JOURNAL OF PLANKTON RESEARCH | VOLUME 25 | NUMBER 2 | PAGES 215–228 | 2003

Effects of mesozooplankton removal and ammonium addition on planktonic trophic structure during a bloom of the Texas 'brown tide': a mesocosm study

EDWARD J. BUSKEY*, HUDSON DEYOE[1], FRANK J. JOCHEM[2] AND TRACY A. VILLAREAL

MARINE SCIENCE INSTITUTE, THE UNIVERSITY OF TEXAS AT AUSTIN, 750 CHANNEL VIEW DRIVE, PORT ARANSAS, TX 78373 AND [1]DEPARTMENT OF BIOLOGY, UNIVERSITY OF TEXAS-PAN AMERICAN, EDINBURG, TX 78539, USA

[2]PRESENT ADDRESS: MARINE BIOLOGY PROGRAM, FLORIDA INTERNATIONAL UNIVERSITY, NORTH MIAMI, FL 33181, USA

*CORRESPONDING AUTHOR: buskey@utmsi.utexas.edu

A bloom of the alga Aureoumbra lagunensis, known as the Texas 'brown tide', persisted in the Laguna Madre of Texas for most of the 1990s. The dominant mesozooplankter in Laguna Madre, Acartia tonsa, does not feed on A. lagunensis, and during blooms there are few other suitably sized phytoplankton cells available to feed on. We hypothesized that these copepods increased their feeding on microzooplankton, thereby reducing grazing pressure by microzooplankton on A. lagunensis and contributing to the persistence of this bloom. A mesocosm experiment was carried out to test this hypothesis during the summer of 1999. Twelve fiberglass corral-type mesocosms were deployed in the field for 16 days, each enclosing ~1.2 m^3 of Laguna Madre water and 1.1 m^2 of natural benthos. Mesozooplankton were removed from six mesocosms with a 153 μm mesh dip net every 4 days; the other six mesocosms were treated in the same way, except that the contents of the net were returned to the mesocosm. For each zooplankton treatment, half of the mesocosms were dosed with ~40 μM NH_4 at 4 day intervals, and half received no additions. Phytoplankton populations in these mesocosms at the start of the experiment were dominated by A. lagunensis and the cyanobacterium Synechococcus spp. Growth rates of A. lagunensis were higher in mesocosms without ammonium additions, providing no evidence for nitrogen limitation. Acartia tonsa populations were reduced by ~50% in the zooplankton removal mesocosms, and ciliate populations were significantly higher. The increase in ciliate population had no significant impact on A. lagunensis population dynamics, however, providing no evidence to support the hypothesis that a trophic cascade reducing microzooplankton populations contributed to the persistence of the brown-tide bloom. In contrast, populations of Synechococcus sp. showed evidence of both 'top–down' and 'bottom–up' control; they grew faster in nutrient addition mesocosms and had lower populations in mesocosms with increased densities of ciliate grazers.

INTRODUCTION

The Laguna Madre is a large (2.15 × 10^5 ha), shallow (average depth 1.2 m) coastal lagoon, whose waters are often hypersaline due to restricted circulation with the Gulf of Mexico, high evaporation rates and low precipitation (Armstrong, 1987). The Texas brown-tide algal bloom persisted in the Laguna Madre of Texas from December 1989 until October 1997 without interruption (Buskey *et al.*, 1997, 2001). The Texas brown tide is a dense, persistent bloom of the alga *Aureoumbra lagunensis*, a small (~4–5 μm diameter) pelagophyte. Under bloom conditions, cell densities range from 0.5 × 10^6 to 5 × 10^6 cells ml^{-1} (Buskey *et al.*, 1996). At these densities, underwater irradiance is severely reduced, and seagrass distribution and biomass has been reduced (Onuf, 1996). Although abnormally high rainfall flushed the brown-tide alga from the Laguna Madre on at least two occasions (October 1997, October 1998), the bloom returned when the salinities increased to hypersaline levels (Buskey *et al.*, 2001). *Aureoumbra lagunensis* has a competitive advantage under hypersaline conditions over many phytoplankton species since it can grow at maximum rates in salinities ranging from 20 to 60 psu (Buskey *et al.*, 1998). Following the initial decline of the extended *A. lagunensis* bloom in 1997, populations of *Synechococcus* sp. bloomed in 1998, with densities of up to 10^7 cells ml^{-1} (Buskey *et al.*, 2001); since then the Laguna Madre has undergone periodic oscillations between *A. lagunensis* and *Synechococcus* sp. populations.

The initiation of the first reported *A. lagunensis* bloom was found to coincide with a period of extreme hypersalinity in the fall of 1989, and an unusually severe freeze in December 1989 (Buskey *et al.*, 1997). Although the bloom initiation was originally thought to have been triggered by an extensive fish kill during the freeze of December 1989 which caused the release of large amounts of nutrients (NH_4^+ concentrations exceeded 15 μM) in the Laguna Madre (Whitledge, 1993; DeYoe and Suttle, 1994), recent examination of archived samples reveals that the bloom had already begun before the freeze (Buskey *et al.*, 1999). The extremely hypersaline conditions prior to the freeze decimated protozoan grazer populations, and the probable release from grazing pressure contributed to the initiation of the bloom in December 1989 (Buskey *et al.*, 1998). The freeze and subsequent release of nutrients after the fish kill fueled the bloom and *A. lagunensis* populations exceeded 5×10^6 cell ml^{-1} during the early bloom (Buskey *et al.*, 1997).

Although the factors leading to the initiation of this extended bloom have been well described, the reasons for the extraordinary persistence of this bloom remain poorly understood. Since the growth of a phytoplankton population represents the balance between growth and various loss factors such as grazing, sinking and advection, it seems likely that one or more of these loss factors are not acting to reduce brown-tide populations in the Laguna Madre. The shallow depth and reduced circulation of the Laguna Madre severely curtail population losses through sinking and advection. Turnover times for the water of the Laguna Madre are thought to exceed 1 year under most conditions (Shormann, 1992), making advective losses much lower than those in most coastal embayments.

There are several lines of evidence from previous studies that support the hypothesis that grazing control of brown tide is not effective. Field studies during the first 2 years of the bloom indicated that both meso- and microzooplankton populations had declined compared with those prior to the bloom (Buskey and Stockwell, 1993). In addition, field samples indicated that adult females of the dominant copepod, *Acartia tonsa*, were smaller, had less chlorophyll in their guts, and had lower egg production rates during the brown-tide bloom, compared with before the bloom began (Buskey and Stockwell, 1993). All three changes in *A. tonsa* suggest that *A. lagunensis* is not a suitable food for these copepods; this may be due in part to its small size, which is outside the preferred size range for *A. tonsa* (Berggreen *et al.*, 1988). Laboratory studies have confirmed that a diet of *A. lagunensis* leads to lower egg production rates and lower survival of nauplii in *A. tonsa* (Buskey and Hyatt, 1995). Since there are few other phytoplankton for these copepods to feed on during a brown-tide bloom, it seems reasonable to expect that *A. tonsa* would feed more on microzooplankton. The low microzooplankton abundance and reduced egg production of field populations of *A. tonsa* suggest that food supplies were limiting their growth (Buskey and Stockwell, 1993; Buskey *et al.*, 1997). Thus it seems possible that microzooplankton populations were kept low by mesozooplankton predation, and, in turn, the microzooplankton exerted less grazing pressure on brown tide, creating a trophic cascade.

Even if grazing pressure is low, it is unclear what nutrient source fueled the extended bloom of *A. lagunensis*. There are no permanent rivers or streams to provide new nitrogen to the upper Laguna Madre. However, an unusual characteristic of *A. lagunensis* is its inability to utilize nitrate as a nitrogen source (DeYoe and Suttle, 1994) so *A. lagunensis* relies on recycled nitrogen in the form of ammonium or dissolved organic nitrogen. Recent studies indicate that *A. lagunensis* has an extremely low phosphate requirement, making the possibility of phosphate limitation less likely (Liu *et al.*, 2001).

Mesocosms are useful tools for investigating the impacts of grazers, nutrients and other factors on the population dynamics of harmful algal blooms. With appropriate manipulations, the numbers of mesozooplankton and microzooplankton can be changed to an extent that should affect overall grazing impact. The advantages of mesocosms are that all components of an ecosystem are allowed to interact in a natural way, while one or more variables can be manipulated in a limited volume of water, without altering an entire system. In order to determine if brown-tide populations were controlled by grazers from the 'top–down', we conducted a mesocosm study during a brown-tide bloom in which mesozooplankton were removed from half of the mesocosms. To determine if the brown-tide bloom was controlled from the 'bottom–up' by the availability of nitrogen we added ammonium to half of the mesocosms with reduced zooplankton and to half of the control mesocosms.

METHOD

Twelve corral-type mesocosms (open-bottom cylinders) were deployed from June 26 to July 12, 1999 in the cooling pond of the Central Power and Light electrical power generating plant. This site was chosen because it contains water pumped from the adjacent upper Laguna Madre, where brown-tide blooms have persisted in the past and a bloom was forming in the early summer of 1999. This pond has physical and biological characteristics that are very similar to those of the upper Laguna Madre, and it provided a secure site where mesocosms would not be tampered with. The power plant pumps ~600 m^3 of sea water min^{-1}, which reaches a maximum temperature of 10°C above ambient for a period of 7 s when passing through the condenser. Although water temperature is ~5°C warmer than ambient when emerging from the electrical plant, the water has cooled completely back to ambient temperatures by the time it reaches our location in the pond 3 km away. More importantly, normal planktonic and benthic communities exist within the cooling pond that are similar to those found in the Laguna Madre. Preliminary studies carried out in previous years indicated that brown tide and zooplankton populations in the cooling pond reflect those of the Laguna Madre (data not shown). The cooling pond is ~3.9 km in length and 1.1 km in width, and is divided into four sections of approximately equal size by three diversion walls that cover ~90% of the width of the pond and force the water to take a circuitous route before emptying into Oso Creek, which in turn flows into Corpus Christi Bay. The mesocosms were deployed behind the third diversion wall in water ~1 m deep; this location protected them from waves that might develop in the prevailing onshore breezes of summer. The mesocosms are fiberglass cylinders, 1.2 m in diameter and 1.5 m in height. These mesocosms had been used in several 2 week deployments in previous years, allowing ample opportunity for any volatile chemicals to leach from the fiberglass. When first deployed in this study the mesocosms were carried to the site, placed on the bottom and pushed ~20 cm into the sandy bottom. Each fiberglass cylinder enclosed ~1.2 m^3 of sea water and ~1.1 m^2 of natural benthos. Each mesocosm was secured to the bottom with rope using three mobile home tie-down stakes.

There were four different treatments, with three replicate mesocosms per treatment. The abundance of mesozooplankton was reduced in six of the mesocosms, and the other six had normal mesozooplankton populations. For the six mesocosms with reduced mesozooplankton, three were given ammonium additions, and three served as controls. Likewise, for the six mesocosms with normal mesozooplankton populations, three were given ammonium additions and three acted as controls. Thus the four treatment conditions were: (i) normal mesozooplankton, normal ammonium (NZ-NA), (ii) normal mesozooplankton, added ammonium (NZ-AA), (iii) reduced mesozooplankton, normal ammonium (RZ-NA), and (iv) reduced mesozooplankton, added ammonium (RZ-AA). Mesozooplankton were removed from six mesocosms with a 153 μm mesh dip net every 4 days; each tank was swept in a figure-of-eight pattern extending through the depth of the mesocosm for a total of 20 sweeps per mesocosm. For the six reduced mesozooplankton mesocosms, the contents of the dip net were back-flushed into the surrounding water. For the normal mesozooplankton mesocosms, the net was back-flushed into the mesocosm, returning any captured zooplankton. This ensured that all mesocosms would be exposed to the same treatment, but only one half would have mesozooplankton removed.

Temperature and salinity were measured near the surface and bottom of each mesocosm before any sampling or manipulation of treatments on every other day with a YSI Model 30 hand-held temperature and salinity meter. Nutrient additions were made at 4 day intervals on June 26, June 30, July 4 and July 8, 1999. Ammonium was added as NH_4Cl in a 1 M stock solution in an amount sufficient to raise the ammonium concentration by ~40 μM. Ammonium concentrations exceeded 15 μM in Laguna Madre in the winter of 1989–90, when the bloom first began (Buskey *et al.*, 1997). Immediately after ammonium addition, all mesocosms were mixed with a paddle. Water samples for nutrient analysis were collected at the beginning, middle and end of the experiment. Samples collected at the beginning of the experiment were taken immediately after nutrient addition and stirring in order to estimate the true concentration of nutrients added. Samples taken during the middle of the experiment were collected before additional nutrients were added that day to assess how much of previous additions had been utilized. No nutrient additions were made on the final day of the experiment when the last set of nutrient samples was collected. Water samples were collected in acid-rinsed polypropylene bottles and stored on ice until return to the laboratory. Water samples were collected for microzooplankton enumeration at 4 day intervals. Whole water samples were collected in 200 ml plastic jars and preserved with 5% acid Lugol's iodine. Whole water samples for flow cytometery counts were collected in acid-cleaned glass scintillation vials and preserved with 2% formaldehyde, and held in the dark at 5°C until enumerated.

Initial mesozooplankton samples were taken by towing a 30 cm diameter, 153 μm mesh plankton net equipped with a General Oceanics flow meter through the cooling pond by hand, in an area adjacent to where the mesocosms were placed. Subsequent mesozooplankton samples were collected by passing 8 l of sea water from each mesocosm through a 153 μm mesh sieve directly over the mesocosm so that the sample water was returned to the mesocosm. The contents of the sieve were then back-washed into a plastic bottle using filtered sea water and preserved with 5% formaldehyde.

Upon return to the laboratory, water samples for nutrient analysis were filtered through 0.45 μm polycarbonate filters (Poretics, Inc.) into 13 ml polystyrene tubes, capped and frozen until analysis. Nitrate + nitrite, phosphate, and ammonia were measured on a Lachat Quikchem 800 ion analyzer with computer-controlled sample selection and peak processing using the manufacturer's recommended chemistries with detection ranges as follows: nitrate + nitrate (0.03–5.0 μM; Quikchem method 31-107-04-1-A), ammonium (0.1–10 μM; Quikchem method 31-107-06-5-A) and phosphate (0.03–2.0 μM; Quikchem method 31-115-01-3-A).

Preliminary studies of the phytoplankton in the Laguna Madre and cooling pond near the time of our study using microscopy and an immumofluorescence method specific for *A. lagunensis* (Lopez-Barreiro *et al.*, 1998) revealed only two major populations: *A. lagunensis* and *Synechococcus* sp. Populations of *A. lagunensis* and *Synechococcus* sp. were enumerated using a Becton-Dickinson (San Jose, CA) FACSort flow cytometer equipped with a 488 nm 15 mW laser at a flow rate of 0.2 μl s^{-1}. Initial studies revealed two distinct clusters in cytometric plots with very few other small eukaryotic phytoplankton scattered outside the *A. lagunensis* cluster. Separation of *A. lagunensis* from other similarly sized eukaryotes was further facilitated by a pronounced fluorescence in the yellow channel, allowing for confidence in our ability to enumerate these two dominant populations using flow cytometry. Due to high cell concentrations, samples were diluted ten-fold with 0.2 μm filtered sea water prior to analysis to extend the measured volume to >10 μl, thereby increasing counting precision. Chlorophyll fluorescence was measured through a 650 nm long-pass filter, and the orange phycoerythrin fluorescence used to distinguish *Synechococcus* spp. was measured through a 585/42 nm band-pass filter. Changes in instrument sensitivity were monitored using 0.993 μm PC red plastic beads (Polysciences, Inc., Warrington, PA) added as an internal standard. Data analysis was performed using WinList® 3.0 software (Verity, Topsham, ME).

Microzooplankton from Lugol's-preserved samples were enumerated using an inverted microscope (Olympus IMT-2). Between 2 and 10 ml of sample (depending on protozoan density) were settled in Utermöhl chambers for enumeration (Gifford and Caron, 2000). Mesozooplankton from the initial net samples were subsampled with a plankton splitter and enumerated under a stereomicroscope (Postel *et al.*, 2000). For the 8 l samples from mesocosms, the entire sample was enumerated.

RESULTS

Temperature, salinity, nutrients and chlorophyll concentrations within the mesocosms were nearly identical to those in the surrounding cooling pond and those in the Laguna Madre where the waters originated at the beginning of the experiment and remained that way during and after the experiment (Table I). The mesocosm waters were slightly hypersaline at the beginning of the experiment (40 psu) and salinities increased slightly over the 16 days of the experiment (Figure 1). Temperatures in the mesocosms ranged from 30 to 32°C over the course of the experiment. On the first day, temperature was not measured until late afternoon, and there was a slight indication of stratification with surface temperatures slightly higher than bottom temperatures (Figure 1). However, stratification is rare in the shallow waters of the Laguna Madre, and was not evident in our mesocosms or in the cooling pond during the rest of the experiment. The sea breeze during summer is typically 20–30 km h^{-1}; this keeps the shallow Laguna Madre, cooling pond and mesocosms (all ~1 m average depth) well mixed.

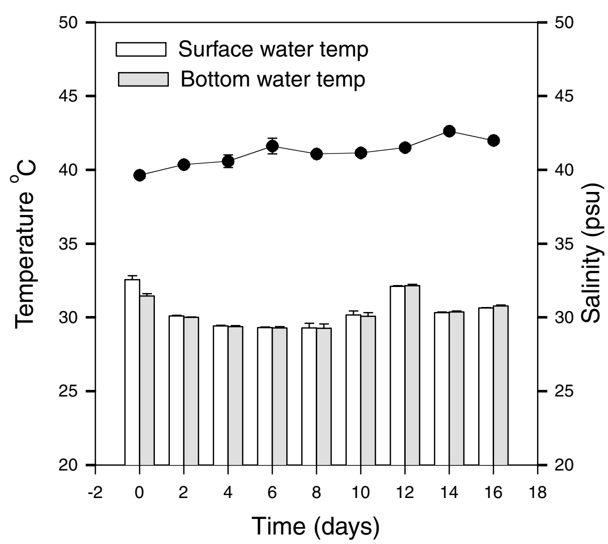

Fig. 1. Water temperature (surface and near bottom) and surface water salinity over the course of the experiments. Each value is the mean (± SD) of all 12 mesocosms on that day.

The ammonia addition was confirmed in N addition mesocosms (NZ-AA; RZ-AA) by increases from the ambient 1–2 μM concentration to 40–45 μM after the addition on the first day of the experiment (day 0; Figure 2). A second addition of ~40 μM of ammonium was made on day 4. When measured on day 8 before a third addition of ammonium, concentrations had decreased to 5–10 μM. This indicates that most of the 80 μM of

Table I: Comparison of physical and chemical characteristics of the Laguna Madre near the power plant intake (LM), the cooling pond (CP) and the mesocosm controls (MC) at 8 day intervals after the beginning of the experiment; a second comparison between Laguna Madre and the cooling pond was made 2 weeks after the end of the experiment

Date	Location	Temp (ºC)	Sal (psu)	NH_4 (μm)	NO_3 (μm)	PO_4 (μm)	Chl a (μgh^{-1})
6/26	LM	32.1	40	0.41	0.06	0.16	36.2
	CP	31.8	39.5	1.24	0.04	0.12	31.6
	MC	32.5	39.6	1.24	0.05	0.13	32.0
7/4	LM	30.1	39.7	–	–	–	–
	CP	29.7	42.9	0.83	0.14	0.13	42.8
	MC	29.3	42.0	1.5	0.24	0.19	38.5
7/12	LM	32.1	40.1	–	–	–	23.4
	CP	31.7	42.5	0.64	0.35	0.22	24.0
	MC	30.7	41.7	1.52	0.22	0.26	24.3
7/29	LM	32.0	40.5	*	0.37	0.07	10.7
	CP	31.9	41.1	1.3	0.55	0.24	8.4

–, no data are available; *, sample collected but nutrient was below detection limit.

Table II: Results of two-way ANOVA testing the effects of zooplankton removal and nutrient (ammonium) addition on the abundance of the two dominant mesozooplankton taxa A. tonsa and Oithona sp. for each of the five sampling dates during the mesocosm experiment

Day	*A. tonsa*			*Oithona* sp.		
	Zooplankton	Ammonium	Interaction	Zooplankton	Ammonium	Interaction
4	0.045*	0.478	0.142	0.669	0.404	0.404
8	0.011*	0.111	0.373	0.453	0.831	0.067
12	0.003*	0.111	0.294	0.363	0.511	0.178
16	0.003*	0.117	0.085	0.351	0.342	0.353

P values with an asterisk indicate significant differences at α = 0.05.

ammonium added on two previous occasions had been taken up by the benthic and planktonic communities. The subsequent additions of 40 μM ammonium on day 8 and day 12 resulted in residual concentrations of ~20 μM on day 16 (Figure 2). In non-addition mesocosms, ammonium concentration was <1.0 μm, with a slight decline evident in the control mesocosms (NZ-NA) from day 8 through day 16. Nitrate + nitrite increased in all mesocosms over the course of the experiment from <0.1 μM to 0.4–0.7 μM. This increase was also noted in the cooling pond water (Table I). Phosphate fluctuated slightly around 0.2 μM and showed no systematic difference between the mesocosms.

Mesozooplankton populations in the Laguna Madre are dominated by the copepod *A. tonsa* with this copepod typically comprising more than 80% of the holoplanktonic mesozooplankton (Buskey *et al.*, 1996), and the same pattern was found in the mesocosms and cooling ponds. Initial populations of *A. tonsa* in the cooling pond were ~10 l^{-1}. In control mesocosms (NZ-NA), there were ~50 l^{-1} on day 4, while those mesocosms from which zooplankton had been removed (RZ-NA) had a lower population of ~35 l^{-1} (Figure 3). *Acartia* populations were consistently higher in the ammonium-addition mesocosms without zooplankton removal (NZ-AA), increasing to over 100 *A. tonsa* per liter on day 8. Also, as expected, *Acartia* populations were consistently lower in ammonium-addition mesocosms in which the mesozooplankton were removed (RZ-AA), with less than 50% of the *Acartia* abundance found in the control mesocosms. *Acartia* populations showed a general de-crease in abundance on days 12 and 16 of the experiment. Two-way analysis of variance revealed that zooplankton removal had a significant effect on *A. tonsa* abundance ($P < 0.05$) on all sampling dates (Table II).

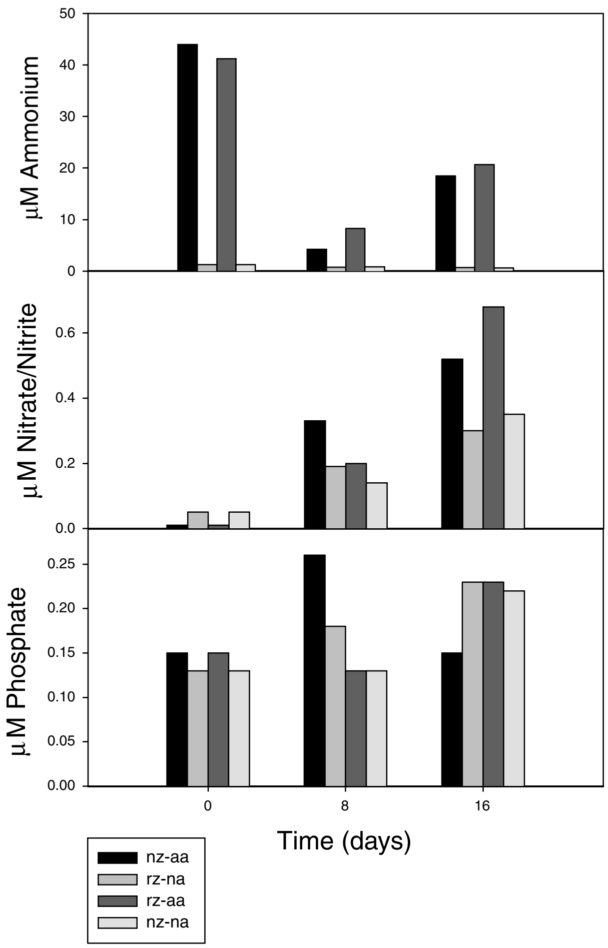

Fig. 2. Major nutrient concentrations (ammonium, nitrate + nitrite and phosphate) measured in water samples from mesocosms at the beginning, middle and end of the experiment. Each value is a mean for the three replicate mesocosms in that treatment. The four mesocosm treatment were: normal mesozooplankton, normal ammonium (NZ-NA); normal mesozooplankton, added ammonium (NZ-AA); reduced mesozooplankton, normal ammonium (RZ-NA) and reduced mesozooplankton, added ammonium (RZ-AA).

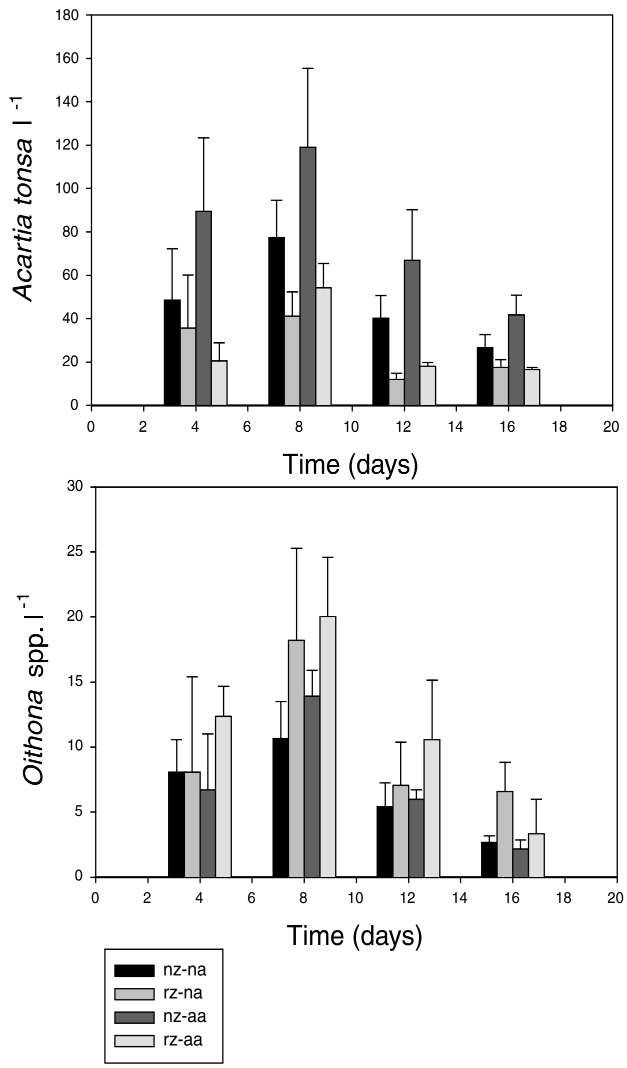

Fig. 3. Abundances of the two major mesozooplankton components of the mesocosms, the calanoid copepod *A. tonsa* (top) and the cyclopoid copepod *Oithona* spp (bottom). Each value is the mean (±SD) for the three replicate mesocosms in that treatment. The four mesocosm treatments were: normal mesozooplankton, normal ammonium (NZ-NA); normal mesozooplankton, added ammonium (NZ-AA); reduced mesozooplankton, normal ammonium (RZ-NA) and reduced mesozooplankton, added ammonium (RZ-AA).

The second most abundant copepod and mesozooplankton taxon was the cyclopoid copepod *Oithona* spp. These copepods are smaller than *A. tonsa*, and are not as efficiently captured with a 153 µm mesh net, especially the juvenile developmental stages. Initial populations of *Oithona* spp. in the cooling pond were $<1\ l^{-1}$, based on net tows taken on the first day. *Oithona* spp. also show a general increase in populations over the first 8 days of the experiment, but there is no consistent pattern of fewer *Oithona* spp. in the mesozooplankton removal mesocosms (RZ-NA) compared with the control mesocosms (NZ-NA). There did appear to be a consistent decrease in *Oithona* populations in nutrient-addition mesocosms (NZ-AA) compared with nutrient-addition mesocosms from which mesozooplankton have been removed (RZ-AA). There was also a general decline in *Oithona* spp. abundance on days 12 and 16 of the experiment (Figure 3). Two-way analysis of variance showed no significant effect of either zooplankton removal or ammonium addition on the abundance of *Oithona* spp. (Table II).

Table III: Results of two-way ANOVA testing the effects of zooplankton removal and nutrient (ammonium) addition on the abundance of ciliates and dinoflagellates (both heterotrophs and autotrophs) for each of the five sampling dates during the mesocosm experiment

Day	Ciliates			Dinoflagellates		
	Zooplankton	Ammonium	Interaction	Zooplankton	Ammonium	Interaction
0	0.236	0.892	0.910	0.266	0.651	0.505
4	0.007*	0.032*	0.886	0.417	0.268	0.261
8	0.019*	0.918	0.831	0.266	0.235	0.057
12	0.001*	0.091	0.064	0.542	0.044*	0.541
16	0.010*	0.409	0.388	0.267	0.001*	0.135

P values with an asterisk indicate significant differences at $\alpha = 0.05$.

Protozooplankton populations were very abundant in all mesocosms at the onset of the experiment, with an average of nearly 250 ciliates ml^{-1} (Figure 4). These samples contained large numbers of small hypotrichs which may have mixed into the water column from bottom sediments when the mesocosms were installed. When mesocosms were sampled again after 4 days, ciliate populations ranged between 25 and 50 ml^{-1}. The mesozooplankton removal mesocosms, which had fewer *A. tonsa*, had significantly more ciliates than those mesocosms from which mesozooplankton were not removed on all sample dates except for the first day of the study (two-way ANOVA, $P < 0.02$; Table III). These differences became quite pronounced by day 8. Following the increase in ciliates on days 8–12, there was a general decline in ciliate populations at the end of the experiment, with fewer than 100 ciliates ml^{-1} in all treatments. Dinoflagellates were enumerated from Lugol's iodine-preserved samples, so autotrophic and heterotrophic dinoflagellates could not be positively distinguished, but dinoflagellate populations appeared to be dominated by a small (~10 μm diameter) *Gymnodinium*-like dinoflagellate that can be common in the Laguna Madre. Initial dinoflagellate populations were between 600 and 700 dinoflagellates ml^{-1} in all mesocosm treatments (Figure 4). By the mid-point of the experiment, dinoflagellate populations had fallen below 200 cells ml^{-1} in all mesocosms, with populations in mesozooplankton-removal mesocosms only slightly greater than those in non-removal mesocosms. On the last two sampling dates, dinoflagellate populations were significantly higher in treatments without ammonium additions, compared with those to which ammonium had been added (two-way ANOVA, $P < 0.05$; Table III).

Initial populations of *A. lagunensis* were ~200 cells μl^{-1}, typical of the Laguna Madre during the brown-tide bloom (Buskey *et al.*, 1996). The population of *A. lagunensis* dropped by more than 50% during the first 4 days of the experiment in mesocosms without added nutrients (NZ-NA, RZ-NA) compared with either treatment in which ammonium was added (NZ-AA, RZ-AA) which showed little change in *A. lagunensis* population in the first 4 days (Figure 5). The *A. lagunensis* populations were significantly lower in mesocosms without added ammonium from day 4 through to the end of the experiment (two-way anova, $P \leq 0.005$; Table IV). After this initial period of decline of brown-tide populations, *A. lagunensis* populations grew at a slow but steady rate in all four mesocosm treatments, although specific growth rates were significantly higher in the mesocosms without added ammonium compared with those with added ammonium over three of the measured growth intervals (two-way ANOVA, $P \leq 0.033$; Figure 6, Table IV). Interestingly, even though mesozooplankton removal both decreased the number of mesozooplankton and increased the number of ciliates in mesocosms with zooplankton removal, this change in grazer populations appeared to have no significant effect on *A. lagunensis* populations (Table IV).

Initial populations of the cyanobacterium *Synechococcus* were comparable with the population of *A. lagunensis* at the onset of the experiment, ~200 cells ml^{-1} (Figure 5), but these populations represent a smaller fraction of the phytoplankton biomass due to their smaller size (cell diameter <2 μm). There was little change in *Synechococcus* populations over the first 4 days of the experiment, but populations increased rapidly between days 4 and 8 in all treatments. During the last three sampling periods, *Synechococcus* populations in the ammonium-addition mesocosms were significantly higher than those with ambient ammonium concentrations (two-way ANOVA, $P \leq 0.007$; Table IV). By the end of the experiment, *Synechococcus* had nearly quadrupled to 800 cells μl^{-1} in the ammonium-addition mesocosms with normal mesozooplankton populations (NZ-AA). *Synechococcus* populations were generally lower in ammonium-addition mesocosms with mesozooplankton removed (RZ-AA), which had larger populations of ciliates (Figure 4) which are potential grazers on *Synechococcus*. However, zooplankton removal only had a significant effect on *Synechococcus* abundance on day 12 (two-way ANOVA, $P = 0.037$; Table IV). Specific growth rates for *Synechococcus* were ~0.1 day^{-1} in the control mesocosms without nutrient additions and ~0.2 day^{-1} in the ammonium-addition mesocosms between days 4 and 8 (Figure 6).

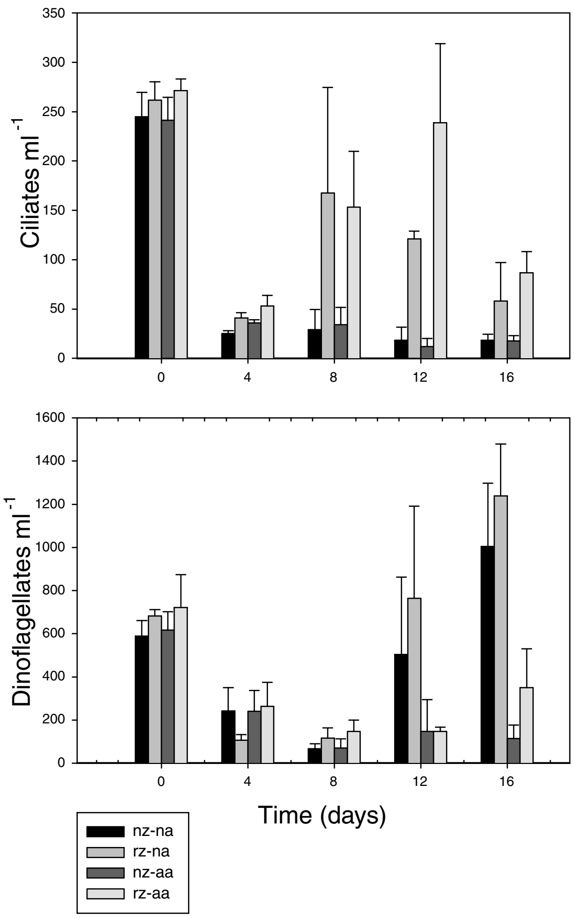

Fig. 4. Abundances of the two major components of the microzooplankton in the mesocosms, ciliates (top) and dinoflagellates, including both autotrophic and heterotrophic forms (bottom). Each value is the mean (±SD) for the three replicate mesocosms in that treatment. The four mesocosm treatments were: normal mesozooplankton, normal ammonium (NZ-NA); normal mesozooplankton, added ammonium (NZ-AA); reduced mesozooplankton, normal ammonium (RZ-NA) and reduced mesozooplankton, added ammonium (RZ-AA).

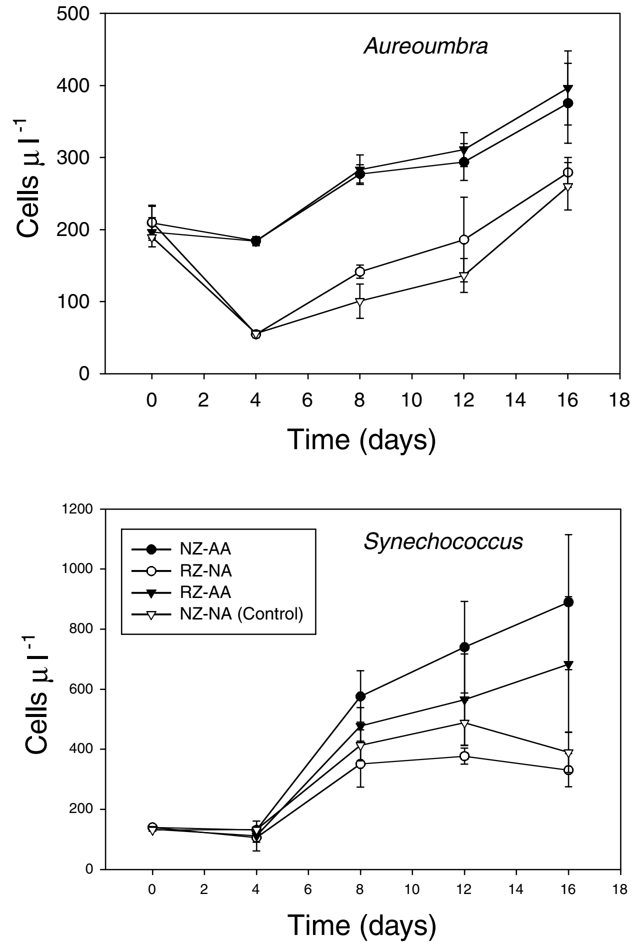

Fig. 5. Abundance of the brown tide alga *A. lagunensis* and the blue-green alga *Synechococcus* spp. in our mesocosms, based on analysis with a flow cytometer. Each value is the mean (±SD) for the three replicate mesocosms in that treatment. The four mesocosm treatments were: normal mesozooplankton, normal ammonium (NZ-NA); normal mesozooplankton, added ammonium (NZ-AA); reduced mesozooplankton, normal ammonium (RZ-NA) and reduced mesozooplankton, added ammonium (RZ-AA).

DISCUSSION

There has been a general increase in the frequency and severity of harmful algal blooms worldwide (Anderson, 1989; Smayda, 1989; Hallegraeff, 1993). During a nearly 8 year period from December 1989 through October 1997 the planktonic food web of the Laguna Madre of Texas was dominated by a high biomass of a single species of phytoplankton, the pelagophyte *A. lagunensis*. One possible explanation for the unusual persistence of this bloom is 'bottom–up' control of phytoplankton biomass through nutrient supply and control of species composition through competition (Tilman, 1976; Sommer, 1985). The Laguna Madre was often characterized by relatively clear, low phytoplankton biomass waters prior to the brown-tide bloom, and this semi-enclosed lagoon might seem to be an unlikely location for a high biomass phytoplankton

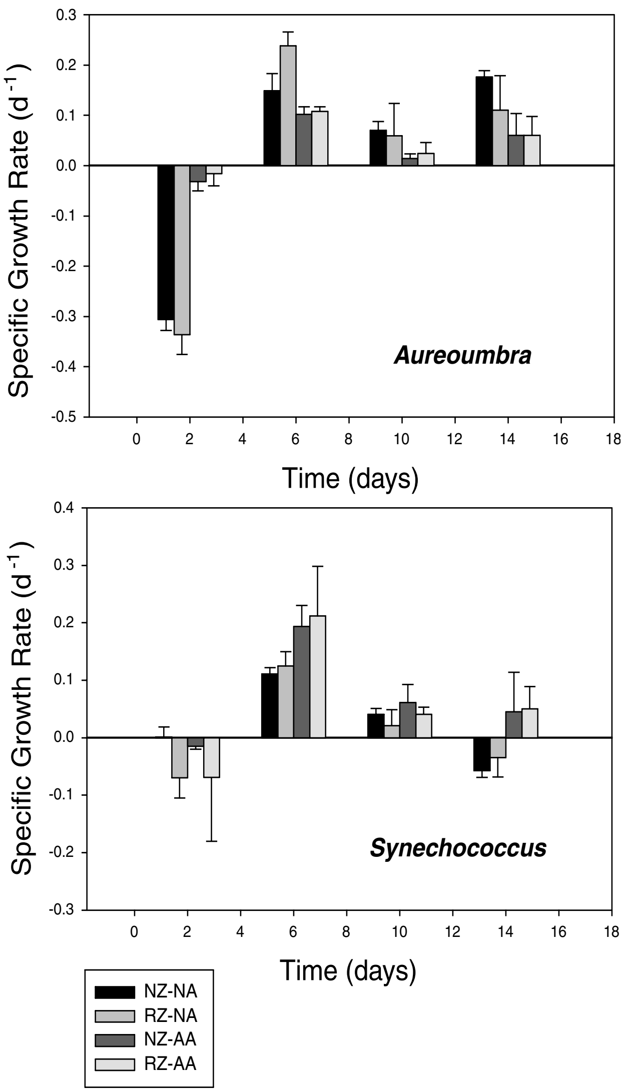

Fig. 6. Specific growth (loss) rates for *Aureoumbra lagunensis* and *Synechococcus* sp based on flow cytometer counts from our mesocosms. Each value is the mean for the three replicate mesocosms in that treatment. The four mesocosm treatment were: normal mesozooplankton, normal ammonium (NZ-NA); normal mesozooplankton, added ammonium (NZ-AA); reduced mesozooplankton, normal ammonium (RZ-NA) and reduced mesozooplankton, added ammonium (RZ-AA).

bloom, since there are no permanent rivers to provide a continual source of new nutrients to this system. This system received a large input of nutrients due to an extensive fish kill near the beginning of this bloom (Whitledge, 1993; DeYoe and Suttle, 1994) and it is possible, although unlikely, that this extended bloom was continually fueled in part by recycled nutrients from this initial pulse. Seawater exchange between the Laguna Madre and adjacent waters is difficult to estimate but is thought to be very low; turnover times for the upper Laguna Madre are estimated to exceed 1 year (Shormann, 1992). During this mesocosm experiment, *A. lagunensis* populations did not appear to be controlled from the 'bottom–up', however. Growth rates of *A. lagunensis* were lower for mesocosms to which ammonium had been added from day 4 through to the end of the experiment, although there was an unexplained decline in cell numbers in tanks without ammonium additions over the first 4 days of the experiment (Figure 5, Table IV). Ammonium additions did stimulate growth of *Synechococcus* (Figure 6), however.

The dominance of *A. lagunensis* during this extended bloom may have been partly a result of this species' ability to grow well in the harsh environmental and unusual nutrient conditions that sometimes exist in the Laguna Madre. Natural populations of *A. lagunensis* have been found to have high alkaline phosphatase activity and N:P ratios of ~70, (considerably higher than the Redfield ratio value of 16) when ambient inorganic phosphate concentrations were very low (Villareal *et al.*, 1998). This species has also been shown to have very low phosphorus requirements in laboratory studies (Liu *et al.*, 2001) which might give it a competitive advantage in this potentially phosphorus-limited coastal lagoon environment (Smith and Atkinson, 1984). *Aureoumbra lagunensis* is also particularly well adapted for living in the often hypersaline conditions of the Laguna Madre; it grows at its maximum rate in salinities up to 60 psu (Buskey *et al.*, 1998) and produces a thick coating of extracellular polymeric substances, especially under hypersaline conditions, that might help buffer cells from salinity stress (Liu and Buskey, 2000a). Conditions during our mesocosm experiment were only slightly hypersaline at about 40 psu (Figure 1) so its adaptations for growing under extreme hypersaline conditions may not have conferred a competitive advantage on *A. lagunensis*. Salinities often ranged between 50 and 70 psu in areas of Laguna Madre with the densest populations of *A. lagunensis* (Buskey *et al.*, 1998).

The extraordinary persistence of the brown-tide bloom in Laguna Madre could also be due to 'top–down' control of phytoplankton species composition through selective grazing on other species of phytoplankton. The dominant mesozooplankter in the Laguna Madre, *A. tonsa*, does not appear to be an important grazer on *A. lagunensis*. At ~4–5 μm diameter, *A. lagunenis* is outside the preferred size range of food particles for *A. tonsa* (Berggreen *et al.*, 1988). When offered a diet of *A. lagunensis*, *A. tonsa* females have similar egg production rates to starved individuals, suggesting that very little *A. lagunensis* is ingested and assimilated (Buskey and Hyatt, 1995). The *A. lagunensis* appears to be difficult for copepods to digest, due to a thick extracellular polysaccharide mucus layer (Liu and Buskey, 2000a), and many cells that are ingested are still viable after passing through copepod guts (Bersano *et al.*,

Table IV: Results of two-way ANOVA testing the effects of zooplankton removal and nutrient (ammonium) addition on the abundance of the two dominant phytoplankton species in the mesocosms, A. lagunensis and Synechococcus sp. for each of the five sampling dates during the mesocosm experiment

Day	*Aureoumbra*			*Synechococcus*		
	Zooplankton	Ammonium	Interaction	Zooplankton	Ammonium	Interaction
Cell density						
0	0.750	0.787	0.174	0.697	0.736	0.061
4	0.799	<0.001*	0.927	0.170	0.872	0.847
8	0.060	<0.001*	0.146	0.08	0.007*	0.663
12	0.147	<0.001*	0.458	0.037*	0.005*	0.591
16	0.682	0.005*	0.748	0.094	<0.001*	0.323
Specific growth rate						
0–4	0.706	<0.001*	0.276	0.169	0.884	0.830
4–8	0.025*	<0.001*	0.041*	0.661	0.039*	0.959
8–12	0.954	0.101	0.676	0.262	0.263	0.993
12–16	0.337	0.033*	0.342	0.677	0.018*	0.800

P values with an asterisk indicate significant differences at $\alpha = 0.05$.

2002). Even earlier developmental stages of *A. tonsa* do not seem to feed on *A. lagunensis*; nauplii will not survive until metamorphosis to copepodid on a diet of *A. lagunensis* (Buskey and Hyatt, 1995). *Acartia tonsa* and other mesozooplankton species would be expected to prey preferentially on larger cells, including diatoms, dinoflagellates and ciliates, and thereby affect size distribution and species composition of phytoplankton (Ryther and Sanders, 1980).

The role of grazers, including zooplankton and benthic organisms, in the population dynamics of harmful blooms remains uncertain, however. In freshwater lakes there is clear evidence for 'top–down' regulation of primary productivity (Carpenter *et al.*, 1985; Sterner, 1986), and there are some examples for marine environments (Riemann *et al.*, 1988; Granéli *et al.*, 1993). Algal blooms are considered to undergo several distinct phases in their population dynamics including initiation, growth, maintenance and dissipation (Steidinger *et al.*, 1998). There are several studies suggesting that a disruption of grazers may aid in the initiation of algal blooms (Smayda and Villareal, 1989; Buskey *et al.*, 1997) and other studies have suggested that grazers may be capable of preventing blooms in their early stages (Uye, 1986). However, the evidence that grazers can affect the growth or maintenance phases of blooms remains equivocal. While some studies indicate that some zooplankton species avoid grazing on algal species that form harmful algal blooms (Ives, 1985; Huntley *et al.*, 1986) other studies show no effects of toxic phytoplankton species on zooplankton grazers (Mallin *et al.*, 1995). There have been few direct studies of the roles of grazers in the dissipation phase of blooms, but it seems reasonable that if other factors such as nutrient limitation are already leading to the decline of a bloom, grazers may help to hasten that decline.

Higher trophic levels could also indirectly affect the persistence of the brown-tide bloom through alterations of the planktonic food web. Protozoan grazers, with their higher growth rates, could respond more quickly to harmful algal blooms and potentially have an important role in bloom control (Nakamura *et al.*, 1992; Jeong and Latz, 1994). It had been hypothesized that at least part of the explanation for the persistence of the brown-tide bloom in the Laguna Madre might be due to increased predation on protozoan grazers by copepods, releasing small-sized phytoplankton from grazer control (Buskey and Stockwell, 1993). *Acartia tonsa* has long been known to feed on protozoa (Stoecker and Egloff, 1987; Gifford and Dagg, 1988) and those mesocosms with fewer *A. tonsa* had more than twice as many ciliates (Figure 4). Even with higher populations of protozoan grazers, these mesocosms did not have significantly fewer *A. lagunensis* than those with more potential grazers (Figure 5, Table IV). Previous studies have demonstrated that several species of ciliates will not survive and grow on diets of *A. lagunensis*, while other species of ciliate such as *Euplotes* sp. and the heterotrophic dinoflagellate *Oxyrrhis marina* can be grown on cultures of *A. lagunensis* (Buskey and Hyatt, 1995). Some ciliates can feed on *A. lagunensis* when it makes up a small fraction of the total phytoplankton population, but their growth and grazing rates decline as *A. lagunensis* increases in relative abundance (Jakobson *et al.*, 2001). Therefore, even though the total population of protozoan grazers was increased in some mesocosms, there may not have been sufficient time for the development of populations of protozoa that graze preferentially on brown tide. It could also be argued that increased numbers of grazers would liberate additional nutrients which could fuel additional phytoplankton growth that might help offset the direct impact of grazers. However, since large ammonium additions in half the mesocosms had little impact on brown-tide growth (Figure 6), this possibility seems unlikely.

The increase in protozoan grazers in mesozooplankton-removal mesocosms did have an impact on populations of the cyanobacterium *Synechococcus* sp., however. Protozoan grazers may have preferentially grazed on *Synechococcus* over *Aureoumbra*. Populations of *Synechococcus* were consistently higher in mesocosms with normal mesozooplankton populations, and hence lower ciliate and heterotrophic nanoflagellate populations. *Synechococcus* came to dominate the phytoplankton populations numerically by the end of our experiment. It has become an important component of the Laguna Madre phytoplankton community since the demise of the extended 1989–1997 bloom (Buskey *et al.*, 2001). *Synechococcus* is also a dominant component of the phytoplankton in parts of Florida Bay, a shallow subtropical system similar to Laguna Madre in several ways (Lavrentyev *et al.*, 1998).

The mesocosms themselves may have had some unexpected effects on the planktonic food web as well. Copepod populations increased in all mesocosm treatments for the first 8 days of the experiment (Figure 3). The *A. tonsa* population density found in the pond at the beginning of the mesocosm experiment ($\sim 10\ l^{-1}$) is within the range of *Acartia* densities normally found in the Laguna Madre and nearby Corpus Christi Bay (Buskey, 1993; Buskey *et al.*, 1996). However, by days 4 and 8 of the experiment, *Acartia* populations were well above normal in the mesocosms without zooplankton removal, especially in the mesocosms with nutrient additions (Figure 3). Several factors may have contributed to these high *Acartia* densities. One factor may have been the difference in sampling methods. Since the mesocosms were stirred before sampling, and *A. tonsa* is known to migrate vertically and remain near the bottom during the day, it may be reasonable to expect more *Acartia* during mesocosm sampling. Previous studies have found about twice as many *Acartia* at night as during the day in surface net tows collected in Corpus Christi Bay near the Laguna Madre, although little difference was found in shallower stations in Nueces Bay (Buskey, 1993). Another possible explanation is that important predators on these copepods, such as gelatinous zooplankton or juvenile and larval fish, were excluded by the mesocosms. Ctenophores and other gelatinous zooplankton have been shown to be important predators on copepods in temperate marine systems (Kremer, 1979; Deason and Smayda, 1982) although gelatinous zooplankton tend to exhibit both temporal and spatial patchiness of distribution in Texas coastal bays (Buskey, 1993). However, larval fish are usually considered too dilute in the environment to affect the density of their prey (Cushing, 1983; Bollens, 1988; Dagg and Govoni, 1996). Another possibility is that buried diapause eggs of *Ac. tonsa* were mixed to the surface when the mesocosms were installed (Marcus, 1984; Marcus and Taulbee, 1992); at 30°C, it would be expected to take about 3 days for these eggs to develop to the first copepodid stage (Heinle, 1966; Miller *et al.*, 1977), so this factor could have an impact within 4 days.

Another potential effect that might be attributed to the installation of the mesocosms was the rapid decline in *A. lagunensis* populations during the first 4 days of the experiment for mesocosms without nutrient addition, with only a slight decline in ammonium addition mesocosms. The reason for this decline is unknown, but added nutrients appeared to minimize the unknown stress associated with mesocosm installation. Addition of ammonium to mesocosms had little or no positive effect on the growth rate of the brown tide (Figure 6) indicating that *A. lagunensis* cells were not nitrogen limited. Over the second and fourth sampling intervals, *A. lagunensis* grew significantly faster in mesocosms without nutrient addition (Figure 6, Table IV). In contrast, *Synechococcus* populations seemed to benefit from ammonium additions, with significantly higher populations in mesocosms with added ammonium by day 12 of the experiment (Figure 5) and significantly higher growth rates over the second and fourth sampling intervals (Figure 6, Table IV). Based on declines in ammonium concentration following our additions, it is clear that nutrient additions were being taken up by some component of the food web (Figure 2).

The mesocosm experiments described in this study were designed to examine the roles of 'top–down' and 'bottom–up' controls of brown-tide population dynamics in the natural environment, and to test specifically for the possibility of a trophic cascade of increased mesozooplankton predation on microzooplankton grazers contributing to the persistence of the bloom. These experiments provided no evidence for 'bottom–up' control of *A. lagunensis* populations. While mesocosms with nutrient additions had more brown-tide cells at the end of the experiment, this was due only to an unexplained decline in their populations during the first few days following installation of the mesocosms. Growth rates of *A. lagunensis* were higher in the mesocosms without nutrient additions. Similarly, the zooplankton removal mesocosms did not clearly demonstrate 'top–down' controls on *A. lagunensis* populations. Mesocosms with reduced mesozooplankton populations resulted in significantly higher ciliate populations, but this increase in ciliates did not result in significantly lower *A. lagunensis* populations. In contrast, populations of *Synechococcus* showed evidence of both 'bottom–up' and 'top–down' controls in these experiments. *Synechococcus* grew significantly faster and ended with higher populations in nutrient addition mesocosms, indicating 'bottom–up' control. However, mesozooplankton removal and the resulting higher ciliate populations produced lower *Synechococcus* populations compared with mesocosms with unmanipulated zooplankton populations, also indicating the potential for 'top–down' control. The inability of increased ciliate populations to control *A. lagunensis* could be related to its general unpalatability to a wide range of protozoan grazers (Buskey and Hyatt, 1995). The results of these experiments suggest that the extraordinary persistence of *A. lagunensis* during its extended bloom in the 1990s may have been due primarily to the unique adaptations of this species to the harsh environment of the Laguna Madre. The thick covering of extracelluar polymeric substance on the surface of brown-tide cells not only allows it to grow under extreme hypersaline conditions (Liu and Buskey, 2000a) but has also been shown to inhibit grazing by ciliates (Liu and Buskey 2000b). The low phosphorus requirements of *A. lagunensis* may also give it a substantial competitive advantage over other phytoplankton species in this potentially phosphorus-limited coastal lagoon (Villareal *et al.*, 1998).

ACKNOWLEDGEMENTS

This research was supported by the Texas Higher Education Coordinating Board under grant 003599-012 and by the National Science Foundation under grant OCE-9529750. We wish to thank Bill Beck, Gary Clark and Howard Fels from the Central Power and Light Company's Barney Davis Power Station for assisting with arrangements and allowing us to carry out this experiment on their property. David Abgrego of the GCCA/CPL Marine Development Center provided temperature and salinity data from the Laguna Madre at the power plant intake. This is University of Texas Marine Science Institute Contribution Number 1224.

REFERENCES

Anderson, D. M. (1989) Toxic algal blooms and red tides: a global perspective. In Okaichi, T., Anderson, D. M. and Nemoto, T. (eds), *Red Tides: Biology, Environmental Science and Toxicology.* Elsevier, New York, pp. 11–16.

Armstrong, N. E. (1987) The ecology of open-bay bottoms of Texas: a community profile. *U.S. Fish. Wildl. Serv. Biol. Rep.*, **85** (7.12).

Berggreen, U., Hansen, B. and Kiørboe, T. (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Mar. Biol.*, **99**, 341–352.

Bersano, J. G. F., Buskey, E. J. and Villareal, T. A. (2002) Viability of the Texas brown tide alga *Aureoumbra lagunensis*, in fecal pellets of the copepod *Acartia tonsa*. *Plank. Biol. Ecol.*, **49**, 88–92.

Bollens, S. M. (1988) A model of the predatory impact of larval marine fish on the population dynamics of their zooplankton prey. *J. Plankton Res.*, **10**, 887–906.

Buskey, E. J. (1993) Annual pattern of micro- and mesozooplankton abundance and biomass in a subtropical estuary. *J. Plankton Res.*, **15**, 907–924.

Buskey, E. J. and Hyatt, C. J. (1995) Effects of the Texas (USA) 'brown tide' alga on planktonic grazers. *Mar. Ecol. Prog. Ser.*, **126**, 285–292.

Buskey, E. J. and Stockwell, D. A. (1993) Effects of a persistent 'brown tide' on zooplankton populations in the Laguna Madre of South Texas. In Smayda, T. J. and Shimizu, Y. (eds), *Toxic Phytoplankton Blooms in the Sea, Proceedings Fifth International Conf. Toxic Marine Phytoplankton*. Elsevier Science Publishers, Amsterdam, pp. 659–666.

Buskey, E. J., Stewart, S., Peterson, J. and Collumb, C. (1996) Current status and historical trends of brown tide and red tide phytoplankton blooms in the Corpus Christi Bay National Estuary Program study area. CCBNEP-07. Corpus Christi, Texas.

Buskey, E. J., Montagna, P. A., Amos, A. F. and Whitledge, T. E. (1997) The initiation of the Texas brown tide algal bloom: disruption of grazer populations as a contributing factor. *Limnol. Oceanogr.*, **42**, 1215–1222.

Buskey, E. J., Wysor, B. and Hyatt, C. (1998) The role of hypersalinity in the persistence of the Texas 'brown tide' bloom in the Laguna Madre. *J. Plankton Res.*, **20**, 1553–1565.

Buskey, E. J., Villareal, T. A. and Lopez-Barreiro, T. (1999) Reconstructing the initiation of the Texas brown tide bloom of *Aureoumbra lagunensis* from archived samples using an immunofluorescence assay. *Plankton Biol. Ecol.*, **46**, 159–161.

Buskey, E. J., Liu, H., Collumb, C. and Bersano, J. (2001) The decline and recovery of a persistent Texas brown tide algal bloom in the Laguna Madre (Texas, USA). *Estuaries*, **24**, 337–346.

Carpenter, S. R., Kitchell, J. F. and Hodgson, J. R. (1985) Cascading trophic interactions and lake productivity. *Bioscience*, **35**, 634–639.

Cushing, D. H. (1983) Are fish larvae too dilute to affect the density of their prey? *J. Plankton Res.*, **5**, 847–854.

Dagg, M. J. and Govoni, J. J. (1996) Is icthyoplankton predation an important source of copepod mortality in subtropical coastal waters? *Mar. Freshwater Res.*, **47**, 137–144.

Deason, E. E. and Smayda, T. J. (1982) Ctenophore-zooplankton-phytoplankton interactions in Narragansett Bay, Rhode Island, USA during 1972–1977. *J. Plankton Res.*, **4**, 203–217.

DeYoe, H. R. and Suttle, C. A. (1994) The inability of the Texas 'brown tide' alga to utilize nitrate and the role of nitrogen in the initiation of a persistent bloom of this organism. J. *Phycol.*, **30**, 800–806.

Gifford, D. J. and Caron, D. A. (2000) Sampling, preservation, enumeration and biomass of marine protozooplankton. In Harris, R. P., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds), *Zooplankton Methodology Manual*. Academic Press, London, pp. 193–221.

Gifford, D. J. and Dagg, M. J. (1988) Feeding of the estuarine copepod *Acartia tonsa* Dana: carnivory vs. herbivory in natural microplankton assemblages. *Bull. Mar. Sci.*, **43**, 458–468.

Granéli, E., Olsson, P., Carlsson, P., Granéli, W. and Nylander, C. (1993) Weak 'top-down' control of dinoflagellate growth in the coastal Skagerrak. *J. Plankton Res.*, **15**, 213–237.

Hallegraeff, G. M. (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia*, **32**, 79–99.

Heinle, D. R. (1966) Production of a calanoid copepod, *Acartia tonsa*, in the Patuxent River Estuary. *Chesapeake Sci.*, **7**, 59–74.

Huntley, M. E., Sykes, P., Rohan, S. and Marin, V. (1986) Chemically-mediated rejection of dinoflagellate prey by the copepods *Calanus pacificus* and *Paracalanus parvus*: mechanism, occurrence and significance. *Mar. Ecol. Prog. Ser.*, **28**, 105–120.

Ives, J. D. (1985) The relationship between *Gonyaulax tamarensis* cell toxin levels and copepod ingestion rates. In Anderson, D. M., White, A. W. and Baden, D. G. (eds), *Toxic Dinoflagellates*. Elsevier, Amsterdam, pp. 413–418.

Jakobsen, H. H., Hyatt, C. J. and Buskey, E. J. (2001) Grazing by the tintinnid *Amphorides quadraliniatus* on the 'Texas brown tide' forming alga *Aureoumbra lagunensis*. *Aquat. Microb. Ecol.*, **23**, 245–252.

Jeong, H. J. and Latz, M. I. (1994) Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. *Mar. Ecol. Prog. Ser.*, **106**, 173–185.

Kremer, P. (1979) Predation by the ctenophore *Mnemiopsis leidyi* in Narragansett Bay, Rhode Island. *Estuaries*, **2**, 97–105.

Lavrentyev, P. J., Bootsma, H. A., Johengen, T. H., Cavaletto, J. F. and Garner, W. S. (1998) Microbial plankton response to resource limitation: insights from the community structure and seston stoichiometry in Florida Bay, USA. *Mar. Ecol. Prog. Ser.*, **165**, 45–57.

Liu, H. and Buskey, E. J. (2000a) Hypersalinity enhances extracellular polymeric substance (EPS) production of the Texas brown tide alga *Aureoumbra lagunensis*. *J. Phycol.*, **35**, 1–7.

Liu, H. and Buskey, E. J. (2000b) The extracellular polymeric substance (EPS) layer surrounding *Aureoumbra lagunensis* cells reduces grazing by protozoa. *Limnol. Oceanogr.*, **45**, 1187–1191.

Liu, H., Laws, E. A., Villareal, T. and Buskey, E. J. (2001) Nutrient limited growth of *Aureoumbra lagunensis*, with implications for its capacity to outgrow other phytoplankton species in phosphate-limited environments. *J. Phycol.*, **37**, 500–508.

Lopez-Barreiro, T., Villareal, T. A. and Morton, S. A. (1998) Development of an antibody against the Texas Brown Tide (*Aureoumbra lagunensis*). In Reguera, B., Blanco, J., Fernadez, M. L. and Wyatt, T. (eds), *Harmful Algae*. Xunta de Galicia and International Oceanographic Commission of UNESCO, Vigo, Spain, pp. 263–265.

Mallin, M. M., Burkolder, J. M., Larsen, L. M. and Glasgow, H. B. Jr (1995) Response of two zooplankton grazers to an ichthyotoxic estuarine dinoflagellate. *J. Plankton Res.*, **17**, 351–363.

Marcus, N. M. (1984). Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. *Mar. Ecol. Prog. Ser.*, **15**, 47–54.

Marcus, N. M. and Taulbee, K. (1992) Potential effects of a resuspension event on the vertical distribution of copepod eggs in the sea bed: a laboratory simulation. *Mar. Biol.*, **114**, 249–251.

Miller, C. B., Johnson, J. K. and Heinle, D. R. (1977) Growth rules in the marine copepod genus *Acartia*. *Limnol. Oceanogr.*, **22**, 326–335.

Nakamura, Y., Yamazaki, Y. and Hiromi, J. (1992) Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, feeding on a red tide flagellate, *Chattonella antiqua*. *Mar. Ecol. Prog. Ser.*, **82**, 275–279.

Onuf, C. P. (1996) Seagrass responses to long-term light reduction by brown tide in upper Laguna Madre, Texas: distribution and biomass patterns. *Mar. Ecol. Prog. Ser.*, **138**, 219–231.

Postel, L., Fock, H. and Hagen, W. (2000) Biomass and abundance. In Harris, R. P., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds), *Zooplankton Methodology Manual*. Academic Press, London, pp. 83–192.

Riemann, B., Nielsen, T. G., Horsted, S. J., Bjørnsen, P. K. and Pock-Steen, J. (1988) Regulation of phytoplankton biomass in estuarine enclosures. *Mar. Ecol. Prog. Ser.*, **3**, 279–283.

Ryther, J. H. and Sanders, J. G. (1980) Experimental evidence of zooplankton control of the species composition and size distribution of marine phytoplankton. *Mar. Ecol. Prog., Ser.*, **3**, 279–283.

Shormann, D. E. (1992) *The Effects of Freshwater Inflow and Hydrography on the Distribution of Brown Tide in South Texas*. MA Thesis. Department of Marine Science, The University of Texas at Austin, 112 pp.

Smayda, T. J. (1989) Primary production and the global epidemic of phytoplankton blooms in the sea: a linkage? In Cosper, E. M., Bricelj, V. M. and Carpenter, E. J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer Verlag, Berlin, pp. 449–483.

Smayda, T. J. and Villareal, T. A. (1989) The 1989 'brown-tide' and the open phytoplankton niche in Narragansett Bay during summer. In Cosper, E. M., Bricelj, V. M. and Carpenter, E. J. (eds), *Novel Phytoplankton Blooms. Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 159–187.

Smith, S. V. and Atkinson, M. J. (1984) Phosphorus limitation of net production in a confined aquatic ecosystem. *Nature*, **207**, 626–627.

Sommer, U. (1985) Comparison between steady state and non-steady state competition: experiments with natural phytoplankton. *Limnol. Oceanogr.*, **30**, 335–346.

Steidinger, K. A., Vargo, G. A., Tester, P. A. and Tomas, C. R. (1998) Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. (eds), *Physiological Ecology of Harmful Algal Blooms*. NATO ASI Series, Vol. 41, Springer Verlag, Berlin, pp. 133–153.

Sterner, R. W. (1986) Herbivores' direct and indirect effects on algal populations. *Science*, **231**, 605–607.

Stoecker, D. K. and Egloff, D. A. (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. *J. Exp. Mar. Biol. Ecol.*, **110**, 53–68.

Tilman, D. (1976) Ecological competition between algae: experimental confirmation of resource-based competition theory. *Science*, **192**, 463–465.

Uye, S. (1986) Impact of copepod grazing on the red-tide flagellate *Chattonella antiqua*. *Mar. Biol.*, **92**, 35–43.

Villareal, T. A., Mansfield, A. and Buskey, E. J. (1998) Growth and chemical composition of the Texas brown tide-forming pelagophyte *Aureoumbra lagunensis*. In Reguera, B., Blanco, J., Fernandez, M. L. and Wyatt, T. (eds), *Harmful Algae*. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo, Spain pp. 359–362.

Whitledge, T. E. (1993) The nutrient and hydrographic conditions prevailing in Laguna Madre, Texas before and during a brown tide bloom In Smayda, T. J. and Shimizu, Y. (eds), *Toxic Phytoplankton Blooms in the Sea. Proceedings 5th International Conference on Toxic Marine Phytoplankton*. Elsevier Science Publishers, Amsterdam, pp. 711–716.

Received on February 8, 2001; accepted on October 24, 2002