

Physiological responses of *Aureoumbra lagunensis* and *Synechococcus* sp. to nitrogen addition in a mesocosm experiment

Hudson R. DeYoe^{a,*}, Edward J. Buskey^b, Frank J. Jochem^c

^aDepartment of Biology, University of Texas-Pan American, 1201 W. University Dr., Edinburg, TX 78541, United States

^bUniversity of Texas Marine Science Institute, Port Aransas, TX 78373, United States

^cFlorida International University, Marine Biology Program, North Miami, FL 33181, United States

Received 7 March 2006; received in revised form 16 May 2006; accepted 6 June 2006

Abstract

Aureoumbra lagunensis is the causative organism of the Texas brown tide and is notable because it dominated the Laguna Madre ecosystem from 1990 to 1997. This species is unusual because it has the highest known critical nitrogen to phosphorus ratio (N:P) for any microalgae ranging from 115 to 260, far higher than the 16N:1P Redfield ratio. Because of its high N:P ratio, *Aureoumbra* should be expected to respond to N additions that would not stimulate the growth of competitors having the Redfield ratio. To evaluate this prediction, a mesocosm experiment was performed in the Laguna Madre, a South Texas coastal lagoon, in which a mixed *Aureoumbra*–*Synechococcus* (a cyanobacterium) community was enclosed in 12 mesocosms and subjected to nitrogen addition (6 controls, 6 added ammonium) for 16 days. After day 4, added nitrogen did not significantly increase *Aureoumbra* specific growth rate but the alga retained dominance throughout the experiment (64–75% of total cell biovolume). In control mesocosms, *Aureoumbra* became less abundant during the first 4 days of the experiment but rebounded by the end of the experiment and was dominant over *Synechococcus*. Despite the lack of a strong positive growth response, *Aureoumbra* did respond physiologically to N addition. By the end of the experiment, the average N:P ratio of the *Aureoumbra*-dominated community was 86 in the N+ treatment and 41 in the control, indicating that the alga became less N-limited in the N+ treatment. The average C:N ratio was 6.6 in the N+ treatment (8.6 in the control) and suggests that the alga was not N-limited, however, C:N ratio may not be a good indicator of nitrogen limitation since this alga can produce significant quantities of carbon-containing extracellular polysaccharides, depending on growth conditions. Both *Aureoumbra* cellular chlorophyll fluorescence and cell size increased in response to added N, indicating a reduction in N limitation. It appeared that the N additions were not large and/or frequent enough to stimulate *Aureoumbra* growth. The main competitor, the unicellular cyanobacterium *Synechococcus*, responded positively to the nitrogen addition by increased specific growth rate. Unlike *Aureoumbra*, no significant effect on *Synechococcus* cellular pigment fluorescence or cell size was noted. Literature data suggest that *Synechococcus*, like *Aureoumbra*, may have a critical N:P ratio much higher than 16:1, which could explain its response.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *Aureoumbra*; Brown tide; Harmful algal bloom; Mesocosm; Nitrogen; *Synechococcus*

1. Introduction

Aureoumbra lagunensis is a small (3–5 µm diameter), single-celled, non-flagellated Pelagophyte (DeYoe et al., 1997) that has significantly impacted the ecosystem of

* Corresponding author. Tel.: +1 956 381 3538;
fax: +1 956 381 3657.

E-mail address: hdeyoe@panam.edu (H.R. DeYoe).

the Laguna Madre, a large hypersaline lagoon on the south Texas coast. *A. lagunensis*, the Texas brown tide organism, became the dominant phytoplankton by the spring of 1990 and remained dominant until 1997 (Buskey et al., 2001). Since 1997, blooms of *A. lagunensis* have recurred but have been of comparatively short duration and restricted distribution (DeYoe, unpublished data). On at least one occasion since 1997, *Synechococcus*-like cells occurred as a subdominant during an *Aureoumbra* bloom (Buskey et al., 2001).

The idea of nutrient supply or more properly nutrient supply ratio with emphasis on N and P, controlling algal community structure has been well established (Schelske et al., 1972; Tilman, 1977; Sommer, 1988; Fong et al., 1993). Deviation from the Redfield ratio of 16N:1P (Redfield, 1958), which is thought to be typical for marine phytoplankton, has been used as evidence of N or P limitation. However, the N:P ratio of some microalgae departs significantly from the Redfield ratio (Suttle and Harrison, 1988; Quigg et al., 2003; Klausmeier et al., 2004), so it is important to know the critical ratio for the species of interest. The critical N:P ratio is the N and P content for a cell equally limited by N and P. Based on chemostat experiments, the critical N:P ratio for *Aureoumbra* varied from a low of 115 at growth rates $>0.45 \text{ day}^{-1}$ to 260 at a growth rate of 0.16 day^{-1} (Liu et al., 2001) and is unusually high compared to other phytoplankton. In addition, *Aureoumbra* has a very low phosphorus requirement (Liu et al., 2001), which would be advantageous in low P environments such as Laguna Madre. *Synechococcus* (three marine strains) N:P ratios have been reported for exponentially growing batch cultures grown in seawater-based media with molar N:P ratios ranging from 8 to 800 (Bertilsson et al., 2003; Heldal et al., 2003). *Synechococcus* cellular N:P ratios ranged from 13.3 when grown in medium with N:P of 8 (N limited) up to 109 (P limited) in medium with N:P of 800. These ratios are not critical N:P ratios but do indicate the physiological N:P range for these strains. Water column total nitrogen to total phosphorus ratios in Baffin Bay, a segment of the Laguna Madre system, range from 5 to 140 (Rhudy et al., 1999), suggesting that growth conditions of *Aureoumbra* could vary from severe N limitation to mild P limitation. Cotner et al. (2004) report seston N:P ratios ranging from 10 to 70 during an *Aureoumbra* bloom in Baffin Bay. They concluded that the plankton were likely to be P limited or well adapted to low P availability.

In addition to the above factor, other physiological features of *A. lagunensis* may pertain to the persistence of the brown tide. *Aureoumbra* has maximum growth

rates at salinities between 20 and 70 (Buskey et al., 1998). Hypersalinity is common in the Laguna Madre and dense blooms develop typically under hypersaline conditions so it is presumed that the alga is a superior competitor under these conditions (Buskey et al., 1998). The alga also has low light tolerance (Buskey et al., 1998; Mansfield, 1997) and produces an extracellular polysaccharide layer that may enhance its ability to tolerate high salinities (Liu and Buskey, 2000) and retard grazing (Liu and Buskey, 2000). It also has the ability to persist for long periods in a dormant stage (DeYoe, unpublished data). Control of *Aureoumbra* population dynamics is likely a combination of several factors including nutrient limitation/competition (bottom-up control) and zooplankton grazing (top-down control). In this study, we examined the effect of nitrogen addition and zooplankton manipulation on *Aureoumbra* dynamics using mesocosms. The focus of this publication is the physiological response of *Aureoumbra* and its competitor *Synechococcus* to nitrogen addition during the mesocosm experiment. In a companion article, Buskey et al. (2003) discuss zooplankton grazing aspects of the experiment. Because of the high critical N:P ratio of *Aureoumbra*, addition of nitrogen, thereby increasing the water column N:P ratio, was expected to stimulate the growth of *Aureoumbra*, although only after physiological responses of competitors having more typical critical N:P ratios have occurred. With the N additions, the competitor would become increasingly P limited while *Aureoumbra*, approaching its critical N:P ratio, would become less N limited.

2. Methods

The study was performed from 26 June to 12 July 1999, in the cooling pond of the Central Light and Power Company Barney–Davis Power Plant located 3 km southeast of Corpus Christi, Texas. The cooling pond was chosen for the study site because it is fenced and wave action is minimal. Cooling water for the plant is drawn from the Upper Laguna Madre and then discharged to the cooling pond, which is ca. 3.9 km long, 1.1 km wide, and has an average depth of 1 m. Mesocosms were placed in water about 1.2 m deep in the third cell of the pond farthest from the point of water inflow. Water passing through the power plant is heated 10°C above ambient for a period of ca. 7 s. Water entering the cooling pond is ca. 5°C warmer than ambient, but by the time it reaches the study site 3 km downstream of the plant it has cooled to ambient temperature. Physicochemical conditions in the cooling

pond at the study site did not differ significantly from those in Upper Laguna Madre (Buskey et al., 2003). Plankton communities of the cooling pond and the Upper Laguna Madre near the power plant intake were similar, based on duplicate net (153 μm mesh) and whole water samples collected in 1995 and 1996 prior to the study (Buskey, unpublished data).

Twelve aged fiberglass cylindrical mesocosms, each approximately 1 m across and 1.5 m in height and open at the top and bottom, were gently rocked into the bottom of the pond and anchored in place. The mesocosms were spaced about 2 m apart. Depth varied slightly at the study site, so mesocosm volumes ranged from 1.27 to 1.44 m^3 . The bottom of the mesocosms enclosed ca. 1.1 m^2 of sand-silt sediment.

The two mesocosm treatments comprised: no nitrogen addition (control; C) and nitrogen addition (N+). Ammonium chloride was added to the N+ mesocosms on days 0, 4, 8, 12 of the 16-day experiment. Nitrogen was added as ammonium because *A. lagunensis* cannot use nitrate (DeYoe and Suttle, 1994). Each 1 M ammonium chloride addition raised mesocosm ammonium levels from initially less than 2 to approximately 40 $\mu\text{mol L}^{-1}$. Nitrogen additions were adjusted according to mesocosm volume to achieve the same final concentration in the treated mesocosms. Immediately after the ammonium addition, all mesocosms were gently mixed with a paddle.

Every other day and prior to mixing or sampling, temperature and salinity were measured in each mesocosm at the surface, middle and bottom with a YSI Model 30 m or a HydroLab Surveyor 4a with datasonde. Light irradiance was measured 2 cm below the surface, at mid-depth, and 10 cm above the bottom of each mesocosm about every 2 days at 08:00 h using a Licor 1100 datalogger with a Licor spherical sensor. To minimize the build up of an attached community on the interior walls of the mesocosms, inside walls were scrubbed approximately every 4 days during the experiment. No significant growth developed on the interior tank walls.

Samples for phytoplankton analysis were collected every 4 days during the experiment. Samples were taken after each mesocosm had been gently mixed. Whole water phytoplankton samples were preserved with Lugol's solution (final concentration 1%). Another set of whole water phytoplankton samples for flow cytometry analysis was collected in foil-wrapped vials and preserved with paraformaldehyde (final concentration 1%) and refrigerated until analysis.

Flow cytometry cell counts of phytoplankton were performed on a Becton-Dickinson FACSsort (San José,

CA). The two major taxa, *A. lagunensis* and *Synechococcus* spp., were distinguished by size (side angle light scatter), chlorophyll autofluorescence measured at >650 nm, and phycoerythrin autofluorescence measured at 585 ± 15 nm. Other phytoplankton taxa were in low numbers ($<20\%$ of total cell abundance). Changes in instrument sensitivity were monitored using 0.993 μm PC red plastic beads (Polysciences, Inc., Warrington, PA) added as internal standard. Data were analyzed by WinList 3.0 (Verity Software, Topsham, ME). For calculation of average cell biovolumes of *Aureoumbra* and *Synechococcus*, cell dimensions of 15–20 cells of selected samples were measured using an ocular micrometer at $400\times$ under bright field illumination. Flow cytometer cell counts were multiplied by average cell biovolume to calculate total cell biovolume for *Aureoumbra* and *Synechococcus*, respectively. To quantify the abundance of other phytoplankton, cell counts of Lugol's preserved samples were performed at $400\times$ using a Palmer–Maloney counting chamber. Typically, 500 units per sample were counted.

Flow cytometry in this study was used in a non-traditional manner. Flow cytometry is a basically a taxonomic method, and assigning taxonomic groups to cytometric clusters relies on crosschecking samples by microscopy. Even after microscopic inspection, small eukaryote phytoplankton cannot be separated since they often cluster closely in one "population" in the cytometric histograms. Unless specific markers, such as taxon-specific antibodies or rRNA probes, are used, the group of small eukaryotic phytoplankton remains taxonomically unresolved by flow cytometry. Even under conditions of quasi-monospecific blooms, other small eukaryotic algae may occur in low abundance.

In the case of Laguna Madre and the here presented study, differentiation of *A. lagunensis* from other small eukaryotic phytoplankton was facilitated by two major circumstances: (a) both cytometric and microscopic analysis revealed that the studied system contained basically only two major populations, i.e. *A. lagunensis* and *Synechococcus*. Cytometric plots of the initial mesocosm communities show only two distinct clusters, and only very few "other small phytoplankton" is seen as scattered dots outside the *A. lagunensis* gate applied for quantification. (b) In contrast to other small eukaryotic phytoplankton, *A. lagunensis* exhibited a pronounced autofluorescence in the yellow fluorescence channel for the FACSsort flow cytometer. This yellow channel is commonly used to detect phycoerythrin fluorescence in *Synechococcus* and cryptophytes. Although the yellow autofluorescence of *A. lagunensis* does not infer the presence of phycoerythrin in this

species, pronounced yellow fluorescence can sometimes be observed in other non-cryptophyte and non-cyanobacterial algae (e.g. some dinoflagellates). In the present study, the pronounced yellow autofluorescence of *A. lagunensis* facilitated separation of this species from other small eukaryotic taxa. This became clear in cytometric histograms of samples from a later state of the mesocosm experiment, when other small eukaryotic algae grew up in minor amounts. In those histograms, these other taxa clustered outside the quantification gate applied to *A. lagunensis*. Specifically, these other small algae exhibited distinctly lower yellow autofluorescence and side angle light scatter than *A. lagunensis*, although cellular chlorophyll (red) fluorescence was comparable.

Samples for analysis of phosphate, ammonium, nitrate–nitrite, silicate, particulate carbon (PC), particulate nitrogen (PN) and particulate phosphorus (PP) were collected at the beginning, middle and end of the experiment. Near-surface water samples were collected from the mesocosms after they had been gently but thoroughly mixed. Samples were collected in acid-rinsed polypropylene bottles and stored on ice until processing. Water for ammonium, phosphate, nitrate–nitrite, and silicate analyses was filtered through 0.2 μm polycarbonate filters prior to freezing and storage. Water for PN, PP and PC analyses was filtered through combusted (470 °C for 3 h) Whatman GF/C filters. Filters for PN and PC analysis were dried at 45 °C while filters for PP analysis were processed according to the procedure of Solorzano and Sharp (1980). Analysis of PN and PC was performed on a Carlo Erba model EA1108 elemental analyzer, which was calibrated with acetanilide standard. Nitrate + nitrite, phosphate, and ammonia were measured on a Lachat Quikchem 800 ion analyzer with computer controlled sample injection and peak processing using the manufacturer's recommended chemistries with detection ranges as follows: nitrate + nitrite (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).

Statistical analyses were performed in StatView ver. 5.01 (SAS Institute, Cary, NC). *t*-Tests and ANOVA were used to detect differences between treatments. Proportion data were arcsine square root transformed prior to statistical analysis to achieve normal distribution.

3. Results

At top and mid-depths, early morning light irradiance ranged from 85 to 1350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and

averaged 478 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Bottom irradiance ranged from 5 to 410 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and averaged 86 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Based on average water column irradiance, irradiance in the N+ mesocosms was not significantly different from the C mesocosms (230 ± 176 S.D. versus 274 ± 180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively, $p > 0.05$).

Trends in mesocosm temperature, salinity, and nutrients can be found in Buskey et al. (2003). The N+ treatment effectively increased ammonium concentration from <1 to 40 μM (Buskey et al., 2003). Mean water column N:P ratio in the N+ mesocosms was 289 on day 0 just after the ammonium addition and 109 on day 16, 4 days after the last ammonium addition. The N:P ratio in the C mesocosms was initially 10 but dropped to 4.8 by day 16. These water column N:P values should be viewed with caution since not all potentially useable forms of N or P such as organic forms are included. N:P ratio based on all useable forms of N and P may be higher or lower than stated above. Average silicate concentrations in the mesocosms ranged from 80 to 157 $\mu\text{mol L}^{-1}$.

The mesocosms were *Aureoumbra*-dominated throughout most of the experiment although *Synechococcus* spp., a 2–3 μm rod- to coccoid-shaped cyanobacterium, was subdominant in all mesocosms and achieved co-dominance in the C mesocosms from days 4 to 12. Diatoms, dinoflagellates, flagellates, and unidentified coccoid cells were present in low numbers varying from 1.6 to 34% and averaging 9% (S.D. = 9) of cell counts. No large phytoplankton species were present, and contributions by taxa other than *Aureoumbra* and *Synechococcus* to total cell biovolume values were low.

Specific growth rates of *Aureoumbra* were low ($<0.2 \text{ day}^{-1}$) or even negative in the C and N+ mesocosms (Fig. 1). From days 0 to 4, growth rates were negative in both the C and N mesocosms but significantly less so in the N mesocosms ($-0.32 \pm 0.039 \text{ day}^{-1}$ versus $-0.02 \pm 0.025 \text{ day}^{-1}$, $p < 0.001$); after day 4, growth rates in both treatments were positive. *Aureoumbra* growth rates were significantly higher in the C than N+ mesocosms between days 4 and 16 ($0.116 \pm 0.012 \text{ day}^{-1}$ versus $0.059 \pm 0.010 \text{ day}^{-1}$, $p < 0.001$). *Synechococcus*-specific growth rates were also low or slightly negative during the experiment but rates for the N+ mesocosms were equal to or significantly higher than rates in the C mesocosms after day 4 ($0.100 \pm 0.032 \text{ day}^{-1}$ versus $0.034 \pm 0.010 \text{ day}^{-1}$, $p < 0.001$, Fig. 1).

Based on combined total cell biovolumes for *Aureoumbra* and *Synechococcus*, the proportion of

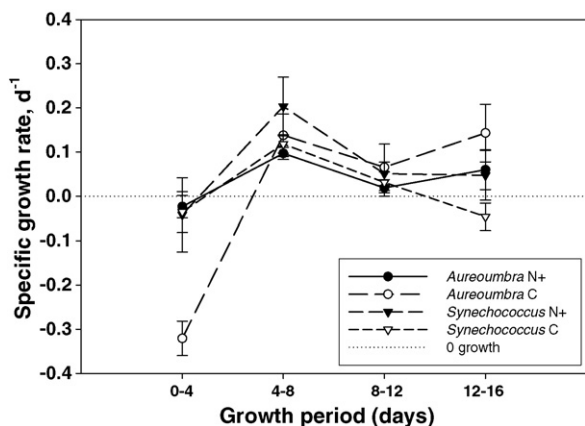


Fig. 1. *Aureobamba* and *Synechococcus* average-specific growth rates (day^{-1}) in the nitrogen amended (N+) and control (C) mesocosms. Data from Buskey et al. (2003).

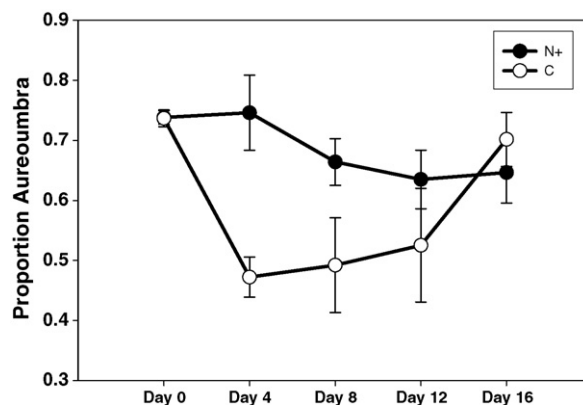


Fig. 2. Mean proportion of *Aureobamba* to total phytoplankton biovolume in the nitrogen amended (N+) and control (C) mesocosms.

Aureobamba to total biovolume remained unchanged in the N+ mesocosms throughout the experiment (Fig. 2). In C mesocosms, initial decline in the *Aureobamba* proportion was followed by an increase, such that by the end of the experiment *Aureobamba* proportions in the C mesocosms had recovered to pre-treatment levels and were indistinguishable from those in the N+ mesocosms (Fig. 2).

Aureobamba was the dominant microorganism, based on total cell biovolume, in all mesocosms at the beginning (range 73–74%) and the end (range 61–71%) of the experiment. As a result, particulate elemental concentrations and ratios at the beginning and end of the experiment largely reflected the status of *Aureobamba* with probably minor influence of *Synechococcus* and other taxa. N+ mesocosm PC and PN concentrations increased while C mesocosm PC and PN concentrations decreased (Table 1a). At the end of the experiment, average PN was significantly higher in the N+ compared

to the C mesocosms ($96 \mu\text{mol L}^{-1}$ versus $41 \mu\text{mol L}^{-1}$, $p < 0.001$; Table 1a). The same pattern applied to average PC when N+ and C mesocosms are compared ($628 \mu\text{mol L}^{-1}$ versus $353 \mu\text{mol L}^{-1}$, $p < 0.001$). However, PP did not differ between treatments.

The average particulate N:P ratio was significantly higher in the N+ than the C mesocosms at the end of the experiment (86 versus 40, $p < 0.005$; Table 1b). The difference between N+ and C mesocosms is largely due to differences in PN rather than PP concentrations, which varied little between treatments (Table 1a). At the end of the experiment, N:P ratios of the C mesocosms were similar to the starting value (40 versus 38). Final mean C:N ratios of the N+ mesocosms were significantly lower than those of the C mesocosms (6.6 versus 8.5, $p < 0.001$) and the initial value (8.2; Table 1b). N+ mesocosm final mean C:P ratio was higher than the C mesocosm mean (562 versus 345, $p < 0.008$) and the initial value (318; Table 1b).

Table 1

Effect of nitrogen addition on mesocosm particulate nitrogen, phosphorus, and carbon concentrations

Mesocosm treatment	Day	PP	PN	PC	Replicates for P, C–N
(a) Average particulate phosphorus (PP), nitrogen (PN), and carbon (PC) concentration ($\mu\text{mol L}^{-1}$) (\pm I.S.D.) in the nitrogen and control mesocosms at the beginning and end of the experiment					
Initial	0	1.65 ± 0.08	63.3 ± 3.3	523 ± 37	3, 3
N+	16	1.16 ± 0.23	95.5 ± 17.5	628 ± 104	6, 6
C	16	1.04 ± 0.14	41.4 ± 5.1	353 ± 44	5, 6
Mesocosm treatment	Day	N/P ratio	C/N ratio	C/P ratio	Replicates for P, C–N
(b) Average particulate nitrogen, phosphorus and carbon molar ratios (\pm one standard deviation) in the nitrogen and control mesocosms at the beginning and end of the experiment					
Initial	0	38.5 ± 2.8	8.2 ± 0.7	318 ± 28	3, 3
N+	16	85.6 ± 23	6.6 ± 0.2	562 ± 142	6, 6
C	16	40.3 ± 5.9	8.5 ± 0.4	345 ± 64	5, 6

A significant difference in *Aureoumbra* cellular chlorophyll autofluorescence as revealed by flow cytometry developed between the N+ and C treatments by day 8; cells from N+ treatment had at least twice the cellular chlorophyll content compared to those in the C treatment from day 8 through 16 ($p < 0.05$) (Fig. 3a). In both treatments, a slight decline was apparent by day 16. Cellular chlorophyll content for *Synechococcus* during the experiment was no more than 5% of *Aureoumbra* values with an initial rise then decline (Fig. 3a). In contrast to *Aureoumbra*, *Synechococcus* cellular chlorophyll content showed no consistent nitrogen effect. Irrespective of treatment, *Synechococcus* cellular chlorophyll increased or remained unchanged from days 0 to 4, then declined sharply from days 4 to 8. After day 8, *Synechococcus* cellular chlorophyll in the N+ treatment declined slightly while it remained unchanged in the control.

As with cellular chlorophyll fluorescence, average *Aureoumbra* cell size (cellular light scatter as measured by flow cytometry) for the N+ and C treatments diverged after day 4 (Fig. 3b). A large drop occurred in

the C mesocosms after day 4, so that by day 8 the average cell size in N+ mesocosms was 1.8 times the C mesocosm average. This size difference persisted through the end of the experiment, while both treatments showed a cell size increase from day 8 onwards. Average *Aureoumbra* cell biovolume, based on microscope measurements, also indicated that cells from the N+ mesocosms were larger than cells from C mesocosms ($33.8 \mu\text{m}^3$ versus $27.7 \mu\text{m}^3$), but this difference was less pronounced than that indicated by flow cytometric light scatter measurements. In *Synechococcus*, cell size decreased an average of 33% during the experiment. *Synechococcus* cells from N+ mesocosms were 14–19% larger than in C mesocosms during the middle of the experiment, but this difference disappeared by the experiment end (Fig. 3b).

4. Discussion

The Texas Brown Tide alga, *A. lagunensis*, exhibits significantly higher critical N:P ratios (115:1 to 260:1, growth rate dependent) than the 16:1 Redfield ratio (Villareal et al., 1998; Liu et al., 2001). The phytoplankton community at the beginning of the mesocosm experiments showed a particulate N:P ratio of 38:1. Based on total cell biovolume, *Aureoumbra* constituted 70% of this phytoplankton community, with *Synechococcus* contributing the bulk of the remainder. The N:P ratio for three strains of marine *Synechococcus* ranged from 13.3 to 33.2 grown under P-replete conditions (Bertilsson et al., 2003; Heldal et al., 2003). Under P limitation, N:P values for two *Synechococcus* strains rose to 96.9 and 109 (Bertilsson et al., 2003; Heldal et al., 2003). If it is assumed that the remainder of the phytoplankton community (30%) was composed of *Synechococcus* and using the minimum and maximum N:P values for *Synechococcus* reported above, the cellular N:P ratio of *Aureoumbra* would range from 8:1 to 49:1 at the start of the experiment. These values are well below the critical N:P ratios reported by Liu et al. (2001), suggesting strong N limitation for this alga at the start of the experiment.

At the end of the experiment, the planktonic community in the mesocosms (N+ or C) was *Aureoumbra*-dominated (61–74% of total cell biovolume). The average N:P ratio in the N+ mesocosms increased to 86, versus 40 in the C mesocosms, suggesting that nitrogen limitation for *Aureoumbra* lessened during the course of ammonium additions. However, it is likely that, except for short periods just after ammonium addition, *Aureoumbra* remained largely N limited. Average *Aureoumbra* growth rates

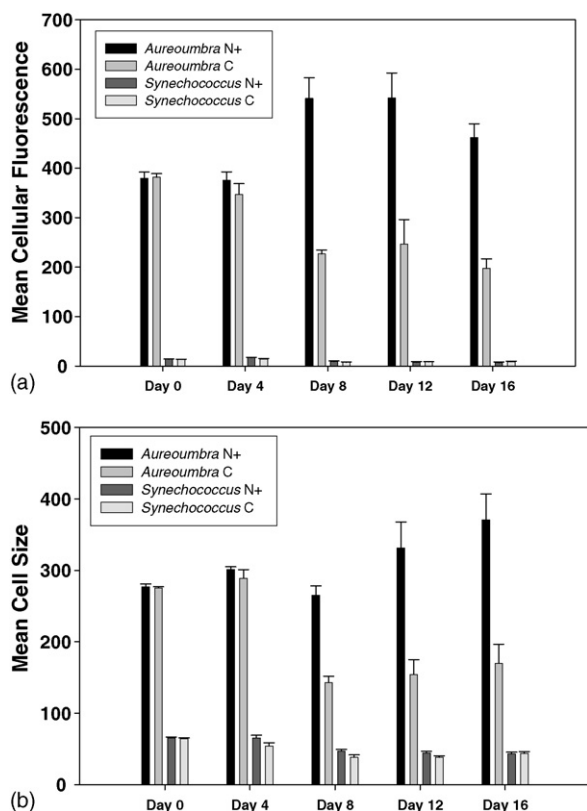


Fig. 3. *Aureoumbra* and *Synechococcus* (a) mean cellular chlorophyll fluorescence and (b) mean cell size (expressed as side angle light scatter) response during the mesocosm experiment.

in the N+ and C mesocosms were significantly different ($p < 0.03$) but both modest (0.06 and 0.14 day^{-1} , respectively). At these growth rates, the critical N:P ratio would be expected to be at or above the highest value (260) determined by Liu et al. (2001). Therefore, it is not surprising that *Aureoumbra* did not respond to the N addition by increased growth. It was still distinctly N limited. *Aureoumbra* did, however, respond to the N addition in subtle ways.

Aureoumbra cellular chlorophyll became elevated in the N+ mesocosms compared to C mesocosm cells and the initial cell condition. Cellular chlorophyll content is inversely related to light irradiance (Falkowski and La Roche, 1991) and directly related to cell nitrogen status (O'Kelley, 1968). Mesocosm light data indicated that light was not significantly lower in the N+ versus the C treatments. Light was probably not a limiting factor in any mesocosm since the mesocosms did not stratify (Buskey et al., 2003) and light saturation for *Aureoumbra* has been estimated to be about $40\text{--}100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Buskey et al., 1998; Villareal et al., 1998) while the average bottom irradiance in the mesocosms was $86 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Higher cellular N:P ratios in the N+ mesocosms, largely due to higher cellular N content, compared to C mesocosms indicate that the cells were less N limited, resulting in increased cellular chlorophyll.

By day 8 of the experiment, *Aureoumbra* mean cell volume was also ca. twice as high in the N+ mesocosms compared to the C mesocosms. For unicellular algae, growth rate and cell volume have been shown to be inversely related (Banse, 1976), although this may not always be the case (Herzig and Falkowski, 1989). *Aureoumbra* net growth rates were higher in C mesocosms compared to N+ mesocosms, which might account for the lower mean cell volume in the C mesocosms. However, cell volume has also been shown to be a positive function of cell nutrient status. Cell volume of four phytoplankton taxa increased with increased nitrogen level (Harrison et al., 1977; Fabregas et al., 1996). The increased volume of N+ mesocosm cells may represent a physiological response to nitrogen addition. Increased cell size would provide more space for N storage.

Based on a previous culture study, the C:N ratio for *Aureoumbra* grown under N-starved conditions (growth rate ~ 0) was ca. 35 whereas N-sufficient cells growing at 0.58 day^{-1} had a C:N ratio of ca. 6 (DeYoe and Suttle, 1994). In the N+ and C mesocosms, the plankton community C:N ratio was 6.6 and 8.7, respectively, both suggesting a lack of N limitation. However, C:N or, for that matter, C:P ratios may be misleading for

Aureoumbra since it can produce large amounts of extracellular polysaccharide as growth slows (Liu and Buskey, 2000).

Synechococcus did respond to the N addition. Despite the higher growth rate upon N addition, *Synechococcus* failed to dominate the N+ mesocosms by the end of the experiment. Irrespective of treatment, *Synechococcus* cellular chlorophyll, after an initial rise, declined significantly after day 4. Cell size also decreased, and both decreases in cellular chlorophyll and cell size were strongest when growth rates were highest. Faster growth resulted in smaller cells, which in turn also contained less chlorophyll. The decrease in cell size was less pronounced under N additions despite higher growth rates compared to C mesocosms, which might reflect the improved nitrogen status of the cells. The influence of growth rate and nitrogen status on *Synechococcus* cell size is not well characterized.

If it was assumed that *Aureoumbra* had a critical N:P ratio near the 16:1 Redfield ratio, there would have been no response to N addition by *Aureoumbra* or any other algae having the Redfield ratio since the starting N:P ratio was 38. However, *Aureoumbra* did respond, not by increased growth rate but by physiological changes. It seems likely that with continued N additions the mesocosm N:P ratios would eventually remain consistently in the range favorable for *Aureoumbra* growth. The main competitor of *Aureoumbra*, *Synechococcus*, did respond to the N addition by increased growth rates. This positive response to N addition suggests that this particular *Synechococcus* strain or population has a critical N:P ratio higher than 16:1 yet lower than that of *Aureoumbra*.

This study illustrates that species-specific information on critical N:P ratios are a prerequisite for accurate predictions of the effect of nutrient additions on a phytoplankton community. Furthermore, consideration should be given to the prokaryotic or eukaryotic nature of phytoplankton competitors as they likely exhibit different physiological properties and reactions.

Acknowledgements

This work was largely supported by the Texas Higher Education Coordinating Board Advanced Research Program Project No. 003599-0012. We wish to thank Bill Beck, Gary Clark and Howard Fels from the Central Power and Light's Barney Davis Power Station for assisting with the arrangements and allowing us to perform this experiment on their property. This is publication CSS 2006-01 of the Center for Subtropical Studies at the University of Texas-Pan American and

contribution 1413 of the University of Texas Marine Science Institute. [SES]

References

- Banse, K., 1976. Rates of growth, respiration and photosynthesis of unicellular algae as related to cell size—a review. *J. Phycol.* 12, 135–140.
- Bertilsson, S., Berglund, O., Karl, D.M., Chisholm, S.W., 2003. Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. *Limnol. Oceanogr.* 48, 1721–1731.
- Buskey, E.J., Wysor, B., Hyatt, C.J., 1998. The role of hypersalinity in the persistence of the Texas brown tide in the Laguna Madre. *J. Plankton Res.* 20, 1553–1565.
- Buskey, E.J., Liu, H., Collumb, C., Bersano, J.G.F., 2001. The decline and recovery of a persistent Texas Brown tide algal bloom in the Laguna Madre (Texas, USA). *Estuaries* 24, 337–346.
- Buskey, E.J., DeYoe, H.R., Jochem, F.J., Villareal, T.A., 2003. Effects of mesozooplankton removal and ammonium addition on planktonic trophic structure during a bloom of the Texas “brown tide”: a mesocosm study. *J. Plankton Res.* 25, 215–228.
- Cotner, J.B., Suplee, M.W., Chen, N.W., Shormann, D.E., 2004. Nutrient, sulfur and carbon dynamics in a hypersaline lagoon. *Estuar. Coast. Shelf Sci.* 59, 639–652.
- DeYoe, H.R., Suttle, C.A., 1994. The inability of the Texas “brown” tide alga to use nitrate and the role of nitrogen in the initiation of a persistent bloom of this organism. *J. Phycol.* 30, 800–806.
- DeYoe, H.R., Stockwell, D.A., Bidigare, R.R., Latasa, M., Johnson, P.W., Hargraves, P.E., Suttle, C.A., 1997. Description and characterization of the algal species *Aureoumbra lagunensis* gen. et sp. nov. and referral of the *Aureoumbra* and *Aureococcus* to the Pelagophyceae. *J. Phycol.* 33, 1042–1048.
- Fabregas, J., Cid, A., Morales, E., Cordero, B., Otero, A., 1996. Discrepancies between cell volume and organic content in semi-continuous cultures of a marine microalga. *Letts. Appl. Microb.* 22, 206–208.
- Falkowski, P.B., La Roche, J., 1991. Acclimation to spectral irradiance in algae. *J. Phycol.* 27, 8–14.
- Fong, P., Zedler, J.B., Donohoe, R.M., 1993. Nitrogen vs. phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnol. Oceanogr.* 38, 906–923.
- Harrison, P.J., Conway, H.L., Holmes, R.W., Davis, C.O., 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular composition and morphology of *Chaetocerus debilis*, *Skeletonema costatum* and *Thalassiosira gravida*. *Mar. Biol.* 43, 19–31.
- Heldal, M., Scanlan, D.J., Norland, S., Thingstad, F., Mann, N.H., 2003. Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. *Limnol. Oceanogr.* 48, 1732–1743.
- Herzig, R., Falkowski, P.G., 1989. Nitrogen limitation in *Isochrysis galbana* (Haptophyceae) I. Photosynthetic energy conversion and growth efficiencies. *J. Phycol.* 25, 462–471.
- Klausmeier, C.A., Litchman, E., Daufresne, T., Levin, S., 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature (London)* 429, 171–174.
- Liu, H., Buskey, E.J., 2000. Hypersalinity enhances the production of extracellular polymeric substance (EPS) in the Texas brown tide alga *Aureoumbra lagunensis* (Pelagophyceae). *J. Phycol.* 35, 1–7.
- Liu, H., Laws, E.A., Villareal, T.A., Buskey, E.J., 2001. Nutrient-limited growth of *Aureoumbra lagunensis* (Pelagophyceae) with implications for its capability to outgrow other phytoplankton species in phosphate-limited environments. *J. Phycol.* 37, 500–508.
- Mansfield, A.D., 1997. Effects of light, nutrients and salinity on the chemical composition of *Aureoumbra lagunensis* Stockwell, DeYoe, et al.: The use of batch cultures to validate field results. M.S. Thesis. Univ. Mass., 94 pp.
- O’Kelley, J.C., 1968. Mineral nutrition of algae. *Ann. Rev. Plant Physiol.* 19, 89–112.
- Quigg, A., Finkel, Z., Irwin, A., Rosenthal, Y., Ho, T.-Y., Reinfelder, J., Schofield, O., Morel, F., Falkowski, P., 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature* 425, 291–294.
- Redfield, A.C., 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46, 205–221.
- Rhudy, K.B., Sharma, V.K., Lehman, R.L., McKee, D.A., 1999. Seasonal variability of the Texas “brown tide” (*Aureoumbra lagunensis*) in relation to environmental parameters. *Estuar. Coast. Shelf Sci.* 48, 565–574.
- Schelske, C.L., Feldt, L.E., Santiago, M.A., Stoermer, E.F., 1972. Nutrient enrichment and its effect on phytoplankton production and species composition in Lake Superior. In: Proceedings of the 15th Conference Great Lakes Research International Association, Madison, Wisconsin.
- Solorzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.* 25, 754–758.
- Sommer, U., 1988. The species composition of Antarctic phytoplankton interpreted in terms of Tilman’s competition theory. *Oecologia* 77, 464–467.
- Suttle, C.A., Harrison, P.J., 1988. Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. *Limnol. Oceanogr.* 33, 186–202.
- Tilman, D., 1977. Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology* 58, 338–348.
- Villareal, T.A., Mansfield, A., Buskey, E.J., 1998. Growth and chemical composition of the Texas brown tide-forming Pelagophyte *Aureoumbra lagunensis*. In: Reguera, B., Fernandez, M.I., Wyatt, T. (Eds.), *Harmful Algae*. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo, Spain, pp. 359–362.