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# Effect of B-vitamins ($B_1$, $B_{12}$) and inorganic nutrients on algal bloom dynamics in a coastal ecosystem

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ABSTRACT: Vitamins play an integral role in the cellular biochemistry of algae, but the effect of these organic metabolites on the growth and diversity of phytoplankton communities has been poorly studied. We integrated newly developed techniques to directly measure vitamins $B_1$ and $B_{12}$ with field-based amendment experiments to elucidate the role of B-vitamins in phytoplankton population dynamics in coastal marine environments. Two sites on Long Island, New York, USA, were monitored from spring through fall: the tidal Old Fort Pond (OFP) and the brackish Peconic River (PR) estuary. Vitamin $B_{12}$ levels were similar between sites (OFP: 1.6 to 21 pM; PR: 1.6 to 17 pM) and were significantly correlated with bacterial densities, dissolved organic nitrogen and dissolved organic phosphorus at OFP, suggesting that $B_{12}$ behaves like regenerated organic nutrients. Concentrations of vitamin $B_1$ were substantially higher in the freshwater dominated site (PR: 12 to 190 pM; OFP: 9 to 43 pM) and were inversely correlated with salinity, suggesting that rivers and groundwater may be an important source of vitamin $B_1$. During dinoflagellate blooms (>$10^4$ cells $ml^{-1}$), occurring in late summer and early fall, vitamin $B_{12}$ and $B_1$ levels in PR decreased 90% relative to pre-bloom levels, while levels temporarily increased to seasonal maxima in OFP, likely reflecting vitamin synthesis and/or regeneration by microbial communities. Nutrient amendment experiments conducted at both sites during summer demonstrated that algal communities were primarily N-limited, while those conducted during early fall showed that vitamins $B_1$ and $B_{12}$ were each capable of significantly enhancing the biomass of larger phytoplankton (>5 µm). The autumnal shift in phytoplankton communities from dinoflagellates to diatoms, as vitamin levels became depleted and algal communities were limited by vitamin $B_{12}$, suggests that B-vitamins may influence the succession of coastal phytoplankton.

KEY WORDS: B-vitamins · Phytoplankton · Harmful algal blooms · Inorganic nutrients



## INTRODUCTION

Autotrophic phytoplankton require dissolved nutrients to grow. Nitrogen (N), phosphorus, and silicon are considered macronutrients, because they are required in relatively large quantities and often control the rate of phytoplankton production. Phytoplankton also have a small, but vital, requirement for micronutrients, such as transition metals (e.g. Fe, Mg, Zn, Mn, Co) and some common ions. Many phytoplankton species also require exogenous vitamins, such as vitamin $B_{12}$ (cobalamin) and vitamin $B_1$ (thiamine). The roles of macro- and micronutrients in phytoplankton community growth have been systematically studied during the past 50 yr. In contrast, the effects of vitamins on the growth and diversity of phytoplankton in aquatic ecosystems have only been examined sporadically.

Phytoplankton species that must assimilate exogenous vitamins because they cannot synthesize them are referred to as auxotrophs. Many microalgal species in nearly all phytoplankton classes are auxotrophic for vitamins $B_{12}$ and $B_1$ (Provasoli & Carlucci 1974, Swift

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1980, Croft et al. 2005). A recent compilation by Croft et al. (2005) indicated that half of the algal species studied in culture (n = 326) cannot grow without an exogenous supply of vitamin $B_{12}$. Sources of vitamins in marine systems include bacterial biosynthesis and allochthonous sources, such as influxes from rivers and streams (Haines & Guillard 1974, Bruno & Staker 1978, Swift 1980).

Few studies have examined the effect of ambient vitamin levels on phytoplankton dynamics in marine ecosystems. These studies have generally suggested that vitamins can be present at picomolar concentrations in seawater and may sometimes be depleted to growth-limiting levels (Swift 1980, Croft et al. 2005, Panzeca et al. 2006, Sañudo-Wilhelmy et al. 2006, Bertrand et al. 2007). Recent independent studies have demonstrated that phytoplankton communities in the Southern Ocean are co-limited by iron and vitamin $B_{12}$ supplies (Panzeca et al. 2006, Bertrand et al. 2007). In the Gulf of Maine, Swift (1981) found that total dissolved $B_{12}$ concentrations decreased in the euphotic zone during the spring diatom bloom while significant amounts of particulate vitamin $B_{12}$ were concurrently measured, presumably due to assimilation by diatoms. However, Menzel & Spaeth (1962) reported that moderate diatom blooms occurred in the Sargasso Sea when vitamin $B_{12}$ concentrations were at their highest. Studies in Chinese coastal waters have suggested that natural and manipulated additions of vitamins may influence phytoplankton species succession (Wang et al. 1995, Yu & Harrison 2000). In south shore estuaries of Long Island, New York, levels of vitamin $B_{12}$ decrease with increasing biomass of large phytoplankton, suggesting active uptake by this size class of algae (Sañudo-Wilhelmy et al. 2006).

Like vitamin $B_{12}$, vitamin $B_1$ is also required for growth by a large number of phytoplankton species (22% of the >300 microalgae surveyed to date; Croft et al. 2006), and the absolute requirement for thiamine has not been universally demonstrated within individual algal classes, but rather has been observed in many unrelated species. The majority of euglenophytes (73%) and prymnesiophytes (83%) surveyed require exogenous $B_1$, while only a minority of species among all other algal classes seem to require this B-vitamin (Croft et al. 2006). Notably, the bloom-forming prymnesiophyte *Phaeocystis globosa* is a $B_1$ auxotroph, and this vitamin has been implicated in bloom occurrence in the North Sea (Peperzak et al. 2000). Vitamin $B_1$ exists at picomolar levels in marine environments (Okbamichael & Sañudo-Wilhelmy 2005), but fewer than a dozen direct measurements of vitamin $B_1$ have been made in marine systems. Studies assessing the effect of this vitamin on phytoplankton in the field have been rare.

In all marine studies published prior to 2004, dissolved vitamin concentrations were estimated indirectly via bioassays, which can be error prone. The B-vitamin bioassay requires a 1 wk incubation period under conditions that could change vitamin availability (Carlucci & Silbernagel 1966, Haines & Guillard 1974). For example, vitamin $B_{12}$ photodegrades and is unstable at alkaline pH (Friedrich 1975, Vandamme 1989), yet bioassay incubations are conducted under fluorescent light banks, and algal culture pH often rises substantially when grown in batch mode. While the original bioassay technique required seawater samples to be passed through a 0.45 µm filter to remove plankton (Carlucci & Silbernagel 1966, Haines & Guillard 1974), this technique permits the passage of a substantial portion of the ambient bacteria population (Kirchman 2000), which could synthesize additional vitamins during the bioassay (Croft et al. 2005). Not surprisingly, assays of the same water mass with different phytoplankton cultures have yielded different vitamin concentrations (Provasoli & Carlucci 1974, Swift 1980). Moreover, vitamin $B_{12}$ concentrations obtained using a radioisotope dilution technique were up to 40 times higher than levels obtained using the bioassay technique (Sharma et al. 1979). Therefore, the indirect bioassay technique may not accurately reflect the availability of ambient dissolved vitamins for phytoplankton in the field (Carlucci & Silbernagel 1969, Carlucci & Bowes 1970, Sharma et al. 1979). Newer, direct methods of quantifying the concentration of vitamins $B_1$ and $B_{12}$ in seawater have been developed, using a solid phase extraction of $B_1$ and $B_{12}$ followed by quantification using reverse-phase high performance liquid chromatography (HPLC) (Okbamichael & Sañudo-Wilhelmy 2004, 2005).

We report on the influence of nutrients and vitamins $B_1$ and $B_{12}$ on phytoplankton growth and diversity by measuring vitamin concentrations using these new HPLC methods at 2 coastal locations. We concurrently documented the diversity and dynamics of the plankton communities and conducted nutrient-vitamin amendment experiments to determine the extent to which nitrogen and vitamins are capable of influencing algal community growth rates. We then statistically examined the biogeochemical relationships among vitamins, nutrients, and plankton communities. This study is the first to report temporal variability of vitamin $B_1$ by direct measurements of this important growth factor in a marine environment.

## MATERIALS AND METHODS

**Study sites.** Field work was conducted at 2 locations from June through November 2005 on eastern Long Island, New York: Old Fort Pond (OFP; 40.87° N,

72.45° W), which exchanges tidally with Shinnecock Bay; and the Peconic River estuary (PR; 40.92° N, 72.62° W), which is a brackish tributary receiving freshwater inputs from the Peconic River and tidal exchange from Peconic Estuary (Fig. 1; Breuer et al. 1999). These 2 study sites represent a contrast in freshwater discharge, as PR is the largest tributary on eastern Long Island while OFP has no point source of surface freshwater. Due to this freshwater discharge, PR typically displays a modest halocline, while OFP is well-mixed with regard to temperature and salinity. Both sites are shallow (~2 m) and have historically been prone to dense summer and fall algal blooms that are often dominated by dinoflagellates (Gobler et al. 2008), a scenario that results in a large nutritional demand for macro- and micronutrients. Furthermore, dinoflagellates have a large vitamin B requirement (Aldrich 1962), and most dinoflagellates (90%) are auxotrophs for vitamin $B_{12}$ (Croft et al. 2005). High levels of algal biomass at both sites promote substantial water turbidity. Based on Secchi disk depths, the mean ± SE depth of the euphotic zone during this study was 2.7 ± 1.1 m (range 0.7 to 2.2 m [site bottom]) for PR and 2.9 ± 1.1 m (range 0.7 to 2.2 m [site bottom]) for OFP, meaning that the entire water column was within the euphotic zone during most of this study.

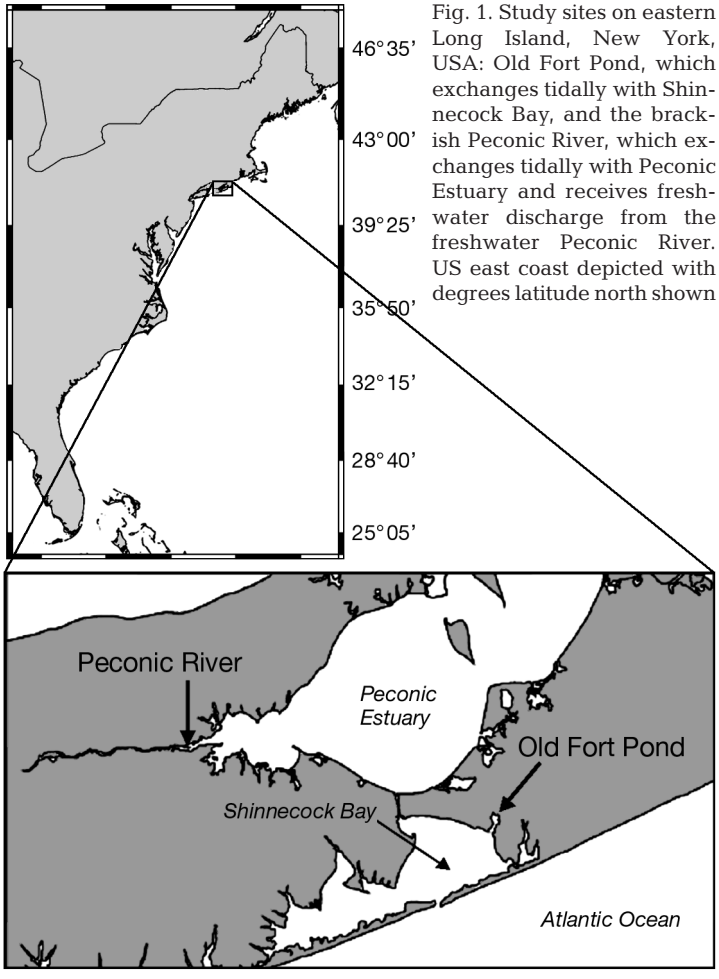


Fig. 1. Study sites on eastern Long Island, New York, USA: Old Fort Pond, which exchanges tidally with Shinnecock Bay, and the brackish Peconic River, which exchanges tidally with Peconic Estuary and receives freshwater discharge from the freshwater Peconic River. US east coast depicted with degrees latitude north shown

While the focus of this study was on the bottom-up effect of organic and inorganic nutrients on phytoplankton populations, zooplankton grazing is also an important factor that likely affected algal communities at these study sites. The most abundant zooplankton grazers present during this study were aloricate ciliates such as *Strombidium* sp. and *Strombidinopsis* sp., which displayed densities of 6 to 100 ind. $ml^{-1}$ in PR (mean ± SE = 44 ± 14) and 21 to 310 ind. $ml^{-1}$ in OFP (120 ± 37). Grazing rates by microzooplankton have previously been measured using the dilution technique (Landry et al. 1995) at both sites during spring, summer, and fall. Grazing rates have generally ranged from 0.3 to 1.0 $d^{-1}$, with the highest rates occurring during warm months with high levels of algal biomass (C. J. Gobler unpubl.).

**Sample collection and analyses.** Sample water was collected in triplicate acid-washed, polycarbonate, 20 l carboys from the surface mixed layer (~0.5 m). Temperature and salinity were measured at each site via a YSI 556 probe. Subsamples from carboys were filtered and analyzed for total, <5 µm, and >5 µm chlorophyll *a* (chl *a*) using GF/F glass fiber filters and 5 µm polycarbonate filters in triplicate (Parsons et al. 1984). Lugol's iodide (5%) and gluteraldehyde (1%) preserved subsamples were also obtained for plankton counts. Larger phytoplankton (>5 µm) were quantified in triplicate in settling chambers and identified to the genus or species level using an inverted microscope (Hasle 1978). Bacteria were DAPI-stained and enumerated in triplicate on an epifluorescent microscope according to Sherr et al. (2001). Water samples for nutrient and vitamin $B_1$ and $B_{12}$ analyses were filtered through acid-washed polypropylene filter capsules (0.2 µm) using a peristaltic pumping system, collected in acid-washed high-density polyethylene bottles, and frozen until analysis. Nitrate, ammonium, ortho-phosphate, silicate, total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) were analyzed in duplicate via spectrophotometric methods (Valderrama 1981, Jones 1984, Parsons et al. 1984). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium), and dissolved organic phosphorus (DOP) was calculated as the difference between TDP and dissolved inorganic phosphate (DIP = orthophosphate). Measurements of standard refer-

ence material for organic and inorganic nutrients were all within 10% of certified values.

Dissolved vitamins $B_1$ and $B_{12}$ were analyzed using reverse-phase HPLC as described by Okbamichael & Sañudo-Wilhelmy (2004, 2005). Prior to sample preconcentration, the pH of the samples was titrated to approximately 6 with HCl. Samples were then passed through a $C_{18}$ resin (pre-treated with methanol) and rinsed with Milli-Q water to eliminate any remaining salt. Sample elution was then carried out with 2 aliquots of 2.5 ml methanol each and allowed to evaporate until dry. Residues were then re-dissolved with 250 µl of Milli-Q water and 0.2 µM syringe filtered before transferring to separate HPLC vials to analyze both vitamin $B_1$ and vitamin $B_{12}$ via reverse-phase HPLC. Methodological relative standard deviation for replicated analyses was ±4.7% for vitamin $B_1$ and ±8.4% for vitamin $B_{12}$.

**Nitrogen-vitamin amendment experiments.** Nitrate and vitamin addition experiments were conducted to establish the potential effect of these compounds on coastal algal communities. Nitrogen was examined in tandem with vitamins, since it is typically limiting to phytoplankton in Long Island estuaries (Ryther & Dunstan 1971, Gobler & Sañudo-Wilhelmy 2001). Within 1 h of collection, 250 ml of seawater were transferred to acid-cleaned polycarbonate flasks. Triplicate flasks were amended with sodium nitrate (20 µM), vitamin $B_{12}$ (500 pM), vitamin $B_1$ (500 pM), and all possible combinations of these additions ($NO_3^-$ + $B_{12}$; $NO_3^-$ + $B_1$; $B_{12}$ + $B_1$; $NO_3^-$ + $B_{12}$ + $B_1$). Nitrate and vitamin additions approximated peak concentrations previously observed in the water column of Long Island embayments (Gobler & Sañudo-Wilhelmy 2001, Okbamichael & Sañudo-Wilhelmy 2004, 2005). Nutrient and vitamin stocks were filter-sterilized (0.2 µm) and stored frozen. Experimental bottles were incubated *in situ* in OFP at the Stony Brook-Southampton Marine Station, allowing for moderate mixing of the bottles. Screening of bottles reduced ambient light penetration by 33% and mimicked a depth of approximately 1 m in the water column (Gobler et al. 2004). After 48 to 72 h, experimental flasks were filtered for size fractionated chl *a* (total, >5 µm, <5 µm) onto GF/F glass fiber and polycarbonate filters. Net growth rates for each size fraction were calculated from changes in chl *a* using the formula: $\mu = [\ln(B_t/B_0)]/t$, where µ is the net growth rate, $B_t$ is the amount of biomass (chl *a*) present at the end of the experiments, $B_0$ is the amount of biomass at the beginning of experiments, and $t$ is the duration of the experiment in days.

**Statistical analyses.** The effect of nutrient amendments on each size fraction of chl *a* during experiments was evaluated using a 3-way analysis of variance (ANOVA) with $NO_3^-$ and vitamins $B_{12}$ and $B_1$ as the main effects ($\alpha = 0.05$). A 3-factor ANOVA consists of 7 significance tests: a test for each of the 3 main effects, a test for each of the three 2-way interactions, and a test of the 3-way interaction. Two independent variables interact if the effect of one of the variables differs depending on the level of the other variable. Three-way interactions exist whenever a 2-way interaction differs depending on the level of a third variable. Nutrient additions that were a significant treatment effect and yielded increased algal growth rates were

Table 1. Water quality data (SE in parentheses) for both study sites during the 2005 sampling season. T: temperature (°C); S: salinity; silicate, DOP, and DON in µM; bacteria are $ml^{-1} \times 10^6$

| | T | S | Silicate | DOP | DON | Bacteria |
|---|---|---|---|---|---|---|
| **Peconic River** | | | | | | |
| 10 Jun | 23.4 | 22.6 | 108 (0.87) | 0.86 (0.13) | 20 (3.59) | 4.25 (0.47) |
| 22 Jun | 25.5 | 11.4 | 122 (13.7) | 0.93 (0.08) | 17.3 (5.82) | 3.90 (0.07) |
| 8 Jul | 21.2 | 10.1 | 93 (12.8) | 0.69 (0.03) | 14.6 (1.84) | 6.06 (0.49) |
| 11 Jul | 24.4 | 10.5 | 73.1 (4.51) | 0.70 (0.18) | 19.9 (2.08) | 6.27 (0.16) |
| 26 Jul | 26.8 | 8.9 | 113 (1.25) | 0.67 (0.50) | 11.7 (6.53) | 6.94 (0.23) |
| 5 Aug | 28.6 | 14 | 124 (1.26) | 1.32 (0.10) | 18.5 (0.89) | 13.3 (0.15) |
| 12 Aug | 28.2 | 18.9 | 151 (1.00) | 1.63 (0.12) | 19.6 (2.62) | 12.4 (1.48) |
| 16 Aug | 26.1 | 13.7 | 128 (3.96) | 1.05 (0.07) | 16.5 (2.87) | 9.06 (0.75) |
| 23 Aug | 25.9 | 16.8 | 95.4 (7.98) | 2.47 (0.14) | 26 (3.15) | 10.6 (1.23) |
| 29 Aug | 26.6 | 13.5 | 79.7 (6.44) | 1.71 (0.04) | 27.1 (1.37) | 9.41 (0.75) |
| 19 Sep | 25.2 | 18.3 | 136 (1.17) | 1.84 (0.17) | 31 (1.79) | 11.0 (0.19) |
| 3 Oct | 20 | 19.6 | 129 (11.1) | 1.53 (0.44) | 26.3 (1.40) | 9.83 (0.43) |
| 17 Oct | 14.5 | 4.1 | 53.2 (1.54) | 0.71 (0.23) | 18 2.09) | 2.97 (0.18) |
| 3 Nov | 11.7 | 10.1 | 108 (0.99) | 0.5 (0.01) | 16.6 (1.44) | 2.58 (0.61) |
| Mean | 23.4 | 13.8 | 108 | 1.19 | 20.2 | 7.76 |
| SE | 1.39 | 1.21 | 7.45 | 0.15 | 1.51 | 0.94 |
| **Old Fort Pond** | | | | | | |
| 2 Jun | 15.8 | 18.3 | 47.2 (0.82) | 0.53 (0.22) | 12.8 (2.11) | 2.17 (0.28) |
| 22 Jun | 19.5 | 27.5 | 44.1 (0.73) | 1.04 (0.15) | 31.8 (21.7) | 3.48 (0.18) |
| 1 Jul | 22 | 25.6 | 98.6 (1.96) | 0.81 (0.06) | 28.8 (7.95) | 3.48 (0.49) |
| 12 Jul | 24.1 | 27.2 | 44 (14.5) | 0.73 (0.43) | 23.5 (1.55) | 6.80 (0.62) |
| 22 Jul | 27.6 | 28.7 | 92.7 (27.5) | 1.07 (0.43) | 18.9 (0.86) | 6.70 (0.28) |
| 1 Aug | 24.2 | 27.3 | 117 (2.98) | 3.56 (0.12) | 73.3 (6.71) | 19.2 (1.60) |
| 16 Aug | 23.5 | 29.1 | 72.4 (3.94) | 1.82 (0.40) | 34.4 (12.6) | 8.76 (2.50) |
| 30 Aug | 25.1 | 25 | 95.8 (1.12) | 3.3 (0.07) | 55 (5.04) | 9.96 (2.13) |
| 13 Sep | 24.7 | 28.4 | 72.6 (1.71) | 1.62 (0.06) | 33 (2.13) | 5.86 (3.26) |
| 27 Sep | 21.1 | 29.2 | 37.4 (0.46) | 1.44 (0.06) | 31 (1.49) | 3.34 (0.07) |
| 11 Oct | 18.1 | 28.2 | 49.2 (0.27) | 1.51 (0.11) | 24.1 (3.32) | 2.40 (0.24) |
| 28 Oct | 10.5 | 24.6 | 49.3 (0.65) | 1.04 (0.12) | 22.2 (1.49) | 1.42 (0.57) |
| 3 Nov | 12.9 | 26.7 | 22.2 (0.47) | 0.68 (0.10) | 21.6 (1.25) | 1.27 (0.07) |
| Mean | 19.6 | 25.0 | 62.2 | 1.41 | 29.7 | 5.59 |
| SE | 1.41 | 0.81 | 8 | 0.26 | 4.47 | 1.36 |

deemed limiting to the accumulation of phytoplankton biomass.

Principal component analysis (PCA) was used as a hierarchical technique to define groups of biological, physical, and chemical variables measured during this study that behaved in a similar manner using the statistical program SPSS (v. 13.0). Varimax rotation with Kaiser normalization was used to assist in the interpretation of composite variables, and only composite variables with eigenvalues exceeding 1 were used for the final analysis (King & Jackson 1999). The degree to which individual parameters were correlated with each other was also evaluated by means of a Spearman rank order correlation matrix.

## RESULTS

### Peconic River estuary

The PR site was physically and chemically dynamic during this study. Water temperatures at this site varied from 11.7 to 28.6°C, peaking in early August, while salinity generally varied from 10.1 to 22.6 (Table 1), reflecting the ephemerally strong freshwater influence at this location and the fact that sampling occurred during different tidal stages. The minimum in salinity (4.13; Table 1) occurred in late October due to heavy precipitation earlier in that month. High DIN levels occurred during the early summer (June, July average ± SE = 11.0 ± 1.5 µM), while DIP levels were lower during this time period (0.7 ± 0.2 µM; Fig. 2C), and the DIN:DIP ratio was close to the Redfield ratio (16 ± 6.2). During late summer (August, September), DIN levels were lower (3.3 ± 1.2 µM), while DIP concentrations were generally higher (2.0 ± 0.4 µM; Fig. 2C), and the DIN:DIP ratio was lower (1.7 ± 1.6). In late September through early October, DIN levels progressively increased until peaking during late October and early November (16 ± 0.5 µM; Fig. 2C) when the DIN:DIP ratio was maximal (29 ± 11). DIP levels at this time were variable but lower than in mid-summer (0.6 ± 0.1 µM; Fig. 2C). Silicate levels in PR were consistently elevated (mean: 110 ± 7.2 µM; Table 1). DON levels were also high, ranging from 12 to 31 µM, while DOP levels were lower (0.5 to 2.5 µM; Table 1). Vitamin $B_{12}$ and $B_1$ levels were dynamic at this site (mean $B_{12}$ = 5.6 ± 1.3 pM; mean $B_1$ = 74 ± 16 pM), with the highest level occurring in late June for both vitamins ($B_{12}$ = 17 pM; $B_1$ = 190 pM). Low levels of vitamin $B_{12}$ (<3 pM) were generally present from early August until mid-October, while vitamin $B_1$ concentrations were an order of magnitude higher at this time (Fig. 2D). Levels of both vitamins increased to near peak levels by the end of the sampling period (November; Fig 2C).

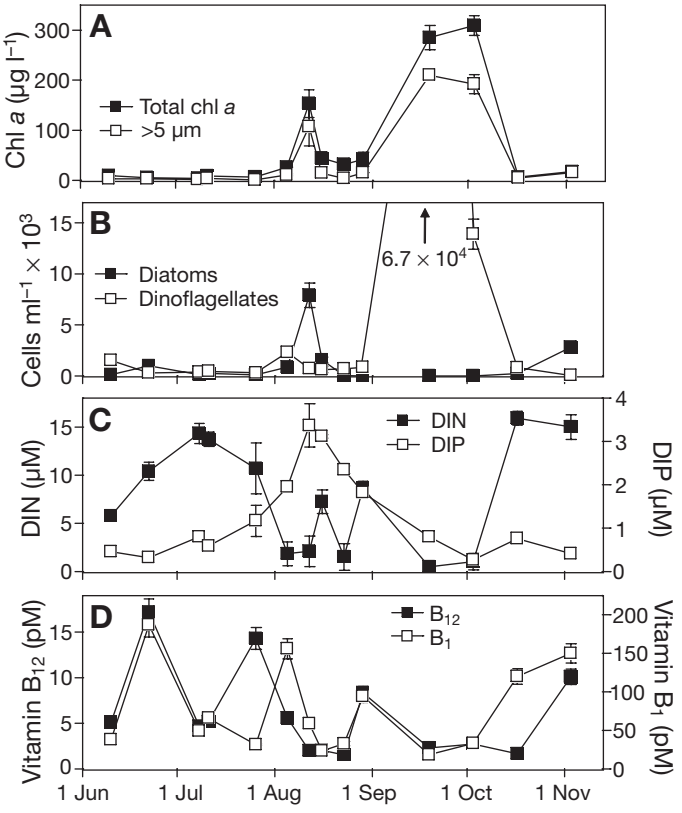


Fig. 2. Time series of parameters measured in the Peconic River, 2005. (A) Levels of total chl *a* and chl *a* >5 µm. (B) Cell densities of diatoms and dinoflagellates. (C) Concentrations of dissolved inorganic nitrogen (DIN) and orthophosphate (DIP). (D) Concentrations of dissolved vitamins $B_1$ and $B_{12}$. Error bars represent ± 1 SD of replicated samples, except for vitamins for which bars represent the analytical precision

Chl *a* concentrations in PR varied widely from 3.8 to 310 µg $l^{-1}$, and were generally dominated by larger phytoplankton (58 ± 26% >5 µm; Fig. 2A). The highest chl *a* levels occurred between August and early October, when levels averaged 130 ± 47 µg $l^{-1}$ (Figs. 3 & 4). Total diatom densities were modest from June through early August (410 ± 170 cells $ml^{-1}$), before peaking on 12 August (7900 ± 120 cells $ml^{-1}$), when *Pseudo-nitzschia* sp. and *Nitzschia closterium* were the dominating species (Fig. 2B, Table 2). After this bloom, diatom levels decreased to undetectable levels from the end of August through early October, when dinoflagellates numerically dominated (Fig. 2B). In mid-September and early October, dinoflagellates bloomed to extremely high levels of 6.8 ± 0.2 × $10^4$ cells $ml^{-1}$ on 19 September and 1.4 ± 0.2 × $10^4$ cells $ml^{-1}$ on 3 October, with these blooms consisting of *Karlodinium veneficum* on 19 September and *K. veneficum* and *Prorocen-*

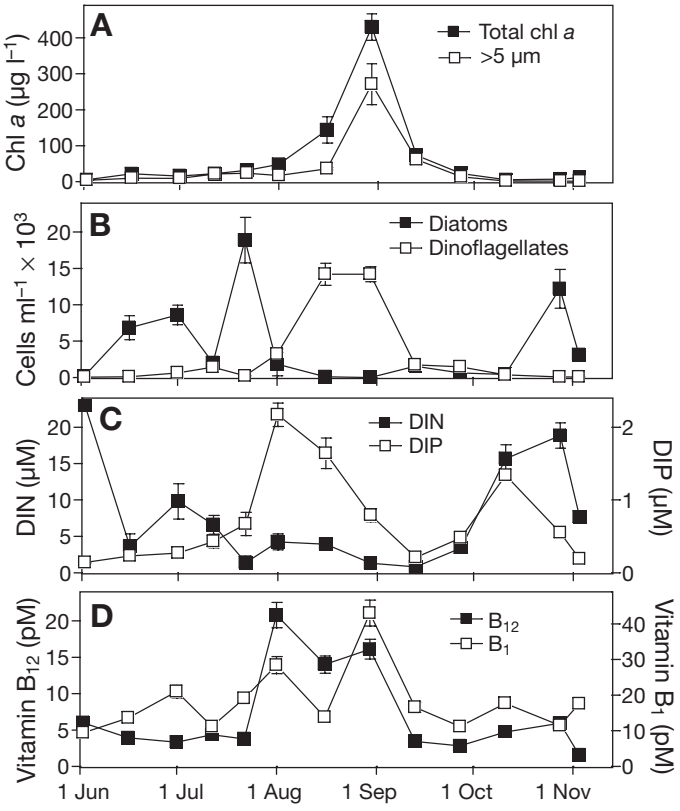


Fig. 3. Time series of parameters measured in Old Fort Pond, 2005. Further details as in Fig. 2

*trum minimum* on 3 October (Fig. 2B, Table 2). Dinoflagellate levels decreased by mid-October (810 ± 46 cells $ml^{-1}$) and were at their lowest level (51 ± 0 cells $ml^{-1}$) in early November, as diatoms returned to dominance (2800 ± 540 cells $ml^{-1}$; Fig. 2B). Bacterial densities increased from 5.5 ± 1.3 × $10^6$ cells $ml^{-1}$ during early summer to 1.1 ± 0.2 × $10^7$ cells $ml^{-1}$ during August through early October, and declined in late October and November (2.8 ± 0.3 × $10^6$ cells $ml^{-1}$; Table 1).

### Old Fort Pond

Water temperatures at OFP varied from 10.5 to 27.6°C, peaking in late July, while salinity levels at this site varied from 18.3 to 29.2 (mean: 26.6 ± 0.81; Table 1). High DIN levels occurred during the early summer (June, July, 8.8 ± 3.8 µM), while DIP levels were lower (0.4 ± 0.1 µM; Fig. 3C). During late summer (August, September), DIN levels in OFP decreased (4.1 ± 2.1 µM), while DIP concentrations were generally higher (1.2 ± 0.4 µM; Fig. 3C). In late September through early October, DIN levels progressively increased until peaking during October (17 ± 1.6 µM; Fig. 3C). DIP levels at this time were more variable and lower than in mid-summer (0.6 ± 0.2 µM; Fig. 3C). Silicate never became depleted at this site, with concentrations ranging from 22 to 120 µM (mean: 65 ± 8.0 µM; Table 1). DON levels ranged from 13 to 73 µM, while DOP levels were lower, ranging from 0.5 to 3.6 µM (Table 1). Vitamin $B_{12}$ was quite dynamic at this site (mean = 6.8 ± 1.7 pM), with the highest levels occurring in August (mean = 17 ± 2.0 pM) and low levels (<5 pM) found before and after this period (Fig. 3D). Vitamin $B_1$ was less variable at OFP (mean = 18 ± 2.5 pM), with the highest level occurring on 30 August (43 pM; Fig 3D). Vitamin $B_1$ levels in OFP were 4 to 5 times lower than in PR (Figs. 2D & 3D).

Chl *a* levels at OFP ranged from 5.1 to 430 µg $l^{-1}$, while the larger fraction of chlorophyll (>5 µm) varied from 1.4 to 270 µg $l^{-1}$ and generally paralleled algal bloom events (Fig. 3A). Total diatom levels were high from mid-June through early August, peaking on

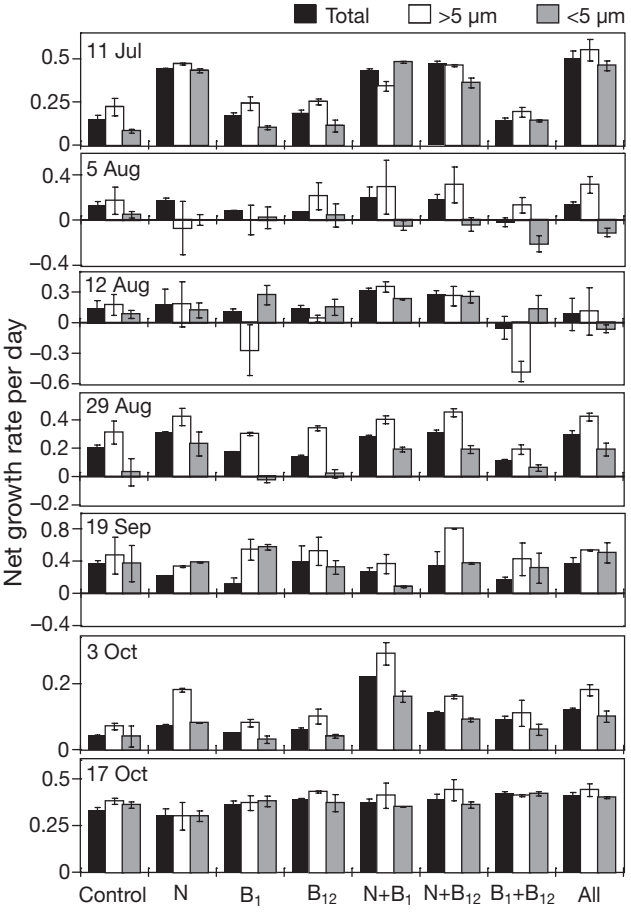


Fig. 4. Net growth rates of total phytoplankton, phytoplankton >5 µm, and phytoplankton <5 µm during nutrient amendment experiments conducted with whole water from the Peconic River. N: nitrate; $B_1$: vitamin $B_1$; $B_{12}$: vitamin $B_{12}$; All: nitrate, $B_1$, and $B_{12}$. Error bars represent ± 1 SE of triplicate experimental bottles

Table 2. Mean diatom and dinoflagellate densities (>5 µm; cells $ml^{-1}$) (SD in parentheses) and the dominating genera and/or species and the relative abundance for each species/genus (as a percentage in parentheses) for each group. Phytoplankton from other classes consistently represented the majority of total phytoplankton counts

| | Density | Dominant diatom species and/or genera |
|---|---|---|
| **Diatoms** | | |
| **Peconic River** | | |
| 10 Jun | 107 (18.2) | *Melosira* sp.[a] (100%) |
| 22 Jun | 999 (166) | *Thalassiosira* sp.[b] (50%), *Guinardia delicatula*[b] (40%) |
| 08 Jul | 121 (13.2) | *Guinardia delicatula*[b] (50%), *Nitzschia seriata*[a] (20%) |
| 11 Jul | 251 (69.6) | *Cylindrotheca* sp.[b] (50%), *Nitzschia seriata*[a] (20%) |
| 26 Jul | 96.4 (6.84) | *Navicula* sp.[b] (70%), *Nitzschia seriata*[a] (10%) |
| 5 Aug | 861 (27.3) | *Nitzschia seriata*[a] (40%), *Pseudonitzschia* sp.[b] (20%), *Nitschia closterium*[a] (10%) |
| 12 Aug | 7910 (120) | *Pseudonitzschia* sp.[b] (70%), *Nitzschia closterium*[a] (20%) |
| 16 Aug | 1560 (280) | *Pseudonitzschia* sp.[b] (60%), *Nitzschia closterium*[a] (30%) |
| 23 Aug | 51 (0) | *Nitzschia closterium*[a] (100%) |
| 29 Aug | 0 (0) | – |
| 19 Sep | 0 (0) | – |
| 3 Oct | 0 (0) | – |
| 17 Oct | 224 (24) | *Navicula* sp.[b] (40%), *Chaetoceros* sp.[b] (30%) |
| 3 Nov | 2820 (54.2) | *Navicula* sp.[b] (30%), *Thalassiosira* sp.[b] (20%), *Chaetoceros* sp.[b] (10%) |
| **Old Fort Pond** | | |
| 02 Jun | 181 (64.3) | *Pseudonitzschia* sp.[b] (60%) |
| 16 Jun | 6840 (1650) | *Pseudonitzschia* sp.[b] (60%), *Guinardia delicatula*[b] (30%) |
| 01 Jul | 8590 (1350) | *Pseudonitzschia* sp.[b] (50%), *Nitzschia seriata*[a] (20%), *Guinardia delicatula*[b] (20%) |
| 12 Jul | 1990 (1.03) | *Nitzschia seriata*[a] (40%), *Pseudonitzschia* sp.[b] (20%), *Guinardia delicatula*[b] (20%) |
| 22 Jul | 18 900 (3100) | *Nitzschia seriata*[a] (40%), *Pseudonitzschia* sp.[b] (20%), *Guinardia delicatula*[b] (20%) |
| 1 Aug | 1840 (1620) | *Pseudonitzschia* sp.[b] (30%), *Cylindrotheca* sp.[b] (30%), *Nitzschia seriata*[a] (10%) |
| 16 Aug | 77.1 (0) | *Thalassionema nitzschioides*[b] (100%) |
| 30 Aug | 0 (0) | – |
| 13 Sep | 1550 (210) | *Pseudonitzschia* sp.[b] (40%), *Rhizoselenia* sp.[b] (30%), *Cylindrotheca* sp.[b] (10%) |
| 27 Sep | 630 (343) | *Skeletonema costatum*[a] (80%), *Navicula* sp.[b] (10%) |
| 11 Oct | 393 (63.4) | *Skeletonema costatum*[a] (50%), *Thalassionema* sp.[b] (20%), *Navicula* sp.[b] (10%) |
| 28 Oct | 12 200 (670) | *Asterionella* sp.[b] (50%), *Skeletonema costatum*[a] (20%), *Chaetoceros* sp.[b] (10%) |
| 3 Nov | 3110 (22.4) | *Asterionella* sp.[b] (40%), *Thalassiosira* sp.[b] (20%), *Skeletonema costatum*[a] (20%) |
| **Dinoflagellates** | | |
| **Peconic River** | | |
| 10 Jun | 1520 (105) | *Scrippsiella* sp.[b] (100%) |
| 22 Jun | 291 (9.81) | *Gymnodinium* sp.[b] (30%), *Amphidinium* sp.[b] (30%), *Dinophysis* sp.[b] (20%) |
| 08 Jul | 375 (14.8) | *Prorocentrum triestinum*[b] (60%), *Scrippsiella* sp.[b] (40%) |
| 11 Jul | 445 (176) | *Prorocentrum triestinum*[b] (80%), *Karlodinium veneficum*[b] (20%) |
| 26 Jul | 308 (54.5) | *Prorocentrum micans*[a] (40%), *Scrippsiella* sp.[b] (30%), *Gymnodinium* sp.[b] (30%) |
| 5 Aug | 2300 (127) | *Karlodinium veneficum*[b] (40%), *Cochlodinium polykrikoides*[U] (30%), *Scrippsiella* sp.[b] (30%) |
| 12 Aug | 717 (112) | *Scrippsiella* sp.[b] (60%), *Gymnodinium* sp.[b] (30%) |
| 16 Aug | 609 (298) | *Scrippsiella* sp.[b] (40%), *Gymnodinium* sp.[b] (40%), *Prorocentrum micans*[a] (20%) |
| 23 Aug | 694 (0) | *Prorocentrum micans*[a] (50%), *Cochlodinium polykrikoides*[b] (30%), *Scrippsiella* sp.[b] (10%) |
| 29 Aug | 848 (36.4) | *Prorocentrum triestinium*[b] (50%), *Cochlodinium polykrikoides*[b] (40%) |
| 19 Sep | 67 800 (1550) | *Karlodinium veneficum*[b] (90%) |
| 3 Oct | 13 900 (1470) | *Karlodinium veneficum*[b] (80%), *Prorcentrum minimum*[b] (10%) |
| 17 Oct | 810 (45.4) | *Prorocentrum triestinium*[b] (50%), *Prorocentrum minimum*[b] (40%) |
| 3 Nov | 51.4 (0) | *Prorocentrum triestinium*[b] (80%) |
| **Old Fort Pond** | | |
| 02 Jun | 46.7 (13.9) | *Prorocentrum micans*[a] (100%) |
| 16 Jun | 85.7 (15) | *Prorocentrum micans*[a] (100%) |
| 01 Jul | 623 (118) | *Prorocentrum triestinum*[b] (50%), *Prorocentrum micans*[a] (30%), *Scrippsiella* sp.[b] (20%) |
| 12 Jul | 1400 (213) | *Prorocentrum triestinum*[b] (60%), *Scrippsiella* sp.[b] (30%), *Prorocentrum minimum*[b] (10%) |
| 22 Jul | 206 (45) | *Prorocentrum micans*[a] (100%) |
| 1 Aug | 3210 (710) | *Prorocentrum minimum*[b] (40%), *Prorocentrum micans*[a] (30%), *Scrippsiella* sp.[b] (20%) |
| 16 Aug | 14 200 (525) | *Prorocentrum micans*[a] (100%) |
| 30 Aug | 14 200 (1040) | *Cochlodinium polykrikoides*[b] (100%) |
| 13 Sep | 1720 (127) | *Cochlodinium polykrikoides*[b] (100%) |
| 27 Sep | 1460 (363) | *Cochlodinium polykrikoides*[b] (70%), *Prorocentrum minimum*[b] (20%) |
| 11 Oct | 353 (8.06) | *Prorocentrum minimum*[b] (80%) |
| 28 Oct | 51.4 (14) | *Prorocentrum minimum*[b] (90%) |
| 3 Nov | 57.8 (9.09) | *Prorocentrum triestinum*[b] (100%) |

[a]Species has a $B_{12}$ requirement (Croft et al. 2005)
[b]$B_{12}$ requirement is unknown (Croft et al. 2005)

22 July ($1.9 \pm 0.3 \times 10^4$ cells $ml^{-1}$), and consisted primarily of *Nitzschia seriata*, *Pseudonitzschia* sp., and *Guinardia deliculata* (Fig. 3B, Table 2). Thereafter, diatom levels decreased, and a period of successive dinoflagellate blooms occurred from August through mid-September, as densities averaged $8.4 \pm 3.4 \times 10^3$ cells $ml^{-1}$ from August to mid-September, peaking on 16 and 30 August ($1.4 \pm 0.1 \times 10^4$ cells $ml^{-1}$ on both dates; Fig. 3B). These dinoflagellate blooms consisted exclusively of *Prorocentrum micans* on 16 August and exclusively of *Cochlodinium polykrikoides* on 30 August (Table 2). In the fall, diatom levels increased from late September through mid-October, peaking again on 28 October when *Asterionella* sp., *Skeletonema costatum*, and *Chaetoceros* sp. were the dominant species ($1.2 \pm 0.1 \times 10^4$ cells $ml^{-1}$; Fig. 3C, Table 2). Bacterial densities were lower during June and July (2.2 to $6.7 \times 10^6$ cells $ml^{-1}$), high during August (8.8 to $19 \times 10^6$ cells $ml^{-1}$), and steadily decreased from early September ($5.9 \pm 3.2 \times 10^6$ cells $ml^{-1}$) through November ($1.3 \pm 0.1 \times 10^6$ cells $ml^{-1}$; Table 1).

### PCA of environmental variables

PCA of the data from both sites extracted 4 composite variables, which captured 73% of the variance of the complied data set (Table 3). The first principal component (PC 1) explained 34% of the variance in the data set. Within PC 1, bacterial densities, temperature, phosphate, and silicate co-varied inversely with nitrate and urea (Table 3). PC 2 accounted for 17% of the variance and comprised chl *a* (>5 and <5 µm) and dinoflagellates, which covaried (Table 3). PC 3 accounted for 11% of data variance and consisted of vitamin $B_1$ and ammonium, which co-varied inversely with salinity and diatom levels. Finally, in PC 4, vitamin $B_{12}$, DON, and DOP were positively correlated with one another and accounted for 10% of the variance (Table 3).

### Peconic River estuary nutrient amendment experiments

During the July experiment at PR, $NO_3^-$ amendments significantly increased net growth rates of all size classes of chlorophyll more than 2-fold ($p < 0.001$; Table 4, Fig. 4). In the same experiment, there was a significant interaction between $NO_3^-$ and vitamin $B_{12}$ amendments ($p < 0.05$; Table 4, Fig. 4) with respect to growth of larger phytoplankton (>5 µm). During the 5 August experiment, $NO_3^-$ addition again had a significant effect on the total phytoplankton community, yielding higher growth rates than other treatments ($p < 0.05$; Table 4, Fig. 4). During the12 August experiment, $NO_3^-$ and $B_{12}$ were both significant treatments for larger phytoplankton ($p < 0.01$, $p < 0.05$, respectively; Table 4, Fig. 4), as $NO_3^-$ additions alone increased growth rates by ~30% compared to controls, and $B_{12}$ additions alone yielded a small decrease in growth compared to the control (Fig. 4). In the same experiment, there were significant interactions between vitamins $B_{12}$ and $B_1$ amendments with respect to smaller phytoplankton growth (<5 µm; $p < 0.01$; Table 4, Fig. 4). During the 29 August experiment, $NO_3^-$ significantly stimulated net growth of all phytoplankton size fractions; total chl *a* = 40% increase, <5 µm chl *a* = 80% increase, and >5 µm chl *a* = 10% increase ($p < 0.01$; Table 4, Fig. 4). During this experiment, ANOVA revealed a significant interaction between $NO_3^-$ and $B_{12}$ with regard to total phytoplankton growth ($p < 0.05$; Table 4, Fig. 4). Although algal communities in PR were nutrient-replete on 19 September, during the 3 October experiment, all individual and combined treatments produced significant effects for total chlorophyll ($p < 0.05$; Table 4, Fig. 4), with individual additions of $NO_3^-$, $B_1$, and $B_{12}$ stimulating net growth rates by 266, 133, and 267% over control treatments, respectively. For growth rates of larger phytoplankton in this experiment, only $NO_3^-$ and vitamin $B_1$ had significant effects ($p < 0.01$, $p < 0.001$, respectively; Table 4, Fig. 4). Significant interactions between $NO_3^-$ + $B_1$, $B_{12}$ + $B_1$, and combined $NO_3^-$ + $B_{12}$ + $B_1$ were apparent for the larger phytoplankton in the 3 October experiment in PR ($p < 0.01$, $p < 0.05$, $p < 0.001$; Table 4, Fig. 4). For the small phytoplankton size fraction

Table 3. Factor loadings for the 4 significant rotated principal components extracted from the Old Fort Pond (OFP) and Peconic River (PR) water quality data set. Values in bold indicate the groupings of the 4 PCs that described 73% of the variance in the PR and OFP data set

| Parameter | PC 1 | PC 2 | PC 3 | PC 4 |
|---|---|---|---|---|
| Bacteria | **0.81** | 0.22 | 0.03 | 0.43 |
| Temperature | **0.81** | 0.05 | 0.01 | 0.09 |
| Phosphate | **0.80** | –0.17 | 0.11 | 0.12 |
| Nitrate | **–0.75** | –0.31 | 0.19 | –0.06 |
| Silicate | **0.71** | 0.29 | 0.36 | –0.01 |
| Urea | **–0.58** | –0.37 | 0.15 | 0.03 |
| Chl *a*, >5 µm | 0.19 | **0.89** | –0.09 | 0.17 |
| Dinoflagellates | 0.09 | **0.84** | –0.01 | –0.03 |
| Chl *a*, <5 µm | 0.20 | **0.78** | –0.08 | 0.33 |
| Salinity | –0.10 | 0.05 | **–0.91** | 0.27 |
| Vitamin $B_1$ | 0.14 | –0.10 | **0.72** | –0.02 |
| Ammonium | –0.22 | –0.37 | **0.66** | 0.02 |
| Diatoms | 0.07 | –0.29 | **–0.55** | –0.28 |
| DON | 0.16 | 0.24 | –0.35 | **0.83** |
| Vitamin $B_{12}$ | 0.03 | –0.04 | 0.28 | **0.82** |
| DOP | 0.45 | 0.32 | –0.23 | **0.74** |
| % of variance explained | 34 | 17 | 11 | 10 |

Table 4. Experimentally significant treatment effects for total, >5 µm, and <5 µm chl *a*-based growth rates as determined by a 3-way ANOVA. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. A negative sign before a nutrient indicates that the treatment yielded a significant decrease in growth rates, whereas all others yielded significantly higher growth rates. On 3 October, all interactions were significant for total chlorophyll ('All'), whereas all interactions except $N+B_{12}$ were significant for chlorophyll >5 µm ('All but $N+B_{12}$'). n/a: not applicable

| | Treatment effects | | | Interactions | | |
|---|---|---|---|---|---|---|
| | Total | >5 µm | <5 µm | Total | >5 µm | <5 µm |
| **Peconic River** | | | | | | |
| 11 Jul | $N^{***}$ | $N^{***}$ | $N^{***}$ | – | $N+B_{12}^{*}$ | – |
| 05 Aug | $N^{*}$ | – | – | – | – | – |
| 12 Aug | – | $N^{**}$, $-B_{12}^{*}$ | – | – | – | $B_{12}+B_1^{**}$ |
| 29 Aug | $N^{***}$ | $N^{**}$ | $N^{***}$ | $N+B_{12}^{*}$ | – | – |
| 19 Sep | – | – | – | – | – | – |
| 03 Oct | $N^{***}$, $B_{12}^{*}$, $B_1^{***}$ | $N^{**}$, $B_1^{***}$ | $N^{***}$, $B_1^{*}$ | All$^{**}$ | All but $N+B_{12}^{*}$ | – |
| 17 Oct | $B_{12}^{*}$ | $B_{12}^{**}$ | – | – | – | – |
| **Old Fort Pond** | | | | | | |
| 22 Jul | $N^{*}$ | – | $N^{***}$ | – | – | $N+B_{12}^{**}$ |
| 01 Aug | – | – | – | – | – | – |
| 16 Aug | $N^{***}$, $-B_1^{*}$ | n/a | n/a | $B_{12}+B_1^{*}$, $N+B_{12}+B_1^{*}$ | n/a | n/a |
| 30 Aug | – | $N^{*}$ | – | – | – | – |
| 13 Sep | $N^{***}$ | – | $N^{*}$ | $B_{12}+B_1^{*}$ | – | – |
| 27 Sep | $N^{***}$, $B_{12}^{**}$ | $N^{***}$, $B_{12}^{***}$ | $N^{***}$ | – | $B_{12}+B_1^{*}$ | – |
| 28 Oct | $B_{12}^{*}$ | – | – | – | – | – |

(<5 µm) in the 3 October experiment, individual additions of $NO_3^-$ or $B_1$ both significantly increased growth rates by 40 and 10%, respectively ($p < 0.001$, $p < 0.05$, respectively; Table 4, Fig. 4). During the 17 October experiment, vitamin $B_{12}$ alone significantly augmented growth of the total and larger phytoplankton size fractions by ~15% ($p < 0.05$ and $p < 0.01$, respectively; Fig. 4, Table 4).

### Old Fort Pond nutrient amendment experiments

During the 22 July experiment, $NO_3^-$ addition significantly increased growth rates of total and smaller phytoplankton by 55 and 76% ($p < 0.05$, $p < 0.001$; Table 4, Fig. 5). Growth of smaller (<5 µm) phytoplankton was significantly stimulated by combined effects of $NO_3^-$ and vitamin $B_{12}$ in this experiment ($p < 0.01$; Table 4, Fig. 5). Although algal communities in OFP were nutrient-replete on 1 August, during the 16 August experiment, $NO_3^-$ and vitamin $B_1$ were both significant treatments for total chlorophyll production ($p < 0.001$, $p < 0.05$; Table 4, Fig. 5). However, the effects of $NO_3^-$ and vitamins differed. $NO_3^-$ additions alone doubled growth rates, while B-vitamin amendments alone depressed growth rates (Fig. 5). In this experiment, interactions among vitamins $B_{12}$ and $B_1$ and $NO_3^-$ were all significant with respect to total chlorophyll growth rates ($p < 0.05$; Table 4, Fig. 5). During the 30 August experiment, $NO_3^-$ was the only treatment that significantly affected the larger (>5 µm) phytoplankton ($p < 0.05$; Table 4, Fig. 5), increasing net growth rates by 45% relative to controls. Similarly, during the 13 September experiment, $NO_3^-$ was the only amendment that significantly stimulated growth of total and the smaller size fraction ($p < 0.001$, $p < 0.05$, respectively; Table 4, Fig. 5), increasing rates by 70 and 30% compared to controls, respectively. In this experiment, ANOVA revealed a significant interaction between vitamins $B_{12}$ and $B_1$ for total algal growth rates ($p < 0.05$; Table 4, Fig. 5). For the 27 September experiment, $NO_3^-$ addition increased net growth by 160% for total chl *a*, 90% for >5 µm chl *a*, and 230% for the <5 µm chl *a* compared to control treatments ($p < 0.001$; Table 4, Fig. 5). In this same experiment, vitamin $B_{12}$ addition also increased net growth rates significantly for both total chlorophyll (20% over control) and the larger phytoplankton (30% over control), but to a lesser extent than $NO_3^-$ ($p < 0.01$, $p < 0.001$; Table 4, Fig. 5). During the 27 September experiment, ANOVA indicated a significant interaction between vitamins $B_{12}$ and $B_1$ for the larger algae ($p < 0.05$; Table 4, Fig. 5). Finally, during the 28 October experiment, vitamin $B_{12}$ addition significantly increased net growth rates of total chlorophyll (by ~20% compared to controls; $p < 0.05$; Table 4, Fig. 5).

## DISCUSSION

During this study, both estuarine sites displayed similar seasonal successions of phytoplankton, macronutrient, and vitamin inventories. We observed lower levels of algal biomass in early summer, followed by intense blooms of dinoflagellates during late summer,

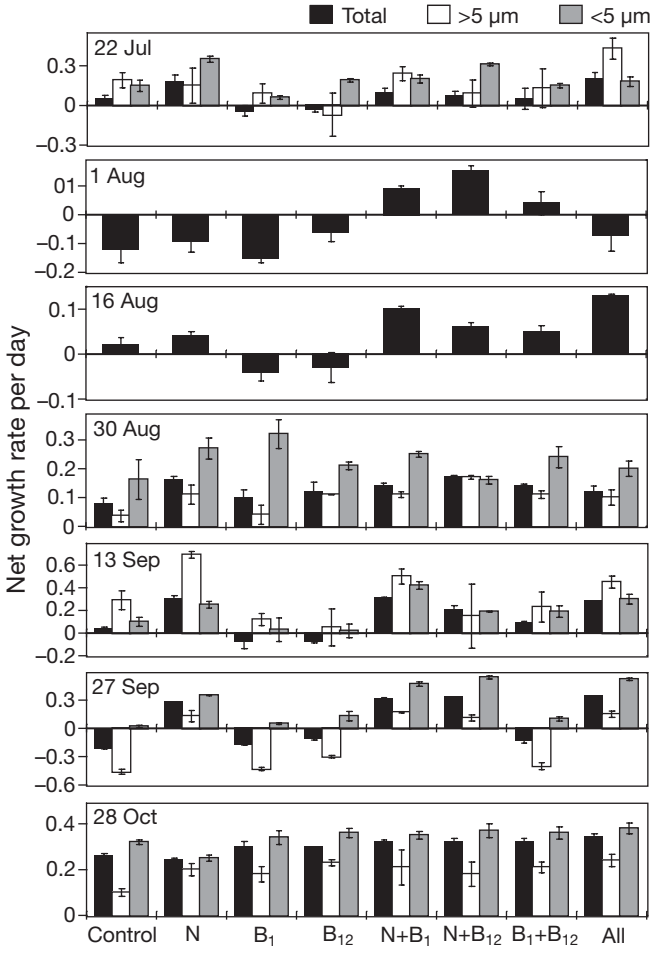


Fig. 5. Net growth rates of total phytoplankton, phytoplankton >5 µm, and phytoplankton <5 µm during nutrient amendment experiments conducted with whole water from Old Fort Pond. Treatment abbreviations as in Fig. 4

succeeded by lower algal biomasses and dominance by diatoms in the fall. Both locations also displayed nutrient-replete periods, as well as periods in which nutrient amendments stimulated growth of the phytoplankton community. For example, $NO_3^-$ additions were stimulatory to the total algal community in 57% of experiments, vitamin $B_{12}$ in 28%, and vitamin $B_1$ in 7% (Table 4). Comparing results from these 2 sites provides new insight into the potential role of vitamins in regulating phytoplankton diversity and biomass in coastal ecosystems.

### Nutrient-vitamin-phytoplankton dynamics

#### Early summer

During early summer (June, July) both study sites hosted moderate levels of phytoplankton biomass (Figs. 2A & 3A), high DIN levels, and lower DIP levels (Figs. 2C & 3C). However, vitamin levels differed between sites at this time; OFP displayed relatively constant, low levels of B-vitamins ($B_1$ = 15 ± 2.2 pM; $B_{12}$ = 3.9 ± 0.6 pM ; Fig. 3D), while PR had higher and more variable levels ($B_1$ = 68 ± 31 pM; $B_{12}$ = 8.5 ± 3.0 pM; Fig. 2D). Levels of vitamin $B_1$ at PR were significantly higher (almost 5 times) than those found in OFP ($p < 0.05$; *t*-test) during this period, as well as throughout the study. Furthermore, a significant, inverse correlation between $B_1$ and salinity was found ($p < 0.001$; Fig. 6A), which was also apparent in PC 3 of our PCA (Table 3). The inverse correlation between salinity and $B_1$ suggests that freshwater input associated with the rivers and/or groundwater may be an important source of $B_1$ to coastal ecosystems.

Nitrate stimulated the accumulation of algal biomass during July at both locations (Figs. 4 & 5). Experiments conducted in OFP on 22 July, when DIN levels were low (1.4 µM), indicated that $NO_3^-$ significantly enhanced algal biomass in the total and smaller phytoplankton (<5 µm) size fractions, but neither $B_1$ nor $B_{12}$ altered

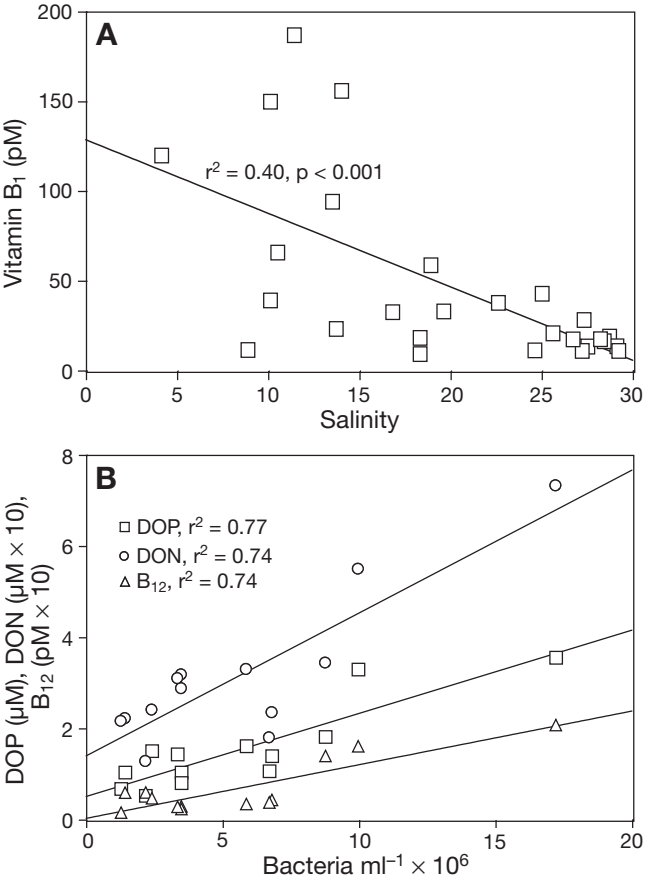


Fig. 6. Covariations between (A) vitamin $B_1$ and salinity, and (B) vitamin $B_{12}$, DON, DOP, and heterotrophic bacteria. All parameters are significantly correlated with each other ($p < 0.001$ for all)

growth rates for any size fraction at this time (Fig. 5, Table 4). Similarly, experiments conducted in PR on 11 July indicated that all size classes of phytoplankton were stimulated by N additions (Table 4, Fig. 4). N limitation has been commonly observed in coastal ecosystems (Ryther & Dunstan 1971, Howarth 1988, Fisher et al. 1992), and in Long Island estuaries in particular (Gobler et al. 2004), especially during summer when high temperatures (>25°C) contribute toward the rapid exhaustion of available N pools (Carpenter & Goldman 1974; Figs. 2C & 3C).

### Late summer

Late summer (August, September) was characterized by a period of high biomass dinoflagellate blooms at both locations (Figs. 2A,B & 3A,B). Compared to early summer, both locales displayed lower levels of DIN, higher DIP concentrations (Figs. 2C & 3C), and DIN:DIP ratios below the Redfield ratio. The temporal coherence among biological, chemical, and physical parameters through the summer was evident within the grouping of these constituents in PC 1, which showed that higher temperatures were associated with higher bacterial densities, phosphate, and silicate concentrations (Table 3), and likely associated with enhanced microbial nutrient regeneration at this time (Boynton et al. 1995, Williams 2000). Higher temperatures were also inversely related to nitrate levels in PC 1 (Table 3), presumably due to rapid uptake by phytoplankton and reduced freshwater delivery of nitrate during warm summer months (Carpenter & Goldman 1974, Gobler & Sañudo-Wilhelmy 2001). Consistent with low DIN levels and low DIN:DIP ratios during late summer, $NO_3^-$ additions enhanced growth rates of all size classes of phytoplankton during approximately half of the experiments conducted during this period (Figs. 4 & 5, Table 4).

In contrast to macronutrients, the dynamics of phytoplankton and vitamins differed markedly between sites during late summer. In PR, vitamin $B_1$ and $B_{12}$ levels were, on average, lower during late summer ($B_1$ = 60 ± 19 pM; $B_{12}$ = 3.50 ± 0.95 pM; Fig. 2D), but were also dynamic, displaying peaks in early and late August prior to the occurrence of large phytoplankton blooms in mid-August and mid-September (Fig. 2). Past research in coastal regions has implicated such increases in nutrients prior to algal blooms as an indication of bloom stimulation (Gobler & Sañudo-Wilhelmy 2001, Berman et al. 2005). Consistent with this hypothesis, the high levels of vitamins $B_1$ and $B_{12}$ present in late August ($B_1$ = 160 ± 4.4 pM; $B_{12}$= 14 ± 0.70 pM) were reduced (by nearly 90%) to their lowest levels of the study ($B_1$ = 18 ± 7; $B_{12}$= 1.7 ± 0.2 pM; Fig. 2D) during the intense bloom of *Karlodinium veneficum* and *Prorocentrum minimum* from mid-September to early October (300 µg chl *a* $l^{-1}$, >$10^4$ cells $ml^{-1}$; Table 2, Fig. 2B). Such a reduction in vitamin levels during the bloom suggests active uptake by dinoflagellates. Moreover, the nutrient-amendment experiment conducted at the end of the bloom (3 October) demonstrated that $B_1$, $B_{12}$, and $NO_3^-$ additions each significantly enhanced phytoplankton growth ($p < 0.05$; 3-way ANOVA; Table 4, Fig. 4). These results suggest that all 3 nutrients were drawn down to limiting levels by the bloom and were responsible, at least in part, for the subsequent bloom collapse (Fig. 2B). The total dissolved N:$B_{12}$ ratio (mol/mol) in PR on 3 October was $1 \times 10^7$, an order of magnitude higher than cellular stoichiometry of exponentially growing phytoplankton (N:$B_{12}$ = $1.4 \times 10^6$; Carlucci & Bowes 1972), suggesting that $B_{12}$ may have been more limiting than N on this date.

Although it is currently unknown whether the dinoflagellates that bloom in PR (*Karlodinium veneficum* and *Prorocentrum minimum*) are vitamin auxotrophs, most dinoflagellates (~90% of those surveyed; Croft et al. 2005), and other *Prorocentrum* species (*P. micans*, in particular), are vitamin $B_{12}$ auxotrophs (Provasoli & Carlucci 1974). Moreover, vitamin $B_{12}$ has been implicated in the occurrence of dinoflagellate blooms around the world (Aldrich 1962, Carlucci 1970, Takahashi & Fukazawa 1982, Yu & Harrison 2000) and in PR (Bruno & Staker 1978). Demand for vitamin $B_{12}$ in dinoflagellates is consistent with the activation of the $B_{12}$-dependent enzyme methylmalonyl-CoA mutase when algae grow heterotrophically and/or phagotrophically (Croft et al. 2006), 2 common nutritional strategies among dinoflagellates (Taylor 1987, Smayda 1997). Consistent with this information, our findings suggest that vitamin inputs could have stimulated initiation of the *K. veneficum–P. minimum* bloom and that vitamin deficiency may have contributed, at least in part, to bloom collapse.

Interactions between dinoflagellate blooms and vitamins were different in OFP. The emergence of massive dinoflagellate blooms in August in OFP (>100 µg chl *a* $l^{-1}$, >$10^4$ cells $ml^{-1}$; Fig. 3B) was accompanied by the highest levels of vitamin $B_{12}$ recorded during this study (15 ± 1.0 pM; Fig. 3D). While these blooms began as a mixed population in early August (*Prorocentrum minimum, P. micans*, and *Scrippsiella* sp.), they were dominated by *P. micans* (Table 2) in mid-August and by *Cochlodinium polykrikoides* from late August through the end of September (70 to 100% of dinoflagellates; Table 2). *P. micans* is a known vitamin $B_{12}$ auxotroph (Croft et al. 2005) and, in a manner consistent with vitamin uptake, when it was the dominant alga (100% of phytoplankton >5 µm on 16 August, Table 2), levels

of both vitamin $B_1$ and vitamin $B_{12}$ were each reduced by 50% compared to the prior sampling date and then rose again when this species was no longer present (30 August; Fig. 3C,D). The requirement for $B_{12}$ by other dinoflagellates blooming in OFP in August is currently unknown. As such, the extremely high levels of vitamin $B_{12}$ occurring during early and late August dinoflagellate blooms in OFP could have been partly due to reduced algal demand and/or higher supply rates. For example, vitamin $B_{12}$ synthesis by co-occurring bacterial populations may have been elevated during these blooms when bacteria reached the highest densities of this study ($>10^7$ cells $ml^{-1}$; Table 1).

Concurrent with high levels of dinoflagellates, bacteria, and vitamin $B_{12}$ in OFP during August, concentrations of dissolved organic matter (DON and DOP) were also elevated (Table 1). Moreover, bacterial densities in OFP were significantly correlated with concentrations of vitamin $B_{12}$ ($p < 0.001$, $r = 0.86$), DON ($p < 0.001$, $r = 0.86$), and DOP ($p < 0.0001$; $r = 0.88$, Fig. 6B). In addition, vitamin $B_{12}$, DON, and DOP comprised PC 4 of our PCA (Table 4). The existence of these correlations in OFP (Fig. 6B), but not in PR, suggests stronger DOM production by bacteria at the former site (Azam et al. 1983, Kirchman 2000) and may account for differences in $B_{12}$ levels found between sites during late summer algal blooms. Together these findings suggest that vitamin $B_{12}$ behaves similarly to recycled organic nutrients in aquatic environments, being generated by bacteria as they grow, degrade organic matter (Williams 2000), and/or experience mortality via microbial grazing or viral lysis (Gobler et al. 1997, Nagata 2000).

## Fall

Following the late summer dinoflagellate blooms that persisted through September in OFP and into October in PR, both sites experienced lower levels of phytoplankton biomass compared to the late summer-early fall months (Figs. 2A & 3A). DIN and vitamin levels progressively increased at this time, perhaps as a function of a reduced algal demand, although levels of vitamins increased at a slower rate than DIN (Figs. 2C,D & 3C,D), a difference reflected in our experimental results. For example, on 27 September in OFP, both $NO_3^-$ and vitamin $B_{12}$ significantly enhanced growth rates for total phytoplankton and the larger phytoplankton (>5 µm), suggesting a co-limitation by these 2 nutrients. While some phytoplankton present during this experiment, such as *Skeletonema costatum*, are known $B_{12}$ auxotrophs (Provasoli & Carlucci 1974), the vitamin requirements of the more abundant dinoflagellates present at the time, such as *Cochlodinium polykrikoides* and *Prorocentrum minimum*, are unknown (Table 2). The experimental response to $NO_3^-$ and vitamin $B_{12}$ by phytoplankton was identical to an experiment conducted almost exactly 1 yr prior in an embayment only 10 km west of OFP (Sañudo-Wilhelmy et al. 2006). As was the case then, $B_{12}$ stimulated growth of larger (>5 µm), but not smaller (<5 µm) phytoplankton in OFP, a finding consistent with the hypothesis that larger eukaryotic algae are often vitamin $B_{12}$ auxotrophs, but smaller, possibly prokaryotic phytoplankton are capable of de novo $B_{12}$ synthesis (Croft et al. 2005, 2006, Sañudo-Wilhelmy et al. 2006).

During October, DIN levels in OFP continued to rise, and the phytoplankton community transitioned from dinoflagellates to domination by diatoms (Fig. 3D). Also at this time (28 October), vitamin $B_{12}$ amendment was the sole treatment capable of significantly stimulating algal growth rates (Table 4, Fig. 5). This finding is consistent with the higher levels of DIN (Fig. 3C) and a high DIN:DIP ratio, more than double the Redfield ratio at this time. It is possible that growth responses among the larger phytoplankton were due to *Skeletonema costatum* and *Chaetoceros* sp., which were abundant and are known vitamin $B_{12}$ auxotrophs (Provasoli & Carlucci 1974; Table 2).

Experimental results in PR during the fall were similar to OFP. On 17 October, larger phytoplankton (>5 µm), particularly the dinoflagellates *Prorocentrum triestinium* and *P. minimum*, dominated the algal community (Table 2, Fig. 3A,B). DIN levels were high (16 µM) and vitamin $B_1$ levels were elevated (120 pM), but $B_{12}$ levels were nearly the lowest of this study (1.7 pM; Fig. 3C,D). During the 17 October experiment in PR, vitamin $B_{12}$ additions significantly stimulated the total phytoplankton community and particularly larger phytoplankton (Table 4, Fig. 5). Similar to the October experiment in OFP, this PR experiment demonstrates that vitamins can stimulate the growth of the microalgal community. Moreover, these results provide another example of larger phytoplankton being more prone to vitamin limitation than smaller phytoplankton (Sañudo-Wilhelmy et al. 2006). In fact, while vitamin amendments enhanced growth of the total or >5 µm size fractions 8 times during this study, vitamins significantly stimulated small phytoplankton only on 1 occasion ($B_1$ at PR: 3 October; Table 3). It is possible that this single exception was due to the growth of small prymnesiophytes, which can be abundant in coastal environments (Seoane et al. 2005) and primarily comprise $B_1$ auxotrophs (83% of species surveyed; Croft et al. 2006).

It is notable that vitamin $B_{12}$ additions significantly increased total algal growth rates only during the 4 experiments conducted during fall (27 September to 28 October) and that vitamin $B_1$ significantly enhanced growth at PR during this period (Table 4, Figs. 4 & 5). These findings are consistent with previous work,

which documented that vitamin $B_{12}$ is capable of enhancing algal biomass during late September (Sañudo-Wilhelmy et al. 2006). The emergence of vitamin $B_{12}$ limitation during early fall may be a seasonal phenomenon that could influence the seasonal succession of coastal phytoplankton from an abundance of auxotrophic species during late summer to species with lower or no vitamin requirements during the early fall. Interestingly, dominance within phytoplankton communities at both study sites shifted from dinoflagellates to diatoms during this period, and a larger percentage of dinoflagellates are $B_{12}$ auxotrophs (90%) compared to diatoms (60%; Croft et al. 2005). It is plausible that variations in prevalence of vitamin auxotrophy or interspecific differences in uptake kinetics ($K_m$ and $V_{max}$) may influence this transition within the phytoplankton community. Other co-occurring processes that may influence the seasonal occurrence of vitamin $B_{12}$ limitation may include the decline in bacterial vitamin synthesis associated with cooler temperatures and lower bacterial densities (Table 1). Another factor that may promote vitamin limitation at this time is the rise in macronutrient concentrations associated with seasonally elevated freshwater discharge (Steenhuis et al. 1985, Gobler & Sañudo-Wilhelmy 2001). With higher levels of DIN, N is less likely to limit algal growth rates, causing other nutrients, such as vitamins, to become limiting. Finally, vitamin-binding factors (VBF) released by some algal species effectively sequester vitamin $B_{12}$ and may make the vitamin unavailable to other species (Droop 1968, Swift 1980, Sahni et al. 2001). VBF production appears to be maximal among algal populations that grow to very high population densities (Swift 1980). Therefore, it is plausible that such binding factors were present at high levels following the collapse of the high biomass blooms observed during this study (>100 µg $l^{-1}$; Figs. 2A & 3A) and could have also contributed to fall vitamin limitation of phytoplankton present following the collapse of these blooms.

## CONCLUSION

Although B-vitamins are essential growth factors for most phytoplankton, no study to date has directly measured vitamin $B_1$ in unison with phytoplankton dynamics, and only one other study has reported direct measurements of vitamin $B_{12}$ along with changes in algal community composition (Sañudo-Wilhelmy et al. 2006). Here we found that vitamin $B_1$ can be supplied by freshwater discharge, while vitamin $B_{12}$ behaved like a recycled organic nutrient with its peak levels associated with elevated bacterial densities. Although these coastal systems were primarily limited by nitrogen during summer, high-biomass blooms of dinoflagellates (>100 µg chl *a* $l^{-1}$) during fall months seemed to reduce vitamin concentrations to levels that limited the accumulation of larger phytoplankton during experiments. This phenomenon likely influenced the seasonal succession of phytoplankton from summer auxotrophs (dominated by dinoflagellates) to non-auxotrophic species (dominated by diatoms) during the fall.

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