{12}, treatment, a response which may have been due to either the added compounds or compounds produced by the phytoplankton which were growing more rapidly. All of these findings demonstrate that vitamin B_{12} can shape plankton community composition in both the coastal and HNLC regions of the Gulf of Alaska. The results further emphasize that different members of the phytoplankton community can be concurrently limited by different nutrients (Saito et al. 2008).

The role of vitamin B_{12} in the cycling of carbon in HNLC, polar seas

Since their characterization in the 1980s by John Martin (Martin and Fitzwater 1988; Martin et al. 1989; Martin et al. 1994), Fe-limited HNLC regions have been extensively studied, particularly with regard to their potential role in climate change. Enhanced deposition of Fe-rich dust during glacial periods putatively led to the alleviation of Fe-stress in HNLC regions, enhanced primary production, and reduced atmospheric CO_2 concentrations (Watson et al. 2000). Although high latitude, HNLC regions generally harbor an active microbial loop (Landry

et al. 1997; Strom et al. 2006) in which elements are recycled rather than exported (Harrison et al. 1999), modern day, large scale Fe enrichments in these regions can result in blooms of diatoms with rapid settling rates and increased export of carbon to deep waters (Buesseler et al. 2004; De Baar et al. 2005). The present study, however, has demonstrated that vitamin B_{12} is capable of enhancing the accumulation of phytoplankton in the Gulf of Alaska, independent of Fe, as well as maximizing Fe-stimulated phytoplankton biomass. Furthermore, B_{12} alone or in conjunction with Fe significantly changed the plankton community composition favoring the growth of putative B_{12} auxotrophs and thus suggesting vitamins may influence the efficiency of the biological pump. The centric diatom *Chaetoceros* sp. and mixotrophic dinoflagellate *Gymnodinium* sp. (both B_{12} auxotrophs; Provasoli and Carlucci 1974 ; Croft et al. 2005) were not present in the Fe only addition but grew robustly in the Fe+B_{12} treatment. The phytoplankton community fostered by B_{12}-enrichment - fewer diatoms and more flagellates - would be seemingly less likely to export C than the predominately diatom community resulting from Fe enrichment (De Baar et al. 2005).

The present study, along with others (Coale et al. 2004; Panzeca et al. 2006; Bertrand et al. 2007), demonstrates other nutrients beyond Fe are vital for shaping community composition of Fe-induced phytoplankton blooms in HNLC regions. In Si-limited regions of the Southern Ocean, the Fe-induced bloom communities have been comprised primarily of non-silicious, autotrophic nanoflagellates (Coale et al. 2004). In the HNLC region of the Gulf of Alaska, vitamin B_{12} alone and in conjunction with Fe significantly altered phytoplankton community composition as well as enhanced algal biomass. While HNLC regions have been considered potential carbon sequestration sites via Fe-stimulated phytoplankton blooms, our findings suggest that the availability of vitamin B_{12} will strongly influence the success of such plans.

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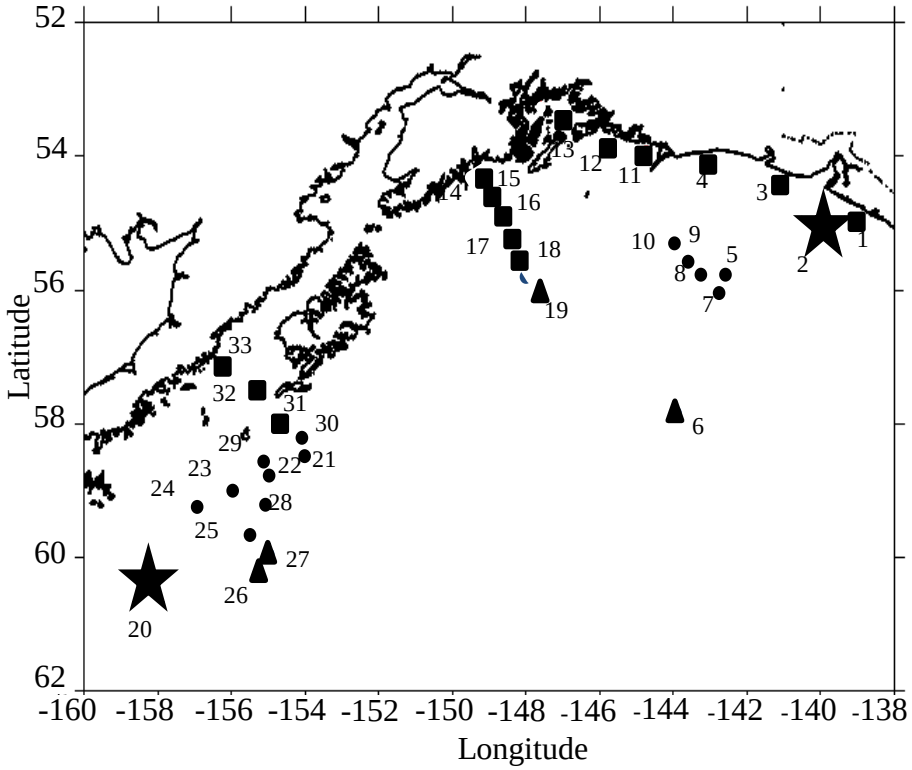

Figure 1. Stations within the Gulf of Alaska sampled. Squares, circles, and triangles represent coastal, off-shore, and HNLC regions, respectively. Stars denote the two stations from which water for amendment experiments was collected.

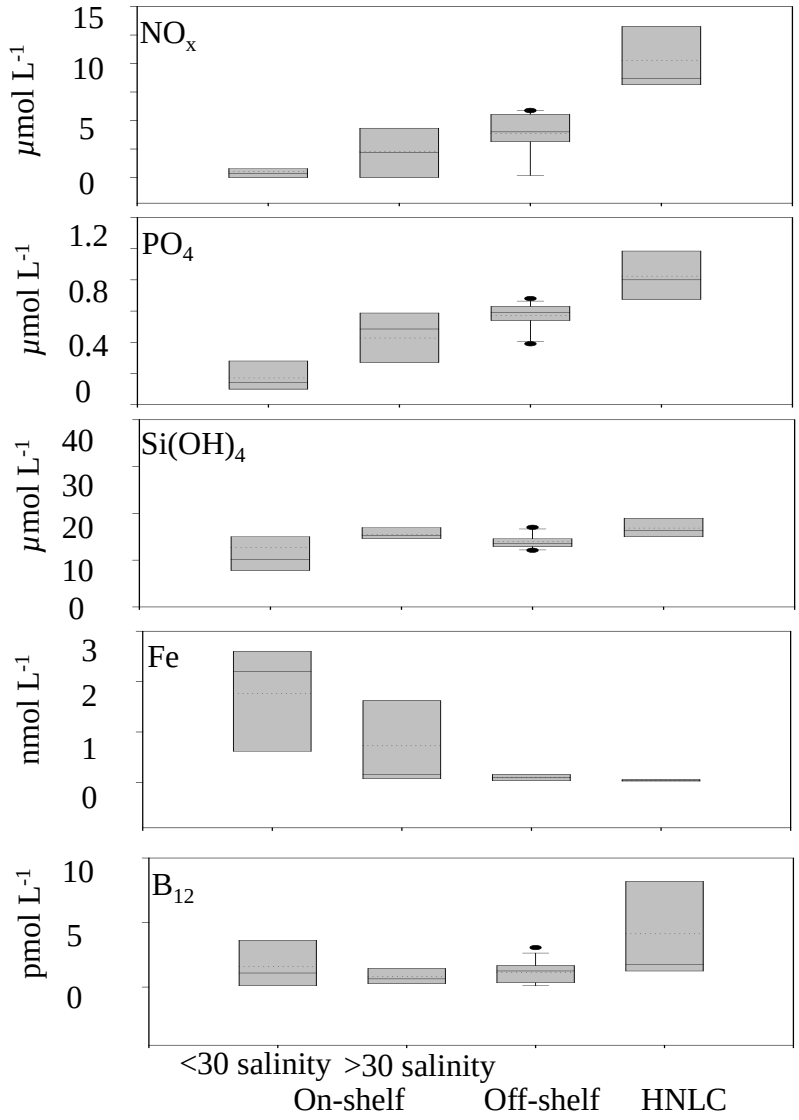

Figure 2. Box-and-whisker plots of nitrate + nitrite (NOx), ortho-phosphate (PO_4^{3-}), silicate ($Si(OH)_4$), vitamin B_{12} and dissolved iron (Fe) within four regions across the Gulf of Alaska. Concentrations are shown as μmol L^{-1} with the exception of B_{12} and Fe which are reported as pmol L^{-1} and nmol L^{-1}, respectively. The solid and dotted lines represent the median and mean, respectively.

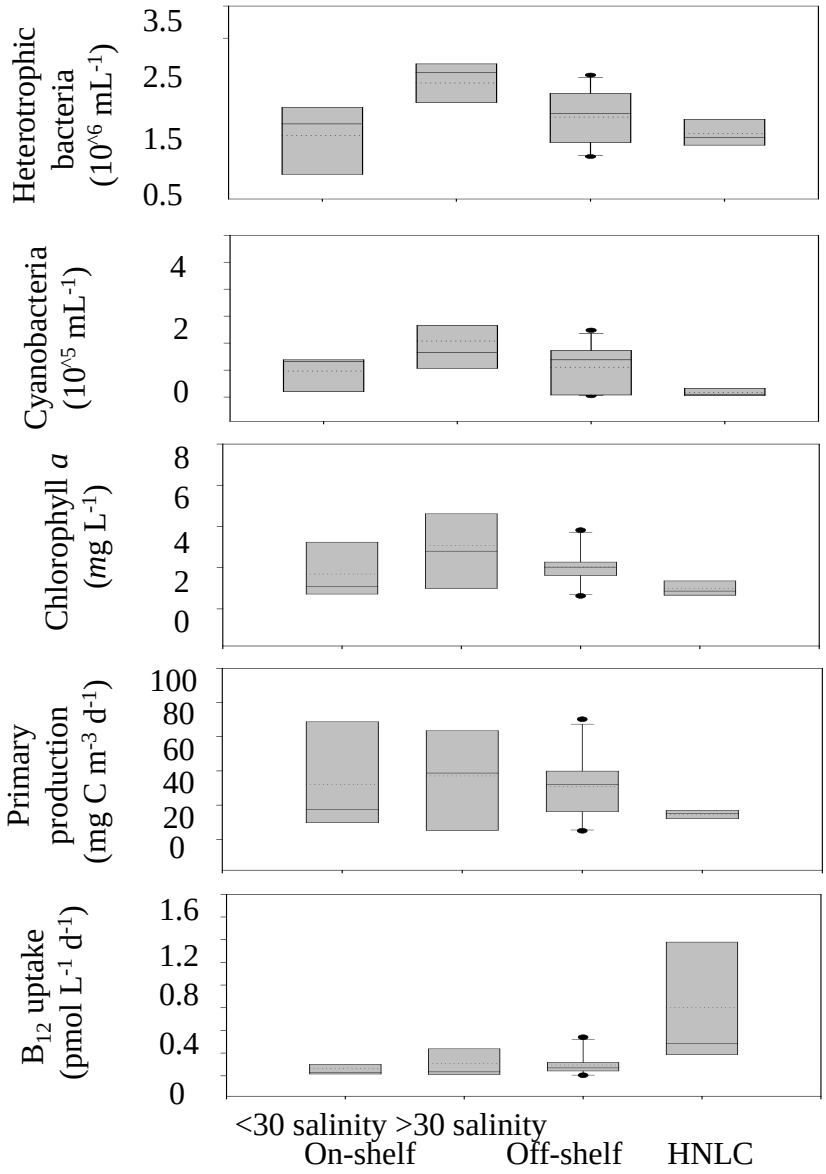

Figure 3. Box-and-whisker plots of densities of heterotrophic bacteria, cyanobacteria, total chlorophyll *a*, primary production, and uptake of vitamin B_{12} within four regions across the Gulf of Alaska. Abundances of heterotrophic bacteria and cyanobacteria are reported as cells mL^{-1}, chlorophyll *a* values are reported as μg L^{-1}, primary production rates are reported as mg C m^3 d^{-1}, and uptake of B_{12} as pmol L^{-1} d^{-1}. The solid and dotted lines represent the median and mean, respectively.

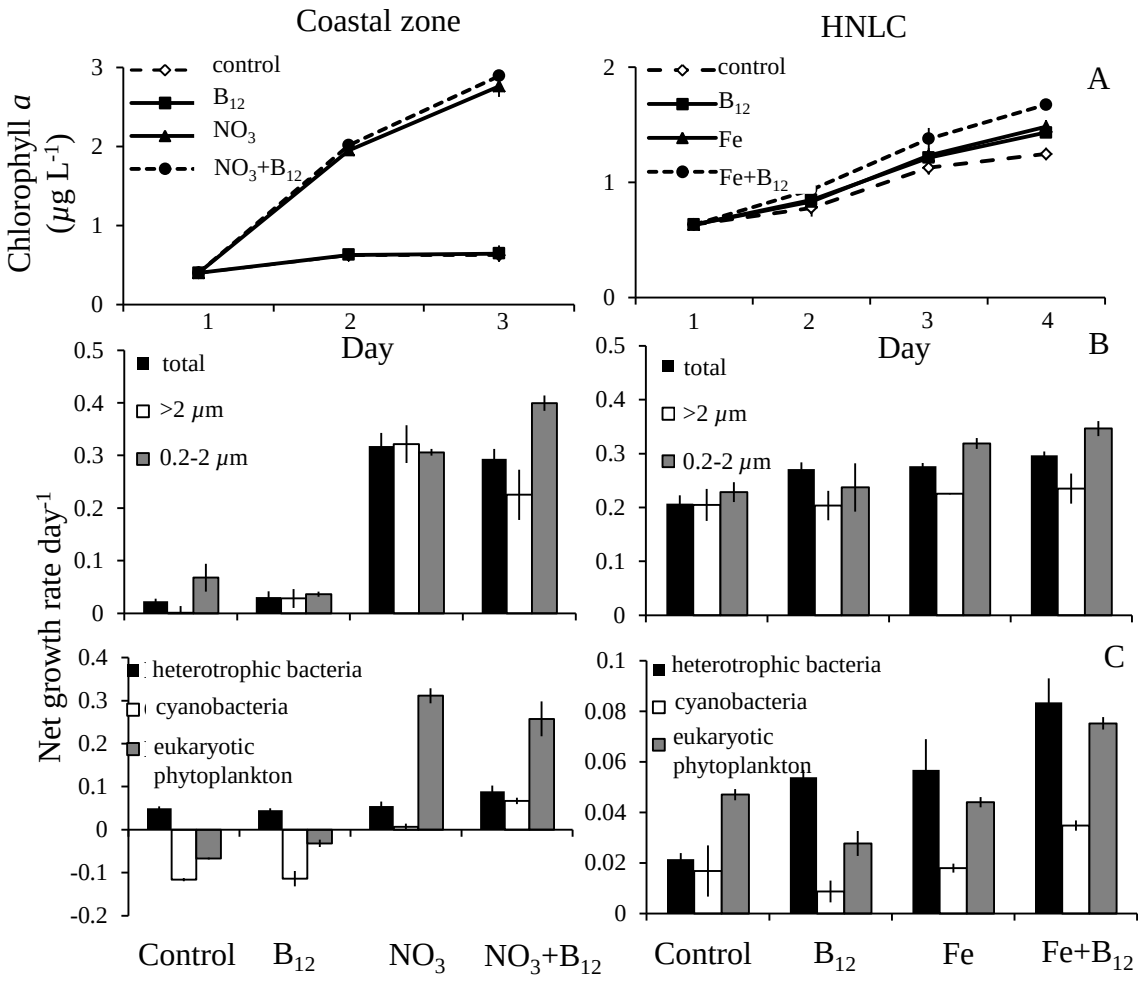

Figure 4. Nutrient amendment experiments conducted within the coastal zone (left) and a HNLC region (right) of the Gulf of Alaska. (A) Accumulation of total chlorophyll *a*, (B) size fractionated net growth rates based on changes in chlorophyll *a*, and (C) net growth rates of cyanobacteria, heterotrophic bacteria, and eukaryotic phytoplankton. Note that in (A) of the coastal experiment the control and B_{12} treatments overlap. Data points are means ± 1 SD error bars.

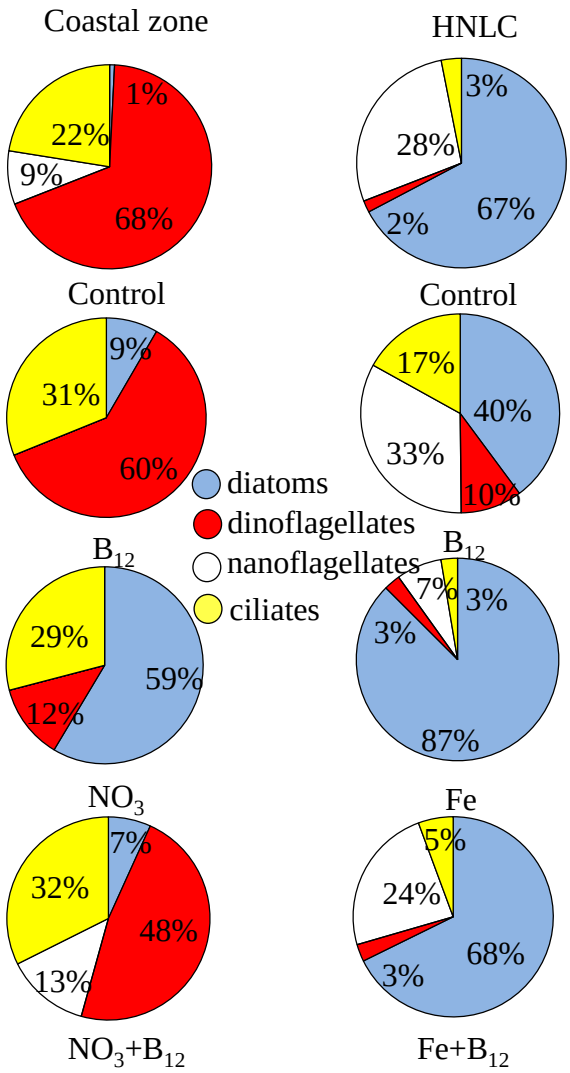

Figure 5. Nutrient amendment experiments conducted within the coastal zone (left) and a HNLC region (right) of the Gulf of Alaska. Displayed are the micro- and nanoplankton community structure for each treatment as a percent of the total number of cells present. Data points are means ± 1 SD error bars.

Table 1. Physical and chemical characteristics of the 33 stations and their characterization as on-shelf (<31 or >31 salinity), off-shelf, or HNLC based on the total water column depth. 'Depth' refers to the depth of the water column, not sampling depth. Water was collected from 1-3 m for all of the stations (*see* Methods). Values for nitrite + nitrate (NO_x), ortho-phosphate (PO_4^{3-}), and silicate ($Si(OH)_4$) are reported as μmol L^{-1} whereas concentrations of B_{12} and Fe are in pmol L^{-1} and nmol L^{-1}, respectively. N:P is the molar ratio of NO_x to PO_4^{3-}. Temperature (Temp) is reported in degrees Celsius. Stations at which water for experiments was collected are shown in bold.

Station	Depth (m)	Temp (°C)	Salinity	NOx	PO_4^{3-}	$Si(OH)_4$	B_{12}	Fe	N:P
On-shelf <31 salinity									
3	73	15.10	30.30	0.00	0.20	15.00	0.10	2.20	0.00
1	80	10.00	10.00	1.40	0.05	33.90	1.09	2.60	28.0
12	118	14.25	27.40	0.35	0.14	10.20	0.10	-	2.50
11	147	14.00	27.80	0.30	0.11	10.10	3.63	-	2.73
33	197	11.12	30.92	0.63	0.32	9.43	3.80	0.75	1.97
14	224	13.30	30.50	0.00	0.28	7.80	1.89	0.22	0.00
13	582	12.30	24.59	0.20	0.10	2.03	0.40	-	2.00
On-shelf >31 salinity									
4	147	15.40	31.26	0.00	0.27	15.00	0.33	1.81	0.00
32	50	9.83	31.83	3.35	0.59	11.70	0.52	1.05	5.68
2	**150**	**14.90**	**31.28**	**0.01**	**0.06**	**15.60**	**0.10**	**2.41**	**0.17**
17	164	11.98	31.38	1.42	0.43	15.50	0.75	0.22	3.30
16	169	12.86	32.20	2.06	0.58	17.40	1.28	0.08	3.55
15	200	12.60	31.60	0.00	0.28	14.70	0.24	0.07	0.00
31	250	10.60	31.97	4.46	0.67	14.60	1.97	0.07	6.66
18	275	13.77	32.30	3.50	0.54	19.00	1.49	0.09	6.48
Off-shelf									
29	684	11.20	32.11	3.90	0.60	13.50	2.00	0.16	6.50
30	968	11.90	32.20	3.63	0.59	12.30	0.58	0.03	6.15
25	1914	11.10	32.26	2.68	0.64	13.20	0.82	-	4.19
23	2032	10.80	32.20	3.20	0.62	14.00	1.42	-	5.16
24	2207	10.90	32.20	2.90	0.58	14.30	1.34	-	5.00
10	3272	13.80	32.50	4.68	0.61	13.55	0.64	-	7.67
9	3564	13.80	32.20	4.70	0.56	16.15	0.10	-	8.39
5	3590	14.30	31.80	0.14	0.39	13.70	1.90	-	0.36
22	3591	12.30	32.40	4.40	0.64	12.10	3.06	-	6.88
7	3600	14.10	31.80	0.14	0.43	13.50	1.25	-	0.33
8	3612	14.20	31.70	3.20	0.52	14.80	0.10	-	6.15
21	5232	12.30	32.40	4.46	0.68	12.60	0.10	-	6.56
28	5493	10.10	31.10	2.34	0.58	17.00	1.39	0.09	4.03
HNLC									
19	1736	13.70	32.50	6.09	0.69	16.20	0.88	0.06	8.83
6	3866	14.10	32.50	6.93	0.66	19.10	1.61	-	10.5
20	**4436**	**11.90**	**32.40**	**6.97**	**0.80**	**13.78**	**1.75**	**0.03**	**8.71**
26	4943	11.80	32.60	10.20	0.97	16.40	8.24	0.03	10.5
27	4586	12.10	32.40	11.00	1.00	18.80	8.13	0.04	11.0

Table 2. Total, 0.2-2 μm, and >2 μm size fraction contribution to chlorophyll *a*, primary production rates, and B_{12} utilization across the different regions of the Gulf of Alaska. While primary production and chlorophyll was, on average, evenly divided among large and small plankton, the majority of vitamin B_{12} assimilation was by the picoplankton.

Location		Chlorophyll *a* (μg L^{-1})	Primary productivity (mg C m^{-3} d^{-1})	ρB_{12} uptake (pmol L^{-1} d^{-1})
On-shelf <31	total	1.87 ± 0.52	28.1 ± 12.7	0.08 ± 0.05
	0.2-2 μm	0.98 ± 0.34	12.4 ± 8.9	0.06 ± 0.04
	>2 μm	0.89 ± 0.28	15.7 ± 5.1	0.02 ± 0.01
	% in 0.2-2 μm	54 ± 8.3	37 ± 12	68 ± 14
On-shelf >31	total	3.09 ± 0.77	37.1 ± 10.3	0.11 ± 0.05
	0.2-2 μm	1.18 ± 0.30	17.7 ± 4.49	0.08 ± 0.04
	>2 μm	1.91 ± 0.62	19.4 ± 6.64	0.02 ± 0.01
	% in 0.2-2 μm	38 ± 12	53 ± 5.4	81 ± 5.3
Off-shelf	total	2.04 ± 0.26	30.9 ± 5.86	0.10 ± 0.03
	0.2-2 μm	0.93 ± 0.12	17.3 ± 3.64	0.08 ± 0.02
	>2 μm	1.11 ± 0.26	13.7 ± 2.76	0.02 ± 0.01
	% in 0.2-2 μm	49 ± 5.1	50 ± 7.3	70 ± 10
HNLC	total	0.98 ± 0.16	14.6 ± 1.24	0.60 ± 0.25
	0.2-2 μm	0.39 ± 0.02	9.10 ± 1.25	0.50 ± 0.22
	>2 μm	0.59 ± 0.14	5.49 ± 0.97	0.10 ± 0.03
	% in 0.2-2 μm	43 ± 5.3	62 ± 6.2	82 ± 4.1

Table 3. Pigment based net growth rates for the coastal experiments. Pigments shown represent dinoflagellates (peridinin), cryptophytes (alloxanthin), chlorophytes (luteine), cyanobacteria (zeaxanthin), and diatoms (fucoxanthin, after DiTullio and Geesey 2002). An asterisk denotes a significant (p<0.001) difference for a treatment compared to the control.

	Total Chl *a*	Alloxanthin	Fucoxanthin	Peridinin	Zeaxanthin	Lutein
Control	0.02 ± 0.01	- 0.36 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	- 0.06 ± 0.01	- 0.08 ± 0.01
NO_3	0.29 ± 0.01*	- 0.18 ± 0.25	0.29 ± 0.02*	0.19 ± 0.06	0.17 ± 0.00*	0.26 ± 0.01*
NO_3+B_{12}	0.29 ± 0.01*	0.19 ± 0.16*	0.23 ± 0.03*	0.31 ± 0.02*	0.15 ± 0.03*	0.24 ± 0.01*

Chapter Three

Vitamin B_1 and B_{12} uptake and cycling by plankton communities in coastal ecosystems

Abstract: While vitamin B_{12} has recently been shown to co-limit the growth of coastal phytoplankton assemblages, the cycling of B-vitamins in coastal ecosystems is poorly understood as planktonic uptake rates of vitamins B_1 and B_{12} have never been quantified in tandem in any aquatic ecosystem. The goal of this study was to establish the relationships between plankton community composition, carbon fixation, and B-vitamin utilization in estuaries. Here I show that, although vitamin concentrations were low (pM), vitamin concentrations and utilization rates were higher within more eutrophic estuaries and that these uptake rates were significantly correlated with rates of primary production. Eutrophic sites hosted larger bacterial and picoplankton abundances with larger carbon normalized vitamin uptake rates. Although the >2µm phytoplankton biomass was often dominated by groups with a high incidence of vitamin auxotrophy (dinoflagellates and diatoms), picoplanktonic heterotrophic bacteria and cyanobacteria (< 2µm) were always responsible for the majority of B-vitamin uptake. Multiple lines of evidence suggest that heterotrophic bacteria were the primary users of vitamins during this study. Nutrient/vitamin amendment experiments demonstrated that, in the summer and fall, vitamin B_{12} occasionally limited or co-limited the accumulation of phytoplankton biomass together with nitrogen. Combined with prior studies, these findings suggest that picoplankton are the primary producers and users of B-vitamins in coastal ecosystems and that rapid microbial cycling of B-vitamins may sometimes deprive larger phytoplankton of these micronutrients and thus influence phytoplankton species succession.

Introduction

Coastal marine ecosystems are amongst the most ecologically and socioeconomically productive on the planet, providing an estimated US$14 trillion, or about 43% of the global total, worth in ecosystem goods and services, annually (Costanza et al. 1997). While coastal areas comprise only 8% of the world's ocean surface they account for over 28% of the annual ocean primary production (Holligan and De Boois 1993). In a manner paralleling global trends, nearly 75% of the US population lives within 75 km of the coastline, making these regions subject to a suite of anthropogenic influences including intense nutrient loading (De Jonge et al. 2002; Valiela 2006) which in turn can lead to ecological perturbations such as harmful algal blooms and hypoxia (Cloern 2001; Heisler et al. 2008). Coastal zone management efforts typically focus on minimizing total nitrogen input since primary production in most coastal marine systems are nitrogen-limited (Nixon 1995). While the absolute magnitude of N entering coastal zones often controls the amount of phytoplankton biomass, the availability, ratio, and/or type (e.g. inorganic vs. organic) of nutrients can also influence algal community composition (Smayda 1997; Koch and Gobler 2009).

B-vitamins such as thiamine (B_1), biotin (B_7) and cobalamin (B_{12}) are important cofactors in a number of cellular processes such as the biosynthesis of methionine (B_{12}), the decarboxylation of pyruvic acid (B_1) and fatty acid synthesis (B_7). While vitamins have long been implicated as growth regulators for microalgae (Droop 1955; Provasoli and Carlucci 1974) their ecological importance has received little attention since early surveys (Vishniac and Riley 1961; Menzel and Spaeth 1962) and laboratory experiments (Droop 1968) suggested that ambient concentration were sufficient to satisfy micro-algal demands (Swift 1980). In addition, prior methods used to estimate vitamin concentrations via bioassays and were tedious, time

consuming, and error prone (Guillard and Ryther 1962; Friedrich 1974). Newly developed methods to directly measure B-vitamins in seawater (Okbamichael and Sanudo-Wilhelmy 2004; Okbamichael and Sanudo-Wilhelmy 2005), have facilitated surveys in several open ocean and coastal ecosystems and have revealed that vitamin concentrations are low, ranging from <0.1 to 40 pmol L^{-1} and <0.1 to 100 pmol L^{-1} (Gobler et al. 2007; Panzeca et al. 2008; Panzeca et al. 2009; Koch et al. 2011) for B_{12} and B_1, respectively.

Surveys of the literature as well as novel laboratory experiments indicate that over half of all phytoplankton species surveyed have an obligate requirement for an exogenous supply of one or more of the B-vitamins with B_{12} being required by the largest number of algae (i.e. are auxotrophic; Croft et al. 2005, Tang et al. 2010). Vitamin enrichment studies in coastal (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007) and open ocean environments (Panzeca et al. 2006; Bertrand et al. 2007; Koch et al. 2011) have shown that B-vitamins can co-limit phytoplankton biomass along with a primary limiting nutrient (i.e. nitrogen or iron). Vitamin availability can also shape coastal phytoplankton community structure. Since centric diatoms which comprise the temperate spring bloom (i.e. *Skeletonema costatum, Thalassiosira pseudonana and Ditylum brightwelli*) require B_{12} while only half of pennate species seem to be B_{12} auxotrophs (Droop 1955; Guillard and Ryther 1962), many have argued that B_{12} concentrations might influence dynamics and composition of the spring bloom (Carlucci and Bowes 1970; Swift 1980). Recently, Koch et al. (2011) demonstrated that vitamin B_{12} concentrations strongly influence algal communities in the coastal Gulf of Alaska, with high concentrations favoring dinoflagellates over diatoms, affirming that B-vitamins can indeed play an important ecological role in plankton succession. Despite this renewed interest in B-vitamins, very little is known how the trophic state of coastal ecosystems influences the cycling of vitamins and only one study

has investigated B-vitamin concentrations and uptake rates by plankton communities in an aquatic ecosystem (Koch et al. 2011).

The goal of this study was to elucidate the relationships between phytoplankton community composition, carbon fixation, and B-vitamin utilization by coastal plankton assemblages. This field study was performed over two years in two contrasting, coastal, marine systems: A hypereutrophic and a mesotrophic estuary. In support of the primary objective of the study, nutrient / vitamin amendment experiments were performed to explore the extent to which the availability of vitamins affected phytoplankton biomass during the study and stocks of organic and inorganic nutrients as well as phytoplankton community assemblages were quantified.

Methods

Study sites – Two estuaries were investigated during this study and were sampled on 18 occasions on a bi-weekly to monthly basis from March 2007 until November 2008 to capture a complete annual cycle of plankton – vitamin dynamics. Water was collected from the mesotrophic portion of Long Island Sound (LIS) near Mount Sinai Harbor (40.97° N, 73.04° W; Fig 1) and a hypereutrophic, tidal tributary on eastern Shinnecock Bay, Old Fort Pond (OFP, 40.87° N, 72.45° W, Fig 1). LIS is a large, urban estuary bordered on the western end by New York City and in the east exchanges with the North Atlantic Ocean, thus displaying a strong east-west eutrophication gradient (Gobler et al. 2006). Our sampling location was located within the eastern, mesotrophic half of this estuary. Old Fort Pond (OFP) exchanges tidally with Shinnecock Bay and is a shallow (<2m), well-mixed, hypereutrophic body of water which, during the summer months, experiences dense algal blooms that create a large demand for micro- and macro-nutrients and may be influenced by the availability of vitamins (Gobler et al. 2007).

This inland, hypereutrophic tributary with high levels of mixed algal biomass (up to 50 µg chl a L^{-1}; Gobler et al 2007) contrasts with the LIS location which typically displays lower levels of algal biomass (mesotrophic; ~ 3 µg chl a L^{-1}; (Gibson et al. 2000)).

Chemical analysis -Water for nutrient analysis was filtered through pre-combusted GF/F filters. Concentrations of nitrate, nitrite, ammonium, and phosphate were determine in duplicate by standard spectrophotometric methods (Jones 1984; Parsons et al. 1984). Total dissolved nitrogen and phosphorus (TDN, TDP) were analyzed in duplicate by persulfate oxidation techniques (Valderrama 1981) and dissolved organic nitrogen and phosphorus (DON and DOP) calculated by subtracting levels of nitrate, nitrite and ammonium or orthophosphate from concentrations of TDN and TDP, respectively. Full recoveries (mean ± 1 S.D.) of samples spiked with SPEX Certi-Prep^{INC} standard reference material for TDN, TDP, nitrate, nitrite and ammonium, and orthophosphate were obtained at environmentally representative concentrations. Vitamin samples were collected and analyzed according to Okbamichael and Sanudo-Wilhelmy (2004, 2005). Briefly, water was filtered through 0.2 µm capsule filters (GE Osmonics, DCP0200006) into 1 L LDPE bottles and stored frozen and in the dark. The samples were then acidified and concentrated at 1 mL min^{-1} onto columns containing 18C beads (Varian, HF BONDESIL), stored frozen, and analyzed via reverse phase HPLC.

Characterization of the plankton community – Several approaches were utilized to characterize resident plankton communities. Size fractionated chlorophyll *a* (Chl *a*) samples were collected by filtering triplicate samples onto 0.2 and 2 μm polycarbonate filters. These filters were stored frozen until analysis via standard fluorometeric methods (Welschmeyer 1994).. Whole seawater was also preserved in 5% Lugols iodine solution for enumeration of plankton (>5 μm) under an inverted microscope. Organisms were identified to the lowest

taxonomic level possible, and were generally grouped as diatoms, dinoflagellates, ciliates, and autotrophic nanoplankton. A minimum of 200 organisms or 100 grids were be counted per sample (Omori and Ikeda 1984). Whole water samples were preserved with 10% buffered formalin (0.5% v/v final) and analyzed flow cytometrically to assess picoplankton densities (Olson et al. 1991). Abundance of heterotrophic bacteria (stained with SYBR Green I; Jochem 2001), phycoerythrin-containing picocyanobacteria, and photosynthetic picoeukaryotes were quantified using a FACScan (BD®) flow cytometer using fluorescence patterns and particle size from side angle light scatter (Olson et al. 1991).

Primary production and vitamin utilization – A ^{57}Co-labeled vitamin B_{12} from MP-Biomedicals (specific activity 212 μCi μg^{-1}; half-life of 272 days) and a ^{3}H-labeled B_1 (specific activity 10 μCi mmol; halflife of 12.3 years) were used to measure planktonic uptake rates of these vitamins largely following the methods described by Koch et al (2011). While vitamin B_{12} assimilation was measured throughout this study, vitamin B_1 assimilation was measured for the second half of this study only (3/7/08 to 11/4/08). Since preliminary studies with these tracers revealed linear uptake rate by multiple types of coastal marine plankton assemblages over 24 hours, incubations were carried out for one day. Measured uptake rates never depleted more than 15 ± 6.3 % and 7.7 ± 2.2% of vitamin B_1 and B_{12} standing stocks during these 24 h incubations. Trace amounts (0.5 pM B_{12}, 0.04 μCi) of ^{57}Co-cyanocobalamine and ^{3}H-thiamine (2 pM, 0.06 μCi) were added to separate sets of triplicate, 300 mL polycarbonate bottles. To assess abiotic binding of radiolabeled vitamin B_1 and B_{12} to particles and, thus, establish a 'blank', several approaches were explored including incubations of natural plankton communities from LIS for 24 h in the dark at 1ºC, and with the addition of the fixatives mercuric chloride and glutaraldehyde at final concentrations of 1%. All of these approaches exhibited

similarly low levels of abiotic binding and 1% glutaraldehyde was ultimately chosen as a 'killed-control' method with one such bottle being spiked with tracer and incubated along the 'live' bottles during all experiments. The background activity for detection of ^{57}Co was generally between 20 and 40 counts per minute while samples ranged from 80 to 1500 counts per minute. In addition preliminary experiments revealed that both, vitamins B_1 and B_{12} were taken up linearly without displaying a diel pattern and thus 24 hour incubations were possible.

To determine primary productivity rates, 10 μCi of ^{14}C-bicarbonate (MP-Biomedicals©, specific activity 55 mCi mmol^{-1}) was added to triplicate bottles according to Joint Global Ocean Flux Study (JGOFS) protocols (1994). All bottles were incubated in an incubator set to mimic ambient light and temperature conditions. Incubations were be terminated after 24 h by filtering up to 100 mL from both live and dead bottles onto 0.2, and 2 µm pore size polycarbonate filters, allowing for the determination of size fractionated uptake of the tracers. At the beginning and end of the incubation, a small aliquot of each bottle (250 μL for ^{14}C and 1 mL for ^{3}H and ^{57}Co) was removed to quantify total activity. The ^{57}Co, ^{14}C, and ^{3}H containing experimental filters and total activities were analyzed on a LKB Wallac 1282 COPMUGAMMA CS universal gamma counter and scintillation counter (Packart-Acanberra Co. PR1-Carb2100TR), respectively. Uptake rates of vitamins B_1 and B_{12} were calculated by using the equation: $((A_f-A_D/A_{tot}) \times [\text{vitamin}])/ t$ where A_f is the activity on the live filters, A_D is the activity on the dead ('killed control') filters, A_{tot} is the total activity added, [vitamin] is the ambient B_1 or B_{12} concentration and t equals the length of the incubation in days. Similarly, uptake of ^{14}C bicarbonate was determined according to the JGOFS (1994) protocol.

Using cell counts obtained via flow cytometry, size fractionated chl *a* concentrations, estimated carbon contents of heterotrophic bacteria and cyanobacteria and previously published

C: chl *a* ratios, carbon specific vitamin uptake rates were calculated for both size classes and systems. For the >2 µm plankton, a carbon : chl *a* ratio of 60 obtained from estuaries including LIS was used (Lorenzen 1968; Riemann et al. 1989; Boissonneault-Cellineri et al. 2001) while previously published values of average carbon contents of 20 fg $cell^{-1}$ and 200 fg $cell^{-}1$ for heterotrophic bacteria (Fukuda et al. 1998; Ducklow 2000), and cyanobacteria and picoeukaryotes (Kana and Glibert 1987; Liu et al. 1999), respectively were used for the <2 µm size class.

Vitamin amendment experiments – On each sampling date, water from both sites was used to conduct nutrient/vitamin amendment experiments. Acid-washed, polycarbonate bottles (1.1 L) were filled and amended in triplicate with either 20 µM nitrate, 100 pM B_{12}, or both nutrients and incubated for 48 hours in OFP mimicking ambient light and temperature conditions (Gobler et al. 2007). To assess phytoplankton responses, bottles were analyzed for size fractionated Chl. *a* (0.2 and 2 µm polycarbonate filters) and net growth rates were estimated for the different size fractions based on changes in pigment concentrations using the formula: $\mu = \ln B_{final} / B_{initial}) /$ incubation time where B_{final} is the Chl. *a* in bottles at the end of experiments, $B_{initial}$is the chl *a* in bottles at the beginning of the experiments, and the incubation time is days.

Data analysis –Relationships between environmental parameters were evaluated by means of a Spearman rank order correlation matrix. *p*-values <0.05 were deemed to be significantly correlated and the correlation coefficient is reported as *r*. For nutrient amendment experiments, differences in growth rates among treatments for each size class of plankton pigments were statistically evaluated using two-way analyses of variance (ANOVA) with N and B_{12} as the main effects. $p<0.05$ was used to establish significant differences among treatments. To assess possible seasonal trends, the data was also grouped seasonally into spring (March 21 -

June 20), summer (June 21 – September 20), fall (September 21 – December 20) and winter (December 21 – March 20).

Results:

Nutrient and vitamin dynamics – Dissolved inorganic nutrients and vitamin concentrations in OFP were seasonally dynamic (Fig. 2, Tables 1). DIN (nitrate+nitrite+ammonium) concentrations varied inversely with temperature being significantly ($p<0.001$) higher in the winter and spring than in summer and fall. Conversely, vitamin B_{12} concentrations (range: <0.1 pmol L^{-1} to 148 pmol L^{-1}; Table 1) were significantly ($p<0.05$) higher in the summer and fall (48.6 ± 32.7 and 21.7 ± 17.3 pmol L^{-1}; seasonal average ± standard error) than the spring and winter (9.95 ± 5.89 and 9.29 ± 4.36 pmol L^{-1}, respectively). Concentrations of B_1 were, on average, higher than vitamin B_{12} (34.9 ± 21.7 – 99.1 ± 7.46; Table 1) and displayed no clear seasonality (Fig. 2). Vitamin B_{12} were higher in 2008 (55.2 ± 30.7 pmol L^{-1}) than 2007 (7.07 ± 1.61 pmol L^{-1}; Table 1). In LIS, levels of DIN, DIP and vitamins were all significantly lower than OFP (Table 1, $p<0.05$ for all) and generally highest is winter and spring. Vitamin B_{12} concentrations in LIS ranged from <0.1 pmol L^{-1} to 43 pmol L^{-1} and B_1 concentrations ranged from <0.1 pmol L^{-1} to 99 pmol L^{-1} (Table 1). Unlike OFP, seasonal averages of B_{12} in LIS were higher in spring and fall, while levels of B_1 were highest in the spring and low (<0.1 pmol L^{-1}) in the fall (Fig. 2, Table 1).

Plankton community succession - Chl *a* concentrations in OFP varied from 1.12 ± 0.04 to 38.9 ± 0.89 µg L^{-1} (mean = 12.7 ± 2.78; 3A). Size fractionation of Chl *a* revealed a seasonal succession larger photoautotrophs being most dominant in the spring, summer, and fall and all size classes contributes equally to the algal biomass during winter (Fig. 3A). Heterotrophic bacteria and cyanobacterial abundances were high, ranging from 0.6 to 11.6 x 10^6 cells mL^{-1} and

0.11 to 98.2 x 10^3cells mL^{-1}, respectively, and displayed maximal densities during summer and fall months with densities of both populations being significantly correlated to temperature ($p<0.01$; Fig. 3B). The microphytoplankton community in OFP was dominated by autotrophic nanoflagellates and dinoflagellates in the summer and fall with diatoms present during the winter and early spring only (Fig. 3C). In contrast to OFP, annual means of plankton biomass in LIS was significantly lower throughout the year (6.55 ±1.33 vs 12.7 ± 2.78 µg chl *a* L^{-1} for OFP), ranging from <1 µg L^{-1} in winter to 18.7 ± 1.79 µg L^{-1} during the spring bloom in 2007 and, with a few exceptions, was dominated by large (>5µm) diatoms (Fig. 3A, C). Heterotrophic bacteria and cyanobacterial abundances in LIS were substantially lower than OFP with peak abundances correlating strongly with high phytoplankton biomass ($p<0.001$) and temperature ($p=0.001$; Fig. 3B).

Primary production and vitamin utilization - Primary production rates in OFP displayed a strongly seasonal signal with extremely high rates (607 ± 118 mgC m^3d^1) in the summer of both years (Fig. 4A, Table 2). With few exceptions, the majority of this productivity occurred in the >2 µm size fraction (Fig 4A). Vitamin B_{12} uptake followed a similar seasonal trend with uptake rates ranging from <0.1 to 27.4 ± 2.32 pmol L^{-1} d^{-1} and the highest rates observed in summer and fall (mean = 3.07 ± 0.57; Fig 4B, Table 2). In contrast, B_1 uptake rates, which were higher, displayed little seasonality, ranging from 0.32 ± 0.01 to 29.5 ± 2.45 pmol L^{-1} d^{-1} (mean = 14.4 ± 0.79; Figs 4C, Table 2). In stark contrast to primary production, picoplankton (0.2-2 µm) were responsible for the majority of vitamin B_1 and B_{12} utilization in OFP (>65% from spring-fall; Table 2, Fig. 4). While there was no relationship between vitamin uptake and primary production among plankton <2 µm, among larger plankton (>2µm) these rates were significantly

correlated (r=0.88; Fig. 5). Notably, the scale of vitamin uptake for the picoplankton was about ten-fold higher than in the >2 µm size fraction.

Mean primary production rates in LIS (343.4 ± 96.8 mgC m^3d^1) were 2-fold lower than OFP, and peak rates were confined to spring and fall of both years (Table 2, Fig 4A). Like in OFP, larger phytoplankton (> 2 µm) accounted for the majority of primary production in LIS (Fig. 4). Vitamin B_{12} (0.22 ± 0.08 pmol L^{-1} d^{-1}) and B_1 (4.39 ± 1.96 pmol L^{-1} d^{-1}) uptake rates were ten- and four-fold lower in LIS than OFP, respectively (Fig. 4B,C). Like OFP, picoplankton were responsible for the majority of vitamin utilization in LIS (> 55% for all seasons, Table 2, Fig 4B,C). Carbon-specific vitamin uptake rates calculated for both size classes and systems revealed striking differences between picoplankton and the >2 µm size class. Specifically, picoplankton utilized an order of magnitude more B_1 and B_{12} per gram of carbon (Fig. 6). In addition, there were 200% and 550% higher vitamin uptake for B_1 and B_{12} normalized to carbon, respectively in OFP compared to LIS.

Vitamin amendment experiments - Experiments conducted using water from OFP and LIS revealed that nitrogen frequently stimulated phytoplankton biomass (56% of experiments, Table 3) and generally did so for both large and small phytoplankton (> and < 2µm). While B_{12} additions only occasionally enhanced larger algal biomass (> 2µm; 11% of experiments) it more frequently enhanced the growth rate of the 0.2-2 μm size fraction (28% of experiments; Table 3). When added together, nitrogen and B_{12} enhanced total algal biomass more than each individual treatment in 20% of experiments, suggesting a co-limitation of the community by both compounds. The >2 μm size class was more frequently enhanced in this treatment and most of these effects were observed in the summer and fall (Table 3).

Discussion

Although the potential for B-vitamins to influence the structure and productivity of phytoplankton communities has been recognized for decades, this study is the first to investigate vitamin B_1 and B_{12} uptake by the plankton communities in an aquatic ecosystem. Performing this investigation in two contrasting estuaries (mesotrophic v. hypereutrophic) this study specifically documented high vitamin concentrations and uptake rates in eutrophic regions that host elevated primary production and heterotrophic bacterial abundances. Although phytoplankton communities were occasionally stimulated by the addition of vitamin B_{12} alone and in tandem with nitrogen, it was the picoplankton community which was responsible for the majority of vitamin utilization during this study. The sum of the data collected suggests that within this size class, it is the heterotrophic bacteria that were responsible for the majority of the vitamin uptake in both systems and in all seasons.

Vitamin availability – The two systems studied here displayed vastly different chemical and biological characteristics, with the OFP containing higher nutrient (Table 1), Chl *a*, and vitamin concentrations than LIS (Fig. 2, 3 and 4). While seasonally averaged primary production values did not vary significantly between the sites (Table 2), OFP had much larger sustained primary productivity rate throughout the summer and fall as evident by the reduced variability among seasonal averages (Table 2). Only prokaryotes possess the genes necessary (to synthesize vitamin B_{12} (Raux et al. 2000; Bertrand et al. 2011) and the two-fold higher heterotrophic bacterial densities in OFP likely resulted in higher rates of *de novo* vitamin synthesis and concentrations. Gobler et al. (2007) found a strong linear relationship between bacterial abundance and B_{12} concentrations in OFP in summer, a time when the highest vitamin concentrations and utilization were observed. Cyanobacteria populations are also important vitamin producers (Raux et al. 2000; Palenik et al. 2003; Bonnet et al. 2010) and were also

present at higher concentrations in OFP (Fig. 3) compared to LIS. Abundances of both of groups of picoplankton were strongly correlated with temperature ($p<0.01$) a fact which likely contributing to the seasonal peaks in B_{12} observed in OFP during summer and fall (Fig. 2; Table 2) but not in LIS where B_{12} concentrations and primary production were less dynamic (Fig. 2; Table 2). For both systems, primary productivity and B_{12} concentrations were strongly correlated ($p<0.005$) suggesting a tight coupling between photoautotrophs and B_{12} producing prokaryotes. In contrast, B_1 was lowest when the bacterial biomass and primary production were at their peak (r=-0.74, $p<0.02$, respectively). Given that vitamin B_1 requirements of phytoplankton exceed those of vitamin B_{12} (Tang et al 2010), vitamin B_1 uptake rates exceeded those of vitamin B_{12}, and that vitamin B_1 is used in muiltiple metabolic pathways (Croft et al. 2006), the inverse correlation between vitamin B_1 and primary production may reflect a larger net microbial uptake of this vitamin. Finally, while some investigators have found apparent positive correlations of vitamin concentrations with macronutrients (Menzel and Spaeth 1962; Cattell 1973) a relationship with macronutrients was found only in LIS (negative with NO_3, r = -0.64, $p<0.01$), likely explained by NO_3 depletion in the summer, the time with the highest B_{12} concentrations but also the highest B_{12} utilization and demand.

Vitamin utilization – Phytoplankton actively utilized vitamins and total uptake rates of B_{12} and total primary production in both systems were highly correlated (r=0.80, $p<0.001$). Examining this trend among size fractions indicated that while primary production and vitamin utilization in the >2µm size were also positively correlated (r=0.83, $p<0.001$), this was not true for the picoplankton (<2µm) despite ten-fold higher vitamin uptake rates in this size class (Fig. 5). The strong correlation between vitamin uptake and primary production in the >2 µm size class is consistent with the fact that more than half of all phytoplankton species surveyed are B_{12}

auxotrophs (Croft et al. 2005). The absence of such a relationship among picoplankton suggests auxotrophic, heterotrophic bacteria were responsible for the majority of the vitamin use. Peak vitamin uptake rates in OFP exceeded those of LIS by an order of magnitude (Table 2) and OFP microphytoplankton were dominated by dinoflagellates through much of this study, a group with a high incidence of vitamin B_{12} and B_1 auxotrophy (91% and 41%, respectively; (Tang et al. 2010). In LIS, phytoplankton communities were generally dominated by diatoms, another group with widespread vitamin B_{12} auxotrophy (Provasoli and Carlucci 1974; Croft et al. 2005) and diatom abundances in LIS were also positively correlated with vitamin B_{12} utilization of the >2µm size class (r=0.65, p<0.02).

This study reports the first ever vitamin B_1 uptake rates by aquatic plankton communities and reveals important similarities and differences between these rates and those of vitamin B_{12} uptake. Like vitamin concentrations (Fig 2), uptake rates of B_1 were an order of magnitude higher than B_{12} (Table 2). Like vitamin B_{12}, B_1 utilization also occurred primarily in the picoplankton size class (60.0 ± 5.60 and 62.8 ± 5.10% for OFP and LIS respectively; Table 2) suggesting that bacteria may hold a competitive advantage over larger phytoplankton for access of this micronutrient. Vitamin B_1 uptake rates in LIS and OFP were generally higher in the spring, summer, and fall, paralleling patterns for vitamin B_1 concentration (Fig. 2), likely due to temperature dependency of microbial metabolisms. Unlike vitamin B_{12}, vitamin B_1 uptake rates were not correlated with any plankton group suggesting that multiple plankton groups were important for B_1 uptake or that groups not definitively documented by this study were important vitamin B_1 users.

A lack of correlation between primary production and vitamin utilization in the picoplankton (Fig.5), ten-to-hundred fold greater heterotrophic bacteria biomass (104 ± 16.0 and

42.2 ± 7.35 µgC L^{-1} for OFP and LIS, respectively) compared to phototrophic picoplankton (3.34 ± 1.48 and 4.44 ± 1.42 µgC L^{-1} for OFP and LIS, respectively), and a strong correlation between heterotrophic bacterial abundances and B_{12} uptake ($p<0.008$) all suggest that heterotrophic bacteria were the primary users of vitamins during this study. Several recently sequenced marine bacterial genomes possess B_{12} dependent enzymes while lacking genes for B_{12} synthesis (Medigue et al. 2005), suggesting that in addition to being B_{12} synthesizers (Bonnet et al. 2010; Bertrand et al. 2011) marine heterotrophic bacteria might also be important vitamin consumers. Cyanobacteria and picoeukaryote populations in OFP for 2008 were low ($<10^4$ cells mL^{-1}) when vitamin uptake rates were the highest recorded for the study (Figs. 3, 4), again implicating heterotrophic bacterial community as the most important group for vitamin utilization. This study highlights the importance of picoplankton in vitamin consumption and suggests that contrary to previous proposals (Karl 2002) vitamins are primarily assimilated by heterotrophic bacteria, even when larger (>2 µm), eukaryotic cells dominate plankton biomass (>85% of the total POC during spring and summer). As such, previously reported paradoxically low vitamin concentrations in areas of high bacterial activity such as the deeper LIS waters during summer hypoxia (Panzeca et al. 2009) may be due to a large vitamin demand by actively growing heterotrophic bacteria outpacing the vitamin supply or low vitamin production. This observation is contrary to Taylor and Sullivan (2008), who observed that nano- and microplankton were the primary (90%) utilizers of B_{12}. Their study, however, was conducted using meltwater from sea-ice, which harbored large mats of phytoplankton, primarily diatoms, a group rich in vitamin B_{12} auxotrophs, and thus is not representative of typical pelagic marine systems as studied in this dissertation.

Vitamins alter plankton community composition – In both OFP and LIS, vitamin B_{12} amendments stimulated total Chl *a* production in 12% of experiments (Table 3) while eliciting size structure changes in nearly half of experiments (47%). In OFP, B_{12} limited phytoplankton solely in the winter and spring (Table 3), times when vitamin concentrations were lowest (Table 1). In contrast to OFP, phytoplankton in LIS were most limited by vitamins in the summer and fall. The high degree of correlation ($r=0.83$, $p<0.005$) between primary production and B_{12} concentrations and uptake for both sites as well as elevated heterotrophic bacteria abundances suggest that it is the supply that drives the degree of vitamin limitation of the phytoplankton. Increases in heterotrophic bacteria, fueled by the high primary production likely leads to increased vitamin production and the alleviation of vitamin limitation. This conclusion is consistent with Bertrand et al (2007, 2011) who found vitamins limited phytoplankton communities in coastal regions of the Ross Sea only when bacterial densities were low. As has been previously observed (Gobler et al. 2007; Koch et al. 2011), the addition of B_{12} in conjunction with nitrogen led to increased growth rates over B_{12} or nitrogen alone, a result that may be elicited by an increase in B_{12} demand due in the presence of extra nitrogen. This occurred most frequently in the >2 μm size fraction. While uptake rates of vitamin B_{12} were tightly coupled to primary production in this larger size class (Fig. 5), the rate of vitamin utilization for that size class was only 10% of the total vitamin consumption suggesting that the uptake of the low concentrations of vitamins is dominated by the picoplankton (Fig. 4), but that both groups are occasionally limited by the availability of vitamins.

A revised notion of B-vitamin cycling - Previously Karl (2002) hypothesized that in marine ecosystems, vitamins are produced by bacteria and utilized by larger phytoplankton and while the importance of B-vitamins in phytoplankton physiology has been well-established, the

ecological relevance of these micronutrients had been dismissed as minimal (Droop 1961; Droop 1968; Droop 1970; Droop 2007). While prior culture studies suggested that vitamins are present at high enough concentrations to satisfy algal demand, these studies relied on bioassays to estimate the levels of vitamins in the world's oceans. Furthermore phytoplankton are often stimulated experimentally by the addition of B-vitamins (Panzeca et al. 2006; Sanudo-Wilhelmy et al. 2006; Bertrand et al. 2007; Gobler et al. 2007; Koch et al. 2011). This paradigm may be best explained by the revelation that, in addition to being the source of B-vitamins (Medigue et al. 2005; Bonnet et al. 2010; Bertrand et al. 2011), prokaryotes are also the main sink for these micronutrients in marine systems (Table 2, Fig 4; Koch et al 2011) and vitamin uptake by these microbes may, on occasion, deprive eukaryotic phytoplankton from a sufficient vitamin supply. The higher vitamin demand by the plankton communities in OFP point to a microbial community adapted to higher vitamin concentrations in this more eutrophic systems with higher ambient vitamin concentrations. The higher carbon normalized B_{12} vitamin demand in OFP may be caused by the denser heterotrophic bacterial populations with a higher percentage of vitamin auxotrophs. Similarly, the dominance of dinoflagellates (Fig. 3), a group comprised almost exclusively of auxotrophs (Tang et al. 2010), among phytoplankton in OFP likely accounted for the large >2 µm B_{12} demand per unit carbon there (Fig 5).

Recent work exploring natural ecosystems (Bertrand et al. 2011), cyanobacterial cultures (Bonnet et al. 2010), and prokaryotic genomes (Raux et al. 2000; Palenik et al. 2003) have highlighted the important role of prokaryotes in ocean vitamin production. While it is unknown whether specific groups of vitamin producers are also responsible for vitamin utilization, this would seem counterintuitive since vitamin biosynthesis is complicated and utilizes numerous genes and enzymatic steps (Raux et al. 2000; Warren et al. 2002). Due to the demanding nature

of this process, B-vitamin auxotrophic bacteria may harbor an energetic advantage over vitamin-producing bacteria and seem to hold a kinetic advantage in assimilating picomolar levels of vitamins over larger, vitamin auxotrophic phytoplankton likely due to their larger surface to volume ratio (Raven and Kubler 2002). Regardless, we hypothesize that vitamin cycling (uptake and production) occurs primarily within the prokaryotic picoplankton community, a factor which may limit the growth of some eukaryotic phytoplankton cells.

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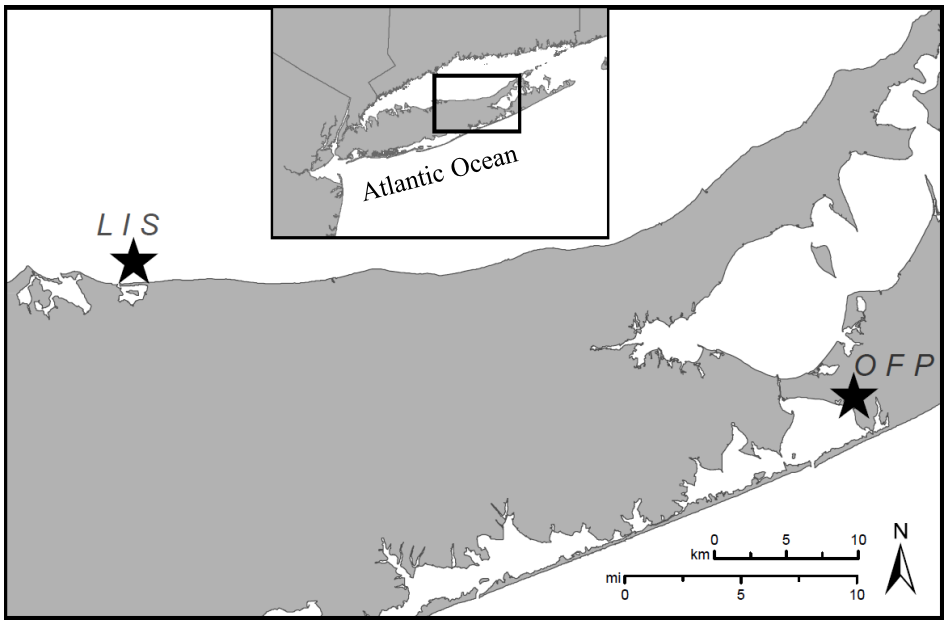

Figure 1. A map of Long Island, NY showing the two study sites (stars). OFP denotes Old Fort Pond, a hypereutrophic systems which exchanges tidally with the Atlantic Ocean through Shinnecock Bay. The second study site, LIS denotes Long Island Sound, was sampled at the incoming tide from a dock at the Mount Sinai Harbor entrance and represents a mesoeutrophic system.

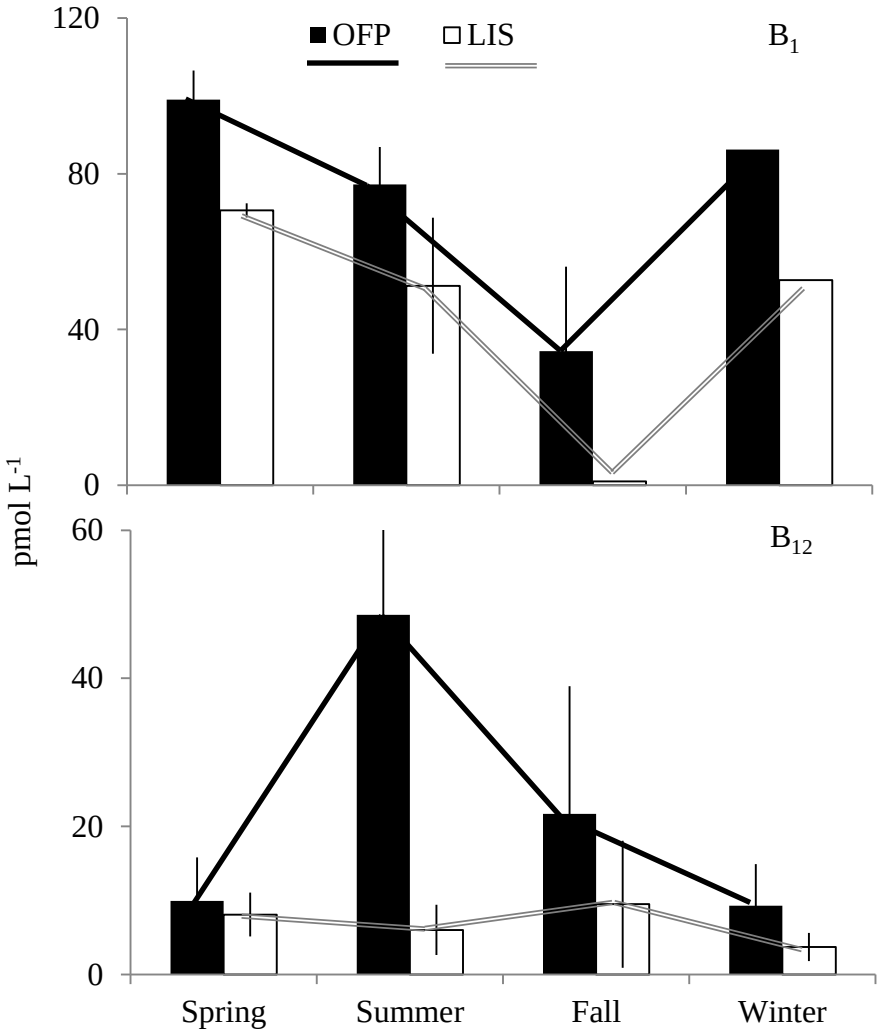

Figure 2. Seasonally averaged concentrations of B_1 (top) and B_{12} (bottom) in Old Fort Pond and Long Island Sound, NY. The solid and hollow lines accentuate the seasonal trend of vitamins in Old Fort Pond and Long Island Sound, respectively. Concentrations are shown in pmol L^{-1}.

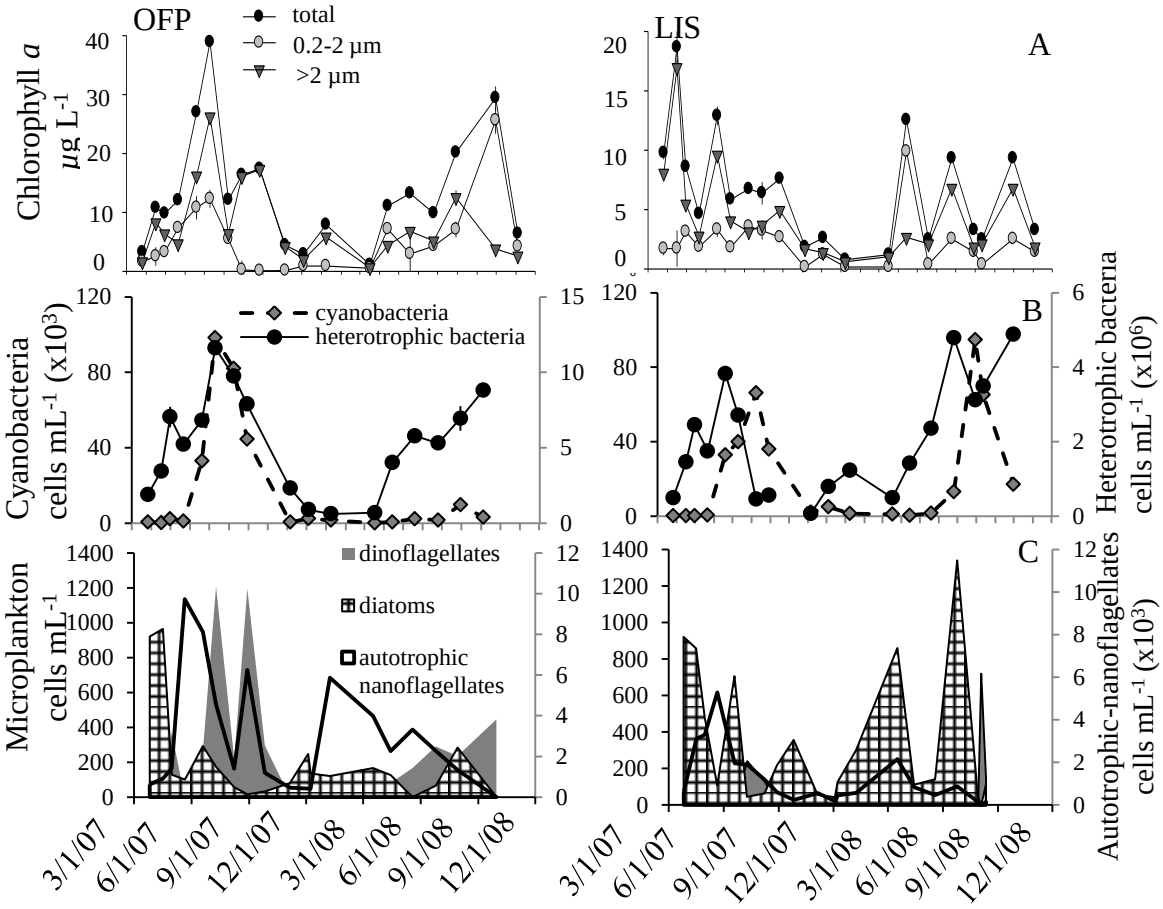

Figure 3. Planktonic community composition from 2007-2008 in Old Fort Pond (left) and Long Island Sound (right), NY. Size fractionated chlorophyll a (A) was obtained using varying pore size polycarbonate filters, cyano- and heterotrophic bacteria concentrations were measured via flow cytometry (B) and the nano- and microplankton community was determined via light microscopy (C).

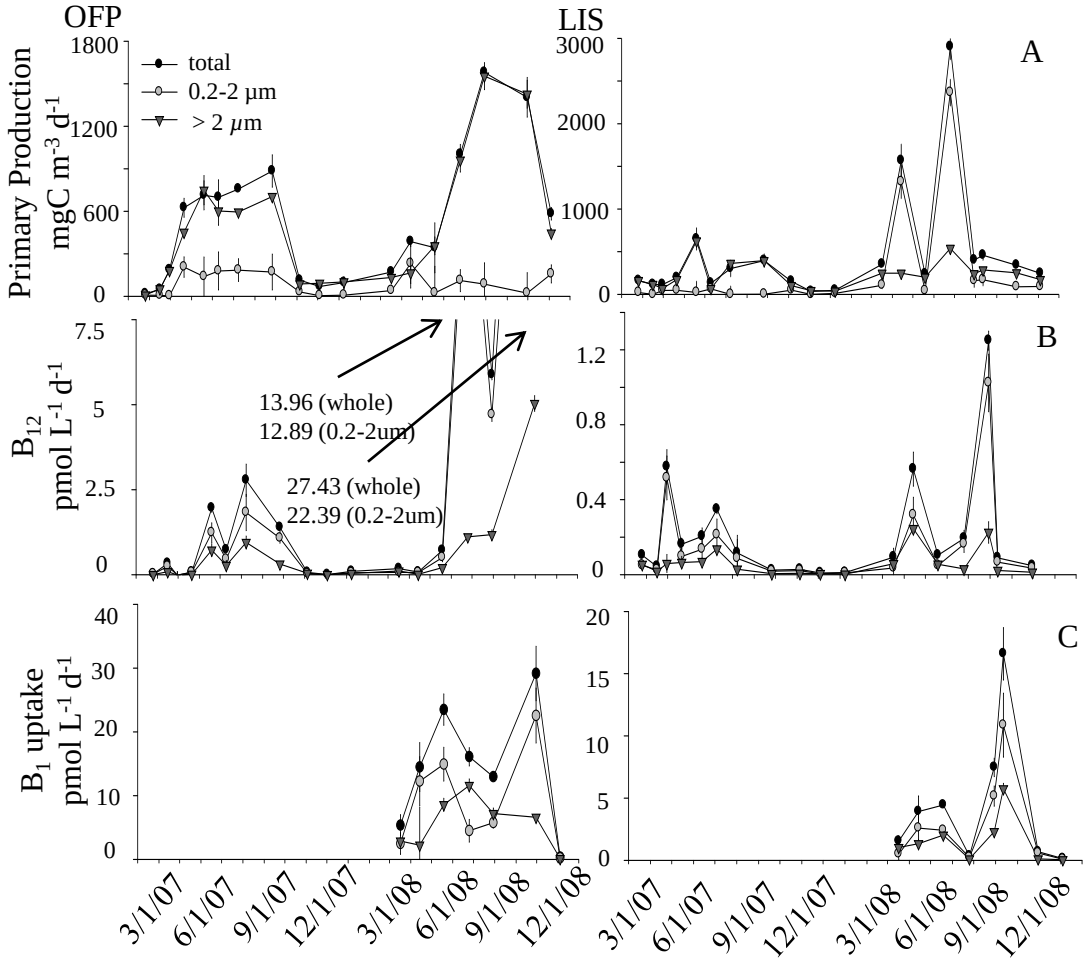

Figure 4. Time series of primary productivity (A), vitamin B_{12} (B) and B_1 (C) uptake dynamics in Old Fort Pond (left) and Long Island Sound (right), NY. Size fractionated primary production and uptake measurements were obtained via the use of polycarbonate filters and are reported in mgC $m^{-3}d^{-1}$ and pmol L^{-1} d^{-1}, respectively.

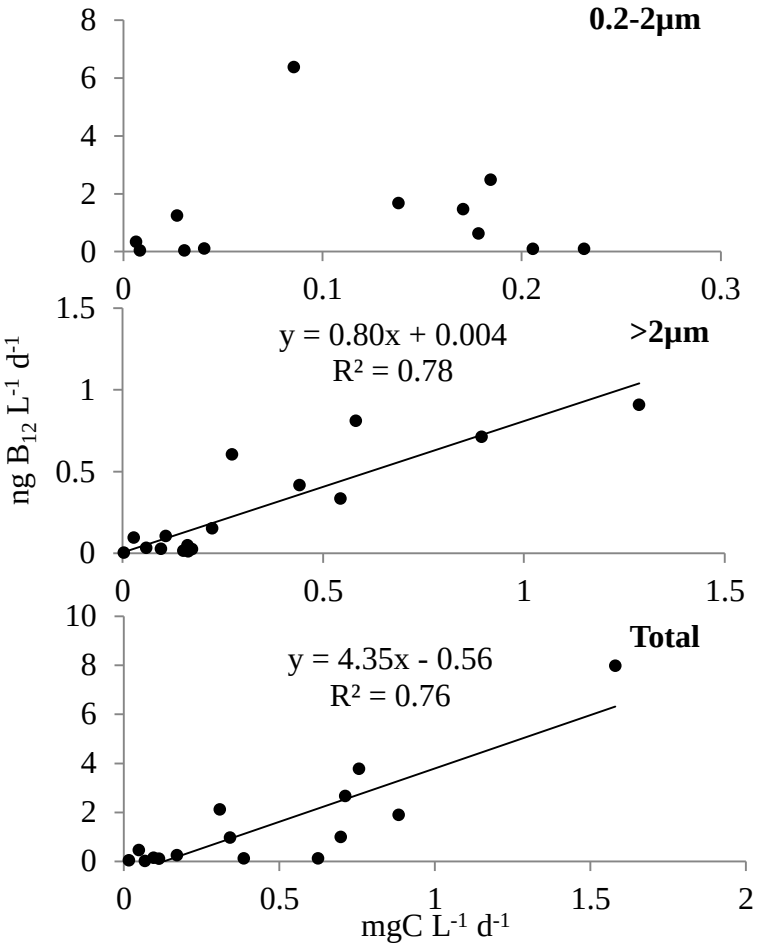

Figure 5. Relationship between B_{12} and carbon utilization in Old Fort Pond. Uptake of B_{12} and bicarbonate are reported as ng L^{-1} d^{-1} and mg L^{-1} d^{-1} respectively. Regressions show that in the larger size class (>2 µm), uptake of B_{12} was driven by the photosynthetic organisms in contrast to the <2 µm size class where B_{12} uptake was not correlated to primary production, with heterotrophic bacteria most likely responsible for the majority of vitamin utilization.

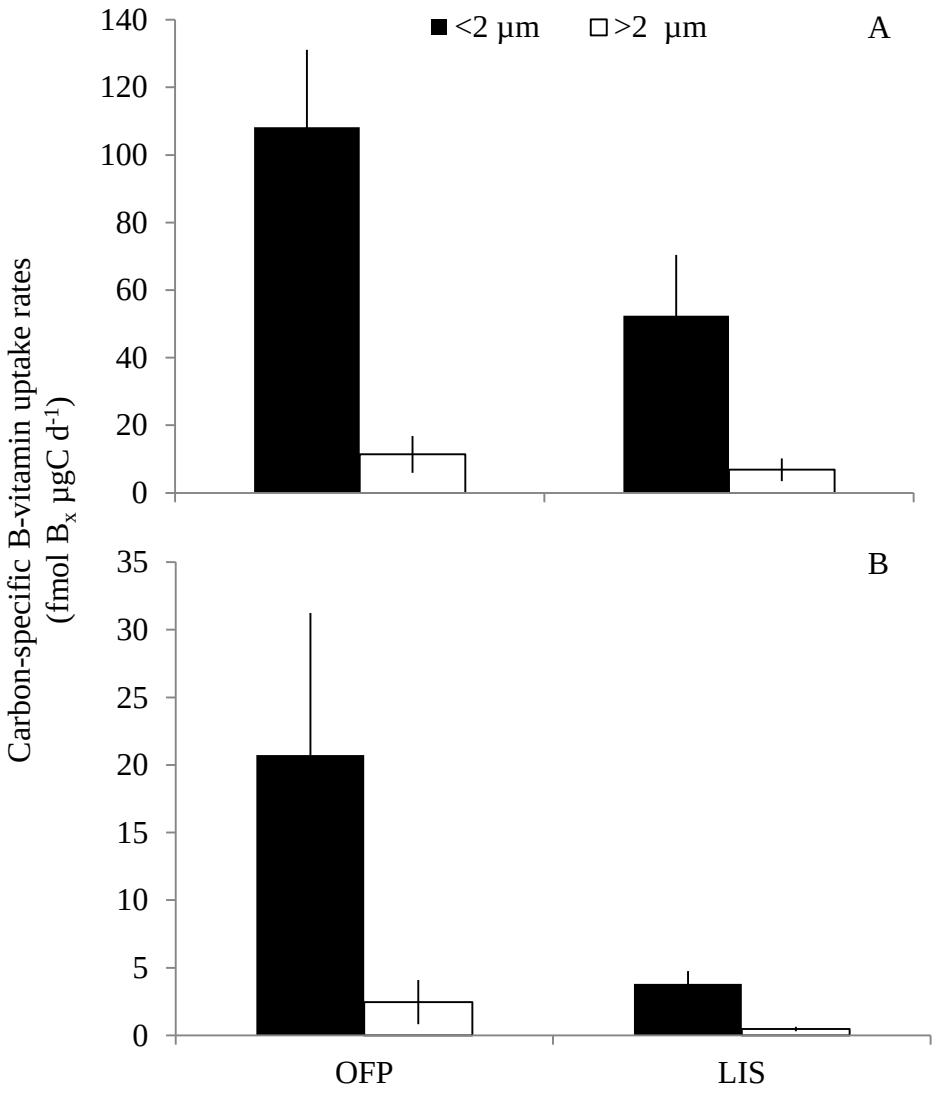

Figure 6. Average B_1 (A) and B_{12} (B) uptake rates normalized to particulate organic carbon (see methods) for each size class and location over the course of the study. Values are shown as fmol of Bx μgC^{-1} d^{-1} where Bx stands for the corresponding B-vitamin.

Table 1. Physical and chemical characteristics of Old Fort Pond and Long Island Sound over the course of the study. Concentrations of dissolved inorganic nitrogen (DIN; nitrate+nitrite+ammonium), ortho-phosphate (PO_4^-) and silicate ($Si(OH)_4$) are shown as μmol L^{-1} where as B-vitamins are depicted in pmol L^{-1}. Note that B_1 concentrations were not measured until the second part of the study (3/17/08-11/4/08).

	Temperature	Salinity	DIN	PO_4^-	$Si(OH)_4$	B_{12}	B_1
OFP	° C		µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	pmol L^{-1}	pmol L^{-1}
3/26/2007	7.99	29.56	11.24 ± 1.86	0.83 ± 0.07	8.43 ± 1.54	0.30	ND
4/16/2007	9.10	24.36	18.92 ± 1.39	0.23 ± 0.11	12.11 ± 0.04	6.82	ND
4/30/2007	12.54	24.61	21.28 ± 1.64	0.27 ± 0.05	17.18 ± 2.11	7.87	ND
5/21/2007	14.79	28.73	2.15 ± 0.22	0.18 ± 0.03	12.95 ± 0.15	2.69	ND
6/19/2007	21.14	20.45	11.98 ± 2.00	0.14 ± 0.04	43.72 ± 0.13	9.04	ND
7/10/2007	24.60	24.30	0.00 ± 0.27	0.15 ± 0.04	54. 79 ± 0.38	3.70	ND
8/8/2007	25.50	26.90	3.49 ± 0.53	0.45 ± 0.04	41.83 ± 0.44	15.49	ND
8/29/2007	ND	ND	1.67 ± 0.32	1.30 ± 0.28	39.14 ± 3.30	17.01	ND
9/26/2007	21.80	28.10	3.50 ± 0.15	0.20 ± 0.01	21.24 ± 6.35	12.98	ND
11/5/2007	11.20	28.49	7.11 ± 2.61	0.34 ± 0.08	10.20 ± 1.33	4.28	ND
12/4/2007	3.90	27.00	3.73 ± 0.19	0.22 ± 0.00	6.02 ± 0.26	0.96	ND
1/8/2008	3.40	25.40	36.21 ± 6.19	0.14 ± 0.01	46.26 ± 4.64	3.66	ND
3/17/2008	5.60	28.20	0.78 ± 0.39	0.09 ± 0.03	3.86 ± 0.46	14.93	86.27
4/14/2008	10.40	27.50	0.78 ± 0.38	0.07 ± 0.00	7.40 ± 0.20	3.15	111.97
5/19/2008	16.20	26.50	29.29 ± 2.45	0.43 ± 0.10	10.93 ± 2.78	38.85	86.14
6/25/2008	23.10	27.80	7.18 ± 0.68	0.89 ± 0.13	30.70 ± 4.97	157.96	78.96
7/30/2008	27.00	25.60	0.78 ± 0.35	0.67 ± 0.09	10.65 ± 0.21	25.09	52.06
9/4/2008	ND	ND	0.35 ± 0.23	0.41 ± 0.07	10.50 ± 0.23	ND	114.61
9/30/2008	20.20	27.20	3.35 ± 0.64	0.42 ± 0.02	21.95 ± 1.12	90.13	68.74
11/4/2008	10.70	28.40	8.91 ± 0.50	0.31 ± 0.02	18.86 ± 0.32	<0.10	0.10
LIS							
3/26/2007	3.92	25.95	13.71 ± 0.91	0.28 ± 0.10	3.09 ± 0.63	5.66	ND
4/16/2007	6.17	25.90	13.44 ± 0.86	0.33 ± 0.13	5.41 ± 1.03	0.30	ND
4/30/2007	9.78	24.00	2.80 ± 0.23	0.22 ± 0.04	8.96 ± 3.24	6.02	ND
5/21/2007	11.60	24.98	2.15 ± 0.21	0.49 ± 0.12	13.22 ± 0.60	10.72	ND
6/19/2007	18.15	25.30	2.07 ± 0.55	0.62 ± 0.04	18.04 ± 2.00	2.76	ND
7/10/2007	20.50	26.80	0.78 ± 0.23	0.36 ± 0.06	22.13 ± 0.27	4.03	ND
8/8/2007	23.10	27.10	3.08 ± 0.26	0.94 ± 0.03	35.87 ± 0.40	4.33	ND
8/29/2007	ND	ND	6.35 ± 0.61	2.09 ± 0.31	66.88 ± 3.03	1.94	ND
9/26/2007	21.80	28.10	1.68 ± 0.42	1.29 ± 0.15	26.30 ± 0.64	0.30	ND
11/5/2007	15.10	28.32	12.07 ± 0.90	1.79 ± 0.13	43.32 ± 1.96	2.49	ND
12/4/2007	7.60	28.20	14.06 ± 1.65	1.72 ± 0.09	42.36 ± 5.31	0.89	ND
1/8/2008	4.80	28.20	14.48 ± 3.47	1.85 ± 0.16	50.72 ± 0.96	1.83	ND
3/17/2008	3.70	26.30	1.92 ± 0.16	0.92 ± 0.02	2.40 ± 0.07	5.64	52.66
4/14/2008	6.90	25.70	0.69 ± 0.09	0.55 ± 0.11	2.74 ± 0.15	21.25	73.72
5/19/2008	12.10	24.70	4.05 ± 0.33	0.44 ± 0.01	4.67 ± 0.25	4.71	67.47
6/25/2008	20.00	25.90	1.13 ± 0.53	0.16 ± 0.03	5.31 ± 0.17	0.64	<0.10
7/30/2008	22.10	26.80	2.26 ± 0.38	0.88 ± 0.04	34.50 ± 0.27	27.51	54.83
8/12/2008	21.80	26.70	3.21 ± 0.15	1.01 ± 0.09	35.86 ± 1.18	0.99	98.77
9/30/2008	19.70	25.70	15.78 ± 1.00	1.61 ± 0.04	33.45 ± 12.47	<0.10	<0.10
11/4/2008	11.90	26.73	9.71 ± 0.94	1.67 ± 0.07	38.93 ± 0.52	43.72	<0.10

Table 2. Seasonal averages of vitamin B_{12} and B_1 concentrations, vitamin B_{12} and B_1 uptake and the percent of uptake occurring in the picoplankton (0.2-2 µm) size fraction for both vitamins. OFP stands for Old Fort Pond while LIS denotes Long Island Sound. Values shown are seasonal means ± standard error.

	Prim. Prod.	%Prim. Prod	B_{12} uptake	B_{12} uptake	B_1 uptake	B_1 uptake
OFP	mgC m^{-3} d^{-1}	in 0.2-2µm	pmol L^{-1} d^{-1}	in 0.2-2µm	pmol L^{-1} d^{-1}	in 0.2-2µm
Spring	267 ± 94	25.1 ± 10.9	0.20 ± 0.11	75.1 ± 4.5	18.9 ± 2.6	74.1 ± 10.3
Summer	923 ± 156	13.3 ± 3.6	4.48 ± 1.76	70.4 ± 4.5	18.7 ± 1.8	51.8 ± 9.3
Fall	574 ± 221	23.5 ± 5.3	7.22 ± 5.61	66.7 ± 10.1	14.7 ± 8.3	71.8 ± 6.6
Winter	134 ± 37	7.6 ± 16.1	0.39 ± 0.04	49.2 ± 11.2	5.3 ± *	46.4 ± *
TOTAL	559 ± 118	20.3 ± 4.4	3.18 ± 1.89	68.9 ± 3.4	17.8 ± 3.2	60.0 ± 5.6
LIS						
Spring	398 ± 234	29.6 ±12.3	0.26 ± 0.10	60.4 ± 6.4	4.2 ± 0.2	60.1 ± 8.2
Summer	806 ± 425	31.6 ± 14.6	0.37 ± 0.16	73.8 ± 3.2	8.1 ± 2.9	67.6 ± 1.8
Fall	233 ± 65	13.5 ± 12.5	0.03 ± 0.01	67.2 ± 7.1	0.4 ± 0.2	74.3 ± 14.1
Winter	200 ± 152	25.5 ± 4.3	0.33 ± 0.04	58.3 ± 20.5	1.5 ± *	32.9 ± *
TOTAL	327 ± 93	31.5 ± 6.5	0.22 ± 0.08	65.9 ± 4.1	4.4 ± 1.7	62.8 ± 5.1

Table 3. Responses to nitrogen and vitamin B_{12} amendments by different size classes of phytoplankton collected from OFP and LIS. Responses for each size fraction were measured via changes in chlorophyll *a* over time and are shown for each sample location. In addition overall effects were examined by looking at all the experiments conducted for each size class (ALL). An effect of the NO_3+B_{12} treatments was deemed significant when $p<0.05$ compared to the NO_3 and B_{12} only additions. Values represent the number of times a treatment resulted in a positive growth response in that size class.

		Total	0.2-2µm	>2 µm
OFP	B_{12}	2	4	5
(n=18)	N	9	5	8
	N+B_{12}	2	4	4
LIS	B_{12}	2	6	4
(n=18)	N	11	11	11
	N+B_{12}	4	2	6
ALL	B_{12}	4	10	9
(n=36)	N	20	16	18
	N+B_{12}	6	6	9

Chapter Four

The effect of vitamins B_1 and B_{12} on the bloom dynamics of the harmful brown tide alga, *Aureococcus anophagefferens* (Pelagophyceae)

Abstract

B-vitamins can significantly influence plankton community structure and/or enhance algal biomass in coastal marine systems and the large majority of harmful algae isolated from coastal zones require vitamins B_1 and B_{12} for growth. I investigated vitamin use by *Aureococcus anophagefferens*, a harmful alga that dominates plankton communities during dense brown tides and is auxotrophic for vitamins B_1 and B_{12}. In culture studies, B_{12}-depleted cultures of *A. anophagefferens* (clone CCMP1984) adapted to lower B_{12} concentrations by reducing half saturation constants (K_s) of B_{12} uptake and increasing maximum uptake rates (V_{max}) compared to vitamin-replete cultures. In contrast, V_{max} of vitamin B_1 was higher in replete compared to the depleted cultures while the K_s values were similar for both. K_s values for B_{12} depleted and replete cultures (5.0-21 pM) were similar to or higher than concentrations measured during brown tides, suggesting B_{12} may restrict the growth of this alga in the field. Over the course of a brown tide in Quantuck Bay, NY, USA, vitamin B_1 and B_{12} concentrations declined from >100 pM to <8 pM, suggesting rapid uptake by *A. anophagefferens* and its associated microbial community. Experiments performed using radioisotope labeled vitamins B_1 and B_{12} and ^{14}C-bicarbonate indicated that plankton in the size range of *A. anophagefferens* (1 – 5µm) were responsible for the majority of primary production and the majority of vitamin B_1 uptake, but a smaller fraction of B_{12} uptake, which was dominated by plankton <1µm. Vitamin uptake rates during brown tide were capable of turning over standing stocks of vitamin B_{12} in 15 h while B_1 depletion was slower with turnover times of 2.8 d. As the brown tide intensified and vitamin B_{12} levels declined, the experimental enrichment of vitamin B_{12} significantly enhanced the growth rates of *A. anophagefferens*. Collectively, this study demonstrates that vitamin B_{12} may control the dynamics of harmful algal blooms caused by *A. anophagefferens*.

Introduction

Over 75% of the US population lives within 75 km of a coastline, a trend which makes these areas susceptible to an array of anthropogenic influences including intense nutrient loading (De Jonge et al. 2002; Valiela 2006) and harmful algal blooms (HABs) (Heisler et al. 2008). In recent decades HABs have caused widespread economic and ecological damage to coastal ecosystems worldwide (Cloern 2001; Hoagland et al. 2002; Sunda et al. 2006). In New York (NY), USA, coastal waters are subject to annual HABs caused by *Aureococcus anophagefferens*, a picoplanktonic pelagophyte (2-3 μm diameter) also known as the brown tide. The first occurrence of brown tide was documented in NY and RI in 1985 and resulted in a mass die-off of eelgrass (*Zostera marina;* Cosper et al. 1989) a critical habitat for juvenile finfish as well as shellfish and other benthic fauna including bay scallop populations (*Argopecten irradians*) (Bricelj et al. 1989). Since 1985, brown tides have recurred almost annually in the different bays on eastern and southern Long Island and has expanded southward to New Jersey, Delaware, Maryland and Virginia and across the globe to South Africa (Gobler et al. 2005) and China (Quingchun et al. submitted). *A. anophagefferens* seems to gain competitive advantage over other phytoplankton by resisting grazing by zooplankton (Gobler et al. 2002; Caron et al. 2004; Deonarine et al. 2006) and bivalves (Bricelj et al. 1989; Bricelj et al. 2001) and exhibiting nutritional flexibility, being able to utilize a variety of nitrogenous compounds for growth (Taylor et al. 2006; Gobler et al. 2011).

While the role of macronutrients in HAB ecology has been extensively studied in recent decades (Smayda 1997; Sunda et al. 2006; Heisler et al. 2008) little attention has been paid to organic micronutrients such as vitamins. This is despite the fact that earlier studies have shown that many phytoplankton have an absolute requirement for exogenous vitamins such as B_{12}

(Droop 1955; Guillard 1968; Swift and Guillard 1977) and that vitamins are present at low, picomolar levels in seawater (Vishniac and Riley 1961; Menzel and Spaeth 1962; Swift 1972). Provasoli and Carlucci (1974) and Swift (1980) indicated that many microalgal species are unable to synthesize vitamin B_{12} (cobalamin), vitamin B_1 (thiamine), and vitamin B_7 (biotin) *de novo* and thus are considered "auxotrophic", meaning they must obtain these vitamins from an external source. Each of these vitamins can play an important role in algal biochemistry. For example, cobalamin (B_{12}) is required for the biosynthesis of methionine, thiamine (B_1) facilitates the decarboxylation of pyruvic acid in the Calvin cycle, and biotin (B_7) plays a critical role in fatty acid synthesis. In the past, B-vitamin have been implicated as a causative factor in some HABs such as occurrences of *Lingulodinium polyedrum* (formerly known as *Gonyaulax polyedra*) blooms off the coast of California (Carlucci 1970) and bloom of *Karenia brevis* (formerly known as *Gymnodinium breve*) in the Gulf of Mexico (Aldrich 1962; Hunter and Provasoli 1964; Stewart et al. 1967). There is a high incident of auxotrophy for B-vitamins among harmful algae (Tang et al. 2010) a finding consistent with the ability of many HABs to utilize organic compounds for nutrition (Taylor 1987; Anderson et al. 2008; Burkholder et al. 2008).

Vitamins may play a key role in the dynamics of HABs caused by *A. anophagefferens.* Vitamins have been found to limit the accumulation of phytoplankton biomass in estuaries where blooms of *A. anophagefferens* frequently occur (e.g. Quantuck Bay, NY; Sanudo-Wilhelmy et al. 2006). Furthermore, recent laboratory experiments (Tang et al. 2010) indicated that *A. anophagefferens* is highly auxotrophic with respect to both, vitamins B_1 (thiamine) and B_{12} (cyanocobalamin), a finding confirmed by the presence of the B_{12}-dependent methionine synthesis gene (met*H*), but not the B_{12}-independent homolog (met*E*) in its recently sequenced

genome (Gobler et al. 2011). No field study has investigated the role of vitamins in HAB events in more than 30 years, including brown tides caused by *A. anophagefferens*.

To assess the role of B-vitamins in the occurrences of brown tides caused by *A. anophagefferens*, I conducted laboratory experiments to quantify the vitamin half saturation constants (K_s), maximum uptake rates (V_{max}) and cellular quotas of *A. anophagefferens* via vitamin B_1 and B_{12} uptake kinetic studies, the first for any HAB species. I quantified B-vitamins dynamics in the field during the development, peak, and demise of high biomass brown tide caused by *A. anophagefferens.* The use of radiotracers allowed for the first measurements of vitamin B_1 and B_{12} uptake during a HAB. Finally, I performed vitamin enrichment experiments to explore the extent to which vitamins may limit or co-limit the growth of *A. anophagefferens* during brown tide blooms.

Methods

Determination of vitamin uptake kinetics constants

Laboratory culture experiments were conducted to assess the manner in which vitamin concentrations impact the uptake rates, cellular vitamin quotas, carbon fixation, and growth rates of *A. anophagefferens* as well as to determine the half saturation constants of uptake (K_s) and maximum uptake rates (V_{max}) for vitamin-replete (B+) and –depleted (B-) cultures. *A. anophagefferens*, strain CCMP1984 was isolated in 1986 from Great South Bay, NY, USA, on Long Island's south shore, and was made axenic in 2003 (Berg et al. 2003). Its vitamin auxotrophy has been previously confirmed in silico and via genomic sequencing (Tang et al 2010; Gobler et al 2011). Cultures were grown in GSe medium (Doblin et al. 1999) made with artificial salts but without the addition of B-vitamins. For all cultures, an antibiotic-antimycotic

solution (final concentrations = 100 I.U. penicillin, 100 μg ml^{-1} streptomycin, and 0.25 μg ml^{-1} amphotericin B; Mediatech. Inc., Hemdon, Virginia) was added into the medium immediately before inoculation to ensure cultures remained free of bacteria and fungi. Cultures were grown in sterilized, 1-L Pyrex™ Erlenmeyer flasks and maintained at 21°C on a 14:10 light:dark cycle in an incubator under ~100 μmol quanta m^{-2} sec^{-1}. Cultures were grown under vitamin replete and depleted conditions (both B_1 and B_{12}) with vitamin concentrations in deplete cultures diluted via successive transfers (Tang et al. 2010). *A. anophagefferens* cultures were deemed limited by a vitamin when growth rates were significantly reduced compared to replete cultures and the addition of the limiting vitamin elicited an elevated growth response.

To assess uptake rates, triplicate 50 mL Pyrex™ culture tubes at eight vitamin concentrations (0, 21, 106, 228, 424, 847, 1271 and 2965 nmol L^{-1} and 0, 7, 15, 23, 31, 82, 184 and 368 pmol L^{-1} for B_1 and B_{12}, respectively) were inoculated with vitamin replete and deplete cultures to a final cell concentration of ~1 x 10^6 cells mL^{-1}, a density typical of *A. anophagefferens* blooms (Gobler et al. 2005; Gobler et al. 2011) and grown for 7-14 days until cells reached stationary phase. Changes in cell densities were determined using a Beckman Coulter Multisizer™ 3 Coulter Counter® with a 50µm aperture allowing for distinct peaks in cell densities to be resolved (Harke et al. 2011) and evaluated every 2 days. Growth rates, µ (d^{-1}), were calculated as ln (N_t/N_0) / t, where N_0 and N_t are at the end and start of exponential phase growth and t is the duration of the exponential growth phase. ^{57}Co-labeled vitamin B_{12} from MP-Biomedicals (specific activity 212 μCi μg^{-1}), ^{3}H-thiamine labeled B_1 (MP-Biomedicals, specific activity 10 μCi mmol) and ^{14}C-bicarbonate (MP-Biomedicals, specific activity 55 mCi mmol^{-1}) were used to quantify uptake kinetics. During exponential growth, five mL of each triplicate tube was transferred to 10 mL glass culture tubes and spiked with

either ^{14}C-bicarbonate (0.25 µCi), ^{3}H-B_1 (0.06μCi) or ^{57}Co-B_{12} (0.04 μCi). The tubes were incubated at 100 μmol quanta m^{-2} sec^{-1} on a 14:10 light:dark cycle and 21°C. Experiments were terminated after 2 hs by filtering each tube onto 1 μm filters, rinsing filters with 0.2 µm filtered seawater, and analyzing them for tracer incorporation. At the beginning and end of the incubation, a small aliquot of each vial (250 μL) was removed to determine total activity (the amount of isotope added). The ^{57}Co and the ^{14}C and ^{3}H containing experimental filters were analyzed on a LKB Wallac 1282 COPMUGAMMA CS universal gamma counter and a scintillation counter (Packart-Acanberra Co. PR1-Carb2100TR) along with the total activities. Uptake (ρ) of vitamins B_1 and B_{12} were calculated by using the equation: $((A_f / A_{tot}) \times [vitamin])/ t$ where A_f is the activity on the life filters, A_{tot} is the total activity added, [vitamin] is the ambient B_1 or B_{12} concentration and t equals the length of the incubation in hours. Uptake of vitamins and primary production rates were normalized to $cell^{-1}$. Cell quotas (i.e. vitamin content per cell) of each B-vitamin and culture were determined via the equation: $V_{quota} = V_{vitamin} / V_{primary\ production} \times$ carbon content where $V_{vitamin}$ is the cell specific vitamin uptake rate in pmol $cell^{-1}$ h^{-1}, $V_{primary\ production}$ is the cell specific carbon fixation rate as pg $cell^{-1}$ h^{-1}. The carbon content per cell of each culture prior to the uptake experiments was determined on a Carlo Erba NA 1500 NCS system according to Cutter and Radford-Knoery (1991) as pg carbon $cell^{-1}$. Vitamin uptake kinetics constants were calculated as described below in the *Data analysis* section.

Field Samples

Water was collected from Quantuck Bay (QB; Fig. 1) which is part of a series of interconnected lagoons bordered to the north by the south shore of Long Island and by barrier islands to the south. A combination of a low mean water depth, ocean winds, and low freshwater

input makes these bays vertically well-mixed (Wong and Wilson 1984). QB has experienced near annual blooms of *A. anophagefferens* since 1985 with cell densities often exceeding 10^6 cells mL^{-1} (Mulholland et al 2002, Deonarine et al 2006, Gobler et al 2011). For this study, samples were obtained from May through August 2009, before, during and after an intense brown tide (>10^6 cells mL^{-1}). Physical parameters (temperature, salinity and dissolved oxygen) were measured using a YSI© 556 sondes and water was collected in acid washed 20 L carboys and brought back to the lab within < 1 h for processing. Size fractionated chlorophyll *a* (Chl *a*) samples were collected by filtering triplicate samples onto 0.2, 2 and 5 μm polycarbonate filters. These filters were stored frozen until subsequent analysis via standard fluorometeric methods (Welschmeyer 1994). Whole seawater was preserved in 5% Lugols iodine solution for subsequent enumeration of plankton under an inverted microscope. Plankton were identified to the highest taxonomic level possible and were generally grouped as diatoms, dinoflagellates, ciliates, and autotrophic nano-flagellates. A minimum of 200 organisms or 100 grids were counted per sample (Omori and Ikeda 1984). Whole water samples were preserved with 10% buffered formalin, flash-frozen in liquid nitrogen and analyzed flow cytometrically to assess picoplankton densities (Olson et al. 1991). Abundance of heterotrophic bacteria (stained with SYBR™ Green I; Jochem 2001), phycoerythrin-containing picocyanobacteria, and photosynthetic picoeukaryotes were determined using a Fluorescence Activated Cell Scan (FACScan; Becton, Dickinson and Company) flow cytometer using fluorescence patterns and particle size from side angle light scatter (Olson et al. 1991). To enumerate *A. anophagefferens*, whole water was preserved with filter sterilized 10% gluteraldehyde solution (1% final v/v), stored in glass tubes at 4°C, and later analyzed using an enzyme-linked immunosorbent assay (ELISA) with a flow cytometrically detected fluorescent, monoclonal antibody (Stauffer et al.

2008). Nutrient samples were pre-filtered with acid-cleaned, polypropylene capsule filters (0.2 μm; GE Osmonics, DCP0200006) and stored frozen. Nitrate, nitrite, ammonium, and phosphate were analyzed in duplicate by standard spectrophotometric methods (Jones 1984; Parsons et al. 1984). Total dissolved N and P (TDN, TDP) were analyzed in duplicate by persulfate oxidation techniques (Valderrama 1981). DON and DOP concentrations were calculated by subtracting levels of nitrate, nitrite and ammonium or orthophosphate from concentrations of TDN and TDP, respectively. Full recoveries (mean ± 1 S.D.) were obtained of samples spiked with SPEX Certi-Prep^{INC} standard reference material at environmentally representative concentrations of TDN, TDP, nitrate, nitrite and ammonium, and orthophosphate. Vitamin samples were collected and analyzed according to Okbamichael and Sanudo-Wilhelmy (2004; 2005). Briefly, samples were collected by filtering 1 L of water through a 0.2 μm Millipore cartridge filter into 4 L amber high density polyethylene bottles (Okbamichael and Sanudo-Wilhelmy 2004). Samples were acidified with 10% HCl to a pH between 6.1 and 6.7 and vitamins were then captured by slowly pumping (1 mL min^{-1}) samples through High Capacity Bondesil C18 (120 μm) beads (BioRad). The columns containing the bound vitamins were then stored in the freezer until subsequent methanol elution and reverse phase HPLC analysis (Okbamichael and Sanudo-Wilhelmy 2004). The detection limit of these analyses were 0.1 pM.

Vitamin utilization and primary production

In a manner similar to the methods described above, ^{57}Co-labeled vitamin B_{12}, ^{3}H-B_1 and ^{14}C-bicarbonate from MP-Biomedicals were used to measure planktonic uptake rates of these compound. Assuming low ambient B_1 and B_{12} concentrations of 15-50 and <5 pM (Gobler et al. 2007) respectively, a trace amount of either ^{3}H-B_1 (2 pM, 0.06μCi) or ^{57}Co-B_{12} (0.5 pM, 0.04 μCi) was added to triplicate, 300 ml polycarbonate bottles. To assess non-specific

binding and/or adsorption of the isotope a 1% gluteraldehyde 'killed-control' bottle was also spiked with tracer and incubated along the 'live' bottles. To determine primary productivity rates, 10 μCi of ^{14}C-bicarbonate was added to triplicate bottles (MP-Biomedicals, specific activity 55 mCi mmol^{-1}) according to JGOFS protocols (1994). Bottles for all isotopes were incubated at temperatures matching ambient conditions found in Quantuck Bay in an incubator with 10 - 100 μmol quanta m^{-2} sec^{-1}, adjusted to ambient light conditions. The length of the light/dark cycle was set to match the natural duration of daylight at the day of sampling.

Incubations were terminated by filtering samples onto 0.2, 1 and 5 μm pore size polycarbonate filters, allowing for the determination of size fractionated uptake of the tracers. *A. anophagefferens* is 2 - 3 μm in size and dominate the eukaryotes during blooms (>95% of biomass; Gobler et al. 2011, this study) thus the 1μm filter in conjunction with the 5 μm filter was used to reflect tracer utilization by the 1 - 5 μm size class and specifically *A. anophagefferens*. Uptake rates for each of the three compounds were determined as described above for culture experiments. Vitamin turnover times were determined by dividing the standing stock of vitamins by vitamin uptake rates. On 22 June 2010 (*A. anophagefferens* densities = 8.35 x 10^5 cells mL^{-1}), bloom water was amended with several levels of vitamin B_{12} (0, 7, 15, 23, 31, 82, 184 and 368 pmol L^{-1}) and size fractionated (0.2-1, 1-5 and >5 µm) rates of primary production and vitamin B_{12} uptake were measured and uptake kinetics constants were calculated as described below in the *Data analysis* section.

Vitamin amendment experiments

On 8 June, 6 July, 13 July, and 27 July, water collected from QB was used to fill 1.1 L, acid washed polycarbonate bottles. Bottles were then amended, in triplicate, with either 10 μmol

L^{-1} ammonium, 100 pmol L^{-1} B_{12} and a combination of ammonium and B_{12} and a combination of ammonium, B_{12} and 100 pmol L^{-1} B_1. Bottles were incubated at a depth of 0.5 m in Old Fort Pond (Stony Brook Southampton Marine Science Center), a water body 15 km east of Quantuck Bay that, like Quantuck Bay, exchanges with Shinnecock Bay, ensuring near ambient light and temperature conditions during experiments (Fig. 1; Gobler et al. 2007). At the end of experiments (24 - 48 h), bottles were processed and analyzed for size fractionated Chl. *a* (0.2, 1, and 5 µm polycarbonate filters) and *A. anophagefferens* densities as described above.

Data analysis

For the culture work, differences in uptake and growth rates between vitamin concentrations in replete and depleted cultures were evaluated using an analyses of variance (ANOVA) where vitamin concentration and culture status (replete or depleted) were the main treatment effects examined and differences among levels were examined using Tukey's multiple comparison tests. A p-value of 0.05 was used to establish significant differences amongst vitamin concentrations. Since the growth and uptake rate response of vitamin auxotrophic phytoplankton cultures as well as natural phytoplankton populations to varying vitamin concentrations follows Michaelis–Menten saturation kinetics (Droop 1968; Tang et al. 2010), half saturation constants (K_s, pM), the concentration at which the uptake rate is $1/2V_{max,}$ and maximum uptake velocities (V_{max}, $pmol_{vitamin}$ $cell^{-1}$ h^{-1}) were derived by fitting the uptake data to Michaelis–Menten equation using the software Kaleidagraph (Synergy software, Maxwell Resources, Inc.) and by using Lineweaver-Burk transformation (Atkins and Nimmo 1975; Greco and Hakala 1979). Since the results of the two methods were nearly identical (± <5% in most cases), only the results from the Michaelis–Menten curve fitting are presented. Affinity constants (α) were calculated as V_{max}/Ks and represented the slope at low Ks and given h^{-1}. The

steeper the slope (higher α), the higher the affinity of the organism to the nutrient in question (Kudela et al. 2008). All of the plots used to calculate kinetics constants were based on curve fits that were statistically significant ($p<0.05$) and included at least 12 data points. The degree to which individual environmental parameters were correlated with each other during the brown tide was evaluated by means of a Spearman rank order correlation matrix. For nutrient amendment experiments, differences in biomass levels among treatments for each size class of plankton and *A. anophagefferens* were statistically evaluated using analyses of variance (ANOVA) as described above.

Results

Culture experiments

Aureococcus anophagefferens (CCMP 1984) exhibited Michaelis-Menten type uptake kinetics for vitamins B_1 and B_{12} (Figs 1, 2). Compared to vitamin replete cultures (B_{12}+), cultures grown under B_{12}-depleted (B_{12}-) conditions exhibited 40% higher maximal uptake rates (V_{max}: 2.65×10^{-9} and 1.95×10^{-9} pmol cell^{-1} h^{-1} for B_{12}- and B_{12}+ respectively) and lower half saturation constants (K_s; 5.16 and 20.8 pM for B_{12}- and B_{12}+ respectively; Fig. 2A, Table 1). Cellular quotas for both B_{12}+ and B_{12}- cultures were not statistically different, ranging from 2.64 -32.4 and 3.58 - 36.1 pmol B_{12} mgC^{-1}, respectively ($p>0.05$). In contrast, vitamin B_1 replete (B_1+) cultures had twice the V_{max} and 20% higher K_s compared to B_1- cultures (Table 1). *A. anophagefferens* had a higher demand for vitamin B_1 displaying K_s and V_{max} values (2.63×10^{3} pM and 3.09×10^{-6} pmol cell^{-1} h^{-1} respectively; (Fig. 3B, Table 1) ~one thousand-fold higher than those for B_{12}. Growth rates of B_{12}- cultures significantly increased from 0.25 ± 0.01 to 0.55 ± 0.03 d^{-1} as B_{12} levels increased from 0.01 to 368 pM (Fig. 2B, Table 1). A similar trend was

observed for B_1 with growth rates increasing from 0.16 ± 0.0.03 to 0.54 ± 0.05 d^{-1} as vitamin concentrations increased from 0.1 to 2965 nM (Fig 3B, Table 1). Growth rates of vitamin replete cultures were not affected by vitamin concentrations (Fig 2).

Nutrient – vitamin – plankton dynamics during a brown tide

Quantuck Bay (QB) experienced a brown tide bloom in 2009 that initiated ~ 26 May when cell densities were 5.81 x 10^4 mL^{-1}. Cell densities gradually increased through June to a peak of 1.22 x 10^6 cells mL^{-1} on 6 July and 1.13 x 10^6 cells mL^{-1} on 13 July (Fig 4C) after which cell densities steadily declined to undetectable levels on 3 August (Fig. 4C). Chl. *a* concentrations generally paralleled *A. anophagefferens* densities, increasing from 7.90 ± 0.63 to 43.2 ± 2.50 μgL^{-1} from May to early July with the majority of Chl *a* (79 ± 13%) occurring in the *A. anophagefferens* size class, 1-5 µm (Fig. 4A). Dinoflagellate densities were highly correlated with *A. anophagefferens* abundances ($r=0.71$, $p<0.003$), peaking in unison (Fig. 4E) and accounting for the majority of the microplankton in QB (Fig. 4B). Heterotrophic bacterial densities, already high at the initiation of the bloom (> 10^7 cells mL^{-1}), increased through the study period and peaked after the brown tide on 17 August (2.8 x 10^7 cells mL^{-1}). With the decline of the brown tide, *Synechococcus sp.* densities increased from < 10^4 cells mL^{-1} to 4 x 10^5 cells mL^{-1} during August (Fig. 4D).

Vitamin concentrations in QB declined precipitously over the course of the brown tide bloom (26 May to 27 July) from 169 ± 18.4 to 7.32 ± 0.87 pM for B_1 and from 121 ± 13.2 to 3.48 ± 0.14 pM for B_{12} (Fig. 4D; Table 2). With one exception (27 May), B_1 concentrations were slightly higher than those of B_{12}. Dissolved inorganic nitrogen (DIN) concentrations were high at the beginning of the bloom (6.58 ± 0.86 µM) and declined below detectable

concentrations during the peak while dissolved organic nitrogen (DON) concentrations were highest immediately before (8 June) and after (20 July) the peak of the bloom (Table 2). Silicate and phosphate concentration were low at the onset of the bloom and increased over the course of the bloom with the former peaking on 6 July (90.8 ± 11.7 µM) while the later peaked on 17 August (0.75 ± 0.03 µM (Table 2).

Vitamin utilization and primary production

Primary production rates in QB during the *A. anophagefferens* bloom were high, peaking at 3,368 ± 86.3 mgC m^{-3} d^{-1} on 1 July with >97% of productivity occurring in the 1-5 µm size class (Fig 5A). There was a significant correlation ($r=0.81$, $p<0.002$) between primary production occurring in the 1-5 µm size class and *A. anophagefferens* cell densities during the study (Fig. 5A). B-vitamins were rapidly assimilated during the bloom. Vitamin B_1 utilization rates were 12.3 ± 2.28 pmol L^{-1} d^{-1} at the beginning of the bloom on 27 May (Fig 5B) with the highest B_1 uptake rates occurring near the peak of the bloom (28.6 ± 0.15 pmol L^{-1} d^{-1} on 7 July). After the bloom, vitamin B_1 uptake rates declined by an order of magnitude after the bloom ended (3.52 ± 0.05 pmol L^{-1} d^{-1} on 27 July; Fig. 5B). Utilization of B_{12} ranged from 0.20 ± 0.05 to 18.3 ± 0.57 pmol L^{-1} d^{-1} with the highest uptake rates coinciding with the peak in *A. anophagefferens* abundances (13 July; Fig. 5C)

During the initiation of the bloom, the majority of B_1 utilization occurred in the 0.2-1 µm size class. However, as densities of *A. anophagefferens* increased, the majority of B_1 uptake shifted to the 1-5 µm size class (65.6 ± 8.41% and 94.7 + 0.05 % of total on 1 July and 13 July, respectively; Fig. 5B). As the brown tide bloom subsided, B_1 uptake rates were again equally distributed between the 0.2-1 and 1-5 µm size classes (Fig 5B). In contrast, during much of the study period, B_{12} utilization was dominated by the 0.2-1 µm size class during the bloom (52% of

total; Fig 5C) even when *A. anophagefferens* densities peaked (97 % of primary production occurred in the brown tide size class (1-5 µm; Fig. 5) and *A. anophagefferens* was >99% of cells >2 µm). After the demise of the bloom, B_{12} uptake was evenly distributed between the three size classes (1.38 ± 0.86, 1.56 ± 0.34, 1.36 ± 0.22 pmol L^{-1} d^{-1} for the 0.2-1, 1-5 and >5 µm size classes respectively; Fig 5C).

During the 22 June vitamin B_{12} gradient experiment, uptake rates were relatively low (0.08 pmol L^{-1} h^{-1} for the total community) and the 0.2-1 and 1-5 µm size classes were responsible for the majority of vitamin utilization (51 ± 4.4 and 40 ± 6.9%, respectively; Fig. 6). Across the gradient of B_{12} concentrations, uptake rates by the total plankton community displayed Michaelis-Menten type uptake kinetics (Fig. 6). Values of K_s and V_{max} for the total community were 47.08 ± 11.4 pM and 0.59 ± 0.02 pmol $L^{-1}h^{-1}$, respectively. The 0.2-1 and 1-5 µm size fraction had a K_s and V_{max} that were statistically similar (36 ±7.6 pM, 0.29 ± 0.02 $L^{-1}h^{-1}$ for 0.2-1 µm and 28 ± 13 pM, 0.23 ± 0.01 $L^{-1}h^{-1}$ for 1-5 µm) while the >5 µm size class had a significantly ($p<0.01$) higher K_s and lower V_{max} as determined via Kaleidagraph (726.80 ± 219 pM and 0.17 ± 0.03 pmol $L^{-1}h^{-1}$, respectively; Table 3).

Vitamin Amendment Experiments

Nutrient amendment experiments demonstrated that the addition of vitamins with and without nitrogen were capable of significantly enhancing the abundance of *A. anophagefferens* and other phytoplankton. While ammonium, ammonium+B_{12}, and ammonium+B_{12}+B_1 treatments enhanced all measured components of the plankton community (chl *a*) at the initiation of the bloom (8 June, $p<001$; Fig. 7), *A. anophagefferens* densities were unaffected. As the bloom peaked (6 July; Fig. 4), the addition of ammonium+B_{12}, and ammonium+B_{12}+B_1 elicited

a statistical ($p<0.01$) increase in all the size classes of chl *a* and also significantly ($p<0.03$) increased *A. anophagefferens* abundances (Fig. 7). On 13 July, the addition of B_{12}, ammonium+B_{12}, and ammonium+B_{12}+B_1, each significantly increased the total and 2-5 µm chl *a* concentrations as well as densities of *A. anophagefferens* ($p<0.01$; Fig. 7). During the demise of the bloom, chl *a* concentrations were unaffected by any treatments while *A. anophagefferens* densities were significantly increased by the addition of B_{12} ($p<0.01$; Fig. 7).

Discussion

Factors promoting brown tides caused by *A. anophagefferens* include the complex interactive effects of grazer inhibition (Lonsdale et al. 1996; Caron et al. 2004; Deonarine et al. 2006) and nutritional flexibility (Taylor et al. 2006; Gobler et al. 2011). The recently discovered vitamin-auxotrophy of this pelagophyte (Tang et al. 2010; Gobler et al. 2011) is consistent with the ability of brown tides to rely on organic compounds for nutrition (Gobler et al. 2004). By utilizing culture experiments, field measurements, and field experiments this study has revealed the key role vitamins play in the occurrence of brown tides caused by *A. anophagefferens*.

Vitamin uptake and growth kinetics

A recent survey of 41 strains of harmful algae found a high incidence of vitamin auxotrophy among species including the brown tide-forming *A. anophagefferens* (Tang et al. 2010). In addition, the recently sequenced brown tide genome (Gobler et al. 2011) indicated that *A. anophagefferens* possesses the genes for B_{12}-dependent methionine synthesis but does not contain the full suite of genes involved in synthesizing B_{12} nor the genes for B_{12}-independent methionine synthesis (Warren et al. 2002), making it an obligate vitamin auxotroph. My study found that vitamin limited cultures of *A. anophagefferens* responded to increasing vitamin

concentrations with increased growth rates. Vitamin utilization rates displayed Michaelis-Menten type uptake kinetics for both depleted and replete cultures, an observation consistent with the findings of Droop (1968, 1970) for *Pavlova Lutherii* (formerly *Monochrisis lutheri*). The maximum vitamin B_{12} uptake rates displayed by *A. anophagefferens* cultures (2.65×10^{-9} pmol $h^{-1}cell^{-1}$) applied to a typical brown tide of 1.25×10^{6} cells mL^{-1} could consume ~79 pmol L^{-1} d^{-1}, suggesting *A. anophagefferens* could have more than accounted for the observed drawndown in vitamin B_{12} over the course of the 2009 brown tide. In addition, since more than half of B_{12} stocks were consumed by the 0.2 – 1 µm size class, the vitamin demand of the total community would likely be much higher. Accordingly, vitamin B_{12} turnover times during blooms were less than a day.

Vitamin B_{12} depleted cultures had a lower half saturation constant and a higher V_{max} than replete cultures suggesting *A. anophagefferens* responded to lower vitamin concentrations by increasing uptake rates. The initial slope (α) of Michaelis-Menten kinetic curves is generally considered to be a robust indicator of efficiency of uptake at low (<Ks) ambient nutrient concentrations since, unlike K_s, it is not dependent on V_{max} (Healey 1980) and has previously been used to assess nutrient affinity in HABs (Kudela et al. 2008). The five-fold higher α for B_{12} limited cultures of *A. anophagefferens* supports the hypothesis that this species adapts to low vitamin concentrations by increasing their affinity and uptake rates at low concentrations, a fact not observed for B_1. This may be accomplished by upregulating recently discovered cobalamin acquisition proteins (CBA1) that diatoms use to acquire B_{12} under limiting conditions and that have been identified in the *A. anophagefferens* genome (E. Bertrand, M. Saito, Woods Hole Oceanographic Institute, pers. comm.). While the kinetic constants changed for B_{12}-starved cultures, the cellular quotas were similar to those reported for the diatoms *Skeletonema costatum*

and *Phaeodactylum tricornutum* (0.07-16.3 and 0.39-1.28 pmol B_{12} mgC^{-1}, respectively; Carlucci and Bowes (1972)). This suggests that in auxotrophic phytoplankton, cellular B_{12} requirements are similar and that competitive advantages emanate from the ability to enhance access to ambient pools, perhaps via cobalamin acquisition proteins, rather than changing their internal demand which may be inflexible due to the critical nature of methionine synthesis. Increased V_{max} in nutrient limited cultures has been previously observed (Parslow et al. 1984) and suggests an increase in transporter sites or the decrease in internal pools (Harrison et al. 1989). The latter, however, was not the case for B_{12} and *A. anophagefferens* since the internal pools for replete and deplete cultures were similar. It has been proposed that, unlike B_1 which is implicated in multiple biosynthetic pathways in algae (Croft et al. 2006), B_{12} is required by plankton primarily for methionine synthesis a notion recently supported by molecular evidence (Helliwell et al. 2011). In addition to the upregulation of cobalamin acquisition proteins and the ability of *A. anophagefferens* to adapt to lower vitamin B_{12} concentrations could also be due the smaller size of cells in deplete cultures (pers. obs.). This would increase surface to volume ratios of cells, and perhaps make them more competitive at lower vitamin concentrations (Raven and Kubler 2002), a notion supported by observations that the vitamin B_{12} K_s and α of the 0.2-1 and 1-5 µm size class of plankton communities during a brown tide bloom were similar.

A. anophagefferens cultures required three orders of magnitude more B_1 than B_{12}. Since B_1 and B_{12} concentrations in the field are similar (Gobler et al 2007; this study), it would seem that B_1 is more likely to be a limiting vitamin than B_{12}. This, however, was not observed in the field where B_1 turnover times were four-fold longer than those observed for B_{12} (67 v 15 hours, respectively) and the addition of vitamin B_1 never enhanced the growth of any population we investigated. These observations suggest that, contrary to laboratory findings, ambient B_1

concentrations were in excess relative to B_{12}. While this was unexpected, there are several facts that may account for this finding. Unlike B_{12}, vitamin B_1 is used in many different metabolic pathways (Provasoli and Carlucci 1974; Swift 1980; Croft et al. 2006) and alternatives compounds found in the field but not present in the GSe growth medium may be substituted by *A. anophagefferens* field populations. In addition the biosynthesis of vitamins is complex and includes many precursor macromolecules (Warren et al. 2002; Begley et al. 2008). Croft et al. (2006) suggested that organisms that possess genes encoding for some of the precursor molecules for vitamins but not the entire molecule may also be able to utilize them and complete synthesis *de novo*. It has also been demonstrated that vitamin auxotrophy can vary even between strains of the same phytoplankton species isolated from the same field population on the same date (Tang et al. 2010). As such, it is possible that unlike *A. anophagefferens* CCMP1984, some strains in the field do not require vitamin B_1 or have a lower cellular requirement than what has been observed in the lab.

Plankton - vitamin dynamics

Over the course of the 2009 *A. anophagefferens* bloom, B-vitamin concentrations declined precipitously and abundance of other plankton groups changed. At the initiation of the 2009 brown tide, the plankton community was dominated by autotrophic nanoflagellates that decreased in abundance by an order of magnitude as the bloom of *A. anophagefferens* intensified. Because brown tides draw down vitamin concentrations to low levels when concentrations in other coastal systems may be at their annual peak (Chapter 3; Panzeca et al. 2009), *A. anophagefferens* may competitively exclude some members of summer algal communities. There was a positive correlation between the abundance of *A. anophagefferens* and dinoflagellates during the bloom and a negative correlation between dinoflagellates and

DON ($r=-0.68$, $p<0.001$). Dinoflagellates are an algal group with an incidence of vitamin B_{12} and B_1 auxotrophy (91% and 41%, respectively) that is substantially larger than most other groups of algae (Tang et al. 2010). Like *A. anophagefferens*, dinoflagellates are mixotrophic (Taylor 1987; Smayda 1997; Gobler et al. 2004) perhaps partly accounting for the co-occurrence of these algae when concentrations of vitamins and DON were high. The substantial requirements for both vitamins and DON by both dinoflagellates and *A. anophagefferens* (Heisler et al 2008; Tang et al. 2010) likely contributed to the order of magnitude reduction in vitamin concentrations and reduction in DON observed over the course of the brown tide (Fig. 4, Table 2). Interesting, the dinoflagellate population that bloomed in tandem with the *A. anophagefferens* population through May and June collapsed in July when the brown tide peaked and vitamin B_{12} levels were reduced to < 10 pM. This succession suggests that *A. anophagefferens* was better adapted to thrive under conditions present at that time (low vitamins, low light) and/or that some dinoflagellates had been consuming *A. anophagefferens* cells and that the brown tide had been released from grazing pressure (Sieraki et al 2004). Finally, elevated levels of vitamins and DON during the brown tide may have given *A. anophagefferens* a competitive edge over strictly autotrophic species that were limited by the N supply early in the bloom when *A. anophagefferens* were nutrient replete (Fig. 7).

During the decline of the brown tide, *Synechococcus* sp. densities in QB increased sharply, an observation consistent with prior studies (Gobler et al. 2004; Sieracki et al. 2004). Vitamins may have influenced this planktonic succession as the dominance of *Synechococcus* sp. occurred when vitamins were reduced to levels limiting for *A. anophagefferens*. Several strains of *Synechococcus* investigated to date possess the genes necessary to synthesize vitamin B_{12} *de novo* (Raux et al. 2000; Palenik et al. 2003; Bonnet et al. 2010), allowing this genus to bloom

when auxotrophic plankton are limited by vitamins. Many bacteria synthesize vitamins (Rocap et al. 2003; Vitreschak et al. 2003; Bertrand et al. 2011) and the bacterial assemblages in QB were significantly higher than what has been reported from other eutrophic Long Island embayments during summer (Gobler and Sañudo-Wilhelmy 2001, Gobler et al. 2007, Chapter 3), suggesting vitamin production could have been high. Despite these dense bacterial assemblages, vitamin concentrations actually decreased to low levels during the brown tide, even as bacterial densities increased indicating that vitamin B_1 and B_{12} utilization exceeded production rates likely due to the elevated algal biomass and a shift in the algal community to one dominated (>95%) by a vitamin auxotroph, *A. anophagefferens*. In addition, bacterial community composition may have shifted towards species that do not synthesize vitamins at high rates during the bloom.

Vitamin utilization by the brown tide community

A previously report of vitamin utilization in marine systems has found that the majority (~70%) of vitamin B_1 and B_{12} uptake is performed by picoplankton, a fact that may contribute to the limitation of some larger phytoplankton by vitamins (Koch et al. 2011, Chapter 3). The presence of *A. anophagefferens* changed this pattern. The *A. anophagefferens* size class (1 – 5 µm) was responsible for half of vitamin B_{12} utilization during the brown tide, a much larger fraction of what has been observed previously for the nanoplankton (<30%, Koch et al. 2011, Chapter 3) . For B_1, the majority of uptake occurred in the 0.2-1 µm size fraction before the brown tide, but diminished to near zero in this size class during the peak of the bloom as the *A. anophagefferens* size class of plankton (1 – 5 µm) was responsible for 97% of B_1 utilization (6 July). As the brown tide subsided the system reverted to pre-bloom conditions with lower B_1 uptake rates, once more primarily in the smallest size class (0.2-1 µm). These trends highlight a

potential competitive strength of *A. anophagefferens* with respect to micronutrients, specifically vitamins. During blooms, the K_s of the >5µm size class was larger than the 1 – 5 µm and < 1µm size classes, while the affinity (α) was >30 times lower, indicating smaller vitamin auxotrophs were better able to access the low (pM) vitamin concentrations than larger phytoplankton, potentially limiting larger eukaryotes, a common finding in coastal ecosystems (Sanudo-Wilhelmy et al 2006, Gobler et al 2007, Koch et al 2011). *A. anophagefferens* may compete effectively with heterotrophic bacteria and other small picoeukaryotes in part due to its similarly large surface to volume ratio (Raven and Kubler 2002) a hypothesis supported by the similar K_s and α of the 0.2-1 and 1-5 µm plankton fractions during brown tides.

Vitamin B_{12} can limit the growth of A. anophagefferens

While the phytoplankton community as a whole was limited by nitrogen during the initiation of the brown tide, *A. anophagefferens* was nitrogen-replete, perhaps due to preferential use of the large DON pool (Gobler et al. 2002). During the peak of the bloom, *A. anophagefferens* was co-limited by N and vitamin B_{12}, perhaps due in reductions in the DON and vitamin B_{12} pools to 13.5 ± 7.60 µM and 12.1 ± 0.65 pM, respectively. As the bloom began to decline, vitamin B_{12} concentrations were further reduced to 3.5 pM, the minimum for the year and lower than nearly all other observations in coastal ecosystems (Panzeca et al. 2009, Koch et al. 2011, Chapter 3). Moreover, the culture generated V_{max} for B_{12} (2.65×10^{-9} pmol $h^{-1}cell^{-1}$) suggests that the potential B_{12} uptake at the peak of the *A. anophagefferens* bloom (densities of 1.25×10^6 cells mL^{-1}) was ~79 pmol L^{-1} d^{-1}, a value well above the observed concentration (3.48 ± 0.14 pM) but consistent with the 15 hr turnover time of this pool at that time. Maximum B_{12} uptake rates for the brown tide community during the 2009 Quantuck Bay bloom were only ~18 pmol L^{-1} d^{-1}, however. Furthermore, this uptake was divided between the small picoplankton (<1

μm) and brown tide size fraction (1-5 μm) indicating that *A. anophagefferens* was utilizing B_{12} well below (nine-fold) its maximum uptake rates and supporting the observation that *A. anophagefferens* was limited by the availability of B_{12}. Furthermore, the K_s for B_{12} from both culture experiments (5.16-20.8 pM) and the natural bloom water kinetics experiment (28.1 pM for the 1-5 μm size class) were all higher than the low levels present after the bloom peak suggesting B_{12} concentrations could have been limiting this alga (Dugdale 1967; Caperon and Meyer 1972). Consistent with all of these observations, *A. anophagefferens* densities were enhanced solely by the addition of B_{12} suggesting a lack of this vitamin may have contributed toward the demise of the bloom. Vitamin B_{12} utilization was shared evenly between *A. anophagefferens* and the 0.2-1 μm size class during blooms indicating that bacteria were effectively competing with *A. anophagefferens* for this micronutrient. This competition was also apparent within the whole community kinetics experiment that produced affinity constants of 8.06 and 8.21 x 10^{-3} hr^{-1}, for the 0.2-1 and 1-5 μm size fractions, respectively, suggesting that each plankton group had similar access to B_{12} (Fig. 6, Table 3). This uptake data, coupled with vitamin amendment experiments that showed *A. anophagefferens* growth was enhanced by B_{12} additions but not B_1, suggests that *A. anophagefferens* effectively outcompetes other plankton for B_1 but must compete for B_{12} with smaller, heterotrophic bacteria.

Conclusions

Recent studies have revealed that vitamins can limit phytoplankton growth (Panzeca et al. 2006; Bertrand et al. 2007) and alter the community composition (Sanudo-Wilhelmy et al. 2006; Koch et al. 2011). Laboratory (Tang et al. 2010) and genomic studies (Gobler et al. 2011) have documented the vitamin B_1 and B_{12} auxotrophy of *A. anophagefferens*, suggesting B-vitamin may be a key nutrient for brown tides. This study has demonstrated that *A. anophagefferens*

utilizes large amounts of vitamins during blooms and can also be limited by the availability of vitamin B_{12}. While much research on brown tides and HABs has focused on the role of organic and inorganic sources of nitrogen, future ecological and modeling studies of HABs need to consider the role of the previously overlooked factors, vitamins, in bloom occurrence.

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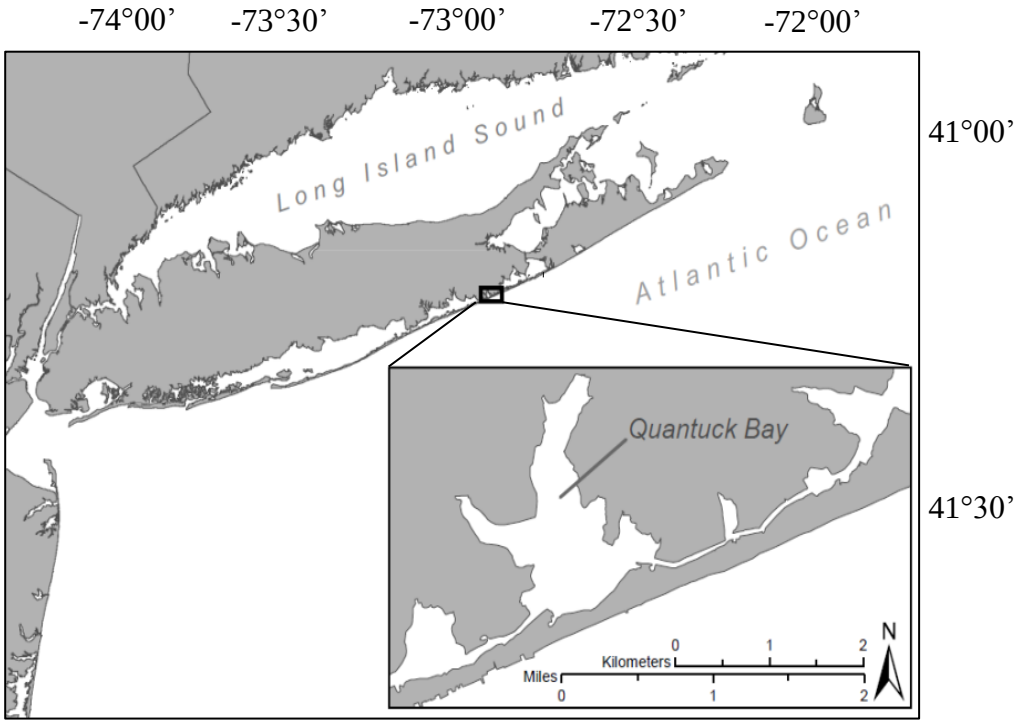

Figure 1. Map of Long Island, NY, USA. Quantuck Bay (QB), part of a series of shallow, well mixed south shore bays is denoted by the star and the site of annual brown tide blooms caused by *A. anophagefferens*.

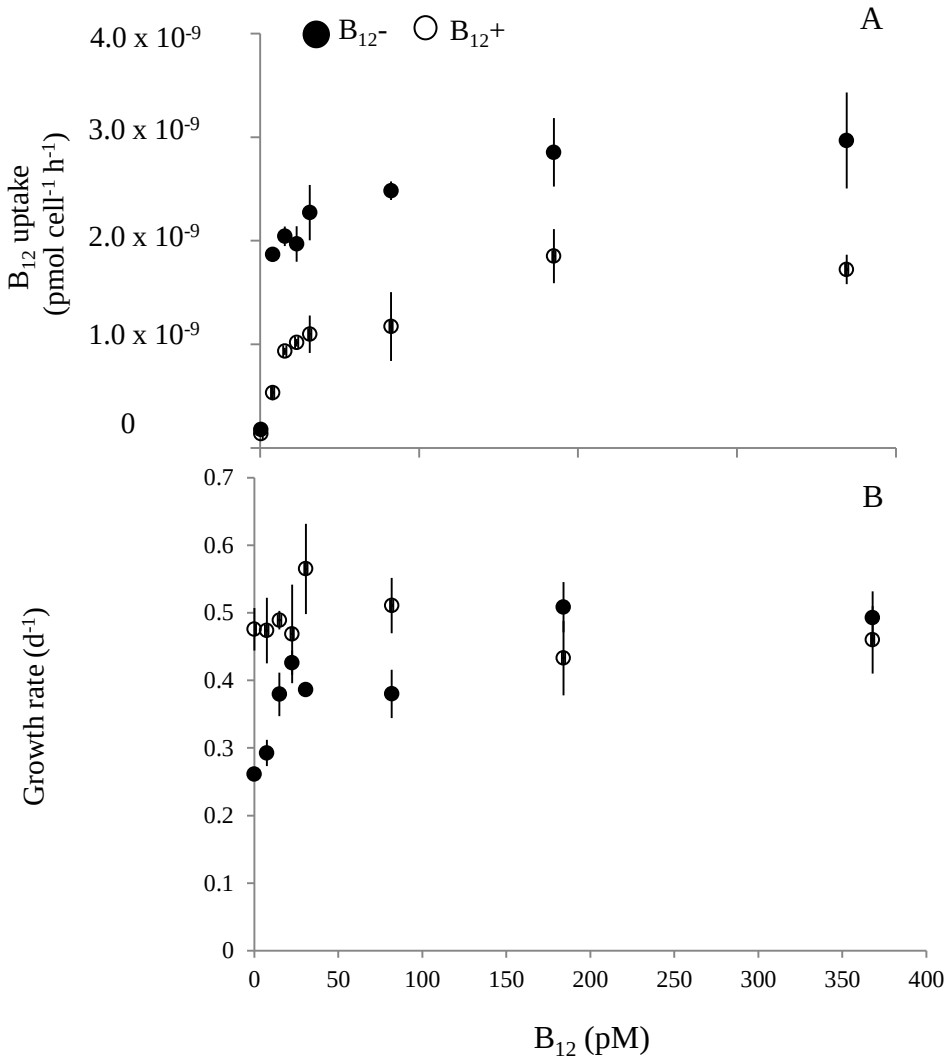

Figure 2. Vitamin B_{12} uptake (A) and growth kinetics (B) for laboratory cultures of *A. anophagefferens* (CCMP1984). Filled circles denote cultures conditioned in vitamin depleted media while the open circles represent cultures which were exposed to vitamin replete conditions until subsequent transfer into varying vitamin concentrations. Points represent mean ± standard deviation with n=3.

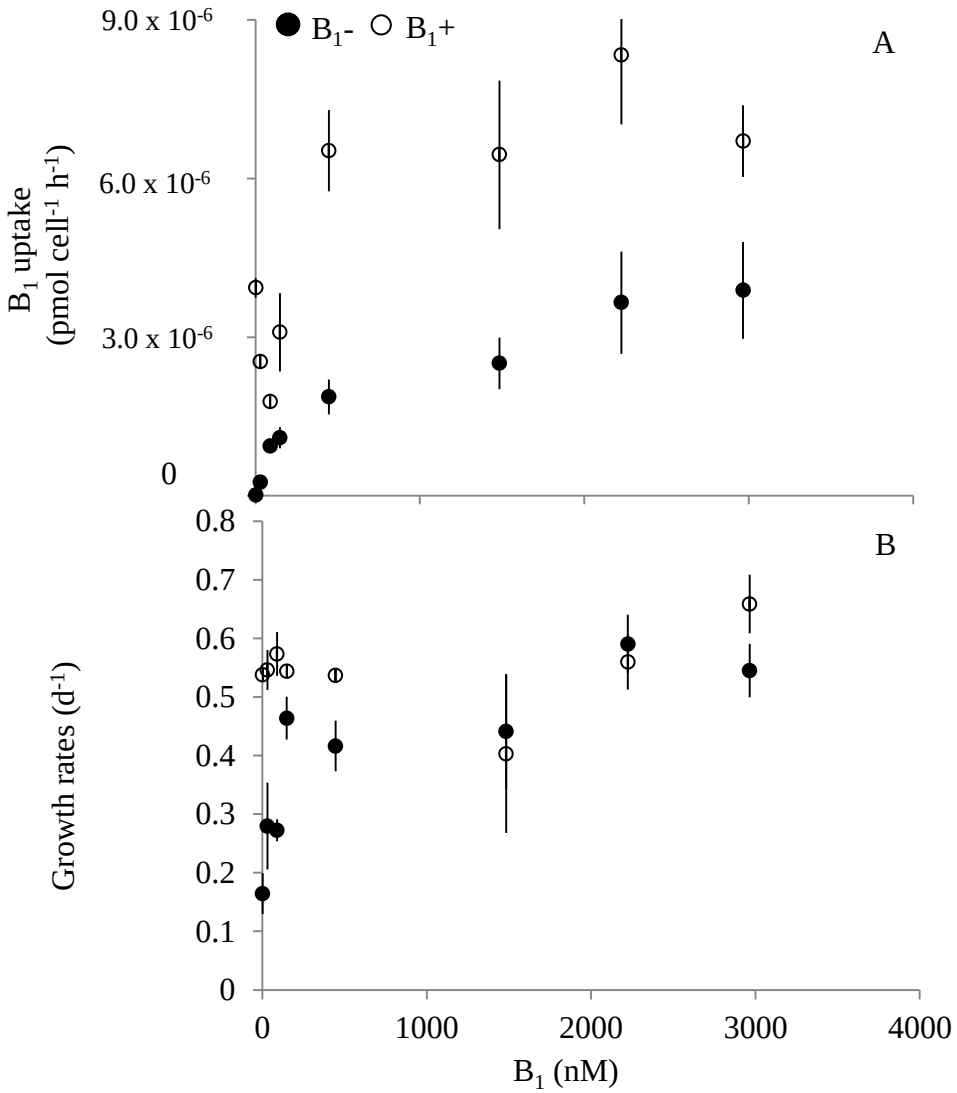

Figure 3. Vitamin B_1 uptake (A) and growth kinetics (B) for laboratory cultures of *A. anophagefferens* (CCMP1984). Filled circles denote cultures conditioned in vitamin depleted media while the open circles represent cultures which were exposed to vitamin replete conditions until subsequent transfer into varying vitamin concentrations. Points represent mean ± standard deviation with n=3.

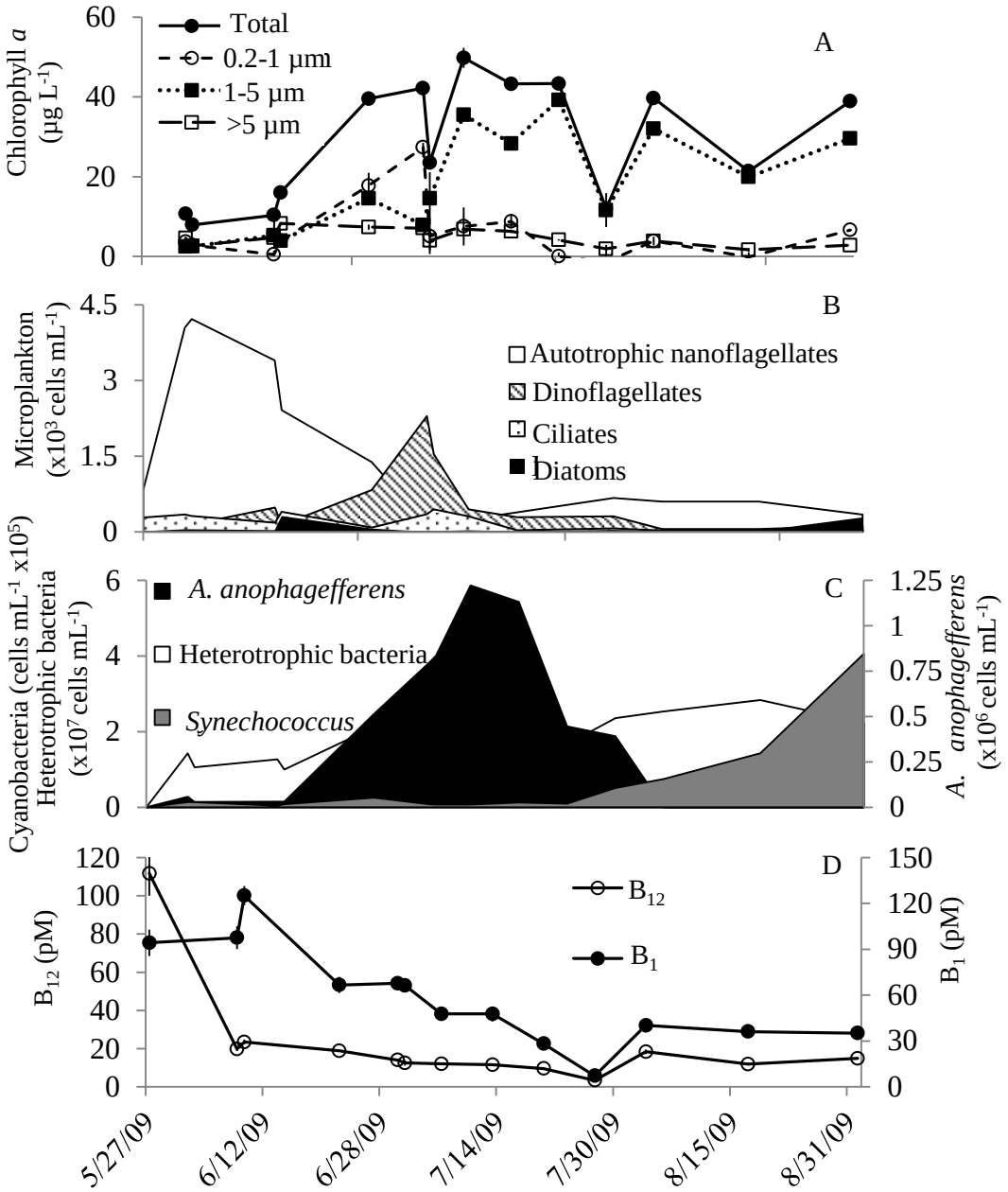

Figure 4. Size fractionated chlorophyll *a* (A) and plankton community dynamics (B and C) as well as Vitamin B_1 and B_{12} (D) concentrations during the 2009 Quantuck Bay brown tide bloom. In A and D data points represent mean ± standard deviation with n= 3 while the mean is represented in B and C.

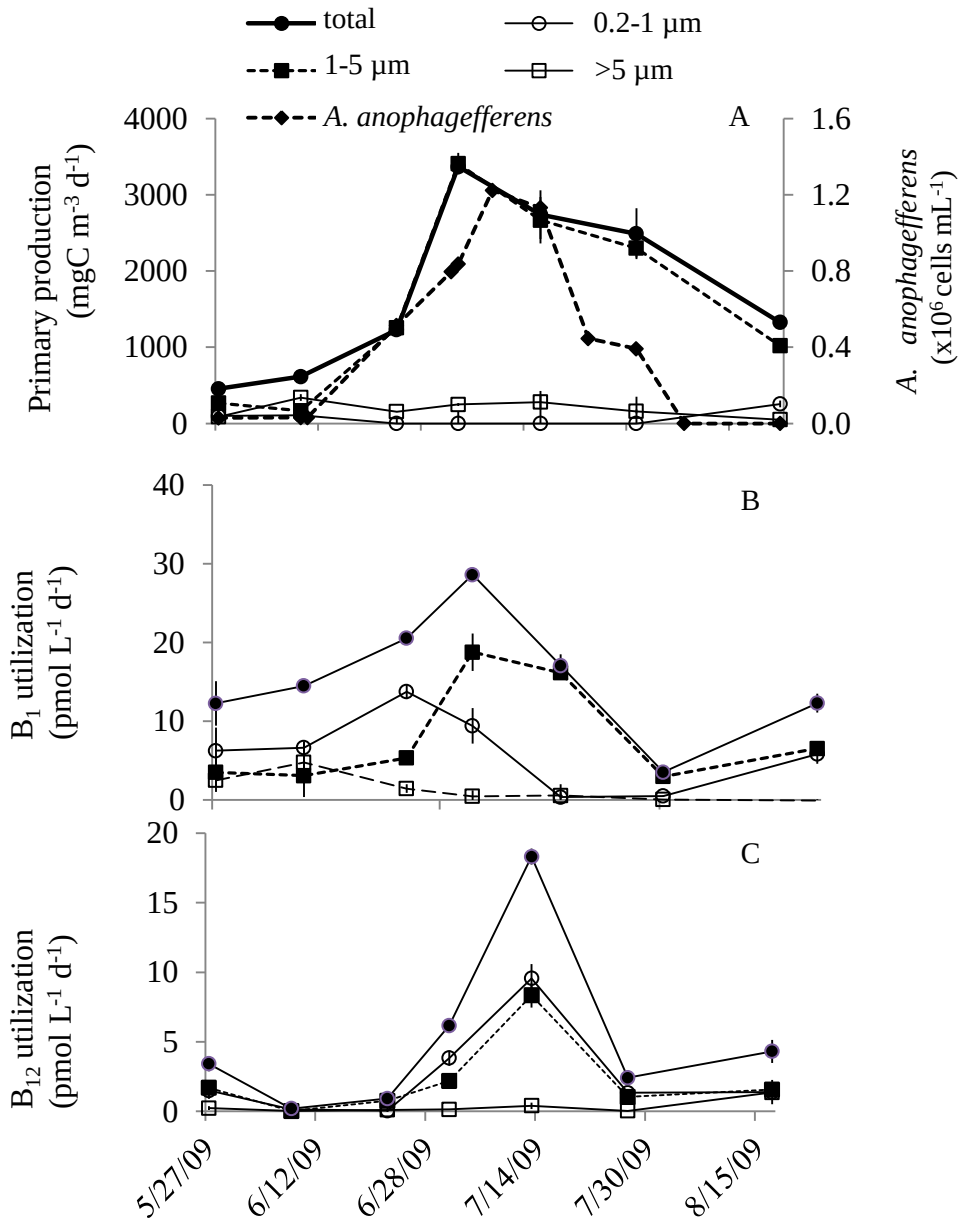

Figure 5. Size fractionated primary production rates and *A. anophagefferens* densities (A), as well as vitamin B_1 (B) and B_{12} (C) uptake rates during the 2009 brown tide bloom in Quantuck Bay, NY. The filled circle, filled square, open circle, open square and filled diamond denote the total, 0.2-1, 1-5, >5 µm and *A. anophagefferns* abundance, respectively. Data points represent mean ± standard deviation of n=3.

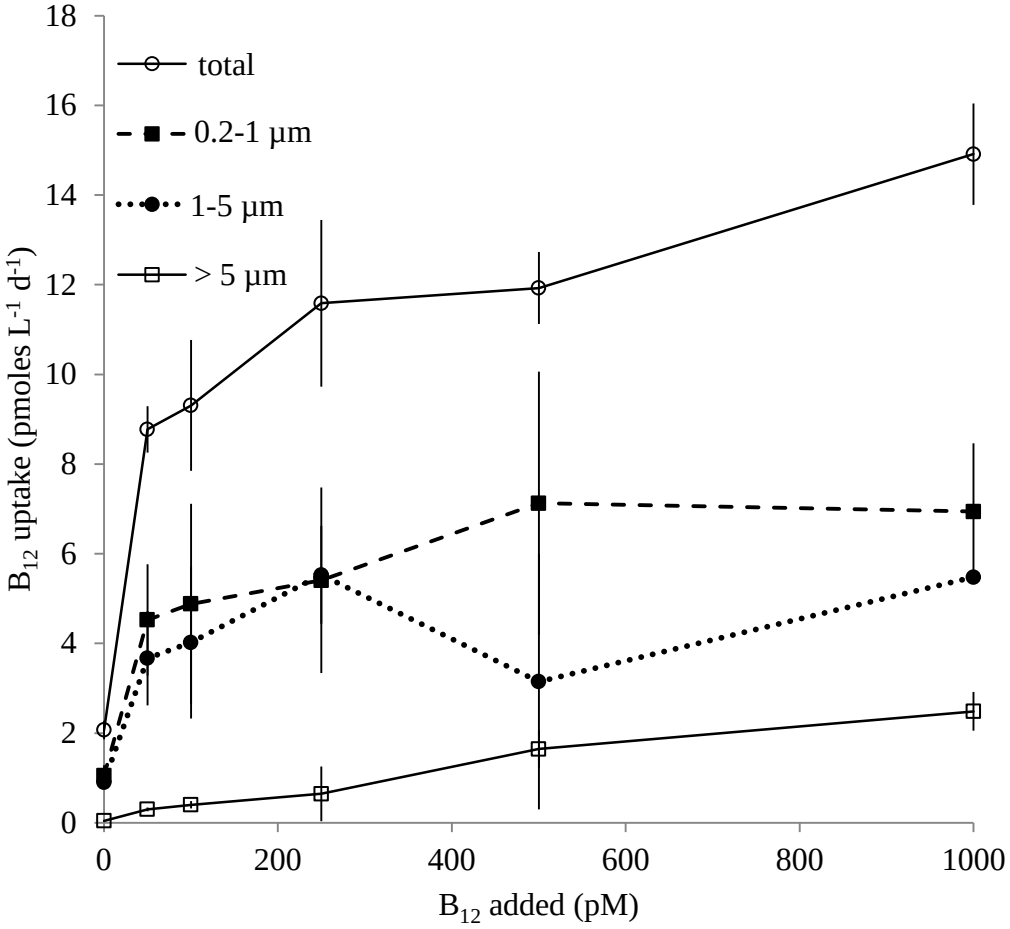

Figure 6. Size fractionated vitamin B_{12} uptake kinetics of the natural plankton community during the 2010 brown tide bloom in Quantuck Bay, NY under a range of different amended vitamin B_{12} concentrations. The initial ambient B_{12} concentration was 3.80 pmol L^{-1}. The open circle, filled square, closed circle and open square denote B_{12} uptake by the total, 0.2-1, and 1-5 and >5 µm size fractions, respectively. Note that uptake in the >5 µm size fraction did not follow Michaelis-Menten uptake kinetics.

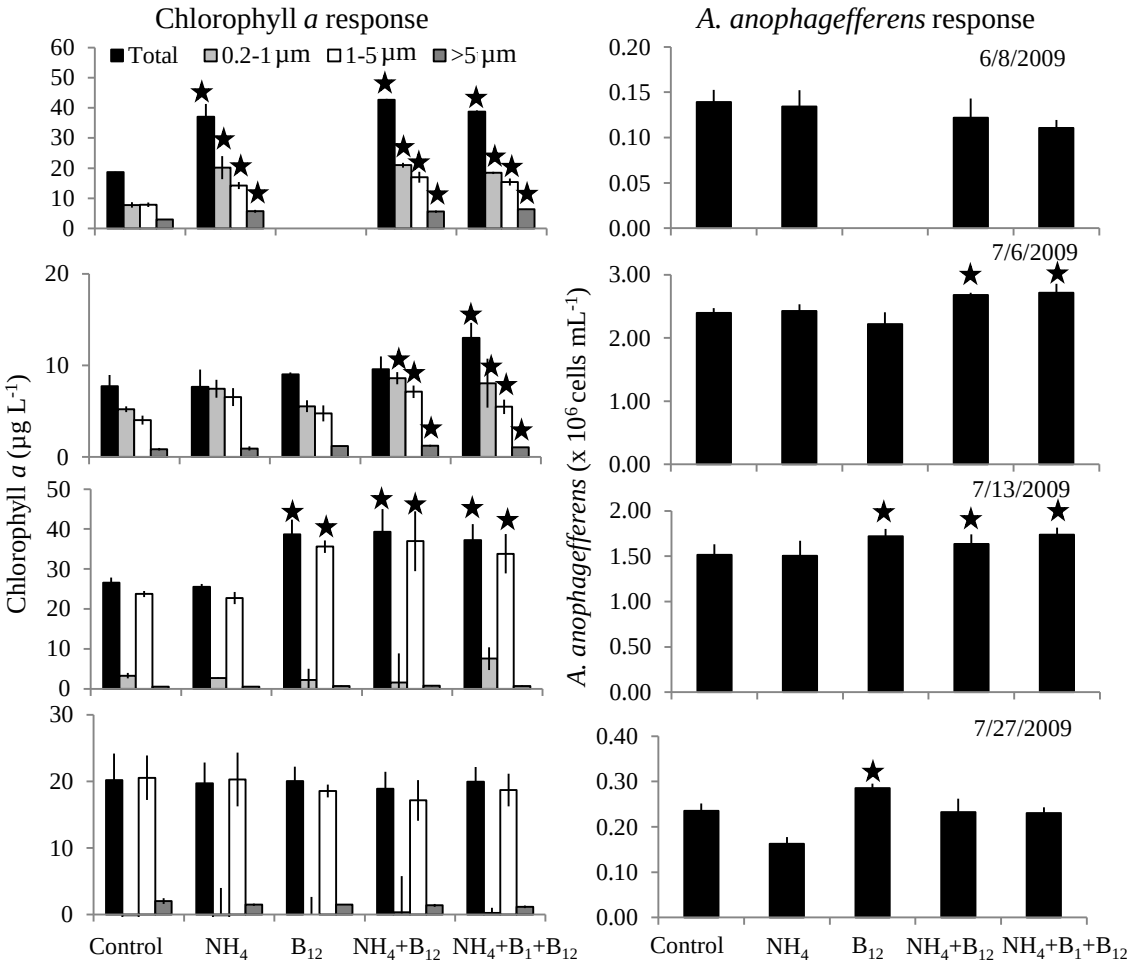

Figure 7. Vitamin/nutrient amendment experiments conducted at the initiation (6/8/09), the peak (7/6-7/13/11)and during the demise (7/27/11) of the 2009 Quantuck Bay brown tide bloom. Responses of the phytoplankton community were assessed via differences in size fractionated chlorophyll *a* (left pannel) while effects of the treatments on *A. anophagefferens* (right pannel) were measured via a monoclonal antibody method. Responses which were significantly ($p<0.05$) different from the controls are marked with a star. The data represents a mean ± standard deviation of n=3.

Table 1. Vitamin kinetics constant for cultures of *A. anophagefferens* (CCMP1984) grown under vitamin replete and depleted conditions. The uptake data (n =18) were fitted to Michalis-Menten curve and were statistically significant as evident in the r^2 (see methods for detailed explanation). The vitamin:carbon ratios were determined for replete and deplete cultures under vitamin deplete (2.0 and 0.5 pM for B_1 and B_{12} respectively) and replete (2965 nM and 370 pM for B_1 and B_{12} respectively) conditions. K_s stands for half saturation constant while V_{max} represent the maximum uptake velocity. K_s is the concentrations in pM where V_{max} is ½. α is the affinity constant and derived from V_{max}/K_s.

	B_{12} replete	B_{12} depleted	B_1 replete	B_1 depleted
K_s (pM)	20.8 ± 3.0	5.2 ± 0.50	2.2 ± 0.3 x 10^5	2.6 ± 0.3 x 10^5
V_{max} (pmol cell^{-1} h^{-1})	1.9 x 10^{-9}	2.7 x 10^{-9}	8.5 x 10^{-6}	3.1 x 10^{-6}
r^2	0.88	0.68	0.53	0.61
α	9.4 x 10^{-11}	51.4 x 10^{-11}	3.9 x 10^{-11}	1.2 x 10^{-11}
B_x:C ratio (pmol B_x : mg C) deplete	2.64 ± 0.41	3.58 ± 0.50	3,057 ± 310	400 ± 25
B_x:C ratio (pmol B_x : mg C) replete	32.4 ± 2.78	36.1 ± 7.32	225,000 ± 650	105,000 ± 400

Table 2. Temperature (Temp), Salinity (Sal), *A. anophagefferens* cell densities, dissolved inorganic (DIN) and organic (DON) nitrogen as well as dissolved inorganic (DIP) phosphorus, silicate (DSi) and vitamin B_1 and B_{12} concentrations in Quantuck Bay during the 2009 brown tide bloom. ND represents 'no data'.

	Temp	Sal	*A. anophagefferens*	DIN	DON	DIP	DSi	B_1	B_{12}
	℃		cells mL^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}		pmol L^{-1}	pmol L^{-1}
5/20/2009	15.7	25.2	0	ND	ND	ND	ND	169 ± 18.4	23.0 ± 0.70
5/26/2009	18.6	26.3	58,000 ± 3,00	1.73 ± 0.08	29.4 ± 4.24	0.11 ± 0.02	17.2 ± 2.17	163 ± 20.2	121 ± 13.2
5/27/2009	16.4	27.4	29,500 ± 2,500	0.73 ± 0.08	30.4 ± 4.49	0.11 ± 0.02	14.8 ± 0.27	94.3 ± 8.60	111 ± 11.6
6/8/2009	19.9	25.8	31,250 ± 2,750	6.58 ± 0.86	40.3 ± 6.70	0.21 ± 0.02	44.1 ± 0.80	97.6 ± 7.50	19.8 ± 1.04
6/9/2009	19.8	25.9	33,500 ± 2,750	3.89 ± 0.43	31.5 ± 1.85	0.11 ± 0.18	30.4 ± 6.37	125 ± 6.20	23.5 ± 1.30
6/22/2009	20.4	24.4	514,250 ± 47,250	0.49 ± 0.10	38.3 ± 3.04	0.24 ± 0.03	62.2 ± 2.98	66.7 ± 5.30	18.9 ± 0.16
6/30/2009	23.5	24.4	796,500 ± 50,500	0.53 ± 0.23	13.0 ± 5.50	0.16 ± 0.01	47.2 ± 10.0	67.8 ± 3.70	14.1 ± 0.70
7/1/2009	23.5	23.3	835,750 ± 20,000	1.40 ± 0.28	24.4 ± 6.50	0.42 ± 0.12	14.1 ± 3.21	66.3 ± 1.87	12.6 ± 1.44
7/6/2009	24.3	25.3	1,222,500 ± 114,500	0.95 ± 0.42	13.5 ± 7.60	0.21 ± 0.04	90.8 ± 11.7	47.8 ± 3.62	12.1 ± 0.65
7/13/2009	23.5	25.5	1,131,750 ± 27,000	0.59 ± 0.15	36.9 ± 6.65	0.30 ± 0.05	66.2 ± 6.19	47.8 ± 4.82	11.6 ± 0.38
7/20/2009	25.8	25.9	445,750 ± 4,000	0.39 ± 0.05	38.8 ± 2.24	0.31 ± 0.24	87.1 ± 8.21	28.3 ± 4.21	9.58 ± 0.27
7/27/2009	24.6	24.8	391,500 ± 37,500	0.17 ± 0.36	32.3 ± 3.56	0.26 ± 0.07	68.6 ± 9.51	7.32 ± 0.87	3.48 ± 0.14
8/3/2009	ND	ND	0	0.83 ± 0.48	30.8 ± 1.59	0.51 ± 0.12	63.8 ± 8.87	40.3 ± 4.60	18.4 ± 1.14
8/17/2009	26.5	23.4	0	0.14 ± 0.04	20.1 ± 2.75	0.75 ± 0.03	62.2 ± 2.04	36.2 ± 2.30	11.9 ± 0.64
9/1/2009	21.6	26.0	0	0.99 ± 0.31	17.8 ± 7.00	0.70 ± 0.10	76.6 ± 5.61	35.2 ± 2.30	14.9 ± 2.50

Table 3. Size fractionated vitamin B_{12} kinetics constants of brown tide bloom water. Ambient bloom water was amended in triplicate with five different B_{12} concentrations, uptake of vitamins measured via radiolabeled B_{12} and the half saturation constants (K_s) and maximum uptake rates (V_{max}) were calculated using KaleidaGraph (see methods). A vitamin affinity coefficient (α) was calculated (V_{max}/K_s) and is represented as $x10^{-3}$ hours. The initial ambient B_{12} concentration was 3.80 pmol L^{-1}. Data represents the mean ± standard deviation of n=3. Note that for the >5 µm size fraction the data did not display Michaelis-Menten style uptake kinetics and thus constants could not be calculated.

	K_s (pM)	V_{max} (pmol L^{-1} h^{-1})	α (x 10^{-3} h^{-1})
Total	47.1 ± 11.4	0.59 ± 0.12	12.5 ± 2.37
0.2-1 µm	36.2 ± 7.60	0.29 ± 0.06	8.06 ± 1.69
1-5 µm	28.1 ± 13.4	0.23 ± 0.05	8.21 ± 1.64

Chapter Five

Effects of harmful *Cochlodinium polykrioides* (Dinophyceae) blooms on plankton communities and the cycling of nitrogen, carbon, and B-vitamins

Abstract

Cochlodinium polykrikoides is a globally distributed, ichthyotoxic, bloom-forming dinoflagellate. Blooms of *C. polykrikoides* manifest themselves as large (kms^2) and distinct patches with cell densities exceeding 10^3 ml^{-1} while water adjacent to these blooms can have < 100 cells ml^{-1}. While the effect of these blooms on fish and shellfish is well-known, their impacts on microbial communities and biogeochemical cycles are poorly understood. Here, we investigated the plankton communities and the cycling of nitrogen, carbon, and B-vitamins within blooms of *C. polykrikoides* and compared them to regions in close proximity (<100m) with low *C. polykrikoides* densities. Within blooms *C. polykrikoides* represented more than 90% of microplankton (> 20µm) cell densities, and heterotrophic bacteria communities were both significantly more abundant and comprised of different species (as indicated by terminal restriction length fragment polymorphisms). Inside the patches, concentrations of nitrate and vitamin B_{12} were lower while levels of ammonium and dissolved oxygen were higher. Carbon fixation and nitrogen uptake rates were up to 10-times higher inside the bloom compared to non-bloom regions. Ammonium was the species of nitrogen primarily assimilated by microplankton within blooms whereas nitrate and urea were assimilated by groups outside of bloom. While uptake rates of vitamin B_1 were similar in bloom and non-bloom regions, vitamin B_{12} was taken up at rates five-fold higher (>100 $pmol^{-1}$ L^{-1} d^{-1}) resulting in turn-over times on a timescale of hours during blooms. This increased vitamin demand likely led to the vitamin B_{12} limitation of *C. polykrikoides* observed during nutrient amendment experiments conducted with bloom water. Collectively, this study demonstrates the ability of *C. polykrikoides* blooms to alter the densities and composition of microbial communities and to rapidly accelerate the cycling of carbon, some nutrients, and vitamin B_{12}.

Introduction

The majority of the World's population live in coastal zones, a situation that has led to a series of anthropogenic stressors such as habitat destruction (Valiela 2006), loss of functionality (Carroll et al. 2008; Koch and Gobler 2009), and accelerated nutrient loading (Cloern 2001; De Jonge et al. 2002). The latter has been implicated as a causative agents for hypoxia and the increasing occurrence of harmful algal blooms (HABs) (Hallegraeff 1993; Glibert et al. 2005; Heisler et al. 2008). While the absolute magnitude of nitrogen entering coastal zones has been the main focus of management efforts since it often controls the amount of phytoplankton biomass in marine systems, it is the availability, ratio, and/or type (e.g. inorganic vs. organic) of nutrients that controls algal community composition (Smayda 1997; Heisler et al. 2008; Koch and Gobler 2009). Many HABs exhibit a diverse range of nutritional strategies including mixotrophy (Smayda 1997; Anderson et al. 2008) and while the role of macronutrients in HAB ecology has been extensively studied in recent decades (Smayda 1997; Sunda et al. 2006; Heisler et al. 2008; Hattenrath et al. 2010) little attention has been paid to organic micronutrients such as vitamins.

Many phytoplankton have an absolute requirement for exogenous B-vitamins such as B_{12} (Droop 1955; Guillard 1968; Swift and Guillard 1977) and are thus considered "auxotrophic". Each of the B-vitamins can play an important role in algal biochemistry. For example, cobalamin (B_{12}) is required for the biosynthesis of methionine, thiamine (B_1) facilitates the decarboxylation of pyruvic acid in the Calvin cycle, and biotin (B_7) plays a critical role in fatty acid synthesis. Earlier estimates (Vishniac and Riley 1961; Menzel and Spaeth 1962; Swift 1972) and more recent direct measurements (Okbamichael and Sanudo-Wilhelmy 2004; Okbamichael and Sanudo-Wilhelmy 2005; Gobler et al. 2007) have found B-vitamins present at

low, picomolar concentrations in seawater and several studies have demonstrated that the enrichment of seawater with B-vitamins can alter both phytoplankton biomass (Sanudo-Wilhelmy et al. 2006; Bertrand et al. 2007) Chapter 3) and community composition (Koch et al. 2011). In addition, a recent study revealed that almost all (>97%) of the 47 strains of harmful algae surveyed required one or more of the B-vitamins for growth, making this group particularly B-vitamin auxotrophic when compared to all other phytoplankton (Tang et al. 2010). However, only one study has focused on vitamin utilization and limitation of a HAB species in the field (Chapter 4) despite the fact that vitamins have previously been implicated in the occurrence of *Lingulodinium polyedrum* (formerly known as *Gonyaulax polyedra*) blooms off the coast of California (Carlucci 1970) and blooms of *Karenia brevis* in the Gulf of Mexico (Aldrich 1962; Hunter and Provasoli 1964; Stewart et al. 1967; Collier et al. 1969).

Cochlodinium polykrikoides is a B-vitamin requiring catenated, athecate, ichthytoxic dinoflagellate. HABs caused by *C. polykrikoides* have plagued Asian coastal waters during the past two decades (Yuki and Yoshimatsu 1989; Kim 1998; Huang and Dong 2000), being responsible for over $100 million in annual fisheries losses in during the 1990s in South Korea (Kim et al. 1999). This dinoflagellate has acute lethal effects on a variety of finfish and shellfish (Kim et al. 1999; Whyte et al. 2001; Tang and Gobler 2009) with high density ($> 10^3$ cells ml^{-1}) exposures resulting in gill hyperplasia, hemorrhaging, squamation, and apoptosis in cells of gills and digestive tracts in fish and shellfish (Gobler et al. 2008; Kudela and Gobler 2012). In addition, a combination of grazing deterrence (Jiang et al. 2009; Jiang et al. 2010) and allelopathy (Tang et al. 2010) are thought to give this species a competitive advantage over other phytoplankton. *C. polykrikoides* and related *Cochlodinium* species are globally distributed (Kudela and Gobler 2012) and have recently been observed in the coastal embayments of Long

Island, NY (Gobler et al 2008). Blooms have occurred annually in the Peconic Estuary and Shinnecock Bay since 2004 during late summer and early fall reaching peak densities of $> 10^4$ cells ml^{-1} (Gobler et al. 2008; Kudela and Gobler 2012) and have recently expanded into the Great South Bay (Koch and Gobler, in prep.). These blooms manifest themselves as large (>100 km^2), and sometimes ephemeral, surface patches of red water that can appear or disappear in a matter of hours when they undergo intense vertical migration (Kudela et al. 2008b). Although the nutrients initiating and sustaining these blooms are still poorly understood, in a manner similar to other dinoflagellates, *C. polykrikoides* seems to display nutritional flexibility, being capable of assimilating and growing on a variety of organic and inorganic N sources (Kim et al. 2001; Kudela et al. 2008a; Gobler et al. 2012). Cultures of *C. polykrikoides* (clone CP-1) isolated from the Peconic Estuary have shown a requirement for extracellular B_{12} and B_1 (Tang et al. 2010) and enrichment of stationary phase cultures of CP-1 with vitamins has been shown to significantly enhance the chain length of *C. polykrikoides* (Jiang et al. 2010) suggesting that vitamins may be important in the ecology of this organism.

While previous studies have investigated various aspects of the ecophysiology of *C. polykrikoides* (reviewed in Kudela and Gobler 2012), no study has investigated the effects of this organism on elemental cycling and the potential role of vitamins in the bloom dynamics of this vitamin auxotroph are unknown. Moreover, while this species has been shown to be strongly allelopathic to other microphytooplankton (Tang and Gobler 2010) its impact on prokaryotic and eukaryotic picoplankton has never been reported. The objectives of this study were to establish the effects of *C. polykrikoides* blooms on plankton community composition and to assess their impacts on the cycling of carbon, nitrogen, and B-vitamins.

Methods

Sampling Location

HABs caused by *C. polykrikoides* can form dense aggregations or bloom 'patches' in which the species dominates the nano- and microplankton (Gobler et al. 2008; Mulholland et al. 2009b; Tang and Gobler 2010). The large, dense, and well-defined bloom patches (>10 km^2) formed by *C. polykrikoides* provide a unique opportunity to investigate the effects of this organism on the ecology and biogeochemistry of coastal systems. Field samples for this study were primarily collected in 2009 and 2010 during the blooms in Shinnecock Bay (SB) which has experienced annual *C. polykrikoides* blooms since 2004 (Fig 1). Samples for terminal restriction length fragment polymorphisms (T-RFLP) analyses of prokaryotic communities were collected from SB during August of 2011. SB is a shallow, well mixed system, which exchanges with the Atlantic Ocean through the Shinnecock Inlet to the south. Water was also collected from several sites on the Peconic Estuary including Meetinghouse Creek (MHC), Flanders Bay (FB) and Great Peconic Bay (GPB) as well as Old Fort Pond (OFP; a tributary within Shinnecock Bay), between 2005 and 2008 for the purposes of performing nutrient/vitamin amendment experiments. All samples were collected between 10:00 and 12:00.

Field samples

For each sampling time, samples were collected inside and immediately adjacent (< 100m) to the bloom patch are henceforth referred to as 'bloom' and 'non-bloom' samples, respectively. At each sampling site, physical parameters (temperature, salinity and dissolved oxygen) were obtained using a YSI© 556 sondes and water was collected in acid washed 20 L carboys and brought back to the lab within < 1 h for processing. Size fractionated chlorophyll *a* (Chl *a*) samples were collected by filtering triplicate samples onto 0.2, 2 and 20 μm polycarbonate filters. These filters were stored frozen until subsequent analysis via standard

fluorometeric methods (Welschmeyer 1994). Whole seawater was preserved in 5% Lugols iodine solution for subsequent enumeration of plankton under an inverted microscope. Plankton were identified to the highest taxonomic level possible, and were generally grouped as diatoms, dinoflagellates, ciliates, and autotrophic nano-flagellates. A minimum of 200 organisms or 100 grids were counted per sample (Omori and Ikeda 1984). Whole water samples were preserved with 10% buffered formalin, flash-frozen in liquid nitrogen and analyzed flow cytometrically to assess picoplankton densities (Olson et al. 1991). Abundance of heterotrophic bacteria (stained with SYBR™ Green I; Jochem 2001), phycoerythrin-containing picocyanobacteria, and photosynthetic picoeukaryotes were determined using a Fluorescence Activated Cell Scan (FACScan; Becton, Dickinson and Company) flow cytometer using fluorescence patterns and particle size from side angle light scatter (Olson et al. 1991). Nutrient samples were pre-filtered with acid-cleaned, polypropylene capsule filters (0.2 μm; GE Osmonics, DCP0200006). Nitrate, nitrite, ammonium, phosphate and silicate were analyzed in duplicate by standard spectrophotometric methods (Jones 1984; Parsons et al. 1984). Total dissolved N (TDN) was analyzed in duplicate by persulfate oxidation techniques (Valderrama 1981). Dissolved organic nitrogen (DON) concentrations were calculated by subtracting levels of nitrate, nitrite and ammonium from concentrations of TDN. Recoveries (mean ± 1 S.D.) of samples spiked with SPEX Certi-Prep^{INC} standard reference material at environmentally representative concentrations were then quantified for TDN, nitrate, nitrite and ammonium, and orthophosphate. Vitamin samples were collected and analyzed according to Okbamichael and Sanudo-Wilhelmy (2004; 2005). Briefly, water was filtered through 0.2 μm capsule filters (GE Osmonics, DCP0200006) into 1 L LDPE bottles to remove any particulate material and stored frozen in the dark. The samples were then acidified in a pH of 6.2-6.9, concentrated at 1 mL min^{-1} onto columns

containing C-18 beads (Varian, HF BONDESIL) and stored frozen until subsequent analysis via HPLC.

T-RFLP analysis

Terminal restriction fragment length polymorphism (T-RFLP) were employed to examine shifts in the composition of the microbial community during *C. polykrikoides* blooms. I specifically collected DNA from bloom and non-bloom sites on 0.2 and 5 µm polycarbonate filters to explore potential differences within the total and attached microbial communities. To extract nucleic acids, 1 mL of 2X CTAB buffer with fresh beta-mercaptonethanol was added to filters, which were vortexed, heated to 50°C for 20 minutes, and frozen at -80°C and extracted following Dempster et al (1999). All PCR reagents were acquired from Promega, and fluorescently labeled primers were synthesized by Integrated DNA Technologies. Triplicate PCR reactions contained 10 µl of 5XGoTaq Flexi DNA Buffer, 3 µl of 25 mM $MgCl_2$, 5 µl of dNTPs at 250 µM each, 4 µl of 5µM of the forward primer 341F, 4 µl of 5µM of the reverse primer 926R (Countway et al., 2005), and 0.5 µl 10 mg/ml acetylated BSA. Reactions were heated to 95^{o}C for 2 min, then held at 80^{o}C while 1U GoTaq Flexi DNA polymerase (Promega) was added, and amplification was performed for 30 cycles of 95^{o}C for 30s, 57^{o}C for 30s, and 72^{o}C for 1 min, followed by a final extension at 72^{o}C for 5 min and a 4^{o}C hold. PCR products were visualized on 1.2% agarose gels to ensure amplification of the correct products. Replicate PCR products were pooled and purified with Promega Wizard SV Gel and PCR Clean-up System. The DNA was quantified by PicoGreen staining (Invitrogen) on a Turner Designs Aquafluor handheld fluorometer. PCR products were treated with Klenow fragment to fill in partially single-stranded amplicons (Egert and Friedrich, 2005). Reactions (50 µl) contained 5 µl Klenow 10X Buffer, 2 µl of dNTPs at 250 µM each, 0.25 µl acetylated BSA 10 µg/µl, and 1U

Promega Klenow per µg DNA (typical reactions contained from 0.5-2.0 µg of DNA). Reactions were incubated at 25^{o}C for 50 min and the reaction stopped by heating to 75^{o}C for 10 min. Products were purified with Promega Wizard SV Gel and PCR Clean-up System and quantified by PicoGreen staining. Klenow-treated PCR product (100 ng) was then digested with the Promega restriction enzymes TaqI in buffers according to manufacturer instructions in duplicate. Digests were ethanol precipitated and resuspended in 10 µl molecular grade water. Hi-Di formamide (10 µl) and 0.5 µl of Genescan 600 LIZ size standard (Applied Biosystems) were added to 1 µl of sample and analyzed on an ABI3130XL capillary electrophoresis DNA fragment analyzer. Electropherograms were processed with Peakscanner version 1.0 software (ABI). Fragments between 30 and 600bp were included in the analysis. Peaks appearing in only one of the duplicate digestions were manually removed. Trials with uncut 16SrDNA showed no artifact peaks in the traces. T-REX™ (Culman et al. 2008) was used to filter noise according to the method of (Abdo et al. 2006), using a one standard deviation cutoff from the mean peak height to identify true peaks. Peaks were binned in T-REX™ using the algorithm developed by (Smith et al. 2005) and allowing more than one peak to be assigned to the same T-RF. T-RFLP data matrices were exported from T-REX™ as relative peak area.

Vitamin and carbon uptake

To establish rates of primary production, as well as uptake rates of vitamin B_1, B_{12} and various nitrogen sources, non-bloom and bloom water was collected. The ability to measure vitamins directly (Okbamichael and Sanudo-Wilhelmy 2004; Okbamichael and Sanudo-Wilhelmy 2005) made possible the use of radio-labeled tracers to measure uptake of vitamins. ^{3}H-B_1, ^{57}Co- B_{12}, and ^{14}C-bicarbonate from MP-Biomedicals were used to measure planktonic uptake rates of vitamins and rates of primary production. Assuming low ambient B_1

and B_{12} concentrations (15-50 and <5 pM, respectively; (Gobler et al. 2007)Chapter 3), a trace amount of either ^{3}H-B_1 (2 pM, 0.06μCi) or ^{57}Co-B_{12} (0.5 pM, 0.04 μCi) was added to triplicate, 300 ml polycarbonate bottles. To assess non-specific binding and/or adsorption of the isotope a 1% gluteraldehyde 'killed-control' bottle was also spiked with tracer and incubated along the 'live' bottles. To determine primary productivity rates, 10 μCi of ^{14}C-bicarbonate was added to triplicate bottles (MP-Biomedicals, specific activity 55 mCi mmol^{-1}) according to JGOFS protocols (1994). Bottles for all isotopes were incubated at ambient temperature conditions in an incubator with 10 - 100 μmol quanta m^{-2} sec^{-1}, adjusted to ambient light intensity and duration. Since uptake rates of vitamins are linear (Chapter 3) and uptake by *C. polykrikoides* cultures and bloom populations was rapid, incubations were terminated after 2h by filtering samples onto 0.2, 2, and 20 μm pore size polycarbonate filters, allowing for the determination of size fractionated uptake of the tracers. *C. polykrikoides* ranges from ~10 - 40 μm in size and dominates the >20 μm phytoplankton community during bloom conditions (>90% of biomass; Gobler et al. 2012, Kudela and Gobler 2012, this study) thus the 20μm filter was used to generally highlight tracer utilization by the >20 μm size class and specifically *C. polykrikoides*. At the beginning and end of the incubation, a small aliquot of each vial (250 μL) was removed to determine total activity (the amount of isotope added). The ^{57}Co and the ^{14}C and ^{3}H containing experimental filters were then analyzed on a LKB Wallac 1282 COPMUGAMMA CS universal gama counter and scintillation counter (Packart-Acanberra Co. PR1-Carb2100TR) respectively along with the total activities. Uptake (ρ) of vitamins B_1 and B_{12} were calculated by using the equation:

$$((A_f - A_{dead} / A_{tot}) \times [\text{vitamin}]) / t$$

where A_f is the activity on the life filters, A_{dead} is the activity on the dead filters, A_{tot} is the total activity added, [vitamin] is the ambient B_1 or B_{12} concentration and t equals the length

of the incubation in hours. To account for the diel variation in photosynthesis, carbon fixation numbers were normalized to h^{-1} and then multiplied by the length of the day at sampling (~14h) while uptake rates of vitamins were simply multiplied by twelve to reflect the linear uptake over 24h as described above. Carbon specific vitamin uptake rates for the different size classes of plankton in both bloom and non-bloom communities were determined. Specifically, carbon:chl *a* ratio of 60 was applied to >2µm cholorophyll *a* values (Lorenzen 1968; Riemann et al. 1989; Boissonneault-Cellineri et al. 2001) while previously published values of average carbon contents of 20 fg $cell^{-1}$ and 200 fg cell-1 for heterotrophic bacteria (Fukuda et al. 1998; Ducklow 2000), and cyanobacteria and picoeukaryotes (Kana and Glibert 1987; Liu et al. 1999), respectively were used for the <2 µm size class. As a comparison to field populations, vitamin B_{12} uptake kinetics of *C. polykrikoides* were determined using a monoculture of strain CP1, isolated from Flanders Bay, NY, USA, in 2006. Cultures were grown in GSe medium (Doblin et al. 1999) made with artificial salts but without the addition of B-vitamins. For all cultures, an antibiotic-antimycotic solution (final concentrations = 100 I.U. penicillin, 100 μg ml^{-1} streptomycin, and 0.25 μg ml^{-1} amphotericin B; Mediatech. Inc., Hemdon, Virginia) was added into the medium immediately before inoculation to ensure cultures remained free of bacteria and fungus. Cultures were grown in sterilized, 1-L Pyrex™ Erlenmeyer flasks and maintained at 21°C on a 14:10 light:dark cycle in an incubator under ~100 μmol quanta m^{-2} sec^{-1}. Cultures were grown under vitamin B_{12} replete and depleted conditions with vitamin concentrations in depleted cultures diluted via successive transfers (Tang et al. 2010). Cultures were deemed limited by a vitamin when the addition of the limiting vitamin elicited an elevated growth response. During exponential growth, five mL vitamin replete and vitamin deplete cultures were transferred to triplicate 10 mL Pyrex culture tubes and spiked with either ^{14}C-bicarbonate (0.25

µCi), ^{3}H-B_1 (0.06μCi) or ^{57}Co-B_{12} (0.04 μCi). The tubes were incubated at 100 μmol quanta m^{-2} sec^{-1} on a 14:10 light:dark cycle and 21°C. Experiments were terminated after 2 h by filtering each tube onto 2 μm filters, rinsing filters with 0.2 µm filtered seawater, and analyzing filtered for tracer incorporation as described above.

Nitrogen uptake

Uptake of nitrate, ammonium and urea was measured using tracer additions (<10% based on measured and added levels of N at the start of 30 minute experiments) of highly enriched (98%) ^{15}N-labeled nitrate, ammonium, and urea (Mulholland et al. 2002; Mulholland et al. 2009a). Incubations were performed in six replicate 50 ml, acid-washed polycarbonate flasks incubated under ambient light and temperature conditions for 30 minutes after which water was filtered on pre-combusted (2 h @ 450ºC) GF/F glass fiber filters with and without pre-filtration with a 20µm mesh (n = 3 for each) to remove a majority of *C. polykrikoides* cells. Bloom water used for experiments contained few other phytoplankton with *C. polykrikoides* representing >80% of cells > 20µm allowing uptake in the >20 μm size to be specifically to *C. polykrikoides* (Gobler et al. 2012). The difference in uptake observed in the total and < 20 µm size fraction was ascribed to cells > 20 µm. The natural abundance of ^{15}N in particulate organic nitrogen (PON) prior to enrichment was also determined. Samples were pelletized in tin discs and were analyzed for at the U.C. Davis Stable Isotope Facility on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Uptake rates were calculated according to the mixing model of Montoya et al. (2002) and using equations from (Orcutt et al. 2001). Rates were considered net uptake as they not corrected for the effects of isotope dilution (Glibert et al. 1982). Uptake by bacteria and production or release of N compounds during incubations were

assumed to be small given the brief incubation periods used (30 minutes). Uptake rates were then normalized to particulate nitrogen.

Vitamin amendment experiments

Nitrogen has recently been shown to limit the growth of *C. polykrikoides* during blooms (Gobler et al. 2012). However, the potential for B-vitamins to be a limiting or co-limiting nutrient while observed in other HAB events (Chapter 4) has never been explored for this species. The potential for vitamin limitation was established as described in Koch et al. (2011) during blooms spanning from 2005-2008. Briefly, triplicate 1.1 L acid washed polycarbonate bottles were filled with bloom water and amended with 10 µM NH_4, 100 pM B_{12}, and a combination of the two nutrients and incubated for 24 - 48 h in OFP after which cell densities in bottles were preserved and enumerated as described above for field samples. OFP exchanges tidally with Shinnecock Bay (Fig 1), one of our collection sites, and thus the experiments experienced near *in situ* light and temperature conditions. The growth rate of *C. polykrikoides* during experiments was calculated according as $\mu = \ln(CP_{final}/CP_{initial})/t$ where µ is the growth rate (d-1), $CP_{initial}$ and CP_{final} are the *C. polykrikoides* abundances in cells mL^{-1} at the start and end of the experiment, respectively and t is incubation times in days.

Statistical analysis

To assess differences in individual parameters inside and outside the bloom, two way analysis of variance (ANOVA) was performed where date of sample and bloom or non-bloom were the treatments and $p<0.05$ signified significant differences. Analysis of similarities (ANOSIM) and similarity percentages (SIMPER) of plankton communities were performed using the PRIMER™ 5.0. The multidimensional scaling (MDS) of patch and non-patch and related community parameters were based on resemblance matrices of square root transformed,

Bray-Curtis similarities. 'Global' R statistics were assessed for significant patch/non-patch effects at $p = 0.05$. Since the growth and uptake rate response of vitamin auxotrophic phytoplankton cultures as well as natural phytoplankton populations to varying vitamin concentrations follows Michaelis–Menten saturation kinetics (Droop 1968; Tang et al. 2010), half saturation constants (K_s) were derived by fitting the uptake data to Michaelis–Menten equation using the software Kaleidagraph (Synergy software, Maxwell Resources, Inc.) and by using Lineweaver-Burk transformation (Atkins and Nimmo 1975; Greco and Hakala 1979). Since the results of the two methods were nearly identical (± <5% in most cases), only the results from the Michaelis–Menten curve fitting are presented. Affinity constants (α) were calculated as V_{max}/Ks and represented the slope at low Ks and given h^{-1}, the steeper the slope (higher α), the higher the affinity of the organism to the nutrient in question (Harrison et al. 1989; Kudela et al. 2008a). All of the plots used to calculate kinetics constants were based on curve with an $r^2>0.90$ and included at least 12 data points. Differences in *C. polykrikoides* growth rates in the nutrient amendment experiments and between the size fractions for the bloom and non-bloom carbon specific uptake rates, were statistically evaluated using an analyses of variance (ANOVA) where ammonium, vitamin B_{12} and ammonium+B_{12} were the treatment factors for the amendment experiments while filter size, bloom and non-bloom where the treatment factors for the carbon specific uptake rates. $p<0.05$ was used to establish significant differences among treatments.

Results

The 2009 and 2010 blooms lasted from late July to early October with *C. polykrikoides* occurring in eastern Shinnecock Bay and all of the Peconic Estuary system and the highest cell densities reaching > 10,000 cells mL^{-1}. Bloom patches generally appeared in the same locations in the late morning and were encountered during a variety of weather conditions (i.e. windy,

calm, sunny, cloudy). With the exception of the vitamin amendment experiments, all samples were collected from Shinnecock Bay (SB in Fig. 1).

Oxygen, nutrients, and vitamins

With two exceptions (1 and 15 September, 2009) total dissolved inorganic nitrogen (DIN, nitrate+nitrite+ammonium) concentrations during the 2010 bloom period were similar inside and outside the blooms (Table 1). Mean ammonium concentrations in bloom samples, however, were higher than non- bloom samples (1.58 ± 0.39 and 0.96 ± 0.19 µM, respectively; mean ± standard error) while the opposite was true for nitrate (0.73 ± 0.17 and 1.74 ± 0.59 µM respectively; Fig. 2, Table 2). Bloom and non-bloom phosphate (0.75 ± 0.09 and 0.84 ± 0.11 µM), silicate (32.3 ± 6.60 and 33.5 ± 6.49 µM) and urea concentrations (0.34 ± 0.03 and 0.37 ± 0.04 µM) were similar. Vitamin B_1 levels during 2009 and 2010 were similar between bloom and non-bloom samples (42.0 ± 2.12 and 40.1 ± 3.87 pM, respectively; Fig. 3A, Table 1, 2). In contrast, B_{12} concentrations were significantly ($p<0.05$) lower in bloom water compared to non-bloom regions in (48.6 ± 3.67 and 56.4 ± 4.07 pM, respectively, for all observations; $p<0.05$; Fig. 3B, Table 1, 2). Temperature and salinity did not vary among sample types while dissolved oxygen concentrations were ~20% higher inside of the blooms ($p<0.05$; Table 1) .

Plankton community composition

The microplankton community outside of blooms varied but was generally dominated by dinoflagellates and small, autotrophic and heterotrophic flagellates (Table 3). Average *C. polykrikoides* densities were 3,932 ± 1331 cells mL^{-1} and 142 ± 63.5 for bloom and non-bloom sites, respectively, in 2010 (Figure 4A) with the highest densities observed on 8 August 2010. Flow cytometric analyses revealed significantly higher picoeukaryotic densities inside the blooms compared to non-bloom regions (18.8 ± 4.84 x 10^5 and 4.35 ± 0.64 x 10^5 cells mL^{-1},

respectively; $p<0.001$; Fig. 4B) while cyanobacterial densities were significantly lower in bloom water ($3.11 \pm 0.63 \times 10^5$ and $5.72 \pm 1.16 \times 10^5$ cells mL^{-1}, respectively; $p<0.05$; Fig. 4C). Heterotrophic bacterial densities inside the blooms were 5- to 10-fold higher than non-bloom regions ($4.91 \pm 0.81 \times 10^6$ and $0.92 \pm 0.04 \times 10^5$ cells mL^{-1}; respectively; $p<0.001$; Fig. 3D;). Terminal restriction length fragment polymorphisms (T-RFLP) analyses of the prokaryotic community (16S rRNA) indicated that, in addition to being more abundant, the richness of the heterotrophic bacterial community within blooms differed with multiple TRFs present only inside bloom (565, 559, 486, 578, 584 base pairs, Fig. 5A). The >5 µm size fraction displayed a similar trend, although there were fewer total fragments than on the 0.2 μm filter (total), with several TRFs were found only in the patch (260, 234, 475, 96 base pairs; Fig. 5B). Analysis of similarities (ANOSIM) revealed that the ambient and bloom plankton communities were significantly different ($r=0.82$, p 0.05; Fig. 6A) even when *C. polykrikoides* was removed from the analysis ($r=0.81$, $p<0.01$; Fig. 6B). On the other hand, when *C. polykrikoides* and the picoplankton community (as quantified via flow cytometer) were both removed, there was no significant difference in the plankton community between the bloom and non-bloom samples ($r=0.23$, $p>0.25$, Fig. 6C).

Nutrient utilization

As expected, the presence of a high densities phytoplankton bloom caused by *C. polykrikoides* resulted in significantly enhanced primary production rates inside blooms compared to non-bloom water. While primary production in non-bloom waters was fairly consistent during the 2009 and 2010 (215 ± 33.5 mgC m^3 d^{-1}, Table 4), rates were one order of magnitude higher and more dynamic inside the blooms (2070 ± 598 mgC m^3 d^{-1}; Fig. 7A, Table 4). There was a significant correlation between primary production in the microplankton

(>20µm) and abundance of *C. polykrikoides* (R = 0.76, p<0.01). Carbon-normalized primary production was dominated by the >20 µm size fraction and was high in bloom water compared to non-bloom communities (2.28 ± 0.57 d^{-1}, v. 1.57 ± 0.37 d^{-1}, respectively; Fig. 8A). Carbon-normalized production rates in the 0.2-2 µm and 2-20 µm size fractions, however, were significantly lower in the bloom compared to the non-bloom water (Fig. 8A) and were many fold lower than the >20µm size fraction in the bloom water.

Vitamin uptake rates obtained during the 2009 and 2010 *C. polykrikoides* blooms revealed that B_1 and B_{12} as well as various nitrogen sources were utilized differently by bloom communities compared to non-bloom regions. With the exception of one date (15 September, 2009) total uptake rates of B_1 in the bloom did not differ from non-bloom water (9.95 ± 2.09 and 10.1 ± 2.61 pmol L^{-1} d^{-1}, Table 4). Size fractionation revealed that the majority of vitamin B_1 uptake in the non-bloom and bloom alike occurred in the <2 µm size class (>60%). In contrast, with one exception (9 September 2010, Fig. 7C) vitamin B_{12} utilization was 2-10 higher within blooms compared to non-bloom communities, averaging 45.7 ± 9.95 and 7.91 ± 1.49 pmol L^{-1} d^{-1}, respectively (Fig. 7C, Table 4). Carbon -normalized vitamin uptake rates were higher for B_{12} than B_1 in the non-bloom water (Fig. 8A, B). While there was no difference between the carbon-specific B_1 uptake rates of the bloom and non-bloom picoplankton communities (<2 µm) there was significantly less carbon specific B_1 uptake in the 2-20 µm (p<0.001) and >20 µm (p<0.05) inside the bloom compared to the non-bloom water (Fig. 8A). In contrast, carbon specific B_{12} utilization by the picoplankton was ~ 40% lower in the bloom than non-bloom water while there was significantly higher carbon specific B_{12} utilization in the >20 µm size fraction (58% higher; p<0.01; Fig. 8B). Mean vitamin B_1 turnover times were similar for non-bloom and bloom samples (5.86 ± 0.62 and 4.99 ± 0.80 d, respectively; Table 4) while vitamin B_{12} was

cycled seven-fold faster inside the bloom (10.3 ± 1.35 d) compared to non-bloom samples (1.53 ± 0.22 d; Table 4). On several occasions, uptake of B_{12} was high enough to deplete the ambient vitamin pool in 0.32, 0.72, and 0.87 days on 9 Aug, 2 Sep, and 7 Sep, respectively (Fig. 7C).

Uptake rates of nitrogenous compounds (nitrate, ammonium and urea) were measured on 2 September, 2010, at a time when non-bloom and patch concentrations of ammonium were 0.52 ±0.12 and 1.31 ± 0.05 µM respectively, nitrate concentrations were 1.39 ± 0.31 and 0.40 ± 0.21 µM, respectively and urea concentrations were 0.38 ± 0.09 and 0.33 ± 0.06, respectively. Nitrogen uptake was more than five-fold higher in the bloom compared to the adjacent non-bloom water (Fig. 9A). Of the three nutrients, ammonium was taken up at seven-fold higher rates by bloom communities (0.19 ± 0.01 µmol L^{-1} h^{-1} in non-bloom community vs. 1.52 ± 0.12 µmol L^{-1} h^{-1}) whereas urea and nitrate utilization rates were only three-fold higher (0.63 ± 0.01 and 0.27 ± 0.02 µmol L^{-1} h^{-1}, respectively for bloom 0.18 ± 0.01 and 0.09 ± 0.01 µmol L^{-1} h^{-1}, respectively, for non-bloom communities; Fig. 9A). Turnover times of all three measured nitrogenous compounds were shorter inside the bloom than adjacent with turnover decreasing from 14.5 to 1.47 h for nitrate, 2.15 to 0.53 h for urea, and from 2.86 to 0.86 h for ammonium indicating increased utilization of all nitrogen sources measured inside the bloom. Nitrogen specific uptake of urea was the same in bloom and non-bloom and did not vary across the different size fractions (Fig. 9B) while nitrogen specific nitrate utilization was slightly lower in the >20 µm size fraction inside the bloom. In stark contrast, total nitrogen specific ammonium uptake was twice as fast in the bloom and >71-fold higher in the >20 µm size fraction compared to the non-bloom water (Fig 9B).

Vitamin amendment experiments

Vitamin amendment experiments conducted over several years from multiple locations across eastern Long Island indicated that B_{12} enhanced the growth of *C. polykrikoides* (Fig. 10, Table 5). Both nitrogen in the form of ammonium and vitamin B_{12} significantly enhanced the growth of *C. polykrikoides* in two-thirds of experiments conducted ($p<0.05$; Fig. 10, Table 5). The combined addition of ammonium and B_{12} elicited a growth rate significantly larger than the individual ammonium and B_{12} only additions in 33% of all experiments ($p<0.05$; Fig. 10, Table 5).

Vitamin uptake kinetics

Cultures of *C. polykrikoides* clone CP1 grown under B_{12} depleted conditions has seven-fold lower vitamin quotas (0.81×10^{-6} pmol cell^{-1}) compared to B_{12} replete cultures (5.26×10^{-6} pmol cell^{-1}). While the Vmax was similar in both cultures ($3.20 \pm 0.13 \times 10^{-6}$ and $2.86 \pm 0.18 \times 10^{-6}$ pmol cell^{-1} h^{-1}, for replete and deplete, respectively) the Ks was ~8 fold higher in the replete culture (41.5 ± 5.02 pM vs. 7.96 ± 1.42 in the deplete culture; Table 6). In addition the affinity constant (α) for the B_{12} deplete culture was five-fold higher (0.10 h^{-1}) than what was observed for the B_{12} replete culture (0.02 h^{-1}, Table 6)

Discussion

While many studies have explored the toxicity of *C. polykrikoides* to a variety of higher trophic level organisms (Gobler et al. 2008; Jiang et al. 2009; Tang and Gobler 2009), the allelopathic effects of *C. polykrikoides* on other phytoplankton groups (Tang and Gobler 2010) and nitrogen-based nutritional ecology in the lab (Kim et al. 2001; Gobler et al. 2012), no study has investigated the effects of this organism on elemental cycling or the potential role of vitamins in the bloom dynamics of this auxotroph. Moreover, while this species has been shown to be allelopathic to some phytoplankton its impact on picoplanktonic prokaryotes and

eukaryotes has never been explored. This study demonstrates that the presence of *C. polykrikoides* not only alters the plankton community composition but also exerts pressure on nutritional resources, increasing vitamin and nitrogen turnover rates, potentially depriving other plankton groups of essential nutrients.

Effects of C. polykrikoides blooms on water chemistry

C. polykrikoides blooms had a discernible impact on water chemistry during this study. Nitrogen uptake rates were five-fold higher in blooms compared to non-bloom regions, indicating there was rapid cycling of labile nitrogen compounds. The nutrient composition of the bloom water (higher ammonium and lower nitrate concentrations), nitrogen uptake dynamics (65-fold higher nitrogen specific ammonium uptake among >20µm plankton compared non-bloom conditions), and turnover time of ammonium (hourly) collectively indicate that ammonium was rapidly cycled and preferred for growth by *C. polykrikoides* during blooms.

During this study, vitamin concentrations were similar to prior observations in similar coastal systems (Sanudo-Wilhelmy et al. 2006; Gobler et al. 2007) and in adjacent bays in NY (Long Island Sound, Old Fort Pond, Quantuck Bay; Chapter 3, 4). Heterotrophic bacteria are hypothesized to be the main source of vitamins to pelagic ecosystems (Raux et al. 2000; Palenik et al. 2003; Rocap et al. 2003; Vitreschak et al. 2003) and recent studies (Koch et al. 2011); Chapter 3) suggest bacteria are also the main utilizers of B-vitamins in these systems. Together with the observation that heterotrophic bacteria and vitamin levels were similar across non-bloom sampling points and that most vitamin uptake occurred in the <2 µm size fraction outside of blooms suggests that normally, production and consumption are balanced and controlled mainly by the prokaryotic community. Inside the blooms, *C. polykrikoides*, a confirmed vitamin-auxotroph (Tang et al. 2010), numerically dominated the nano- and microplankton community

and altered the cycling of vitamin B_{12}. Uptake rates of vitamin B_{12} were >7 fold higher inside the bloom compared to outside the bloom and likely contributed to the lower concentrations in bloom compared to non-bloom water. In contrast, B_1 concentrations during blooms were generally similar to non-bloom regions likely due to the fact that *C. polykrikoides*, although a confirmed B_1 auxotroph (Tang et al. 2010), did not discernibly increase B_1 utilization suggesting either a lower cellular requirement for this vitamin compared to B_{12} or that cells were replete with regard to this vitamin. In fact, the three-fold higher carbon-specific vitamin B_1 uptake rates outside the bloom compared to non-bloom indicates *C. polykrikoides* had a smaller vitamin demand than both, the nano- and microplankton in adjacent non-bloom regions.

Effects of C. polykrikoides blooms on the plankton community

Studies have shown that cultures and wild bloom populations of *C. polykrikoides* can alter the phytoplankton community composition by inhibiting or lysing other phytoplankton via the secretion of allelochemicals (Tang and Gobler 2010). While we did not observe differences among the microplankton communities of bloom and non-bloom samples there were significant differences between patch and non-patch picoplankton communities ($p<0.01$) . The significantly lower cyanobacterial abundances ($p<0.05$) suggests this population was negatively affected by the presence of *C. polykrikoides*, either by direct allelopathy (Tang and Gobler 2010) or indirectly by the altered picoplankton community (higher levels of heterotrophic bacteria and picoeukaryotes) associated with *C. polykrikoides* blooms. The presence of ten-fold higher heterotrophic bacterial and picoeukaryote abundances indicates that a microbial community 'bloom' developed in concert with *C. polykrikoides* blooms. This is, to my knowledge, the first documentation of such a microbial community but is not surprising given the abundance extracellular polysaccharide compounds excreted by *C. polykrikoides* (Kim et al. 2002; Gobler et

al. 2008) that likely enhances bacterial densities (Cole et al. 1982; Hedges 1987; Kirchman et al. 1991).

T-RFLP analyses indicated that in addition to harboring a denser microbial community, *C. polykrikoides* blooms harbor a unique microbial community. There were multiple, unique TRFs found in suspension and attached to *C. polykrikoides* during blooms that were not present in non-bloom water. Compounds produced by *C. polykrikoides* (both toxins and polysaccharides) likely account, at least in part for this altered prokaryotic community, with some bacteria being susceptible to toxins, others being immune, and yet other benefitting from organic carbon compounds. In addition, the large B_{12} requirement of *C. polykrikoides* (highest observed for any HAB sepecies; Tang et al. 2010) and its ability to efficiently utilize this vitamin likely shifts the bacterial community from one where picoplankton are the primary B_{12} consumers to one dominated by producers as evident by the decrease in carbon-specific vitamin B_{12} uptake rate in the <2µm size fraction during blooms. Since bacteria require B_{12} but they take up less during blooms, are more likely to be B_{12} producers and thus supply the large quantities of vitamin B_{12} required by *C. polykrikoides* blooms.

Effects of C. polykrikoides blooms on resource utilization

Carbon fixation in *C. polykrikoides* bloom patches was dominated by nano- and microplankton, but not to the extent expected considering the five- to ten-fold increase in total primary production rates. The large picoeukaryotic plankton community was responsible for the ~20% of total primary production in the bloom water, a finding consistent with the significantly higher abundances of picoeukaryotes during blooms. Primary production rates inside the bloom patches were some of the highest observed in eastern Long Island estuaries (Chapter 3, Bruno et al. 1983), rivaling those observed during high biomass blooms of the brown tide forming *A.*

anophagefferens (Chapter 4). The high rates of carbon specific primary production rates in the >20 µm size fraction and the high levels of dissolved oxygen within patch communities suggested that, similar as what has been previously observed in Chesapeake Bay (Mulholland et al. 2009b), *C. polykrikoides* is an efficient primary producer. This suggests that, despite its phagotrophic abilities (as reviewed by Kudela and Gobler 2012), *C. polykrikoides* obtained much of its cellular carbon via photosynthesis during this study. The recent revelations that harmful algae have a much high incidence of vitamin auxotrophy (Tang et al. 2010), that vitamins can enhance the chain length of *C. polykrikoides* (Jiang et al. 2010), and that vitamin B_{12} can influence brown tides formed by *A. anophagefferens* (Chapter 4) have all added vitamins to the list nutritional factors that may contribute to the formation of HABs. Previous studies (Koch et al. 2011, Chapters 3, 4) have shown that vitamin utilization, even during algal blooms events (Chapter 4), is dominated by the picoplankton. In contrast, during *C. polykrikoides* blooms B_{12} utilization occurred primarily in larger plankton (>2µm; 64.4 ± 7.10%) suggesting that *C. polykrikoides* is better able to gain access to the pM concentrations of vitamins than *A. anophagefferens*, other phytoplankton, and bacteria (Chapter 2,3,4).

There are multiple factors that may account for the significantly higher B_{12} uptake within the larger plankton sizes during *C. polykrikoides* blooms. Carbon-specific B_{12} utilization by picoplankton inside blooms was lower than outside and suggesting that the altered prokaryotic community (as indicated by T-RFLP) had a lower B_{12} requirement, thus may be comprised of a larger proportion of B_{12} producers. Such a change would alleviate competitive pressure for vitamin B_{12} uptake typically experienced by other eukaryotic phytoplankton (Chapters 2, 3, 4). Alternatively, it is possible that these bacteria are simply outcompeted for vitamin uptake by *C. polykrikoides*. While it has recently been discovered that several eukaryotic phytoplankton have

inducible vitamin B_{12} transport proteins, homologs of these proteins have yet to be identified in dinoflagellates (E. Bertrand, M. Saito, Woods Hole Oceanographic Institute, pers. comm.). The ability of vitamin depleted cultures of *C. polykrikoides* to lower their half saturation constants and increase their affinity constants five-fold for B_{12} does suggest *C. polykrikoides* has an inducible mechanism to enhance its vitamin B_{12} uptake under limiting conditions. Maximum B_{12} uptake rates were the same for limited and replete cultures, supporting the notion that this species can rapidly assimilate this vitamin even under low levels while its high affinity constant values suggest that depleted cultures adapt to lower B_{12} concentrations and were more effective at utilizing this vitamin under sub-K_s conditions. Finally, it is possible that some of the vitamin uptake measured in *C. polykrikoides* size class was due to attached bacteria. However, each *C. polykrikoides* cell would need to harbor >2,000 heterotrophic bacterial cells to match the planktonic densities of the heterotrophic bacterial community and thus vitamin utilization was likely mainly due to *C. polykrikoides*. In summary, *C. polykrikoides'* ability to outcompete bacteria for vitamin B_{12} uptake may be due to its associated picoplankton community having a lower B_{12} requirement than other microbes, its ability to reduce its K_s under B_{12} deplete conditions, its high uptake rate even at low concentrations, and/or attached bacteria.

While *C. polykrikoides* displayed a large vitamin B_{12} requirement during blooms, vitamin B_1 seems to play a less important role in the bloom dynamics of this HAB, despite its absolute requirement for this vitamin (Tang et al. 2010). Although primary production rates during blooms were 10-fold higher than parallel non-bloom samples, B_1 uptake was equal or lower. Carbon specific uptake rates of vitamin B_1 in the >20µm size class inside the bloom were lower compared non-bloom plankton, indicating there was a lower B_1 requirement for *C. polykrikoides* compared to comparably sized plankton in non-bloom water. As such, vitamin B_1 seemed to be

a less important nutritional factor than B_{12} for *C. polykrikoides* blooms. Interestingly, unlike vitamin B_{12}, there was no significant difference ($p>0.05$) between the carbon specific B_1 uptake of the 0.2-2 µm size fraction between the bloom and non-bloom despite the ten-fold higher biomass (1553 ± 246 and 147 ± 20.5 µgC L^{-1}, respectively). The similarity of carbon specific B_1 uptake by picoplankton between bloom and non-bloom samples may be due to an absence of competition for assimilation of this vitamin with *C. polykrikoides* and thus supporting the hypothesis that the disproportionately lower carbon specific B_{12} uptake of picoplankton in blooms was to due competition with *C. polykrikoides*. Like *C. polykrikoides*, *A. anophagefferens* also displayed a a larger requirement for vitamin B_{12} compared to B_1 in culture but a lower requirement for vitamin B_1 compared to B_{12} during blooms (Chapter 4; Tang et al 2010) Unlike B_{12}, vitamin B_1 is used in many different metabolic pathways (Provasoli and Carlucci 1974; Swift 1980; Croft et al. 2006) and alternatives compounds found in the seawater not present in synthetic growth medium may be substituted by *A. anophagefferens* field populations. The biosynthesis of vitamins is complex and includes many precursor macromolecules (Warren et al. 2002; Begley et al. 2008). Croft et al. (2006) suggested that organisms that possess genes encoding for some of the precursor molecules for vitamins but not the entire molecule may also be able to utilize them and complete synthesis *de novo*. It has also been demonstrated that vitamin auxotrophy can vary even between strains of the same phytoplankton species isolated from the same field population on the same date (Tang et al. 2010). As such, it is possible that unlike *C. polykrikoides* clone Cp1, some strains in the field do not require vitamin B_1.

Total uptake rates of nitrogenous compounds inside *C. polykrikoides* bloom patches were similar to those previously been observed in adjacent Great Peconic Bay, NY by Gobler et al. (2012) and in Chesapeake Bay by Mulholland et al. (2009b). Not only did total nitrogen uptake

increase five-fold in blooms compared to non-bloom samples, but the relative importance of regenerated nitrogen sources (ammonium) also increased. The relative preference of the different nitrogen sources in the non-bloom community was ammonium>urea>nitrate (42, 37 and 21% of total measured nitrogen uptake). While this hierarchy remained the same in bloom water, the relative importance of ammonium increased by 20% while the relative nitrate and urea uptake decreased by 10% each indicating that *C. polykrikoides* preferred ammonium. Further evidence of *C. polykrikoides'* preference for ammonium was seen in the two-fold higher nitrogen-specific ammonium uptake rates in the bloom compared to adjacent non-bloom water, with nitrogen specific ammonium uptake in the >20 µm size fraction increasing nearly three orders of magnitude, from <0.00045 to >0.034 h^{-1}. *C. polykrikoides* has been shown to grow well on various nitrogen sources, including ammonium (Gobler et al. 2012; Kudela and Gobler 2012) and the high heterotrophic bacterial abundances in the bloom patches likely lead to the increased ammonium concentrations observed in the blooms. Not only does this alga have the ability to utilize various nitrogenous compounds (Mulholland et al. 2009b) but consistent with the findings presented in this study Gobler et al. (2012) observed that it tends to take up the most abundant source the quickest.

Nitrogen and vitamin limitation of C. polykrikoides

While nitrogen imitation of *C. polykrikoides* blooms has recently been described in eastern Long Island embayments (Gobler et al. 2012) and elsewhere (Kudela et al. 2008a), this research shows that vitamin B_{12} can also limit the proliferation of blooms. Just as vitamin B_{12} had the ability to restrict the growth of *A. anophagefferens* during the peak and demise of brown tide blooms (Chapter 4), this vitamin limited the growth of *C. polykrikoides* in multiple locations across eastern Long Island. This is consistent with culture work that measured Ks values that

fell well into the range of ambient B_{12} concentrations and the observation that cellular quotas of *C. polykrikoides* are amongst the highest measured for any harmful alga. *C. polykrikoides* blooms occur when primary production rates and vitamin utilization are highest while inorganic nitrogen concentrations are low (Chapter 3). The occurrence of a high biomass bloom of a vitamin auxotroph under such conditions accounts for vitamin B_{12} turning over several times daily during blooms and limiting the growth of *C. polykrikoides* nearly as much as nitrogen, usually the limiting nutrient in these systems (Chapter 3, Bruno et al 1983, Gobler et al. 2007, 2012). Although *C. polykrikoides* takes up B_{12} faster than other microplankton present during blooms, its large cellular demand and the more than daily turnover B_{12} during blooms likely leads to the limitation frequently observed since incubations were 24 - 48 h. In addition, the ten-fold higher abundance of heterotophic bacteria and five-fold higher picoeukaryotes likely enhances the planktonic demand for B_{12} during incubations. Hence, despite the efficient use of B_{12} by *C. polykrikoides*, when isolated from some ambient vitamin sources during incubations, bloom communities were likely to have quickly exhausted the B_{12} supply. In the field, this species undergoes intense diel vertical migration (Kudela and Gobler 2012), behavior that has been linked to nutrient acquisition by other dinoflagellates (Holmes et al. 1967; Tyler and Seliger 1981). Intense microbial activity likely make sediments a rich source of vitamin B_{12} (Qingzhi et al. 2011) and, as such, *C. polykrikoides* may partially fill some of its vitamin quota during migrations to the benthos. Similarly, the low B_1 utilization may also be related to a preferential fulfillment of B_1 requirement near the sediment water interface.

Conclusion

C. polykrikoides blooms photosynthesize at high rates, outcompete all other plankton for vitamin B_{12}, typically the limiting vitamin (Chapters 3, 4), and more rapidly assimilate

ammonium than other plankton. These attributes provide this harmful algal with a competitive edge that contributes likely toward bloom persistence. While the presence of *C. polykrikoides* has been shown to alter the microplankton community via the release of allelopathic and toxic compounds, this study demonstrates that *C. polykrikoides* harbors its own picoplanktonic consortium including picoeukaryotes and heterotrophic bacteria with lower requirements for vitamin B_{12}. *C. polykrikoides* blooms turnover labile nitrogen species (ammonium, nitrate, and urea) and vitamin B_{12} in the bloom in 1.5 and <8 h respectively, compared to 14h and >10 days, respectively, outside the bloom, making acquisition of these limiting elements more difficult for other plankton. *C. polykrikoides* may rely its ability to vertically migrate and a B_{12} producing and nitrogen re-mineralizing, microbial community to replenish its nitrogen and vitamin requirements. In conclusion, the presence of *C. polykrikoides* blooms fundamentally changes the microbial community and resource cycling of the coastal marine ecosystem.

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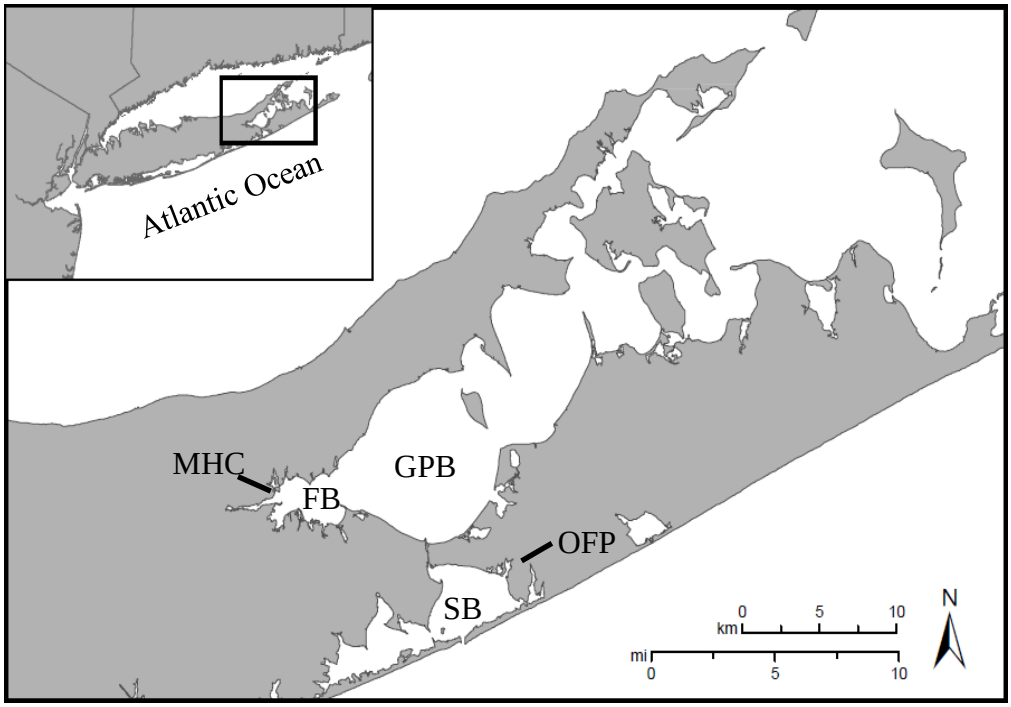

Figure 1. Map of eastern Long Island showing sites sampled during the 2009/2010 blooms of *C. polykrikoides* .

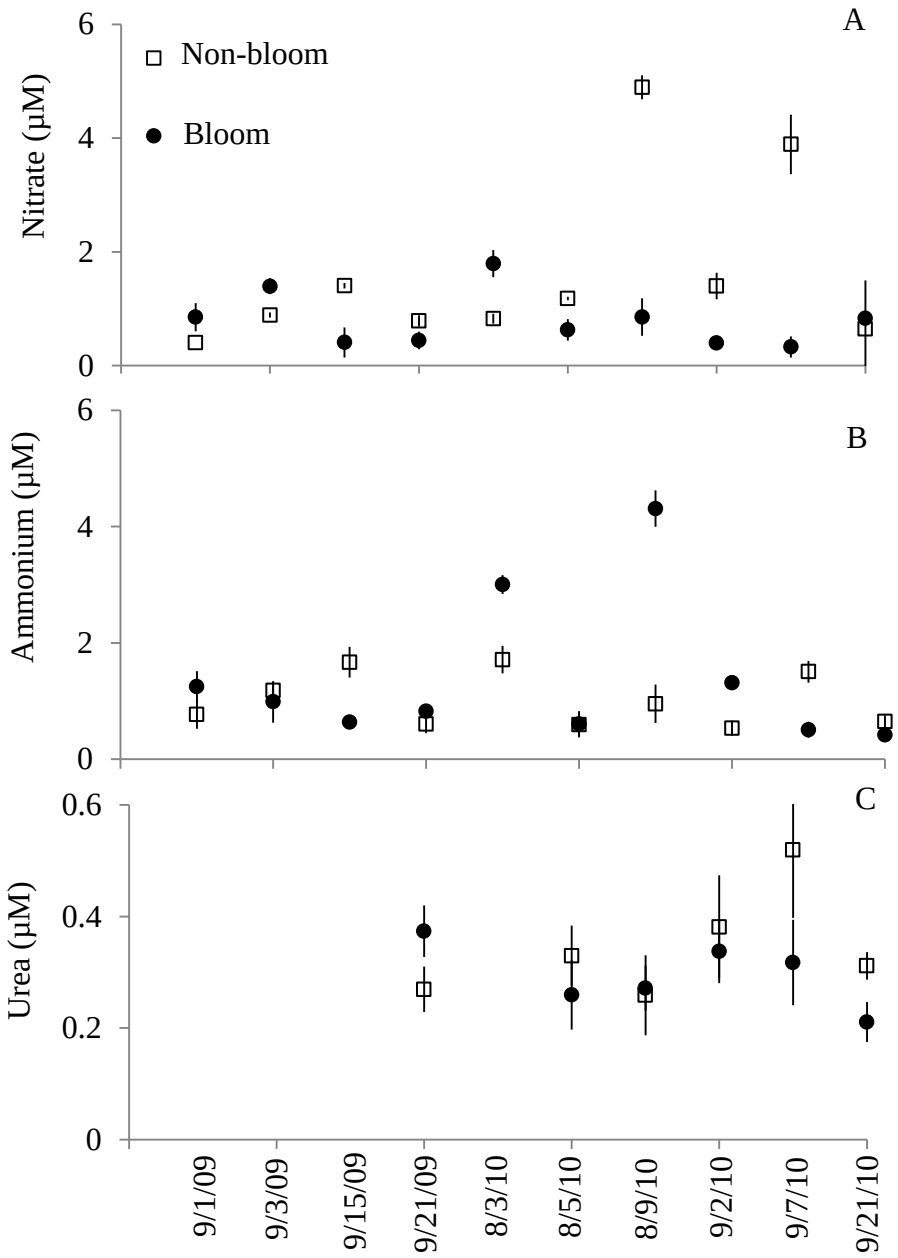

Figure 2. Nitrate (A), ammonium (B) and urea (C) concentrations inside (solid circles) and adjacent (open squares) to *C. polykrikoides* blooms during 2009/2010. Concentrations are in µM and samples were collected from Shinnecock Bay.

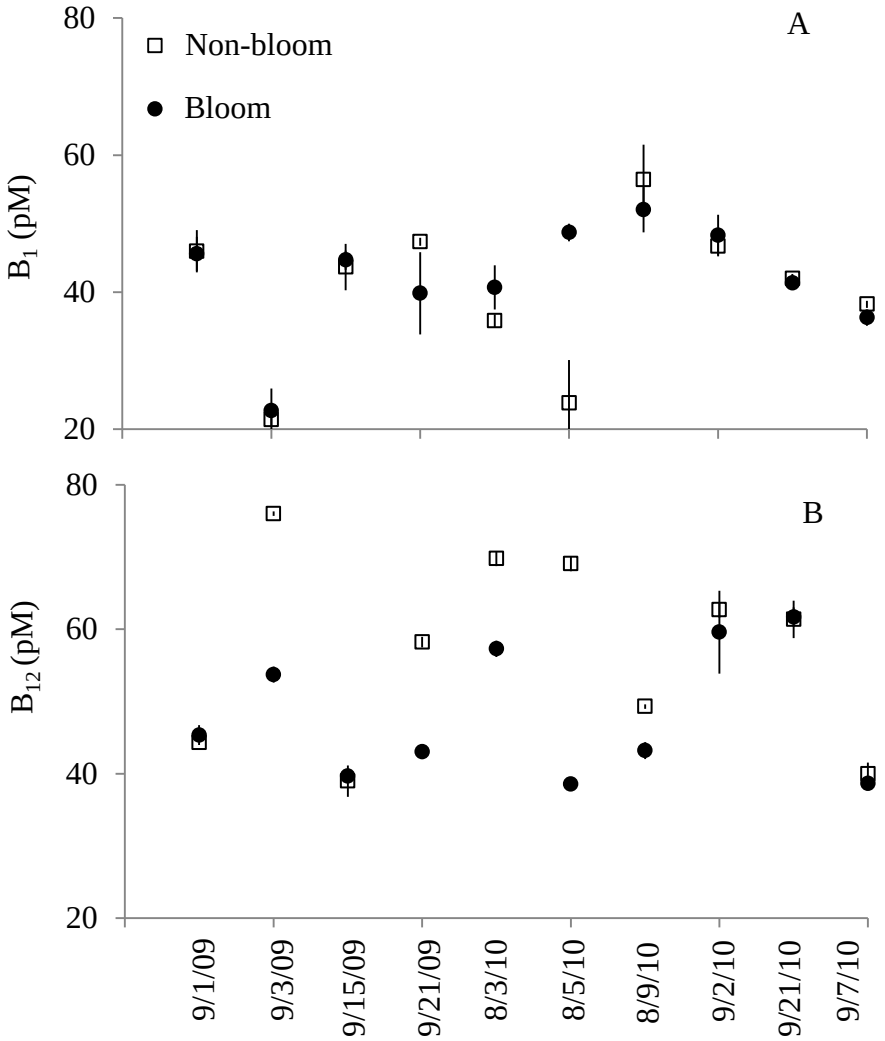

Figure 3. Vitamin B_1 (A) and B_{12} (B) concentrations inside (solid circles) and adjacent (open squares) to *C. polykrikoides* blooms during 2009/2010. Concentrations are in pM and with one exception B_1 concentrations did not differ significantly ($p<0.05$) between patch and non-patch. In contrast B_{12} concentrations were significantly lower inside the patches a majority of the time (5 of 9 sampling times). All samples were collected from Shinnecock Bay.

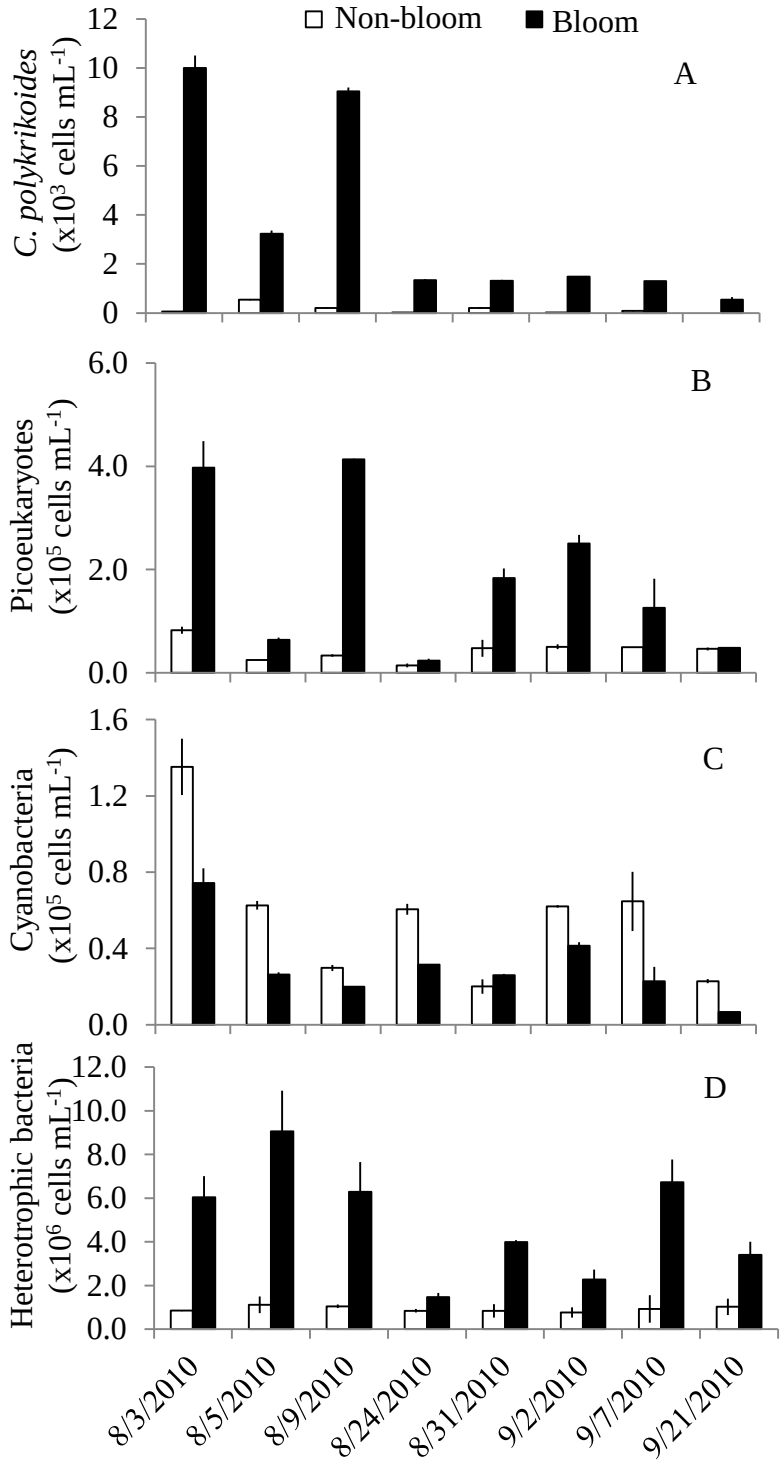

Figure 4. Plankton distribution inside and outside of *C. polykrikoides* blooms Shinnecock Bay, NY, during 2010. Samples are depicted as mean ± standard deviation (n=3).

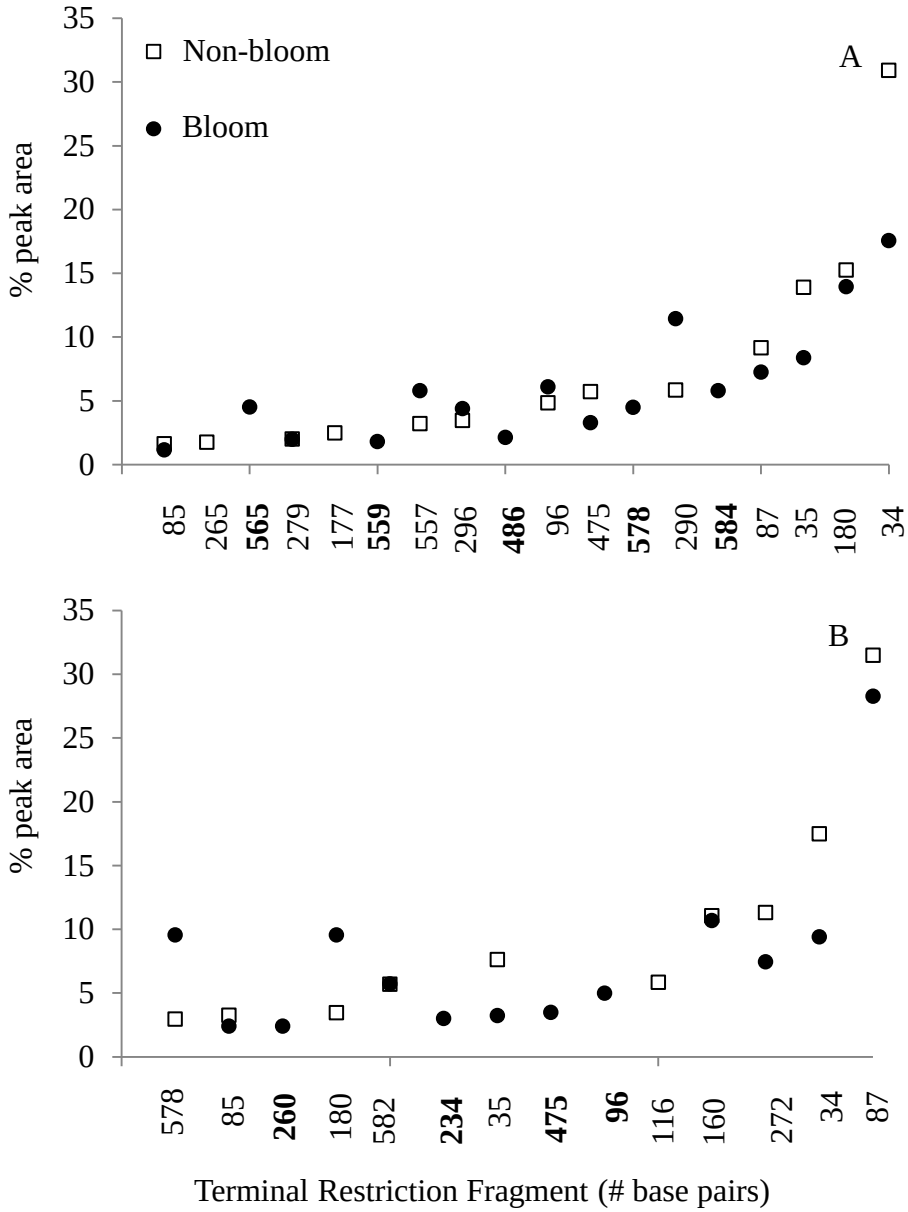

Figure 5. Terminal Restriction Fragment Polymorphism (T-RFLP) analysis of bloom (dark circles) and non-bloom (open squares) water collected on 8 August, 2011 from SB. The y-axis represents the relative peak area presumably indicative of the relative abundance of each TRF while the x-axis shows TRFs based on increasing base pair in length. Data is for the 16S rRNA gene of the total (A) and >5µm community. Bold TRFs denote those fragments which were found only in patch water.

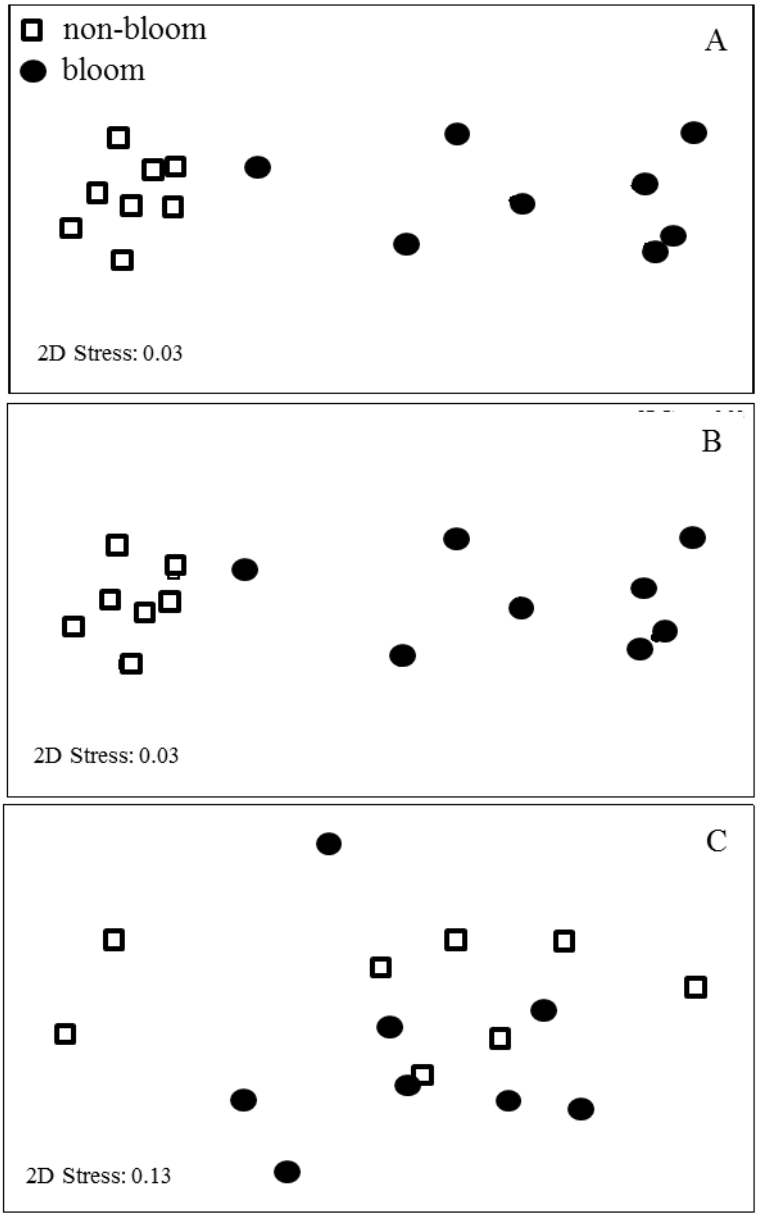

Figure 6. Multiple dimensional scaling (MDS) plot of plankton community composition during the 2009/2010 *C. polykrikoides* blooms as quantified via microscopy and flow cytometry. Two dimensional ordinates produced from 4th-root transformed, Bray-Curtis similarity matrices are presented with samples delineated by bloom (dark circles) and non-bloom (open squares). In addition to the MDS plot for the total plankton community (A), plots for the plankton community without *C. polykrikoides* (B) and without *C. polykrikoides* and picoplankton (C) are also shown. 'Stress' indicates how faithfully the high-dimensional relationships among the samlples are represented in the 2-d orientation plots.

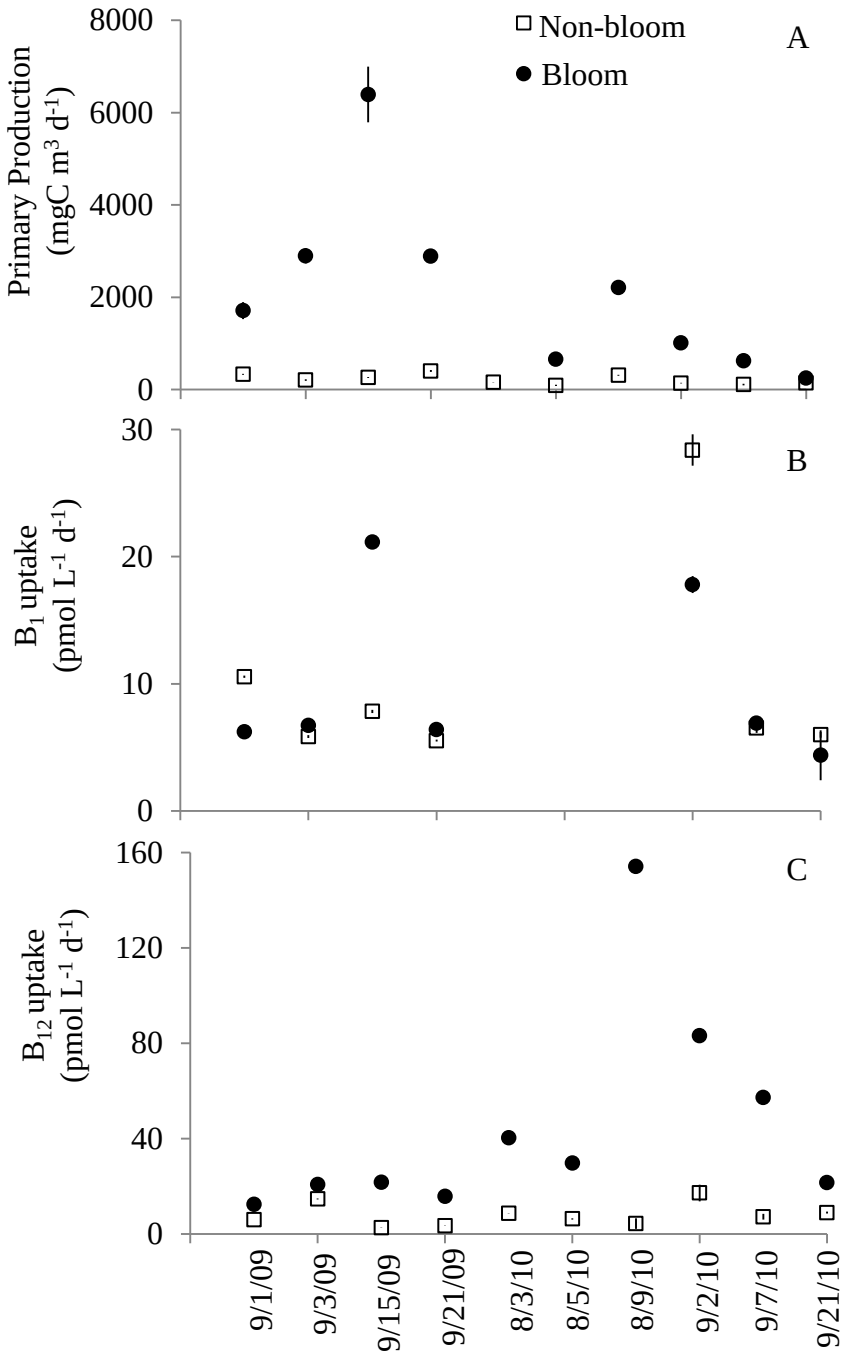

Figure 7. Rates of total primary production (A), and vitamins B_1 (B) and B_{12} (C) uptake rates inside of (solid circles) and adjacent (open squares) to *C. polykrikoides* blooms during the 2009/2010 bloom seasons. All rate measurements represent the mean ± standard deviation (n=3).

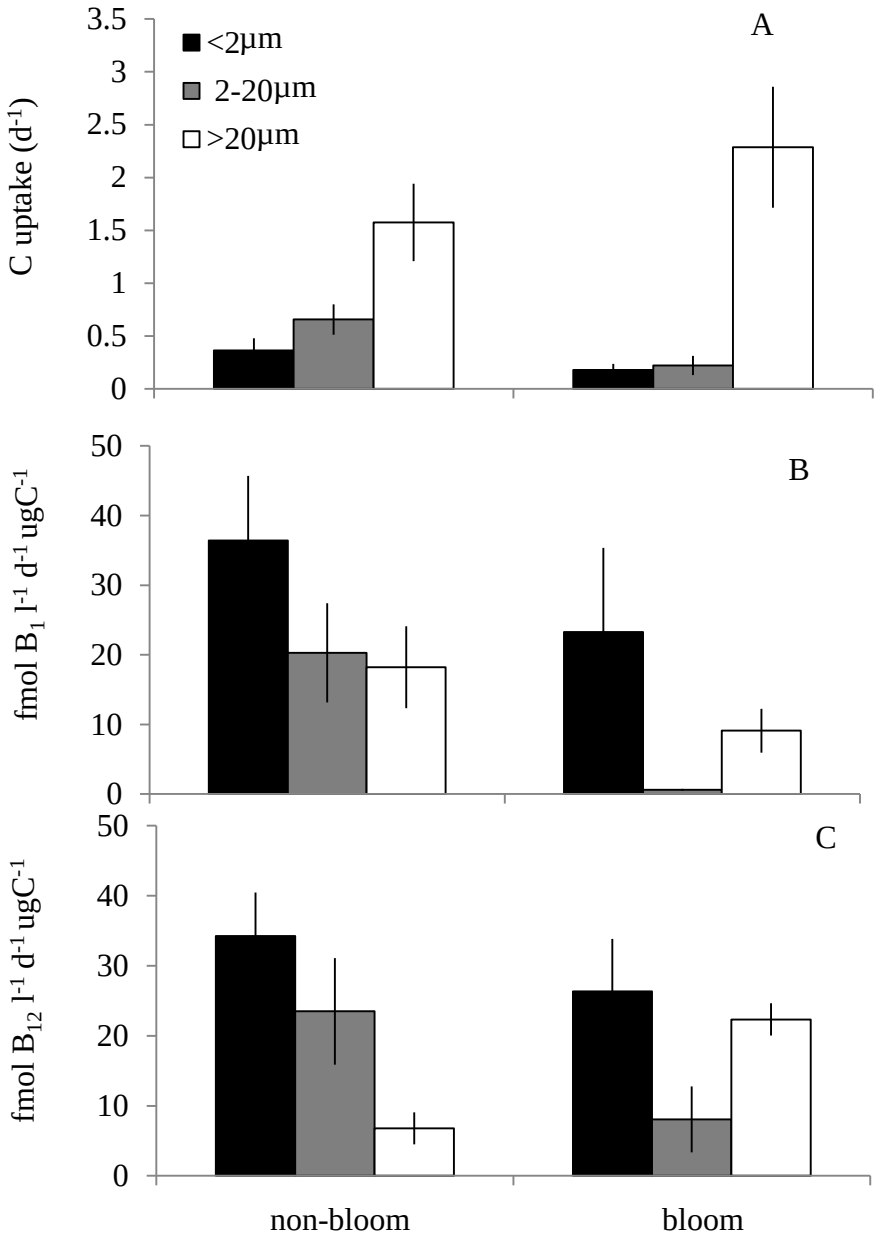

Figure 8. Carbon-specific vitamin Primary production (A), B_1 (B) and B_{12} (C) uptake rates for three size fractions of plankton within bloom and non-bloom water. Bars are the mean ± standard error from all available uptake data for the 2009 and 2010 bloom season (n=13).

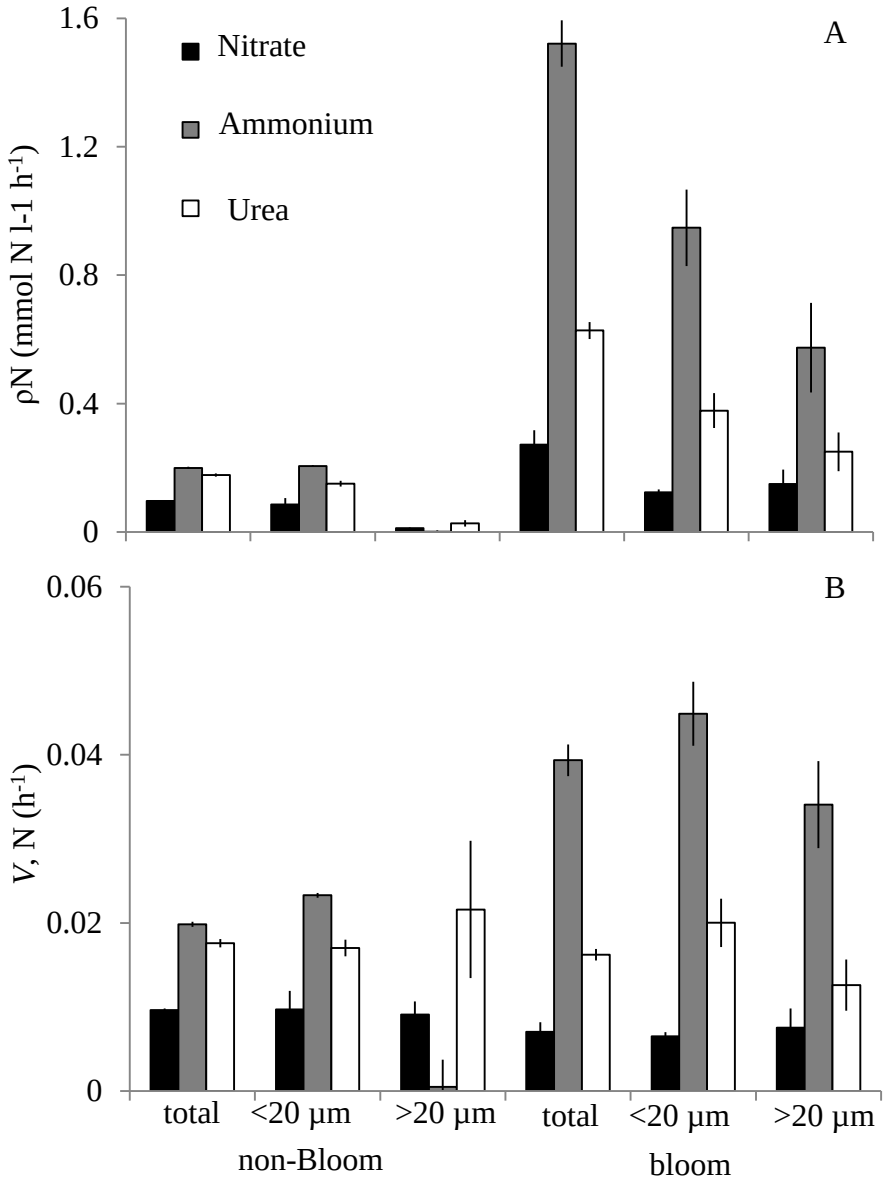

Figure 9. Absolute (A) and nitrogen-specific (B) uptake rates of various nitrogenous compounds within *C. polykrikoides* bloom water and water collected adjacent to the bloom on 9/2/2010. Values represent mean ± standard deviation (n=3).

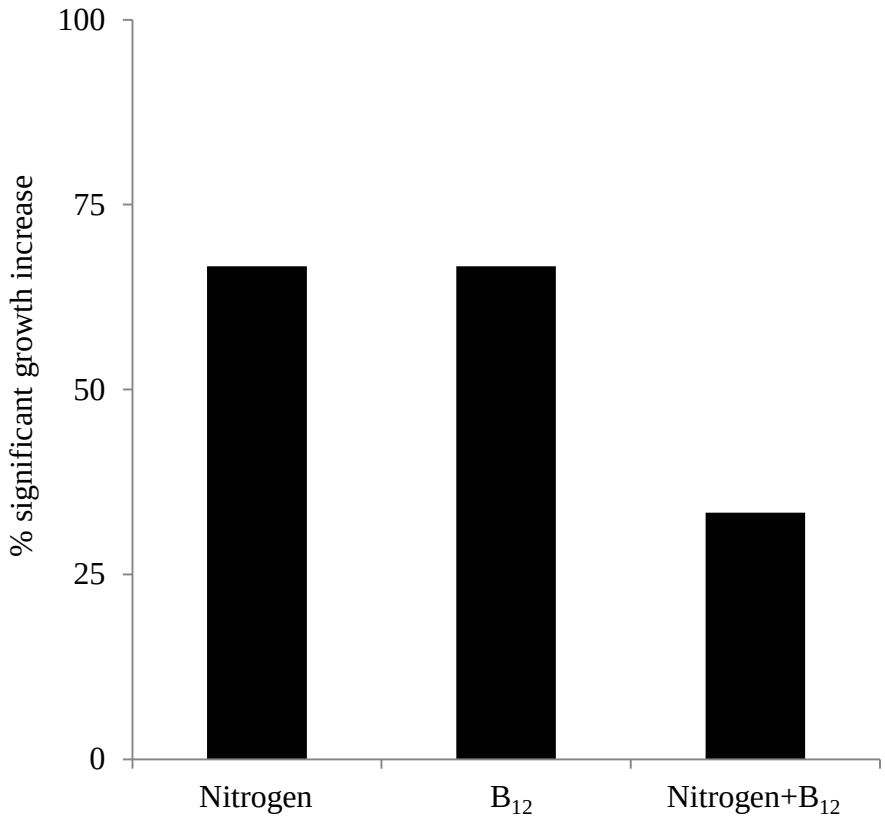

Figure 10. Summary of vitamin and nitrogen amendment experiments showing the percent of positive responses for each treatment for all experiments (n=15). A response for nitrogen+B_{12} was deemed significant when the growth rate was greater ($p>0.05$) over the B_{12} or nitrogen treatments alone as determined by an ANOVA.

Table 1. Physical and chemical parameters non-bloom and bloom water during the 2009/2010 *C. polykrikoides* (CP) blooms. Temp denotes temperature in degrees Celsius, while DO is dissolved oxygen and is shown as mg L^{-1}. For dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and vitamin concentrations, values shown represent the mean ± standard deviation.

		Temp	Salinity	DO	DIN	DON	B_1	B_{12}	CP
		°C		mgL^{-1}	µM	µM	pM	pM	cells mL^{-1}
9/1/2009	non-bloom	28.2	22.5	6.30	1.17 ± 0.25		45.7 ± 2.45	44.3 ± 1.37	52 ± 5
	bloom	28.3	22.8	7.41	2.10 ± 0.26		45.6 ± 3.08	45.3 ± 3.65	4567 ± 109
9/3/2009	non-bloom	28.1	22.9	6.50	2.07 ± 0.14		21.4 ± 3.27	76.1 ± 1.12	245 ± 28
	bloom	28.3	22.8	7.97	2.38 ± 0.43		22.7 ± 1.04	53.7 ± 2.16	7080 ± 134
9/7/2009	non-bloom	29.1	21.8	6.90	2.14 ± 0.35				37 ± 17
	bloom	29.2	21.8	8.90	2.13 ± 0.18				2472 ± 160
9/15/2009	non-bloom	28.9	21.0	8.58	3.07 ± 0.38		43.7 ± 1.41	38.9 ± 0.14	13 ± 3
	bloom	29.0	21.3	8.69	1.05 ± 0.17		44.7 ± 3.37	39.9 ± 0.72	3637 ± 42
9/21/2009	non-bloom	27.9	21.1	8.54	1.39 ± 0.17		47.4 ± 5.99	58.2 ± 1.01	264 ± 28
	bloom	28.1	20.9	9.90	1.26 ± 0.13		39.9 ± 0.60	43.1 ± 1.04	1813 ± 35
8/3/2010	non-bloom	28.6	24.6	6.50	2.54 ± 0.25	18.1 ± 4.69	35.8 ± 3.23	69.8 ± 1.14	58 ± 4
	bloom	28.4	24.4	7.25	2.79 ± 0.48	14.3 ± 2.88	40.7 ± 0.95	57.3 ± 1.04	10000 ± 301
8/5/2010	non-bloom	28.4	25.6	6.68	1.77 ± 0.28	46.8 ± 4.82	23.8 ± 1.29	69.1 ± 0.90	540 ± 35
	bloom	28.5	26.0	7.78	1.23 ± 0.31	23.8 ± 3.87	48.7 ± 6.31	38.5 ± 0.32	3235 ± 125
8/9/2010	non-bloom	28.8	25.8	8.08	5.84 ± 0.40	30.1 ± 2.18	56.4 ± 3.29	43.2 ± 1.17	199 ± 15
	bloom	28.7	25.9	9.94	5.16 ± 0.36	29.5 ± 2.89	52.0 ± 5.10	49.3 ± 1.07	9046 ± 157
8/29/2010	non-bloom	29.3	22.4	5.72	2.14 ± 0.18	32.6 ± 8.97			23 ± 5
	bloom	29.4	22.2	6.26	2.38 ± 0.31	16.1 ± 11.2			1340 ± 41
8/31/2010	non-bloom	30.6	23.7	6.68	1.73 ± 0.15	13.3 ± 3.25			210 ± 38
	bloom	30.5	23.5	7.28	1.05 ± 0.19	18.0 ± 1.93			1315 ± 26
9/2/2010	non-bloom	28.4	24.2	5.03	1.93 ± 0.54		46.7 ± 3.04	62.7 ± 5.73	19 ± 1
	bloom	28.3	24.1	5.07	1.71 ± 0.21	13.4 ± 1.11	48.3 ± 1.33	59.6 ± 2.61	1480 ± 28
9/7/2010	non-bloom	29.6	21.8	6.96	5.39 ± 0.87	12.9 ± 0.95	42.0 ± 0.66	61.4 ± 1.35	85 ± 21
	bloom	29.7	23.1	7.61	4.84 ± 0.14	16.7 ± 2.37	41.4 ± 0.66	61.7 ± 1.58	1295 ± 7
9/21/2010	non-bloom	30.3	19.4	5.48	1.29 ± 0.16	38.9 ± 0.38	38.3 ± 1.19	39.7 ± 0.31	0
	bloom	30.2	19.5	8.90	1.24 ± 0.16	19.1 ± 4.18	36.3 ± 0.51	38.6 ± 0.28	545 ± 65

Table 2. Average concentrations of macronutrients and vitamins for non-bloom and bloom water during the 2009/2010 *C. polykrikoides* blooms. Values shown are mean ± standard error for each constituent for all 2009 and 2010 sampling dates (n= 13)

	Nitrate µM	Ammonium µM	Urea µM	Phosphate µM	Silicate µM	B_1 pM	B_{12} pM
NB	1.74 ± 0.59	0.96 ± 0.19	0.34 ± 0.03	0.84 ± 0.11	32.3 ± 6.60	40.1 ± 3.87	56.4 ± 4.57
B	0.73 ± 0.17	1.58 ± 0.49	0.37 ± 0.04	0.77 ± 0.09	33.5 ± 6.49	42.0 ± 2.12	48.6 ± 3.67

Table 3. Microplankton composition outside (NB) and inside (B) *C. polykrikoides* blooms during 2009/2010. Values are in cells mL^{-1}. ANAN refers to autotrophic-nanoflagellates.

		Diatoms						Dinoflagellates					Ciliates			ANAN
		Guinardia	*Thalassiosira*	*Chaetoceros*	*Rizozolenia*	*Asteriolenopsis*	*Nitszchia*	*Girodinium*	*Prorocentrum*	*Scripsiella*	*Cochlodinium*	*Others*	*Strombidium*	*Strobilidium*	*Tinntinds*	
9/1/2009	NB	0	88	200	0	0	308	0	84	36	52	20	0	44	4	1120
	B	0	0	433	0	493	673	0	100	107	4567	0	0	0	0	773
9/3/2009	NB	0	19	0	0	360	0	0	31	23	245	0	0	30	0	300
	B	0	14	0	0	424	0	0	24	12	7080	0	0	13	0	528
9/7/2009	NB	0	1	14	0	0	1	8	8	8	37	6	0	12	2	51
	B	0	3	12	0	0	1	4	6	1	2472	0	1	5	0	41
9/15/2009	NB	5	0	25	0	10	9	8	11	7	13	7	0	23	0	325
	B	0	2	19	0	7	6	4	11	0	3637	3	0	16	0	299
9/21/2009	NB	213	0	108	0	26	0	0	0	0	264	9	0	4	9	582
	B	175	0	98	0	14	17	0	0	0	1813	0	0	1	0	541
8/3/2010	NB	0	0	0	0	0	0	0	0	0	58	5	0	0	3	0
	B	0	0	0	0	0	0	85	0	0	10000	120	0	0	3	90
8/5/2010	NB	0	0	0	0	0	0	0	39	0	540	75	0	0	2	395
	B	0	0	0	0	0	0	0	20	0	3235	150	0	0	0	0
8/9/2010	NB	0	0	0	0	0	0	1	7	9	199	0	0	5	7	440
	B	0	0	0	0	0	0	0	180	0	9047	0	0	0	0	227
8/29/2010	NB	0	0	0	0	0	0	0	0	0	23	12	0	15	0	0
	B	0	0	0	0	0	0	0	0	0	1340	225	0	0	0	250
8/31/2010	NB	390	0	0	0	275	0	95	195	60	210	160	85	0	0	590
	B	0	45	0	0	0	0	0	395	0	1315	490	0	0	0	0
9/2/2010	NB	0	0	0	0	0	0	15	0	0	19	0	0	0	0	16
	B	0	0	125	0	0	0	0	0	0	1480	0	0	0	0	205
9/7/2010	NB	315	0	0	0	0	0	70	55	0	85	0	60	0	0	875
	B	0	0	0	0	0	0	205	0	0	1295	55	990	0	0	0
9/21/2010	NB	40	0	0	0	0	0	3	0	23	0	0	37	0	0	57
	B	0	0	0	0	0	0	0	0	17	545	0	24	0	17	168

Table 4. Mean total primary production, and B_1 and B_{12} uptake rates as well as B_1 (τB_1) and B_{12} (τB_{12}) turnover times in days for non-bloom (NB) and bloom (B) samples collected during the 2009-2010 *C. polykrikoides* bloom seasons. Numbers represent means ± standard error for all sampling dates (n=13) from 2009 and 2010.

	Primary Production mgC m^{-3} d^{-1}	B_1 uptake pmol L^{-1} d^{-1}	τ B_1 days	B_{12} uptake pmol L^{-1} d^{-1}	τ B_{12} days
NB	215 ± 33.5	10.1 ± 2.61	5.83 ± 0.62	7.91 ± 1.49	10.3 ± 1.36
B	2070 ± 599	9.95 ± 2.09	4.99 ± 0.80	45.7 ± 9.95	1.53 ± 0.22

Table 5. Results of nutrient amendment experiments conducted using *C. polykrikoides* bloom water during the 2005-2008. Shown are the mean ± standard deviation of net growth rates (μ) as d^{-1} for each treatment. The treatments consisted of 10 μM NH_4^+, 100 pM vitamin B_{12} and a combination of the two. Bloom water was collected from two hypereutrophic systems, Old Fort Pond (OFP) and Meetinghouse Creek (MHC), and three open embayments, Flanders Bay (FB), Shinnecock Bay (SB) and Great Peconic Bay (GPB). Bold numbers denote a significant ($p<0.05$) treatment effect over the control for NH_4 and B_{12} while for the NH_4+B_{12} treatment it signifies a greater effect than expected for each constituent by itself.

DATE	Site	Control	NH_4	B_{12}	NH_4+B_{12}
8/30/2005	OFP	-0.09 ± 0.35	**0.63 ± 0.21**	**0.65 ± 0.04**	0.74 ± 0.02
9/13/2005	OFP	-0.001 ± 0.04	0.06 ± 0.04	0.01 ± 0.06	**0.09 ± 0.02**
9/27/2005	OFP	-0.23 ± 0.16	**0.32 ± 0.04**	**0.21 ± 0.05**	**0.63 ± 0.07**
8/30/2007	OFP	0.33 ± 0.02	**0.43 ± 0.01**	**0.62 ± 0.05**	0.17 ± 0.03
9/5/2006	MHC	0.13 ± 0.05	----	**0.45 ± 0.09**	----
8/30/2007	MHC	0.13 ± 0.03	**1.08 ± 0.03**	**1.15 ± 0.05**	----
10/3/2008	MHC	0.59 ± 0.05	**0.88 ± 0.11**	----	0.66 ± 0.53
8/23/2006	FB	-0.08 ± 0.01	**0.13 ± 0.05**	0.11 ± 0.11	0.10 ± 0.02
9/11/2006	FB	-0.27 ± 0.13	0.17 ± 0.10	0.09 ± 0.14	----
8/22/2007	FB	-0.09 ± 0.03	**0.14 ± 0.01**	**0.11 ± 0.04**	0.12 ± 0.01
9/29/2008	FB	0.14 ± 0.02	**0.42 ± 0.11**	----	0.40 ± 0.01
9/24/2008	FB	0.20 ± 0.08	0.15 ± 0.09	----	**0.31 ± 0.12**
8/25/2008	SB	0.06 ± 0.04	**0.12 ± 0.04**	----	0.01 ± 0.09
9/5/2008	SB	0.05 ± 0.02	0.02 ± 0.04	----	0.09 ± 0.01
9/4/2008	GPB	-0.06 ± 0.07	**0.18 ± 0.06**	----	**0.27 ± 0.04**

Table 6. Vitamin B_{12} uptake kinetics and cellular quota ranges for cultures of *C. polykrikoides* grown under B_{12} replete (B_{12} +) and depleted (B_{12} -) conditions.

	Cellular Quota	V_{max}	K_s	r^2	α
	pmol cell^{-1}	pmol cell^{-1} h^{-1}	pM		h^{-1}
B_{12} +	0.52 - 1.13 x 10^{-6}	3.20 ± 0.13 x 10^{-6}	41.5 ± 5.02	0.98	0.02
B_{12} -	0.08 - 1.16 x 10^{-6}	2.86 ± 0.18 x 10^{-6}	7.96 ± 1.42	0.92	0.10

Chapter Six

Dissertation summary and conclusions

During the middle of the last century, B-vitamins were recognized as important growth factors for some species of phytoplankton (Droop 1955; Droop 1957; Droop 1958; Ford 1958; Provasoli 1958). Cobalamin (B_{12}) is required for the biosynthesis of methionine, thiamine (B_1) facilitates the decarboxylation of pyruvic acid in the Calvin cycle and biotin (B_7) plays a critical role in fatty acid synthesis. In the following two decades, the oceanographic community focused their attention on the role of vitamins in the ocean by surveying various coastal (Vishniac and Riley 1961; Ohwada and Taga 1972; Cattell 1973; Bruno and Staker 1978), and open ocean (Menzel and Spaeth 1962; Natarajan and Dugdale 1966; Propp 1970; Swift 1972; Carlucci and Cuhel 1975) environments. Droop explored nutrient uptake mechanisms and kinetics not using one of the macronutrients, such as nitrate or phosphate, but rather using vitamin B_{12} as a model nutrient (Droop 1961; Droop 1968; Droop 1970). A multitude of studies of hundreds of phytoplankton species demonstrated that a majority of microalgae had an obligate requirement for an exogenous source of one or more of the B-vitamins, making the majority of phytoplankton B-vitamin auxotrophs (Provasoli and Carlucci 1974). In addition, some culture experiments explored the importance of bacteria in supplying B-vitamins to phytoplankton. Provasoli (1963) and Fogg (1966) observed that B_{12} auxotrophic phytoplankton grew better in the presence of bacteria than in the axenic, B_{12}-depleted media. Consistent with this concept, a later study demonstrated that the B_{12} auxotrophic red algae *Porphyridium purpureum* could be grown in B_{12}-deficient media in the presence of the marine bacteria *Halomonas* sp. (Croft et al. 2005). Taylor and Sullivan (2008) also argued that the microbial communities in sea ice are likely a source of vitamins to its algal inhabitants since melt-water from the ice had 40-fold higher B_{12} concentrations than seawater. Besides being directly supplied by prokaryotes, other sources of

vitamins to phytoplankton likely include viral lysis and zooplankton grazing.(Provasoli 1963; Croft et al. 2006; Taylor and Sullivan 2008).

Two factors likely contributed to the relative dearth of continued research on the role of vitamins in algal ecology from 1970s - 2004: 1) The lack of a method to accurately, precisely and directly measure vitamins in seawater – measurements prior to 2004 were made using laborious and error-prone bioassays to estimate concentrations (Provasoli and Carlucci 1974; Sharma et al. 1979; Swift 1980), and 2) The hypothesis that the cellular quotas of phytoplankton were small and ambient concentrations (surveys before 2004; Table 1) sufficient to meet their estimated requirements, rendering B-vitamins ecologically irrelevant (Droop 2007). For example, the handful of half saturation constants (Ks; Droop 2007) available (0.1 - 8.7 pM mean of 1.7; Table 1) were most often below the range measured in the ocean (1-12 pM), further supporting the notion of a vitamin-replete oceanic plankton community. The development of methods to measure vitamins B_1 and B_{12} in seawater directly (Okbamichael and Sanudo-Wilhelmy 2004; Okbamichael and Sanudo-Wilhelmy 2005) and evidence that the addition of vitamins can limit or co-limit phytoplankton in the coastal and open ocean systems (Panzeca et al. 2006; Sanudo-Wilhelmy et al. 2006; Bertrand et al. 2007) lead to a renewed interest in these cofactors. Other recent studies have investigated the concentrations vitamin B_{12} across the World's Oceans (Panzeca et al. 2009) and the role of prokaryotes in supplying vitamins to the water column (Panzeca et al. 2008; Bonnet et al. 2010; Bertrand et al. 2011). Vitamin utilization by the plankton community, however, had been assessed only in one lake and in sea-ice (Parker 1977; Taylor and Sullivan 2008). As such, the seasonal dynamics of vitamin concentration and utilization by plankton and the impact of these micronutrients on plankton succession in the marine environment were unexplored. Finally, although B-vitamin have been implicated as a

causative factor in some harmful algal blooms or HABs (Aldrich 1962; Hunter and Provasoli 1964; Stewart et al. 1967), the role of vitamins in the ecology of vitamin-auxotrophic harmful algal bloom species (HABs) had never been established.

This dissertation strove to elucidate the extent to which B-vitamins influence phytoplankton community growth, composition, and succession in marine ecosystems. My research specifically focused on vitamin utilization and primary production by plankton communities and the role of vitamins in the occurrence of high biomass HABs, using as models two B-vitamin auxotrophic HAB species, the pelagophyte *Aureococcus anophagefferens* and the dinoflagellate *Cochlodinium polykrikoides*. In addition to field studies, I evaluated the cellular requirement and kinetics of B-vitamins for growth and uptake of these two harmful algae. My general approach included the use of novel, radiolabeled tracers to measure size fractionated uptake of B-vitamins (^{57}Co-B_{12} and ^{3}H-B_1) and primary production (^{14}C-bicarbonate) in conjunction with a detailed characterization of the plankton community. The ability to measure vitamin concentrations directly allowed for the first ever direct measurements of vitamin uptake in pelagic marine environments.

Collectively my dissertation demonstrated that B-vitamins are actively utilized by the plankton community with the highest uptake rate being observed during times of peak primary production. For all regions surveyed in the Gulf of Alaska (Chapter 2) across estuaries on Long Island, NY (Chapter 3), picoplankton (<2 µm) were responsible for the majority of vitamin uptake. Combined with concurrent ancillary data, this suggested that prokaryotes, especially heterotrophic bacteria, are not only the primary source of B-vitamins to marine environments but are also the main utilizers. Vitamin amendment experiments (Chapters 2-5) revealed that vitamin B_{12} can alter plankton community composition and limit the growth of larger, eukaryotic

phytoplankton in a variety of ecosystems from the HNLC North Pacific to hypereutrophic coastal systems of NY. Together these observations implied that smaller cells with larger surface area-to-volume ratios likely have better access to the low, picomolar concentrations of vitamins and thus contribute toward the vitamin B_{12} limitation of larger phytoplankton. In addition the presence of vitamin auxotrophic harmful algae greatly increased the ambient vitamin demand (Chapters 4, 5) sometimes to the point where their growth was limited by the availability of vitamins B_{12} when isolated from their coastal ecosystems during incubation experiments. While Tang et al. (2010) highlighted the much higher incidence of vitamin auxotrophy among harmful algae my dissertation quantified the high B-vitamin requirements of harmful algae. B-vitamins, especially B_{12}, are key nutrients that can exert competitive pressure on various plankton groups and thus, like other nutrients (N or P), should be considered in plankton ecology and biogeochemical and ecological models.

As previously described, research on the role of vitamins in marine ecology initially peaked in the 1960s. Although there were a number of culture studies at this time, notably growth kinetics work by Droop (1961; 1968; 1970), much research focused on performing vitamin surveys in various marine environments. Vitamin concentrations, mostly B_{12}, were estimated via bioassays, a laborious and error prone process involving the use of various vitamin-auxotrophic organisms in grow-out experiments. Estimates all showed sub-picomolar to picomolar concentrations ranging from 0.01pM in the oligotrophic Sargasso Sea to 11.8 pM in mesotrophic Long Island Sound (Table 1). Even though these values represent estimates via bioassay they are within the range of values recently reported via direct measurement, indicating that, although the methodologies of bioassays are laborious and potentially error-prone, they are generally a robust way to estimate vitamin concentrations in marine systems. This includes low

concentrations observed by Panzeca et al. (2009) and Koch et al. (2011) in the North Atlantic and North Pacific with concentrations ranging from 0.2-4 pM and 0.1-3 pM, respectively and high (>250 pM, Chapter 3) concentrations reported in coastal regions, especially within Long Island, NY's south shore estuaries. Measurements of vitamins within Long Island Sound in the 1960's via bioassay (Vishniac and Riley 1961) were quite similar to values measured by Panzeca (2009) and this dissertation (Chapter 3). The data generated to date regarding B-vitamin concentrations (Table 1) indicate the existence of a geographical vitamin gradient with highest values found in coastal areas and decreasing concentrations away from land. Since coastal systems, although comprising only 8% of the world's ocean surface, account for >25% of the oceans primary productivity and vitamin concentrations have been shown to be significantly correlated with primary productivity (Chapter 3), this trend is not surprising. An intermediary agent in this process is likely heterotrophic bacteria that are present at higher abundances in coastal systems compared to the open ocean (Chapter 2, 3). Gobler et al. (2007) reported that, during the summer, vitamin B_{12} concentrations in a hypereutrophic embayment (Old Fort Pond, Chapter 3) were positively correlated with heterotrophic bacterial abundances. To date, the genes necessary to synthesize B_{12} have been found only in prokaryotes (Palenik et al. 2003; Rocap et al. 2003; Vitreschak et al. 2003; Bertrand et al. 2011), suggesting that the abundance of heterotrophic bacteria and other prokaryotes should play a key role on the supply of vitamins and thus ambient concentrations.

The Gulf of Alaska broke from the trend of high vitamin B_{12} concentrations in coastal zones as, along with nitrate and phosphate concentrations, vitamin B_{12} levels were lowest in coastal stations and highest in HNLC stations in this region (Chapter 2). This was likely due to iron limiting primary production in this HNLC region, a scenarios that leads to a subsequent

buildup of other nutrients, including vitamin B_{12}, that were unused by the algal community. Turnover times for vitamin B_{12} in the HNLC zone (5.5 days) were, however, more rapid than at coastal sites (>11 days), but similar to the duration of incubation experiments during which vitamin B_{12} limited the growth of phytoplankton in the Gulf of Alaska. Collectively, that work demonstrated that bacteria dominated the cycling of this micronutrient, accounting for its ability to limit the growth of larger phytoplankton in this region (Chapter 2).

Vitamin B_1 concentrations were, on average, higher than B_{12}, ranging from 0.1 pM during winter in Long Island Sound (Chapter 3) to 169 pM in Quntuck Bay in the summer (Chapter 4) and consistent with estimates from 35 years ago (Vishniac and Riley 1961; Ohwada and Taga 1972; Swift 1973; Carlucci and Cuhel 1975). The clearest relationship between concentrations and utilization of B_1 was observed during the 2009 brown tide bloom in Quantuck Bay when concentrations of B_1 fell from 169 pM to <7 pM as densities of *A. anophagefferens* climbed to > 1.25 x 10^6 cells mL^{-1} (Chapter 4). The lack of correlation between B_1 concentrations and uptake and the sundry biological, chemical and physical parameters measured during this study was puzzling. One possible explanation is that the incidence of B_1 auxotrophy among phytoplankton is much lower compared to B_{12} (22% vs. 51%, respectively; Croft et al. 2006) which might also account for the higher ambient concentrations, relative to vitamin B_{12}. Vitamin B_1 apparently is a less important resource for many phytoplankton. This was most clearly demonstrated during blooms of *C. polykrikoides* during which many nutrients, including vitamin B_{12}, were cycled at a significantly faster rate compared to regions outside of blooms. In contrast, vitamin B_1 concentrations and uptake rates were unchanged. In contrast, however, the steady depletion of B_1 during the brown tide and the uptake of vitamin B_1

exclusively within the *A. anophagefferens* size fraction during the bloom suggested that this nutrient can be important at least for some auxotrophic harmful algae.

This dissertation is the first comprehensive study of vitamin utilization in the marine environment, generating the first direct uptake measurements of both vitamin B_1 and B_{12} (Fig. 1, 2, Table 2). Previous studies have inferred vitamin utilization by plankton by the disappearance of the ambient vitamin pool or the presence/absence of confirmed vitamin-auxotrophs (Menzel and Spaeth 1962; Gobler et al. 2007). This approach in the absence of measuring rates is flawed since the ambient pool represents the sum of production and removal. In addition, vitamin auxotrophy has been shown to vary greatly between species and even clones of a given species (Tang et al. 2010), making it difficult to ascribe vitamin utilization based on the presence/absence of a group/species alone. As summarized above, the majority of vitamin utilization was carried out by the picoplankton community (Chapter 2, 3). It is this fact that Droop (2007) and others have failed to consider when dismissing the ecological role of vitamins in the marine environment. The conclusion that cellular vitamin quotas of phytoplankton are met by ambient concentrations is flawed since the large majority of this ambient pool is consumed by heterotrophic bacteria (Chapter 2-5) and thus unavailable to eukaryotic algae. It is thus more likely that the relative abundance of vitamin auxotrophic bacteria and vitamin-producing prokaryotes and their rates of production and consumption that governs the availability of vitamins to eukaryotes. Further study of their vitamin auxtrophy among bacteria, vitamin production among bacteria, and their dynamics and vitamin uptake and production rates is clearly warranted.

Quantifying carbon-specific vitamin uptake rates was a useful way to consider vitamin utilization across ecosystems in time and space (Fig 1). Carbon-specific vitamin uptake was

highest among the picoplankton for all studies with the exception of blooms of *C. polykrikoides* for which carbon-specific uptake in the >20 µm size class (representative of this HAB) was three-fold higher than the picoplankton (Fig. 1). The lowest carbon-specific uptake rates were observed in coastal and off-shelf regions of the Gulf of Alaska while, with the exception of the *C. polykrikoides* blooms, the next fastest were in the hypereutrophic Long Island estuary, Old Fort Pond. This indicates that more productive systems, including during HAB events, possess a greater ratio of B_{12} utilizers to B_{12} producers within the prokaryotic community or that the microbial community present has a greater cellular vitamin demand. If this trend was universal, it would imply that both a bacterial communities with larger B_{12} demands and high productivity rates contribute to the B_{12} limitation of phytoplankton in coastal zones (Sanudo-Wilhelmy et al 2006; Gobler et al 2007; Chapter 2 – 5). One exception to this was the relatively high carbon-specific uptake rates of B_{12} found in HNLC regions of the Gulf of Alaska (Fig. 1). Even though biomass and primary production rates in HNLC regions were lower than coastal sites, carbon-specific uptake rates (Fig. 1) indicated that the microbial, picoplankton community present used more vitamin B_{12} compared to onshore sites. The higher B_{12} concentrations found in HNLC regions suggest Fe-replete prokaryotic picoplankton ($>10^6$ cells mL^{-1}; Fig. 3) actively grow and produce B_{12} (Parker 1977; Rodionov et al. 2003; Bonnet et al. 2010) a hypothesis supported by Bertrand et al. (2011) who discovered a new group of B_{12} producing bacteria in polar regions. These vitamins are then effectively utilized by prokaryotic bacteria, in part due to the inability of Fe-limited nano- and micropankton to grow (Martin and Fitzwater 1988; Martin et al. 1989; Boyd et al. 1996).

In a manner similar to vitamin B_{12}, carbon-specific B_1 uptake was always faster for picoplankton and was twice as fast in the hypereutrophic Long Island estuary compared to the

mesotrophic Long Island Sound (Fig 2). With the exception of *C. polykrikoides* blooms, the carbon-specific uptake rate of B_1 in the larger size classes did not vary greatly between sites (Fig. 2) while varying by an order of magnitude among picoplankton populations. While a cross-ecosystem comparison of all ecosystems investigated during my dissertation demonstrated that there was no relationship between carbon-specific vitamin B_1 uptake rates by picoplankton and the nano/microplankton (Fig. 3A), there was a highly significant ($p<0.0003$) positive relationship between carbon specific vitamin B_{12} uptake by the picoplankton and the nano/microplankton community (Fig. 3B). Together these results suggest ecosystems that harbor vitamin B_{12} auxotrophic phytoplankton (~ > 2µm) are also more likely to host microbial communities that have an elevated requirement for vitamin $B_{12,}$ a finding that would account for the frequent B_{12} limitation of phytoplankton in ecosystems with large B_{12} uptake by picoplankton communities.

To assess the extent to which vitamin B_1 requirements among natural plankton communities investigated during this dissertation might be related to requirements for vitamin B_{12}, a second cross-ecosystem comparison was performed. While there was no relationship between the carbon specific uptake rates of vitamin B_1 and vitamin B_{12} within the picoplankton community (Fig. 4A),there was a strong, significant ($r=0.99$; $p<0.003$) positive correlation between rates for the nano/microplankton community when the *C. polykrikoides* bloom community, which had anomalously low B_1 requirements, were not considered (Fig. 4B). This finding suggests that, with the exception of the *C. polykrikoides* blooms, the vitamin B_1 and B_{12} requirement of phytoplankton communities trended together. While the prevalence of vitamin B_{12} auxotrophy among phytoplankton is approximately double that of vitamin B_1 (Croft et al 2005), most phytoplankton that require B_{12} also have an absolute requirement for B_1 (Tang et al

2010). The slope of the regression between the carbon-specific vitamin B_1 and vitamin B_{12} uptake rates (0.5) for phytoplankton made be indicative of an ideal ratio for uptake and thus can be compared to ambient concentrations to assess the relative abundance of these vitamins relative to algal requirements. Given that the ratios of vitamin B_1: vitamin B_{12} measured in all systems (Table 1) ranged from 0.8 to 16, this analysis suggests that B_{12} is the B-vitamin in shortest supply for phytoplankton relative to their requirement. This is also consistent with observations that B_1 never stimulated phytoplankton growth in experiments, even during high biomass HAB events (Chapters 4, 5), that the turnover times of vitamin B_{12} were much quicker than vitamin B_1, and that vitamin B_{12} auxotrophy is twice as prevalent vitamin B_1 auxotrophy among phytoplankton. Collectively, all of this information leads me to conclude that B_{12} is the B-vitamin most likely to limit the growth of phytoplankton in the ocean.

Tang et al.'s (2010) finding that harmful algae are especially auxotrophic with respect to vitamins is consistent with observations that a majority of HABs are formed by dinoflagellates, a group rich in osmotrophs (Taylor 1987; Smayda 1997) and observations that like dinoflagellates, the pelagophyte responsible for brown tide events, *A. anophagefferens* also relies heavily on osmotrophy (Taylor et al. 2006; Gobler et al. 2011). This dissertation is the first to investigate the use of vitamins by harmful algae in an ecosystem setting. Overall, vitamin utilization was an order of magnitude higher during high biomass blooms of vitamin-auxotrophic HAB species compared to coastal settings without blooms (Table 2). During the *C. polykrikoides* bloom, vitamin B_{12} utilization was higher by an order of magnitude compared to non-bloom regions while B_1 utilization was only 25% higher and similar other locales (Table 2). In the case of *A. anophagefferens* blooms, the maximum potential uptake rate of 2.65×10^{-9} pmol B_{12} cell^{-1} h^{-1} (Table 3), suggested that a bloom of 1.25×10^{6} cells mL (peak of the 2009 bloom) could

potentially consume >79 pM of B_{12} d^{-1}, a calculation consistent with the 100 pM drawdown of vitamins during the 2009 bloom (Chapter 4). Maximum B_{12} uptake rates for the brown tide community during the 2009 Quantuck Bay bloom were only ~18 pmol L^{-1} d^{-1}, however. Furthermore, this uptake was divided between the small picoplankton (<1 µm) and brown tide size fraction (1-5 µm) indicating that *A. anophagefferens* were utilizing B_{12} well below (nine-fold) their maximum uptake rates and supporting the observation that *A. anophagefferens* was limited by the availability of B_{12}. Conversely, a bloom of *C. polykrikoides* of 4000 cells mL^{-1} (mean of blooms during this study) with a maximum laboratory observed uptake rate of 2.86 x 10^{-6} pmolesB_{12} $cell^{-1}$ h^{-1} could take up 275 pmoles of B_{12} L^{-1} d^{-1} similar to the maximum uptake displayed during a *C. poykrikoides* bloom (150 pmol L^{-1} d^{-1}). The uptake kinetics parameters calculated for both species, fell well within the range of values observed for other HAB species, with *C, polykrikoides* having the largest cellular requirement of any phytoplankton species surveyed to date (Table 3).

Unlike blooms of *A. anophagefferens* and other plankton communities, during blooms of *C. polykrikoides*, carbon-specific uptake rates were dominated by the microplankton while the carbon-specific uptake rate in the picoplankton were actually less than non-bloom conditions. These differences may be related to the dynamics of bacterial population associated with each HAB. *C. polykrikoides* harbored a unique microbial consortium that seemed to consist of a larger proportion of vitamin producers than consumers as evident in the lower carbon-specific B_{12} uptake rate (Chapter 5). While brown tides might also have unique bacterial communities, preliminary findings (S. Bell, SoMAS, Stony Brook University, pers. comm.) suggest that the heterotrophic community in Quantuck Bay and other bays experiencing brown tides is remarkably similar between bloom and non-bloom periods (also determined via T-RFLP). As

such, the unique bacterial community present during *C. polykrikoides* blooms could give this species a competitive advantage having alleviated some of the competition for vitamin B_{12} uptake from the picoplankton community that is present during brown tides (Chapter 4).

In contrast to vitamin B_{12}, vitamin B_1 was utilized heavily by *A. anophagefferens*, but not by *C. polykrikoides*. The high, nM half saturation and cellular requirements of B_1 for *A. anophagefferens* (Chapter 4) although on a different scale than observed in the field, support the notion that the brown tide actively requires and utilizes B_1. Conversely, as evident by the lower carbon-specific B_1 uptake rates for microplankton (Fig. 2), *C. polykrikoides* utilized B_1 in the field at a slower rate than B_{12}. It is possible that wild populations of *C. polykrikoides* are comprised of fewer B_1 auxotrophic strains (Tang et al 2010) or that their cellular requirement for B_1 is low. This was further evident in the regression of carbon-specific vitamin B_1 and vitamin B_{12} uptake for the nano/microplankton in which the data associated with the *C. polykrikoides* bloom fell outside of the otherwise very strong relationship (Fig. 4B) due to the low utilization of vitamin B_1 relative to vitamin B_{12} inside the bloom.

This dissertation examined planktonic vitamin utilization, the manner in which vitamins shape plankton communities, and sheds new light on the importance of heterotrophic bacteria in both processes. While the ability to directly measure vitamin utilization by plankton has led to a better understanding of the role of vitamins in plankton ecology, the absence of a method to measure net vitamin production is a major shortcoming in this field of study. Such measurements would allow for the development of vitamin budgets and enable a quantitative assessment of the relative importance of uptake and ambient concentrations. In addition, while this dissertation offers numerous lines of evidence suggesting that heterotrophic bacteria are important in oceanic vitamin cycling, it has only preliminarily characterized this community.

Characterization of prokaryotes important for producing vitamins in the ocean and assessing the relative abundance of these individuals in comparison to the total bacterial community would begin to facilitate an understanding of the relative importance of vitamin production and consumption among picoplankton. My dissertation work also discovered ~20 different prokaryotes present during blooms of *C. polykrikoides* (determined via T-RFLP analysis). Isolation and/or sequence analysis and subsequent identification of these bacteria would permit a better characterization of the differences in prokaryotic community associated with this harmful algae that seemed to have different vitamin requirements than those associated with non-bloom water.

A recent study examined a newly discovered protein important in vitamin B_{12} utilization under B_{12} starved condition in diatoms (E. Bertrand and M. Saito, WHOI, pers. comm.). In addition the authors examined different nutritional strategies in dealing with B_{12} deprivation, including the lowering of cellular requirements and increases of uptake machinery, both strategies observed in *A. anophagefferens* (Chapter 4) with increased uptake rates and affinity constants and decreased cellular requirements and half saturation constants. Importantly, a homolog of this protein has been found in *A. anophagefferens* (E. Bertrand and M. Saito, WHOI, pers. comm.). A study of the expression of these genes coupled with uptake rates especially during a bloom of a known vitamin B_{12} auxotroph such as *A. anophagefferens* would help highlight trends regarding in situ vitamin concentrations and the physiological status of *A. anophagefferens* as a function of those concentrations. Similar proteins have not been identified in picoplankton, the main vitamin utilizes in the marine environment, but could be of great interest given their dominance of vitamin assimilation in many marine ecosystems (Chapters 2 - 5).

In summary, this dissertation has revealed many important aspects of B-vitamins in a variety of marine environments, including their impacts on planktonic species succession, the first recorded vitamin uptake measurements by marine plankton, the importance of picoplankton as vitamin consumers, and the role of vitamins in the occurrence of HABs. These findings collectively demonstrate that, like the well-studied macronutrients (N and P) and mirconutrients (Fe), B-vitamins can play a central role in ecology of the ocean plankton. As such, more research is needed to clarify this role.

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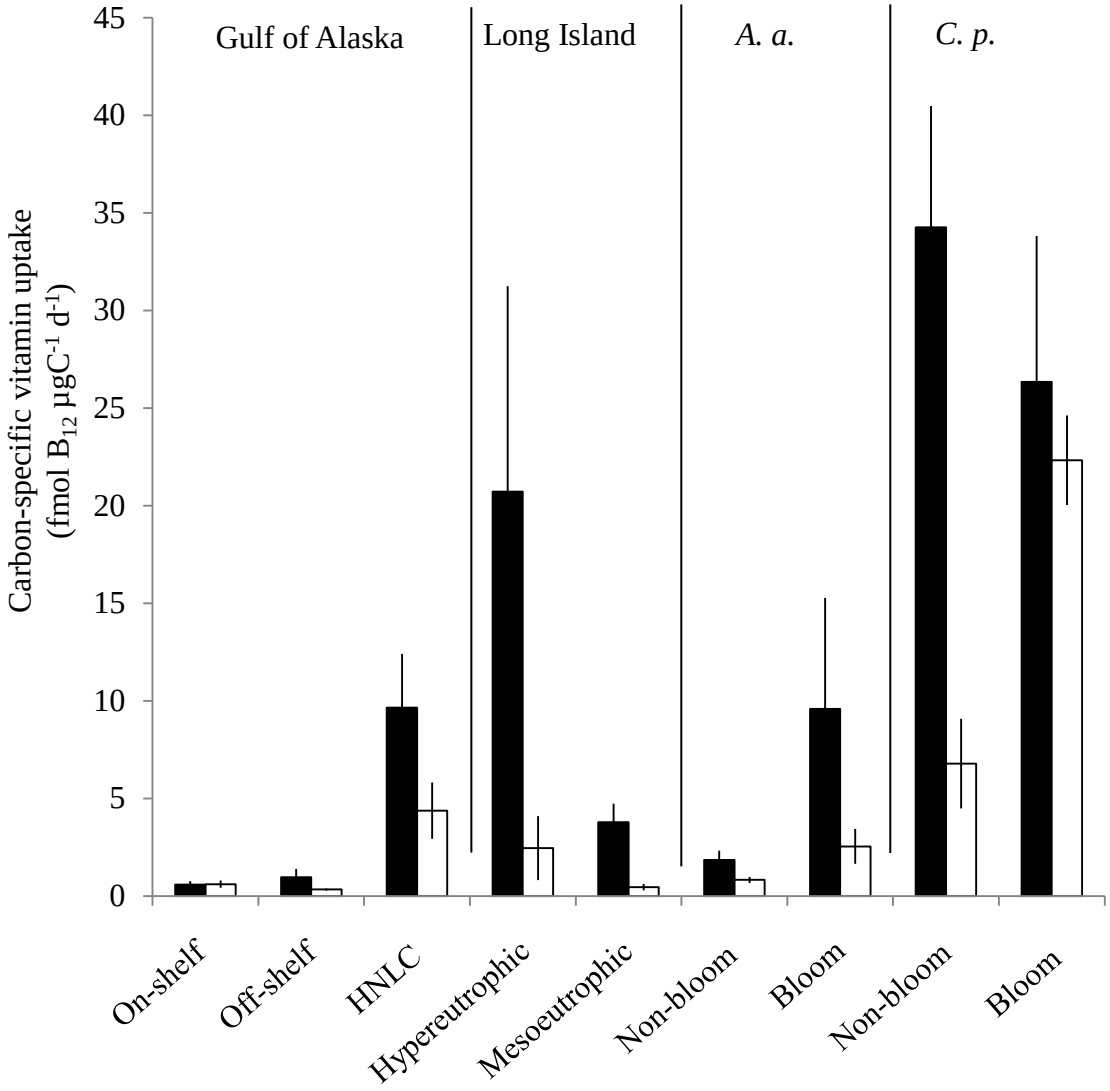

Figure 1. Carbon normalized vitamin B_{12} uptake rates collected in two Long Island, NY, ecosystems as well as during HABs caused by *A. anophagefferens* (A.a.; Chapter 4) and by *C. polykrikoides* (C.p.; Chapter 5). The hypereutrophic and mesotrophic systems sampled were Old Fort Pond and Long Island Sound, respectively (Chapter 3). Black and white bars for Long Island represent the 0.2-2 and >2 µm size fractions respectively while they represent the 0.2-1 and >1 µm for *A. anophagefferens* respectively and 0.2-2 and >20 µm for *C. polykrikoides*, respectively. Values shown are averages ± standard error and represent the first ever direct vitamin B_{12} uptake measurements in the marine environment.

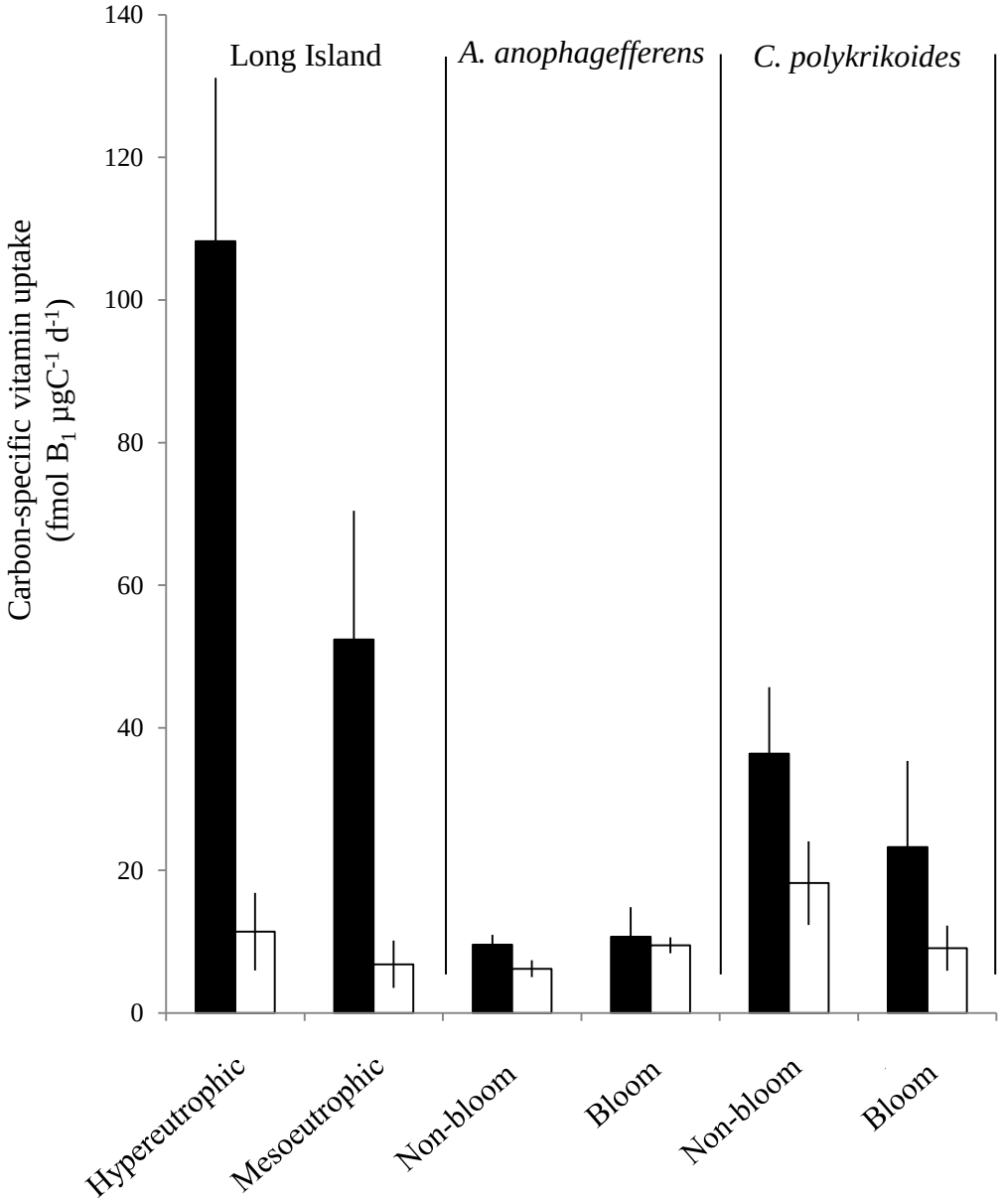

Figure 2. Carbon normalized vitamin B_1 uptake rates collected in two Long Island, NY, ecosystems as well as during HABs caused by *A. anophagefferens* (Chapter 4) and by *C. polykrikoides* (Chapter 5). The hypereutrophic and mesotrophic systems sampled were Old Fort Pond and Long Island Sound, respectively (Chapter 3). Black and white bars for Long Island represent the 0.2-2 and >2 µm size fractions respectively while they represent the 0.2-1 and >1 µm for *A. anophagefferens* respectively and 0.2-2 and >20 µm for *C. polykrikoides*, respectively. Values shown are averages ± standard error and represent the first ever direct vitamin B_1 uptake measurements in the marine environment.

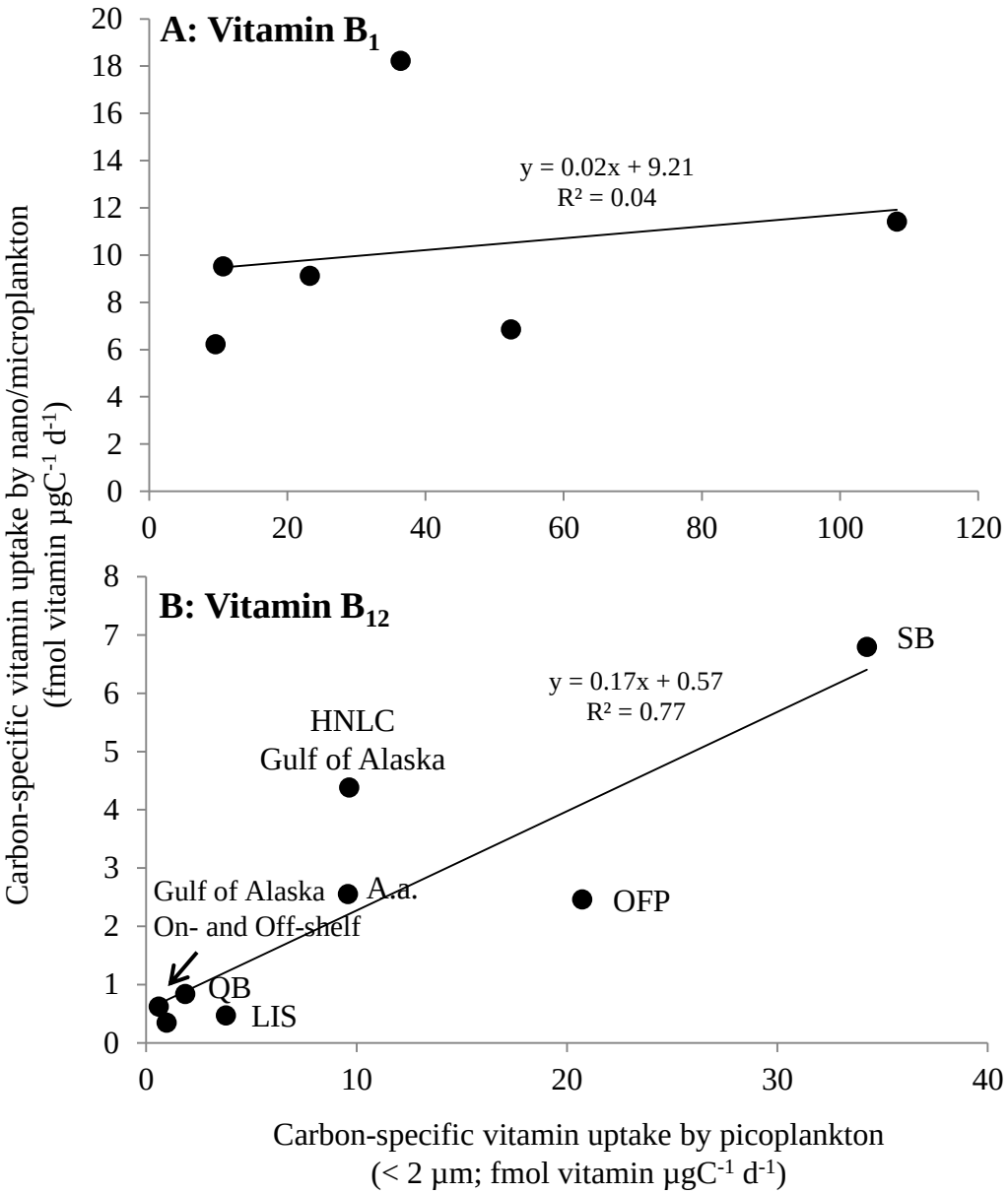

Figure 3. Linear regressions of the mean carbon-specific (A) vitamin B_1 and (B) vitamin B_{12} uptake rates for the picoplankton v. the nano/microplankton for all the sites investigated during this dissertation. Pico-, nano- and microplankton are defined as described in figures 1 &2. SB, QB, OFP, and LIS refer to Shinnecock Bay, Quantuck Bay, Old Fort Pond and Long Island Sound, respectively. QB and SB were sampled during HABs caused by *A. anophagefferens* (A.a.; Chapter 4) and by *C. polykrikoides* (C.p.; Chapter 5).

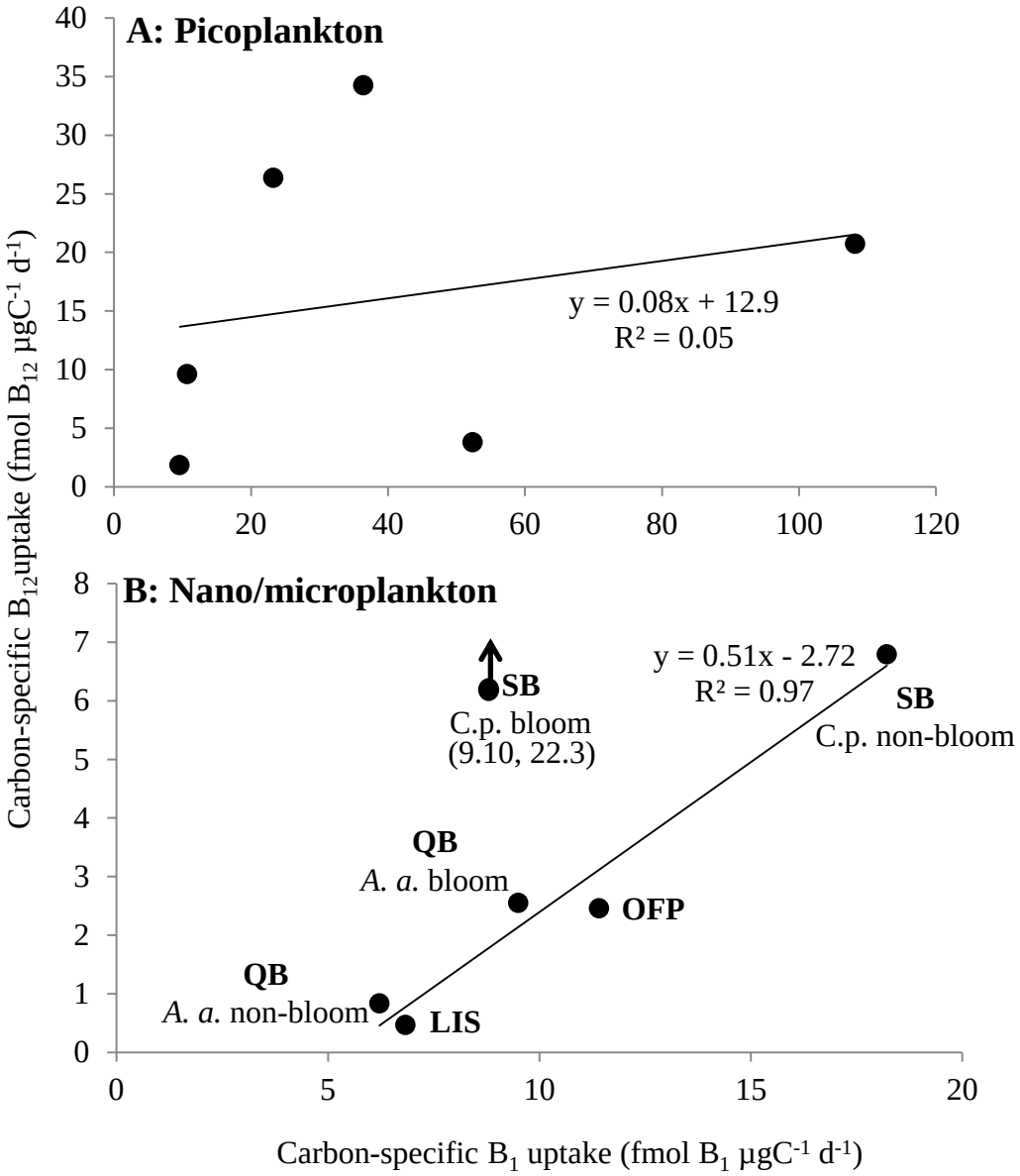

Figure 4. Linear regressions of the carbon-specific vitamin B_1 v. the carbon-specific vitamin B_{12} uptake rates for the (A) picoplankton and (B) the nano/microplankton for all the sites investigated during this dissertation. SB, QB, OFP, and LIS refer to Shinnecock Bay, Quantuck Bay, Old Fort Pond and Long Island Sound, respectively. QB and SB were sampled during HABs caused by *A. anophagefferens* (A.a.; Chapter 4) and by *C. polykrikoides* (C.p.; Chapter 5). Pico-, nano- and microplankton are defined as described in figures 1 &2.

Table 1. Concentrations of B_{12} and B_1 as well as ratios of the B_1:B_{12} in a variety of marine systems. Prior to the development of a method to directly measure vitamins in the ocean by Okbamichael and Sañudo-Wilhelmy (2004, 2005) concentrations were estimated via bioassays. For *A. anophagefferens*, the brown tide forming species, pre-bloom denotes a time when *A. anophagefferens* cell densities were <3 x10^5 cells mL^{-1} while for the red tide forming *C. polykrikoides*, samples were collected either inside the dense bloom patch (mean cell densities = 4000 cells mL^{-1}) or adjacent (100m) outside of the bloom patch. Values are depicted as a range or as the mean ± standard error.

Location	Ecosystem type	B_{12} pM	B_1 pM	B_1:B_{12}	Source
Early estimates					
Long Island Sound	Coastal	2.95 - 11.8	7.55 - 75.5		Vishian and Riley 1961
Straight of Georgia	Coastal	0.74 - 8.86			Cattell 1960
Napeague	Coastal	0.22 - 5.17			Bruno and Staker 1963
Sagami Bay, Japan	Coastal	0.44 - 3.54	22.6 - 347		Ohwada and Taga 1972
Sargasso Sea	Open Ocean	0.01 - 0.07			Menzel and Spaeth 1962
Barents Sea	Open Ocean	0.07 - 1.48			Propp 1970
Gulf of Maine	Open Ocean	0.22 - 1.18	30.2 - 124		Swift 1973
South Polar Seas	Open Ocean	0.07 - 2.44	7.55 - 26.4		Carlucci and Cuhel 1975
North Pacific	Open Ocean	0.07 - 2.51			Natarajan and Dugdale 1966
Direct measurments					
L.I. southshore bays	Coastal	15.0 - 87.0			Sanudo-Wilhelmy et al. 2006
Todos Santos Bay	Coastal	2.00 - 60.0			Panzeca et al. 2009
San Pedros Basin	Coastal	0.2 - 1.80			Panzeca et al. 2009
Long Island Sound	Coastal	0.20 - 15.0			Panzeca et al. 2009
Baja California	Coastal	0.2 - 7.0			Panzeca et al. 2009
North Atlantic	Open Ocean	0.2 - 4.0			Panzeca et al. 2009
Gulf of Alaska	Coastal	0.10 - 3.80			Koch et al. 2011
Gulf of Alaska	Open Ocean	0.10 - 3.06			Koch et al. 2011
Gulf of Alaska	HNLC	0.88 - 8.24			Koch et al. 2011
Old Fort Pond	Hypereutrophic	0.10 - 257	0.10 - 114	0.77 ± 0.33	Chapter 3
Long Island Sound	Coastal	0.10 - 43.7	0.10 - 98.7	16.3 ± 10.8	Chapter 3
Harmful Algal Blooms					
pre-bloom	A.a. bloom	23.0 - 121	94.3 - 169	3.01 ± 0.21	Chapter 4
bloom	A.a. bloom	3.48 - 18.9	7.32 - 67.8	3.22 ± 0.16	Chapter 4
non-bloom	C. p. bloom	38.9 - 69.1	21.4 - 56.4	0.78 ± 0.42	Chapter 5
bloom patch	C. p. bloom	38.5 - 61.7	22.7 - 52.0	0.89 ± 0.08	Chapter 5

Table 2. Average rates of primary production, B_{12} and B_1 uptake in a variety of systems sampled during this dissertation. Represented are the mean ± standard error for each system. For *A. anophagefferens,* a bloom was defined as cell densities exceeded 3 x 10^5 cells mL^{-1} while for *C. polykrikoides* a bloom was defined as samples collected inside the dense bloom water (mean cell densities = 4,000 cells mL^{-1}) while non-bloom samples were collected immediately adjacent (100 m) to the bloom.

Location		Primary Production mgC m^3 d^{-1}	B_{12} Uptake pmol L^{-1} d^{-1}	B_1 uptake pmol L^{-1} d^{-1}
Gulf of Alaska	On-shelf	37.1 ± 10.3	0.11 ± 0.05	
	Off-shelf	30.9 ± 5.89	0.09 ± 0.03	
	HNLC	14.6 ± 1.23	0.60 ± 0.25	
Long Island	hypereutrophic	559 ± 118	3.18 ± 1.89	17.8 ± 3.16
	mesotrophic	327 ± 92.8	0.22 ± 0.08	4.39 ± 1.96
HAB events				
A. anophagefferens	Non-bloom	789 ± 268	2.65 ± 1.24	13.0 ± 0.74
	Bloom	2456 ± 448	6.95 ± 3.94	17.4 ± 5.22
C. polykrikoides	Non-bloom	214 ± 33.5	7.91 ± 1.49	10.1 ± 2.61
	Bloom	2109 ± 626	45.7 ± 13.9	9.94 ± 2.09

Table 3. Summary of available vitamin B_{12} kinetics data. For studies referenced 1-8 half saturation constants (K_s), and cellular quotas were measured via growth and cell yield while constants including the maximum uptake rate (V_{max}) for this dissertation were obtained via the use of radiolabeled ^{57}Co-B_{12}. References include: 1-Ford 1958, 2-Droop 1961, 3-Droop 1968, 4-Swift and Taylor 1974, 5-Wood 1962, 6-Carlucci and Bowes 1972, 7-Carlucci and Bowes 1974, 8-Tang et al. 2010.

	K_s	**Quota**	**V_{max}**	**Reference**
Species	pM	pmol B_{12} $cell^{-1}$	pmol B_{12} $cell^{-1}$ h^{-1}	
O. malhamensis	8.7			1
P. lutheri	0.1-1.87			2,3,4,5
I. galbana	1.13			4
S. costatum	0.19-5	$0.79 - 8.07 \times 10^{-9}$		5, 6
T. pseudonana	0.17-0.26			4
T. oceanica	0.13-0.26			7
P. minimum	0.02 ± 0.01	3.98×10^{-10}		8
K. mikimotoi	13.1 ± 1.11	1.66×10^{-6}		8
C. marina	0.19 ± 0.11	3.00×10^{-9}		8
F. japonica	0.28 ± 0.02	1.64×10^{-8}		8
R. salina	0.36 ± 0.02	1.79×10^{-10}		8
A. anophagefferens	3.49 ± 0.75	3.25×10^{-9}		8
deplete	5.16 ± 0.50	$5.75 \pm 0.91 \times 10^{-9}$	$2.65 \pm 0.17 \times 10^{-9}$	Chapter 4
replete	20.8 ± 3.00	$7.55 \pm 1.63 \times 10^{-9}$	$1.95 \pm 0.14 \times 10^{-9}$	Chapter 4
C. polykrikoides				
deplete	7.96 ± 1.42	$0.81 \pm 0.16 \times 10^{-6}$	$2.86 \pm 0.18 \times 10^{-6}$	Chapter 5
replete	41.5 ± 5.02	$5.26 \pm 0.64 \times 10^{-6}$	$3.20 \pm 0.13 \times 10^{-6}$	Chapter 5

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