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Speciation and concentrations of dissolved nitrogen as determinants of brown tide *Aureococcus anophagefferens* bloom initiation

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ABSTRACT: Growth responses of the brown tide organism *Aureococcus anophagefferens* and 4 co-occurring microalgal species to varying concentrations of nitrate, ammonium, urea, and glutamate were assayed in laboratory experiments. Analogous to seasonal shifts in nutrient regimes in local bays, growth functional responses were used to predict relative species abundances in simulated communities supported by a reduced nitrogen source after cultivation on NO_3^-. Simulations were based on Monod kinetic parameters, lagged growth responses and threshold nutrient concentrations. Presented with NO_3^- only, rank order of biomass production was *Thalassiosira pseudonana* > *Nannochloris atomus* > *Synechococcus bacillaris* > *Prorocentrum minimum* > *A. anophagefferens* at all concentrations. In contrast, model communities offered NH_4^+ or glutamate at most environmental concentrations (1 to 50 µM N) tended to be dominated by *A. anophagefferens* and *P. minimum*. Over environmental ranges of urea concentrations (1 to 50 µM N), *T. pseudonana* grew fastest, followed by *A. anophagefferens*. In field validation experiments, bay water with bloom concentrations of *A. anophagefferens* (10^5 cells ml^{-1}) amended with equimolar (total N) nitrate-rich groundwater or reduced N-rich sediment porewater supported equal increases in total chlorophyll *a* through time. However, porewater selectively stimulated *A. anophagefferens* growth more than groundwater, and the converse was observed for cyanobacteria. Extrapolation of all experimental results to conditions in Long Island embayments suggests that phytoplankton communities supplied primarily with NO_3^- will be dominated by diatoms, such as *T. pseudonana*, and perhaps by chlorophytes and cyanobacteria like *N. atomus* and *S. bacillaris*. Conversely, phytoplankton communities primarily supplied with low-to-moderate concentrations of reduced N-species, NH_4^+, and dissolved organic nitrogen (DON) will be dominated by *A. anophagefferens* and, to a lesser extent, dinoflagellates like *P. minimum*. Our results are consistent with published field observations of *A. anophagefferens* bloom dynamics in Long Island estuaries.

KEY WORDS: *Aureococcus anophagefferens* · Bottom-up control · Brown tide · Nitrogen uptake · Pelagophyceae · Submarine groundwater discharge

INTRODUCTION

Brown tide (BT), a harmful algal bloom caused by proliferation of the pelagophyte *Aureococcus anophagefferens* Hargraves et Sieburth has afflicted the Peconic and Great South Bay estuaries of Long Island (New York, USA) almost annually since 1985. These bays possess the requisite physico-chemical conditions to support massive phytoplankton blooms: long hydrologic residence times, shallow and well-lit water columns, and substantial nutrient loadings from a densely populated watershed. However, a robust explanation for why almost unialgal blooms of *A. anophagefferens* recur with such regularity in these embayments, but only sporadically elsewhere along the mid-Atlantic M.S. seaboard, has yet to be validated. Uncoupled spatial and temporal distributions of nutrients and *A. anophagefferens* argue against BT

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merely resulting from coastal eutrophication (Cosper et al. 1989b). Clearly, subtle selective pressures are operative, permitting faster growth and/or slower removal of *A. anophagefferens* relative to other competing phytoplankters that commonly inhabit these embayments.

Hypotheses that invoke either bottom-up or top-down control have been advanced to explain onset and persistence of the BT, but none have proven to be entirely satisfactory (reviewed in Bricelj & Lonsdale 1997). Among the bottom-up hypotheses, connections between BT and meteorological and hydrological processes have been inferred (Nixon et al. 1994, LaRoche et al. 1997). Cosper et al. (1987) reported that selected blooms coincided with unusual drought periods. LaRoche et al. (1997) noted an inverse correlation between BT outbreaks and submarine groundwater discharge (SGD) in an 11 yr series of field observations. SGD is the primary pathway for freshwater entry into many estuaries, similar to the Peconic and Great South Bays, and carries with it high concentrations of nutrients—especially NO_3^-—from the watershed (LaRoche et al. 1997, Paerl 1997, Gobler & Sañudo-Wilhelmy 2001a). These observations are consistent with the hypothesis that SGD introduces materials that alter selective pressures on phytoplankton in these embayments and thereby controls phytoplankton dynamics, although the primary mechanism(s) is yet to be fully elucidated. Materials introduced into these embayments may preferentially stimulate or inhibit growth of populations within the phytoplankton community directly, or set up environmental conditions so that a succession of events leads to BT onset (Berg et al. 1997). A SGD-related explanation is appealing because quantifiable changes in these land-linked ecosystems between the pre-BT (before 1985) and BT eras can be related to human population growth and land use (LaRoche et al. 1997).

LaRoche et al. (1997) observed ebbing SGD in the summer of 1995 prior to build up of dissolved organic nitrogen (DON) concentrations, which subsequently became depleted as *Aureococcus anophagefferens* abundances rose at 2 stations within the Peconic estuary. Their time-series analyses led to postulation that *A. anophagefferens* gained competitive advantages over other microalgae because of its efficient DON utilization (Dzurica et al. 1989, Berg et al. 1997, 2002, Lomas et al. 2001, Mulholland et al. 2002). Gobler & Sañudo-Wilhelmy (2001a) observed that BT was a secondary bloom in West Neck Bay, Long Island, apparently supported by reduced forms of nitrogen. Consistent with LaRoche et al.'s (1997) observations, these authors hypothesized that in late spring/early summer, a mixed algal bloom was stimulated by accelerated input of nitrate-laden SGD that was hydraulically forced into these bays by spring rains. As the bloom depleted NO_3^-, trophic interactions in the water column, in addition to heterotrophic metabolism and diagenetic processes in sediments, regenerated reduced forms of dissolved nitrogen, NH_4^+, urea and other DON, which then became the dominant N-species available to phytoplankton as SGD ebbed. In turn, the shift in available N-species exerted a new set of selective pressures favoring onset of BT and perhaps competitive exclusion of other species.

It is well established that relative availabilities of macronutrients (N:P:Si stoichiometries) can shape phytoplankton community composition based on individual species' nutrient requirements, uptake capabilities, and inter-specific competition for resources (Tilman 1976, 1982, Smayda 1989, Schöllhorn & Granéli 1996, Carlsson & Granéli 1999). Field studies have demonstrated that microalgal taxa prefer particular forms of a given nutrient element (Berg et al. 1997, 2001, 2003a, Lomas et al. 1996, 2001). For example, *Aureococcus anophagefferens* has been shown to proliferate faster in NH_4^+ or urea-amended water than in water with NO_3^- amendments, while diatoms tend to grow fastest in waters with high NO_3^- fluxes (Berg et al. 1997, Collos et al. 1997). The present study examines the hypothesis that nitrogen speciation in Long Island embayments controls phytoplankton community composition through resource competition. Specifically, we tested the hypothesis that, barring other resource limitations, *A. anophagefferens* has a competitive advantage over other indigenous phytoplankton species when reduced nitrogen species (NH_4^+, urea, and other DON) supplant NO_3^- as the dominant dissolved nitrogen species. We compared growth rates of *A. anophagefferens* cultures with 4 other algal species incubated in parallel over concentration ranges of nitrate, ammonium, urea, and glutamate (an example of DON). Analogous to field conditions described in LaRoche et al. (1997) and Gobler & Sañudo-Wilhelmy (2001a), we examined algal response to transition from NO_3^--supported growth to that supported by reduced N species. Algae used in these experiments span broad phylogenetic distances and include a chlorophyte, dinoflagellate, cyanobacterium, diatom, and pelagophyte, all of which are common to these coastal embayments. Monod functional responses were derived from growth curves, and simple predictive simulations were run to determine which species would dominate a phytoplankton community under constant nutrient availability and in the absence of top-down control or other limitations. Using bay water experiencing a BT, we then compared model predictions with actual community responses to equimolar amendments of nitrate-rich groundwater and reduced N-rich benthic porewater.

MATERIALS AND METHODS

Phytoplankton cultures. All phytoplankton clones used in this study were originally isolated from coastal waters of Long Island and were obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP; http://ccmp.bigelow.org). Although maintained as unialgal cultures, they were never axenic. Epifluorescent microscopic inspection of DAPI-stained preparations confirmed that bacterial abundances in cultures usually remained an order of magnitude lower than those of algae from one transfer to the next until the late stationary phase, and were equivalent to ~0.4 to 3% of algal N-biomass. *Aureococcus anophagefferens* (CCMP 1708) was originally isolated from West Neck Bay (Fig. 1). The chlorophyte and dinoflagellate, *Nannochloris atomus* Butcher (CCMP 509; Clone Synonym: GSBNANNO) and *Prorocentrum minimum* Schiller (CCMP 696; Clone Synonym: NEPCC541), respectively, were isolated from Great South Bay. The cyanobacterium *Synechococcus bacillaris* Butcher (CCMP 1333; Clone Synonyms: WH5701 and NEPCC539) was collected from Long Island Sound off Milford, Connecticut. The centric diatom *Thalassiosira pseudonana* Hasle et Heimdal (CCMP 1335; Clone Synonym: NEPCC58) was isolated from Moriches Bay, Long Island (Fig. 1).

All microalgal cultures were grown in BT medium, a modified f/2 medium (Guillard & Ryther 1962). Maintenance medium was prepared from filtered (0.22 µm) Atlantic Ocean seawater collected 8 km southeast of the Shinnecock Inlet, near the east end of Long Island, and had a salinity of 34 psu. BT medium differed from the published f/2 medium in that it contained 10^{-8} M selenium as selenite, citric acid was substituted for EDTA as the metals chelator, and Fe concentrations were 10^{-6} M (Cosper et al. 1993). Semi-complete medium was autoclaved for 15 min in 125 ml Pyrex erlenmeyer flasks. After cooling, filter-sterilized vitamin and phosphate stocks were aseptically transferred to each flask. Final concentrations of nitrate, phosphate and silicate in the maintenance medium were 883, 36, and 110 µM, respectively. Using spectrophotometric techniques (Valderrama 1981, Jones 1984, Parsons et al. 1984), we determined that background levels of DON, NO_3^-, NO_2^-, and NH_4^+ in our basal seawater were 8.9 ± 0.8, 0.15 ± 0.05, 0.0 ± 0.0, and 0.23 ± 0.05 µM N (±1 SD), respectively. Cultures were maintained at 20°C on a 14:10 h light:dark cycle, illuminated by a bank of 6 × 20 W fluorescent lights that provided 48 to 63 µmol quanta m^{-2} s^{-1} to culture flasks in a highly reflective incubator. These conditions approximated temperature and light exposures found in Long Island estuaries during early summer months when *Aureococcus anophagefferens* blooms occur (Cosper et al. 1989a, Milligan & Cosper 1997). Light exposures approximated the half-saturating irradiances (K_{lt}) determined for *A. anophagefferens* cultures (69 µmol quanta m^{-2} s^{-1}; Milligan & Cosper 1997), but surpassed K_{lt} observed in field blooms (1.1 to 12.8 µmol quanta m^{-2} s^{-1}; Lomas et al. 1996).

Grow out experiments. Algal responses to nitrogen speciation and concentration were examined in parallel incubations of all 5 species, each provided with 6 concentrations of a primary N-source in 100 ml of the BT medium described above. For the nitrate experiment, a separate series of 125 ml Pyrex flasks was prepared for each algal species with 6 diminishing NO_3^- concentrations from 1500 to 10 µM. Subsequent experiments with NH_4^+, urea (2 N atoms per urea molecule),

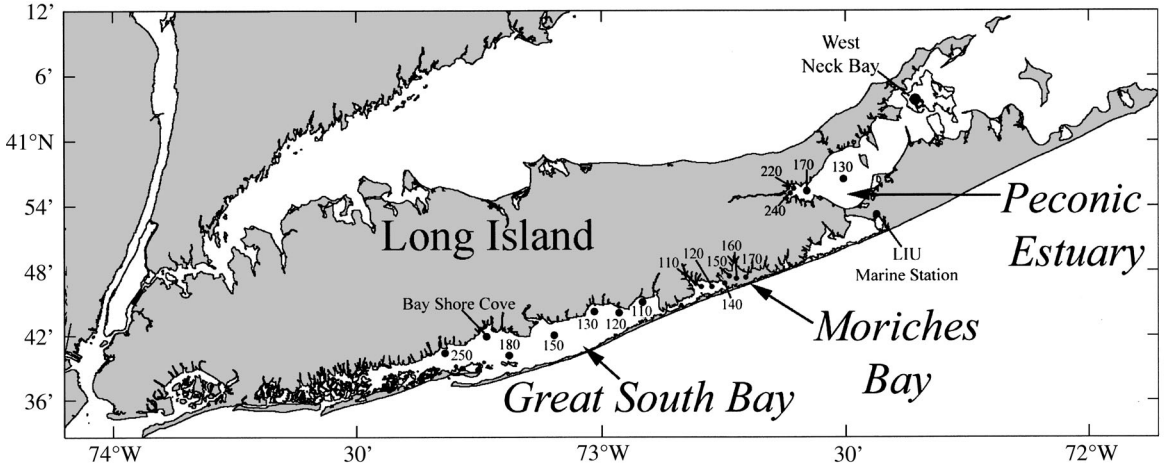

Fig. 1. Long Island embayments (New York, USA), including sampling sites for field validation experiment and Suffolk County Department of Health Services monitoring stations

and glutamate utilized 6 diminishing nitrogen concentrations from 900 to 1 µM N for each algal species. To assure nutrient saturation, highest nitrogen concentrations were those of standard phycological media, exceeding ranges found in BT-prone and BT-free embayments by 10 to 100-fold (Ryther & Dunstan 1971, Carpenter & Dunham 1985, Chang & Carpenter 1985, Cosper et al. 1990, LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a, Gobler & Boneillo 2003). Each set of flasks received a 1 ml inoculum from a single microalgal culture, previously grown in NO_3^- medium for 8 d (early stationary phase). This equated to final cell concentrations of ~0.6 to 3.4 × 10^5 ml^{-1} or approximately 37 to 218 ng N ml^{-1} of biomass at the experiment's initiation, depending on the species. During each experiment, samples were incubated under a fluorescent light bank, which was randomly arranged on a reflective white rotating platform (6 rpm) to cancel effects of spatial variations in illumination. Growth experiments were conducted under the same incubation conditions described above for maintenance cultures for ~2 wk.

Accumulation of cell biomass through time was estimated by *in vivo* fluorescence, measured the same time each day to account for diel fluctuations in cell fluorescence. After gently swirling each culture, 3.5 ml subsamples were removed from each flask and fluorescence was measured in a Turner Designs fluorometer. Previous research demonstrates that *in vivo* fluorescence is proportional to cell densities of a variety of cultured phytoplankton species (Fogg & Thake 1987), including *Aureococcus anophagefferens* (Gobler & Cosper 1996). Cellular chlorophyll *a* (chl *a*) content and consequently *in vivo* fluorescence obviously vary among species, and also vary within a species depending upon nitrogen source and physiological status (Carlsson et al. 1998). However, *in vivo* fluorescence is used here to generate biomass production rate constants (d^{-1}) under stable environmental conditions, so the actual chl *a* cell quotients are irrelevant to the calculation as long as they are relatively constant during early to mid-exponential growth phase.

Within-treatment reproducibility observed among triplicate flasks in these (Fig. 2a,b) and previous unreported bioassays was relatively high; mean relative standard deviation (SD $mean^{-1}$) within treatments for >800 individual observations was 16% among all 5 algal species. Therefore, the practice of triplicating all treatments was discontinued because of limitations in incubator space and processing time.

At selected time points, subsamples were preserved in 2% buffered formaldehyde, then stained with DAPI and prepared for epifluorescent enumeration of algae and bacteria (Porter & Feig 1980). Algal surveys were used to calibrate *in vivo* fluorescence measurements for each species and bacterial surveys used to assess contamination level. For elemental analysis, cells from each culture were harvested at mid-exponential phase, filtered onto precombusted GF/F filters, rinsed with filtered seawater, and stored frozen until analysis. Particulate organic carbon and nitrogen were analyzed on filters by combustion in a Carlo Erba EA1108 CHNS-O analyzer and corrected with filter and reagent blanks (Sharp 1991). Algal biomass estimates were also corrected for bacterial biomass contribution using DAPI counts and extrapolations of 149 × 10^{-15} g C $cell^{-1}$ and 35 × 10^{-15} g N $cell^{-1}$ (Vrede et al. 2002).

Growth performance simulations. Growth curves were produced from daily *in vivo* fluorescence data. Growth rate, µ (d^{-1}), was estimated from the slope of ln (N_t/N_0) plotted against time, where N_0 and N_t equal fluorescence unit accumulation, between times 0 and t. Regressions were based on 3 to 6 time points over which ln fluorescence increased linearly and most rapidly. The time interval employed varied between experiments, depending upon length of growth lags (≤3 d) and onset of nutrient exhaustion (stationary phase). All regressions were statistically significant ($p < 0.05$) and typically yielded coefficients of determination (r^2) exceeding 0.90. Errors associated with determination of µ were represented by the standard error of the regression slope. Growth rates in most experiments were saturating functions of dissolved nitrogen concentration, S ($\mu = \mu_{max}\,[S/K_s + S]$), permitting calculation of the Monod kinetic parameters K_s (half-saturation constant) and μ_{max} (maximum growth rate). In 2 instances, an extended form of the Monod model ($\mu = \mu_{max}\,[(S - S_{min})/(K_s + S - S_{min}]$) was employed because negative growth was observed below a finite nutrient concentration (S_{min}; Kovarova et al. 1996). Monod constants were estimated by 2 methods; indirectly by Eadee-Hofstee linear transformation and directly by iterative non-linear curve fitting (Marquardt-Levenberg method: SPSS SigmaPlot version 8.0). As evaluated in Berges et al. (1994), non-linear curve fitting is more appropriate for geometrically distributed data and this technique systematically yielded higher coefficients of determination (r^2) than linear transforms in our study. Therefore, only Monod constants derived from non-linear curve fitting are reported.

Growth performance simulations incorporated lag periods and Monod constants (μ_{max}, K_s, S_{min}) and assumed an arbitrary initial condition of equivalent biomasses for all 5 species tested (N_0 = 0.1 ng N ml^{-1}), representing an early spring field condition of 60 to 300 cells ml^{-1}, depending on species. To predict how a fixed nutrient concentration determines relative abundances of the 5 species through time within a simplified phytoplankton community, concentration-

dependent growth rates ($\mu = \mu_{max}\ [(S - S_{min})/(K_s + S - S_{min})]$) were applied to the logistic growth equation ($N_t = N_0 e^{\mu t}$) for each species, where N_t equals biomass at time t. As long as a lag period (l) was apparent, N_t was assumed to equal N_0: i.e. if $(t - l) \leq 0$, then $N_t = N_0$. Simulations were run for 7 d because rank order of biomass production among species did not change beyond that time, although relative abundances did. Our model assumed no removal and that nutrient supply kept pace with plankton uptake, resulting in steady-state nutrient concentrations for the duration of the simulation.

Field experiment. Variations in groundwater flow and porewater seepage alter nutrient chemistry in Long Island bays and are believed to influence brown tide blooms (LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001b, Lomas et al. 2004). Therefore, growth of *Aureococcus anophagefferens* within natural phytoplankton assemblages was examined in baywater amended either with local groundwater or benthic porewater. Subtidal groundwater was collected from an intertidal piezometer on the shoreline of a bloom-prone embayment, West Neck Bay (Fig. 1) then filtered and stored frozen (see Gobler & Sañudo-Wilhelmy 2001b for procedures). Benthic porewater was isolated from subtidal surface sediments (0 to 20 cm) collected from western Great South Bay via centrifugation at $1990 \times g$ (Aller & Benninger 1981). Immediately following centrifugation, benthic porewater was filtered (0.2 µm) and stored frozen. Nitrate, nitrite, ammonium, phosphate, and total dissolved nitrogen (TDN) content of all water samples were determined by standard spectrophotometric techniques (Valderrama 1981, Jones 1984, Parsons et al. 1984). DON was calculated as the difference between TDN and dissolved inorganic nitrogen (nitrate, nitrite, and ammonium).

Baywater was collected in HCl-cleaned polycarbonate bottles on 24 June 2001 from Bay Shore Cove, in western Great South Bay (Fig. 1) during a brown tide bloom (>10^5 *Aureococcus anophagefferens* ml^{-1}). The experiment was initiated by amending triplicate flasks with groundwater or benthic porewater to achieve final TDN (DIN + DON) additions of 7.5 µM N in all flasks. To ensure volumes and salinities remained constant among all treatments, control and porewater-amended flasks received an equivalent volume of Milli-Q water, and groundwater–amended flasks received additions of 0.2 µm-filtered, aged seawater from Bay Shore Cove. Freshwater additions decreased final salinity from ~27 to ~25 psu, remaining within the physiological optimum for *A. anophagefferens* (Cosper et al. 1989a). The 7.5 µM TDN porewater and groundwater amendments were repeated at 24, 48, and 72 h to maintain nutrient inventories semi-continuously. Amended TDN concentrations and final salinities were similar to those previously observed in the water column of BT prone-embayments on Long Island (LaRoche et al. 1997). Incubations were conducted by immersing flasks in Old Fort Pond at the Southampton College (Long Island University [LIU]) Marine Station (Fig. 1), closely mimicking ambient light and temperature conditions found in Great South Bay (Gobler et al. 2002).

At 0, 24, 48 and 72 h, subsamples from all flasks were filtered through GF/F glass fiber filters for chl *a* determinations using standard fluorometric techniques (Parsons et al. 1984). At 72 h, 10 ml aliquots were preserved with 1% glutaraldehyde (final conc.) and stored at 5°C for enumeration of algal cell densities. Cyanobacteria were enumerated by autofluorescent microscopy on slides prepared within 24 h of preservation and stored frozen until analysis (MacIsaac & Stockner 1993). *Aureococcus anophagefferens* densities were enumerated by the immunofluorescent direct count method from the preserved samples (Anderson et al. 1989).

RESULTS

Response of *Aureococcus anophagefferens* to inorganic N-speciation

The growth rate of *Aureococcus anophagefferens* was stimulated by increasing NO_3^- concentrations up to 200 µM, but not at higher concentrations (Fig. 2a). During exponential growth phase, initial slopes for 200, 500, 900 and 1500 µM NO_3^- curves were not significantly different from one another ($p > 0.05$; ANCOVA), suggesting saturating growth rates at these concentrations. The medium's initial o-PO_4^{3-} concentration was 36 µM, so that N:P ratios surpassed 16 at 570 µM. Hence cell yields in the highest nitrate exposures were similar, and were probably limited by final o-PO_4^{3-} availability. P-limitation may have been operative for all algal species tested at high N concentrations, regardless of chemical form. Cultures reached stationary phase after 4 d at the lowest concentration and 7 d at the highest, illustrating the time required to exhaust useable NO_3^- and reach nutrient limitation, whether limited by N or P.

Growth response of *Aureococcus anophagefferens* to increasing concentrations of NH_4^+ was markedly different to its response to NO_3^- (Fig. 2b). At the lowest concentrations, 1 and 10 µM, cultures grew immediately and reached stationary phase within 5 d. At 50 µM, *in vivo* fluorescence measurements at Days 0 and 3 were equivalent, suggesting that *A. anophagefferens* growth lagged after transfer from NO_3^- medium. Exponential phase was then sustained for 3 d

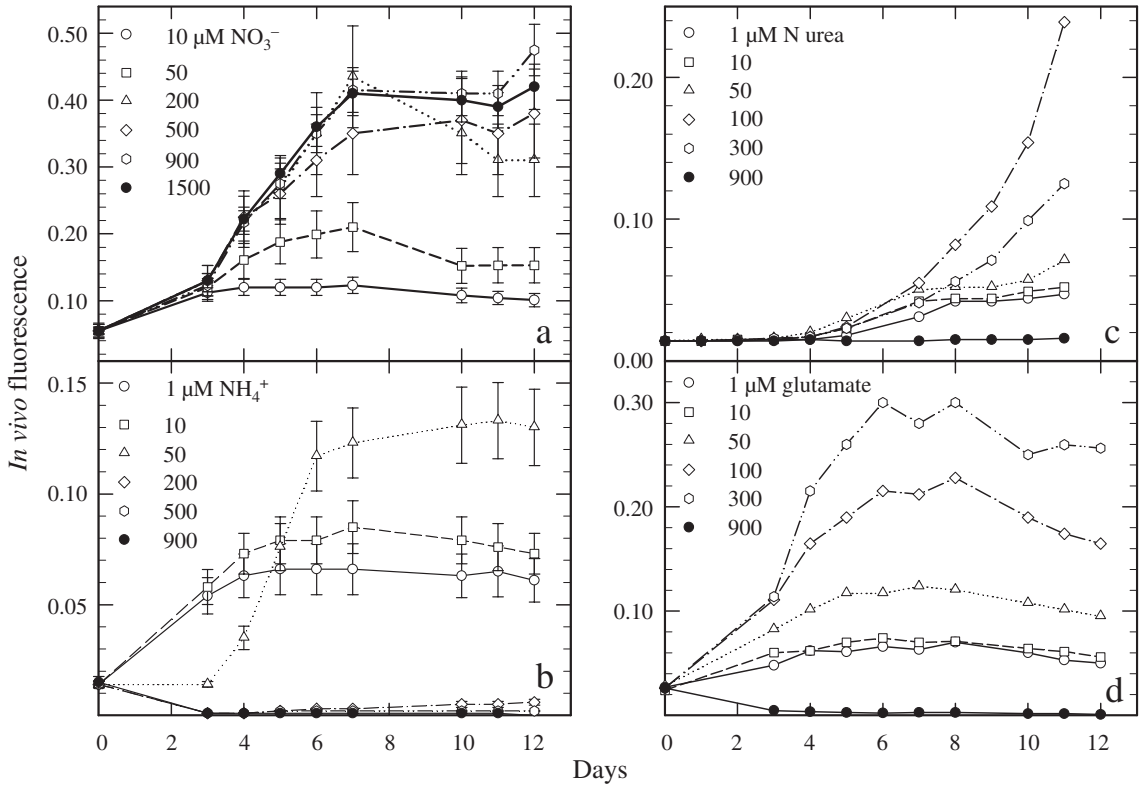

Fig. 2. *Aureococcus anophagefferens*. Growth curves under a range of concentrations of (a) nitrate, (b) ammonium, (c) urea or (d) glutamate as primary N sources in standard BT medium. Samples incubated on a 14:10 h light:dark cycle. Cell biomass determined using proxy of *in vivo* fluorescence. (a,b) Error bars in represent 1 SD from triplicate incubation flasks. Analytical precision of *in vivo* fluorescence measurements was ±1.4% relative SD, so errors were smaller than symbols

before reaching stationary phase. Exposures of *A. anophagefferens* to NH_4^+ concentrations ≥200 µM appeared to be strongly inhibitory, if not lethal (see lower 3 curves, Fig. 2b).

Transfer from NO_3^- to urea-based media delayed initiation of detectable growth in cultures of *Aureococcus anophagefferens* by 2 to 3 d at all concentrations (1 to 900 µM N) (Fig. 2c). Growth lags observed in all treatments of this experiment and in some treatments of other experiments revealed that carry-over of residual NO_3^- with the original inoculum was insufficient to support detectable growth. Lags also demonstrated that acclimation of cultures was sometimes necessary for growth after transfer to a new nitrogen source. Similar to ammonium, rates of *A. anophagefferens* accumulation increased over the lower range in urea concentrations (≤100 µM N), decreased at 300 µM N, and were essentially zero at 900 µM N.

Cultures transferred to media with the amino acid glutamate as the sole source of nitrogen appeared to grow almost immediately (Fig. 2d). In contrast to results from other reduced forms of N, cultures responded positively to increasing glutamate concentrations up to 300 µM N. However, exposures to 900 µM N of glutamate appeared to be lethal, as was also observed for ammonium and urea (but not nitrate).

Growth rate comparisons

Growth curves similar to those presented in Fig. 2 were simultaneously generated for the other 4 algal species (not presented), allowing calculation of specific growth rates of all species at each nutrient concentration. Analysis of growth rates for all species illustrated that when NO_3^- was the predominant nitrogenous nutrient available, *Aureococcus anophagefferens* was only capable of relatively meager growth rates, 0.17 to 0.30 doublings d^{-1} (simply 'd^{-1}' hereafter), even after being maintained in nitrate-based media for many generations (Fig. 3a). *Aureococcus anophagefferens* grew slower on NO_3^- (μ_{max} = 0.30 d^{-1}) and had a higher half saturation constant (K_s = 8.6 µM) than any other microalga tested, independent of nutrient concentration (Table 1). In sharp contrast, nitrate-fed *Thalassiosira pseudonana* cultures outgrew all species tested, followed by *Nannochloris atomus*. Growth rates of *Synechococcus bacillaris* and *Prorocentrum minimum*

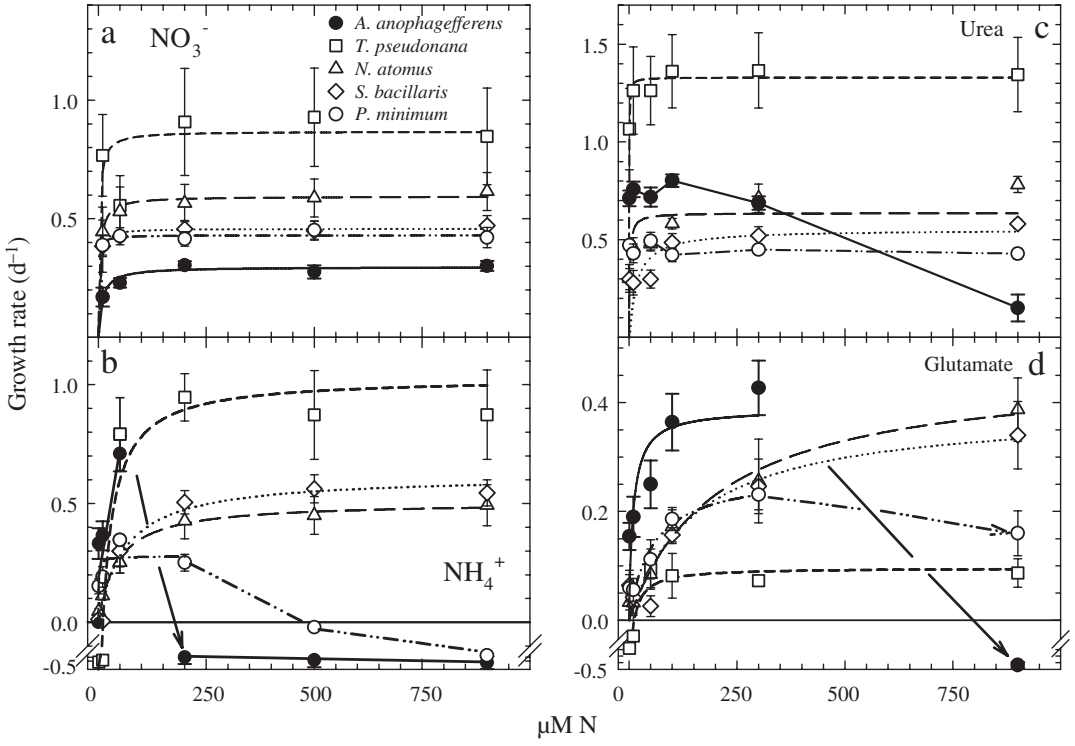

Fig. 3. Growth rates for 5 species under ranges of (a) nitrate, (b) ammonium, (c) urea, or (d) glutamate. Symbols represent slope of $\ln[N_t/N_0]$ versus t in growth curves; error bars are SE of regression slopes

were similar over the entire NO_3^- concentration range tested. Even though *T. pseudonana* possessed the highest μ_{max} (0.87 d^{-1}), its K_s for NO_3^- (2.5 μM) was slightly higher than those of *S. bacillaris* and *P. minimum* (Table 1). Use of an approximation of the Monod equation's slope (μ_{max}/K_s) as an index of competitive advantage (Healey 1980) clearly demonstrated that *A. anophagefferens* is a poor competitor for NO_3^-; the μ_{max}/K_s ratio for *A. anophagefferens* was 5- to 10-fold lower than for other species (Table 1). For all species, growth rates approached their maxima at NO_3^- concentrations between 50 and 200 μM N. In this concentration range, P limitation on growth is unlikely for any species (see above), but Si availability may have limited *T. pseudonana* yields at higher NO_3^- concentrations; silicic acid in the media was 110 μM, and while the stoichiometry of Si:N can vary among diatom species, a 1:1 stoichiometry is generally assumed (Brzezinski 1985).

In NH_4^+-fed cultures, our microalgal populations responded very differently (Fig. 3b). At NH_4^+ concentrations below 50 μM, *Aureococcus anophagefferens* was capable of growth rates (± SE) of about 0.35 ± 0.06 d^{-1}; growth rates among other species varied from negative at S_{min} below 13 μM (*Thalassiosira pseudonana*) to 0.19 ± 0.04 d^{-1} (*Prorocentrum minimum*). At 50 μM, *A. anophagefferens* and *T. pseudonana* grew at least twice as fast as other species and achieved comparable growth rates (0.71 to 0.79 d^{-1}), but both species exhibited growth lags of ≤3 d when acclimating to high concentrations of NH_4^+ after being in NO_3^- maintenance cultures. Over the remaining concentration range, *T. pseudonana* achieved higher growth rates than all other species; however, the 3 d lag phase was still present at all concentrations. *Nannochloris atomus* and *Synechococcus bacillaris* were similarly stimulated by increasing ammonium concentrations, and exhibited comparable growth rates except at the lowest concentrations. Similar to NO_3^- trials, maximum growth rates were approached at NH_4^+ concentrations above 100 μM for *T. pseudonana*, *N. atomus*, and *S. bacillaris*. In contrast to NO_3^- trials, inhibitory effects of NH_4^+ were evident at concentrations above 50 μM for *A. anophagefferens*, and at concentrations above 200 μM for *P. minimum*; populations experienced die off after being transferred from NO_3^- maintenance cultures to media containing high concentrations of NH_4^+. These 2 species appeared to be most competitive at low NH_4^+ concentrations, $\mu_{max}/K_s \geq 0.1$ and 0.3, respectively, but exact determination of the kinetics constants was difficult given the existing data (Fig. 3b; Table 1).

Urea supported superior growth rates (1.1 to 1.4 d^{-1}) of *Thalassiosira pseudonana* at all concentrations

Table 1. Monod kinetic parameters for all species and nutrients estimated by iterative, non-linear curve-fitting to the formula $\mu = \mu_{max}\,[(S - S_{min})(K_s + S - S_{min})^{-1}]$. Standard errors in parentheses. r^2 = coefficient of determination or curve's goodness of fit to data; lag = time interval over which chl *a* remained constant for all concentrations. Values in **bold** indicate principal kinetic parameters in determining population response

Variable	*Aureococcus anophagefferens*	*Thalassiosira pseudonana*	*Nannochloris atomus*	*Synechococcus bacillaris*	*Prorocentrum minimum*
NO_3^-					
K_s (µM N)	8.6 (2.0)	2.5 (2.8)	3.7 (0.6)	2.1 (0.3)	1.1 (0.4)
μ_{max} (d^{-1})	0.30 (0.01)	0.87 (0.07)	0.59 (0.01)	0.46 (0.01)	0.43 (0.01)
μ_{max}/K_s	**0.04**	**0.40**	**0.20**	**0.20**	**0.40**
r^2	0.98	0.86	0.99	0.99	0.99
Lag (d)	0	0	0	0	0
NH_4^+					
K_s (µM N)	6.4[a]	34 (21)[b]	44 (7.3)	61 (21)	0.7 (0.4)
μ_{max} (d^{-1})	0.71[a]	1.02 (0.13)	0.51 (0.02)	0.62 (0.05)	0.23 (0.02)
μ_{max}/K_s	**0.10**	**0.03**	**0.01**	**0.01**	**0.30**
r^2	0.99	0.91	0.99	0.97	0.97
Lag (d)	0–3	3	0	0	0
Urea					
K_s (µM N)	<0.4[c]	0.3 (0.06)	2.2 (1.5)	18 (9.2)	<0.5[c]
μ_{max} (d^{-1})	0.75[d]	1.33 (0.02)	0.64 (0.06)	0.55 (0.05)	0.45[d]
μ_{max}/K_s	**1.90**	**4.40**	**0.30**	**0.03**	**0.90**
r^2	–	0.99	0.86	0.91	–
Lag (d)	1	0	0	1	3
Glutamate					
K_s (µM)	9.8 (8.4)	22 (19)[b]	203 (44)	151 (70)	53 (31)
μ_{max} (d^{-1})	0.39 (0.06)	0.10 (0.02)	0.46 (0.04)	0.39 (0.06)	0.27 (0.05)
μ_{max}/K_s	**0.040**	**0.005**	**0.002**	**0.003**	**0.005**
r^2	0.81	0.95	0.98	0.95[e]	0.91[e]
Lag (d)	0	0	3	3	0

[a]Estimates determined from linear regression analysis between minimum and maximum observations (lack of observations in critical concentration range precluded using Monod formalism); [b]only assays where S_{min} was apparent: 13 µM for NH_4^+ and 12 µM for glutamate; [c]maxima estimated between origin and saturating concentration; [d]mean value; [e]1 outlier removed from analyses

(Fig. 3c). At urea concentrations of 50 µM (100 µM N) or less, *Aureococcus anophagefferens* achieved the second highest growth rates, 0.69 to 0.80 d^{-1}, but growth performance diminished at higher urea concentrations. In fact, *A. anophagefferens* was the only microalga to experience inhibition at high urea concentrations. Growth rates of *Nannochloris atomus* and *Synechococcus bacillaris* exhibited Monod kinetics over the urea concentration range examined (Fig. 3c). In contrast, *Prorocentrum minimum*'s growth rates (0.42 to 0.49 d^{-1}) appeared to be independent of the urea concentrations employed ($p > 0.05$), which suggested nutrient saturation near 1 µM N urea. Lags in growth were evident for *A. anophagefferens* (≤1 d), *S. bacillaris* (≤1 d), and *P. minimum* (≤3 d), which suggested acclimation to a new N source in these cultures that was not apparent for *T. pseudonana* nor *N. atomus*. Estimates of μ_{max} were highest on a urea diet for all species except *S. bacillaris* (Table 1). Competitive performance (μ_{max}/K_s) for urea appeared to be highest in *T. pseudonana*, *A. anophagefferens*, and *P. minimum*, although accurate K_s determinations for the last 2 species were not possible because sub-micromolar concentrations were not tested (Table 1).

Glutamate favored growth of *Aureococcus anophagefferens* over the other 4 microalgae at all concentrations below 900 µM N (Fig. 3d). From 1 to 300 µM N, growth rates of *A. anophagefferens* followed Monod kinetics, and exhibited modest maximal growth and half-saturation constants: μ_{max} = 0.39 d^{-1} and K_s = 9.8 µM (Table 1). However, growth rates of *A. anophagefferens* were negative at the highest glutamate concentration. Similar to NH_4^+, only glutamate concentrations above 12 µM N (S_{min}) supported growth of *Thalassiosira pseudonana*, and then only slowly at best (<0.10 ± 0.03 d^{-1}). *Nannochloris atomus* and *Synechococcus bacillaris* responded similarly to glutamate as a primary N source, yielding μ_{max} of 0.46 and 0.39 d^{-1} and high K_s values of 203 and 151 µM N, respectively (Table 1). Both species exhibited delays in growth of ≤3 d when glutamate was the primary nitrogen source. Growth rates of *Prorocentrum minimum* were not detectably different between 1 and 10 µM N (0.06 d^{-1}), but did increase hyperbolically (r^2 = 0.91)

between 10 and 300 µM N, attaining a μ_{max} of only 0.27 d^{-1} (Table 1). Like *A. anophagefferens*, growth of *P. minimum* was inhibited at the highest glutamate concentration.

Growth performance simulations

Growth performance simulations were run to predict which algal species would dominate a hypothetical phytoplankton community in a nitrogen-limited system after nitrogen speciation shifted from nitrate-domination, as it might seasonally in coastal embayments. Simulations incorporated Monod kinetics estimates (Table 1), lag periods, and threshold concentrations to generate growth curves for each species at a specific nutrient concentration. Simulations assumed an initial state of low but equivalent biomass and constant nutrient supply, examples of which are presented in Fig. 4. Outcomes of modeled growth were similar at NO_3^- concentrations of 5 and 50 µM; *Thalassiosira pseudonana* would rapidly outgrow the other 4 species and *Aureococcus anophagefferens* would be the slowest grower (Fig. 4a,b). At 50 µM NO_3^-, *Nannochloris atomus* appeared to have a slight competitive advantage over *Synechococcus bacillaris* and *Prorocentrum minimum* that was not apparent at 5 µM NO_3^- (Fig. 4a,b).

In the absence of other N-sources, low concentrations of NH_4^+ (5 µM) clearly favored a community dominated by *Aureococcus anophagefferens*, distantly followed by *P. minimum* (Fig. 4c). After 7 d, the other 3 species would be rare. At higher NH_4^+ concentrations (≥50 µM), selective advantage initially favored *Synechococcus bacillaris*, *Nannochloris atomus*, and *Prorocentrum minimum*. However, *A. anophagefferens* and *Thalassiosira pseudonana* may gain dominance after a growth lag, if other factors do not become limiting (Fig. 4a).

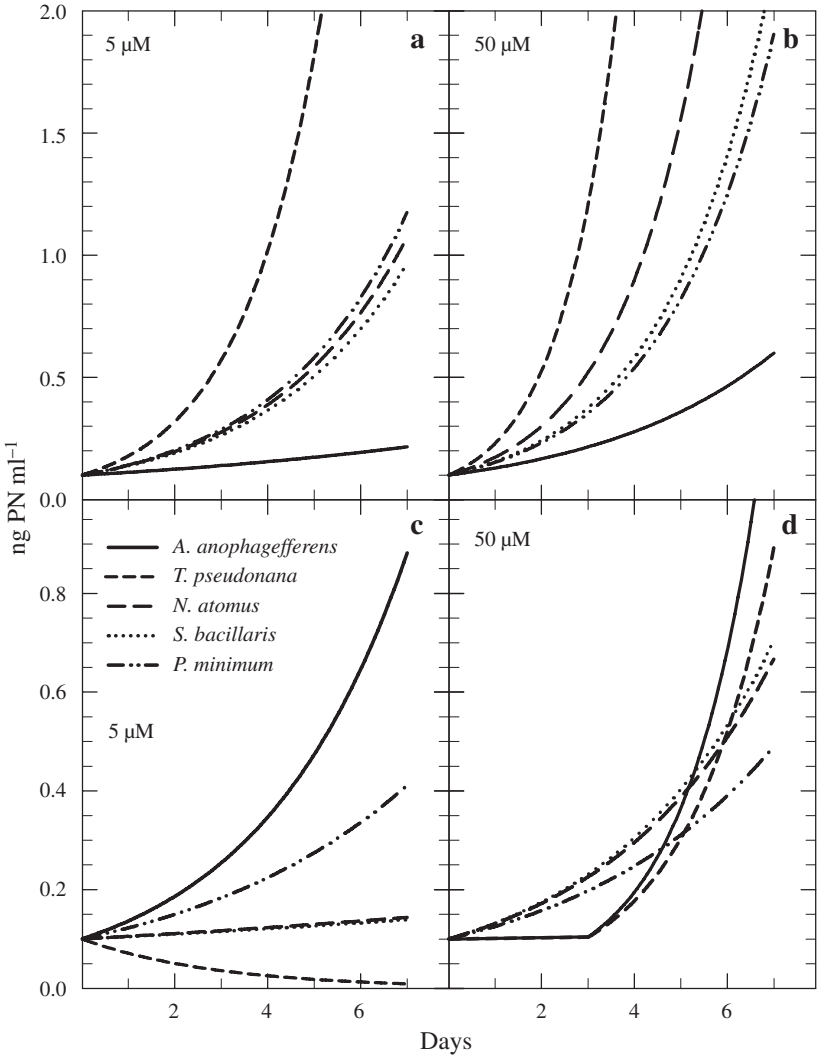

Fig. 4. Examples of growth performance simulations, expressed as particulate nitrogen (PN) for 5 species offered (a) 5 or (b) 50 µM NO_3^-, and (c) 5 or (d) 50 µM NH_4^+. Model assumed biomass of each species was 0.1 ng N ml^{-1} at Day 0, nitrogen was the only growth-limiting nutrient, and its concentration remained constant. Growth rates and lag times applied to the logistic equation

Rank order outcomes of all simulation runs were tabulated as ratios of biomass yields after 1 wk, and normalized to the least abundant species in that trial. Results for low, intermediate, and high concentrations are summarized in Table 2 to represent general trends along trophic gradients. Results for NO_3^- were the most straightforward and consistent. At all concentrations tested, *Thalassiosira pseudonana* dominated this simulated phytoplankton community, outweighing *Aureococcus anophagefferens* by 5- to 55-fold and the other species by at least 1.7- to 7-fold (Table 2).

Results for NH_4^+ simulations were more varied (Table 2). At 1 µM, *Prorocentrum minimum* was a slightly better competitor than *Aureococcus anophagefferens* and growth of both species far outpaced the other 3 species. At 10 µM, *A. anophagefferens* and *P. minimum* biomasses were highest after 7 d, with *A. anophagefferens* > 40-fold more abundant than *Thalassiosira pseudonana*, the poorest performer under these conditions. At

Table 2. Results from simulations of 5 microalgal species competing for nitrogen available in varying forms and concentrations. Model outcome based on equal initial cell biomass (0.1 ng particulate nitrogen ml^{-1}), constant nutrient supply, predicted growth rates (μ d^{-1}) and observed lag periods (0 to 3 d). Reported in descending rank order as relative cell yields, normalized to the species with the lowest yield at the end of a 1 wk incubation under constant environmental conditions. A = *Aureococcus anophagefferens*; N = *Nannochloris atomus*; P = *Prorocentrum minimum*; S = *Synechococcus bacillaris*; T = *Thalassosira pseudonana*

Nitrogen species	Relative yields after 7 d
NO_3^-	
1 µM	5T:3P:2S:2N:1A
10 µM	42T:7N:5P:5S:1A
50 µM	55T:8N:4S:3P:1A
NH_4^+	
1 µM	205P:156A:86N:85S:1T
10 µM	44A:10P:4N:4S:1T
50 µM	3A:2T:1S:1N:1P
Urea	
1 µM N	1083T:21A:5P:3N:1S
10 µM N	2592T:23A:12N:2P:1S
50 µM N	1472T:12A:10N:2S:1P
Glutamate	
1 µM	3A:2P:2S:2N:1T
10 µM	4A:1P:1S:1N:1T
50 µM	7A:2P:1T:1S:1N

higher NH_4^+ concentrations (50 µM), *T. pseudonana* became more common, but competitive advantage for any single species eroded. Despite *T. pseudonana*'s delayed growth when exposed to NH_4^+ (3 d lag), its unresponsiveness to low NH_4^+ concentrations (S_{min} = 13 µM) and its relatively weak functional response (μ_{max}/K_s = 0.03), its high μ_{max} allowed it to outgrow all but *A. anophagefferens* within the 1 wk simulation (Fig. 4d, Tables 1 & 2).

Over the entire simulated range in urea concentrations (1 to 50 µM N), the hypothetical community would be dominated by *Thalassiosira pseudonana* and *Aureococcus anophagefferens*, but *T. pseudonana* was favored 50- to 110-fold over the BT organism after 1 wk (Table 2). The combination of weak functional response for urea (μ_{max}/K_s = 0.03), modest growth rate, and 1 d lag in growth when exposed to urea placed *Synechococcus bacillaris* at a competitive disadvantage at all urea concentrations. Simulations suggested that relative abundances of *Nannochloris atomus* would increase with urea concentrations, displacing *Prorocentrum minimum* as third most abundant species.

Glutamate conferred a clear competitive advantage to *Aureococcus anophagefferens* over the other 4 species, consistently followed by *Prorocentrum minimum* at all concentrations (Table 2, Fig. 3d). However, biomass yields for *A. anophagefferens* after 1 wk were low compared to yields from NH_4^+ or urea, because only a moderate μ_{max} was achieved (0.39 d^{-1}) on a glutamate diet (Table 1). While its functional response (μ_{max}/K_s) was an order of magnitude more sensitive to changes in glutamate concentration than those of other algae, *A. anophagefferens*' competitive advantage appeared to be modest as evidenced by small biomass ratios apparent at low and intermediate glutamate concentrations compared to other nutrients (Table 2). While *Nannochloris atomus* and *Synechococcus bacillaris*' growth potentials were among the 3 highest (Table 1), their growth lags and low μ_{max}/K_s ratios prevented them from effectively competing for resources.

Natural assemblages

Results from our growth experiments and simulations strongly supported the hypothesis that nitrogen speciation can exert selective pressure on phytoplankton species in Long Island embayments. Nitrogen speciation in the source waters of these shallow bays may be strongly influenced by the residence time of subtidal groundwater passing through sediments. In fact, the porewater and groundwater we isolated from coastal Long Island has similar TDN levels, but very different N-speciation (Table 3). In our groundwater sample, 97% of the dissolved N was NO_3^- (320 µM) and most of the remaining 3% was DON (Table 3). In contrast, benthic porewater was depleted in NO_3^- and enriched in NH_4^+ (130 µM; 56% of TDN) and DON (100 µM; 43% of TDN) (Table 3). Nitrogen inventories in Great South Bay during a brown tide outbreak were clearly dominated by DON, which comprised 89% of TDN (Table 3).

Amending seawater from western Great South Bay with groundwater and porewater had a clear impact on the BT-dominated algal community. Control treatments exhibited a steady decrease in chl *a* from 16 to 10 µg l^{-1} during the 72 h incubation, which indicated

Table 3. Nutrient concentrations in Great South Bay (GSB) during BT bloom and in subtidal groundwater and sediment porewater from BT-prone embayments. Values reported as means and SD of analytical triplicates in µM N or µM P. TDN = total dissolved nitrogen; DON = dissolved organic nitrogen

	TDN	NO_3^-	NH_4^+	DON	o-PO_4^{3-}
GSB	29	2.8 (0.16)	0.27 (0.03)	26 (0.17)	0.28 (0.05)
Groundwater	330	320 (3.9)	0.83 (0.05)	9.1 (1.2)	0.55 (0.12)
Porewater	230	0.6 (0.85)	130 (21)	100 (6.4)	3.9 (0.60)

that the entire phytoplankton community declined without nutrient amendments (Fig. 5). In contrast, chl *a* increased to 21 and 23 µg l^{-1} in samples amended with groundwater or porewater (Fig. 5) and was at significantly greater levels than in control treatments by 72 h ($p < 0.05$; Tukey test). While groundwater and porewater treatments contained similar amounts of chl *a* ($p > 0.05$), suggesting comparable community growth, microscopic observations revealed a differential effect between these treatments (Fig. 6). Net production rates (growth – mortality) of cyanobacteria during 72 h incubations with groundwater were significantly faster than those with porewater ($p < 0.05$; Tukey test). The response of *Aureococcus anophagefferens* to these amendments was just the opposite. Its growth in the porewater-amended treatment was significantly greater than its rate in the groundwater-amended treatment and control ($p < 0.05$). Net production of both *A. anophagefferens* and cyanobacteria was stimulated by additions of porewater to a greater degree than the total phytoplankton community (chl *a*), which suggested that these plankters would numerically dominate over time.

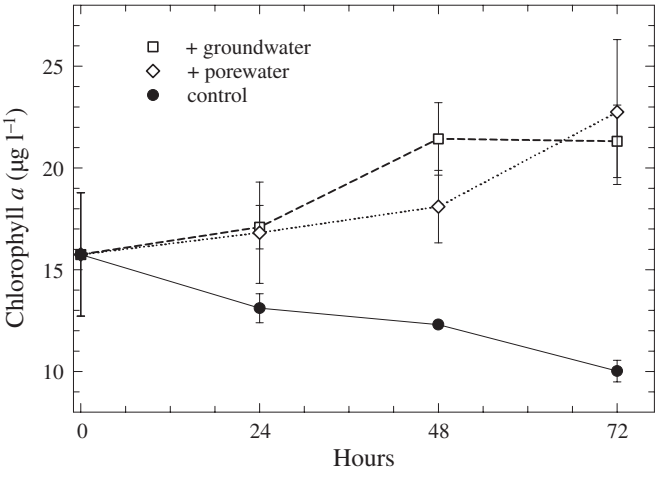

Fig. 5. Time courses of chl *a* production in water samples collected from Bay Shore Cove and Great South Bay on 24 June 2001, amended with coastal groundwater or benthic porewater or not amended. Error bars represent SD from triplicate filtrations

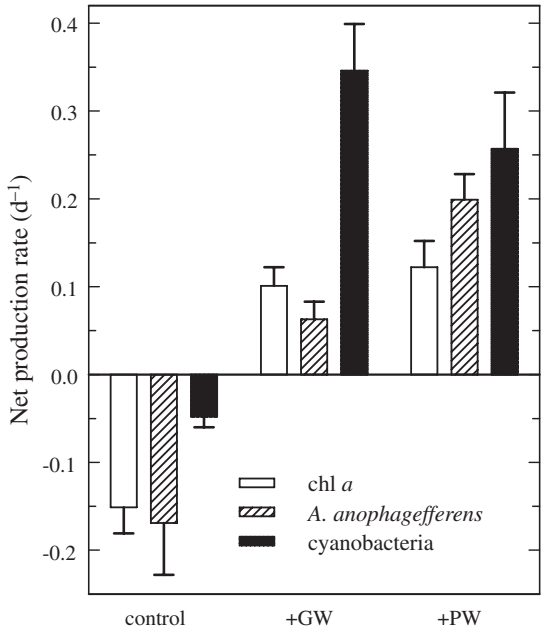

Fig. 6. Net production rates of total phytoplankton community (chl *a*), *Aureococcus anophagefferens*, and cyanobacteria in unaltered bay water and replicate samples amended with coastal groundwater (GW) or benthic porewater (PW). Net production rate = growth rate – mortality rate. Error bars represent 1 SD

DISCUSSION

Dissolved nitrogen in Long Island embayments

Coastal eutrophication has often been cited as the primary cause for escalating global frequencies of harmful algal blooms (Smayda 1990, Paerl 1997, Anderson et al. 2002). Antithetically, temporal and spatial mismatches observed between *Aureococcus anophagefferens* blooms and dissolved nutrient distributions argue against a direct link between eutrophication and BT onset (Cosper et al. 1989a,b, Keller & Rice 1989, LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a). In bays which host BT, NO_3^- concentrations may be as high as 30 µM in spring, but decline well below 5 µM for most of the summer and fall when BT typically becomes apparent. In these same embayments, DON concentrations vary between 10 and 50 µM N throughout the warm seasons (LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a, Gobler et al. 2002), and typically comprise 60 to 90% of the long-term averages for dissolved nitrogen inventories (SCDHS 1976–2001). However, composition of these DON pools is not known and bioavailability may be highly variable (Antia et al. 1991).

Is it possible that changes in nitrogen loadings or speciation could be responsible for recurring BT in Long Island embayments since 1985? An instructive approach is to evaluate whether nitrogenous nutrients in coastal waters were systematically different prior to 1985 (pre-BT era) relative to a comparable time interval after the onset of BT (post-1985). Median DIN and DON concentrations in 2 affected Long Island embayments (Peconic and Moriches; Fig. 1) have not been systematically higher in the post-BT era than they were prior to 1985, even though DON concentrations at 2 stations in the Peconic Bay were elevated

(Table 4). In contrast, DIN concentrations at 4 Great South Bay stations were significantly higher from 1985 to 1993 than from 1976 to 1984 (no such differences were observed in DON pools) (Table 4). On average, NH_4^+ comprised 21 to 70% of DIN inventories at stations listed in Table 4, and significant differences were observed between pre- and post-BT datasets for 70% of the stations. However, stations were as likely to show decreases in NH_4^+ concentrations as they were to exhibit increases (data not shown). Median Kjeldahl total N concentrations varied from 16 to 57 µM N, and only 2 of the 17 stations exhibited marginally higher total N concentrations (dissolved + particulate) in the post-BT era (SCDHS 1976–2001). Total N concentrations were not significantly different between these periods at the remaining stations (not presented). The fact that total N concentrations did not statistically vary between sampling periods argues that the post-BT water column is not supporting larger N inventories than it did before 1985, regardless of precise N-speciation. No compelling evidence for shifts in total nitrogen inventories emerged from our analysis of historical nutrient data.

Variations in water column nutrient concentrations are modulated by removal processes, such as trophic transfer, advection, sedimentation, and denitrification, all of which may change in response to nutrient loadings. Therefore, better assessments of historical changes in removal and nutrient loadings are required to adequately evaluate the eutrophication issue. Most freshwater and nutrients entering these embayments is via SGD (Bokuniewicz 1980, Capone & Bautista 1985, LaRoche et al. 1997), which is diffuse and highly variable in space and time. Unfortunately, these loadings are poorly quantified due to infrequent and geographically-limited measurements of SGD.

Nitrogen loadings probably have increased as a consequence of increasing human populations, cesspool leaching, and fertilizer application to developed lands (LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a). From the limited sites for which data are available, nitrogen nutrients in local groundwaters differed dramatically from those measured in the receiving waters, as exemplified in Table 3. For example, groundwater in monitoring wells on the northern margin of the Peconic Estuary may have as much as 800 and 10 µM N of NO_3^- and DON, respectively, under spring high-flow conditions (Montluçon & Sañudo-Wilhelmy 2001). Under low flow conditions, maxima of NO_3^- and DON shift to 170 and 32 µM N, respectively. However, nitrogen loadings and speciation in SGD passing through sediments are altered by microbial processes, depending upon flow rates, temperature, and organic content of sediments. For exam-

Table 4. Comparison of dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + NH_4^+) and dissolved organic nitrogen (DON = dissolved Kjeldahl nitrogen – DIN) prior to first reported BT ('pre' = 1976 to 1984) and a comparable period after onset of BT ('post' = 1985 to 1993). Data from the 2 collection periods compared using Mann-Whitney rank sum test. Those yielding values of $p > 0.05$ were considered statistically indistinguishable. Peconic, Moriches and GSB (Great South Bay) are major embayments routinely monitored by Suffolk County Department of Health Service's surface water quality program; station numbers are a subset of those monitored. (pre:post) = number of samples analyzed in pre- and post-brown tide periods

Station	Median DIN pre	Median DIN post	n (pre:post)	p	Median DON pre	Median DON post	n (pre:post)	p
Peconic								
130	0.86	1.29	(33:352)	ns	13.29	17.36	(32:352)	ns
170	1.21	1.36	(35:491)	ns	13.22	18.07	(32:491)	0.0001
220	1.12	1.87	(37:24)	ns	20.84	30.38	(34:10)	ns
240	3.93	5.11	(41:37)	ns	16.59	29.16	(39:37)	0.003
Moriches								
100	1.64	1.64	(34:48)	ns	21.50	21.43	(33:48)	ns
110	2.14	2.21	(31:38)	ns	26.12	19.21	(30:38)	ns
120	1.50	0.93	(33:46)	ns	24.07	17.61	(32:46)	ns
130	1.50	1.50	(31:46)	ns	18.68	16.61	(30:46)	ns
140	2.00	1.00	(30:38)	0.035	12.86	16.43	(29:38)	ns
150	2.07	2.25	(34:50)	ns	15.67	16.03	(33:50)	ns
160	2.14	3.04	(29:46)	ns	18.25	14.46	(28:46)	ns
170	3.18	3.92	(30:48)	ns	16.86	18.36	(28:48)	ns
GSB								
110	1.35	5.43	(65:55)	0.0001	25.76	24.75	(63:55)	ns
120	1.21	2.50	(64:79)	0.0001	26.79	21.79	(62:58)	ns
130	1.71	3.79	(68:60)	0.0001	28.08	22.82	(66:60)	ns
150	1.14	2.43	(70:60)	0.002	25.79	16.07	(77:60)	0.03
250	8.86	4.00	(86:48)	ns	12.11	17.07	(86:86)	ns

ple, when SGD is high in the spring, N-speciation may change little due to short residence times in organic-rich sediments, releasing substantial amounts of NO_3^- to the bays. In contrast, under summer low flow conditions, denitrifying bacteria may significantly reduce the high NO_3^- concentrations in SGD as it passes through sediments, releasing N_2 and NH_4^+ to overlying waters (Seitzinger 1988). This is exemplified by the nitrogen inventories reported for porewater (Table 3).

Algal responses to N-speciation

Clearly, relative contributions of inorganic and organic nitrogen species vary seasonally in Long Island embayments (Gobler & Sañudo-Wilhelmy 2001a). Our experimental results were consistent with the hypothesis that the dominant nitrogen species influences whether BT blooms will occur in Long Island embayments (Keller & Rice 1989, Nixon et al. 1994, LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a). Unlike many co-occurring microalgal species, *Aureococcus anophagefferens* has qualities consistent with phytoplankton adapted to a more oligotrophic, deep-water environment, such as relatively sensitive functional responses to reduced nitrogenous nutrients (NH_4^+, glutamate, and urea) and relatively low light requirements (Dzurica et al. 1989, Yentsch et al. 1989, Lomas et al. 1996, Milligan & Cosper 1997, this study). While photoautotrophic and capable of growing solely on inorganic nutrients, *A. anophagefferens* also appears adept at utilizing a variety of dissolved organic nutrients, including proteins, which qualifies it as a mixotroph (Dzurica et al. 1989, Lomas et al. 1996, 2001, Berg et al. 1997, 2002, 2003b, Mulholland et al. 2002). Despite *A. anophagefferens'* rather modest growth potentials ($\mu_{max} \leq 0.80$ d^{-1}), its unique combination of attributes may provide competitive advantages over other species and permit the occupation of a turbid niche dominated by reduced nitrogen species.

Consistent with published field and mesocosm observations (Keller & Rice 1989, Berg et al. 1997, LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a, Kana et al. 2004), our simulations predicted that *Aureococcus anophagefferens* would be the poorest performer of 5 species tested when NO_3^- overwhelms nitrogen inventories, irrespective of absolute NO_3^- concentration. This result agrees well with ^{15}N experiments that demonstrated that this species obtains only a minute fraction of its total N requirement from NO_3^- (Berg et al. 1997, Mulholland et al. 2002). While *A. anophagefferens* does grow when provided NO_3^- as sole N-source, its μ_{max} was only 0.30 d^{-1}; in contrast, *Thalassiosira pseudonana* exhibited a μ_{max} of 0.87 d^{-1} under the same conditions (Table 1). If we extrapolate these results to field conditions, we might expect that phytoplankton communities would consist largely of diatoms, chlorophytes, and cyanobacteria during periods of rapid SGD and high NO_3^- loadings to embayments, i.e. during the spring and early summer.

Barring other resource limitations, *Aureococcus anophagefferens* appeared to have a competitive advantage over 4 common co-occurring phytoplankton species when NH_4^+ and DON species (such as glutamate) dominated dissolved nitrogen inventories at low to moderate concentrations. For example, *Thalassiosira pseudonana* appeared to be unable to grow if only supported by low NH_4^+ or glutamate concentrations, i.e. S_{min} of 12 to 13 µM N. This diatom appeared to possess substrate thresholds below which growth was not supported. We speculate that these thresholds may be a manifestation of low affinities of uptake systems or regulation of uptake system expression.

Our findings agreed well with observations of N uptake by *Aureococcus anophagefferens*, which have shown that reduced N compounds are taken up preferentially, even when nitrate is more abundant (Lomas et al. 1996, Berg et al. 1997, Mulholland et al. 2002). In the case of NH_4^+, this advantage existed throughout typical coastal concentration ranges. However, ammonium concentrations above 50 µM actually repressed growth and thus appeared to be toxic to *A. anophagefferens*, whereas glutamate only became inhibitory above 300 µM. These inhibitions may clarify why Cosper et al. (1990) and Fan et al. (2003) were unable to grow *A. anophagefferens* in media provided with millimolar NH_4^+ concentrations as the sole N-source. The dominance of *A. anophagefferens* when glutamate was the primary nitrogen source might reflect its ability to obtain cellular carbon, energy, and nitrogen from this compound (Berg et al. 1997, Mulholland et al. 2002). Under low light conditions typical of bloom periods, a phytoplankter such as *A. anophagefferens* that possesses the ability to supplement photosynthetic C-fixation with heterotrophic uptake of dissolved organic matter would have a clear advantage over strictly autotrophic species (Gobler & Sañudo-Wilhelmy 2001b, Lomas et al. 2001).

Observed growth responses of these 5 phytoplankton species to ammonium and glutamate were consistent with field observations of phytoplankton communities within Long Island estuaries. For example, BT typically occur when ammonium levels are at low to moderate levels (≤10 µM) and DON compounds are moderately abundant (20 to 100 µM N) (SCDHS 1976–2001, Berg et al. 1997). This is more than sufficient nitrogen to support a typical BT bloom of ~1.5×10^6 cells ml^{-1}. We determined that our cultured *Aureococcus anophagefferens* had an average cell content of 0.32 pg N, so a bioavailable pool of only about 35 µM

dissolved N is required to fuel bloom conditions. This same amount of nitrogen would support half as much *Synechococcus bacillaris* (0.64 pg N cell^{-1}) or *Prorocentrum minimum* (0.66 pg N cell^{-1}), 4-fold less *Nannochloris atomus* (1.21 pg N cell^{-1}), and 5-fold fewer *Thalassiosira pseudonana* (1.69 pg N cell^{-1}). Interestingly, within the more eutrophied zones of Long Island estuaries where ammonium concentrations can exceed 100 µM, BT are absent and phytoplankton communities are dominated by diatoms and dinoflagellates (Gobler & Boneillo 2003).

Prior to 1983, urea appeared to dominate dissolved nitrogen inventories in Long Island embayments, accounting for ≤85% of total dissolved N and attaining concentrations as high as 7.5 µM (Kaufman et al. 1983). However, concentrations reported more recently have been typically <0.5 µM N (Berg et al. 1997, Gobler & Sañudo-Wilhelmy 2001b). Long-term urea abatements probably reflect the abandonment of intensive duck farming along coasts of bays and tributaries, and decimation of shellfish beds (hard clams, bay scallops) (LaRoche et al. 1997). Urea also enters groundwater through application of fertilizer and deicing agents within the watershed (Lomas et al. 2001). However, urea concentrations in the few groundwater samples analyzed in this system appear to be very low near submarine groundwater discharge areas (C. J. Gobler unpubl. data).

Responses of our simulated phytoplankton community to environmentally realistic concentrations of urea (≤10 µM N) differed from its response to other N-species. *Thalassiosira pseudonana* dominated the model community, followed by *Aureococcus anophagefferens* (Table 2). Field populations of *A. anophagefferens* have been shown to have high affinities and rapid specific uptake rates for urea (Lomas et al. 1996). However, Dzurica et al.'s (1989) and our results demonstrated that an acclimation period is necessary for *A. anophagefferens* to express maximal growth when presented with urea after growing on NO_3^-. While intracellular hydrolytic ureases appear to be constitutive in *A. anophagefferens* and *Prorocentrum minimum* (Fan et al. 2003), transmembrane transporters may not be. Regardless of N-source (NO_3^-, NH_4^+, urea) used in the study by Fan et al. (2003), urease activity in these algae increased proportionately with growth rate; this did not hold for the diatom *Thalassiosira weissflogii*. Delays in *A. anophagefferens*' response to increasing urea concentrations may simply result from regulation of uptake systems rather than kinetic properties of intracellular urease. Such delays may place *A. anophagefferens* at a competitive disadvantage compared with other microalgae, which possess lower induction thresholds or constitutive high-affinity uptake pathways.

It is notable that, in addition to *Aureococcus anophagefferens*, 2 other algal species that we examined (*Prorocentrum minimum* and *Nannochloris atomus*) are known to cause harmful algal blooms. In the 1950s and later, green tide blooms of *Nannochloris* spp. plagued numerous embayments along the south shore of Long Island and eliminated the once profitable oyster fishery there (Ryther 1954). Our demonstration that *N. atomus* was quite competitive at the highest concentrations of all forms of nitrogen is consistent with previous studies that concluded that green tide blooms arose in highly eutrophied sub-estuaries contaminated by duck farm effluents (Ryther 1954). Moreover, our results argued that subsequent abandonment of duck farms and transition of these bays to a less eutrophic condition in recent decades (Ryther 1989) set the stage for a switch in the dominant alga from *Nannochloris* spp. to *A. anophagefferens* (LaRoche et al. 1997).

Recently, *Prorocentrum minimum* was grouped along with *Aureococcus anophagefferens* and other phytoplankton into a category of harmful algae that seem to initiate blooms under high DOC:DON ratio conditions (Glibert et al. 2001, Anderson et al. 2002). Our observation of high *Prorocentrum minimum* and *A. anophagefferens* growth performance under low to moderate levels of glutamate (Table 2) supports this categorization.

Field validation

Our field validation experiments were conducted to determine how the dominant nutrient reservoirs (groundwater and benthic porewater), distinguished by their N-speciation, influence a natural algal assemblage already dominated by *Aureococcus anophagefferens*. Although our experiments were designed to deliver equal amounts of nitrogen, both porewater and groundwater obviously contain a host of non-nitrogenous constituents that may have also influenced algal growth rates. The indistinguishable chl *a* yield in baywater amended with porewater or groundwater argues against P-limitation in our samples, because benthic porewater contained 7-fold more o-PO_4^{3-} than groundwater (Fig. 5, Table 3). Furthermore, chronic N-limitation has been reported for these Long Island estuaries (Ryther & Dunstan 1971, Gobler & Sañudo-Wilhemly 2001a, Gobler et al. 2002). Therefore, nitrogen within the porewater and groundwater appears to be primarily responsible for growth in these treatments.

Results from these field experiments are coherent with our laboratory results and growth performance simulations. Specifically, porewater amendments (which contained primarily reduced N species) yielded higher cell densities of *Aureococcus anophagefferens*

relative to groundwater amendments (which contained nitrate almost exclusively). Conversely, cyanobacteria, which were likely dominated by *Synechococcus* sp. at this time of year (Campbell et al. 1983, Gobler et al. 2004), grew more rapidly in nitrate-rich groundwater amendments than in benthic porewater amendments. All results reported above are consistent with the hypothesis that moderate effluxes of reduced N from sediments favor BT proliferation in Long Island estuaries over blooms of competing phytoplankters (LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a, Lomas et al. 2004, MacIntyre et al. 2004). In addition to reduced nitrogen, *A. anophagefferens* growth may also be stimulated by organic carbon enrichments provided by porewater amendments (Burdige & Homstead 1994, Lomas et al. 1996, 2001, Berg et al. 1997, 2002, 2003b).

CONCLUSIONS

Our experimental approach did not account for other ecological factors that all undoubtedly influence BT dynamics, such as: prey selectivity by suspension feeders, toxic contaminants, micronutrients, allelopathic inhibition, or variability in nutrient supply (Bricelj & Lonsdale 1997, Caron et al. 2004). Nonetheless, our results were consistent with previous field, mesocosm and laboratory observations, which suggested that nitrogen speciation is a significant determinant in establishing dominance of *Aureococcus anophagefferens*. Behavior of *A. anophagefferens* in our grow out experiments supported its portrayal as a mixotroph and a relatively oligotrophic member of coastal phytoplankton communities. Our simulations suggested that proliferation of *A. anophagefferens* is favored in environments with low to moderate loadings of reduced DIN and organic nitrogen species (1 to 50 µM NH_4^+, glutamate, and possibly urea), but not in those dominated by NO_3^-. In fact, at moderate to high nutrient concentrations, *A. anophagefferens* grows at significantly slower rates than all other co-occurring microalgal species tested.

Characterization of BT as a secondary bloom fueled by nitrogen regenerated by planktonic and sedimentary processes is mostly consistent with available data (LaRoche et al. 1997, Gobler & Sañudo-Wilhelmy 2001a, Kana et al. 2004, Lomas et al. 2004, MacIntyre et al. 2004). However, if we accept nitrogen speciation as a major factor influencing BT onset, then the following question still remains: how different were system-wide nitrogen dynamics in the pre-BT era compared to today? We can only speculate that perhaps these bays responded non-linearly to alterations in coastal eutrophication, whereby they shifted to a new operational state after some threshold in nutrient input or nutrient compositional change was surpassed. In this scenario, increased nutrient loadings may have caused intensification of primary spring blooms (mixed algal assemblages) beyond the system's abilities to denitrify or export fixed nitrogen offshore. In this state (post-1985?), residual reduced nitrogen attained sufficient levels to support secondary BT blooms in the summer. Historical nutrient and chl *a* datasets (SCDHS 1976–2001) neither support nor refute this hypothesis, because temporal sampling resolution is too coarse and very little information on loading or removal rates is available. Furthermore, this interpretation still begs the question: what sets Long Island embayments apart from other coastal sites in the western North Atlantic that are only subject to sporadic BT outbreaks? With incomplete nitrogen inventories, sparse nitrogen loading data, and no denitrification measurements, answering questions regarding altered nitrogen dynamics is presently beyond our reach.

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