Limnol. Oceanogr., 45(5), 2000, 1187–1191
© 2000, by the American Society of Limnology and Oceanography, Inc.

The exopolymer secretions (EPS) layer surrounding *Aureoumbra lagunensis* cells affects growth, grazing, and behavior of protozoa

Abstract—"Texas brown tide", a nuisance algal bloom formed by the marine pelagophyte *Aureoumbra lagunensis,* persisted without interruption from 1990–1997 in Laguna Madre, Texas. We hypothesize that, in addition to other factors, the presence of a mucus layer of exopolymer secretions (EPS) outside its cell wall may have played a role in reducing microzooplankton grazing on *A. lagunensis,* and therefore contributing to the persistence of the brown tide bloom. Three protozoan species common to the bloom waters fed *A. lagunensis* with low or high EPS showed significantly reduced growth rates with a high EPS diet. An additional experiment with the ciliate *Aspidisca* sp. revealed that the lower growth rate was caused by a reduced grazing rate on *A. lagunensis* with high EPS. *A. lagunensis* cells with high EPS might be unpalatable to *Aspidisca,* hence forced it to feed on bacteria as an alternate food source. Also, the EPS mucus might have affected the feeding mechanics of ciliates by adhering to their cilia and clogging their feeding apparatus. Motion analysis of swimming behavior of *Aspidisca* fed high and low EPS *A. lagunensis* revealed altered behavior in the presence of high EPS that could affect their grazing efficiency.

A nuisance algal bloom called the "Texas brown tide" persisted without interruption from 1990–1997 in Laguna Madre, Texas. The bloom was formed by *Aureoumbra lagunensis* (Stockwell, DeYoe, Hargraves, and Johnson), a small pelagophyte that shares some similarities to the species that forms brown tides along the coast of Long Island (DeYoe et al. 1997). It has been suggested that hypersaline conditions in the Laguna Madre may favor brown tide blooms (Buskey et al. 1998). Several lines of evidence support this hypothesis. First, *A. lagunensis* can grow at its maximum rate at salinities as high as 70 PSU (Buskey et al. 1998). Second, fewer phytoplankton species were found under hypersaline conditions, hence there were few potential competitors. Third, the high salinity caused a decline of microzooplankton and benthic filter-feeders; therefore, grazing pressure was reduced in hypersaline waters (Buskey et al. 1997). *A. lagunensis* possesses a mucus layer of exopolymeric secretions (EPS) outside its cell wall (DeYoe et al. 1997). Liu and Buskey (2000) recently reported that *A. lagunensis* produces more EPS under hypersaline conditions. They also found that total carbohydrates in the stationary and declining phases of the cultures were several times higher than for cultures in the exponential growth phase, mainly due to the high abundance of so-called slime-EPS, material disassociated or loosened from aged cells. These findings lead us to hypothesize that the increased EPS mucus layer surrounding *A. lagunensis* cells may prevent effective grazing by microzooplankton. Previous studies (Buskey and Hyatt 1995) found lower growth rates of protozoa fed *A. lagunensis* compared to other similarly sized algal species, but the mechanism causing the reduced growth was unknown.

We used three species of protozoa fed *A. lagunensis* cells with low or high EPS mucus layer, to test our hypothesis. All three species are common components of the microzooplankton community in the brown tide impacted waters of the Laguna Madre. The heterotrophic dinoflagellate *Oxyrrhis marina* was isolated from the Aransas Ship Channel near the University of Texas Marine Science Institute in Port Aransas as described in Buskey et al. (1998). The two species of ciliates, *Euplotes* sp. and *Aspidisca* sp., were isolated near the Kennedy Causeway that spans the connection between the Laguna Madre and Corpus Christi Bay. One-liter samples of whole seawater were enriched with *A. lagunensis* culture to promote growth of protozoans that feed on brown tide. After the enrichment was incubated at 20°C at low light intensity for several days, individual cells were isolated under a stereomicroscope and transferred to tissue culture plates filled with ciliate media (Gifford 1985). Cultures of the three protozoans were fed *A. lagunensis* and transferred to new media at weekly intervals. *A. lagunensis* was cultured in modified f/2 media (*see* Buskey et al. 1998) at 22°C in a 12 : 12 light : dark cycle at approximately 120 μM photons m^{-2} s^{-1}.

We conducted experiments that fed *A. lagunensis* with high and low EPS to three protozoan species. In the first set of experiments, high EPS cells were provided from *A. lagunensis* cultures in stationary phase, whereas the low EPS cells were produced by removing some of the EPS layer from the same batch of culture using chemical treatment and physical force (Decho and Lopez 1993). To remove EPS from *A. lagunensis* cells, a final concentration of 0.05 mM ethylenediaminetetraacetic acid (EDTA) was added to the culture, which was then centrifuged three times at high speed (21,000 $\times$ *g*, 15 min). After the treatment, the cells were resuspended in filtered (0.2 μm pore size) and sterilized seawater for immediate experimental use. EPS in the cultures were measured with the alcian blue assay method using gum xanthan as a standard (Passow and Alldredge 1995). This method only measures the relative abundance of EPS because alcian blue only stains carboxyl and sulfated polysaccharides, but not neutral sugars (Horobin 1988). Tests revealed that *A. lagunensis* cells treated by the procedure described above have less than half of the EPS compared to the untreated cells.

All protozoan grazing experiments were carried out in triplicate in 250-ml acid cleaned polycarbonate bottles, incubated under dim light at 22°C and rotated on a bottle roller at 1 rpm. Specific growth rates of three protozoan species were measured by adding a controlled number of protozoa to 100 ml of ciliate media containing treated (low EPS) and untreated (high EPS) *A. lagunensis* cells at two different concentrations ($\sim$1 and 5 $\times$ 10^5 cells ml^{-1}, which is the equivalent to 1 and 5 mg carbon L^{-1}, respectively). Samples of

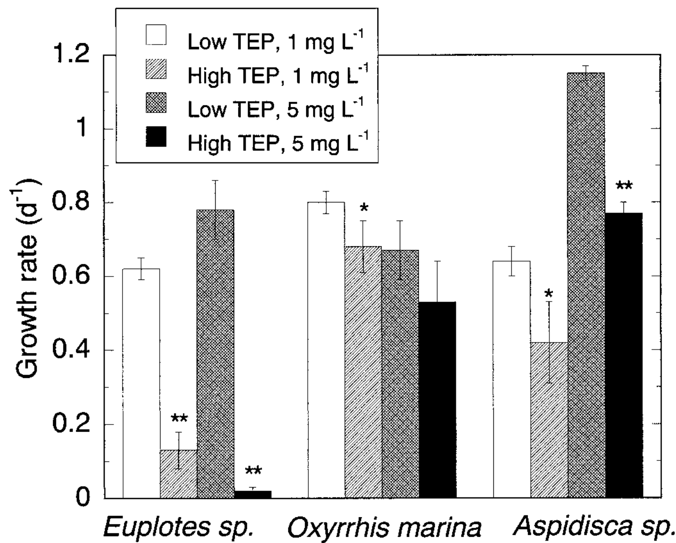

Fig. 1. Average growth rates in 4 days for *Euplotes* sp., *O. marina,* and *Aspidisca* sp. fed with 1 and 5 mg C L^{-1} of low and high EPS *Aureoumbra lagunensis,* respectively. The error bars show the standard error of triplicates. Asterisks indicate the significance levels of student *t*-test; $*P < 0.05$, $**P < 0.001$.

10 ml (5 ml in some cases) were withdrawn from each experimental bottle at the start of the experiment and daily at the same time for 4 days. Samples were preserved with 0.5 ml (0.25 ml for 5 ml samples) acid Lugol's solution, settled in Utermöhl chambers and enumerated using an inverted microscope (Olympus IMT-2). Protozoan grazing rates were measured by counting the changes in the concentration of cells over 24 h in the controls (*A. lagunensis* only) and the bottles containing protozoan grazers using a Z1 Coulter Counter. Protozoa clearance rates were calculated following the formula of Omori and Ikeda (1984).

We were not able to obtain accurate grazing rates for three protozoan species fed on low and high EPS *A. lagunensis* cells in the first set of experiments because some low EPS cells were damaged during the treatment and started to lyse during the experiment, indicated by the decrease in cell concentrations in the controls (data not shown). In order to assess whether the reduction in protozoa growth rates in the presence of high EPS was due to reduction in grazing on *A. lagunensis* cells, we tried a different treatment for achieving low EPS *A. lagunensis* cells without damaging them. We found that increasing the final EDTA concentration to 0.5 mM and reducing the centrifuge speed by one-half produced viable cells with ~40% EPS removed. We used these treated cells and untreated cells to feed *Aspidisca* sp. (32 PSU, ~1 mg C L^{-1}). We measured the growth rate of *Aspidisca* and monitored the number of *A. lagunensis* and heterotrophic bacteria in the *Aspidisca* cultures and controls for four days. Bacteria were enumerated using DAPI stain on 0.2 μm black membrane filters (Porter and Feig 1980). More than 200 cells were counted for each sample under ultraviolet (UV) light using epifluorescence microscope.

In addition, *Aspidisca* swimming behavior in low and high EPS *A. lagunensis* cultures were recorded on a video cassette recorder using a Cohu model 3315 monochrome CCD video camera mounted on an Olympus SZH stereoscope at $\times$ 100 magnification (Buskey 1997). Low EPS *A. lagunensis* obtained from the gentler treatment described above had 41% less EPS than the high EPS untreated cells. Three replicates of each treatment were prepared with ca. 1,000 ciliates ml^{-1} in 1 mg C L^{-1} of high and low EPS *A. lagunensis* held within transparent tissue culture flasks. These cultures were held at the same conditions described above and we videotaped ciliates for 5 min each at 4, 24, and 48 h after the beginning of the experiment. A water bath of clear acrylic plastic containing about 500 ml of filtered seawater was used to keep the temperature in the tissue culture flasks unchanging while videotaping. Only ciliates swimming freely in the water and away from surfaces were recorded. The field of view while videotaping was ca. 2 $\times$ 1.5 mm. One minute of videotape from each recording was analyzed at a rate of 10 frames s^{-1} using an Expertvision Cell-Trak video-computer motion analysis system and a minimum of 2,000 measures of each parameter was used to calculate the mean values of each parameter (*see* Buskey 1997 for more details on motion analysis). Parameters that were calculated included the swimming speed (mm s^{-1}), rate of change of direction (RCD, degrees s^{-1}), and net-to-gross displacement ratio (NGDR, dimensionless).

Results from the first set of experiments showed that the growth rates of *Euplotes* and *Aspidisca* fed with high EPS *A. lagunensis* cells were significantly lower than those fed

Table 1. *Aspidisca* growth rates and clearance rate and digestion rate estimates based on the disappearance rate of *Aureoumbra lagunensis* and bacteria when fed with 1 mg C L^{-1} low and high EPS *A. Lagunensis* cultures. Numbers in parenthesis are standard errors.

	Aspidisca growth rate (d^{-1})		*A. Lagunensis* Clearance rate (μl protist^{-1} h^{-1})		*A. Lagunensis* Ingestion rate (*a. Lagunensis* protist^{-1} h^{-1})		Bacteria Clearance rate (μl protist^{-1} h^{-1})		Bacteria Ingestion rate (bacteria protist^{-1} h^{-1})	
	Day-1	Day-2	First 6-h	Day–1	First 6–h	Day–1	First 6–h	Day–1	First 6-h	Day-1
Low EPS	0.64	1.13	1.55	0.85	149	76	0.63	−0.03	271	−29
	(0.06)	(0.18)	(0.46)	(0.25)	(45)	(21)	(0.63)	(0.03)	(272)	(25)
High EPS	0.76	0.66	0.06	0.32	6	27	1.30	0.50	689	350
	(0.14)	(0.07)	(0.19)	(0.02)	(23)	(14)	(0.41)	(0.28)	(225)	(199)

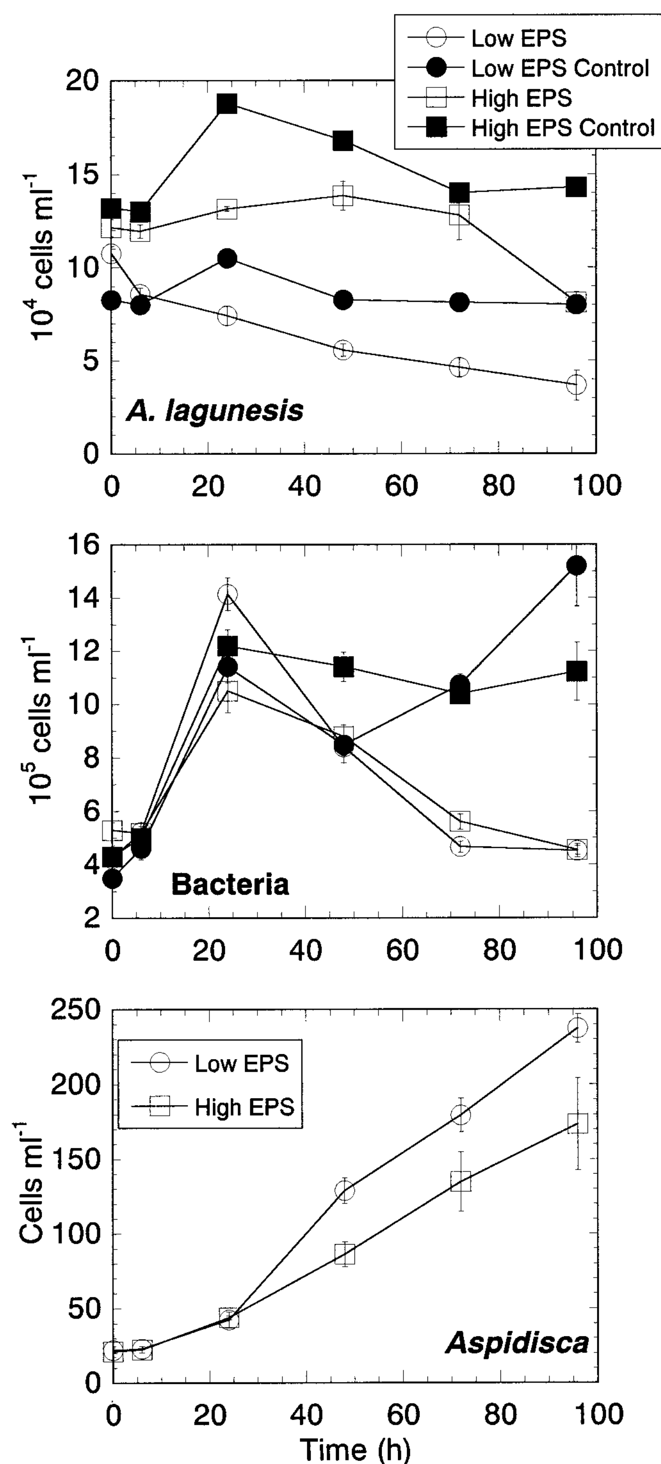

Fig. 2. Changes in concentrations of *Aspidisca* sp., *A. lagunensis,* and heterotrophic bacteria during the time course of 4 days. All data points are the mean values from three replication bottles. The error bars show the standard error.

with low EPS cells (Fig. 1). The growth rate for *Aspidisca* grown with 1 and 5 mg C L^{-1} high EPS *A. lagunensis* were about 2/3 of the growth rates measured for those grown with a low EPS diet. The reductions of growth rate in *Euplotes* cultures fed with high EPS *A. lagunensis* were even more apparent (Fig. 1). The most severe impact was seen for *Euplotes* fed with 5 mg C L^{-1} *A. lagunensis,* in which the *Euplotes* under a high EPS diet had almost no growth (Fig. 1). The effect of EPS on the growth of *O. marina* was, however, not so obvious (Fig. 1), although the reduction of growth rate for *O. marina* in 1 mg C L^{-1} high EPS cultures was statistically significant ($P < 0.05$, Student's *t*-test).

Results from the follow-up experiment indicate that the reduction in growth rate for protozoa fed on high EPS *A. lagunensis* is indeed due to the reduction in grazing on *A. lagunensis* cells (Table 1). The concentrations of *A. lagunensis* and bacteria in the experimental and control bottles, as well as the numbers of *Aspidisca* in the low and high EPS diet, are plotted in Fig. 2. We have only used the data from the first day of the experiment to calculate *Aspidisca* clearance rate, because the growth rate of *A. lagunensis* and bacteria in the controls and the bottles with predators may differ after long incubation. There was very heavy grazing in the first 6 h when *Aspidisca* were fed low EPS *A. lagunensis.* In contrast, no grazing on the high EPS *A. lagunensis* was observed during the first 6 h. *Aspidisca* clearance rates and grazing rates on low EPS cells during the first day were also significantly higher than those on high EPS cells (0.85 μl protist^{-1} h^{-1} and 76 *A. lagunensis* protist^{-1} h^{-1} vs. 0.26 μl protist^{-1} h^{-1} and 21 *A. lagunensis* protist^{-1} h^{-1}, respectively). There was a sharp increase in heterotrophic bacteria on the first day of the experiment, probably due to the release of nutrients caused by the dilution effect (adding *Aspidisca* cultures to the treatment bottles and media to the control bottles). The effect of reduced grazing on high EPS *A. lagunensis* on *Aspidisca* growth had a time lag of about one day. *Aspidisca* growth rates were similar during the first day, but were much higher under low EPS diet than high EPS diet on the second day (Table 1).

Motion analysis of *Aspidisca* swimming behavior revealed that its swimming speed was not strongly affected by the amount of EPS associated with *A. lagunensis* cells (Table 2). However, *Aspidisca* in the high EPS cultures have a significantly higher RCD than those with low EPS cells during all three measurement times (Table 2). We observed a higher incidence of ciliates just swimming around in small circles with high EPS *A. lagunensis,* rather than the spiraling and zigzagging behavior that was more normally observed. The NGDR is the ratio of the linear distance between the starting and ending points of a path of travel (net displacement) and the total distance covered by the path (gross displacement). A low NGDR indicates a circuitous path, whereas a high NGDR indicates a more direct path. NGDR was lower under high EPS treatment, but the difference was not always statistically significant (Table 2).

We have developed two hypotheses to explain our findings. First, the low grazing of *Aspidisca* on high EPS *A. lagunensis* may have resulted from the effect of the EPS mucus layer on protozoan feeding mechanics. The EPS mucus layer might coat the cilia, and interfere with their motion, and therefore alter their feeding current. Slime EPS may also clog the feeding apparatus of protozoa grazers. Since the same cilia are used for swimming and feeding, interference with feeding mechanisms should also be reflected in a

Table 2. Observed swimming behavior of *Aspidisca* and high and low EPS *A. Lagunensis*. Speed, swimming speed (mm s^{-1}); RCD, rate of change of direction (degrees s^{-1}); NGDR,–net-to-gross displacement ratio (dimensionless). Asterisks indicate the significance levels of student *t*-test for two-tailed distribution; *$P<0.1$, **$P<0.02$.

	T = 4h			T = 24h			T = 48h		
	Speed	RCD	NGDR	Speed	RCD	NGDR	Speed	RCD	NGDR
LoEPS-1	0.17	211	0.61	0.27	226	0.62	0.26	319	0.59
LoEPS-2	0.20	268	0.58	0.25	295	0.53	0.27	318	0.56
LoEPS-3	0.18	179	0.77	0.24	294	0.64	0.26	328	0.57
Mean	0.183	219	0.653	0.253	272	0.597	0.263	322	0.573
SD	0.015	45**	0.102	0.015*	40*	0.059	0.006	6**	0.015**
HiEPS-1	0.18	311	0.55	0.22	322	0.49	0.22	546	0.38
HiEPS-2	0.15	358	0.43	0.24	418	0.46	0.22	506	0.47
HiEPS-3	0.23	351	0.54	0.2	361	0.57	0.28	406	0.48
Mean	0.187	340	0.507	0.22	367	0.507	0.24	486	0.443
SD	0.040	25	0.067	0.02	48	0.057	0.035	72	0.055

change of swimming pattern. Results from the study of *Aspidisca* behavior support the idea of cilia impaired by EPS. Although high EPS did not always significantly reduce the swimming speed of *Aspidisca,* it caused a significant increase in RCD. Impairment of some of the oral ciliature could cause *Aspidisca* to alter its swimming pattern and swim more frequently in small tight circles, and reduce the apparent clearance rates. Both *Euplotes* and *Aspidisca* are hypotrichous filter-feeding ciliates that do not expend cellular organelles to capture food, whereas *Oxyrrhis* is a raptorial feeder that uses its flagella to capture prey (Fenchel 1987). It appears that the EPS mucus has a more severe impact on the feeding mechanics of hypotrichous ciliates than on the heterotrophic dinoflagellate. The present study confirmed the previous finding that *O. marina* was able to grow well when fed on *A. lagunensis* (Buskey and Hyatt 1995). In contrast, Caron et al. (1989) found that *Euplotes* were not able to grow with *Aureococcus anophagefferens,* another brown tide-forming alga that is also covered by an extracellular polysaccharide layer (Sieburth et al. 1988). Our results show that *A. lagunensis* coated with thick EPS layer was also not an adequate food for *Euplotes.*

Secondly, high EPS *A. lagunensis* cells may be simply unpalatable to *Aspicisca,* which then switched its feeding preference to bacteria as a response to a perceived change in the food environment. Our results show that the pattern of ingestion of bacteria for *Aspidisca* is opposite to the pattern of ingestion of *A. lagunensis,* i.e., high bacterial ingestion with low ingestion of *A. lagunensis* in high EPS *A. lagunensis* prey (Table 1). This opposite trend supports the dietary switching hypothesis. Bacterivory is common in marine ciliates and phagotrophic flagellates (e.g., Fenchel 1984; Sieburth 1984; Albright et al. 1987). All three protozoa species used in this study are reported as being capable of feeding on bacteria (Capriulo 1990; Schumann et al. 1994; Zubkov and Sleigh 1996). However, both *Euplotes* and *Oxyrrhis* have been reported to favor larger particles and consume them more efficiently (Fenchel 1986; Schumann et al. 1994). It is also possible that *Aspidisca* feed selectively on large slime EPS particles that are more abundant in the high EPS *A. lagunensis* cultures, although we did not observe a significant portion of our bacteria counts in association with the EPS slime. Like *Euplotes, Aspidisca* is more adapted to benthic feeding and is reported to prefer to feed on bacterial aggregates over free-living bacteria (Albright et al. 1987). Furthermore, the negative bacterial ingestion rate observed for *Aspidisca* with low EPS diet may be caused by the faster (than the control) bacterial growth in these bottles, due to higher nutrient availability resulting from enhanced *Aspidisca* grazing on less EPS coated *A. lagunensis* (Fig. 2).

Among the various proposed functions of EPS (*see* review by Decho 1990), its key advantage is thought to be enhancing the survival and competitive success of microbial cells under varying natural environments. *A. lagunensis* can grow in a wide range of salinities, but produces more EPS under hypersaline conditions (Buskey et al. 1998; Liu and Buskey 2000). High *A. lagunensis* EPS production in hypersaline conditions is probably a protective adaptation to buffer the great osmotic disequilibrium across the cell membrane and prevent the cells from dehydration. The EPS capsule might also help the cells to resist digestion and remain viable after passage through a grazer's gut (Porter 1976; Epp and Lewis 1981). Results from the present study show that it also reduces the chance of being grazed by certain protozoan grazers. The capsule-EPS layer surrounding *A. lagunensis* cells affects the grazing and growth of *Euplotes* and *Aspidisca,* while the growth of *O. marina* was not severely affected. The mechanisms for the negative effect of EPS on protozoan grazing efficiency are unclear. We speculate that the EPS mucus may interfere with feeding activities of ciliates by adhering to or even clogging up their feeding apparatus. Another explanation may be that high EPS simply caused *Aspidisca* to become more bacterivorous. Because of these two scenarios, cessation in feeding vs. dietary switching, have very different ecological consequences, further studies are needed to reveal the details in changes of feeding behavior for ciliates fed with particles covered with a thick EPS layer. We certainly cannot rule out the possibility that the EPS of *A. lagunensis* may contain some inhibitory compounds that can reduce the feeding activities of protozoan grazers. The EPS layer of *A. anophagefferens* contains some sort of inhibitory neurotransmitter-like substance that appears to cause

reduced ciliary activity in some bivalves (Gainey and Shumway 1991). Nevertheless, our findings, together with other evidence, show that the hypersaline condition in the Laguna Madre favor the formation and maintenance of *A. lagunensis* blooms (Buskey et al. 1997, 1998; Liu and Buskey 2000), and may help to explain the extraordinarily long persistence of the Texas brown tide (1990–1997).

Hongbin Liu[1] *and Edward J. Buskey*

Marine Science Institute
The University of Texas at Austin
750 Channel View Drive
Port Aransas, Texas 78373

References

ALBRIGHT, L. J., E. B. SHERR, B. F. SHERR, AND R. D. FALLON. 1987. Grazing of ciliated protozoa on free and particle-attached bacteria. Mar. Ecol. Prog. Ser. **38:** 125–129.

BUSKEY, E. J. 1997. Behavioral components of feeding selectivity of the heterotrophic dinoflagellate *Protoperidinium pellucidum.* Mar. Ecol. Prog. Ser. **153:** 77–89.

———, AND C. J. HYATT. 1995. Effect of the Texas (USA) ''brown tide'' alga on planktonic grazers. Mar. Ecol. Prog. Ser. **126:** 285–292.

———, P. A. MONTAGNA, A. F. AMOS, AND T. E. WHITLEDGE. 1997. Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide bloom. Limnol. Oceanogr. **42:** 1215–1222.

———, B. WYSOR, AND C. HYATT. 1998. The role of hypersalinity in the persistence of the Texas 'brown tide' in the Laguna Madre. J. Plankton Res. **20:** 1553–1565.

CAPRIULO, G. M. 1990. Feeding-related ecology of marine protozoa, p. 186–259. *In* G. M. Capriulo [ed.], Ecology of marine protozoa. Oxford Univ. Press.

CARON, D. A., E. L. LIM, H. KUNZE, E. M. COSPER, AND D. M. ANDERSON. 1989. Trophic interactions between nano- and microzooplankton and the ''brown tide'', p. 265–294. *In* E. M. Cosper, V. M. Bricelj, and E. J. Carpenter [eds.], Novel phytoplankton blooms. Springer-Verlag.

DECHO, A. W. 1990. Microbial exopolymer secretions in ocean environments: Their roles in food webs and marine process, p.73–153. *In* M. Barnes [ed.], Oceanography and marine biology annual review (V. 28). Aberdeen Univ. Press.

———, AND G. R. LOPEZ. 1993. Exopolymer microenvironments of microbial flora: Multiple and interactive effects on trophic relationships. Limnol. Oceanogr. **38:** 1633–1645.

DEYOE, H. R., AND OTHERS. 1997. Description and characterization of the algal species *Aureoumbra lagunensis* gen. et sp. nov. and referal of *Aureoumbra* and *Aureococcus* to the Pelagophyceae. J. Phycol. **33:** 1042–1048.

EPP, R. W., AND W. M. LEWIS. 1981. Photosynthesis in copepods. Science **214:** 1349–1350.

FENCHEL, T. 1984. Suspended marine bacteria as a food source, p. 301–305. *In* M. J. Fasham [ed.], Flows of energy and materials in marine ecosystems. Plenum.

———. 1986. Protozoan filter feeding. Prog. Protistol. **1:** 65–113.

———. 1987. Ecology of protozoa. Springer-Verlag.

GAINEY, L. F., JR., AND S. E. SHUMWAY. 1991. The physiological effect of *Aureococcus anophagefferens* (''brown tide'') on the lateral cilia of bivalve mollusks. Biol. Bull. **181:** 298–301.

GIFFORD, D. J. 1985. Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotricha). Mar. Ecol. Prog. Ser. **23:** 257–267.

HOROBIN, R. W. 1988. Understanding histochemistry. Selection, evaluation and design of biological stains. Wiley.

LIU, H., AND E. J. BUSKEY. 2000. Hypersalinity enhances extracellular polymeric substance (EPS) production of Texas brown tide alga, *Aureoumbra lagunensis* (Pelagophyceae). J. Phycol. **35:** 71–77.

OMORI, M., AND T. IKEDA. 1984. Methods in marine zooplankton ecology. Wiley.

PASSOW, U., AND A. L. ALLDREDGE. 1995. A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnol. Oceanogr. **40:** 1326–1335.

PORTER, K. G. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science **192:** 1332–1334.

———, AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

SCHUMANN, R., B. MUNZERT, J.-U. WÜNSCH, AND H.-P. SPITTLER. 1994. The feeding biology of *Oxyrrhis marina* Dujardin (Flagellata). Limnologica **24:** 29–34.

SIEBURTH, J. MCN. 1984. Protozoan bacterivory in pelagic marine waters, p.405–444. *In* J.E. Hobbie, and P.J. Williams [eds.], Heterotrophic activity in the sea. Plenum Press.

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

ZUBKOV, M. V., AND M. A. SLEIGH. 1996. Bacterivory by the ciliate *Euplotes* in different states of hunger. FEMS Microbial. Ecol. **20:** 137–147.

Received: 5 June 1999
Accepted: 18 February 2000
Amended: 24 March 2000

[1] To whom correspondence should be addressed. Present address: Institute for Hydrospheric-Atmospheric Sciences, Nagoya University, Chikusa-ku, Nagoya 464–8601, Japan.
(hliu@ihas.nagoya-u.ac.jp)

Acknowledgments

This study was supported by National Science Foundation grant OCE-9529750 to E.B. and a postdoctoral fellowship from the Japan Society for the Promotion of Science to H.L. We thank Megan Black for assisting in protozoan isolation, Chris Collumb for assistance with bacterial counts, Peter Lavrentyev for helping in taxonomic identification for the two ciliates, and Cammie Hyatt for assistance with the second *Aspidisca* growth and grazing study. Comments from David Caron and two anonymous reviewers improved the quality of the manuscript. This is University of Texas Marine Science Institute Contribution number 1135.