

Urease activity in cultures and field populations of the toxic dinoflagellate *Alexandrium*

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Abstract

Nitrogen availability is an important factor controlling phytoplankton abundance and species composition in marine waters. In addition to inorganic nitrogen, some phytoplankton species can use dissolved organic nitrogen sources such as urea for growth. Herein we demonstrate that axenic laboratory cultures of the toxic dinoflagellate, *Alexandrium fundyense* strain CB301A and *A. catenella* strain TN9A were able to grow on urea as a sole nitrogen source in the presence of nickel. This nickel dependence suggests that these *Alexandrium* species hydrolyze urea into ammonia with the enzyme urease rather than adenosine triphosphate urea amidolyase. Cells grown on urea had lower toxin content (15%–30%) than f/2-grown cells. In *A. fundyense* the urease enzyme appears to be nitrogen-regulated. In culture experiments, enzyme activity was highest in nitrate-starved and urea-grown (replete) cells, whereas activity was undetectable in f/2-grown (replete) and phosphate-starved cells. Urease activity in ammonia-grown (replete) cells was also depressed. Urease activity also appeared to increase with decreasing nitrate-limited growth rate in semicontinuous cultures. May and June cruises in the Gulf of Maine followed the yearly bloom of *A. fundyense*. On average, inorganic nitrogen concentrations in May were higher than in June, whereas cell abundances, urea concentrations, and urease activity in May were lower than in June. The latter measurements relied on an immunomagnetic bead separation to isolate living *A. fundyense* cells from mixed phytoplankton samples for analysis. The differences between May and June suggest that urea may be important for *Alexandrium* nutrition as inorganic nitrogen concentrations in surface water decline.

Harmful algal blooms cause serious public health and economic impacts on a global scale (Hallegraeff 1993; Anderson et al. 2000). For example, species within the marine dinoflagellate genus *Alexandrium* are responsible for paralytic shellfish poisoning (PSP) along the coastlines of the United States, Canada, and many other countries (Hallegraeff 1993; Anderson 1997). PSP can result in serious illness or death if humans ingest sufficient shellfish contaminated with the dinoflagellate toxins (Van Dolah 2000). Observations of *Alexandrium fundyense* and PSP outbreaks in the northeastern United States indicate that populations accumulate in a variety of oceanographic habitats (Anderson 1997). There is considerable interest in the environmental factors that affect the growth of this genus in field populations. For example, *A. fundyense* populations in the western Gulf of Maine are often found in surface waters where inorganic nitrogen concentrations are low ($<2 \mu\text{mol L}^{-1}$) to undetectable, particularly in the late spring (Martorano 1997; Poulton 2001). In this system it is unclear whether *A. fun-*

dyense populations may be constrained by the lack of inorganic nitrogen or if they are able to supplement their nitrogen demand through organic sources.

The availability of different forms of nitrogen can significantly affect the abundance of different species and the composition of marine phytoplankton communities. For instance, dinoflagellates have been associated with low nitrate and high ammonium or high dissolved organic nitrogen (DON) concentrations, which suggests that some species within this phytoplankton class may selectively use organic nitrogen sources (Paerl 1988; Berg et al. 1997; Glibert and Terlizzi 1999). DON often represents a significant proportion of the total dissolved nitrogen pool (reviewed in Antia et al. 1991). To predict how harmful phytoplankton, such as *A. fundyense*, may respond to DON, it is critical to assay the availability of different nitrogen forms within the DON pool and the extent to which these compounds may be utilized.

Dinoflagellates are able to take up and assimilate a variety of DON compounds, including humic substances (Doblin et al. 1999), purines such as hypoxanthine (reviewed in Antia et al. 1991), amino acids (John and Flynn 1999), and urea (Kudela and Cochlan 2000). For example, recent laboratory work has shown that *A. fundyense* is able to take up dissolved free amino acids but could not use these compounds as a sole nitrogen source for growth (John and Flynn 1999). Urea may be a particularly important component of the DON pool for dinoflagellate nutrition; in a recent field study, the dinoflagellate *Lingulodinium polyedra* had high urea uptake rates relative to nitrate (Kudela and Cochlan 2000). Last, increased dinoflagellate abundance has been linked to increased urea concentrations in aquaculture facilities (Glibert and Terlizzi 1999). Taken together, these data suggest that DON, and particularly urea, may be important for nitrogen nutrition in dinoflagellates.

Urea is often the dominant component of DON in marine

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Acknowledgments

We thank Dave Kulis, Jody Donahue, Mike Lomas, Bruce Keafer, Liz Jablonski, and Dave Townsend for technical assistance and sample processing. Special thanks also go to Uwe John and Linda Medlin of the Alfred Wegener Institute for supplying the *A. ostenfeldii* oligonucleotide probe. We also thank the Captain and crew of the R/V *Cape Hatteras* and the R/V *Oceanus*. The manuscript was improved with valuable comments from two anonymous reviewers. Our research was supported by the Woods Hole Postdoctoral Scholar Program with funds from the Stanley Watson Chair (to D.M.A.), by NSF grant OCE-9808173 (to D.M.A.), and by NOAA grant NA96OP0099 (to D.M.A.). This is contribution 10679 from the Woods Hole Oceanographic Institution and 59 from the ECOHAB program. The ECOHAB program is sponsored by the NOAA and the US EPA, NSF, NASA, and ONR.

waters, where urea concentrations typically range from 0.1 to 1.0 $\mu\text{mol L}^{-1}$ (Antia et al. 1991). Growth on urea, urea uptake, or urease activity have been demonstrated for many different phytoplankton species (Carpenter et al. 1972; Antia et al. 1991; Collier et al. 1999; Palinska et al. 2000; Berg et al. 2002), although not for many dinoflagellates. Urea is assimilated by hydrolysis of ammonia via either the enzyme urease or adenosine triphosphate (ATP) urea amidolyase in marine phytoplankton (Antia et al. 1991). With the exception of chlorophytes, marine phytoplankton appear to use the nickel-dependent urease enzyme (Bekheet and Syrett 1977).

Studying the in situ physiological ecology of *A. fundyense* presents particular challenges because *Alexandrium* species are often in low abundance in the environment. Yet, even at these very low cell densities, *A. fundyense* can cause toxicity. In the present study, we investigate how *A. fundyense* cultures respond to and process urea. We also use an immunomagnetic procedure to separate and concentrate *A. fundyense* cells from mixed field populations for species-specific physiological analyses of *A. fundyense* populations during two cruises in the western Gulf of Maine.

Materials and methods

Cell culture—Axenic *A. fundyense* strain CB301A, isolated from the Gulf of Maine, and axenic *Alexandrium catenella* strain TN9A, isolated from Tanabe Bay, Japan, were used in this study. Cell cultures were grown in f/2 medium at 20°C in cool white fluorescent light ($\sim 200 \mu\text{mol}$ of quanta $\text{m}^{-2} \text{s}^{-1}$) on a 16:8 light:dark cycle. Sterility was confirmed by light microscopy and by periodic inoculation into a tryptone-fortified media to check for bacterial growth (Anderson et al. 1991). Replete cells were grown in f/2 media made with 0.2- μm filtered local seawater (salinity ~ 31 psu) autoclaved with inorganic f/2 nutrients except silica (Guillard 1975). Filter-sterilized f/2 vitamins were added after autoclaving. For low nitrate conditions, f/2 was modified by changing from 853 to 50 $\mu\text{mol L}^{-1}$ nitrate or for low phosphate conditions from 36.3 to 1 $\mu\text{mol L}^{-1}$ phosphate. Cells harvested at stationary phase from these cultures are referred to as nitrogen- or phosphorus-starved, respectively. For cultures grown on urea, nitrate was omitted and the media was supplemented with 50 $\mu\text{mol L}^{-1}$ urea and 100 nmol L^{-1} nickel. For ammonium-grown cultures, the nitrate was again omitted and the media was supplemented with 50 $\mu\text{mol L}^{-1}$ ammonia. Growth in the cultures was evaluated using a fluorometer (Turner Designs) or via cell counts on Utermohl preserved samples.

Urease assays—Urease assays were performed as described elsewhere (Peers et al. 2000) with the following modifications: 100 mmol L^{-1} KPO_4 buffer was used instead of 150 mmol L^{-1} KPO_4 buffer, and the enzyme reactions were stopped by boiling each sample for 2 min. Cultures were always harvested at the same time of day to minimize potential diurnal effects on the enzyme activity.

Toxin analyses—Toxin content and composition of triplicate cultures grown in different media were analyzed by high-performance liquid chromatography (HPLC) as de-

scribed elsewhere (Oshima 1995). In brief, $0.4\text{--}1.0 \times 10^5$ cells were harvested into preweighed 15-ml centrifuge tubes. Tubes were centrifuged at $5,000 \times g$ for 5 min, the supernatant aspirated, and 1.0 ml acetic acid added to each tube. Samples were sonicated for 20 s on ice. After sonication, extracts were stored frozen at -20°C until further analysis. At the time of analysis, extracts were thawed and centrifuged at $5,000 \times g$ for 5 min to separate cellular debris, and 150 μl of sample extract was transferred to spring-loaded HPLC glass tubes and sequentially analyzed for C, neosaxitoxin, saxitoxin, and gonyautoxin (GTX) toxins according to the methods described elsewhere (Anderson et al. 1996).

Semicontinuous experiments—Duplicate cultures were inoculated with equivalent cell numbers and allowed to grow for 5 d, at which point they were diluted at rates of 0.35, 0.3, 0.21, and 0.1 d^{-1} . Starting medium was modified f/2 with 83 $\mu\text{mol L}^{-1}$ nitrate and the dilution medium was 83 $\mu\text{mol L}^{-1}$ in all treatments but the fastest dilution rate (0.35 d^{-1}). In this 0.35 d^{-1} treatment, the starting and diluting media were both f/2 to ensure nutrient-replete conditions. Once cell number and relative fluorescence had stabilized, dilutions were maintained for 9 d, and then the cultures were harvested for analyses of urease activity, cellular C and N content, and toxin content. Because of low cell numbers, cellular C and N content and toxin content were not assessed in the 0.21 d^{-1} treatment. Urease assays were performed as described above. For the cellular C and N measurements, $\sim 100,000$ cells were collected onto precombusted (6 h at 400°C) 25-mm A/E filters. These filters were analyzed on a Perkin Elmer 2400 CHN analyzer using precombusted filters without cells as a blank. The total toxin content was determined as described above.

Immunomagnetic bead separation—To assess urease activity in field populations of *A. fundyense* in the western Gulf of Maine, where this genus is typically a small component of the total community, we pursued an Immunomagnetic bead separation (IMBS) method. *Alexandrium* cells were isolated according to the indirect method described in Aguilera et al. (1996) and adapted for living cells (Aguilera et al. 2002). In short, this involves an indirect coupling of *Alexandrium* cells to magnetic beads via the monoclonal antibody M-8751-1. This antibody reacts with cells of the *A. fundyense/tamarensis* complex and with *Alexandrium ostensefeldii* (Adachi et al. 1993; Anderson et al. 1999; unpubl. data). Bead-labeled cells were then separated from the unwanted material using a magnet. Isolated cells were drawn down onto 25-mm GF/F filters and stored in liquid nitrogen prior to analyses of urease activity.

Field sampling—Two saxitoxin-producing species of *Alexandrium* occur in the Gulf of Maine: *A. fundyense* and *A. tamarensis* (Anderson et al. 1994). We consider these to be varieties of the same species (Anderson et al. 1994; Scholin et al. 1995), and neither antibody nor oligonucleotide probes can distinguish between *A. fundyense* and *A. tamarensis* from this region. Only detailed analysis of the thecal plates on individual cells can provide this resolution, but this is not practical for large numbers of field samples. Accordingly,

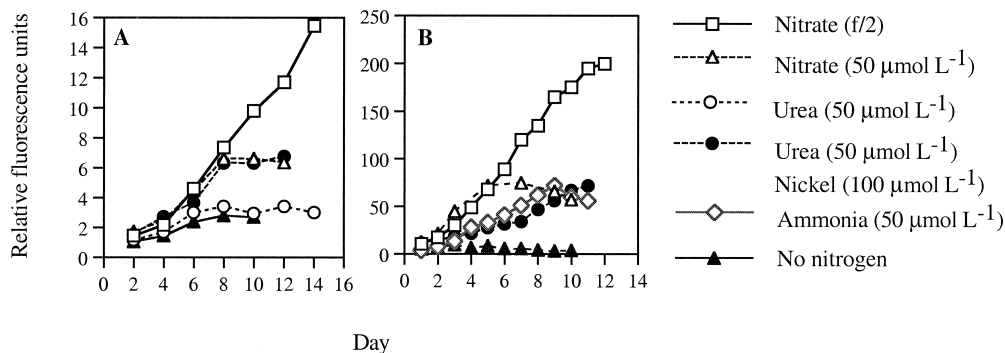


Fig. 1. Growth of (A) *A. fundyense* and (B) *A. catenella* on different nitrogen sources.

for the purpose of this and other field studies, we use the name *A. fundyense* to refer to both forms. *A. ostensfeldii* also occurs in the Gulf of Maine; however, it was not abundant at our sites during this study.

Two process studies were conducted in May and June 2001 as part of the ECOHAB-Gulf of Maine program (see Fig. 4 below for map of study area). Samples for *A. fundyense* abundance and inorganic nutrient concentrations were collected from surface Niskin bottles. For the *A. fundyense* counts, 8 liters of water were sieved onto a 20- μm mesh Nitex screen, and the cells retained on the screen were washed back into a 15-ml tube and preserved in 5% formalin. After 20 min, the cells were centrifuged for 10 min at $3,000 \times g$, the supernatant was aspirated, and the cell pellet was transferred into methanol for storage. *A. fundyense* can often be at very low densities in the Gulf of Maine. To maximize cell detection and quantification, an oligonucleotide labeling procedure (described in Anderson et al. 1999) was used to fluorescently tag cells. We used a dual labeling protocol, using the oligonucleotide probe NA1 (Anderson et al. 1999) specific for North American *A. fundyense/tamarense* and conjugated to fluorescein isothiocyanate (FITC) and the oligonucleotide probe Ao (5' ATT CCA ATG CCC ACA GGC 3') specific for *A. ostensfeldii* and conjugated to CY3. Although the relative intensity of the fluorescent labeling can differ with changes in temperature or cellular nutritional status (Anderson et al. 1999), previous work has demonstrated that this labeling procedure can be used to accurately determine environmental cell counts. The labeled cells were counted using a Zeiss Axioscope fitted with a FITC/CY3 filter set, excitation 450 nm and emission 750 nm (Chroma Tech).

Macronutrient samples were collected from the surface Niskin bottles. Samples were collected and analyzed using the protocols described by Townsend et al. (2001). Urea concentrations were determined by hydrolyzing the sample urea into ammonia. In brief, urea samples were collected as above and stored frozen until analysis. For each station, one subsample was thawed and then treated with 36 units of jack bean urease (Sigma) as described elsewhere (McCarthy 1970). The other subsample was treated with 36 units of heat-inactivated jack bean urease as a blank. These samples were incubated for 30 min at 50°C. These processed samples were stored frozen for ~1 week, at which point ammonia concentrations were determined at the Ocean Data Facility

of the Scripps Institution of Oceanography, as described elsewhere (Dyhrman and Palenik 1999). Urea concentrations were calculated on the basis of the difference in the ammonia concentration of the hydrolyzed samples and heat-inactivated blank controls.

IMBS samples for determining urease activity were obtained by pumping 1,800 liters of surface water through a 20- μm mesh net. The resulting cell sample was prescreened through a 100- μm sieve and then collected on a 20- μm sieve. Retained material was washed back into a final volume of 3 ml using filtered local seawater. Triplicate 1-ml samples were then immediately processed with the IMBS method (Aguilera et al. 1996, 2002) as discussed above. After the cells were isolated, 900 μl of each sample was collected onto a GF/F filter and stored in liquid nitrogen for the urease assays. The remaining 100 μl was diluted with 900 μl of sterile seawater and Utermohl solution for cell counts. Cell counts were used to assess contamination of the samples from other species, to normalize the urease activity to *A. fundyense* cell number and to determine whether the sample was within the detection limit of the urease assay. As with the culture work, all sampling for urease activity was done at roughly the same time of day to minimize potential diurnal variation in *A. fundyense* nitrogen physiology.

Results

Growth experiments—*A. fundyense* was able to grow on urea as a sole nitrogen source in the presence of nickel (Fig. 1A). In our media, the cultures did not grow in the presence of urea without added nickel or with no nitrogen added. A related dinoflagellate, *A. catenella* was also able to grow on urea in the presence of nickel (Fig. 1B). Both strains reached similar cell densities ($\sim 4,000 \text{ cell ml}^{-1}$) on the 50- $\mu\text{mol L}^{-1}$ nitrate, urea, and ammonia treatments. The difference in relative fluorescence units (RFU) is a function of calibration for the two different fluorometers used in this study.

Urease activity—To examine the regulation of the urease enzyme, triplicate cultures grown in different media types were assayed for urease activity. Enzyme activity was detected in the nitrogen-starved culture and in the urea grown culture, but no activity was detected in replete cultures or phosphate-starved cultures (Fig. 2). Activity was also depressed when cells were grown on ammonia as the sole ni-

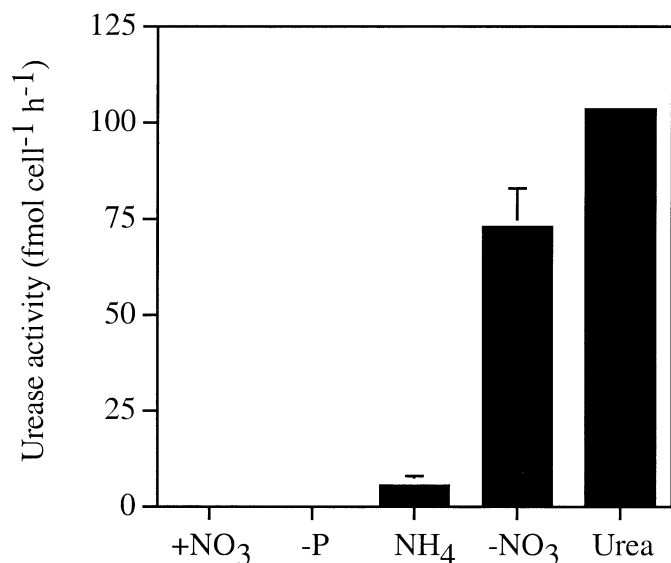


Fig. 2. Urease activity detected in *A. fundyense* cultures. Cultures were tested from f/2 media (+NO₃), low phosphate media (-P), media with ammonia (HN₃) as the nitrogen source, low-nitrate (-NO₃) media, and media with urea as the nitrogen source.

trogen source at replete levels (Fig. 2). These assays were repeated several times with similar results. Notably, urease activity was not detected in f/2 cultures grown with 100 nmol L⁻¹ supplemental Ni (data not shown).

Toxin analyses—Total toxin content (fmol cell⁻¹) of *A. fundyense* grown on urea and tested in both exponential and stationary phase was lower than the toxin content of replete cells, nitrate-starved cells, or ammonia (stationary phase)-grown cells (Fig. 3A). The total toxin content of *A. catenella* was substantially lower than *A. fundyense* but also showed a similar relationship with respect to urea, nitrate, and ammonia (Fig. 3B). Growth on urea was also characterized by changes in the toxin composition of each species, particularly in the case of *A. catenella*. The concentration (fmol cell⁻¹) of the epimer pair C1, two in the urea-grown cells

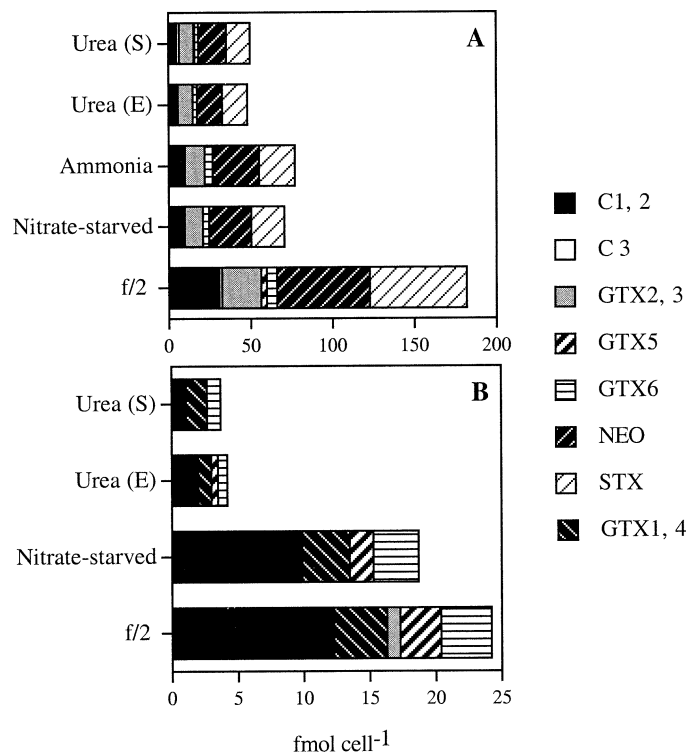


Fig. 3. Toxin content and composition for (A) *A. fundyense* and (B) *A. catenella* grown on urea and harvested in either the stationary (S) or exponential (E) phase. Toxin composition and content were also determined in medium with ammonia as the nitrogen source as well as in low-nitrate media (nitrogen-starved) and f/2 media (f/2).

decreased 50% compared with the nitrate-starved sample in *A. fundyense* (Fig. 3A). In *A. catenella* C1, 2 decreased 85% compared with the nitrate-starved sample (Fig. 3B). If the data are transformed to reflect the relative abundance of each derivative as a percentage of total toxin, these differences are not as striking for *A. fundyense* (Table 1). However, there are compositional changes reflected in the mole percentage of each derivative for *A. catenella*. Notably, the relative per-

Table 1. Relative mole percentage toxin composition of *A. fundyense* and *A. catenella* grown in different media types. For urea-grown cultures, both stationary phase (S) and exponential phase (E) cultures were tested.

	C1, 2	C3	GTX 1, 4	GTX 2, 3	GTX5	GTX6	NEO	STX
<i>Alexandrium fundyense</i> (mol %)								
Urea (S)	12	ND	.3	18	.5	ND	34	28
Urea (E)	10	2	ND	19	ND	ND	32	32
Ammonia	13	ND	ND	15	ND	ND	37	28
Nitrate-starved	14	ND	ND	16	ND	1	37	29
f/2	17	2	ND	13	2	3	31	33
<i>Alexandrium catenella</i> (mol %)								
Urea (S)	29		42	ND	ND	27		
Urea (E)	47		24	ND	12	17		
Nitrate-starved	53		19	ND	9	18		
f/2	53		17	4.3	13	16		

ND, not detectable.

Table 2. A table of parameters from semi-continuous experiments with *A. fundyense*.

Dilution rate (d ⁻¹)	Urease activity (fmol cell ⁻¹ h ⁻¹)	C:N	Total toxin content (fmol cell ⁻¹)
0.35 (replete)	ND	6.3	377
0.30 (low nitrate)	77.01	6.9	299
0.21 (low nitrate)	100.5	—	—
0.10 (low nitrate)	140.17	10	51

centage of the primer pair C1, two decreased 46% in the urea grown cells harvested in stationary phase compared with nitrate-starved cells also harvested in stationary phase (Table 1). There was a corresponding 55% increase in GTX1, 4, and a 34% increase in GTX6 (Table 2) in urea-grown cells compared with nitrate-starved cells.

Semicontinuous culture experiments—In semicontinuous cultures, there was a general trend of increasing urease activity per cell with decreasing nitrate-limited growth rate (Table 2). Other potential indicators of nitrogen physiology include the cellular C:N ratio and the toxin content per cell. With decreasing nitrate-limited growth rate, the C:N ratio increased and the total toxin content declined. The total toxin content of the replete (0.35 d⁻¹) and 0.3 d⁻¹ treatments were somewhat higher in the semicontinuous cultures than in the batch cultures; however, toxin content of the 0.1 d⁻¹ treatment was similar to the concentration seen in low nitrate media.

Field data—Using the IMBS method, urease activity was detected in *Alexandrium*-enriched field samples. Initial testing of the IMBS method on culture controls indicated that the isolation procedure did not significantly alter the urease activity per cell (data not shown). Cell counts from field samples after IMBS indicated that the samples were substantially enriched in *Alexandrium* (on average, 75% of the cells in the sample consisted of *A. fundyense* and *A. ostenfeldii*), and in only one case (Sta. 115) was there significant contamination by other phytoplankton. At a number of stations, the cell numbers in the IMBS processed samples were too low to be assayed for urease activity, so data from these stations are not reported. At the remaining stations cell-specific urease activities were highly variable, ranging over 100-fold. In general, the average urease activities detected in June (92.5 ± 63.9 fmol cell⁻¹ h⁻¹) were higher than in May (8.5 ± 0.5 fmol cell⁻¹ h⁻¹) (Fig. 4). For example, the June activities at stations 15 and 67 were 141.7 fmol cell⁻¹ h⁻¹ and 115.7 fmol cell⁻¹ h⁻¹, respectively, whereas the highest activity detected at any station during May was 8.91 fmol cell⁻¹ h⁻¹.

The total average *Alexandrium* densities including both *A. fundyense* and *A. ostenfeldii*, were lower in May (32.0 ± 19.4 cell L⁻¹) than in June (199.8 ± 173.2 cell L⁻¹) (Fig. 4). The highest cell abundances for the stations tested were centered near Penobscot Bay. The density of each individual species also followed these patterns, although the *A. ostenfeldii* concentration was low compared with *A. fundyense*,

comprising <15% of the total *Alexandrium* population at the stations where urease activity was detected (data not shown).

Although variable, the average urea concentration was lower in May (0.07 ± 0.1 μmol L⁻¹) than in June (0.26 ± 0.17 μmol L⁻¹) (Fig. 4). In contrast, combined nitrate, nitrite, and ammonia (DIN) concentrations in surface samples were on average higher during the May cruise (0.63 ± 0.58 μmol L⁻¹) than the June cruise (0.27 ± 0.21 μmol L⁻¹) (Fig. 4). The average surface DIN:dissolved inorganic phosphorus (DIP) ratio was 4.76 in May and 0.77 in June. The average surface temperature in May (7.57°C) was somewhat lower than in June (9.34°C).

Discussion

Herein we demonstrate that both *A. fundyense* and *A. catenella* are able to grow on urea as a sole nitrogen source. These species would not grow on urea without added nickel, which suggests that their urea metabolism is nickel dependent. Of the two enzymes able to hydrolyze urea into ammonia, the urease enzyme (urea amidohydrolase, EC 3.5.1.5) requires a nickel cofactor, whereas ATP:urea amidolyase does not. Similar to the dinoflagellate *Amphidinium carterae* (Antia et al. 1991), *A. fundyense* and *A. catenella* appear to process urea with the enzyme urease.

In marine phytoplankton, data on the regulation of urea uptake and urease activity by nitrogen are inconsistent, even among species, with little information on urease regulation in dinoflagellates (Antia et al. 1991; Peers et al. 2000). However, in many microbes, the production of urease is tightly regulated by nitrogen. For example, urease synthesis can be repressed by the presence of ammonia or other nitrogen rich compounds and derepressed by nitrogen-limiting or nitrogen-starvation conditions (Moblely and Hausinger 1989). In *A. fundyense*, urease activity was not detected under nitrate-replete or phosphate-starved conditions, which suggests that the enzyme is not constitutively expressed. The depression of activity in ammonia-grown (replete) cells compared with nitrogen-starved or urea grown (replete) cells is also consistent with nitrogen regulation of urease activity.

The toxin content and composition of a given *Alexandrium* strain can vary significantly with nutritional status (Anderson et al. 1990a,b; Flynn et al. 1994; John and Flynn 2000). In a review of data from both semicontinuous and batch cultures, phosphate limitation, or starvation, in *A. fundyense* and *A. tamarensis* typically increases toxin content, whereas nitrogen limitation or starvation decreases toxin content (Anderson et al. 1990a,b; Taroncher-Oldenburg et al. 1999; John and Flynn 2000; Poulton 2001). Consistent with these observations, the toxin content per cell in both species was lower in nitrogen-starved cells than in nitrogen-replete cells in our experiments. In *A. fundyense*, the toxin content was somewhat lower in cells grown on urea (an average of 48.5 fmol cell⁻¹) than nitrogen-starved cells (70.1 fmol cell⁻¹) or cells grown on ammonia (77.0 fmol cell⁻¹). Similarly, the toxin content in *A. catenella* grown on urea was lower than for nitrogen-starved cells, decreasing from 18.7 to 4.2 fmol cell⁻¹. The decreased toxin content in these *Alexandrium* species may be a function of urea specifically

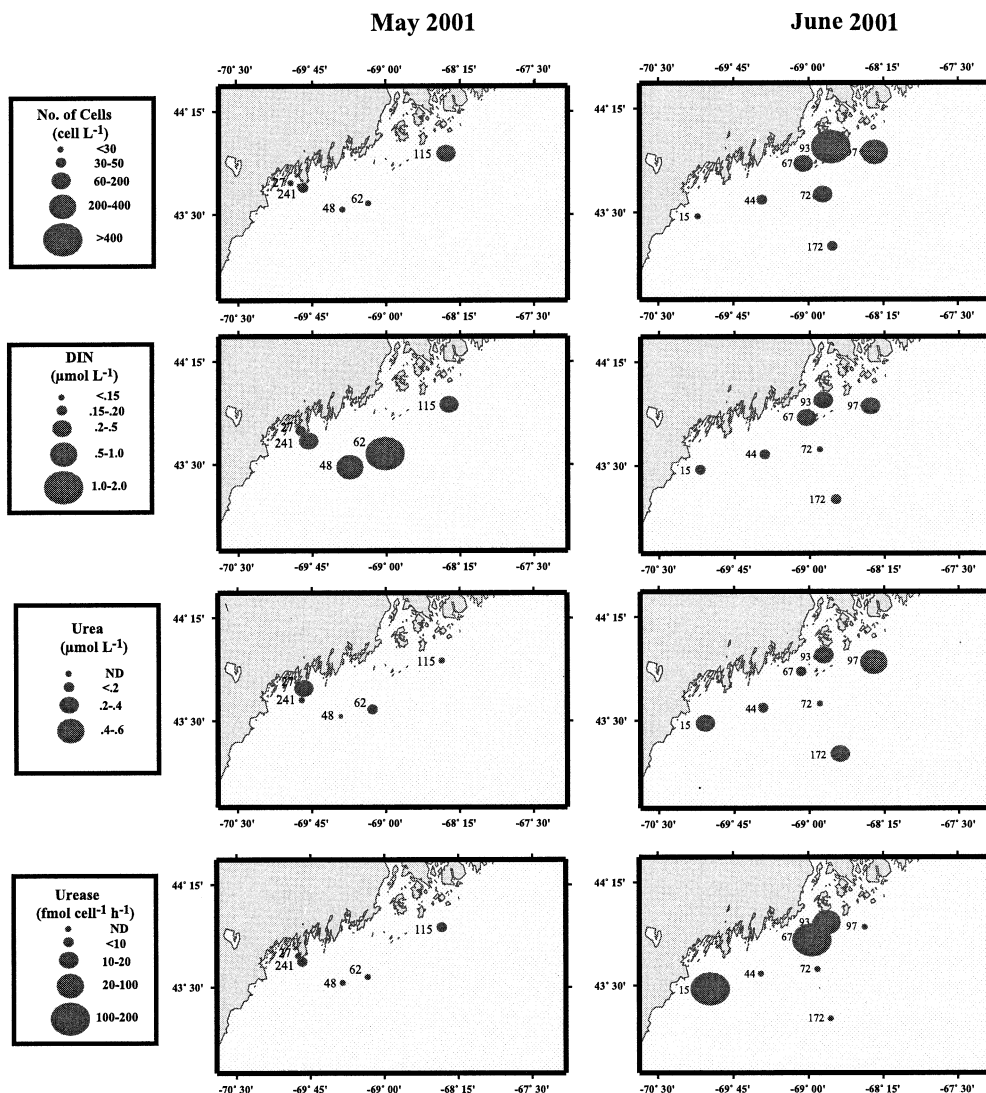


Fig. 4. Maps of the western Gulf of Maine study area plotted with the number of *Alexandrium* cells, combined nitrate, ammonia and nitrite (DIN) concentration, urea concentration, and urease activity.

rather than growth on DON in general. In other studies with *A. fundyense* that have used natural concentrations of dissolved free amino acids (DFAA) as a DON source, toxin content was not significantly altered compared with controls (John and Flynn 1999). At high concentrations of DFAA, toxin content increased compared with controls (John and Flynn 1999). A decreased toxin content in the presence of urea has also been observed in the saxitoxin-producing cyanobacterium *Planktothrix* sp. FP1 (Pomati et al. 2001).

Growth on urea also was characterized by variability in toxin composition. In *A. fundyense*, the largest change between nitrate-starved and urea-grown cells was in the concentration of the epimer pair C1, 2, which decreased. However, this decrease is not reflected in the mole percentage, or the relative amount, of this derivative. It has been pointed out by others that the relative amounts of the different toxin derivatives need to be contrasted with the absolute concentrations because differences in composition can be obscured

when composition is reflected as relative abundance (Taronecher-Oldenburg et al. 1999). In *A. catenella*, there was also a decrease in the concentration of C1, 2, from nitrate-starved cells to urea-grown cells. In this case, the change in the absolute concentration was also reflected in the relative percentage, because there was a 46% decrease in the relative percentage of C1, 2 in urea-grown *A. catenella* (stationary phase) compared with stationary phase cells from nitrate-starved conditions. This was coincident with an increase in the relative percentage of both GTX1, 4, and GTX6. In a review of past work on toxin composition changes, decreases in C1, 2 and increases in GTX1, 4 were observed in response to nitrogen limitation for *A. fundyense* (Anderson et al. 1990a), although these trends were not always consistent between isolates (Poulton 2001).

The changes we observed in toxin composition may be related to growth on urea, although the production of C1, 2 toxin may also be influenced by the nickel supplemented to

the culture medium. Yoshida et al. (1998) identified a sulfotransferase enzyme putatively responsible for sulfation of GTX2, 3 to C1, 2 (Yoshida et al. 1998). The activity of the N-sulfotransferase purified from *A. catenella* and *Gymnodinium catenatum* cultures was inhibited in the presence of nickel (Yoshida et al. 1998; Yoshihiko et al. 2002). Because we were unable to grow *Alexandrium* on urea in the absence of nickel we could not control for a potential nickel effect on the toxin composition.

In short, the toxin content and composition of a given *Alexandrium* strain can vary dramatically with nutritional physiology (discussed in Taroncher-Oldenburg et al. 1999). This is significant for studies of field populations where the physiology of the cells, in addition to their abundance, can affect shellfish toxicity and PSP. Our results suggest that field populations using urea as a nitrogen source in low nitrate environments could be less toxic than populations growing in higher nitrate environments.

To further examine the relationship between nitrogen physiology and urease activity in *Alexandrium*, we assayed urease activity in cells from semicontinuous cultures. Semicontinuous culture methods that adapt a population of cells to pulses of nitrogen (nitrate) may better mimic conditions cells experience in natural waters. This contrasts with low nitrate cultures studied in stationary phase, which represent nitrogen-starved populations. In our semicontinuous experiments, urease activity increased with decreasing nitrogen-limited growth rate. Others have observed a similar response for the nutrient-regulated enzymes of other species. For example, cell-specific alkaline phosphatase activities increased as a function of decreasing phosphate-limited growth rate in semicontinuous culture experiments with *Emiliania huxleyi* (Riegman 2000). In our experiments, an increase in urease activity was accompanied by a decrease in total toxin per cell and an increase in the C:N ratio. Both of these parameters are common indicators of nitrogen status, and these data suggest that urease activity in *A. fundyense* is regulated by nitrogen supply.

One of the challenges in studying the ecology of *Alexandrium* is obtaining species-specific physiological measurements. To assay urease activities representative of the *A. fundyense* population and not the entire phytoplankton community, we used an IMBS method to enrich samples for *A. fundyense* during May and June cruises in the western Gulf of Maine. These cruises occurred during the yearly spring bloom of this species. Although the IMBS technique has not previously been used in the field, it has been extensively tested on cultures and culture-spiked field samples (Aguilera et al. 1996, 2002; Poulton 2001). Furthermore, in culture experiments measures of protein concentration and other physiological parameters were not significantly different in untreated controls versus IMBS treatments (Poulton 2001; Aguilera et al. 2002). With this approach, we were able to obtain data on *A. fundyense* urease activity for a number of stations. To our knowledge, this is the first attempt to assay species-specific enzyme activity from *Alexandrium* in field samples. Although there was significant spatial variability, the urease activity was typically quite low in May compared with June. At two June stations, urease activities were consistent with the activities seen during the

semicontinuous experiments at the lowest nitrate-limited growth rate (e.g., 100–140 fmol cell h⁻¹). In only one case (Sta. 115) was there substantial contamination of the sample with other species of phytoplankton. This station was sampled during the May cruise, when *A. fundyense* urease activities were typically low, and in this case the activity we detected may even have been an overestimation of the *A. fundyense* activity if the contaminating phytoplankton also have urease activity. The shortcomings of the IMBS approach in this system are that it is difficult to isolate enough cells to exceed the detection limit of the urease assay and that this approach does not result in truly species-specific measurements because there is some contamination of the sample by other phytoplankton. The method is not perfectly able to separate out contaminating phytoplankton species, most likely because of their high concentrations in field samples relative to *Alexandrium*. Furthermore, the monoclonal antibody we employ cross-reacts with *A. ostenfeldii*, a species that occurs with *A. fundyense* in the Gulf of Maine. Although we cannot rule out the possibility that urease activity was detected from *A. ostenfeldii* in our field samples, cell counts indicate that this species was, on average, <15% of the total *Alexandrium* population in the samples where urease activity was detected. Likewise, contamination with other phytoplankton species was, on average, 25% and typically lower. Despite the drawbacks, the IMBS approach can provide unique and valuable information, as demonstrated here.

Past studies of *A. fundyense* dynamics in the Gulf of Maine during 1993, 1994, and 1998 (Martorano 1997; Poulton 2001; Townsend et al. 2001) often identified *A. fundyense* populations in low DIN surface water, with DIN:DIP well below Redfield ratios (Redfield 1958), particularly in the later stages of the bloom. These studies suggest that nitrogen physiology may be important to the dynamics of *A. fundyense* populations in this environment. Although high-resolution spatial and temporal analyses of our 2001 cruises are ongoing, we have analyzed select stations for key parameters to compare with our data on urease activities.

During the May cruise, *A. fundyense* abundances were low compared with abundances during the June cruise, when concentrations were >600 cells L⁻¹ at one station. In June, this area of high cell number was centered along the interface of the Eastern Maine Coastal Current (EMCC) and the Western Maine Coastal Current (WMCC) near Penobscot Bay (described in Anderson 1997). The overall increase in cell numbers between May and June was accompanied by a general decline in the DIN concentration at our stations. DIN:DIP concentrations during both cruises were below the Redfield ratio and decreased more than sixfold between May and June. In a previous 1998 field study, a decline in DIN and DIN:DIP ratios in surface water was also observed through the course of the bloom season (roughly April–June) (Poulton 2001). Although DIN concentrations are typically higher deeper in the water column (Townsend et al. 2001), Poulton (2001) was unable to induce diel vertical migration in an isolate from the Gulf of Maine, suggesting that *A. fundyense* in this environment may not have access to the increased DIN at depth (Poulton 2001). In short, these data suggest that DIN may become unavailable to *A. fundyense*

populations in the Gulf of Maine as the bloom season progresses into June.

With our limited data set we are unable to establish a significant inverse correlation between DIN and urease activity, although a comparison of data in May with June revealed a general decline in DIN and an increase in urease activity. It is important to note that urease activity reflects the nutritional history of the cell. In the Gulf of Maine where surface DIN concentrations can be patchy, particularly near the EMCC/WMCC boundary, and where *A. fundyense* distributions can be heavily influenced by quickly changing hydrographic regimes, it is not surprising that there is variability in urease activity. Nevertheless, there is an apparent relationship between decreased DIN concentration and increased urease activity in a comparison of data from the two cruises.

Average urea concentrations in June were 3.5 times higher than in May. Cell numbers in June were also higher than May, in association with the observed increase in urea concentration. These results are consistent with a previous study that showed increased dinoflagellate abundance to be associated with increased urea concentrations (Glibert and Terlizzi 1999), suggesting that urea may be an important nitrogen source for this group.

In summary, our field data comparing DIN, urea, urease activity, and *A. fundyense* abundance are consistent with past studies of the Gulf of Maine (Martorano 1997; Poulton 2001; Townsend et al. 2001) and those linking dinoflagellate abundance with increased urea availability (Glibert and Terlizzi 1999). We were successful in targeting our urease assays to the *A. fundyense* population and documented consistent differences between the two cruises in May and June, suggesting a progressive decrease in DIN availability and increased hydrolysis of urea. We would predict from culture data that urease activity should be highest during periods of decreased DIN availability, as we observed in the field during our June cruise. Although more detailed temporal and spatial analyses are necessary to fully elucidate the impact of nitrogen physiology on the dynamics of the *A. fundyense* population, our data are intriguing and suggest that urea may be important to the nitrogen nutrition of *A. fundyense* toward the end of the western Gulf of Maine bloom, when DIN is depleted in surface waters.

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Received: 25 April 2002

Accepted: 8 October 2002

Amended: 14 November 2002