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Microbial herbivory on the brown tide alga, *Aureococcus anophagefferens*: results from natural ecosystems, mesocosms and laboratory experiments

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Abstract

Experiments were conducted with natural plankton assemblages from two areas in Great South Bay (GSB) and the Peconic Bays Estuary System, NY, to compare the rates of growth and pelagic grazing mortality of *Aureococcus anophagefferens* with co-occurring phytoplankton. We hypothesized that *A. anophagefferens* would experience low mortality rates by microbial herbivores (relative to feeding pressure on other algae) thus providing it with a competitive advantage within the phytoplankton community. In fact, substantial rates of mortality were observed in nearly every experiment in our study. However, mortality rates of *A. anophagefferens* were less than intrinsic growth rates of the alga during late spring and early summer in Great South Bay, resulting in positive net growth rates for the alga during that period. This timing coincided with the development of a brown tide in this estuary. Similarly, growth rates of the alga also exceeded mortality rates during bloom development in natural plankton assemblages from the Peconic Bays Estuary System held in mesocosms. In contrast to the situation for *A. anophagefferens*, growth rates of the total phytoplankton assemblage, and another common picoplanktonic phytoplankter (*Synechococcus* spp.), were frequently less than their respective mortality rates. Mortality rates of *A. anophagefferens* in both systems were similar to growth rates of the alga during later stages of the bloom. Laboratory studies confirmed that species of phagotrophic protists that consume *A. anophagefferens* (at least in culture) are present during brown tides but preference for or against the alga appears to be species-specific among phagotrophic protists. We conclude that two scenarios may explain our results: (1) protistan species capable of consuming the brown tide alga were present at low abundances during bloom initiation and thus not able to respond rapidly to increases in the intrinsic growth rate of the alga, or (2) the brown tide alga produced substance(s) that inhibited or retarded protistan grazing activities during the period of bloom initiation. The latter scenario seems less likely given that significant mortality of *A. anophagefferens* was measured during our field study and mesocosm experiment. However, even a minor reduction in mortality rate due to feeding selectivity among herbivores might result in a

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mismatch between growth and grazing of *A. anophagefferens* that could give rise to significant net population growth of this HAB species. Either scenario infers an important role for trophic interactions within the plankton as a factor explaining the development of brown tides in natural ecosystems.

Keywords: Brown tide; *Aureococcus anophagefferens*; Herbivory; Feeding selectivity; Protozoa; Protists; Algal mortality; Dilution technique

1. Introduction

Harmful algal blooms (HABs) caused by the pelagophyte alga *Aureococcus anophagefferens* have recurred sporadically since 1985 throughout estuaries of the Middle Atlantic United States and caused considerable environmental damage and economic loss. Numerous hypotheses and scenarios have been proposed to explain the initiation of these massive blooms (Bricelj and Lonsdale, 1997). Despite considerable effort to correlate physical and chemical factors with these massive accumulations of algae, no single factor appears to be responsible for brown tides. This inability to reveal a 'silver bullet' that can explain and predict these HABs has resulted in a consensus that a number of factors may be working in concert to explain the development of brown tides (Cosper et al., 1995).

Most observational and experimental work to date on blooms of *A. anophagefferens* has focused on factors that might stimulate the intrinsic growth rate of the alga in natural assemblages of phytoplankton. The objectives of those studies have been to determine conditions that might selectively favor growth of *A. anophagefferens* over other algal species (Dzurica et al., 1989; Keller and Rice, 1989; Cosper et al., 1993; Gobler and Cosper, 1996; Lomas et al., 1996; Berg et al., 1997; LaRoche et al., 1997; Milligan and Cosper, 1997; Gobler and Sañudo-Wilhelmy, 2001). This information has provided insight into the conditions under which *A. anophagefferens* population growth might take place, but it has not been sufficient to predict the occurrence of brown tides. This lack of a predictive understanding implies that factors inherent in the removal processes (e.g. trophic coupling) within the plankton may also be important determinants in the development of brown tides.

One hypothesis for explaining the occurrence of blooms of this noxious alga is that selective grazing by planktonic microbial consumers (phagotrophic protists) provides a competitive advantage for *A. anophagefferens*. That is, consumption of other phytoplankton by herbivorous protists removes palatable algae from the water, while rejection of *A. anophagefferens* as suitable prey by pelagic consumers allows accumulation of the latter species in planktonic communities. The result of this feeding selectivity is a net growth rate of the brown tide alga that may be greater than rates for other algal species, thus promoting the development of a brown tide. Thus far, there has been relatively little observational or experimental information to draw firm conclusions regarding this hypothesis.

The potential importance of selective grazing within pelagic microbial food webs for explaining the dominance of *A. anophagefferens* in estuarine waters of the Middle Atlantic states is supported by studies of brown tides in the Laguna Madre region of Texas, that are caused by the pelagophyte alga *Aureoumbra lagunensis*. Field and laboratory studies of *A. lagunensis* have demonstrated an inhibition of feeding by pelagic protists on that alga due to the secretion of an exopolymeric material (Buskey and Hyatt, 1995; Buskey et al., 1997; Liu and Buskey, 2000). Feeding inhibition may play an important role in lowering mortality rates of the alga. Therefore, development of the Texas brown tides may be explained, in part, by a reduction in the mortality rate for *A. lagunensis* relative to other algae.

The role of pelagic microbial consumers in the mortality of *A. anophagefferens* has been examined in a few studies (Caron et al., 1989; Mehran, 1996; Boissonneault-Cellineri et al., 2001). The results of these studies indicated that populations of the brown tide alga are consumed in the plankton, but the significance of these losses relative to the rates of removal of other phytoplankton species is still poorly characterized. Only a single study conducted during a fall and winter brown tide has demonstrated differences in the removal rates of *A. anophagefferens* cells relative

to other phytoplankton cells in natural ecosystems (Gobler et al., 2002). Therefore, the overall contribution of selective grazing losses to the development of brown tides remains uncertain.

Performing experiments to examine grazing by microbial consumers on *A. anophagefferens* in natural samples has been hindered, in part, because *A. anophagefferens* cells are difficult to count in natural assemblages. *A. anophagefferens* is small, spherical and possesses few morphological characteristics that aid in distinguishing the species from other minute co-occurring algae. An antibody-based, epifluorescence microscopical method (Anderson et al., 1989) has been used to count brown tide cells in water samples, but the method is time-consuming and thus has limited the breadth of ecological studies that have been possible. The development of a rapid technique for accurately counting *A. anophagefferens* cells in natural samples based on the application of a highly specific monoclonal antibody in an enzyme linked immunosorbent assay (ELISA) format has greatly reduced this problem in recent years (Caron et al., 2003).

This study examined growth and mortality of *A. anophagefferens* and co-occurring phytoplankton assemblages in natural, unperturbed ecosystems commonly afflicted with brown tides, and in natural plankton assemblages held in 300 l mesocosms and enriched with nutrients to stimulate brown tides. Substantial mortality rates for *A. anophagefferens* were measured throughout our study, although these rates were less than the intrinsic growth rates of the alga during periods of bloom initiation. In contrast, net growth rates of another group of picoplanktonic phytoplankton (*Synechococcus* spp.) were generally low and frequently negative. Species of phagotrophic protists capable of consuming the brown tide alga were readily enriched and cultured from natural plankton assemblages. Some species actually grew well when *A. anophagefferens* was the only prey available, indicating that low mortality rates of *A. anophagefferens* could not be explained by an absence of species capable of consuming the alga. We speculate that either these microbial consumers were present at low abundances during bloom initiation, or that the brown tide alga was capable of inhibiting grazing by these species during certain phases of its life cycle.

2. Material and methods

Three types of experiments were conducted to examine the importance of phagotrophic protists in affecting the fate of *A. anophagefferens* in natural water samples and laboratory cultures. (1) Field experiments were carried out during 2000 in two estuarine ecosystems using the dilution technique (Landry and Hassett, 1982; Landry et al., 1995) to measure growth and mortality rates of the brown tide alga and other co-occurring phytoplankton by planktonic microbial consumers. (2) Dilution experiments were conducted from late May to early June during a 9-day mesocosm experiment using natural seawater from the Peconic Bays Estuary System to compare growth rates and mortality rates of the brown tide alga and other co-occurring phytoplankton as a brown tide developed under controlled conditions. (3) Experiments were carried out to examine the ability of cultured heterotrophic protists to consume *A. anophagefferens* under laboratory conditions.

2.1. Field studies

Field experiments were carried out nine times from May through September with seawater collected from Bay Shore Cove (BSC; 40°42′08″N, 73°14′12″W) and Patchogue Bay (PB; 40°44′03″N, 73°01′23″W) of Great South Bay (GSB), NY, to examine phytoplankton growth and pelagic microbial herbivory. The study period spanned the development and decline of a brown tide in those environments. All materials used for sampling and incubations were soaked in 10% HCl prior to use. Water was collected with a HDPE bucket from the surface at each site on each date, transferred into 20 l polyethylene carboys, and returned in coolers to the laboratory at the Natural Science Division, Southampton College of Long Island University at Southampton, NY, where the incubations were conducted. Ancillary measurements of temperature, salinity (measured with a YSI® 85 probe), and secchi depth were made at the time of water collection.

Samples were also collected for analyses of dissolved inorganic nitrogen (DIN) and phosphorus (DIP), silicate (DSi) and chlorophyll a. Seawater for chlorophyll analyses was filtered onto GF/F glass fiber filters and stored frozen until analyzed. Seawater for nutrient analyses was pumped by peristaltic

pump from a depth of 1 m using Teflon® tubing held 3 m from the boat into the prevailing wind (Gobler and Sañudo-Wilhelmy, 2001). Samples were filtered through an acid-cleaned polypropylene filter capsule (0.2 μm, MSI) and frozen until analyzed. Chlorophyll and nutrients were analyzed by standard methods (Parsons et al., 1984).

Experiments to examine microbial herbivory were conducted using a modified version of the dilution method of Landry and Hassett (1982) and Landry et al. (1995). The design of the dilution experiments conducted on Great South Bay water (specific dilutions and nutrient enrichments) was consistent with previous work in this environment (Gobler et al., 2002). The dilution series for these experiments consisted of treatments containing 1.1 l aliquots of 100, 70, 40 and 15% unfiltered seawater diluted with 0.2 μm filtered seawater, performed in triplicate and incubated in clear 1.2 l polycarbonate bottles. Controls consisted of bottles containing only filtered seawater. All bottles in the dilution series were enriched with nutrients (10 μM nitrate and 1 μM phosphate) to ensure nutrient replete growth of the phytoplankton community. An additional set of bottles with 100% unfiltered seawater received no nutrient enrichment in order to examine net phytoplankton growth in the samples under natural (unenriched) conditions. All experimental bottles were incubated without mixing in Old Fort Pond at the Southampton College Marine Station. Water temperature in the pond was always within 2 °C of the collection sites. Neutral density screening was used to reduce light intensity to approximately 65% of surface values (corresponding to approximately 0.5 m depth in Great South Bay, based on secchi disc values).

Measurements of total phytoplankton biomass (chlorophyll a concentration), *A. anophagefferens* (Caron et al., 2003), and *Synechococcus* spp. (chroococcoid cyanobacteria exhibiting phycoerythrin fluorescence; determined by epifluorescence microscopy (MacIsaac and Stockner, 1993)) were obtained from each bottle at the beginning of the experiments and after 24 h of incubation. These measurements were used to estimate intrinsic growth rate (d^{-1}), net growth rate (d^{-1}) and mortality rate (d^{-1}) of the taxa (*A. anophagefferens*, *Synechococcus* spp.) or assemblage (total phytoplankton) assuming exponential growth and mortality rates and employing standard protocols for the dilution technique. Briefly, net growth rates were obtained from changes in abundance/biomass in the undiluted, unenriched bottles during incubations. Mortality rates were obtained from the slopes of the regressions of apparent growth rates at each dilution versus the fraction of unfiltered seawater (i.e. relative grazing pressure). The *Y*-intercepts of these regressions yielded intrinisic growth rates under the nutrient replete conditions provided in the dilution series. These 'enriched' intrinisic growth rates were corrected for the effect of enrichment by comparing growth rates in the undiluted bottles (100% unfiltered seawater) with and without nutrient enrichment to yield intrinsic growth rates in the natural (i.e. unenriched) sample.

2.2. Mesocosm studies

Studies of microbial herbivory were conducted within the context of a mesocosm experiment carried out from 26 May to 4 June 2000 at the Suffolk County Marine Environmental Learning Center, Southold, NY, located on a small inlet (Cedar Creek) along the northern boundary of the Peconic Bays Estuary System. The design and performance of the experiments was based on a setup that we have employed previously using nutrient enrichment of natural seawater to enrich phytoplankton abundance and shift the composition of the assemblage to dominance by the brown tide alga, *A. anophagefferens*. This approach was employed in the present study to induce brown tides in the mesocosms and then employ water from the mesocosms in dilution experiments in order to characterize growth and grazing during bloom development.

All mesocosms were soaked overnight with 10% HCl and rinsed with seawater from Cedar Creek prior to the experiment. The incubations were conducted using approximately 280 l of natural, unfiltered seawater pumped from Cedar Creek at a depth of 0.5 m into twelve 300 l cylindrical, translucent polyethylene tanks (Nalgene®; inside diameters of 60 cm and depth of 122 cm). The experiment was performed in batch mode (i.e. no exchange with seawater outside the mesocosms), and acidified at the end of each experiment to eliminate any chance of stimulating a brown tide in Cedar Creek. Water temperature was maintained within 1 °C of the temperature of Cedar Creek by placing all mesocosms into an enclosure (5.5 m diameter holding tank) through which water from

Cedar Creek was continually pumped (approximately 400 l min^{-1}). Mesocosm placement in the enclosure was randomized. The translucent lids were left on the mesocosms throughout the experiments (except for sampling) in order to reduce light intensity and minimize evaporation. Light intensity measured 5 cm below the water surface in the mesocosms with lids in place ranged from 140 to 210 μmol quanta m^{-2} s^{-1} (equivalent to approximately 10% of surface intensity at the water surface).

All mesocosms were enriched daily with nitrogen as urea (2.5 μM nitrogen per day) and phosphorus (NaH_2PO_4; 0.16 μM phosphorus per day) to stimulate phytoplankton growth (N:P in approximate Redfield Ratio). Six of the mesocosms ('nutrients + pump' treatment) were mixed by means of small (<10 cm), submersible aquarium pumps with plastic impellers (Rio Mini 180, TAAM Inc.) which circulated water at a rate of approximately 450 l h^{-1}. We established previously by empirical trial that this mixing regime was likely to stimulate dominance of the phytoplankton assemblage by *A. anophagefferens*. Therefore, we mixed half of the mesocosms in order to maximize our chances of stimulating brown tides in some of the mesocosms. The pumps were suspended 5–10 cm below the water surface and ran continuously. The remaining half of the mesocosms received nutrients but were not mixed ('nutrients' treatment).

The abundance of *A. anophagefferens* in Cedar Creek at the beginning of the experiment was below the limit of detection of our method (<5000 cells ml^{-1}) (Caron et al., 2003). In order to begin each experiment with measurable populations of brown tide cells in the mesocosms, seawater was collected in acid-washed containers from Great South Bay at Patchogue, NY, for use as an inoculum in the mesocosms. The abundance of *A. anophagefferens* in GSB exceeded 10^6 cells ml^{-1} at the time of the experiment (Caron et al., 2003). The concentration of *A. anophagefferens* in all 12 of the mesocosms was increased at the beginning of the experiment to approximately 10^4 cells ml^{-1} by adding an appropriate volume of GSB seawater to each mesocosm.

Temperature was recorded daily near the surface (10 cm) and at mid-depth in all mesocosms prior to water sampling. A slight gradient (2–3 °C) developed in the mesocosms that were not continuously mixed. Temperature in all mesocosms increased by approximately 5 °C from the start to the end of the experiment (16.0–20.7 °C). All mesocosms were mixed thoroughly just prior to sample collection using a plastic paddle. Water was removed daily from each mesocosm and processed for measurements of total chlorophyll a and the abundance of *A. anophagefferens*. Seawater samples for counts of *A. anophagefferens* were preserved with 10% gluteraldehyde prepared with 0.22 μm filtered natural seawater (Sherr and Sherr, 1993) for a final preservative concentration of 1%. *A. anophagefferens* cells were enumerated using the monoclonal antibody colorimetric technique of Caron et al. (2003). Replicate 25–72 ml samples were filtered onto Gelman GF/F glass fiber filters for the measurement of total chlorophyll a concentration using a Turner Designs fluorometer, Model TD-700 (Arar and Collins, 1992). Water samples were collected initially and every second day, and preserved with acid Lugol's solution (Stoecker et al., 1994) for counts of the dominant microplankton. Microplankton were counted in settling chambers using an inverted microscope (Utermöhl, 1958). Metazoan zooplankton (predominantly, copepods and polychaete larvae) were counted at the beginning of the experiment by sacrificing two additional mesocosms filled at the start of the experiment, filtering the zooplankton from the water onto 64 μm Nitex screening, collecting the material on the filter by rinsing it into a container, and preserving with 4% formalin. Counts of metazoa were performed using a dissecting microscope.

One mesocosm from each of two treatments ('nutrients' and 'nutrients + pump') was sacrificed for use in dilution experiments on day 3, 5 and 7 based on the progression of the brown tide that developed during the experiment (Section 3). Our intent was to conduct grazing experiments during periods as the bloom developed and during peak abundances of the brown tide alga. Daily measurements of chlorophyll and *A. anophagefferens* abundance allowed us to monitor the progression of the phytoplankton assemblages in the mesocosms. In reality, our sampling times coincided with bloom initiation (experiments 1 and 2), the period when the abundance of *A. anophagefferens* peaked (experiments 3 and 4), and 3–4 days after maximal abundance of the alga was attained (experiments 5 and 6).

The protocol for the dilution experiments performed with mesocosm water differed slightly from the pro-

tocol used for the field experiments conducted with Great South Bay water. Experiments with mesocosm water were performed by removing sufficient seawater from a single mesocosm to prepare all treatments. The same volumes and bottle types were used for the field and mesocosm experiments, but the latter experiments consisted of 100, 80, 60, 40 and 20% unfiltered seawater treatments with nutrient enrichment, 100% unfiltered seawater without nutrient enrichment and diluent (as a control). Nutrient enrichment for these dilution experiments consisted of the addition of 10 μM nitrate and 0.62 μM phosphate. All treatments were performed in triplicate. Bottles were incubated for 24 h without mixing in Cedar Creek at 0.5 m depth. Biological parameters were measured at the beginning and end of the incubation. Measurements of intrinsic growth rates, net growth rates and mortality rates of total phytoplankton biomass (i.e. chlorophyll a concentration) and *A. anophagefferens* (Caron et al., 2003) were obtained as described above.

2.3. Laboratory studies

Experiments were carried out in the laboratory to examine the ability of cultured phagotrophic protists to consume and grow using *A. anophagefferens* as prey. These experiments addressed the fundamental question of whether or not phagotrophic protists existed that could utilize the brown tide alga as food. If so, it might be expected that these consumers could significantly impact *A. anophagefferens* populations in nature if the consumers were present at sufficient abundances. We specifically enriched natural seawater experiencing a brown tide to select for any phagotrophic protists capable of growth using *A. anophagefferens* as prey.

Three species of the protists were enriched and isolated from brown tides occurring in south shore bays of Long Island during May/June of 1996 (a heterotrophic dinoflagellate, *Oxyrrhis marina*, isolated from Shinnecock Bay; a scuticociliate, *Uronema* spp., isolated from Moriches Bay; a hypotrich ciliate, *Euplotes* spp., isolated from East Islip). Clonal cultures were established by micropipetting cells from natural seawater samples to which cultured *A. anophagefferens* (Culture Collection of Marine Phytoplankton (CCMP 1784), Boothbay Harbor, Maine) was added periodically at high abundance ($>10^6$ ml^{-1}) for a period of 2–3 weeks to enrich the abundances of phagotrophic protists that might consume the brown tide alga. Established cultures were transferred into fresh cultures of *A. anophagefferens* ($>10^6$ ml^{-1}) every 7–10 days.

Gross growth efficiencies of these protists were determined when they were grown on pure cultures of *A. anophagefferens*. *A. anophagefferens* was cultured to high abundance (5.5×10^6 cells ml^{-1}) and the culture split into aliquots. *O. marina*, *Uronema* spp. and *Euplotes* spp. were added separately at low abundance ($<4 \times 10^3$ cells ml^{-1}), and their abundances were determined periodically using light microscopy until each consumer attained the stationary growth phase. Volume estimates of the prey and predators were obtained from microscopical measurements of cell dimensions. Volume estimates of prey and predators, starting abundances and abundances at the beginning of stationary growth phase of the phagotrophic protists were used to estimate gross growth efficiency for each consumer from the total cell volume of phagotrophic protists produced divided by the total cell volume of prey consumed. Treatments were performed in triplicate but samples were merged for analysis. The experiment was performed at 20 °C in dim light (14:10 h, light:dark cycle).

An experiment was also conducted to examine the ability of *Uronema* spp. to grow in pure cultures of *A. anophagefferens*, and in mixtures of the brown tide alga and a minute (2 μm × 3 μm) diatom, *Minutocellus polymorphus*, which is often a co-dominant with *A. anophagefferens* in natural brown tides (Sieburth and Johnson, 1989; Smayda and Villareal, 1989). Cultures of the two phytoplankton were grown to late exponential growth phase in enriched natural seawater (Guillard, 1975), and mixtures of *A. anophagefferens* and *M. polymorphus* were prepared in the proportions 0:1, 1:4, 1:1, 4:1 and 1:0 (v/v of culture). Initial cell densities from which the mixtures were prepared were 5.5×10^6 cells ml^{-1} of *A. anophagefferens* and 6.7×10^5 cells ml^{-1} for *M. polymorphus*. The experiment was performed at 20 °C in dim light (14:10 h, light:dark cycle). Cell abundances of *Uronema* spp. were determined periodically by light microscopy throughout the growth cycle of the cultures. Rates of increase in cell abundance throughout the growth cycle of the ciliate were compared for the various algal mixtures examined.

A fourth phagotrophic protist was isolated from Flanders Bay, Peconic Bays Estuary System (an oligotrich ciliate, *Strombidium* spp.) during a period of relatively low *A. anophagefferens* abundance in December of 1995. *Strombidium* spp. was maintained on a diet of *Isochrysis galbana* and *Heterocapsa triquetra* cultured in natural seawater enriched with nutrients and trace metals (Guillard, 1975). The ciliate was transferred into fresh medium every five days to maintain population growth. Maintenance cultures and experimental treatments were kept under dim light at 18–20 °C.

Growth responses of *Strombidium* spp. were studied under five initial ratios of *A. anophagefferens* (strain CCMP 1708) to the prymnesiophyte, *I. galbana*. The approximate initial ratios of *A. anophagefferens*:*I. galbana*, based on cellular volumes, were 0:1, 1:4, 1:1, 4:1 and 1:0. Each treatment was run in triplicate. Sterile seawater and algae (150 ml total volume), and 20 individuals of actively growing *Strombidium* spp. were added to each experimental microcosm (sterile 250 ml, polycarbonate Erlenmeyers). The experiment was performed at 18 °C in dim light (14:10 h, light:dark cycle). Growth rates were calculated based on changes in ciliate abundance determined during the exponential growth phase of the ciliate.

3. Results

3.1. Field studies in Great South Bay

Chlorophyll a concentrations varied approximately four- to seven-fold throughout the spring-fall period at the two study sites in GSB (overall range of ≈6–42 $\mu g\,l^{-1}$; Fig. 1). Seasonal maxima of chlorophyll were observed in June in both environments (26 June in BSC, 13 June in PB). Abundances of the brown tide alga also were several-fold greater in late spring and early-mid summer than during late summer and fall. Chlorophyll maxima at both sampling sites coincided with seasonal maxima in the abundance of *A. anophagefferens* (Fig. 1). These maxima also coincided with minima in light transmission and low nutrient concentrations (Table 1). Peak abundances of *A. anophagefferens* (≈1.5×10^6 ml^{-1} in BSC and ≈1.4×10^6 ml^{-1} in PB) were characteristic of some of the most intense brown tides that have been observed in natural ecosystems (Gastrich and Wazniak, 2002). Abundances of the alga decreased dramatically during late summer and early fall.

Synechococcus spp. also attained high abundances and exhibited similar seasonal trends at the two study sites in GSB during the study period, but maxima in cyanobacteria were offset temporally from maxima in the abundances of *A. anophagefferens* (Fig. 1). Cyanobacteria reached similar overall abundances as the brown tide alga, but maximal abundances of the former population occurred on 22 August in BSC (≈1.3×10^6 cells ml^{-1}) and 20 July in PB (≈1.6×10^6 cells ml^{-1}). Relatively high abundances of cyanobacteria persisted until the end of the study period in GSB.

Intrinsic growth rates, mortality rates and net growth rates were determined using the dilution technique at the two study sites in GSB for the total phytoplankton assemblage (based on changes in the concentration of chlorophyll a; Fig. 2), *Synechococcus* spp. (Fig. 3) and *A. anophagefferens* (Fig. 4). Nearly all dilution experiments yielded significant regressions. The regression between apparent growth rate and relative grazing pressure for brown tide was not significant for the experiment conducted on 6 July in PB, and no growth rate or mortality rate information was obtained for that population in that experiment.

A percentile bootstrap method for comparing the trimmed means of two populations was employed for comparing growth and mortality rates of the total phytoplankton assemblage, *A. anophagefferens* and *Synechococcus* spp. (Wilcox, 2003). That test was chosen over a standard ANOVA because the percentile bootstrap method minimizes bias and achieves high power, without the assumption of normal distribution or equal variance.

Intrinsic growth rates for the total phytoplankton assemblages were consistently high throughout the study period (Fig. 2A and B). Overall, rates ranged from approximately 0.8 to >2 d^{-1}. Mortality rates for the total phytoplankton assemblage were also consistently high in GSB (overall range ≈ 1.0–1.9 d^{-1}; Fig. 2C and D) and the general seasonal patterns of the mortality rates were similar to the patterns for intrinsic growth rates of the whole assemblage. Thus, mortality losses closely matched growth rates for most of the samples. Intrinsic growth rates averaged for all experiments were 1.4 d^{-1} in BSC and 1.3 d^{-1} in PB, while mortality rates were

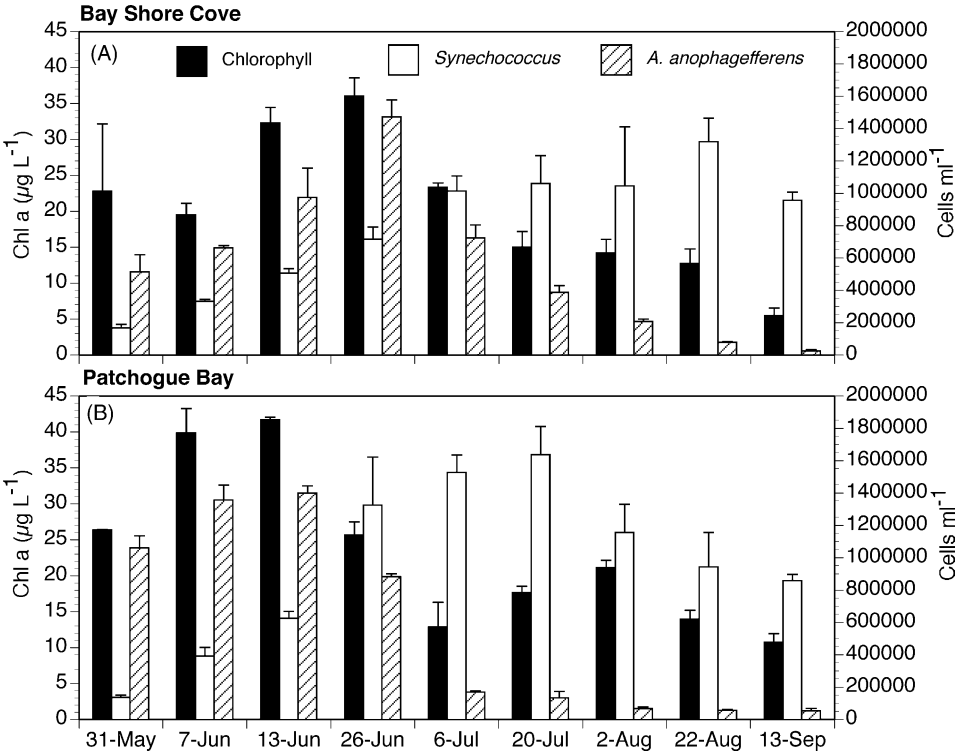

Fig. 1. Chlorophyll a concentrations, *Synechococcus* spp. and *Aureococcus anophagefferens* abundances on nine sampling days in Bay Shore Cove (A) and Patchogue Bay (B), Great South Bay, Long Island, NY, during 2000. Error bars are ±1 standard deviation.

1.4 and 1.5 d^{-1}, respectively. This close match between growth and mortality was reflected in very low net growth rates for the phytoplankton assemblage for most experiments (Fig. 2E and F). Averaged over all nine experiments, net growth rates of the total phytoplankton assemblage were approximately zero at both study sites.

Growth and mortality rates of *Synechococcus* spp. were determined in the dilution experiments because this taxon represents an assemblage that can be readily identified by its unique autofluorescence, and because this group may occupy a niche similar to *A. anophagefferens* in planktonic food webs due to its small size and photosynthetic nutrition (Smayda and Villareal, 1989). Intrinsic growth rates of *Synechococcus* spp. were high throughout the study period at both sites (Fig. 3A and B). The overall average intrinsic growth rate was 1.8 d^{-1} for both sites. Mortality rates were commensurately high (Fig. 3C and D), averaging 1.7 d^{-1} for BSC and 1.6 d^{-1} for PB over the entire study period. Despite substantial intrinsic growth rates, net growth rates for this taxon determined from changes in abundance in the dilution experiments were low because of the impact of grazing mortality (Fig. 3E and F). This result was in contrast to the observed increases in the abundances of cyanobacteria at the two sampling sites over the study period (Fig. 1).

Intrinsic growth rates of *A. anophagefferens* in GSB also were consistently high at both sampling stations throughout the study period (Fig. 4A and B). These rates ranged from 0.8 to 2.3 d^{-1}, and the overall averages for the two sites were 1.5 and 1.7 d^{-1} for BSC and PB, respectively. Averaged over the study period, intrinsic growth rate of the brown tide alga in GSB was significantly greater than the intrinsic growth rate of the total phytoplankton assemblage in PB ($p = 0.014$) but the rate was not significantly different in BSC ($p = 0.105$). The average intrinsic growth rates for *A. anophagefferens* were not significantly different from those of *Synechococcus* spp. at either of the two study sites in GSB.

Table 1
Chemical and physical parameters at the Bay Shore Cove (BSC) and Patchogue Bay (PB) sampling sites in Great South Bay (GSB) during 2000

Date	Temperature	Salinity	1% light	DIN	DIP	DSi
BSC						
31 May	19.5	26.16	2.0	0.78 (0.21)	0.14 (0.02)	7.58 (0.91)
7 Jun	19	25.61	2.2	0.67 (0.12)	0.20 (0.00)	10.8 (0.77)
13 Jun	22.3	24.63	1.9	0.97 (0.18)	0.23 (0.01)	10.3 (4.34)
26 Jun	25.9	26.30	1.4	0.22 (0.15)	0.24 (0.03)	32.0 (4.07)
6 Jul	25.7	27.47	2.3	0.34 (0.13)	0.18 (0.02)	39.6 (0.83)
20 Jul	24.1	28.77	2.4	0.87 (0.15)	0.62 (0.01)	37.7 (0.76)
2 Aug	24.6	27.05	2.4	0.52 (0.22)	0.80 (0.01)	38.6 (2.26)
22 Aug	21.8	26.21	2.6	0.63 (0.16)	0.60 (0.02)	47.2 (0.64)
13 Sep	20.6	25.40	2.7	0.84 (0.25)	0.49 (0.04)	40.9 (10.8)
PB						
31 May	16.2	24.50	1.5	1.25 (0.26)	0.07 (0.00)	10.9 (1.68)
7 Jun	18.5	24.08	1.5	0.81 (0.25)	0.10 (0.01)	13.8 (0.19)
13 Jun	21	24.45	1.4	0.82 (0.24)	0.05 (0.02)	22.3 (5.74)
26 Jun	25.9	24.39	1.4	0.30 (0.04)	0.12 (0.10)	38.3 (0.57)
6 Jul	26.1	24.65	2.4	0.28 (0.12)	0.30 (0.00)	49.2 (7.87)
20 Jul	24.2	24.85	2.4	0.95 (0.11)	1.12 (0.09)	60.5 (0.49)
2 Aug	24.3	24.60	2.2	1.22 (0.22)	0.88 (0.00)	70.1 (1.73)
22 Aug	22.7	24.51	2.2	0.61 (0.11)	0.79 (0.05)	74.7 (0.21)
13 Sep	20.4	24.50	2.7	0.66 (0.21)	0.77 (0.06)	59.1 (0.76)
GSB						
Mean	22.4 (3.00)	25.5 (1.30)	2.1 (0.5)	0.71 (0.32)	0.43 (0.33)	33.2 (19.0)

Units are as follows: temperature in °C; salinity in PSU; 1% light depth was calculated from the extinction coefficient; dissolved inorganic nitrogen is DIN (μM); dissolved inorganic phosphorus is DIP (μM); dissolved silicate is DSi (μM). Values in parentheses are for standard deviation.

Mortality rates determined for the brown tide alga in the field experiments exceeded $1\,d^{-1}$ in most cases (Fig. 4C and D). The overall averages at the two sampling sites were $0.94\,d^{-1}$ for BSC and $1.5\,d^{-1}$ for PB. Averaged over the study period, mortality rate for *A. anophagefferens* was significantly less than the rates for both total phytoplankton and *Synechococcus* spp. in BSC ($p = 0.023$ and 0.038, respectively). Averaged mortality rates were not significantly different among the three assemblages in PB. Mortality rates obtained for total phytoplankton and *Synechococcus* spp. were not significantly different in either environment.

Net growth rates of *A. anophagefferens* determined in dilution experiments were significantly greater than net growth rates determined for the total phytoplankton assemblage and *Synechococcus* spp. ($p < 0.001$ for both study sites; Fig. 4E and F). The greatest net growth rates were observed in both environments during late May and early June during the period when brown tides were developing in both locations. Net growth rates of *A. anophagefferens* averaged over the period when its standing stock increased at the sampling sites (31 May to 26 June in BSC and 31 May to 13 June in PB) were significantly greater than net growth rates for the alga averaged over the remaining sampling dates ($p = 0.036$ and <0.001, respectively).

3.2. Mesocosm studies in the Peconic Bays Estuary System

Our mesocosm experiment was predicated on the knowledge that development of a brown tide bloom in an enclosure would allow study of the phenomenon under more controlled conditions than would be possible in nature where the movement of water due to tides and wind make it difficult to repeatedly sample the same body of water. We performed two treatments in our mesocosm experiments ('nutrients' and 'nutrients + pumps'). Our previous success in shifting the composition of the phytoplankton assemblage in our mesocosms to dominance by *A. anophageffer-*

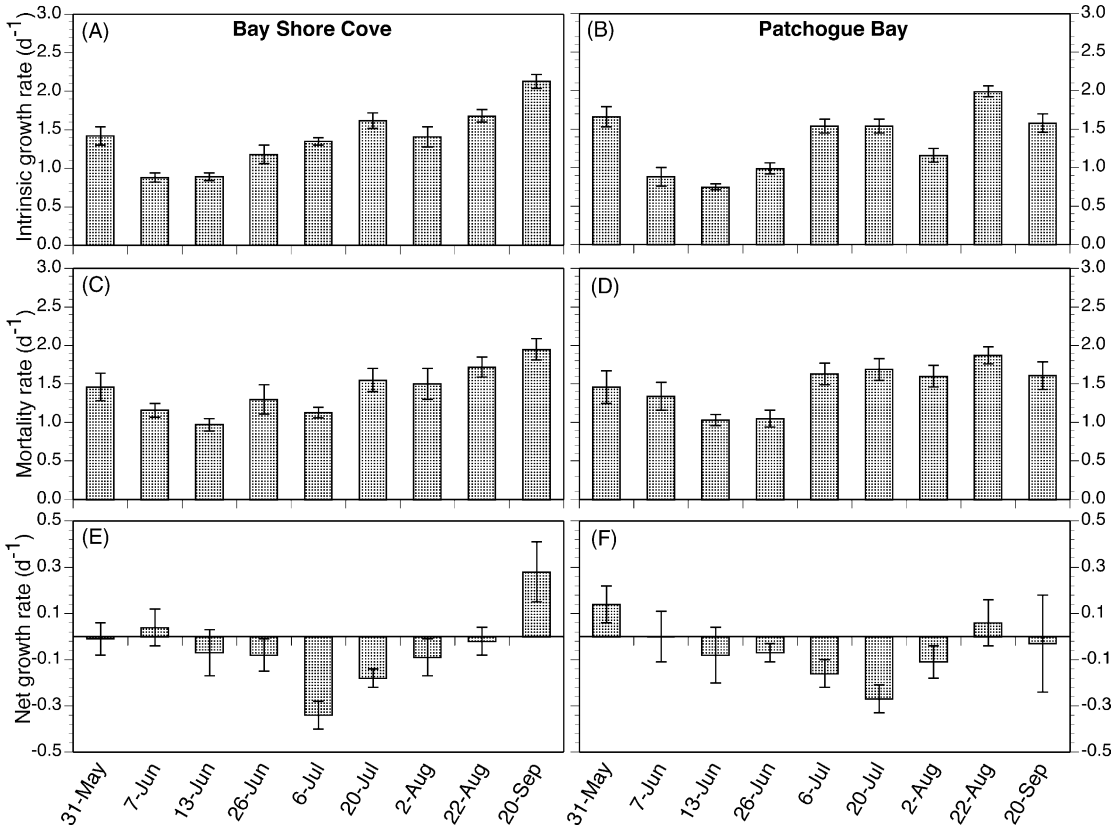

Fig. 2. Intrinsic growth rate (A and B), mortality rate (C and D) and net growth rate (E and F) of total phytoplankton assemblages in Bay Shore Cove (A, C and E) and Patchogue Bay (B, D and F), Great South Bay, Long Island, NY during 2000 determined from dilution experiments. Rates are based on the changes in chlorophyll a concentration during the incubations. Error bars are ±1 standard deviation.

ens through the manipulation of nutrients and mixing was the basis for the protocols used in this study. We performed two different treatments in order to increase our chances that we would form a brown tide in at least one treatment. Brown tides actually formed in both treatments, and we conducted dilution experiments in both treatments.

Chlorophyll a concentrations and abundances of *A. anophagefferens* increased in a similar manner in all mesocosms over the course of the 9-day experiment (Fig. 5A and B). Chlorophyll concentrations decreased to approximately half of their starting concentrations during the first 2 days of the experiment, but subsequently increased rapidly through day 7/8 (Fig. 5A). Abundances of *A. anophagefferens* showed no lag in growth and began to increase rapidly in all mesocosms from the beginning of the experiment (Fig. 5B). Diatom species dominated the phytoplankton assemblages at the beginning of the experiment but *A. anophagefferens* rapidly gained dominance and diatom abundances diminished over the course of the experiment (Fig. 5C). Ciliates increased markedly in the nutrients + pump treatment, but showed no such increase in the nutrients treatment that had no mixing. Metazoan zooplankton at the beginning of the experiment were primarily meroplanktonic polychaete larvae but copepods also contributed significantly (Fig. 5D). Most polychaetes settled during the course of the experiment (data not shown).

Dilution experiments were carried out three times during the mesocosm experiment. Two mesocosms were sampled (one from each treatment) and two experiments performed at each time point. The first two experiments were performed during the period when

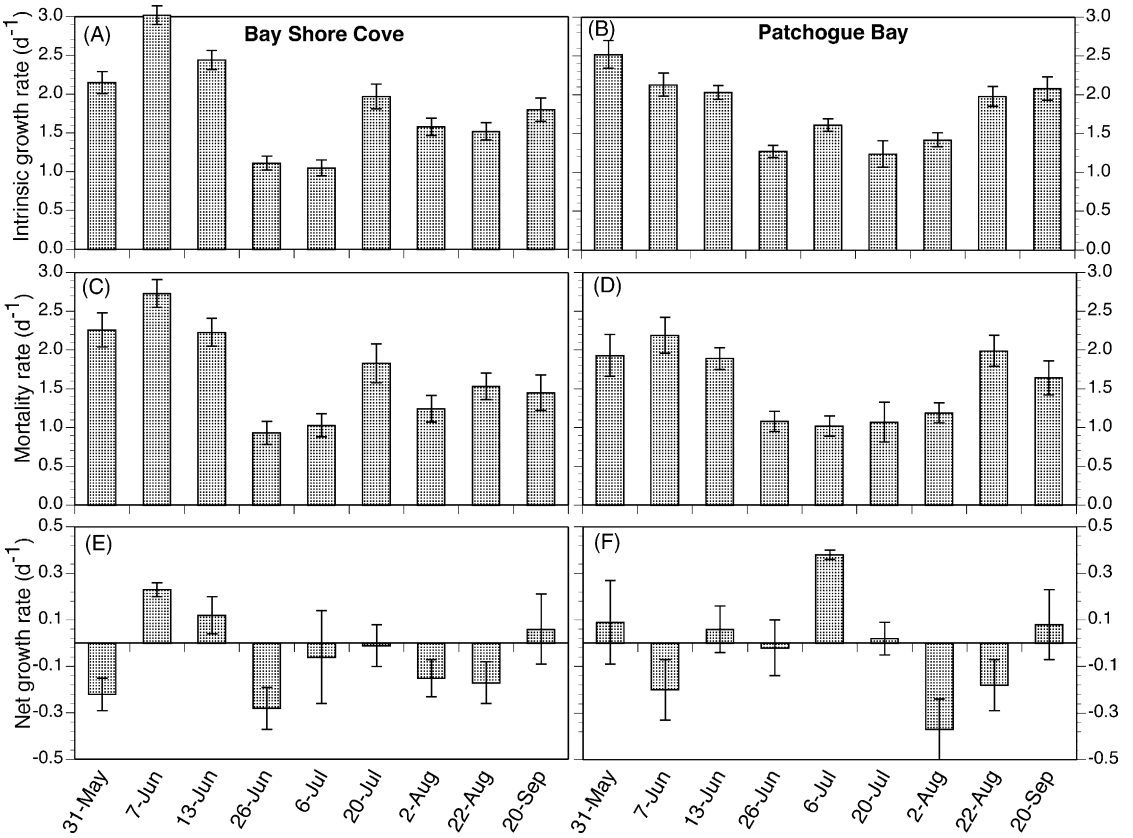

Fig. 3. Intrinsic growth rate (A and B), mortality rate (C and D) and net growth rate (E and F) of *Synechococcus* spp. in Bay Shore Cove (A, C and E) and Patchogue Bay (B, D and F), Great South Bay, Long Island, NY, during 2000 determined from dilution experiments. Error bars are ±1 standard deviation.

the abundance of *A. anophagefferens* was approximately 10^5 cells ml^{-1} and its abundance in the mesocosms was increasing rapidly (Fig. 5B). The second set of experiments was performed when the brown tide alga had just attained its maximal abundance (3×10^5 cells ml^{-1}), and the third set was performed 2 days after maximal abundance had been attained. Apparent growth rates of the total phytoplankton assemblage were aberrantly low in one set of dilution bottles in two experiments (experiment 2, nutrients treatment, 20% unfiltered seawater; experiment 4, nutrients treatment, 60% unfiltered seawater) and were excluded from the regressions based on the results of an outlier test, $p < 0.1$ (Sokal and Rohlf, 1995).

Mortality rates ranging from ≈0.2 to 1.0 d^{-1} were obtained for the total phytoplankton assemblage and for *A. anophagefferens* specifically (Fig. 6A). Significant regressions were obtained in all experiments, but one sample set was lost (*A. anophagefferens*, nutrients treatment at day 5). Mortality rates for total phytoplankton and *A. anophagefferens* were compared on each experimental date using a percentile bootstrap method for comparing the slopes of two regressions (Wilcox, 2003). None were found to be significantly different.

Intrinsic phytoplankton growth rates based on changes in the concentration of chlorophyll yielded negative results due to strong photoadaptation of the assemblage in Cedar Creek during the incubations, and thus are not presented. However, net growth rates of the total phytoplankton assemblage and *A. anophagefferens* were determined directly from changes in chlorophyll and population abundance of *A. anophagefferens* in the mesocosms over the course

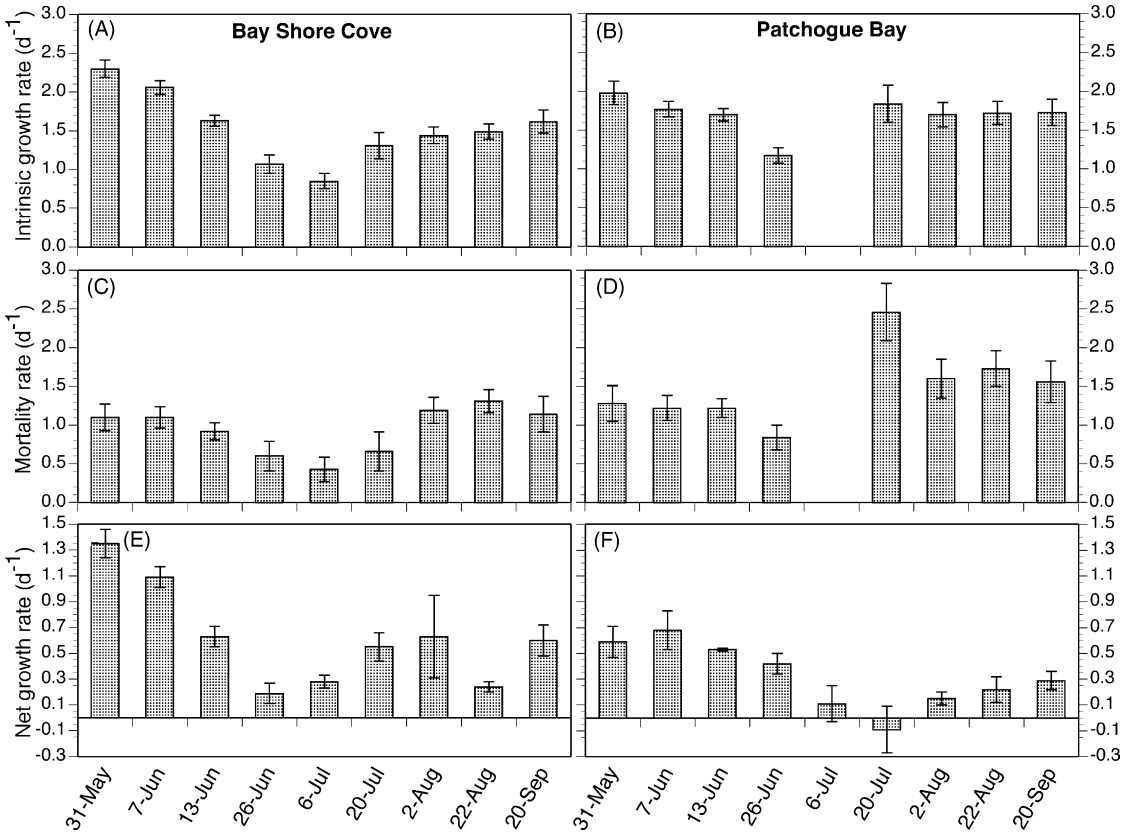

Fig. 4. Intrinsic growth rate (A and B), mortality rate (C and D) and net growth rate (E and F) of *Aureococcus anophagefferens* in Bay Shore Cove (A, C and E) and Patchogue Bay (B, D and F), Great South Bay, Long Island, NY, during 2000 determined from dilution experiments. Error bars are ±1 standard deviation.

of the experiment assuming exponential growth. Net growth rates determined in this way for *A. anophagefferens* were approximately 0.8 d^{-1} in both treatments between days 1 and 3 (Fig. 6B). This rate was equivalent to some of the highest net growth rates of the alga observed in the experiments in BSC and PB during this study (Fig. 4E and F). Net growth rates of *A. anophagefferens* in the mesocosms decreased to ≈0.4–0.5 d^{-1} in the two treatments for the interval between days 3 and 5, and then to essentially zero for the remainder of the experiment.

Net growth rates of *A. anophagefferens* and the total phytoplankton assemblage (Fig. 6B) were compared using a percentile bootstrap method for comparing the trimmed means of two populations (Wilcox, 2003). The net growth rates of *A. anophagefferens* in both treatments were significantly greater than rates for the total phytoplankton assemblage between days 1 and 5 ($p < 0.001$). In contrast, net growth rates of *A. anophagefferens* were significantly less than rates for the total phytoplankton assemblage between days 5 and 7 ($p < 0.001$). Rates for the two assemblages were not significantly different between days 7 and 9.

3.3. Laboratory studies of phagotrophic protists

Phagotrophic protists that are capable of consuming cultured *A. anophagefferens* were easily isolated and cultured from natural seawater samples. Three species isolated from sites of brown tides in south shore bays were able to grow and reproduce readily on a diet of the alga alone (Fig. 7A). Growth of these three phagotrophic protists on the brown tide alga was ro-

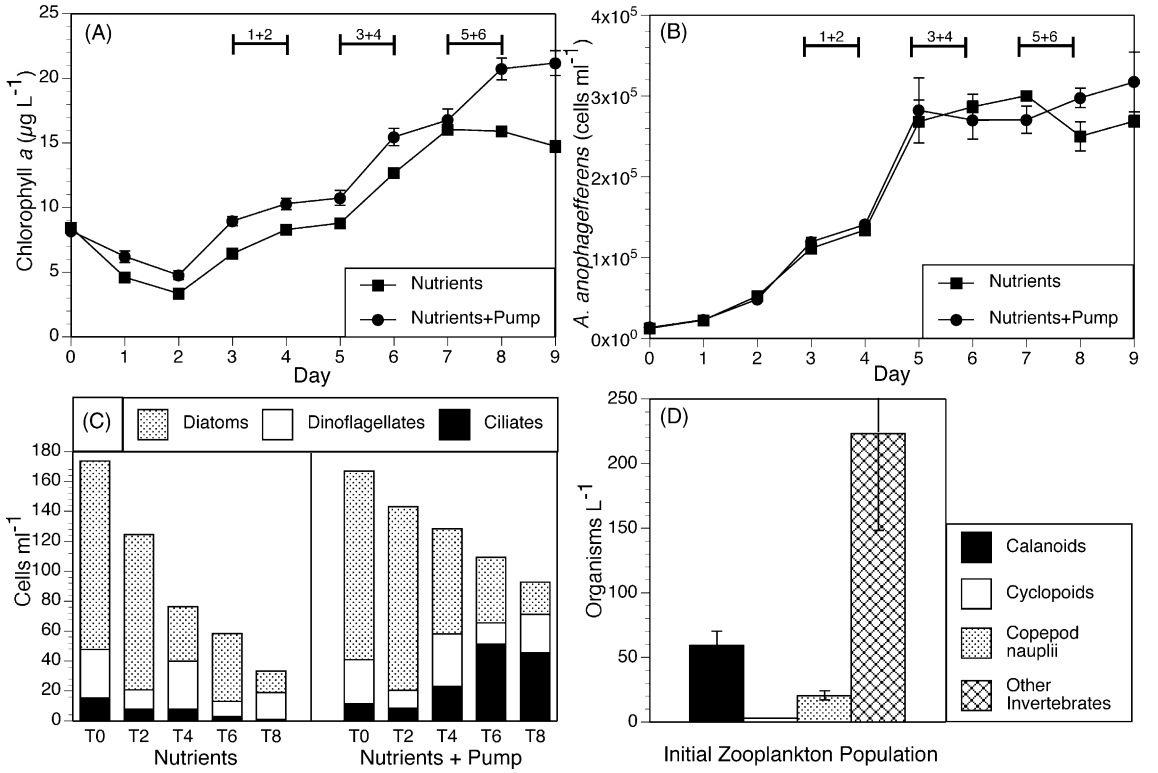

Fig. 5. Changes in biological parameters during a mesocosm experiment performed during 2000 using seawater from the Peconic Bays Estuary System. Changes in the concentration of chlorophyll a (A) and *Aureococcus anophagefferens* abundance (B). Treatments included nutrient-enriched mesocosms with mechanical mixing (circles) and without mixing (squares). Horizontal bars with numbers indicate the timing of dilution experiments on water taken from the mesocosms. Changes in major microplanktonic taxa (C) and initial abundances of metazoan zooplankton (D) during the mesocosm experiment. Error bars for panels A, B and D are ±1 standard deviation. Some errors are smaller than the symbols. For panel C, coefficients of variation for each component of the stacked histograms averaged less than 10%.

bust, and cultures of all three species attained relatively high abundances (Fig. 7B). Gross growth efficiencies of *Oxyrrhis marina*, *Uronema* spp. and *Euplotes* spp. grown using *A. anophagefferens* as the sole food source yielded values of 32, 48 and 37%, respectively. *Uronema* spp. grown in mixtures of *A. anophagefferens* and the minute diatom *Minutocellus polymorphus* grew at similar growth rates (i.e. similar slopes of cell number versus time) regardless of the percentage of the two algae in the mixed cultures (and also on pure cultures of both algae). Differences that were observed in the final yield of ciliates in the experiment were due to differences in the biomass available as food in the two cultures. *M. polymorphus* did not grow to the density that *A. anophagefferens* cultures attained in the laboratory, and no attempt was made to normalize biomass among the mixtures. Nevertheless, *Uronema* spp. grew on all mixtures (and pure cultures) and attained final abundances commensurate with the total amount of prey biomass present in the cultures (Fig. 7B).

Feeding and growth experiments performed with *Strombidium* spp. in the laboratory tested the effects of varying concentrations of *A. anophagefferens* and the prymnesiophyte *I. galbana*. *Strombidium* is an abundant, non-loricate ciliate genus in the Peconic Bays Estuary System and thus a likely consumer of picoplanktonic algae in these ecosystems. *Strombidium* spp. fed and grew on mixtures of the algal species even at relatively high percentages of brown tide (4:1 ratio of *A. anophagefferens*:*I. galbana*; Fig. 8). Growth rates were calculated separately for the time intervals be-

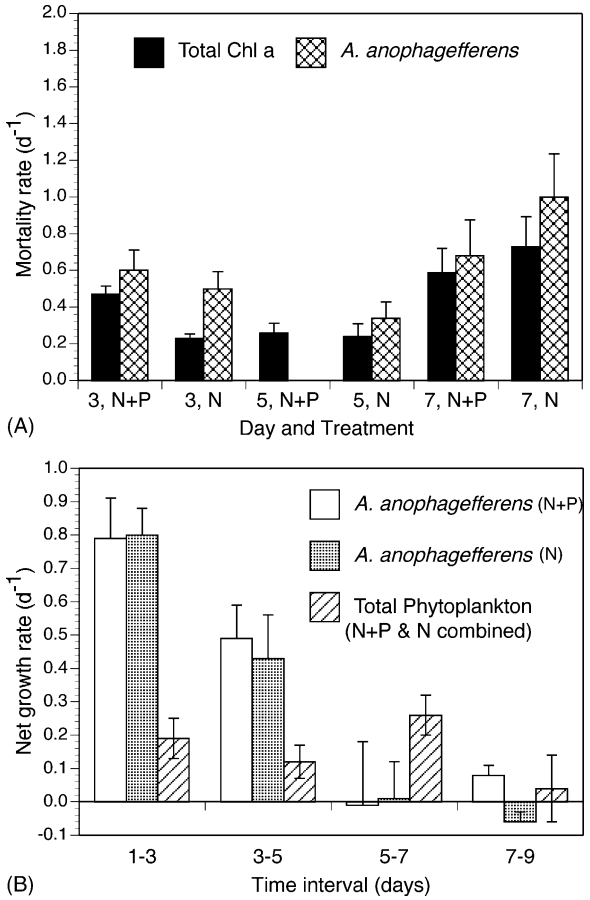

Fig. 6. Results of dilution experiments conducted with water taken from two treatments of a mesocosm experiment performed during 2000 using seawater from the Peconic Bays Estuary System. (A) Mean (±1 standard deviation) mortality rates of *Aureococcus anophagefferens* and total phytoplankton in both treatments (see Fig. 5 for timing of dilution experiments relative to population abundances of total phytoplankton and *A. anophagefferens* specifically). (B) Net growth rates of *A. anophagefferens* in both treatments and net total phytoplankton growth rates (treatments combined). Error bars are ±1 standard deviation.

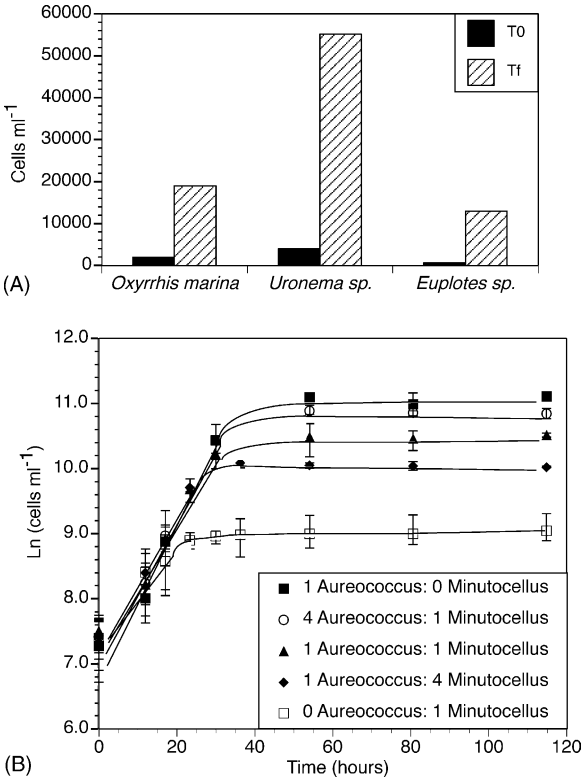

Fig. 7. (A) Yield of a heterotrophic dinoflagellate, *Oxyrrhis marina* and two ciliates (*Uronema* spp. and *Euplotes* spp.) when offered only *Aureococcus anophagefferens* as prey. Columns indicate the density of phagotrophic protists at the time of inoculation (T_0) and at the beginning of their stationary growth phase (T_f). (B) Growth of *Uronema* spp. (A) on five mixtures of the brown tide alga, *Aureococcus anophagefferens* and the diatom *Minutocellus polymorphus*. The key indicates the ratios (v/v) of the two cultured algae. Error bars are ±1 standard deviation.

tween 0 and 48 h, and between 48 and 72 h of incubation in the algal mixtures and pure cultures. Growth rates of the ciliate were high (≈0.9 to >3.0 d^{-1}) in all cultures except the cultures with *A. anophagefferens* as the only food source. Growth rates of the ciliate fed only *A. anophagefferens* were approximately one-third to one-sixth the rates observed when the ciliate was offered a mixture of the two algae.

4. Discussion

The three types of experiments carried out in this study were designed to contribute complementary pieces of information on the trophic interactions between the brown tide alga, *A. anophagefferens*, and potential microbial consumers in the water column of Long Island estuaries. We employed the dilution technique as our main experimental protocol for examining this issue in our field and mesocosm studies because it allowed the simultaneous estimation of phytoplankton growth and mortality rates in our samples. This method is not without problems. Photoadaptation of the phytoplankton assemblage during

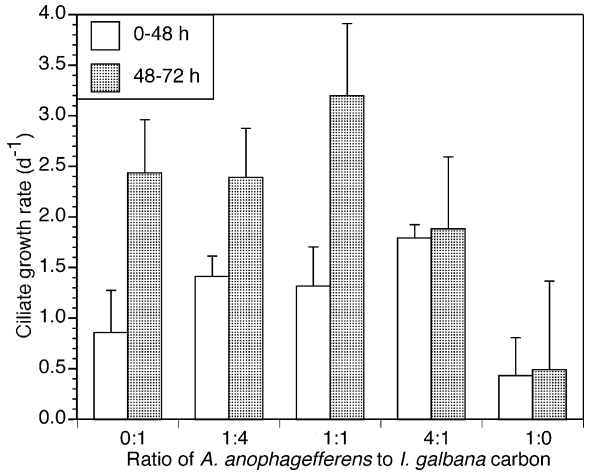

Fig. 8. Growth of the oligotrich ciliate *Strombidium* spp. on five mixtures of the brown tide alga, *Aureococcus anophagefferens* and the prymnesiophyte, *Isochrysis galbana*. Error bars are ±1 standard deviation.

the incubations can affect apparent growth rate of the assemblage, and differences in the growth of the consumer assemblage among the dilutions can also affect the results (McManus, 1995; Dolan et al., 2000). Indeed, photoadaptation of the phytoplankton in our mesocosm experiment negated the measurement of intrinsic growth rates of the phytoplankton assemblage based on changes in total chlorophyll concentration. This issue was not observed in our experiments conducted in Great South Bay, and would not affect growth determined by changes in cell number (e.g. with *A. anophagefferens* and *Synechococcus* spp.). Despite these caveats, the dilution method remains an effective and widely used method for assessing microbial growth and mortality in natural water samples (Caron, 2000).

Studies of intrinsic growth rates and mortality rates in GSB indicated rapid rates of both processes for the total phytoplankton assemblage and for the two taxa examined (*Synechococcus* spp. and *A. anophagefferens*; Figs. 2–4). Averaged over the study period, intrinsic growth rates of *A. anophagefferens* and *Synechococcus* spp. were not significantly different (averages for all experiments were 1.6 d^{-1} versus 1.8 d^{-1}, respectively). Thus, changes in the relative abundances of the brown tide alga and *Synechococcus* spp. that occurred at the study sites (Fig. 1) do not appear to have been due to drastic differences in their intrinsic growth rates. The average intrinsic growth rate of *A. anophagefferens* also did not differ significantly from intrinsic growth rate for the total phytoplankton assemblage in BSC (1.5 and 1.4 d^{-1}, respectively) although average intrinsic growth rate for the alga was significantly greater in PB (1.7 d^{-1} versus 1.3 d^{-1}, respectively).

We speculated that the dramatic increase in *A. anophagefferens* abundance in GSB during the spring and early summer of 2000 might be explained by much lower mortality rates of the brown tide alga relative to rates for other phototrophic populations. That is, if intrinsic growth rates were similar for both the brown tide alga and other phytoplankton species, then the taxon with the lowest mortality rate should increase more rapidly in the ecosystem. Indeed, mortality rates for *A. anophagefferens* averaged over the study period were significantly less than mortality rates for *Synechococcus* spp. or for the total phytoplankton assemblage in BSC (0.94 d^{-1} versus 1.7 d^{-1} and 1.4 d^{-1}, respectively), and thus could explain the preferential increases in the abundance of the brown tide alga in that environment. In PB, conversely, the average mortality rate for *A. anophagefferens* was not significantly different from mortality rates for *Synechococcus* spp. or the total phytoplankton assemblage in PB. However, higher averaged intrinsic growth rates of *A. anophagefferens* (relative to rates for the total phytoplankton assemblage, as noted above) would also have favored population growth of the brown tide alga over competing species.

The net growth rates of *A. anophagefferens* measured in our dilution experiments were consistent with the findings noted above. Net growth rates of the brown tide alga were greater than net growth rates of *Synechococcus* spp. and the total phytoplankton assemblage at both study sites (Figs. 2E and F, 3E and F, 4E and F). Moveover, net growth rates of *A. anophagefferens* during the late spring and early summer (the period when brown tide developed at both study sites; Fig. 1) were significantly greater than net growth rates later in the season ($p = 0.036$ and <0.001 for BSC and PB, respectively; Fig. 4E and F). Overall, these findings are consistent with the results of previous experimental investigations in this environment (Gobler et al., 2002), and indicate that subtle changes in the ratio between intrinsic growth rate and grazing mortality rate of *A. anophagefferens*

can have important implications for the spatial and temporal dynamics of brown tides.

Most net growth rates of *Synechococcus* spp. were quite low during our study (Fig. 3E and F), a result that is not consistent with the significant increases in the abundance of this taxon observed in GSB during the study period (Fig. 1). The reason for this inconsistency is unclear but may relate to the underestimation of *Synechococcus* spp. abundance by epifluorescence microscopy due to chlorosis of cells during the incubations. Similarly, positive net growth rates were observed for *A. anophagefferens* in nearly all dilution experiments in this study, although standing stocks of this alga clearly declined in GSB during July. This inconsistency may indicate that factors not accounted for in the dilution experiments contributed to the removal of these cells. This discrepancy may also relate to spatial or temporal variability in rate processes, and the limited resolution that any field study can provide.

A distinct advantage of our mesocosm experimental approach was the ability to repeatedly sample the same plankton assemblage sequentially through time. Advective processes make this task very difficult when a time series study of a natural ecosystem is desired. Daily measurements of biological parameters in the mesocosms enabled us to know the status of the plankton community at the time of sampling (standing stocks) as well as the recent history of the assemblages (e.g. if *A. anophagefferens* was increasing or decreasing in abundance). In this way, we were able to examine directly the possibility of a link between changes in the mortality rate of *A. anophagefferens* and changes in net population growth.

The mesocosm experiment performed using natural seawater from Cedar Creek in the Peconic Bays Estuary System confirmed our findings with natural assemblages from GSB that *A. anophagefferens* suffers significant microbial mortality in nature (Fig. 6A). Nevertheless, net growth rates of the brown tide alga were high ($\approx 0.8\,d^{-1}$) during the first few days of the experiment (Figs. 6B). These high net rates, combined with the mortality rates measured by the dilution technique indicated that the intrinsic growth rates of *A. anophagefferens* in the mesocosms were $\approx 1.2\,d^{-1}$ during the period when the biomass of the brown tide alga increased rapidly.

Negligible net growth rates were observed after day 5 in the mesocosm experiment. This situation may have been, in part, a consequence of modest increases in mortality rate near the end of the experiment, but decreases in the intrinsic growth rate of the alga presumably also played a role. No significant differences were observed between the mortality rates for *A. anophagefferens* and for the total phytoplankton assemblage for any dilution experiment in the mesocosm study (see paired rates in Fig. 6A). However, net growth rates between these two entities differed markedly over the course of the experiment (Fig. 6B). Net growth rates of *A. anophagefferens* were much greater than the rate observed for the total phytoplankton assemblage between day 1 and 5, while net growth rates of the total phytoplankton assemblage were equal to or greater than rates for *A. anophagefferens* after day 5. Given that mortality rates were similar for *A. anophagefferens* and other phytoplankton for all experiments, a decrease in the intrinsic growth rate of *A. anophagefferens* was the most likely explanation for the decreases observed in the net growth rates of the alga during the second half of the mesocosm study. The ability of this species to utilize a great diversity of organic and inorganic constituents (Berg et al., 2002, 2003), and the ability to grow under a wide range of irradiances (MacIntyre et al., 2004) are two likely factors that contribute to the high intrinsic growth rate of this species observed during blooms (Fig. 4A and B).

It should be noted that the net growth rates determined in the mesocosm experiment take into account all losses of the alga, not just grazing mortality. Viral lysis or settling may contribute to losses of *A. anophagefferens* and thus cause an underestimation of the intrinsic growth rate of the alga. Nevertheless, a rate of $1.2\,d^{-1}$ is substantial, and is consistent with the intrinsic growth rates of *A. anophagefferens* that we observed for actively growing populations in GSB during this study (Fig. 4A and B).

The significant mortality rates that we observed for *A. anophagefferens* in GSB and mesocosms indicated that consumers of the brown tide alga are present and active in natural ecosystems. The small size of this alga implicates phagotrophic protists as potential consumers of this species, and previous work to isolate these species has been successful (Caron et al., 1989). Therefore, it was not surprising that we were able to enrich for and isolate species of phagotrophic protists that consumed *A. anophagefferens* in the laboratory in this study. The information presented here expands

our knowledge of the range of species that are capable of consuming this alga.

The ability of the three species of protists isolated from a brown tide in bays along the south shore of Long Island to consume cultured *A. anophagefferens* indicated that the alga can serve as a suitable food source for at least some phagotrophic protists. These consumers were grown using *A. anophagefferens* strain CCMP 1784 as prey. This strain has been in culture for more than a decade, however, and it is not as noxious as other more recently isolated strains (Bricelj et al., 2001). Nevertheless, the strain that we used supported robust growth of three protistan species (Fig. 7). Gross growth efficiencies of *Oxyrrhis marina*, *Uronema* spp. and *Euplotes* spp. grown on *A. anophagefferens* as the sole food source are consistent with values that would be expected for protists growing on a good food source (Caron and Goldman, 1990; Hansen et al., 1997).

The species of *Strombidium* spp. isolated from Flanders Bay exhibited reduced growth rates when *A. anophagefferens* was the sole food source (Fig. 8). The ciliate grew well on mixtures of the brown tide alga and *I. galbana* up to ratios of the two alga of 4:1 (*A. anophagefferens*:*I. galbana*; Fig. 8), and it is possible that the ciliate fed selectively on *I. galbana* when it was present in the cultures. The poor growth of *Strombidium* spp. on the brown tide alga observed in this study (Fig. 8) whereas *O. marina*, *Uronema* spp. and *Euplotes* spp. grew well on it (Fig. 7) may indicate that while some phagotrophic protists are capable of using *A. anophagefferens* as food, other species of consumers are less able to cope with the noxious nature of this alga. Poor growth of phagotrophic protists on *A. anophagefferens*, and/or selective feeding on other algal species in the presence of *A. anophagefferens*, could explain the inability of the grazer community to respond rapidly to increases in the growth rate of *A. anophagefferens* in nature. Alternatively, our results with cultured consumers may indicate differences in the noxious character of different strains (or different physiological states) of the brown tide alga. The strain used for this study (CCMP 1708) was isolated nearly a decade later than strain CCMP 1784 employed with the other three phagotrophic protists, and there is some evidence that strain CCMP 1708 may be more noxious than strain CCMP 1784 (Bricelj et al., 2001). It is also possible that the toxicity of *A. anophagefferens* may vary with its physiological state. This speculation is presently untested as the causative agent for the toxicity of this species has not yet been characterized.

5. Conclusions

Experimental investigations of microbial herbivory on the brown tide alga, *A. anophagefferens*, have demonstrated substantial albeit variable rates of grazing mortality for this alga in natural ecosystems, manipulated natural assemblages and laboratory cultures. Mortality rates in excess of $1.0\,d^{-1}$ were often observed in natural plankton communities, but lower rates were also observed. Both rapid seasonal increases in the intrinsic growth rate of the alga in nature as well as seasonal minima in mortality rate (relative to growth and mortality rates for co-occurring algae) lead to disparities between growth rates and mortality rates that resulted in large net increases in the standing stock of *A. anophagefferens*. Our studies demonstrate a fundamental role for pelagic food web interactions in explaining the development (and demise) of brown tides in coastal waters of Long Island.

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