TROPHIC INTERACTIONS BETWEEN NANO- AND MICROZOOPLANKTON AND THE "BROWN TIDE"

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INTRODUCTION

The topic of nuisance algal blooms such as the brown tide' has become a focus of attention in recent years for both the general public and the scientific community (Nuzzi, 1986; Olsen, 1986; Smayda, 1986, 1988; Okaichi *et al.*, 1988; Wise, 1988). Attention has been drawn to the possibility of a correlation between man's activities in the coastal zone and the occurrence and possible increasing abundance of massive algal accumulations (Anderson, 1988). As the input of allochthonous materials to our coastal waters continues to expand with coastal development, there is an increasing need to understand the processes that lead to the formation of these phenomenal algal blooms.

Considerable effort has been directed at determining the physical and chemical conditions that initiate and promote problem algal blooms. Notwithstanding some advances, it remains largely enigmatic why particular species of algae, but not others, are able to bloom under a given set of environmental conditions. In particular, the mechanism(s) by which many of

these species escape predation pressure that normally controls algal density remains unknown. In order for a species to attain bloom densities, it is not only necessary for conditions to be favorable for growth of the alga but it is also necessary for the rate of removal of algal biomass to be less than the rate of production. Interestingly, few studies have examined factors affecting the dissipation or removal of algal biomass during problem blooms. In addition to sinking or senescence of the algae, grazing by herbivorous organisms is a primary consideration.

It is intuitively obvious that to allow algal density to increase, grazing by herbivores must be less than algal production. Two obvious mechanisms exist whereby the interaction of algal growth and herbivore grazing could result for bloom formation. On the one hand, algal growth may be sufficiently rapid that the algae reproduce faster than grazers can remove them from the water. This situation is probably the case for blooms of relatively large microalgae whose major consumers are the larger metazoa. Because metazoan life cycles are typically more complex and lengthy than those of the microalgae, grazer density may not increase as rapidly as the alga. Grazing would thus be inadequate to control the algal population until the metazoa could reproduce (or migrate into the environment). A second possibility is that production of a substance or substances that could reduce grazing could also result in a net increase in algal density. In this instance, an enhanced algal growth rate is not required for bloom initiation.

Providing an explanation for the massive blooms of minute, non-toxic algae such as the brown tide is more problematic. The causative organism in these blooms is *Aureococcus anophagefferens*, a small, 2-3 μm chrysophyte alga (Sieburth *et al.*, 1988). Because of its small size, metazoan grazing would be expected to be ineffective (Nival and Nival, 1976). However, *A. anophagefferens* is an ideal size to be efficiently grazed by bacterivorous and herbivorous protozoa that typically abound in coastal waters (Davis and Sieburth, 1982; Fenchel, 1980a; 1982b; Johnson *et al.*, 1982; Caron, 1983; Goldman and Caron, 1985; Parslow *et al.*, 1986; Sherr *et al.*, 1986; Suttle *et al.*, 1986; Turley *et al.*, 1986). In addition, many of these protozoa can feed and grow at rates that exceed the maximal observed growth rate of 3 day^{-1} for *A. anophagefferens* (Fenchel, 1980b, 1982a; Caron *et al.*, 1986; Cosper, 1987). Thus, the recent blooms of this alga in coastal waters along the Northeastern U.S. present an apparent paradox. How can this alga attain enormously high densities in the presence of protozoan grazers?

One possible explanation is a reduction of grazing as a consequence of some material produced by the organism. Although *A. anophagefferens* does not appear to produce a toxin, the organism does cause a depression or cessation in the rate of filtration of some filter feeding organisms (Tracey *et al.*, 1988; Gallager *et al.*, 1988). The underlying reason for this behavior is not yet clear, but may relate to the polysaccharide-like material that occurs on the surface of the alga. We hypothesized that this same effect could reduce the activity of phagotrophic protozoa and other microbial grazers of *A. anophagefferens*, thus allowing virtually unrestricted growth of the alga. In support of this hypothesis, Sieburth *et al.* (1988) noted that during the 1985 bloom in Narragansett Bay, ingestion of *A. anophagefferens* by phagotrophic protists was apparent in electron micrographs only during the decline of the bloom.

We have examined the ability of five species of protozoa to consume *A. anophagefferens* in laboratory cultures. In addition, we have performed preliminary experiments in the field to identify potential consumers of this brown tide alga, and to measure microbial grazing rates at several sites in the inshore waters of Long Island.

MATERIALS AND METHODS

Laboratory Grazing Experiments

Studies were conducted with five clonal cultures of protozoa to test their ability to consume *A. anophagefferens* and to grow on this algal biomass. All protozoa were isolated from Buzzards Bay or Vineyard Sound, Massachusetts, by enriching the bacterial flora of natural seawater samples by adding sterile rice grains, or by adding aliquots of microalgal cultures (*Isochrysis galbana*, *Dunaliella tertiolecta*). Monospecies cultures of protozoa were started from these enrichments by micropipetting individual protozoa from these mixed assemblages. The resulting clonal cultures were maintained on a mixed bacterial assemblage in natural seawater with 0.05% yeast extract added (for bacterivorous species of protozoa), or in natural seawater with f/2 nutrient enrichment (Guilliard, 1975) and *I. galbana* (for herbivorous species).

The protozoan species examined in this study were representative of several taxonomic and trophic groups; an omnivorous chrysomonad microflagellate (*Monas* sp.), a bacterivorous bodonid microflagellate (*Bodo* sp.), an omnivorous hypotrichous ciliate (*Euplotes* sp.), an unidentified bacterivorous pleuronematid ciliate that possessed a limited ability to consume small algae, and an unidentified bacterivorous scuticociliate. Inocula for the experiment were taken from cultures that were growing exponentially on bacteria. Vineyard Sound seawater was employed in all cultures.

The prey species for the experiment were the heterotrophic bacterium *Pseudomonas halodurans* and *A. anophagefferens* (clone BT3P). Bacteria were grown to stationary phase in 0.1% yeast extract in sterile natural seawater, centrifuged at 10,000 rpm, and resuspended as a 'bacterial concentrate' in sterile natural seawater. *A. anophagefferens* was grown in natural seawater with f/2 nutrient enrichment. The culture was not axenic, and bacteria were always present in these cultures at a density of 5×10^6 to 10^7 ml^{-1}.

Three culture treatments were examined for each protozoan species; sterile natural seawater to which concentrated *P. halodurans* had been added at a final density of $\approx 2 \times 10^8$ ml^{-1}, an aliquot of the culture of *A. anophagefferens* (algal density $\approx 10^6$ ml^{-1}), and an aliquot of this algal culture to which *P. halodurans* was added at a density of $\approx 2 \times 10^8$ ml^{-1}. Protozoa were inoculated (Time = 0), and samples were removed periodically thereafter for determinations of cell densities. Population counts were performed by epifluorescence microscopy (Porter and Feig, 1980; Davis and Sieburth, 1982). Protozoan growth rates were determined for each of the treatments from the rate of increase in their densities during the exponential growth phase of the protozoa. All grazing experiments were carried out at 20°C in continuous darkness on a rotary shaker (≈60 rpm).

Field Studies

Preliminary field studies of microbial grazing in areas affected by the brown tide were conducted in the coastal bays of Long Island, NY, on three separate dates during the summer, 1988. The sampling stations are shown in Figure 1. Samples were obtained by aerial overflights on two occasions (June 13 and August 17; courtesy of the New York Air National Guard, 106

ARRG, Westhampton Beach, Long Island) and from the shore on a third occasion (July 19). Two liter samples were collected from a depth of 0 to 1 m using a Niskin bottle. The samples were transferred immediately to polycarbonate bottles and maintained at approximately ambient temperature in the dark during their return to the laboratory. The entire sampling operation required <3 hours.

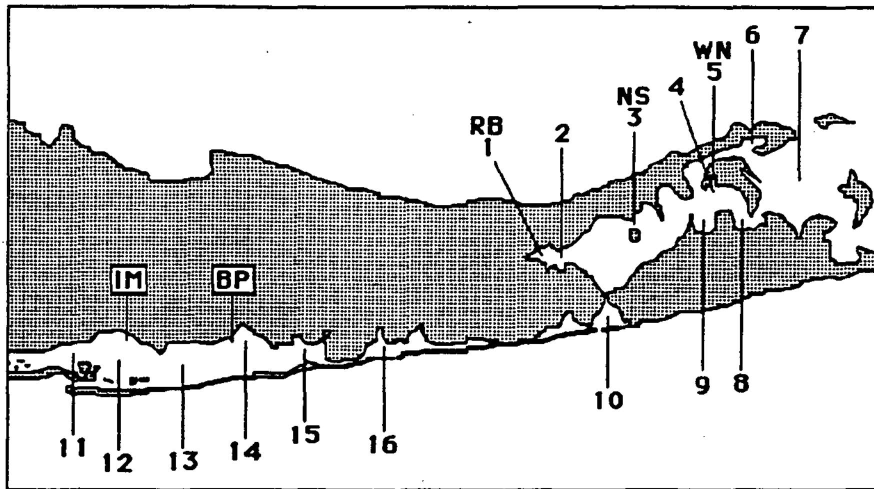

Figure 1. Location of sampling stations along eastern and southern Long Island, New York.

Aliquots of the samples were removed and preserved with filtered, neutralized glutaraldehyde (1% final concentration) for the enumeration of bacteria, *A. anophagefferens*, phototrophic nanoplankton and heterotrophic nanoplankton. Samples for microplankton counts were preserved with Lugol's fixative. Cell densities of the phototrophic and heterotrophic picoplankton (0.2-2.0 μm) and nanoplankton (2.0-20 μm) were obtained using epifluorescence microscopy (Porter and Feig, 1980; Caron, 1983; Hobbie and Cole, 1984; Bloem *et al.*, 1986). The densities of *A. anophagefferens* were also determined using epifluorescence microscopy and DAPI-stained preparations. This method has been routinely used for counting *A. anophagefferens*. It is not as accurate as other methodologies that are now emerging (see Anderson *et al.*, this volume), but a preliminary comparison of cell densities based on DAPI-stained

preparations with the fluorescent antibody technique resulted in reasonably good agreement for natural seawater samples (Anderson and Caron, unpubl. data). Microplankton were counted from settled samples using an inverted microscope.

The remainder of the samples were used to determine grazing rates of the more common microplankton and larger nanoplankton (>10 μm). Our primary goal in this work was to identify the major consumers of pico- and nanoplankton in water samples containing *A. anophagefferens*, and to determine if the grazing rates of specific groups of protozoa were adversely affected by the presence of this alga. Grazing rates were not determined for the small nanoplankton or for all of the samples collected because of the considerable amount of work required.

Microbial grazing in the field was examined using the fluorescently labeled bacteria (FLB) technique of Sherr *et al.* (1987), and the fluorescently labeled algae (FLA) technique of Rublee and Gallegos (1989). In this method, fluorescently stained prey are used as tracers to identify consumers of specific microbial populations. In addition, the grazing rates of the consumers can be determined from the rate of uptake of the labeled prey during short-term time-course incubations. FLB were prepared using the bacterium P. *halodurans* grown to late stationary growth on 1.0% yeast extract in natural seawater. FLA were prepared using a 2-3 μm nonmotile chlorophyte (clone BT3) isolated in 1987 from Great South Bay, Long Island. We attempted to produce FLA from *A. anophagefferens* (clone BT3P). However, these attempts have been ineffective because of the presence of contaminating bacteria in these cultures and because of the large amount of particulate material produced in these cultures. FLB and FLA were added at densities that were 10-25% of the concentration of bacteria and phototrophic nanoplankton, respectively.

Grazing experiments were carried out in the sampling containers at 15°C (June), 20°C (July) or 25°C (August) in constant subdued light (fluorescent light at an intensity of <100 μEinsteins m^{-2} sec^{-1}). Samples were removed periodically during the incubations and preserved with neutralized glutaraldehyde (1% final concentration). FLA and FLB uptake was quantified for the most abundant consumers in settled samples using an inverted microscope. A combination of phase microscopy and epifluorescence microscopy was used to locate and identify cells, and to quantify ingested FLA and FLB.

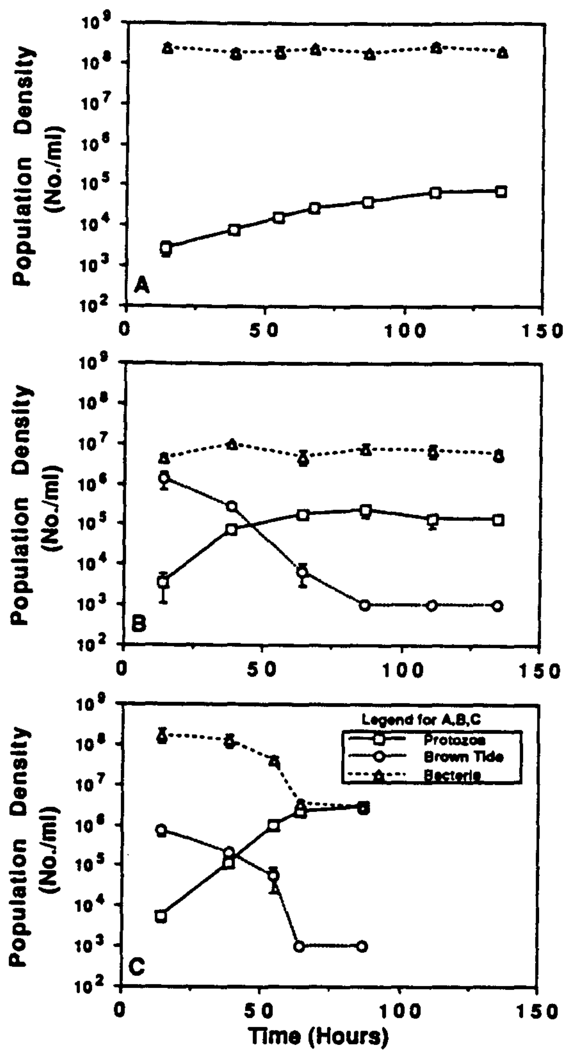

Figure 2. Changes in the population density of bacteria, *A. anophagefferens*, and the microflagellate *Monas* sp. during a laboratory grazing experiment. The protozoan was fed P. *halodurans* at a density of $\approx 2x10^8$ ml^{-1} (A), *A. anophagefferens* at a density of $\approx 10^6$ ml^{-1} (B), and a mixture of P. *halodurans* and *A. anophagefferens* (C). Bacteria were present in (B) at a background concentration of $\approx 0.5-1.0x10^7$ ml^{-1}. All cultures were inoculated with the protozoan at time = 0.

TABLE 1. Growth rates (day^{-1}) of five species of cultured bacterivorous protozoa and a mixed natural protozoan assemblage grown on cultured heterotrophic bacteria (*Pseudomonas halodurans*), the brown tide alga (*Aureococcus anophagefferens*), and a mixture of these two organisms. Initial bacterial and/or algal concentrations were approximately $2x10^8$ ml^{-1} and $1x10^6$ ml^{-1}, respectively. A mixed bacterial assemblage was present in the culture of *A. anophagefferens* at a density of approximately $5x10^6$-10^7 ml^{-1}. For the mixed natural protozoan assemblage, cultured bacteria and algae were added to a natural seawater sample from Vineyard Sound, MA. *P. halodurans* was not added to the mixed natural assemblage, but some bacteria were added with the alga.

	Protazoan Species					
Prey Species	*Monas* sp.	*Bodo* sp.	*Euplotes* sp.	Unidentified Pleuronematid Ciliate	Unidentified Scuticociliatid Ciliate	Mixed Natural Assemblage
P. halodurans	0.60	1.1	0.29	3.2	<0.10	-
A. anophagefferens	3.0	0.49	<0.10	3.8	<0.10	-
P. halodurans + *A. anophagefferens*	2.9	2.1	<0.10	3.4	<0.10	1.1

RESULTS

Laboratory Grazing Experiments

Two of the five species of protozoa examined in the laboratory study displayed an ability to grow in the presence of *A. anophagefferens* and to consume this alga (Figs. 2 and 3, Table 1). The densities of *A. anophagefferens* decreased in the treatments with *Monas* sp. (Fig. 2) and the pleuronematid ciliate (Fig. 3). These decreases indicated a consumption of the alga by these protozoa, particularly when bacteria were also present in high densities. Ingested algae were observed during the experiment using epifluorescence microscopy. Replicate cultures of *A. anophagefferens* incubated in the dark, but not inoculated with protozoa, showed slow steady decreases in cell density. These decreases, however, were two orders of magnitude less than the rapid population decline observed in the grazed cultures.

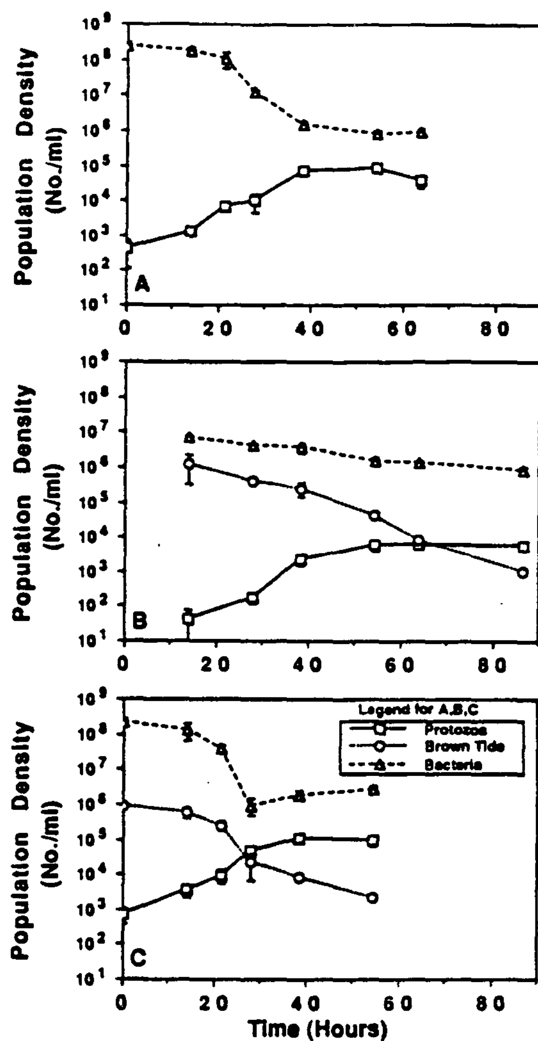

Figure 3. Changes in the population density of bacteria, *A. anophagefferens*, and a pleuronematid ciliate during a laboratory grazing experiment. The protozoan was fed *P. halodurans* at a density of $\approx 2\times10^8$ ml^{-1} (A), *A. anophagefferens* at a density of $\approx 10^6$ ml^{-1} (B), and a mixture of *P. halodurans* and *A. anophagefferens* (C). Bacteria were present in (B) at a background concentration of $\approx 0.5\text{-}1.0\times10^7$ ml^{-1}. All cultures were inoculated with the protozoan at time = 0.

The presence of *A. anophagefferens* increased the growth of at least one of the protozoa relative to its growth rate on bacteria alone (*Monas* sp.; Table 1). The growth rates of this microflagellate in culture vessels containing the alga were 5X the growth rate in the vessel containing only P. *halodurans*. The pleuronematid ciliate, although it consumed the alga, grew at similar rates in all three treatments. *Bodo* sp. also grew more rapidly in the culture vessel containing algae and P. *halodurans* (relative to bacteria alone) but it grew more slowly in the algal culture with no P. *halodurans* added (Table 1).

Two protozoan species (*Euplotes* sp. and the scuticociliate) did not show any significant increases in density during the incubation period in the treatments that contained *A. anophagefferens* (Table 1). *Euplotes* sp. was able to grow slowly on P. *halodurans* alone, but it did not grow on this bacterium when the alga was present. The scuticociliate did not grow in any of the treatments including P. *halodurans* alone, although it has been maintained in the laboratory on a mixed bacterial assemblage for ≈2 years.

The addition of *A. anophagefferens* to a natural seawater sample from Vineyard Sound resulted in a rapid increase in the density of protozoa in this sample and a decrease in the density of the added alga (Fig.4). Heterotrophic microflagellates dominated the protozoan population that developed in this sample. A net growth rate of 1.1 day^{-1} was estimated for the assemblage based on the rate of increase in the population density (Table 1).

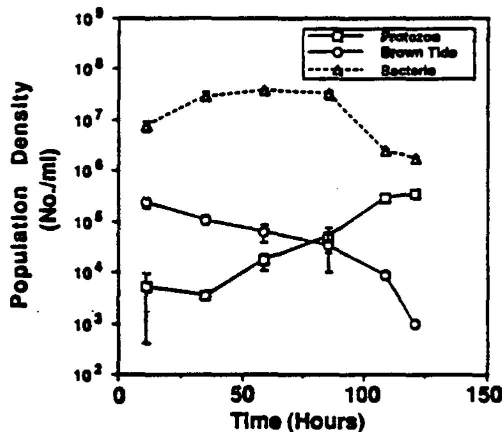

Figure 4. Changes in the population densities of bacteria, *A. anophagefferens* and nanoplanktonic protozoa in a natural seawater sample from Vineyard Sound, Massachusetts to which a culture of *A. anophagefferens* had been added.

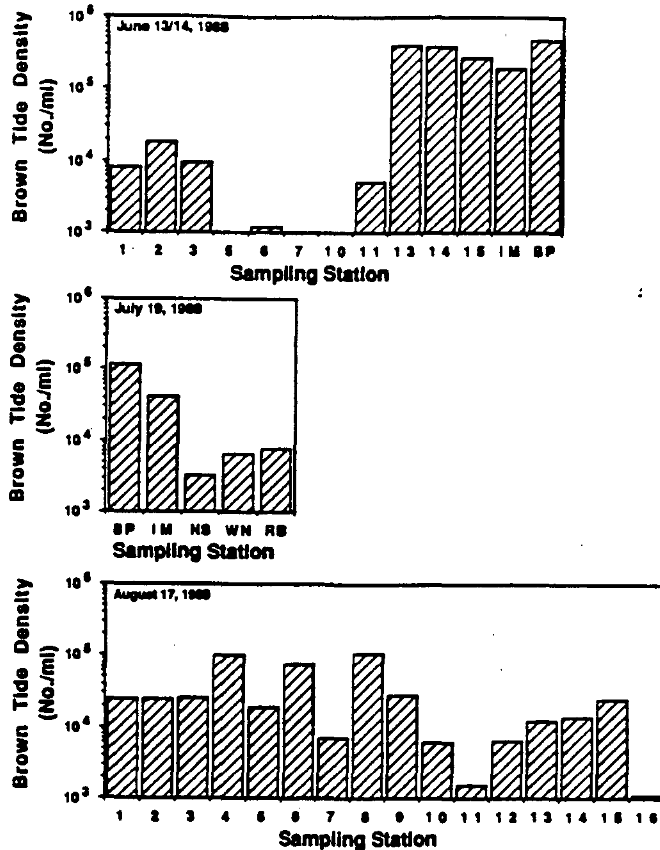

Figure 5. Abundances of the brown tide alga at sampling stations throughout the Peconic Bay system and Great South Bay system, Long Island, New York at three sampling times in 1988. See Figure 1 for station locations.

Field Studies

Population Abundances

The density of the brown tide alga varied by at least three orders of magnitude both geographically and temporally during the field study in Long Island coastal waters (Fig. 5). "Bloom" densities were observed during only one of the three sampling periods (June 13/14), and only at some of the Great

South Bay stations during this period. Concentrations of *A. anophagefferens* were $>2\times10^5$ cells ml^{-1} at five sampling stations at this time. Overall, however, 19 out of the 34 samples enumerated had densities of the brown tide alga that were $\geq10^4$ cells ml^{-1}. *A. anophagefferens* densities less than $\approx10^3$ cells ml^{-1} were not counted during this study, because of the presence of other more numerous picoplanktonic and nanoplanktonic algae.

We enumerated other microorganisms in the field samples and tested these densities for correlations with the brown tide alga to determine if *A. anophagefferens* affected the distributions or abundances of these assemblages (Fig. 6). In general, the densities of all microbial assemblages were high in all samples relative to the densities of these microorganisms in other marine planktonic environments. A significant correlation was found between the density of *A. anophagefferens* and only the phototrophic nanoplankton (correlation coefficient = 0.74). This correlation reflected the fact that the brown tide alga dominated the phototrophic assemblage in the nanoplankton size fraction (2.0-20 µm) in these waters. Although bacterial, ciliate and heterotrophic nanoplankton densities were not positively correlated with the density of *A. anophagefferens*, the concentrations of these assemblages were not reduced in samples containing 'bloom' concentrations of *A. anophagefferens*. Several of the highest concentrations of these plankton assemblages occurred in samples containing the highest density of *A. anophagefferens* (Fig. 6).

The occurrence of the brown tide had no discernable effect on the composition of the heterotrophic microplankton assemblage (Fig. 7). Changes in species occurrence and abundance between sampling stations and sampling periods were observed, but these changes showed no consistent trends with the abundance of the brown tide. The heterotrophic microplankton was always dominated numerically by protozoa, and ciliates comprised the largest percentage of this size fraction. Non-loricate choreotrich ciliates were consistently the most abundant ciliate group. Some microplanktonic flagellates (predominantly heterotrophic dinoflagellates) were also observed in some samples.

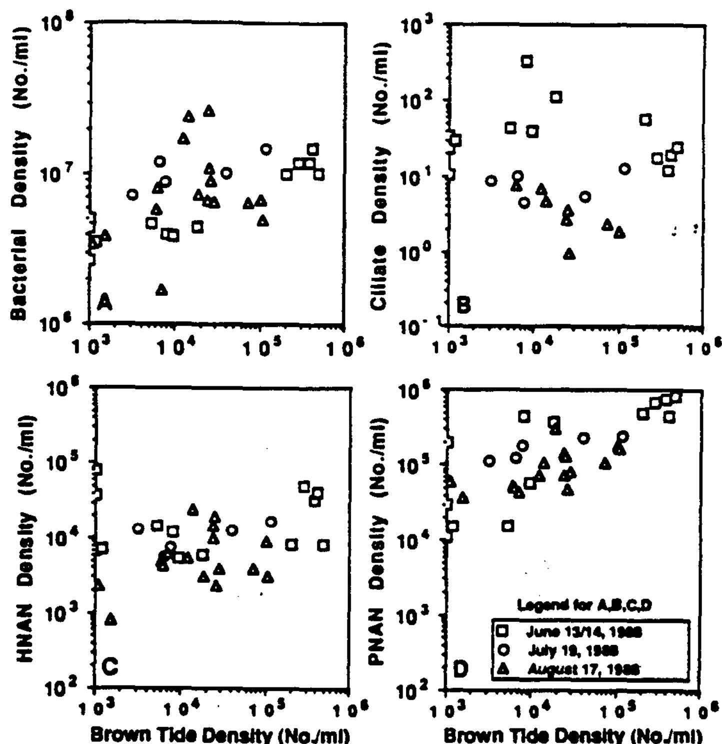

Figure 6. Densities of bacteria (A), microplanktonic ciliates (B), and phototrophic (HNAN;C) and heterotrophic (PNAN;D) nanoplankton in the Peconic Bay system and Great South Bay system, Long Island, New York, as a function of the density of the brown tide alga. Station locations and sampling times are presented in Figures 1 and 5.

Microbial Grazing Impact

Fluorescently labeled algae and bacteria were used to investigate the grazing of consumers of picoplankton and small nanoplankton. The advantage of this method is that it makes it possible to distinguish between microorganisms that ate primarily bacteria and those that ate primarily small nanoplankton using FLB and FLA, respectively, as tracers for these populations. In addition to identifying the major consumers of bacteria-sized and brown tide-sized particles, the FLA/FLB method was used to quantify the grazing rates of the numerically dominant microbial groups. An example of the results of this method is shown in Figure 8. Grazing rates (expressed

as clearance rate = volume filtered protozoan^{-1} unit time^{-1}) were calculated from a regression between time and the rate of increase in the average number of ingested FLA or FLB in the protozoa (Fig.8).

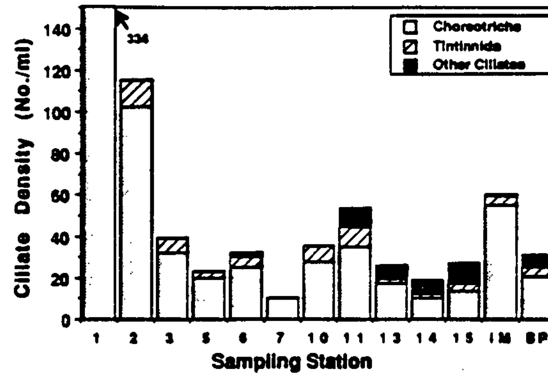

Figure 7. Density and broad taxonomic composition of the heterotrophic microplanktonic ciliates in Long Island waters on June 13/14, 1988. 'Choreotrichs' refers to non-loricate choreotrichs. Station locations are given in Figure 1. Refer to Figure 5 for the densities of the brown tide alga in these samples.

The linear portion of the uptake curve in these figures varied with prey type (FLA or FLB) and with the protozoan group investigated. For most protozoa, the uptake of FLA remained linear over the entire length of the two hour incubations. On the other hand, the average number of ingested FLB protozoan^{-1} reached a maximum during this time for most bacterial consumers, and grazing rates for these organisms were determined using more closely spaced samples and shorter overall incubation periods.

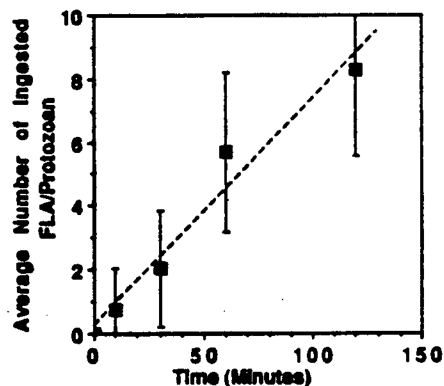

Figure 8. Uptake of fluorescently labeled algae (FLA) by ebridians at station #13 on August 17, 1988.

Larger nanoplanktonic (>10 μm) and microplanktonic flagellates were consistently the major consumers of fluorescently labeled algae similar in size to *A. anophagefferens* (Fig. 9E,F). Heterotrophic dinoflagellates were by far the numerically dominant consumers within this protozoan group. Ebridian flagellates were common in some samples and consumed significant numbers of FLA in those instances (Fig. 9C,D). Tintinnid ciliates (Fig. 9A,B) and choreotrich ciliates also consumed FLA.

The protozoan groups mentioned above were also observed with FLB within their food vacuoles. However, selective feeding was apparent for some of these groups. Larger nanoplanktonic and microplanktonic flagellates, tintinnid ciliates and ebridian flagellates all preferentially ingested FLA to FLB. Small scuticociliates showed the opposite selectivity (Fig. 10C,D). These ciliates rapidly ingested FLB but were never observed with ingested FLA. Non-loricate choreotrich ciliates, however, showed little selectivity. These protozoa consumed FLA, but they also captured significant quantities of FLB (Fig. 10A,B).

Clearance rates calculated from the uptake of FLB or FLA were highly variable overall (Table 2). These rates generally varied by more than an order of magnitude for any protozoan group, and similar ranges of these rates were obtained regardless of whether FLB or FLA were used as prey. These rates represent averages of a number of species that are morphologically similar, but they undoubtedly combine several slightly different feeding preferences and possibly active and inactive cells. These factors may explain, at least in part, a significant portion of the variability of the observed clearance rates.

Feeding preference of the protozoan groups for bacterial or nanoplanktonic prey that were apparent from visual observations were examined by a comparison of the clearance rates determined on the same samples from the uptake of FLA and FLB (Fig. 11). Ebridian flagellates, other flagellates >10 μm and tintinnid ciliates had higher clearance rates for FLA than for FLB, while scuticociliates had higher clearance rates for FLB than for FLA. Non-loricate choreotrich ciliate assemblages had roughly similar clearance rates for either type of prey.

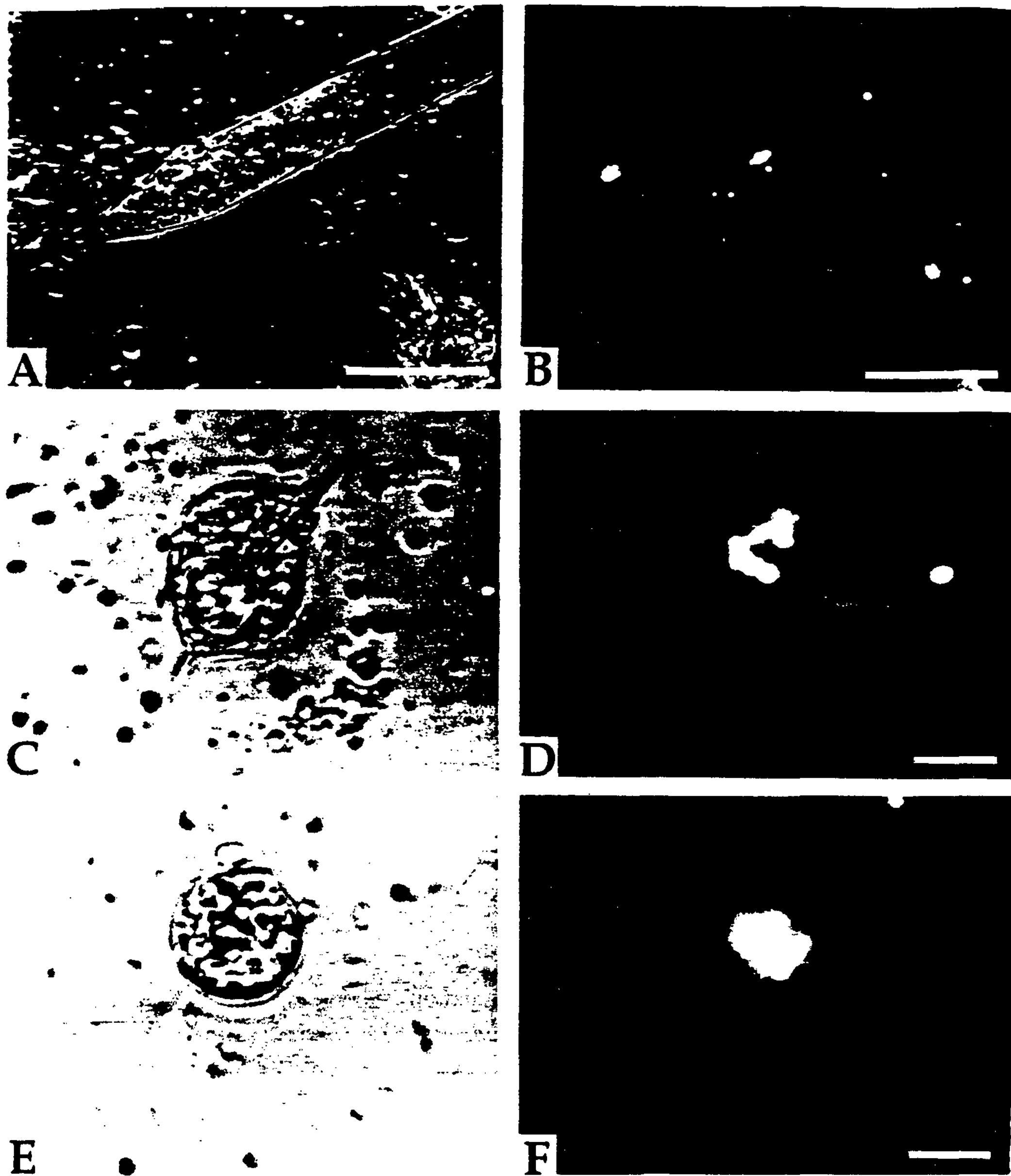

Figure 9. Transmitted light photomicrographs of protozoa with ingested fluorescently labeled algae (A, C and E) and epifluorescence photomicrographs of the same organisms (B, D and F, respectively) showing the fluorescent prey. The protozoa are a tintinnid ciliate (A,B), an ebridian flagellate (C,D) and a small unidentified heterotrophic flagellate (E,F). Marker bars are 60 μm (A,B) and 15 μm (C-F).

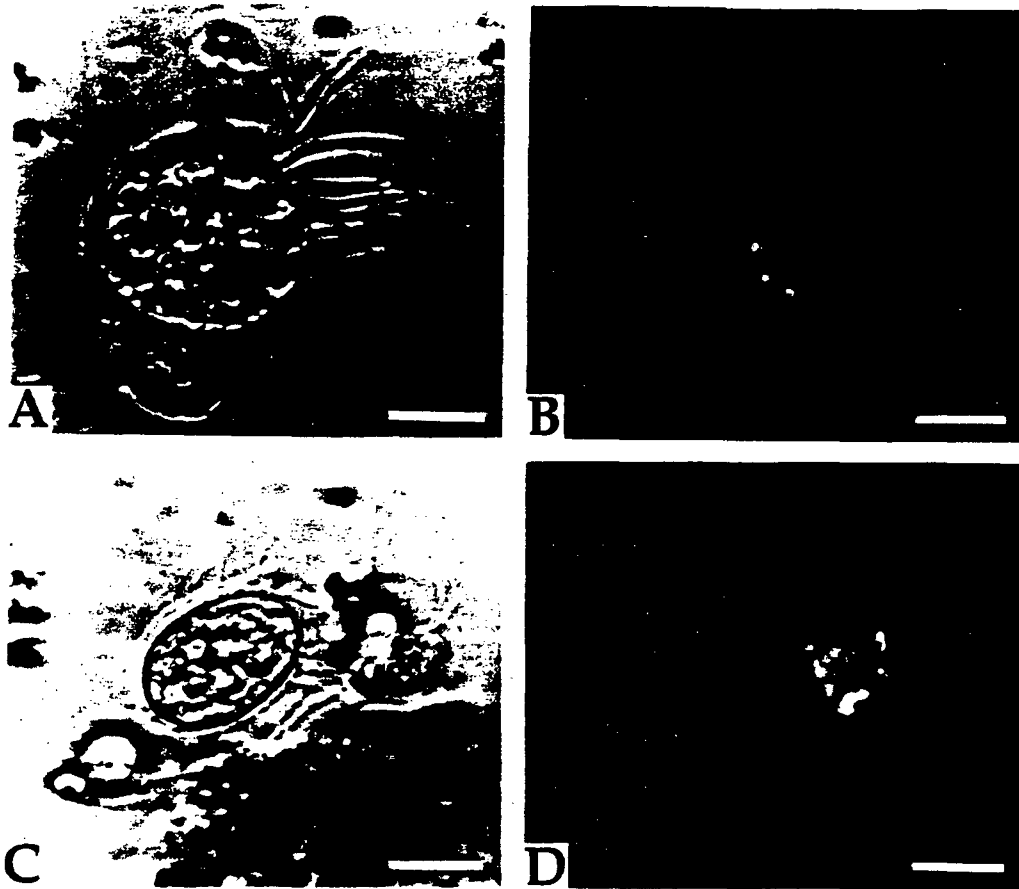

Figure 10. Transmitted light photomicrographs of protozoa with ingested fluorescently labeled bacteria (A and C) and epifluorescence photomicrographs of the same organisms (B and D, respectively) showing the fluorescent prey. The protozoa are a non-loricate choreotrich ciliate (A,B) and a small scuticociliate (C,D). Marker bars are 15 μm.

No clear correlation was apparent between the clearance rates of the various protozoan groups and the density of *A. anophagefferens*. The individual clearance rates measured in this study are summarized in Figure 12. Low rates were observed in samples that had relatively high densities of this alga, but low rates were also observed in samples that had relatively low densities.

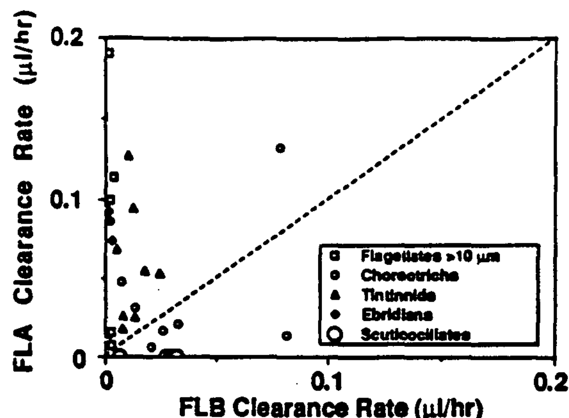

Figure 11. Comparison of the clearance rates of protozoan groups determined from the uptake of FLB and FLA by populations in the same seawater samples. The dotted line indicates the line of 1:1 correspondence between the two clearance rates. Values above this line indicate a preference for FLA, while values below this line indicate a preference for FLB. 'Choreotrichs' refers to non-loricate choreotrichs.

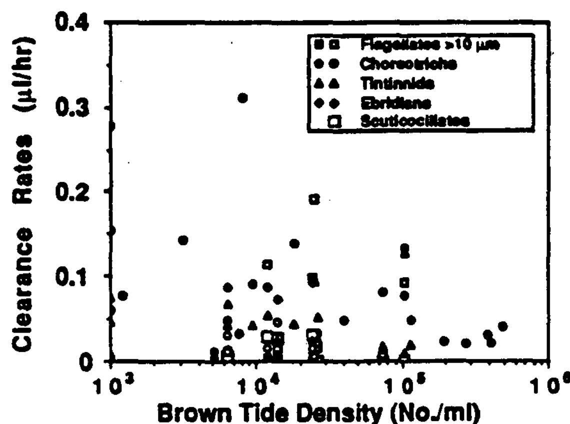

Figure 12. Summary of all of the clearance rates for protozoan groups observed in the field study in Long Island inland waters as a function of brown tide density. See Figures 1 and 5 for station locations and sampling dates. Open symbols are rates calculated from the uptake of FLB, closed symbols are rates determined using FLA. 'Choreotrichs' refers to non-loricate choreotrichs.

Summed Grazing Impact of Microbial Consumers

The clearance rates determined for the numerically dominant protozoan taxa (Table 2) and the densities of these groups of protozoa were used to

estimate the grazing pressure of these assemblages on FLB-sized particles and FLA-sized particles (Fig. 13). In nearly all the samples, non-ebridian heterotrophic flagellates were the major consumers of FLA for grazers >10 μm (Fig 13B). These protozoa clearly constituted the bulk of the grazing pressure on FLA in the size range of *A. anophagefferens*. This overwhelming importance was due to the high ingestion rates of these organisms for FLA (Fig. 9E,F) and the generally high abundances of these protozoa in most samples. Although tintinnid ciliates and ebridians were also voracious consumers of FLA, they were only occasionally important in the overall rate of ingestion of FLA because they usually occurred at much lower abundances than the non-ebridian heterotrophic flagellates.

Table 2. Clearance rates (ml filtered organism^{-1} hr^{-1}) for several taxonomic groups of protozoa from inshore waters of Long Island. Clearance rates were determined from the rate of uptake of fluorescently labeled algae (FLA) and fluorescently labeled bacteria (FLB) only for samples for which sufficient numbers of organisms were obtained. Samples were collected June 13 and 14, July 19 and August 17, 1988. "UN" indicates that no ingested FLA or FLB were observed in any specimens (undetected).

	Clearance Rate			
Protozoan Group	Range	Mean	Prey	Number of Trials
Flagellates >10 μm	0.07-1.91x10-4	6.82x10^{-5}	FLA	8
(except Ebridians)	UN-3.65x10^{-6}	1.85x10^{-6}	FLB	8
Non-loricate	0.06-1.33x10^{-4}	3.54x10^{-5}	FLA	8
Choreotrich Ciliates	0.02-3.11x10^{-4}	7.08x10^{-5}	FLB	26
Tintinnid Ciliates	0.24-1.27x10^{-4}	5.80x10^{-5}	FLA	8
	0.54-7.61x10^{-5}	2.42x10^{-5}	FLB	16
Scuticociliates	UN	-	FLA	4
	0.64-3.16x10^{-5}	2.37x10^{-5}	FLB	4
Ebridians	7.32-9.23x10^{-5}	8.49x10^{-5}	FLA	4
	UN-2.64x10^{-6}	1.52x10^{-6}	FLB	4

Total grazing pressure on FLB by organisms >10 μm was less dominated by non-ebridian heterotrophic flagellates (Fig. 13A). The clearance rates of these protozoa for FLB averaged approximately 1.5 orders of magnitude less

than the clearance rates of these organisms for FLA. The contribution of these protozoa to the total grazing pressure on FLB was still important, however, because of their high population densities. Non-loricate choreotrich ciliates constituted a much greater fraction of the total grazing pressure on FLB than they did for FLA. Scuticociliate grazing was also a significant fraction of the total grazing pressure at some stations. In contrast, this latter group of protozoa did not contribute to FLA ingestion. Tintinnids and ebridians contributed only modestly to total grazing pressure on FLB.

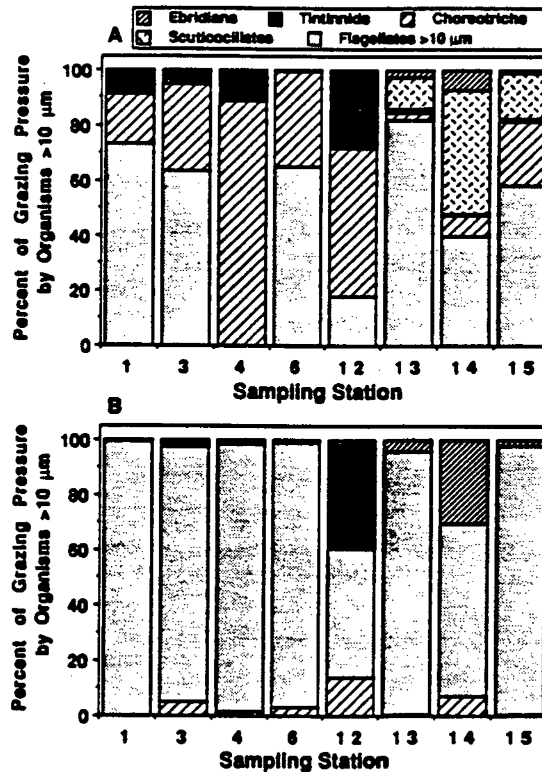

Figure 13. Grazing pressure of various protozoan taxa as a percentage of the total grazing pressure of the microbial consumers >10 µm on August 17, 1988. Grazing pressure was determined as the product of the clearance rate (ml filtered $cell^{-1}$ hr^{-1}) and the densities (cells ml^{-1}) of these organisms. (A) Grazing on FLB-sized particles. (B) Grazing on FLA-sized particles. 'Choreotrichs' refers to non-loricate choreotrichs. Station locations are given in Figure 1.

We employed a fluorescently labeled alga in this study that was the same size as *A. anophagefferens* in order to determine the clearance rates of the

numerically important nano- and microplankton for prey of this size. By assuming that these protozoa removed *A. anophagefferens* from the water at the same rate that they removed the FLA, it was possible to calculate the rate of removal of the brown tide alga from the water as a consequence of the grazing of these organisms (Fig. 14). The estimated removal rate of the brown tide alga (percent of the population removed day^{-1}) by the microorganisms >10 µm in size was only a minor percentage of the total population for all but two of the samples examined. These removal rates were calculated using the higher of the two rates (either the FLB or FLA rate) calculated for a given protozoan assemblage. Except for one observation in July, the highest removal rates were observed in samples that had intermediate densities of *A. anophagefferens*.

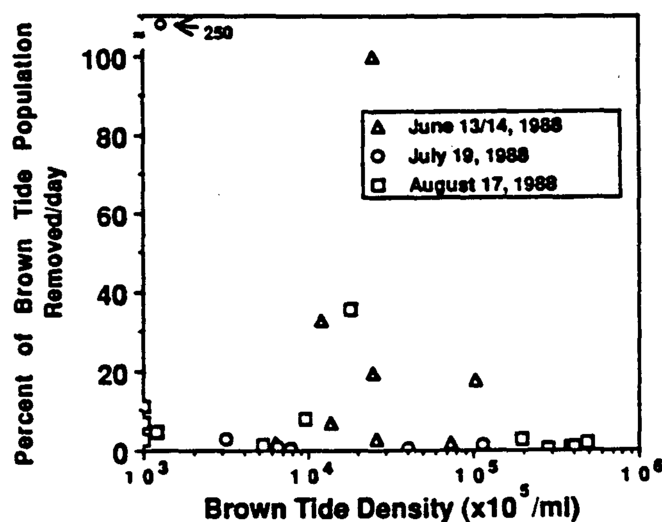

Figure 14. Estimated rates of removal of *A. anophagefferens* by microbial consumers >10 µm as a function of population density. Removal rates were calculated from the average clearance rate of each consumer group (Table 2) multiplied by the density of the protozoan population and the brown tide density.

DISCUSSION

Based on the results of our laboratory and field grazing studies, the high densities of *A. anophagefferens* that have been observed in nature cannot be easily explained. It is puzzling that an alga that was an acceptable food for some commonly occurring species of protozoa in laboratory cultures can attain such phenomenally high densities in marine plankton communities. Two of the five species of cultured protozoa that were tested in the laboratory demonstrated an ability to consume *A. anophagefferens* and to grow on this biomass at rates that were at least equivalent to the estimated growth rate of this alga in nature (Cosper *et al.*, 1987).

The alga apparently did not produce any dissolved substance in culture that depressed the feeding rates of these two protozoa. Protozoa were added directly to the culture vessels used to grow *A. anophagefferens* for those treatments that contained the alga. Therefore, any effect of dissolved substances released by the alga would still have been present in the culture medium during the protozoan grazing phase of the experiment. In addition, these species, and a natural protozoan assemblage from Vineyard Sound, were able to graze the alga down to relatively low abundance in laboratory cultures ($\approx 10^3$ cells ml^{-1}). This density is approximately three orders of magnitude less than 'bloom' densities of *A. anophagefferens* in nature (Cosper *et al.*, 1987; Sieburth *et al.*, 1988). Therefore, protozoa should, in theory, be able to restrain the population growth of the brown tide. The protozoa tested in our laboratory experiments were commonly occurring bacterivorous species. We made no attempt to culture species that might be specifically adapted to consuming *A. anophagefferens*. Therefore, species may exist that can utilize this alga even more efficiently than we have demonstrated.

It is not surprising that some of the protozoan species did not grow well when offered *A. anophagefferens* as prey. The value of individual species of microorganisms as food varies among protozoan species (Rubin and Lee, 1976). For example, it has been demonstrated that some bacteria can support the growth of some but not all protozoa tested (Curds and Vandyke, 1966). Particle size selection can also be quite narrowly defined for some protozoa, thereby limiting the type of prey organisms that can be captured efficiently (Fenchel, 1980a). Given these considerations, it is noteworthy that *A. anophagefferens* was an acceptable food for two of the species that we tested. However, it is possible that our laboratory experiments with the brown tide

alga have not adequately imitated the natural environment. For example, *A. anophagefferens* in nature may produce a substance(s) that reduces or eliminates grazing by planktonic microorganisms and that the clonal culture that we used in our studies did not produce this substance. At present, however, there is still only one clone of *A. anophagefferens* in laboratory culture and we are therefore unable to test this possibility. The isolation and culture of more clones of this species are imperative.

Our analysis of the densities of potential grazers of *A. anophagefferens* and the grazing rates of these organisms in areas affected by the brown tide also indicated that the presence of this alga caused no significant depression in microbial grazing pressure. All microbial populations that were enumerated occurred at high densities during the brown tide. This observation is consistent with a previously published report of a brown tide in Narragansett Bay, Rhode Island during 1985 (Sieburth *et al.*, 1988). The densities of these microbial assemblages indicated a highly eutrophic ecosystem, and all were near the upper limit of published reports for the densities of heterotrophic and phototrophic pico-, nano- and microplankton (Davis and Sieburth, 1982; Davis *et al.*, 1985; Caron, 1983; Linley *et al.*, 1983; Fenchel, 1982b; Sherr and Sherr, 1983; Landry *et al.*, 1984; Pick and Caron, 1988; Sieburth *et al.*, 1988).

The range of clearance rates measured using FLA and FLB in this study did not indicate a depression in individual rates of filtration by protozoa consuming particles the size of bacteria and *A. anophagefferens*. These rates are not high compared with the clearance rates reported in the literature for some protozoa, but they are well within the range of values from cultures and eutrophic environments (Heinbokel, 1978; Conover, 1982; Fenchel, 1982a; 1987; Stoecker and Evans, 1985; Verity, 1985; Sherr *et al.*, 1987). Low clearance rates were observed in samples that had high concentrations of the brown tide (Fig. 12), but low rates were also observed in samples that had relatively low densities of this alga. Somewhat lower individual clearance rates would be expected for microbial consumers in samples that had high concentrations of brown tide because all pico- and nanoplankton prey occurred in high concentration in these samples. Clearance rates typically vary inversely with prey concentration. We have calculated ingestion rates (prey consumed protozoan^{-1} unit time^{-1}) from our FLB and FLA uptake data and have found that ingestion rates of bacteria-sized and small nanoplankton-sized particles increased with increasing concentration of brown tide because of

the generally higher densities of all microbial populations in these latter samples.

If grazing is not substantially curtailed in the presence of *A. anophagefferens*, and if this alga can be consumed by commonly occurring protozoa, then how is this organism able to bloom? Undoubtedly, the unique physiology of this alga is part of the answer. Field studies of *A. anophagefferens* have demonstrated a very rapid growth rate (Cosper *et al.*, 1987), yet this growth potential alone cannot explain the occurrence of blooms. Other factors that affect the grazing pressure of microbial consumers must be involved. We speculate that a critical factor in explaining the explosive growth of *A. anophagefferens* may be the density of potential consumers of the brown tide alga *at the time of bloom initiation*. We demonstrated that once the bloom was established, grazing by larger nanoplankton and microplankton would only remove a small fraction of the algal population each day (Fig. 14). However, when the alga was at 'moderate' densities, a significant fraction could be consumed daily by this assemblage. If low densities of the consumers of *A. anophagefferens* were present at the time that conditions for the rapid growth of that alga occurred, then a high net growth rate could result. Once the bloom became established, removal by grazing, even in the absence of continued growth of the alga, would require a substantial amount of time because of the slow rate of removal (Fig. 14).

The scenario hypothesized above entails several assumptions. We have assumed that FLA are an adequate tracer of the grazing rates of microbial consumers for *A. anophagefferens*. For this assumption to be correct it is necessary that no selectivity occurs for or against the FLA or *A. anophagefferens* in the samples. At present we have no mechanism for examining this possibility. Fluorescent labeling of cultures of *A. anophagefferens* has not been effective because of the unavailability of an axenic culture of the alga and the production of large amounts of particulate material by the alga in laboratory cultures. Immunological techniques presently being developed may alleviate this shortcoming (Anderson *et al.*, 1988) by allowing labeling of natural populations of *A. anophagefferens*. A thorough examination of grazing selectivity will be necessary to verify the results of our FLA and FLB studies because selection by planktonic protozoa is possible (Stoecker *et al.*, 1981).

It also is assumed that the grazing rates measured in our study are accurate. The validity of the FLA and FLB methods for measuring microbial grazing rates has been previously studied (Sherr *et al.*, 1987; Rublee and Gallegos, 1989). However, we did not determine the summed grazing rates of all of the microbial assemblages. Measurements of the grazing rates of small nanoplankton were not performed because these small protozoa require special precautions to prevent the release of ingested particles during fixation (Sieracki *et al.*, 1987). The potential importance of small nanoplankton as consumers of small algae has been clearly demonstrated in several recent studies (Haas, 1982; Goldman and Caron, 1985; Parslow *et al.*, 1986; Suttle *et al.*, 1986). Therefore, our estimates of removal of *A. anophagefferens* from natural samples (Fig. 14) might significantly underestimate the actual losses due to grazing. A 'community level' approach to estimating grazing will be required to address this issue.

Most importantly, our hypothesized explanation for the occurrence of brown tides assumes that the consumption of small nanoplankton and picoplankton was reduced at the time of rapid *A. anophagefferens* growth. The most logical manner in which this situation could have occurred (other than feeding selectivity by consumers) is if the consumers of nanozooplankton and microzooplankton occurred at high abundance or were feeding actively at that time. As the available food supply for the larger zooplankton shifted to a size class that was too small to be efficiently removed from the water, these organisms might have increased predation rates on the nano- and microzooplankton. The removal of the protozoan consumers would further enhance competitive advantage of *A. anophagefferens* and ultimately result in a picoplankton bloom.

We speculate that this situation is more likely than a chemical inhibition of microbial grazing simply because we have been unable to demonstrate a reduction in cell-specific clearance rates in seawater samples containing high abundances of the brown tide. Interestingly, the abundances of crustacean zooplankton observed in Long Island coastal waters during the massive brown tide blooms in 1985 and 1986 were among the highest ever observed in coastal waters of the eastern U.S. (Duguay *et al.*, 1988). This observation is not a confirmation of our hypothesis but it is consistent with our expectations, and it presents a possible mechanism whereby the densities of nano- and microzooplankton might be reduced in this environment. However, the exact timing that would be required to produce the sequence of events

described above is difficult to predict. These relationships would be even more difficult to demonstrate in the field because they would entail a long-term study with high temporal resolution in order to link cause and effect.

Is an interruption of grazing the key to explaining how *A. anophagefferens* is able to attain such high densities in nature? If this speculation is true, then it would be expected that other pico- and nanoplankton would also increase their densities in response to a decrease in grazing pressure. Given this expectation, it may be noteworthy that bacteria and other minute algae also have been observed at very high concentrations in conjunction with brown tides (Cosper *et al.*, 1987; Sieburth *et al.*, 1988). The dominance of *A. anophagefferens* in these situations might then be related to its ability to grow faster than competing microalgae.

The conjecture that we present for the establishment and maintenance of the brown tide is based on the limited data that are presently available concerning the trophic interactions of *A. anophagefferens*. More information will be required before any firm conclusions can be formed concerning the exact conditions leading to the formation of these extraordinary blooms. However, our study presents the first testable hypothesis for explaining the high densities of this alga, and thereby a framework for further studies involving alga-herbivore interactions in this and other problem algal blooms. The importance of trophic interactions in the establishment of algal blooms has been largely overlooked in the past. It is imperative that this work proceed concurrently with research on the physiology of the algal species causing these blooms if we are to gain a clear understanding of the factors leading to these phenomena.

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