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Growth and grazing on the 'Texas brown tide' alga *Aureoumbra lagunensis* by the tintinnid *Amphorides quadrilineata*

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ABSTRACT: Growth and ingestion by the loricate ciliate *Amphorides quadrilineata* exposed to increasing dietary doses of the Texas brown tide alga *Aureoumbra lagunensis* were investigated. The ciliate grew at a maximum rate of 0.38 d^{-1}, ingesting 0.032 ppm (~6.4×10^2 cells) prey d^{-1}on a diet consisting only of *Isochrysis galbana*. When *A. quadrilineata* was offered a mixed diet of *I. galbana* and *A. lagunensis*, the growth rate decreased when *A. lagunensis* made up more than ca 40% of the diet. Ingestion rate by *A. quadrilineata* remained constant with increasing *A. lagunensis* proportion in the diet, which indicates that *A. quadrilineata* did not avoid feeding on *A. lagunensis*. The behaviour of *A. quadrilineata* was largely unchanged when ciliates were fed *A. lagunensis*. However *A. quadrilineata* did perform a significant transient response approx. 60 min after *A. quadrilineata* was exposed to *A. lagunensis* by increasing the turning rate per unit time. This study suggests that efficient top-down control of *A. lagunensis* by heterotrophic protozoans such as the studied ciliate may not happen as long as phytoplankton organisms other than *A. lagunensis* make up a minor part of the standing phytoplankton stock.

KEY WORDS: Texas brown tide · Tintinnid · Growth · Ingestion · Harmful algae · Ciliate · Prey selection

INTRODUCTION

In the Laguna Madre, Texas, USA, an almost monospecific bloom of the alga *Aureoumbra lagunensis* Stockwell, DeYoe, Hargraves, et Johnson 1997 existed without interruption from 1990 to 1997, and has reappeared intermittently since then. Compared to most other algal blooms, this bloom is remarkable in its duration. Before the onset of the *A. lagunensis* bloom, the planktonic food web in Laguna Madre was characterised by heterotrophic dinoflagellates and ciliates of various genera including tintinnid ciliates (Buskey 1993, Buskey & Stockwell 1993). After the onset of the bloom, the concentration and species diversity of heterotrophic protists was dramatically reduced, indicating that few, if any, protists were able to prey upon and control the stock of *A. lagunensis* (Buskey & Stockwell 1993). There may be several reasons for the absence of predatory control on *A. lagunensis* by heterotrophic protozoans. Buskey & Hyatt (1995) conducted a series of growth experiments with heterotrophic protists feeding on *A. lagunensis*. They found that growth typically decreased with increasing concentration of *A. lagunensis*, suggesting that the alga was toxic to some grazers or had a low nutritional value for others. A mucus layer consisting of polysaccharides covers *A. lagunensis* (EPS: exopolomeric secretion). The amount of EPS around *A. lagunensis* is dependent on the age of the cells (Villareal et al. 1998) and ambient salinity (Liu & Buskey 2000a). In another study, Liu & Buskey (2000b) demonstrated that the effect of *A. lagunensis* on heterotrophic protist grazers was dependent on the amount of EPS per *A. lagunensis* cell.

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Liu & Buskey (2000b) hypothesised that EPS may interfere with the feeding organelles of grazers, due to the stickiness of the mucus cover.

In this study we used a ciliate that was growing well when feeding on prey particles of a similar size to *Aureoumbra lagunensis*. We used a loricate ciliate because there is growing evidence on the importance of tintinnids relative to other oligotrich ciliates in controlling coastal waters with high chlorophyll levels (reviewed by Suzuki & Taniguchi 1998). The loricate tintinnid ciliate *Amphorides quadrilineata* Clapréde & Lachmann 1858 fulfilled these requirements and it was used to study the effects of increasing dietary proportion of *A. lagunensis* on growth, feeding, prey selection and behaviour of a microzooplankton grazer. We offered *A. quadrilineata* a mixed diet of *A. lagunensis* and *Isochrysis galbana* at a constant biomass in food concentrations markedly lower than those used in the previous study of Buskey & Hyatt (1995). We also investigated the behaviour of *A. quadrilineata* exposed to *A. lagunensis* to see if the ciliate performed any transient or permanent changes in behaviour in order to avoid ingestion of *A. lagunensis* cells.

MATERIALS AND METHODS

Isolation and cultivation of the tintinnid. *Amphorides quadrilineata* was isolated in April 1999 from 20 µm mesh plankton net tows sampled at the Port Aransas south jetty (Texas, USA) during an incoming tide. Tintinnids were picked individually with a micropipette and added to 7 ml micro-wells containing microwave sterilised 0.2 µm filtered 30 to 32 ppt seawater (Keller et al. 1988) with F/2 nutrients added. A mixture of the microalgae *Heterocapsa rotundata* and *Isochrysis galbana* were added to each micro-well as prey for the isolated ciliates. After a couple of weeks, a few tintinnids were transferred to 50 ml polystyrene tissue culture flasks and the cells were maintained in dim light at 21°C feeding on a diet of *I. galbana* until they were needed in experiments. The light was supplied from light fluorescent tubes in a 12:12 h light:dark cycle. The light intensity was kept low (25 µmol photons m^{-2} s^{-1}) in order to avoid excessive growth of *I. galbana* in containers with *A. quadrilineata*. No attempts were made to make either ciliate or prey cultures free of bacteria.

Description of organisms used. *Amphorides quadrilineata* measures ca 75 × 22 µm, with a cell volume of 2×10^4 µm^3 ± SD = 0.66×10^4 (N = 30) measured on cells fixed in 5% acid Lugol's. The tintinnid cell is housed in an amphora-shaped lorica with length × width dimensions of 131.83 × 29 µm. The diameter of the anterior opening of the lorica is 41 µm. Prey cell sizes were estimated from linear dimensions measured in an inverted microscope at a magnification of 1000× (N = 30), cells fixed in 5% acid Lugol's) and converted into volumes using appropriate volumetric formula. The volume of *Isochrysis galbana* was 65 SD ±12 and 50 SD ±14 µm^3 for *Aureoumbra lagunensis*. This volume was used throughout all calculations in the present paper.

Aureoumbra lagunensis used as prey was grown and aged for >1 mo in order to build up EPS (Villareal et al. 1998), thus resembling *A. lagunensis* during natural Laguna Madre bloom conditions. The alga was grown in aerated Fernbach flasks in microwave sterilised seawater. Because it uses ammonium as a nitrogen source, ammonium was substituted for nitrate in F/2 nutrients added to the media (DeYoe & Suttle 1994). *Isochrysis galbana* used for prey were grown in 250 ml Erlenmeyer flasks using regular F/2 nutrients added to the medium. Only cells from exponentially growing culture were used in the experiments. Both prey species were cultivated at an irradiance of ca 30 to 50 µm mol photons m^{-2} s^{-1} under the same day:night and temperature regime as with *Amphorides quadrilineata.*

Mortality. The mortality of *Amphorides quadrilineata* due to starvation and sudden exposure to monospecific cultures of *Aureoumbra lagunensis* was compared. Ciliate cells were harvested from a single culture of *A. quadrilineata* growing exponentially on *Isochrysis galbana* as prey. *A. quadrilineata* cells were added either to a triplicate series with sterilised seawater with F/2 nutrients without food or to a triplicate series containing *A. lagunensis* added in excess (corresponding to: 5×10^4 cells ml^{-1}/0.34 µg C l^{-1}/2.5 ppm). Physical conditions regarding light, salinity, plankton wheel set-up and temperature were as stated below in the '*Ciliate growth and grazing*' section. *A. quadrilineata* cell counts were carried out on samples collected at intervals of ca 24 h.

Ciliate growth and grazing. The following protocols apply for all growth and grazing experiments: grazing experiments were carried out in a climate room at a constant temperature of 21 ± 1°C. All experiments were run in triplicate in 250 ml polycarbonate flasks. Triplicate controls without predators were run in parallel. The flasks were mounted inside a transparent polystyrene tube (length: 30 cm, diameter: 15 cm). The tubes with the bottles inside were rotated on a bottle roller board (0.5 to 1 rpm). Samples were fixed in acid Lugol's solution (final concentration of 4%) and counted using an inverted microscope on the same or the following day as samples were taken. A minimum of 400 prey cells were counted in a Sedgwick-Rafter chamber. Ciliates were allowed to settle out and counted in 7 ml micro-wells; 200, and if possible up to 400, tintinnids were counted in each treatment.

Ciliates in the grazing experiments were allowed to acclimate to the desired prey concentration approx.

1 d before the initial sampling was done. The time interval between sampling was 24 h, equivalent to 1 day:night cycle, thus excluding potential diurnal variations in measured grazing and growth rates. Average prey concentration was calculated as the geometric mean between starting and ending concentrations of prey cells. Prey cell concentration and ingestion rates were converted into either biovolume or carbon using the size:carbon relationship given in Montagnes et al (1994).

The growth rates of both predators and prey were calculated assuming constant logistic growth ($N = N_0 e^{(t\mu)}$) while feeding with a constant ingestion rate (I). The ingestion rates of tintinnids were estimated as the decrease in prey cells in grazing flasks compared to controls. The model used to estimate the grazing rate was the iterative model used by, e.g., Jakobsen & Hansen (1997):

$$\frac{dx}{dt} = \mu_x x - Iy \quad (1)$$

$$\frac{dy}{dt} = \mu_y y \quad (2)$$

The iterative model assumes that the growth rates (μ) of predator (y) and prey (x) are constant and exponential, with rate constants μ_y and μ_x respectively. The prey mortality induced by predators, $I \times y$, is calculated iteratively using a computer with time steps of 0.01 h. In order to measure predator growth and ingestion rates as closely as possible to balanced growth we only used data from experiments where prey concentrations changed no more than ±30% of initial prey concentration.

Two series of grazing experiments were run. The first series of experiments was done to establish a 'standard' functional and numerical response for tintinnids with *Isochrysis galbana* as prey. In the second experimental series, tintinnids were fed a mixed diet of *Aureoumbra lagunensis* and *I. galbana* at ratios from high to low in biovolumes corresponding to the biovolume of *I. galbana* sustaining maximum growth. In order to assure that ingestion and growth were saturated throughout the mixed diet experiments, a prey bio-volume ranging from 0.9 to 3.4 ppm was used (mean 1.6 ± 0.96 SD). A prey biovolume of 1.6 ppm of *A. lagunensis* corresponds to 0.22 µg C ml^{-1} (estimated from Villareal et al. 1998), while the corresponding biovolume of *I. galbana* is equivalent to 0.168 µg C ml^{-1} based on the size:carbon relationship given in Montagnes et al. (1994). The pH was occasionally measured in the grazing experiments with an Orion portable 200 series pH meter to ensure that pH never changed outside the level found in the field (approx. 8.1 to 8.2).

Behaviour experiment. Four replicates of 150 *Amphorides quadrilineata* were pipetted and transferred to 4 separate 4 ml multidish micro-well plates. *Isochrysis galbana* was added to each well, making a final prey concentration of 2 ppm. The ciliate cells were allowed to feed and acclimate in the climate room for 12 h. After 12 h the ciliates in each well were videotaped in order to estimate the behavioural parameters of well-fed *A. quadrilineata* in an undisturbed environment (control). The cells were then transferred by micropipetting to a new set of 4 micro-wells containing F/2 enriched seawater with a total of 150 *A. quadrilineata* cells in each well. To each well *Aureoumbra lagunensis* cells were added as prey, making a final concentration of 2 ppm. After 10 min of acclimation, the first video recording of swimming cells was made. Subsequently, the cells were taped after 60 min and again 20 h after addition of *A. lagunensis*. The behavioural pattern of *A. quadrilineata* was videotaped in micro-wells covered with a microscope cover glass slide using an Olympus dissection microscope with dark-field illumination. The dissection microscope was fitted with a monochrome video camera connected to a Panasonic AG-1960 SVHS video tape recorder. The videotape was digitised using Motion Analysis VP-110 as described by Buskey & Stoecker (1988).

RESULTS

Response of *Amphorides quadrilineata* to *Aureoumbra lagunensis*

Amphorides quadrilineata underwent approx. 1 residual cell division after cells were transferred to a well without food (Fig. 1). After 40 h the population growth ceased and subsequently *A. quadrilineata* began to die after approx. 75 h with a mortality rate (μ d^{-1}) of -9.9×10^{-2}. When *A. quadrilineata* was added to *Aureoumbra lagunensis*, no residual cell division was observed and the number of *A. quadrilineata* remained almost constant for the first 40 h, after which the cells died at a rate (μ d^{-1}) of -9.5×10^{-2}. The mortality rates of each treatment were compared, but no significant differences were found ($t = 0.0807$, $p < 0.005$).

Functional and numerical responses

The growth (μ, d^{-1}) of *Amphorides quadrilineata* (Fig. 2) exhibited a steep increase when *Isochrysis galbana* were offered as prey in concentrations above 0.225 ppm (1 ppm = 1.5×10^4 *I. galbana* ml^{-1}) and reached maximum growth (0.38 d^{-1}) at around 1 ppm *I. galbana* ml^{-1}. The predator:prey ratio in terms of cell

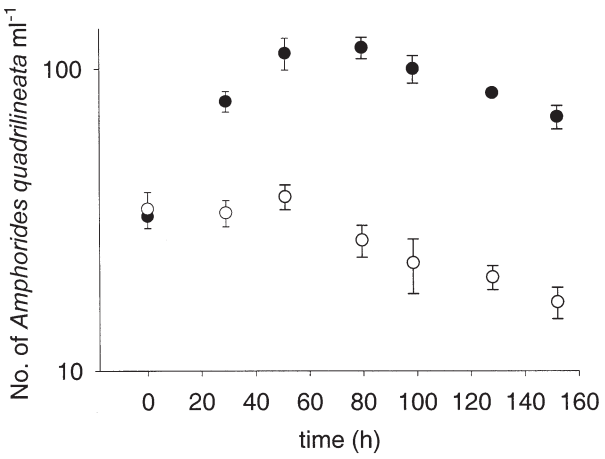

Fig. 1. Number of *Amphorides quadrilineata* (ciliates ml^{-1}) during food depletion (●) and during long-term exposure to a diet consisting solely of *Aureoumbra lagunensis* (○). Error bars = ±SD

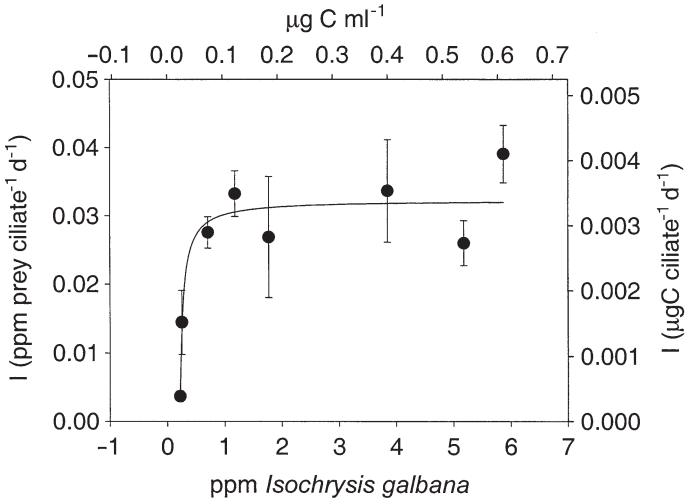

Fig. 3. *Amphorides quadrilineata* ingestion rate (*I*, prey ciliate^{-1} d^{-1}) feeding on *Isochrysis galbana*. Prey concentrations were calculated as geometric mean between start and end prey concentrations. Error bars = ±SD

diameter was ca 7. Growth rates were fitted to a second order curve to provide a general description of the numerical response for suspension-feeding heterotrophic protozoans (Fenchel 1987). Using Sigma Plot® we were able to iteratively fit our experimental data to the following model:

$$\mu = \frac{\mu_{max}(X-Z)}{k/2+(X-Z)} \quad (3)$$

where X is the actual prey cell concentration, $k/2$ is the food concentration that sustains 50% of maximum growth, μ_{max} is the maximum growth rate and Z is the threshold for growth prey concentration μ, where $\mu = 0$. The result of the fit yielded the following characteristics of *A. quadrilineata* preying on *I. galbana*:

$$\mu(d^{-1}) = \frac{0.38(X-0.225)}{0.256+(X-0.225)} \quad (4)$$

The ingestion rate (I) (Fig. 3) increased until a constant maximum level was reached. It can be argued that his type of feeding response follows a Holling type II response for predation (Fenchel 1980) which can be

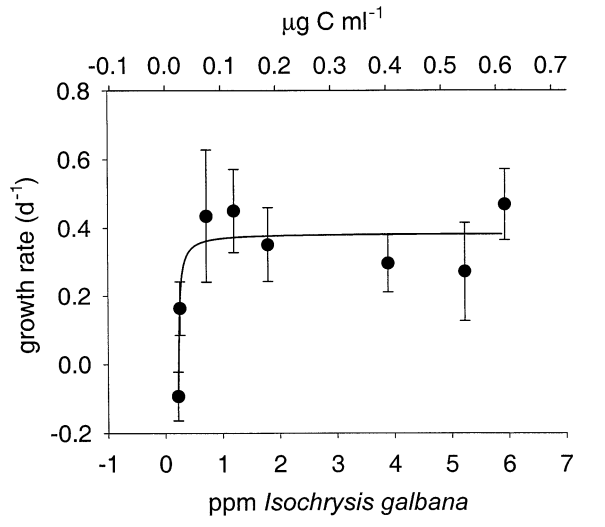

Fig. 2. *Amphorides quadrilineata* growth rate (μ, d^{-1}) feeding on *Isochrysis galbana*. Prey concentrations were calculated as geometric mean between start and end prey concentrations. Error bars = ±SD

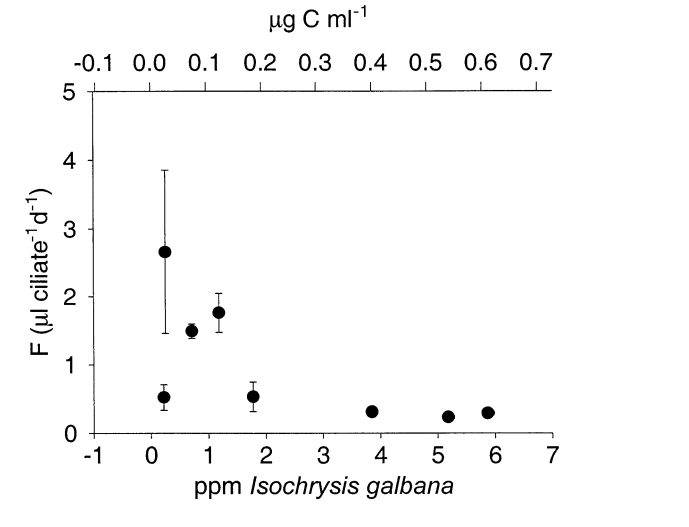

Fig. 4. *Amphorides quadrilineata* clearance rate (*F*, µl ciliate^{-1} d^{-1}) feeding on *Isochrysis galbana*. Prey concentrations were calculated as geometric mean between start and end prey concentrations. Error bars = ±SD

described by the second order math used to describe growth (μ) and was similarly determined using Sigma Plot except that I substitutes for μ

$$I(\text{ppm d}^{-1}) = \frac{0.032(X-0.211)}{0.265+(X-0.211)} \quad (5)$$

When a regression of specific growth against specific ingestion was performed (data not shown), the regression gave a gross growth efficiency (GGE) of 30.2% $R^2 = 0.92$. However it must be kept in mind that resources spent forming the lorica are not included in this estimate of the yield. The threshold for growth corresponds with a daily threshold consumption of 140 *Isochrysis galbana* d^{-1}.

Clearance (Fig. 4) decreased with increasing *Isochrysis galbana* concentration from a maximum value of 2.7 µl d^{-1} to a minimum of approx. 0.5 µl d^{-1} per *Amphorides quadrilineata*. Specific clearance in terms of body volumes ranged from 0.25×10^5 to 1.4×10^5 d^{-1}.

Mixed diet experiment

When *Amphorides quadrilineata* was feeding on *Isochrysis galbana* and *Aureoumbra lagunensis* in a mixed diet experiment, population growth (Fig. 5) was largely unaffected when *A. lagunensis* made up less than 40% of the diet. Transient growth responses were found when the amount of *A. lagunensis* varied between 40 and 60% of the diet. When *A. lagunensis* consisted of more than 60% of the diet, the population growth rate became negative (Fig. 5). The corresponding ingestion rate (Fig. 6) measured was almost constant at approx. 0.026 ppm $d^{-1,}$ as long as growth of *A. quadrilineata* was positive. However when the amount of *A. lagunensis* in the diet exceeded 60% and growth became negative, the corresponding ingestion rate decreased.

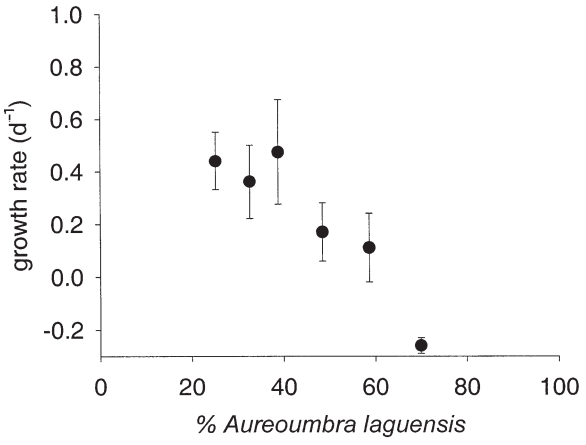

Fig. 5. *Amphorides quadrilineata* growth rate (μ, d^{-1}) in the mixed diet experiment; changes with increasing content of *Aureoumbra lagunensis* in the diet. Error bars = ±SD

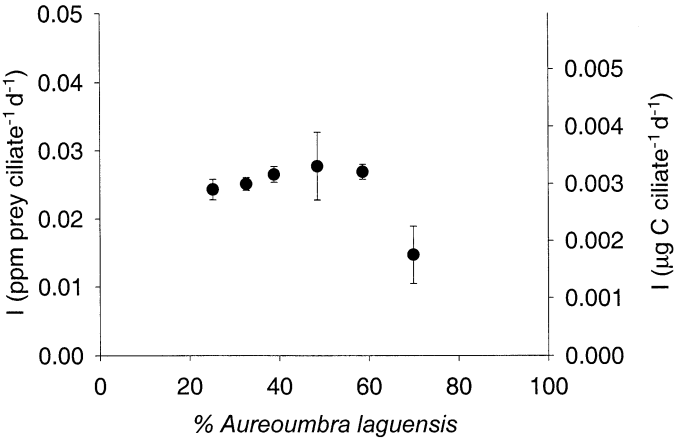

Fig. 6. *Amphorides quadrilineata* ingestion rate (I, prey ciliate^{-1} d^{-1}) in the mixed diet experiment; changes with increasing content of *Aureoumbra lagunensis* in the diet. Error bars = ±SD

Behaviour

The 3 behavioural parameters investigated were swimming speed (mm s^{-1}), turning rate (RCD, ° s^{-1}) and net to gross displacement ratio (NGDR). The speed of *Amphorides quadrilineata* was unaffected by *Aureoumbra lagunensis* relative to *Isochrysis galbana* and no significant differences between treatments were found (Table 1; 1-way ANOVA $\alpha = 0.05$; $p = 0.695$). The differences of NDGR between treatments did not yield any significant difference (Kruskal-Wallis test $\alpha = 0.05$; $p = 0.579$). The only behavioural parameter that gave a significant difference between treatments was a transient increase in RCD after 60 min (1-way ANOVA $\alpha = 0.05$; $p = 0.005$).

Table 1. Behavioural parameters for *Amphorides quadrilineata* estimated when cells were grazing on *Isochrysis galbana* (control) and after 10 min, 60 min and 20 h after transfer to addition to wells with *Aureoumbra lagunensis*.. *Significant differences between treatments and control were found (SD = standard deviation). RCD: turning rate; NGDR: net to gross displacement ratio

Treatment	Swimming speed ± SD (cm s^{-1})	RCD ± SD (° s^{-1})	NGDR ± SD
Control	0.0242 ± 0.04	110 ± 14	0.851 ± 0.02
10 min	0.0247 ± 0.05	125 ± 9	0.843 ± 0.01
60 min	0.0213 ± 0.05	163* ± 16	0.867 ± 0.01
20 h	0.0227 ± 0.04	146 ± 26	0.872 ± 0.05

DISCUSSION

The ciliate *Amphorides quadrilineata* grew well on the alga *Isochrysis galbana* with a growth rate close to that expected from scaling relationships between size and growth of ciliates (Hansen et al. 1997). Also, since *A. quadrilineata* was isolated from an area unaffected by *Aureoumbra lagunensis* close to Laguna Madre, we believe that tintinnid ciliates like *A. quadrilineata* are among the potential pioneer species in controlling growth of *A. lagunensis* since it can feed on prey sizes similar to *A. lagunensis*. Before the onset of the *A. lagunensis* bloom, oligotrich loricate ciliates were seasonally among the most abundant protozoan grazers near the Laguna Madre (Buskey 1993). However, the blooming of the brown tide alga *A. lagunensis* negatively affected the growth of heterotrophic microzooplankton (Buskey & Hyatt 1995), thus decreasing the abundance of oligotrich ciliates such as tintinnids in Laguna Madre (Buskey & Stockwell 1993). The results of this study correspond well with these observations.

When *Amphorides quadrilineata* grown on *Isochrysis galbana* was exposed to *Aureoumbra lagunensis*, the effect was immediate and on a similar time scale to the effect of food depletion (Fig. 1). Although *A. quadrilineata* was affected by *A. lagunensis*, the effecting component of *A. lagunensis* did not kill *A. quadrilineata*, but instead arrested residual cell division. The differences in the pattern of mortality for *A. quadrilineata* due to starvation and for *A. quadrilineata* exposed to *A. lagunensis* suggest that the effects of *A. lagunensis* arise from more than 1 factor. Our study suggests that either cell division or some biochemical processes associated with growth of *A. quadrilineata* is blocked immediately upon exposure to *A. lagunensis*. After a period corresponding to the length of the residual growth of starving *A. quadrilineata*, starvation or ageing of cells exposed to *A. lagunensis* may come into effect since *A. quadrilineata* populations exposed to *A. lagunensis* declined at the same rate as food-depleted cells. Although the growth-effecting agent of *A. lagunensis* could not be identified per se, it must arise from an internal cell component, since the inhibition of ciliate population growth was diluted out with the alternative prey, *I. galbana*, regardless of the *A. lagunensis* concentrations used in the mixed diet experiment. When *A. quadrilineata* cells were offered a mixed diet of *A. lagunensis* and *I. galbana* as prey, the growth of *A. quadrilineata* decreased with the proportion of added *A. lagunensis* when it made up more than 40% of the diet (Fig. 5). However, ingestion rates were constant until the growth of *A. quadrilineata* became negative at *A. lagunensis* concentrations exceeding 60%. Liu & Buskey (2000b) found that the amount of EPS in *A. lagunensis* affected feeding rates of ciliates and suggested that EPS may block the feeding organelles of ciliates. Using video techniques Kamiyama & Arima (1997) found that blocking of the feeding organelles in the tintinnid *Favella taraikaensis* prevented the ciliate from feeding as long as *Heterocapsa circularisquama* adhered to the feeding organelles. However blocking of the cytostome in *F. taraikaensis* was followed by a subsequent rejection of the prey dinoflagellate. In contrast *A. quadrilineata* in our study ingested *A. lagunensis* cells at the same rate as the good food, *I. galbana*, in the mixed diet experiment thus excluding prey-handling problems for *A. quadrilineata* feeding on *A. lagunensis*. However, the prey concentrations that we used in the mixture experiment were >1 ppm prey, and it may very well be that ingestion is negatively affected at sub-maximum prey concentrations due to effects on the feeding apparatus of *A. quadrilineata*.

Amphorides quadrilineata ingested *Aureoumbra lagunensis* until its growth ceased, making it unlikely that *A. lagunensis* had cell surface properties alerting the ciliate of its potential unsuitability as prey. The presence of *A. lagunensis* does not induce any selection against the good food, since there is a linear relationship between ingestion of *A. lagunensis* and the relative concentration of *A. lagunensis* available (Fig. 7), nor does *A. lagunensis* induce any avoidance behaviour in *A. quadrilineata*. In fact, the behaviour experiment suggests a weak positive response to *A. lagunensis* since the RCD transiently increases after 60 min. A transient increased turning rate of tintinnids has previously been interpreted as a swimming response performed in order to exploit food patches (Buskey & Stoecker 1988, Fenchel & Jonsson 1988). Lui & Buskey

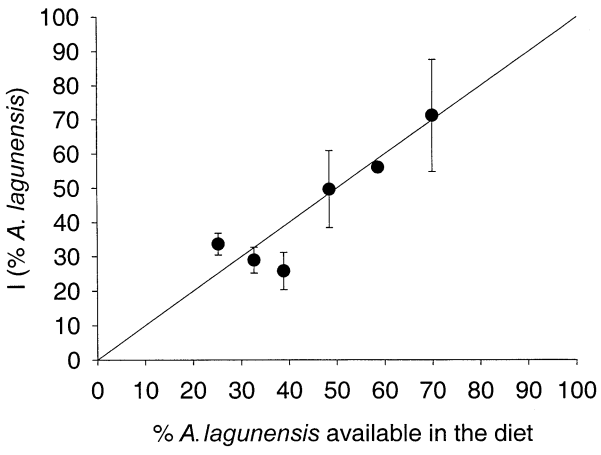

Fig. 7. *Aureoumbra lagunensis* ingestion (*I*) in percent of total biovolume ingested versus percent *A. lagunensis* available in the mixed diet experiment. Data are normalised by prey volume. Error bars = ±SD

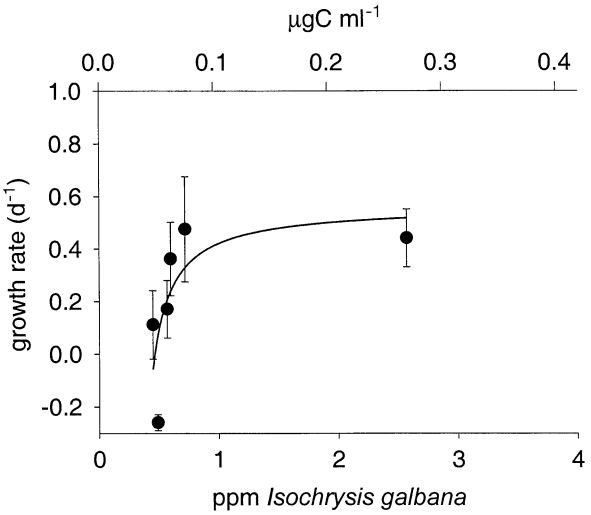

Fig. 8. *Amphorides quadrilineata* growth rate (μ, d^{-1}) versus *Isochrysis galbana* concentration in the mixed diet experiment. Prey concentrations were calculated as geometric mean between start and end prey concentrations. Error bars = ±SD

(2000b) also observed an increase in RCD in the benthic ciliate *Aspidisca* sp. when they offered it a diet of *A. lagunensis* with high EPS compared to low EPS cells. They speculated that their finding was a result of a negative interference of EPS with the swimming and feeding organelles of *Aspidisca* sp.

Villareal et al. (1998) found that the proportion of carbon increased relative to nitrogen and phosphorus in *Aureoumbra lagunensis* with the age of the cells, due to a build-up of EPS. The increased C:N may turn *A. lagunensis* into a poor food source and reduce growth of protists feeding on *A. lagunensis*. Comparing the C and N intake of *Amphorides quadrilineata* feeding on mono-specific *Isochrysis galbana* and a mixed diet of *A. lagunensis* and *I. galbana* suggests that the nitrogen supply in the mixed diet is sufficient to maintain positive growth of *A. quadrilineata* (Table 2). Even when growth of *A. quadrilineata* = 0 in the mixed diet experiment, the C and N intake are of the same magnitude as the corresponding food intake when feeding on *I. galbana* alone (Table 2). However it cannot be ruled out that EPS or the high C:N content make *A. lagunensis* indigestible, since the growth of *A. quadrilineata* in the mixed diet largely followed the concentration of *I. galbana* (Fig. 8) and the mortality of *A. quadrilineata* after ca 60 h of exposure to *A. lagunensis* was similar to the mortality rate of starving *A. quadrilineata* cells, indicating that starvation played a role in the post exposure effect of *A. lagunensis*. Also, nutrients other than C and N may be limiting for predators feeding on *A. lagunensis* but data on this issue are presently limited.

ECOLOGICAL IMPLICATIONS

There is a well-documented historical record of the heterotrophic protist abundance in the Laguna Madre before the onset of the *Aureoumbra lagunensis* bloom, and from these records it is evident that it was not the *A. lagunensis* per se which originally disrupted the microzooplankton food web (Buskey et al. 1997,1998). The heterotrophic protist biomass had already been drastically reduced before the onset of the bloom. The reduction of grazers has been suggested to be due to hypersaline conditions (>60 ppt) caused by a severe drought (Buskey et al. 1997). The absence of grazers may subsequently have allowed the bloom to develop undisturbed, since *A. lagunensis* seems better fit to cope with hypersalinity than other algae (Buskey et al. 1998, Lui & Buskey 2000a). Our findings showed that growth of *Amphorides quadrilineata* is reduced by *A. lagunensis*, but ingestion rate, in terms of volume or carbon, is constant in the mixed diet experiment. We believe that lack of prey selection for *A. quadrilineata* feeding on *A. lagunensis* (Fig. 7) may explain the general absence of tintinnid ciliates, and possibly other oligotrich ciliate species, in waters dominated by high-density blooms of the brown tide alga *A. lagunensis.* In an environment with physical conditions such as those found in Laguna Madre before the bloom of *A. lagu-*

Table 2. Carbon and nitrogen uptake by *Amphorides quadrilineata* feeding on mono-specific *Isochryis galbana* or when feeding on a mixed diet (60% *A. lagunensis* and 40% *I. galbana*)

Feeding on *I. galbana*				Feeding on mixture	
Maximum ingestion for growth (pg d^{-1} *A. quadrilineata*$^{-1}$)		Threshold ingestion for growth (pg d^{-1} *A. quadrilineata*$^{-1}$)		Threshold ingestion for growth of μ = 0 (pg d^{-1} *A. quadrilineata*$^{-1}$)	
Carbon[a]	Nitrogen[a]	Carbon[a]	Nitrogen[a]	Carbon[a,b]	Nitrogen[a,b]
3275	590	955	172	3307	559

[a]Calculated from size:carbon or size:nitrogen relationships given by Montagnes et al. (1994)
[b]Calculated from Villareal et al. (1998)

nensis, or in adjacent Corpus Christi Bay, blooms will rarely develop due to protist grazers feeding on a diverse assemblage of phytoplankton cells. However when the *A. lagunensis* bloom is established, heterotrophic grazers like tintinnids are unable to graze it down since the ratio between *A. lagunensis* and other nanoflagellates will be periodically unfavourable for ciliates such as the studied *A. quadrilineata*. In addition, in semi-enclosed systems like Laguna Madre, phytoplankton species may have difficulties in inoculating to establish viable populations. Admittedly, protist other than those that typically occurred before the bloom, such as the species studied by Lui & Buskey (2000b), may benefit from the lack of planktonic protist species and thus take their place in the planktonic food web feeding on *A. lagunensis*. Some protist species are found to be able to grow on mono-specific blooms of *A. lagunensis* (Buskey & Hyatt 1995, Lui & Buskey 2000b) although these heterotrophic protists are apparently not able to efficiently control and graze down the *A. lagunensis* bloom. The reasons for this may be complex, but the elevated salinity may play a substantial role since the hypersaline conditions may depress growth of protist predators to a level that is too low to outbalance predation from metazoans such as copepods (Buskey et al. 1998). In addition, the present or absence of other phytoplankton species may play an important role as an alternative prey that can dilute *A. lagunensis* and make them edible to protists. Re-inoculation of oligotrich microzooplankton grazers and subsequent top-down control of the *A. lagunensis* bloom will, according to this study, only occur when alternative prey nanoflagellates are available at at least approximately equal ratios of *A. lagunensis.*

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