Limnol. Oceanogr., 47(1), 2002, 129–141

Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom

Christopher J. Gobler,[1] *Marc J. Renaghan, and Nathaniel J. Buck*
Natural Science Division, Southampton College of Long Island University, Southampton, New York 11968

Abstract

Although nutrients and grazing both contribute to the formation of harmful algal blooms, research on these events has rarely considered both factors simultaneously. To ascertain the impact of nutrients and grazing on brown tides of *Aureococcus anophagefferens*, nutrient bioassays were conducted in parallel with dilution-style microzooplankton grazing experiments during an intense bloom that occurred throughout Great South Bay (GSB), New York, in fall of 1999. During the study, *Aureococcus* represented between 25 and 85% of phytoplankton biomass and attained peak cell densities $>6 \times 10^5$ cells ml^{-1}. Concentrations of dissolved organic carbon (DOC) and nitrogen (DON) in GSB were high (mean = 430 μM and 32 μM, respectively) during the bloom, while dissolved inorganic nitrogen (DIN) levels were low (mean = 2.5 μM). Although the experimental additions of nitrogen (nitrate or urea) typically enhanced the growth rates of the non–brown tide phytoplankton community, such additions often had no impact on, or decreased, growth rates of *Aureococcus* relative to unamended control treatments. These observations suggest that growth of non–brown tide phytoplankton depended on ambient N supply rates, while *Aureococcus* experienced nutrient replete growth. Dilution experiments indicated that microzooplankton grazing rates on *A. anophagefferens* were significantly lower than those on other algal populations. This reduced grazing pressure contributed toward higher net growth rates for *Aureococcus* relative to non–brown tide phytoplankton. In sum, these results demonstrate that both top-down (low grazing mortality rates) and bottom-up (a high DOC/DON, low DIN nutrient regime) factors can contribute to the proliferation of brown tide blooms in New York waters.

The level of phytoplankton biomass in a body of water at any given time is a function of cooccurring algal growth and removal processes. Algal blooms, therefore, result when these processes are imbalanced, with phytoplankton growth significantly outpacing removal rates. To date, most research on harmful algal blooms has tended to focus singularly on factors that either enhance or remove phytoplankton biomass but has seldom considered both simultaneously. This is particularly true of research on brown tide blooms caused by *Aureococcus anophagefferens* (Pelagophyceae), since every hypothesis developed to explain bloom initiation and sustenance to date has invoked an exclusively bottom-up (Cosper et al. 1989; Nixon et al. 1994; LaRoche et al. 1997; Gobler and Sañudo-Wilhelmy 2001*a*) or top-down hypothesis (Caron et al. 1989; Lonsdale et al. 1996; Mehran 1996).

There exists a substantial body of evidence that organic nutrients play an important role in the nutrition of brown tide blooms. Laboratory cultures and field populations of *A. anophagefferens* have demonstrated the ability to assimilate organic carbon and nitrogen compounds (Dzurica et al. 1989; Berg et al. 1997). Field observations of brown tides indicate that blooms often occur when dissolved organic nitrogen (DON) levels are high (LaRoche et al. 1997) or when inorganic nutrient levels are low (Keller and Rice 1989). Moreover, natural and manipulated inputs of dissolved organic nitrogen and carbon (DOC) have been observed to stimulate the growth of *A. anophagefferens* during brown tide events (Gobler and Sañudo-Wilhelmy 2001*a*,*b*).

The impact of microzooplankton grazing on *A. anophagefferens* blooms is unclear. Caron et al. (1989) demonstrated that some cultured protozoan grazers are capable of growing in the presence of *A. anophagefferens*, while Mehran (1996) found that a cultured ciliate isolated from a brown tide prone embayment experienced significantly reduced growth rates in the presence of moderate *A. anophagefferens* densities ($>10^5$ cells ml^{-1}). Field studies have also come to differing conclusions with regard to *A. anophagefferens*–protozoan interactions. While some studies have observed decreased grazing and growth rates of microzooplankton during brown tide events (Lonsdale et al. 1996; Mehran 1996), others have concluded that grazing rates are not impacted by *A. anophagefferens* densities (Caron et al. 1989). To date, grazing rates on *A. anophagefferens* during brown tide events have not been quantified.

To assess the relative importance of top-down and bottom-up processes in controlling the abundance of *A. anophagefferens*, an observational and experimental field campaign was established in Great South Bay, New York, during a brown tide in the fall of 1999. We conducted bioassays experiments with organic (urea, glycine, glucose) and inorganic (nitrate, phosphate) nutrients (Gobler and Sañudo-Wilhemly 2001*a*) in parallel with dilution-style microzooplankton grazing experiments (Landry et al. 1995). Since it has been hypothesized that cyanobacteria compete with *A.*

[1] Corresponding author (cgobler@southampton.liu.edu).

Acknowledgements

This paper is dedicated to the memory of Jason R. Vercelletto, who assisted in dilution experiments conducted during this study. This project was funded by New York Sea Grant award R/CMB-25 to C.J.G. and was supported by a start-up award from Southampton College of Long Island University to C.J.G. We thank A. Freidank, C. Jewett, and M. Roemer for assistance during experiments and field sampling. We thank the Southampton College Marine Station for boat usage and assistance and the Fire Island National Seashore's Patchogue facility for boat dockage. We are grateful to H. Cyr, S. Shumway, and two anonymous reviewers for useful comments on earlier versions of this manuscript.

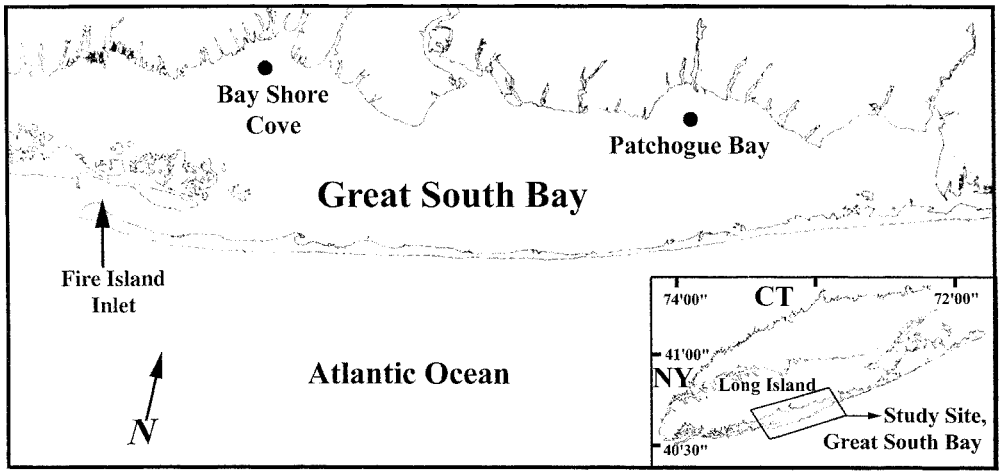

Fig. 1. Great South Bay, New York, USA, with study sites, Bay Shore Cove and Patchogue Bay, noted.

anophagefferens to fill a picoalgal niche in Long Island embayments (Sieracki et al. 1999), the responses of both populations, as well as the total phytoplankton community, were monitored during these experiments. This work represents the first experimental field study of brown tides in GSB, as well as the first study to simultaneously evaluate the impact of nutrients and grazing on brown tide blooms of *Aureococcus anophagefferens.*

Methods

Great South Bay (GSB) is a long (40 km), shallow (2 m), barrier island estuary located along the south shore of Long Island, New York, USA (Fig. 1). GSB is one of the most productive estuaries in the world (Lively et al. 1983) and has been plagued with brown tide blooms nearly every year since 1985 (Bricelj and Lonsdale 1997). Our research was conducted during a brown tide event that occurred during the fall of 1999. Using a Boston Whaler, we collected samples on 14, 21, 28 October; 4, 12, 22 November; and 8 December from two sites in GSB: Patchogue Bay, located in the eastern portion of GSB (40°44′03″N, 73°01′23″W; Fig. 1), and Bay Shore Cove, located in the western portion of GSB (40°42′08″N, 73°14′12″W; Fig. 1). On station, whole water was collected with a high density polyethylene (HDPE) bucket and was transferred with minimal bubbling to three 20-liter polyethelene carboys that were kept in coolers during transport to the lab. The shallow, well-mixed nature of GSB (Wilson et al. 1991) ensured that sample water collected was representative of the entire water column at each station. Carboys, plastic buckets, and all other materials associated with the sampling, handling, and storage of seawater during this project were submerged in 10% HCl for 1 month before the project began, stored in 10% HCl between sampling dates, and rinsed liberally with distilled-deionized water before use. Ancillary measurements made at each station included temperature and salinity (measured with a YSI© 85 probe), as well as secchi disk depth. Field salinity measurements were confirmed by analysis of samples on a Beckman© induction salinometer (model RS 7B).

Filtered seawater was collected in the field using a peristaltic pumping system equipped with an acid-cleaned polypropylene filter capsule (0.2 μm; MSI) and acid-washed Teflon tubing that extended on a boom at a 45° angle, 3 m into prevailing winds and waves to a depth of 1 m (Gobler and Sañudo-Wilhemly 2001*a*). From this system, organic nutrient, inorganic nutrient, and salinity samples were collected and immediately stored on ice. Within 1 h of collection, DOC samples were acidified with quartz-distilled nitric acid and frozen along with nutrient samples. Ten liters of filtered seawater for dilution experiments were also collected in fluorinated HDPE carboys. Levels of dissolved organic nutrients in filtered seawater collected by this method were not statistically different from levels determined in water that was gravity filtered (combusted GF/F glass fiber filter). Triplicate chlorophyll *a* (Chl *a*) samples were collected on station using GF/F glass fiber filters (nominal pore size = 0.7 μm) and stored frozen. Chl *a* was also size fractionated using a 5 μm Nitex© mesh (Gobler and Sañudo-Wilhemly 2001*a*). Samples for the enumeration of cyanobacteria and *A. anophagefferens* were preserved to a final concentration of 1% glutaraldehyde in sterile polycarbonate test tubes using a 10% stock solution made from 0.2-μm filtered GSB seawater.

Nutrient addition experiments were conducted to identify the type of nutrient regime that promotes brown tide proliferation. Within an hour of collection, 1 liter of seawater was transferred to acid-cleaned 1.2-liter polycarbonate flasks. Triplicate flasks were amended with sodium nitrate (10 μM), urea (5 μM = 10 μM N), glycine (10 μM = 10 μM N), glucose (17 μM = 100 μM C), and phosphate (1 μM), or were left unamended as a control treatment. The concentrations of these additions were similar to previously observed increases of these nutrients in the water column of brown tide prone embayments on Long Island (Carpenter et al. 1991; Gobler and Sañudo-Wilhemly 2001*a*). Nutrient stocks were filter sterilized (0.2 μm) and stored frozen. High levels of silicate (30–60 μM) in the Great South Bay during the course of our experiments assured silicate-replete conditions for diatoms.

Dilution experiments (Landry et al. 1995) were conducted

to establish the grazing rates of microzooplankton on *A. anophagefferens*, cyanobacteria, and the total phytoplankton community. Care was taken to minimize bubbling during experiments and thus prevent destruction of fragile protozoan species. One-liter experimental dilutions of 100, 70, 40, and 15% whole seawater were incubated in triplicate, 1.2-liter polycarbonate flasks. Dilution bottles were amended with 10 μM nitrate and 1 μM phosphate to ensure nutrient replete growth. An additional set of unfiltered triplicate bottles were incubated without nutrients to assess the impact of nutrient amendments on microbial communities. A control bottle of 100% filtered seawater was also incubated and consistently maintained chlorophyll levels of <1% of levels found in GSB.

Experimental bottles were incubated at a depth of ~0.25 m under two layers of neutral density screening in Old Fort Pond (OFP) at the Southampton College, Long Island University, Marine Station, located 40 km east of GSB. Open tidal exchange with Shinnecock Bay keeps OFP well flushed; temperatures during incubations were always within 2°C of stations in GSB. Screening reduced ambient light penetration by 65%. Since the average extinction coefficient in the GSB during experiments was 2.8 (based on secchi disc readings), the light used in our experiments was similar to the light levels found at 0.5 m in the GSB water column. After 24 h, dilution experiment flasks were filtered for Chl *a* onto GF/F glass fiber filters and a 10-ml aliquot was preserved to a final concentration of 1% glutaraldehyde for cell counts. After 48 h, nutrient addition experiments were processed in an identical manner.

DOC samples were analyzed in duplicate by high temperature catalytic oxidation using a Shimadzu TOC-5000 total organic carbon analyzer (Benner and Strom 1993). Chl *a* was analyzed by standard fluorometeric methods (Parsons et al. 1984). Standard spectrophotometric methods were used to analyze nitrate/nitrite (Jones 1984), urea (Newell et al. 1967), ammonium, phosphate and silicate (Parsons et al. 1984) in duplicate. Total dissolved nitrogen and phosphorus concentrations (TDN and TDP) were determined in duplicate using persulfate oxidation methods (Valderrama 1981). DON was calculated as the difference between TDN and dissolved inorganic nitrogen (nitrate, nitrite, ammonium), while DOP was calculated as the difference between TDP and orthophosphate. Measurements of J. Sharp's (U. Delaware) intercalibration DOC samples were within 5% of the consensus value. Recoveries (mean ± 1 SD) of SPEX Certi-Prep inorganic nutrient standard reference material at environmentally representative concentrations were 97 ± 8% for nitrate, 106% ± 8% for ammonium and 101 ± 4% for phosphate. Recoveries of SPEX Certi-Prep organic nutrient standard reference material at environmentally representative levels were 94 ± 12% for total nitrogen and 109 ± 8% for total phosphorus. Precombustion of glassware and acid-cleaned plasticware provided adequately low blanks for DOC, DON, and DOP (<10% of lowest sample).

A. anophagefferens and cyanobacteria densities in preserved samples were determined by direct count methods employing an epifluorescent microscope. *A. anophagefferens* cells were enumerated by staining cells that were gently filtered (<5 kPa) onto a 0.8-μm black polycarbonate filter with an immunofluorescent label as described by Anderson et al. (1989). Modifications of the original technique included increasing the amount of primary and secondary antibody used by more than twofold. A minimum of 100 cells were counted per sample in at least 10 fields to yield a relative standard deviation of 12% for replicate counts of the same sample ($n = 12$) at cell densities of 3×10^5 cells ml^{-1}, approximating average densities during experiments. To ensure accurate results, the immunofluorescent technique was compared to counts performed with a hemacytometer on a light microscope. The two techniques yielded statistically identical results using *A. anophagefferens* clone 1708 at cell densities of 3×10^5 cells ml^{-1}, the approximate mean densities found in GSB during this study.

Cyanobacteria cells containing phycoertithrin type II and phycocyannin were enumerated with an epifluorescent microscope possessing green light with a longwave pass filter set at 560 nm, a 520 nm shortwave pass filter, and an emission filter of 590 nm (MacIsaac and Stockner 1993). The unicellular, <1-μm cells quantified with this filter set likely included phycoerythrin and phycocyannin containing *Synechcococcus* sp., as well as *Synechcocystis* sp., all cyanobacterial species previously observed in GSB by Campbell et al. (1983). These cells will be referred to simply as cyanobacteria from this point onward. Within 24 h of collection, a small volume of glutaraldehyde-preserved sample (0.5 to 1 ml) was gently filtered (<5 kPa) onto 0.2-μm black polycarbonate filters. Sample filtration was followed by a 1-ml rinse of 1% glutaraldehyde made with 0.2-μm filtered GSB water. Analysis of blank filters ensured this rinse process did not add additional cyanobacteria cells to filters. Filters were stored dark and frozen and were counted within 48 h of filtration. A minimum of 200 cells were counted per sample in at least 10 fields to yield a relative standard deviation of 10% for replicate counts of the same sample ($n = 10$) at cell densities of 4×10^5 cells ml^{-1}, approximating average densities during experiments. Direct epifluorescent counts of a cyanobacteria culture (*Synechococcus bacillaris* Butcher, strain WH5701) at concentrations of 8×10^5 cells ml^{-1} yielded cell densities statistically identical to those obtained independently with a hemacytometer.

The amount of Chl *a* due to the presence of brown tide in samples was estimated by assuming a constant Chl *a* per cell value for *Aureococcus* (0.035 ± 0.003 pg cell^{-1} for nutrient replete cultures; Gobler 1995), and multiplying this value by the *Aureococcus* densities. Levels of Chl *a* from non–brown tide phytoplankton (NBTP) were calculated as the difference between total Chl *a* and Chl *a* due to *Aureococcus.* Clearly, such calculations could introduce bias from variability of cellular chlorophyll concentrations due to changes in light and nutrient levels. However, such approximations have been used successfully in the past to compare *Aureococcus* biomass to that of the total algal community (Schaffner 1999; Gobler and Sañudo-Wilhemly 2001*a*) and offer a means by which the growth of non-*Aureococcus* populations can be monitored in experimental treatments. Such an analysis is invaluable during brown tide blooms such as the one presented here, where *Aureococcus* represented a large portion (25–90%) of the total algal biomass (Chl *a*). The relative abundance of *Aureococcus* within the phytoplankton community was calculated as a per-

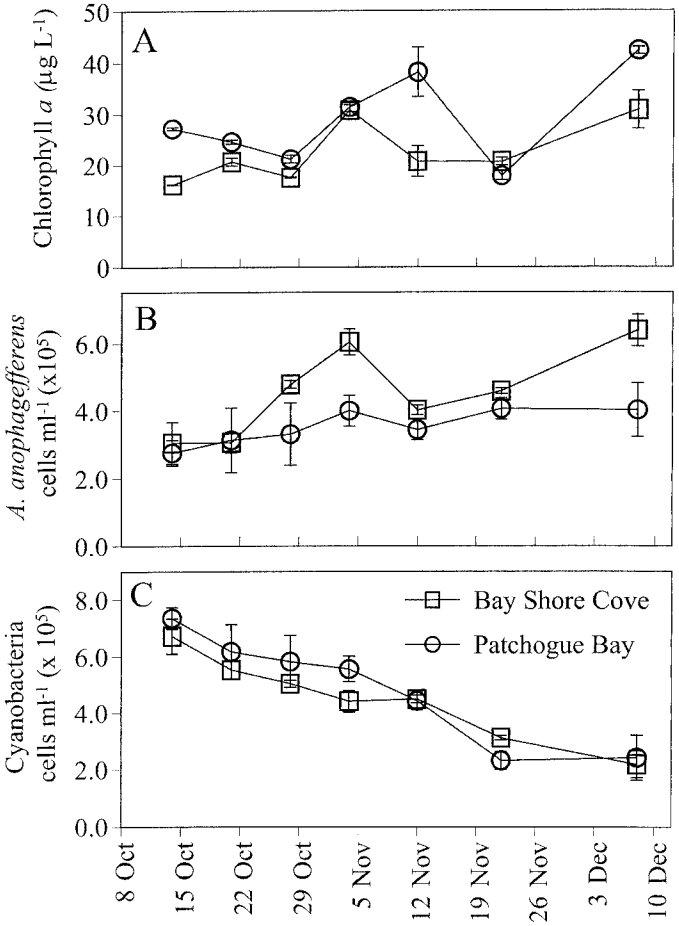

Fig. 2. Variation in (A) chlorophyll *a*, (B) *Aureococcus anophagefferens*, and (C) cyanobacteria in Bay Shore Cove and Patchogue Bay of Great South Bay during the fall of 1999. Error bars represent ±1 SD of triplicate measurements.

centage of total phytoplankton biomass [(*Aureococcus* Chl *a*/total Chl *a*) × 100].

Net growth rates of *A. anophagefferens*, cyanobacteria, the total phytoplankton community, and NBTP were calculated from changes in cell densities and Chl *a* using the formula $k = [\ln(B_t/B_0)]/t$ where k is the net growth rate, B_t is the amount of biomass (cell density or Chl *a*) present at the end of the experiments, B_0 represents the amount of biomass at the beginning of experiments, and t is the duration of the experiment in days. Grazing mortality rates (m) of phytoplankton were determined using the slope of a linear regression of the dilution of seawater (x-axis) versus apparent net growth rates (y-axis), while nutrient-enriched growth rates (μ_n) were determined from the y-intercept of these plots (Landry et al. 1995). Phytoplankton growth rates in unenriched, undiluted bottles without nutrients added (μ_0) were derived from net growth rates in unenriched, undiluted bottles and grazing rates ($\mu_0 = k + m$; Landry et al. 1995). The ratios of phytoplankton growth rates in nutrient unenriched and enriched bottles from dilution experiments (μ_0/μ_n) were used to assess the impacts of inorganic nutrient additions (nitrate and phosphate) on various phytoplankton populations (Landry et al. 1995). In general, μ_0/μ_n ratios of 1 indicate nutrient replete conditions,

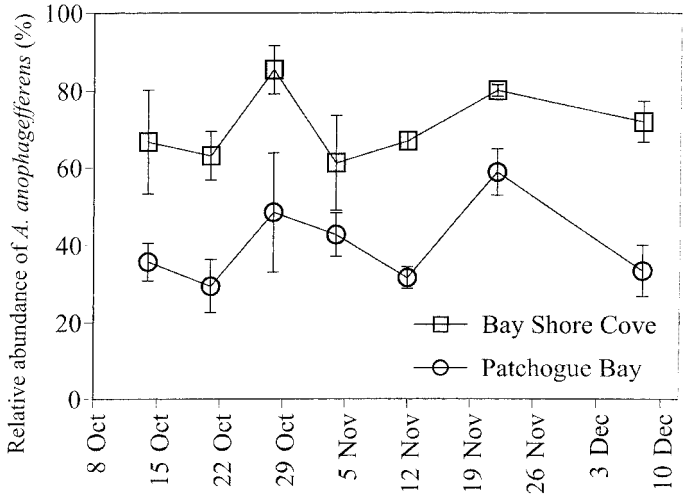

Fig. 3. Variation in the relative abundance of *Aureococcus anophagefferens* expressed as a percentage of the total phytoplankton community in Bay Shore Cove and Patchogue Bay of Great South Bay during the fall of 1999.

while ratios <1 indicate inorganic nutrient deficiency (Landry et al. 1995). The portion of phytoplankton standing stock consumed per day was calculated as percentage = $(1 - e^{-g}) \times 100$. Three-point regressions of dilution curves during this study did not indicate saturation of grazing during experiments (Gallegos 1989).

Results

Phytoplankton and nutrient dynamics—High levels of phytoplankton biomass were found in Great South Bay during the fall of 1999, as levels of total Chl *a* fluctuated between 16 and 42 μg L^{-1}, with higher levels found in Patchogue Bay (PB) relative to Bay Shore Cove (BSC; Fig. 2A). Size fractionation indicated that >80% of this Chl *a* was consistently <5 μm. Concentrations of brown tide in GSB varied from 2.2 to 6.4 × 10^5 cells ml^{-1} during this study (Fig. 2B). *Aureococcus* levels were higher later in the fall and higher at BSC than at PB (Fig. 2B). In contrast, cyanobacteria densities steadily decreased during the study period at both sites from >6 × 10^5 cells ml^{-1} in early October to <2.0 × 10^5 cells ml^{-1} in December (Fig. 2C). Brown tide was a larger fraction of total phytoplankton biomass (as estimated using a cellular chlorophyll quota of 0.035 ± 0.003 pg cell^{-1} for *Aureococcus*; Gobler and Sañudo-Wilhemly 2001*a*) at BSC (60–90%) compared to PB (30–50%; Fig. 3). Similar calculations for cyanobacteria (~2 fg Chl *a* per cell; Kana and Glibert 1987) suggest this population comprised a much smaller proportion of total phytoplankton biomass (<0.1–10%) during the fall of 1999.

Concentrations of most dissolved inorganic nutrients in GSB during the fall of 1999 were rather low (Table 1). Dissolved inorganic nitrogen (DIN: nitrate, nitrite, and ammonium) concentrations averaged 2.5 μM, with mean levels in PB (average ± standard deviation = 3.8 ± 1.9 μM) exceeding those in BSC (1.2 ± 0.3 μM) (Table 1). While orthophosphate levels in GSB were also low during this study

Table 1. Levels of inorganic nutrients, organic nutrients, and temperature in Great South Bay during the fall of 1999. All nutrient concentrations are in μM. BSC is Bay Shore Cove. PB is Patchogue Bay. GSB is Great South Bay (PB and BSC) and represents the mean of all observations for each parameter during this study. Standard deviations (SD) of duplicate measurements are in parentheses. SD within the mean row represents the standard deviation of all measurements at BSC, PB, or GSB. DIN is dissolved inorganic nitrogen (nitrate, nitrite, and ammonium), DIP is orthophosphate, DOC is dissolved organic carbon, DON is dissolved organic nitrogen, DOP is dissolved organic phosphorus, *T* is temperature in degree Celsius.

	DIN	DIP	DOC	DON	Urea	DOP	*T*
BSC							
14 Oct	1.74 (0.06)	0.66 (0.13)	497 (19.9)	39.3 (3.97)	1.29 (0.12)	0.59 (0.15)	17
21 Oct	1.07 (0.03)	0.34 (0.09)	404 (12.5)	26.9 (3.13)	0.47 (0.01)	0.42 (0.07)	14
28 Oct	1.00 (0.07)	0.47 (0.16)	500 (19.5)	35.9 (3.25)	0.82 (0.05)	0.29 (0.18)	12
4 Nov	1.37 (0.20)	0.43 (0.20)	449 (9.23)	34.5 (3.08)	0.40 (0.10)	0.51 (0.13)	12
12 Nov	0.93 (0.20)	0.21 (0.01)	412 (42.7)	28.0 (1.76)	0.15 (0.06)	0.49 (0.11)	9
22 Nov	1.14 (0.25)	0.10 (0.01)	318 (2.57)	20.5 (2.62)	0.11 (0.01)	0.49 (0.06)	9
8 Dec	1.20 (0.16)	0.09 (0.00)	300 (0.64)	18.8 (0.72)	0.10 (0.01)	0.24 (0.07)	6
mean	1.21 (0.28)	0.33 (0.21)	412 (79.0)	29.1 (8.20)	0.48 (0.44)	0.43 (0.13)	11
PB							
14 Oct	5.22 (0.03)	0.15 (0.19)	507 (17.6)	38.9 (3.53)	0.22 (0.03)	0.83 (0.02)	17
21 Oct	4.1 (0.07)	0.23 (0.11)	389 (3.90)	30.9 (0.98)	0.55 (0.03)	0.21 (0.12)	14
28 Oct	1.28 (0.10)	0.56 (0.38)	444 (13.5)	37.2 (2.25)	0.59 (0.05)	0.28 (0.07)	12
4 Nov	6.16 (0.19)	0.77 (0.09)	424 (1.14)	31.0 (0.19)	0.56 (0.04)	0.23 (0.07)	12
12 Nov	2.04 (0.07)	0.25 (0.01)	502 (55.6)	34.5 (3.62)	0.37 (0.01)	0.79 (0.09)	9
22 Nov	2.60 (0.26)	0.27 (0.01)	429 (6.94)	32.0 (3.22)	0.09 (0.03)	0.67 (0.07)	9
8 Dec	5.44 (0.96)	0.27 (0.00)	368 (56.3)	33.5 (1.21)	0.22 (0.08)	0.60 (0.10)	5
mean	3.83 (1.88)	0.36 (0.22)	438 (52.0)	34.0 (3.10)	0.37 (0.20)	0.51 (0.27)	11
GSB							
mean	2.52 (1.88)	0.34 (0.21)	425 (66.0)	31.5 (6.20)	0.42 (0.33)	0.47 (0.21)	11

(0.3 ± 0.2 μM; Table 1), silicate levels were consistently high (49 ± 5.0 μM). In contrast to inorganic nutrient levels, DOC and DON concentrations in GSB were high during this study. DON levels ranged from 39 μM in BSC and PB in early October 1999 to 19 μM in BSC in December and averaged 31.5 μM during this study (Table 1). DOC concentrations were $\sim$500 μM at both locations on 14 October and decreased during the fall to 300 and 370 μM on 8 December at BSC and PB, respectively (Table 1). Concentrations of other organic constituents measured during this study were low; urea and dissolved organic phosphorus averaged 0.4 and 0.5 μM, respectively (Table 1). While salinities in GSB during our sampling remained fairly consistent (26.5 ± 0.62‰ for BSC and 25.2 ± 0.97‰ for PB), temperatures at both stations steadily dropped during the fall from 17°C on 14 October to 5°C on 8 December (Table 1).

Nutrient addition experiments—The responses of the non–brown tide phytoplankton population (NBTP) and cyanobacteria to experimental nutrient additions were fairly similar during the fall of 1999 and did not change dramatically during the course of the brown tide bloom (Figs. 4, 6). Specifically, the growth rates of these populations were most frequently enhanced above unamended controls by additions of simple nitrogen compounds, such as nitrate and/or urea. In contrast, these same compounds often had no effect on or significantly decreased growth rates of *Aureococcus* populations.

Nutrient addition experiments: Bay Shore Cove—At Bay Shore Cove, nitrate additions increased growth rates of NBTP significantly above control treatment during all seven experiments, while urea additions did so in three out of seven experiments (28 October, 12 November, 8 December; Fig. 4; $P < 0.05$; *t*-test). With the exception of the 12 November experiment, cyanobacteria populations at BSC also experienced growth rates significantly higher than controls whenever nitrate was added (Fig. 4; $P < 0.05$; *t*-test). Urea also augmented cyanobacteria growth rates beyond controls on 14 October and 28 October (Fig. 4; $P < 0.05$; *t*-test). Some nutrient additions significantly reduced the growth rates of NBTP and cyanobacteria at BSC during experiments. Specifically, the addition of glucose on 22 November decreased NBTP growth significantly below control treatments (Fig. 4; $P < 0.05$; *t*-test), while both glucose and phosphate additions significantly lowered cyanobacteria growth below unamended treatments on 8 December (Fig. 4; $P < 0.05$; *t*-test).

Urea additions enhanced brown tide growth rates beyond control treatments during the first two experiments conducted at BSC (14 October, 21 October; Fig. 4; $P < 0.05$; *t*-test). However, experimental nutrient additions after these dates failed to significantly augment *Aureococcus* growth rates above control treatments (Fig. 4). Brown tide growth rates were significantly lowered by the addition of nitrate or urea on 12 November (Fig. 4; $P < 0.05$; *t*-test). Simultaneous consideration of changes in brown tide and NBTP population densities during nutrient addition experiments indicates how nutrient treatments affected the relative abundance of *Aureococcus* within the phytoplankton community. At BSC, only additions of glucose on 4 November and 22 November increased the relative abundance of brown tide compared to control treatments (Fig. 5; $P < 0.05$; *t*-test). In contrast, additions of nitrate during all experiments except 14 October, as well as additions of urea on 28 October and

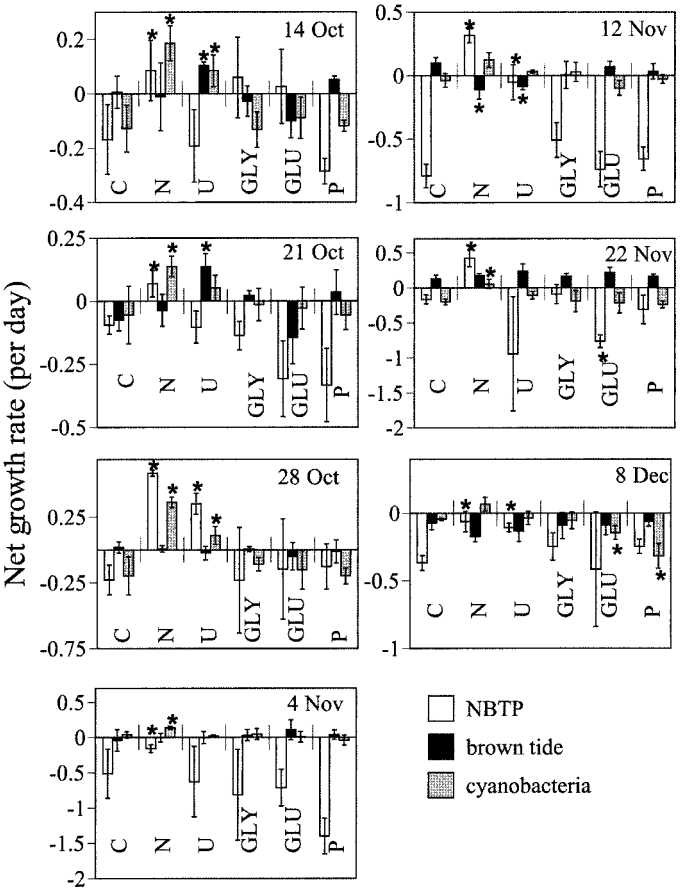

Fig. 4. Net growth rates of NBTP, brown tide (*Aureococcus anophagefferens*), and cyanobacteria during experiments using water from Bay Shore Cove, Great South Bay. Within each experiment, abbreviations for treatments are as follows: C = control, N = nitrate, U = urea, Gly = glycine, Glu = glucose, and P = phosphate. Error bars represent ±1 SD of triplicate measurements. Asterisk indicates treatments that yielded growth rates that were significantly different from control treatments for each phytoplankton population.

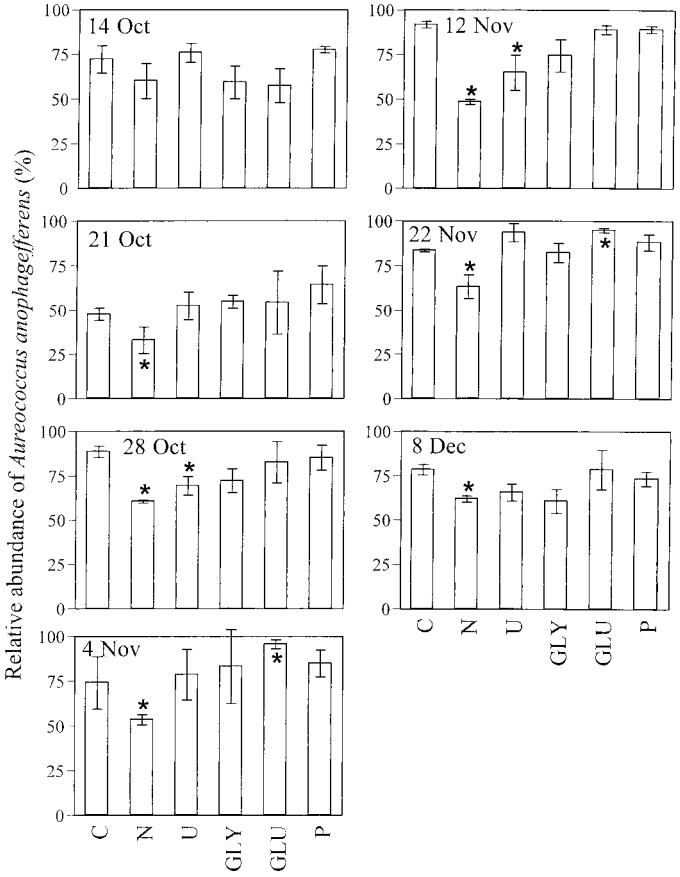

Fig. 5. Relative abundance of *Aureococcus anophagefferens* expressed as a percentage of the total phytoplankton community at the end of the experiment conducted using water from Bay Shore Cove, Great South Bay. Within each experiment, abbreviations for treatments are as follows: C = control, N = nitrate, U = urea, Gly = glycine, Glu = glucose, and P = phosphate. Error bars represent ±1 SD of triplicate measurements. Asterisk indicates treatments that yielded a relative abundance of *A. anophagefferens* that was significantly different from the control treatment.

12 November, resulted in a significant reduction in the relative abundance of *Aureococcus* among phytoplankton (Fig. 5; $P < 0.05$; *t*-test), due largely to enhanced NBTP growth and unchanged brown tide growth rates in these treatments (Fig. 5).

Nutrient addition experiments: Patchogue Bay—At Patchogue Bay, nitrate additions enhanced growth rates of NBTP beyond controls treatments on 21 October, 28 October, 12 November, 22 November, and 8 December, and urea did so on 28 October (Fig. 6; $P < 0.05$; *t*-test). Cyanobacteria also had significantly increased growth rates during nitrate additions 14 October, 21 October, 28 October, and 4 November (Fig. 6; $P < 0.05$; *t*-test). Significant reductions in cyanobacteria growth rates compared to control were experienced during phosphate additions on 8 December (Fig. 6; $P < 0.05$; *t*-test).

Surprisingly, brown tide growth rates were augmented by phosphate additions on 14 October and 21 October (Fig. 7; $P < 0.05$; *t*-test). *Aureococcus* populations experienced significantly reduced growth rates relative to unamended treatments during several nitrate (28 October, 12 November, 22 November, 8 December) and urea (28 October, 12 November) additions (Fig. 7; $P < 0.05$; *t*-test). These lowered brown tide growth rates coupled with the frequently enhanced NBTP growth in the same treatments led to a significant reduction in the relative abundance of *Aureococcus* during nitrate additions on 21 October, 28 October, 12 November, 22 November, and 8 December and during urea additions on 28 October and 12 November (Fig. 7; $P < 0.05$; *t*-test). Only the addition of phosphate on 21 October was capable of increasing the relative abundance of *Aureococcus* within the phytoplankton community during experiments conducted at PB (Fig. 7; $P < 0.05$; *t*-test).

Dilution experiments: grazing mortality and phytoplankton growth rates—Significant rates of microzooplankton grazing as determined by dilution experiments were measured throughout the fall of 1999 at both Bay Shore Cove

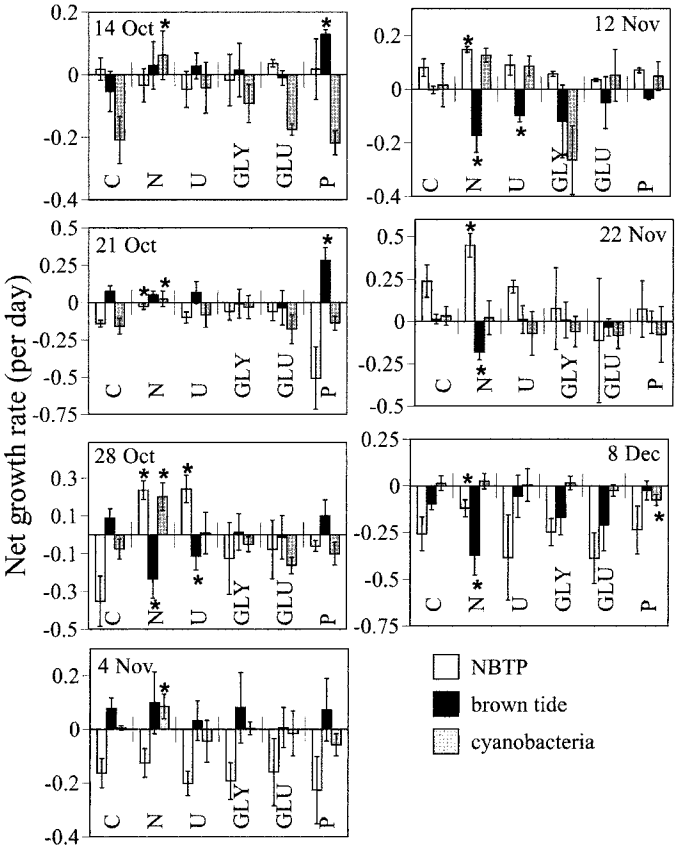

Fig. 6. Net growth rates of NBTP, *Aureococcus anophagefferens*, and cyanobacteria during experiments using water from Patchogue Bay, Great South Bay. Within each experiment, abbreviations for treatments are as follows: C = control, N = nitrate, U = urea, Gly = glycine, Glu = glucose, and P = phosphate. Error bars represent ±1 SD of triplicate measurements. Asterisk indicates treatments that yielded growth rates that were significantly different from control treatments for each phytoplankton population.

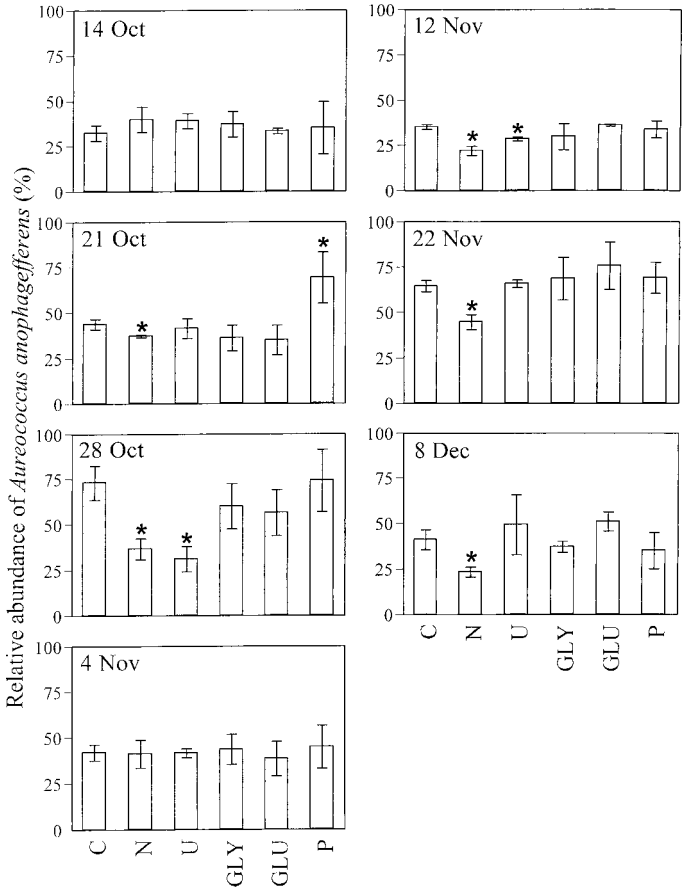

Fig. 7. Relative abundance of *Aureococcus anophagefferens* expressed as a percentage of the total phytoplankton community at the end of the experiment conducted using water from Patchogue Bay, Great South Bay. Within each experiment, abbreviations for treatments are as follows: C = control, N = nitrate, U = urea, Gly = glycine, Glu = glucose, and P = phosphate. Error bars represent ±1 SD of triplicate measurements. Asterisk indicates treatments that yielded a relative abundance of *A. anophagefferens* that was significantly different from the control treatment.

and Patchogue Bay in Great South Bay (Table 2). Specifically, grazing mortality rates of NBTP were significantly different from zero, and thus detectable, during 13 out of 14 experiments conducted in GSB. Grazing rates on all phytoplankton populations monitored were not significantly different from zero on 8 December at BSC (Table 2). Grazing rates on the total phytoplankton community in GSB (BSC and PB) ranged from 0.36 to 1.02 d^{-1} and averaged 0.69 ± 0.22 d^{-1} (mean ± 1 SD) during this study, while grazing rates on NBTP ranged from 0.47 to 1.58 d^{-1} and averaged 1.03 ± 0.37 d^{-1}. Grazing rates on the total phytoplankton community and on NBTP were generally lower later in the fall (Table 2). Grazing rates on cyanobacteria in GSB ranged from 0.39 to 0.86 d^{-1}, averaged 0.54 ± 0.18 d^{-1}, and did not evidence a seasonal trend (Table 2). Significant grazing rates on *A. anophagefferens* were measured during 11 out of 14 experiments. *A. anophagefferens* grazing mortality rates ranged from 0.19 to 0.49 d^{-1} and averaged 0.31 ± 0.09 d^{-1} (Table 2). Nonsignificant grazing rates on *A. anophagefferens* were measured on 14 October and 8 December in Bay Shore Cove and on 21 October in Patchogue Bay. The percentages of standing stocks removed per day by microzooplankton in GSB were similar between BSC and PB but varied among populations. For example, while 49 ± 11% (average ± 1 SD) of the total phytoplankton population was consumed on a daily basis by microzooplankton, 62 ± 14%, 41 ± 10%, and 26 ± 7% of the NBTP, cyanobacteria, and brown tide populations, respectively, were consumed per day.

A comparison of grazing mortality rates of phytoplankton populations in Great South Bay during the fall of 1999 revealed significant differences between NBTP, cyanobacteria, and *Aureococcus anophagefferens.* Strikingly, mean grazing mortality rates of brown tide (0.31 ± 0.09 d^{-1}) during the fall of 1999 in GSB were significantly lower than those on both NBTP (1.03 ± 0.37 d^{-1}; Table 2; $P < 0.0001$; *t*-test) and on cyanobacteria (0.54 ± 0.18 d^{-1}; Table 2; $P < 0.001$; *t*-test). Moreover, mean grazing mortality rates of cyanobacteria in GSB were significantly lower than those on NBTP (Table 2; $P < 0.01$; *t*-test). These statistical differences in

Table 2. Grazing mortality and phytoplankton growth rates in Great South Bay during the fall of 1999. r^2 is the correlation coefficient of the regression of dilution versus net growth rate from dilution experiments (Landry et al. 1995); m is the grazing mortality rate per day as determined by the slope of the regression of dilution versus net growth rate during dilution experiments; k is the net growth rate per day of populations undiluted, unenriched bottles during dilution experiments; and μ_0/μ_n is the ratio of phytoplankton growth rates in unenriched (μ_0) and enriched (with nitrate and phosphate) bottles (μ_n) during dilution experiments. Values are included for the total phytoplankton population, non–brown tide phytoplankton, *Aureococcus anophagefferens*, and cyanobacteria. Values in parentheses are standard errors and standard deviations for m and k, respectively. Means calculated as the average of values determined on each date are presented for BSC, PB, and GSB (mean of observations at BSC and PB.) Dates without data presented indicate the measurement of grazing rates not significantly different from zero.

	Total phytoplankton				Non–brown tide				*A. anophagefferens*				Cyanobacteria			
	r^2	m	k	μ_0/μ_n	r^2	m	k	μ_0/μ_n	r^2	m	k	μ_0/μ_n	r^2	m	k	μ_0/μ_n
BSC																
14 Oct	0.90	0.67 (0.07)	−0.13 (0.06)	0.78	0.90	1.29 (0.14)	−0.75 (0.08)	0.42	0.27	—	0.05 (0.10)	—	0.71	0.86 (0.17)	−0.13 (0.04)	0.81
21 Oct	0.85	0.94 (0.12)	−0.20 (0.03)	0.90	0.83	1.29 (0.18)	−0.50 (0.08)	0.72	0.48	0.35 (0.12)	−0.02 (0.08)	1.43	0.49	0.39 (0.13)	−0.16 (0.08)	0.72
28 Oct	0.93	0.78 (0.07)	0.01 (0.03)	0.87	0.90	1.58 (0.17)	0.06 (0.38)	0.75	0.66	0.19 (0.04)	−0.02 (0.13)	0.84	0.70	0.63 (0.13)	−0.14 (0.11)	0.71
4 Nov	0.86	0.73 (0.09)	−0.23 (0.05)	0.73	0.83	1.20 (0.17)	−0.61 (0.09)	0.53	0.37	0.22 (0.09)	0.07 (0.09)	2.67	0.64	0.37 (0.09)	−0.12 (0.05)	0.70
12 Nov	0.89	0.56 (0.06)	−0.13 (0.03)	0.75	0.78	1.04 (0.17)	−0.17 (0.04)	0.64	0.52	0.39 (0.12)	0.07 (0.07)	1.04	0.88	0.84 (0.10)	−0.28 (0.13)	1.05
22 Nov	0.92	0.36 (0.03)	−0.09 (0.15)	0.51	0.52	0.50 (0.15)	−0.23 (0.44)	0.22	0.56	0.28 (0.08)	0.04 (0.10)	0.93	0.70	0.43 (0.09)	0.04 (0.07)	0.85
8 Dec	0.23	—	−0.16 (0.05)	—	0.19	—	−0.19 (0.15)	—	0.02	—	−0.01 (0.08)	—	0.00	—	0.12 (0.12)	—
mean	—	0.67 (0.20)	−0.13 (0.08)	0.76	—	1.15 (0.36)	−0.34 (0.29)	0.55	—	0.29 (0.08)	0.03 (0.04)	1.38	—	0.59 (0.23)	−0.10 (0.13)	0.81
PB																
14 Oct	0.64	0.74 (0.18)	0.27 (0.06)	1.13	0.53	0.86 (0.26)	0.25 (0.05)	1.20	0.40	0.49 (0.19)	0.30 (0.09)	1.02	0.67	0.49 (0.11)	−0.15 (0.13)	0.87
21 Oct	0.94	1.02 (0.08)	−0.05 (0.01)	0.86	0.93	1.28 (0.11)	−0.22 (0.06)	0.85	0.18	—	0.02 (0.22)	—	0.62	0.40 (0.10)	−0.24 (0.09)	0.45
28 Oct	0.90	1.02 (0.11)	−0.15 (0.06)	0.99	0.90	1.53 (0.15)	−0.33 (0.03)	0.86	0.55	0.26 (0.06)	0.08 (0.08)	2.13	0.63	0.46 (0.11)	−0.21 (0.08)	0.50
4 Nov	0.89	0.73 (0.08)	0.01 (0.05)	0.92	0.86	0.87 (0.11)	−0.16 (0.04)	0.85	0.72	0.28 (0.08)	−0.08 (0.08)	1.89	0.74	0.47 (0.09)	−0.21 (0.06)	0.74
12 Nov	0.86	0.51 (0.07)	−0.12 (0.10)	0.67	0.68	0.55 (0.12)	−0.45 (0.14)	0.21	0.58	0.37 (0.10)	0.21 (0.07)	1.23	0.86	0.81 (0.10)	0.24 (0.23)	1.15
22 Nov	0.73	0.56 (0.11)	0.21 (0.01)	0.88	0.65	0.98 (0.23)	−0.06 (0.22)	0.61	0.59	0.33 (0.09)	0.20 (0.07)	1.19	0.59	0.39 (0.10)	−0.09 (0.17)	0.99
8 Dec	0.90	0.37 (0.04)	−0.22 (0.01)	0.55	0.80	0.47 (0.07)	−0.37 (0.03)	0.35	0.49	0.20 (0.07)	0.02 (0.06)	0.96	0.75	0.46 (0.08)	0.06 (0.10)	1.12
mean	—	0.71 (0.25)	−0.01 (0.19)	0.86	—	0.93 (0.37)	−0.19 (0.23)	0.70	—	0.32 (0.10)	0.11 (0.13)	1.40	—	0.50 (0.14)	−0.09 (0.18)	0.83
GSB																
mean	—	0.69 (0.22)	−0.07 (0.15)	0.81	—	1.03 (0.37)	−0.27 (0.26)	0.63	—	0.31 (0.09)	0.06 (0.10)	1.39	—	0.54 (0.18)	−0.09 (0.15)	0.82

grazing rates on these populations were also present at each site (BSC and PB) during the fall of 1999 (Table 2).

To determine what factors might have influenced microzooplankton grazing on each phytoplankton population observed in GSB during the fall of 1999 (total phytoplankton, NBTP, brown tide, and cyanobacteria), rates were regressed against factors that have been previously cited to affect microzooplankton in New York embayments: temperature, chlorophyll levels, and brown tide concentrations (Lonsdale et al. 1996). Analysis revealed grazing mortality rates of the total and NBTP phytoplankton communities were both significantly correlated with temperature in GSB during this study ($P < 0.05$; $n = 13$ for both). In contrast, grazing rates on *A. anophagefferens* and cyanobacteria were not. Moreover, levels of Chl *a* or concentrations of *A. anophagefferens* were not significantly correlated with grazing rates on any phytoplankton population.

Dilution experiments also provided information about phytoplankton growth rates in GSB during the fall of 1999. Specifically, during these experiments, net growth rates (k) of *A. anophagefferens* (mean ± SD = 0.06 ± 0.10; Table 2) were significantly higher than those of NBTP (−0.27 ± 0.26; Table 2; $P < 0.01$; *t*-test) and cyanobacteria (−0.09 ± 0.15; Table 2; $P < 0.05$; *t*-test). Also during dilution experiments, the addition of inorganic nutrients (nitrate and phosphate) consistently yielded higher growth rates relative to unamended samples for NBTP ($\mu_0/\mu_n = 0.63 \pm 0.28$; Table 2). Cyanobacteria also displayed nutrient enhanced growth rates during dilution experiments conducted from 14 October to 4 November ($\mu_0/\mu_n = 0.69 \pm 0.14$; Table 2) but were unaffected by nutrient addition during the final three dilution experiments ($\mu_0/\mu_n = 1.03 \pm 0.12$ for 4 November, 12 November, and 8 December; Table 2). By contrast, inorganic nutrient additions had no effect on or occasionally yielded lower growth rates for *A. anophagefferens* ($\mu_0/\mu_n = 1.39 \pm 0.41$; Table 2).

Discussion

This paper presents the first research to simultaneously characterize the importance of both nutrients and microzooplankton grazing in the formation of brown tides caused by *Aureococcus anophagefferens.* During the fall 1999 brown tide bloom in Great South Bay, levels of DOC and DON were high (mean ± 1 SD = 430 ± 66 μM and 32 ± 6.5 μM, respectively; Table 1), dissolved inorganic nitrogen levels were low (2.5 ± 1.9 μM; Table 1), and additions of simple nitrogen compounds (nitrate, urea) typically decreased the relative abundance of *Aureococcus* within the phytoplankton community (Figs. 5, 7). Concurrently, microzooplankton grazing rates on *A. anophagefferens* were significantly lower than those on cyanobacteria and other NBTP. Such differences in grazing mortality contributed toward higher net growth rates for the brown tide compared to other phytoplankton populations (Table 2). Together, these results characterize an ecological niche in which both top-down (low *Aureococcus* specific grazing mortality rates) and bottom-up (enriched organic nutrients) factors contribute to the sustenance of brown tide blooms.

Nutrients and brown tides—Organic nutrients are hypothesized to provide *A. anophagefferens* with the nutritional resources necessary to bloom (Dzurica et al 1989; Berg et al. 1997; LaRoche et al. 1997; Gobler and Sañudo-Wilhemly 2001*a*,*b*). Moreover, brown tides are often observed when DIN levels are low (Keller and Rice 1989; Gobler and Sañudo-Wilhemly 2001*a*). During the fall 1999 brown tide event in GSB, levels of DOC and DON averaged 430 ± 66 μM and 32 ± 6.5 μM, respectively (mean ± 1 SD), while DIN levels averaged 2.5 ± 1.9 μM (Table 1). These DIN levels are within the range of those previously observed in GSB (Carpenter et al. 1991) and during *Aureococcus anophagefferens* blooms on Long Island (Cosper et al. 1989; Gobler and Sañudo-Wilhemly 2001*a*) and in Rhode Island (Keller and Rice 1989). Since concentrations of DOC and DON in GSB have not been previously published, comparison to historic values is not possible. However, levels of DOC and DON present in GSB during the fall of 1999 were significantly greater than those reported during previous New York brown tides in the Peconic Estuary (195 μM DOC; Breuer et al. 1999), Shinnecock Bay (3.5 μM DON; Berg et al. 1997), and West Neck Bay (mean DOC and DON = 244 μM and 16.5 μM; Gobler and Sañudo-Wilhemly 2001*b*). It is possible that the high levels of DOC and DON present in GSB during the fall of 1999 provided *Aureococcus* populations with adequate nutritional substrate to bloom through the fall and into the winter.

Results from nutrient addition and dilution experiments also indicate that the nutrient regime present during much of fall 1999 in GSB was nearly ideal for the brown tide, particularly with regard to N. Growth rates of NBTP and cyanobacteria populations during the majority of nutrient addition experiments seemed dependent on N, as additions of simple nitrogen compounds such as nitrate and urea typically enhanced their growth rates relative to unamended treatments (Figs. 4, 6). Similarly, the addition of nitrate in tandem with phosphate in dilution experiments yielded growth rates that frequently exceeded unamended samples for NBTP and cyanobacteria (Table 2). In contrast, nitrate or urea additions during nutrient addition experiments or additions of nitrate with phosphate during dilution experiments had no impact on or occasionally decreased *Aureococcus* growth rates (Figs. 4, 6; Table 2). These additions thus resulted in a significant reduction in the relative abundance of *Aureococcus* within the phytoplankton community (Figs. 5, 7). Such results are consistent with the conclusion that relatively large (10 μM) inputs of simple N compounds (nitrate, urea), with or without phosphate, can hinder the formation of brown tide blooms (Gobler and Sañudo-Wilhemly 2001*a*).

While *Aureococcus*'s growth seemed nutrient replete during most of this study, some nutrients did significantly enhance brown tide growth rates beyond unamended treatments during experiments conducted on 14 October and 21 October. For example, urea enhanced brown tide growth rates above control treatments at BSC on 14 October and 21 October. These results, along with the low levels of DIN observed at BSC (average ± standard deviation = 1.2 ± 0.3 μM Table 1), suggest the N supply may have influenced brown tide growth rates at this station on these two dates. This result is consistent with previous research indicating

that *Aureococcus* can rely on organic nitrogen compounds, such as urea, to form blooms (Berg et al. 1997; LaRoche et al. 1997; Gobler and Sañudo-Wilhemly 2001*b*). Failure of glycine to enhance brown tide growth rates could indicate bacteria had a higher affinity for such amino acids (Wheeler and Kirchman 1986) or that *Aureococcus*'s amino acid oxidase enzymes were not active at the low temperatures present in GSB during this study (Pantoja and Lee 1992).

In contrast to BSC, additions of phosphate in Patchogue Bay significantly enhanced brown tide growth rates above controls on 14 October and 21 October (Table 1). This was a surprising result, since P stimulation of *Aureococcus* growth rates during a brown tide has not previously been reported. However, the ambient nutrient regime present during these experiments and the physiology of *Aureococcus anophagefferens* may account for such a result. While the total dissolved N supply at Patchogue Bay on these dates was large (DON and DIN = 44 μM on 14 October, 35 μM on 21 October), levels of P were relatively low (DOP and DIP = 0.98 μM on 14 October, 0.44 μM on 21 October). Therefore, additional P may have been required for *Aureococcus* to use the copious amounts of dissolved N (DIN and DON) in Patchogue Bay. In contrast, other autotrophic species, which may use only DIN, would have a smaller relative P demand.

During this study, ambient DIN concentrations in Patchogue Bay (3.8 $\pm$ 1.9 μM = average $\pm$ 1 SD) were significantly greater than those in Bay Shore Cove (1.2 $\pm$ 0.3 μM; $P < 0.01$; *t*-test). This difference could be due to more rapid flushing of western portions of GSB, such as BSC, with the more oligotrophic Atlantic Ocean water through the Fire Island inlet (Fig. 1; Wilson et al. 1991). This gradient in DIN levels across GSB seemed to have a discernable impact on the results of nutrient addition and dilution experiments. For example, during Patchogue Bay nutrient addition experiments, nitrate failed to enhance NBTP growth rates above control treatments when ambient concentration of DIN exceeded 5 μM (14 October, 4 November, and 8 December; Fig. 6, Table 1). Similarly, the addition of inorganic nutrients during Patchogue Bay dilution experiments discernibly increased NBTP growth rates ($\mu_0/\mu_n < 0.85$) during only three of seven experiments (Table 2). Moreover, during many Patchogue Bay experiments, additions of nitrate (with or without phosphate) and/or urea significantly deceased brown tide growth rates relative to control treatments (Fig. 6; Table 2). These results contrast with BSC, where DIN levels were lower and nitrate additions (with or without phosphate) consistently caused significant enhancement of NBTP growth rates and only decreased *Aureococcus* growth rates once (Fig. 4, Table 2). Significant reductions in *Aureococcus* growth rates at PB during nitrate and urea additions could be due to the already high DIN levels found there (Table 1) and support the hypothesis that brown tide bloom proliferation can be prevented by eutrophic, high DIN conditions (Keller and Rice 1989; Gobler and Sañudo-Wilhemly 2001*a*).

In contrast to nitrogen, additions of phosphate (PB, 21 October) or glucose (BSC, 4 November, 22 November) occasionally increased the relative abundance of *Aureococcus* within the phytoplankton community (Figs. 5, 7). This was due to decreases in NBTP growth rates (22 November at BSC; Fig. 5), increases in *Aureococcus* growth (21 October at PB; Fig. 7), or a combination of both occurrences (4 November at BSC; Fig. 5). As discussed above, additional P may allow *Aureococcus* to exploit the large DON and DIN pools at PB, whereas other phytoplankton would require less P, since they may only use DIN. Regarding the impact of glucose, such additions have led to significant decreases in NBTP and thus increases in the relative abundance of *Aureococcus* during previous blooms in the Peconic Estuary (Gobler and Sañudo-Wilhelmy 2001*a*). Those decreases in NBTP growth rates were hypothesized to be caused by a trophic interaction with bacteria, which can also experience enhanced growth during glucose additions (Gobler and Sañudo-Wilhelmy 2001*a*). To balance their internal stoichiometry, organic carbon additions may have caused bacteria to use ambient DIN pools (Goldman and Dennett 1991) causing N starvation and decreased growth rates in N-limited NBTP. By contrast, *Aureococcus* was not N limited during most fall 1999 experiments in GSB, and thus would be unaffected by this process.

Grazing mortality and brown tides—Dilution experiments conducted during the fall 1999 brown tide bloom indicated that microzooplankton grazing rates on *Aureococcus anophagefferens* were significantly lower than those on cyanobacteria and other phytoplankton species (Table 2). This finding suggests that during brown tide blooms, microzooplankton may preferentially avoid *Aureococcus* and instead choose to consume other species of phytoplankton. This conclusion is consistent with Lonsdale et al. (1996), who found that during peak monospecific brown tide densities in GSB, microzooplankton experienced negative growth rates. Moreover, laboratory studies have established that multiple cultured microzooplankton are unable to grow on a diet of *Aureococcus* (Caron et al. 1989; Mehran 1996). Reduced microzooplankton grazing observed during Texas brown tides has been linked to extracellular polysaccharide secretions (EPS) from the causative species, *Aureoumbra lagunensis* (Buskey and Hyatt 1995; Liu and Buskey 2000). *Aureococcus* is also known to have such EPS (Sieburth et al. 1988), which contains a dopamine-like compound that is capable of inhibiting ciliary motion in bivalves (Gainey and Shumway 1991). It is possible that this compound could have a similar effect on the cilia of microzooplankton and thus contributes to the observed lowered grazing rates on the brown tide. Alternatively, the substantial cellular levels of dimethylsulphoniopropionate (DMSP) found in *Aureococcus* (Keller et al. 1989) may also inhibit protozoan grazing (Wolfe et al. 1997). Regardless of the precise cause, the results presented here indicate, for the first time, that reduced grazing pressure on *Aureococcus* is a factor that can contribute to the proliferation of brown tide blooms in New York waters.

Despite lower grazing mortality rates of *A. anophagefferens* during the fall of 1999, microzooplankton grazing rates on the total phytoplankton and NBTP communities in GSB were well correlated with temperature but not with brown tide densities. Similarly, Lonsdale et al. (1996) found that annual growth rates of microzooplankton in GSB were cor-

related with temperature, but predicted an inhibition of microzooplankton grazing when brown tide densities exceeded 1×10^6 cells ml^{-1}. *Aureococcus* densities remained below this level during the present study (Fig. 2B). Moreover, the fall 1999 brown tide was never monospecific, as *Aureococcus* represented between 30 and 85% (mean $\pm$ SD = 55 $\pm$ 19%) of the total phytoplankton biomass (Fig. 3). This indicates alternative food sources were available for consumption by microzooplankton. It seems, therefore, that during nonmonospecific brown tide blooms of $<1 \times 10^6$ cells ml^{-1}, microbial food web processes, such as microzooplankton grazing, remain intact.

Levels of phytoplankton biomass increase when algal growth rates exceed cooccurring mortality grates. During dilution experiments, *A. anophagefferens* growth rates were slightly greater than grazing mortality rates, as brown tide populations generally displayed small, positive net growth rates (mean $\pm$ SD = 0.06 $\pm$ 0.10; Table 2), which were statistically identical to those in control treatments of nutrient addition experiments (0.04 $\pm$ 0.07; Figs. 4, 6). By contrast, other phytoplankton groups (total phytoplankton, NBTP, cyanobacteria), which were more heavily grazed, experienced negative net growth rates that were significantly lower than those of *A. anophagefferens* during both types of experiments ($P < 0.05$ for each; *t*-test; Table 2; Figs. 4, 6). These experimental observations are consistent with the dynamics of phytoplankton populations in GSB during the fall of 1999, when *A. anophagefferens* displayed a slow increase in densities while other populations gradually declined (Fig. 2). Higher grazing pressure on NBTP and cyanobacteria requires these species to grow at a faster pace to maintain their population densities during brown tide events. In contrast, reduced grazing pressure affords *A. anophagefferens* the luxury of growing slower and possibly allows it to expend more cellular energy processing complex and difficult to metabolize sources of organic carbon and nitrogen (Mulholland et al. 1998).

Fall/winter brown tide blooms—The occurrence of the fall 1999 brown tide was somewhat unusual in its timing, as *Aureococcus* blooms more commonly initiate during summer months (Cosper et al. 1989; LaRoche et al. 1997; Gobler and Sañudo-Wilhelmy 2001*b*). Although this study terminated in December, it is noteworthy that the brown tide that began in the fall of 1999 in GSB remained at levels of $\sim 10^5$ cells ml^{-1} through March 2000 (SCDHS 2000). While it is difficult to ascertain what factors observed during this study are universal contributors to *Aureococcus* blooms, there are multiple factors that may be uniquely important for promoting fall and winter brown tides blooms.

Light availability has been hypothesized to seasonally limit photosynthetic rates in GSB (Lively et al. 1983). During this study, the mean 1% light level was only 1.8 m (based on secchi disc readings) and the amount of daily irradiance available for photosynthesis was decreasing to the winter solstice minimum. Under such circumstances, a phytoplankton species possessing the ability to supplement photosynthetic C-fixation with heterotrophic uptake of DOC, such as *Aureococcus* (Dzurica et al. 1989; Gobler and Sañudo-Wilhelmy 2001*a*), would have a clear advantage over strictly autotrophic species. The extremely high levels of DOC present in GSB during the fall 1999 brown tide (mean = 425 μM) exceeded any level previously reported during an *Aureococcus* bloom (Breuer et al. 1999; Gobler and Sañudo-Wilhelmy 2001*b*) and thus provided an abundant organic carbon supply for heterotrophic use.

The seasonal succession of plankton communities in Long Island embayments may also favor the proliferation of brown tide blooms during winter months. It has been well documented that both phytoplankton and zooplankton communities undergo a seasonal shift toward larger sized organisms during winter months in Long Island bays (Lively et al. 1983; Turner et al. 1983). Moreover, these cold-temperature grazing communities are less likely to consume small phytoplankton cells than summer populations (Turner et al. 1983; Lonsdale et al. 1996). This is consistent with the observed grazing rates on small *Aureococcus* ($\sim$2 μm) and cyanobacteria ($\sim$1 μm) cells during this study, which were both significantly lower than those on other, presumably larger, phytoplankton (Table 2). Therefore, smaller cells that are capable of surviving colder temperatures and eluding grazing pressure, such as *Aureococcus*, may have a competitive advantage during fall and winter months in Long Island embayments.

Seasonal changes in cyanobacterial densities may also favor the occurrence of fall–winter brown tide blooms. During the fall of 1999, decreases in cyanobacteria densities and increases in *Aureococcus* concentrations in GSB (Fig. 2B,C) were significantly correlated with temperature and with one another ($P < 0.05$ for all; Table 1, Fig. 2B,C). The observed decline in cyanobacteria numbers is consistent with previously documented dynamics of these populations, which typically parallel changes in water temperatures (Waterbury et al. 1986). Moreover, the contrasting dynamics of cyanobacteria and *Aureococcus* may represent cyanobacteria vacating and *Aureococcus* filling the putative picoalgal niche in New York waters of Sieracki et al. (1999). In addition to temperature, dominance in this niche by either species may also be a function of the prevailing nutrient regime or microzooplankton community. For example, since these populations contain similarly sized cells (1–2 μm), they are likely to have similar nutrient uptake kinetics (Chisholm 1992) and thus may compete for nutrients. Moreover, if summer dominance by cyanobacteria (Sieracki et al. 1999) selects for a specific microzooplankton community, those grazers may not be capable of efficiently removing brown tide cells (Caron et al. 1989). Be it for top-down or bottom-up reasons, the decline of cyanobacteria densities in the fall may contribute toward a window of opportunity for *Aureococcus* to form winter blooms in New York waters.

References

ANDERSON, D. M., D. M. KULIS, AND E. M. COSPER. 1989. Immunofluorescent detection of the ''brown tide'' organism, *Aureococcus anophagefferens.* Lecture Notes Coastal Estuarine Studies. **35:** 213–228.

BENNER, R., AND M. STROM. 1993. A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. Mar. Chem. **41:** 153–160.

BERG, G. M., P. M. GILBERT, M. W. LOMAS, AND M. BURFORD.

1997. Organic nitrogen uptake by the chrysophyte *Aureococcus anophagefferens* during a brown tide bloom event. Mar. Biol. **129:** 377–387.

BREUER, E., S. A. SAÑUDO-WILHELMY, AND R. C. ALLER. 1999. Trace metals and dissolved organic carbon in an estuary with restricted river flow and a brown tide. Estuaries **22:** 603–615.

BRICELJ, V. M., AND D. J. LONSDALE. 1997. *Aureococcus anophagefferens:* Causes and ecological consequences of brown tides in U.S. mid-Atlantic coastal waters. Limnol. Oceanogr. **42:** 1023–1038.

BUSKEY, E. J., AND C. J. HYATT. 1995. Effects of a persistent ''brown tide'' alga on planktonic grazers. Mar. Ecol. Prog. Ser. **126:** 285–292.

CAMPBELL, L., E. J. CARPENTER, AND V. J. IACONO. 1983. Identification and enumeration of marine chroococcoid cyanobacteria by immunofluorescence. Appl. Environ. Microbiol. **46:** 553–559.

CARON, D. A., E. L. LIM, H. KUNZE, E. M. COSPER, AND D. M. ANDERSON. 1989. Trophic interactions between nano- and microzooplankton and the brown tide. Lecture Notes Coastal Estuarine Studies **35:** 263–294.

CARPENTER, E. J., B. M. BRINKHUIS, AND D. G. CAPONE. 1991. Primary production and nitrogenous nutrients in Great South Bay, p. 33–42. *In* J. R. Schubel, T. M. Bell, and H. H. Carter [eds.] The Great South Bay. SUNY Press.

CHISOLM, S. W. 1992. Phytoplankton size. *In* P. G. Falkowsi and A. D. Woodhead [eds.], Primary production and biogeochemical cycles in the sea. Plenum.

COSPER, E. M., W. C. DENNISON, A. J. MILLIGAN, E. J. CARPENTER, C. LEE, J. HOLZAPFEL, AND L. MILANESE. 1989. An examination of the environmental factors important to initiating and sustaining ''brown tide'' blooms. Lecture Notes Coastal Estuarine Studies **35:** 317–340.

DZURICA, S., C. LEE, E. M. COSPER, AND E. J. CARPENTER. 1989. Role of environmental variables, specifically organic compounds and micronutrients, in the growth of Chrysophyte *Aureococcus anophagefferens*, the ''Brown Tide'' microalage. Lecture Notes Coastal Estuarine Studies **35:** 229–252.

GAINEY, L. F., AND S. E. SHUMWAY. 1991. The physiological effect of *Aureococcus anophagefferens* (''brown tide'') on lateral cilia of bivalve mollusks. Biol. Bull. **181:** 298–306.

GALLEGOS, C. L. 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: Non-linear feeding kinetics. Mar. Ecol. Prog. Ser. **57:** 23–33.

GOBLER, C. J. 1995. The role of iron in the occurrence of *Aureococcus anophagefferens* blooms. Master's thesis. SUNY-Stony Brook.

———, AND S. A. SAÑUDO-WILHELMY. 2001*a*. Effects of organic carbon, organic nitrogen, inorganic nutrients, and iron additions on the growth of phytoplankton and bacteria during a brown tide bloom. Mar. Ecol. Prog. Ser. **209:** 19–34.

———, AND ———. 2001*b*. Temporal variability of groundwater seepage and brown tide blooms in a Long Island embayment. Mar. Ecol. Prog. Ser. **217:** 299–309.

GOLDMAN, J. C., AND M. R. DENNETT. 1991. Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. Mar. Biol. **109:** 369–378.

JONES, M. N. 1984. Nitrate reduction by shaking with cadmium: Alternative to cadmium columns. Water Res. **18:** 643–646.

KANA, T. M., AND P. M. GLIBERT. 1987. Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus.* Deep-Sea Res. **34:** 497–516.

KELLER, A. A., AND R. L. RICE. 1989. Effects of nutrient enrichment on natural populations of the brown tide phytoplankton *Aureococcus anophagefferens* (Chrysophyceae). J. Phycol. **25:** 636–646.

KELLER, M. D., W. K. BELLOWS, AND R. R. L. GUILLARD. 1989. Dimethylsulfide production and marine phytoplankton: An additional impact of unusual blooms. Lecture Notes Coastal Estuarine Studies **35:**101–115.

LANDRY, M. R., J. KIRSHTEIN, AND J. CONSTANTINOU. 1995. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. Mar. Ecol. Prog. Ser. **120:** 53–63.

LAROCHE, J., R. NUZZI, R. WATERS, K. WYMAN, P. G. FALKOWSKI, AND D. W. R. WALLACE. 1997. Brown tide blooms in Long Island's coastal waters linked to variability in groundwater flow. Global Change Biol. **3:** 397–410.

LIU, H., AND E. J. BUSKEY. 2000. The exopolymer secretions (EPS) layer surrounding *Aureoumbra lagunensis* cells affects growth, grazing, and behavior of protozoa. Limnol. Oceanogr. **45:** 1187–1191.

LIVELY, J. S., Z. KAUFMAN, AND E. J. CARPENTER. 1983. Phytoplankton ecology of a barrier island estuary: Great South Bay, New York. Estuar. Coast. Shelf Sci. **16:** 51–68.

LONSDALE, D. J., E. M. COSPER, W.-S. KIM, M. H. DOALL, A. DIVADEENAM, AND S. H. JONASDOTTIR. 1996. Food web interactions in the plankton of Long Island bays, with preliminary observations on brown tide effects. Mar. Ecol. Prog. Ser. **134:** 247–263.

MACISAAC, E. A., AND J. G STOCKNER. 1993. Enumeration of phototrophic picoplankton by autofluorescence microscopy, p. 187–197. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, [eds.] Handbook of Methods in Aquatic Microbial Ecology. Lewis.

MEHRAN, R. 1996. Effects of *A. anophagefferens* on mircozooplankton grazing and growth rates in the Peconic Bays system, Long Island, New York. MS thesis, SUNY Stony Brook.

MULHOLLAND, M. R., P. M. GLIBERT, G. M. BERG, L. VAN HEUKELEM, S. PANTOJA, AND C. LEE. 1998. Extracellular amino acid oxidation by microplankton: A cross-ecosystem comparison. Aquatic Microbial Ecol. **15:** 141–152.

NEWELL, B. S., B. MORGAN, AND J. CUNDY. 1967. The determination of urea in seawater. J. Mar. Res. **25:** 201–202.

NIXON, S. W., S. L. GRANGER, D. I. TAYLOR, P. W. JOHNSON, AND B. A. BUCKELY. 1994. Subtidal volume fluxes, nutrient inputs and the brown tide—an alternative hypothesis. Estuar. Coast. Shelf Sci. **39:** 303–312.

PANTOJA, S., AND C. LEE. 1992. Cell-surface oxidation of amino acids in seawater. Limnol. Oceanogr. **39:** 1718–1725.

PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.

SCDHS. 2000. Suffolk County Department of Health Services, Office of Ecology's annual report of water quality in the Peconic Estuary. Riverhead, New York.

SCHAFFNER, R. 1999. The role of suspension feeding bivalves in the initiation and control of *Aureococcus anophagefferens* blooms. Master's thesis. SUNY Stony Brook.

SIEBURTH, J. M., P. W. JOHNSON, AND P. E. HARGRAVES. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. Et. sp. nov. (Chrysophyceae): The dominant picoplankter during a bloom in Naragansett Bay, Rhode Island, summer 1985. J. Phycol. **24:** 416–425.

SIERACKI, M. E., M. D. KELLER, T. L. CUCCI, AND E. THEIR. 1999. Plankton community ecology during the bloom initiation period of the Brown Tide organism *Aureococcus anophagefferens* in coastal embayments of Long Island, N.Y. EOS **80:** 285.

TURNER, J. T., S. F. BRUNO, R. J. LARSON, R. D. STAKER, G. M. SHARMA. 1983. Seasonality of plankton assemblages in a temperate estuary. Mar. Ecol. **4:** 81–99.

VALDERRAMA, J. C. 1981. The simultaneous analysis of total nitrogen and phosphorus in natural waters. Mar. Chem. **10:** 109–122.

WATERBURY, J. B., S. W. WATSON, F. W. VALOIS, AND D. G. FRANKS. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium, *Synechococcus*, p. 71–121. *In* T. Platt and W. K. W. Li [eds.], Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. publications.

WHEELER, P. W., AND D. L. KIRCHMAN. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. **31:** 998–1009.

WILSON, R. E., K. C. WONG, H. H. CARTER. 1991. Aspects of circulation and exchange in Great South Bay, p. 33–42. *In* J. R. Schubel, T. M. Bell, and H. H. Carter [eds.] The Great South Bay. SUNY Press.

WOLFE, G. V., M. STEINKE, AND G. O. KIRST. 1997. Grazing-activated chemical defence in a unicellular marine alga. Nature **387:** 894–89.

Received: 19 March 2001
Accepted: 17 September 2001
Amended: 16 October 2001