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# Urease activity in five phytoplankton species

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ABSTRACT: Growth rates, internal nitrogen (N) pools of $NH_4^+$ and urea, and urease, the enzyme responsible for the catabolism of urea to $NH_4^+$ and $CO_2$, were investigated in laboratory cultures of 5 species of estuarine phytoplankton grown on $NO_3^-$, $NH_4^+$, and urea. The phytoplankton used were the dinoflagellates *Prorocentrum minimum*, *Karlodinium veneficum*, *Heterocapsa triquetra*, the cryptophyte *Storeatula major*, and the haptophyte *Isochrysis* sp. All dinoflagellates had larger internal pools of $NH_4^+$ than did the other species. With the exception of *H. triquetra* grown on $NO_3^-$, this was also true for internal pools of urea. Two of the harmful dinoflagellates, *P. minimum* and *K. veneficum*, had significantly higher urease activity rates on both a per cell basis and a per cell volume basis than the other species. These dinoflagellates had different rates of urease activity when grown on different N sources, suggesting that urease may be down-regulated by $NH_4^+$ or up-regulated by urea and/or $NO_3^-$. In all dinoflagellates, the intracellular urea concentrations were greater than the half-saturation constant for enzyme activity, suggesting that *in vivo* urease activity rates were nearer to maximal in those species. Results from this study and previous studies suggest that harmful dinoflagellates may be better adapted to utilize urea than other species based on rates of high urease activity and large intracellular urea pools, providing some insight as to why these species may proliferate when urea is a significant N source in the environment.

KEY WORDS: Phytoplankton · Urea · Urease · Harmful algae · Dinoflagellates · Enzyme activity



## INTRODUCTION

Phytoplankton and bacteria can use a variety of nitrogenous substrates to meet their metabolic demands, including inorganic forms such as $NO_3^-$ and $NH_4^+$, and organic forms such as urea and amino acids. In estuarine ecosystems, urea concentrations are typically lower than concentrations of $NO_3^-$ (e.g. Glibert et al. 2006) and thus generally contribute only a small percentage of total nitrogen (N) uptake relative to $NO_3^-$ and $NH_4^+$. However, in some coastal regions and periods of the year, urea uptake can exceed 50% of total N uptake by phytoplankton (e.g. Kudela & Cochlan 2000, Twomey et al. 2005). Moreover, urea fertilizer use is increasing globally, leading to elevated urea concentrations due to runoff in many coastal and estuarine environments (Glibert et al. 2006).

Some species of phytoplankton, including many harmful algal species, use urea at higher rates than $NO_3^-$ (Kudela & Cochlan 2000, Fan et al. 2003, Collos et al. 2004). In fact, many harmful dinoflagellates prefer urea both in culture and in the field. For example, a large bloom of *Lingulodinium polyedrum*, a red tide dinoflagellate, off Newport Beach, California, USA, had higher urea than $NO_3^-$ and $NH_4^+$ uptake rates (Kudela & Cochlan 2000). A study of *Alexandrium catanella* N kinetics also reported higher urea uptake rates compared to inorganic N uptake rates in culture (Collos et al. 2004). In addition, after elevated levels of urea were observed in aquaculture ponds, a consortium of harmful dinoflagellates, including *Karlodinium veneficum* (reported as *Gyrodinium galatheanum*), *Gymnodinium nelsonii*, *Prorocentrum minimum*, and *Katodinium* sp. increased in biomass (Glibert & Terlizzi 1999). Furthermore, the percent contribution of urea to

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total N uptake was highly correlated with the percentage of dinoflagellates in the plankton community of Moreton Bay, Australia (Glibert et al. 2006).

Dinoflagellates are not the only taxon that uses urea at high rates. Blooms of the pelagophyte *Aureococcus anophagefferens* can be fueled by high urea concentrations in some environments (Kana et al. 2004, Mulholland et al. 2004). In Florida Bay and on the western Florida shelf, USA, both higher rates of urea uptake and urease activity were found when the cyanobacterium *Synechococcus* sp. dominated the assemblage than when diatoms dominated the assemblage (Glibert et al. 2004, Heil et al. 2007). Another cyanobacterium, *Trichodesmium* sp. (strain NIBB1067), had higher urea uptake rates than $N_2$ fixation rates when grown on urea (Mulholland et al. 1999). The estuarine species *Chloromorum toxicum* (formerly the rhaphidophyte *Chattonella* cf. *verriculosa*), grows better on urea or $NH_4^+$ than on $NO_3^-$ (Tomas 2005).

In order for a phytoplankton cell to use urea, it must first be transported into the cell via either passive or active transport. Most phytoplankton have active urea transport systems (Antia et al. 1991, Mulholland & Lomas 2008). Urea is also produced intracellularly as the byproduct of the ornithine-urea cycle of arginine biosynthesis and catabolism (Antia et al. 1991) or catabolism of purines (Allen et al. 2006, Berg & Jørgensen 2006). Inside the cell, urea must then be catabolized by urease or ATP:urea amidolyase (UALase) before urea-N enters the GS/GOGAT pathway as $NH_4^+$ (Antia et al. 1991, Berges & Mulholland 2008). The urease catabolism pathway for converting urea into $CO_2$ and $NH_4^+$ is more common in phytoplankton than is UALase, which appears to be present only in the Chlorophyceae (Syrett & Leftley 1976, Antia et al. 1991).

In the past decade, the study of urease activity has been limited to a few representative phytoplankton species from the Bacillariophyta (diatoms), Dinophyta (dinoflagellates), Pelagophyta, and Cyanophyta (Collier et al. 1999, Peers et al. 2000, Dyhrman & Anderson 2003, Fan et al. 2003, Lomas 2004). The urease gene has been detected in many species of other taxonomic groups, but the rates of urease activity are unknown (Bruhn et al. 2002, Collier & Baker 2004). Due to the paucity of urease activity data, it is difficult to determine which taxonomic groups of phytoplankton are better competitors for urea and whether this ability is regulated by different N sources. Urease activity is hypothesized to be higher in species that now commonly proliferate in harmful algal blooms. To test this, growth rates, internal N pools, and urease activity rates were determined in laboratory cultures of 5 species from 3 taxonomic groups grown on different N substrates.

## MATERIALS AND METHODS

**Species studied.** Five phytoplankton species were investigated under controlled laboratory conditions. These included 3 harmful or toxic dinoflagellates (*Prorocentrum minimum*, *Karlodinium veneficum* [formerly *K. micrum*; Bergholtz et al. 2006], and *Heterocapsa triquetra*), the common cryptophyte *Storeatula major*, which is often a prey species for *K. veneficum* (e.g. Li et al. 2001), and the haptophyte *Isochrysis* sp. Three species were derived from strains isolated from the Chesapeake Bay, USA: *P. minimum* by M. Johnson (PM-1, Horn Point Laboratory, Cambridge, MD), *K. veneficum* (Leadbeater et Dodge) Larsen (strain GE) by A. Li and D. Stoecker (Provasoli-Guillard National Center for Culture of Marine Phytoplankton, CCMP 1974), and *S. major* Butcher ex Hill (strain g) by A. Lewitus (Baruch Marine Laboratory, Georgetown, SC). *H. triquetra* was obtained from CCMP (CCMP 449) and was originally from the St. Lawrence estuary in Canada. *Isochrysis* sp. was isolated from near Providenciales Island in the Turks and Caicos Islands (Milford strain, C-ISO) and was obtained from G. Wikfors (NOAA, National Marine Fisheries Service Laboratory, Milford, CT).

**Culture conditions.** The 5 species of phytoplankton were grown under identical nutrient and light conditions. Non-axenic cultures were grown in f/20 media (Guillard & Ryther 1962) with nitrogen ($NO_3^-$, $NH_4^+$, or urea) and phosphate ($PO_4^{3-}$) substrates added at f/20 concentrations (88 µM N, 3.6 µM N, respectively; N:P = 24). All species were acclimated to the culture conditions described above for a period of several weeks to months before the experiments were conducted. Duplicate or triplicate cultures were grown in 2 l glass bottles in a 20°C incubator room at 300 µmol photons $m^{-2}$ $s^{-1}$ on a 12:12 h light:dark cycle over the course of the experiment. Culture preparation and sampling were done under sterile conditions to reduce introduction of new bacterial strains into the cultures.

**Phytoplankton and bacteria biomass.** During the 2 wk of each experiment, 10 ml samples for phytoplankton and bacterial counts were taken each morning after swirling the culture. These were preserved in 4% glutaraldehyde. Depending on the density of the culture, samples varying from 1 to 20 ml (and diluted with artificial seawater [salinity of 15] to a final volume of 20 ml) were also collected to determine cell counts and cell diameters (except *Prorocentrum minimum*) using a Coulter Counter (Coulter Multisizer II). *P. minimum* cell diameter was determined using epifluorescent microscopy. Bacterial biomass was calculated from bacterial counts made on a flow cytometer (Beckman Dickinson FACSCalibur) using the DNA stain SYTO 13 (del Giorgio et al. 1996) and an estimate of

19.5 fg C per bacterial cell for estuarine bacteria (Ducklow 2000).

**Nutrient analysis.** When each culture reached the mid- or late exponential phase as determined by cell counts, samples were collected for the analyses of particulate carbon (PC) and nitrogen (PN), and of internal cell N pools. No residual N remained in the *Storeatula major* and *Isochrysis* sp. cultures at the time of sampling, although approximately 44 µM N remained in the dinoflagellate cultures at the time of sampling (data not shown).

For all analyses, phytoplankton were filtered (25 to 75 ml) onto a combusted (1 h at 450°C) GF/F filter. Samples for PC and PN were stored in a –20°C freezer, dried at 50°C for 3 d, and then analyzed on a Control Equipment CHN elemental analyzer. Internal cell N samples were placed into 5 ml of boiling water to break apart the cell walls, then immediately frozen (Raimbault & Mingazzini 1987). After thawing, samples were separated into two 2 ml subsamples and diluted to 5 ml for $NH_4^+$ analyses and 4 ml for urea analyses. $NH_4^+$ internal cell concentrations were measured using the indophenol method of Parsons et al. (1984), while urea internal cell concentrations were analyzed using the diacetylmonoxime method (Revilla et al. 2005). Total N concentrations were corrected by adding intracellular $NH_4^+$ concentrations to PN to account for loss of $NH_4^+$ from volatilization when filters were dried for measurement by CHN elemental analyzer.

**Urease activity.** Subsamples (n = 15) from each culture for urease activity were collected at mid- to late exponential phase by filtering 25 to 75 ml of culture onto combusted GF/F filters and then immediately freezing them in liquid $N_2$. The urease activity samples were transferred to a –80°C freezer for overnight storage. An exception to this procedure occurred for *Karlodinium veneficum* samples, which were immediately frozen in a –80°C freezer.

One day following sample collection, urease activity was assayed on the filters according to Solomon et al. (2007). To assess Michaelis-Menten kinetic parameters for urease enzyme activity, rates were determined on triplicate subsamples that were enriched with urea concentrations of 0.25, 0.5, 1.36, and 3 mM N. One set of triplicate tubes had 0 addition.

**Data analysis.** The kinetic parameters $K_m$ (half-saturation constant for enzyme activity) and $V_{max}$ (maximum rate of enzyme activity) are defined by the Michaelis-Menten equation (Michaelis & Menten 1913):

$$V = V_{max} \frac{S}{(K_m + S)}$$

where $S$ is the concentration of the substrate urea. To make comparisons among species and with other published studies, specific urease activity ($V_{max}$, µM N $h^{-1}$) was normalized on both a per cell ($V_{max\text{-}cell}$, fmol N $cell^{-1}$ $h^{-1}$) and a per cell volume basis ($V_{max\text{-}vol}$, fmol N $\mu m^{-3}$ $h^{-1}$).

The mean urease activity at each assayed urea concentration was calculated for each species and for each growth N substrate. First, the subsamples (n = 3) of urease activity from each individual culture were averaged at each urea concentration. Next, sets of averages for each species (n = 2 or 3 depending on species) grown on the same N source were combined to obtain an overall mean urease activity at each urea concentration. Using the overall mean urease activity data, both $K_m$ or $V_{max}$ were calculated by using SigmaPlot software (SYSTAT), using the best fit to the Michaelis-Menten kinetic curve.

Statistical testing was done to determine whether there were differences in growth rate and intracellular N concentrations between species grown on $NO_3^-$, $NH_4^+$, or urea. Significant differences among N sources were determined by 1-way analysis of variance (ANOVA) and post-hoc comparisons (Tukey HSD) using data from each individual species.

ANOVAs were also conducted to determine significant differences in $K_m$ and $V_{max}$ among species or N sources. Values of $K_m$ and $V_{max}$ for each culture were obtained using the best fit to the Michaelis-Menten curve using SigmaPlot software (SYSTAT). The calculated $K_m$ and $V_{max}$ values were checked to determine if the data had a normal distribution using the S-PLUS statistics program (Insightful). Because the original data did not have a normal distribution, they were transformed using the $\log_{10}(x+1)$ function. One-way ANOVAs and post-hoc comparisons (Tukey HSD), using the transformed $K_m$ or $V_{max}$ data, were run to test for effect of species or N source.

## RESULTS

### Growth rates and biomass

Phytoplankton and bacterial growth rates in the cultures varied depending on the species and growth N source. Among the phytoplankton studied, the dinoflagellate *Heterocapsa triquetra* had the lowest growth rates (0.21 ± 0.04 to 0.24 ± 0.00 $d^{-1}$) while the haptophyte *Isochrysis* sp. had the highest growth rates (0.72 ± 0.01 to 0.85 ± 0.01 $d^{-1}$; Table 1). *Isochrysis* sp. had higher growth rates on $NO_3^-$ than on the other N substrates (ANOVA, Tukey HSD, $p < 0.05$). The cryptophyte *Storeatula major* had a significantly higher growth rate on $NH_4^+$ (0.71 ± 0.03 $d^{-1}$) than on $NO_3^-$ (0.65 ± 0.02 $d^{-1}$; $p < 0.05$). For all other species, differences in growth rate between N substrates were not significant (ANOVA, $p > 0.05$).

Table 1. Growth rates ($d^{-1}$ ± SD) of 5 phytoplankton species grown on $NO_3^-$, $NH_4^+$, and urea. n: number of replicate cultures

| Phytoplankton species | n | Growth substrate – Nitrogen | | |
|---|---|---|---|---|
| | | $NO_3^-$ | $NH_4^+$ | Urea |
| **Dinoflagellates** | | | | |
| *Prorocentrum minimum* | 3 | 0.34 ± 0.02 | 0.31 ± 0.04 | 0.29 ± 0.01 |
| *Karlodinium veneficum* | 3 | 0.42 ± 0.06 | 0.52 ± 0.06 | 0.49 ± 0.07 |
| *Heterocapsa triquetra* | 2 | 0.21 ± 0.04 | 0.24 ± 0.00 | 0.23 ± 0.01 |
| **Cryptophyte** | | | | |
| *Storeatula major* | 3 | 0.65 ± 0.02 | 0.71 ± 0.03 | 0.69 ± 0.02 |
| **Haptophyte** | | | | |
| *Isochrysis* sp. | 2 | 0.85 ± 0.01 | 0.72 ± 0.01 | 0.78 ± 0.00 |

Bacteria net growth rates in the *Karlodinium veneficum* and *Heterocapsa triquetra* cultures were less than 0.22 $d^{-1}$ (data not shown), while in the *Prorocentrum minimum*, *Storeatula major*, and *Isochrysis* sp. cultures, bacteria net growth rates were comparable to or greater than the phytoplankton growth rates, varying from 0.25 to 0.91 $d^{-1}$. Regardless of growth rates, bacterial density was low (900 to $5 \times 10^4$ bacteria $ml^{-1}$), and bacterial carbon biomass contributed only 0.01 to 0.3% to the total carbon biomass in all cultures.

## Biochemical state of cells

All dinoflagellates had larger internal pools of $NH_4^+$ than did the other species (Table 2). With the exception of *Heterocapsa triquetra* grown on $NO_3^-$, this was also true for internal pools of urea (Table 2). Intracellular concentrations of urea in *Prorocentrum minimum*, *Karlodinium veneficum*, and *H. triquetra* were 3- to 40-fold higher in cells grown on urea than those grown on $NO_3^-$, while in *Storeatula major* and *Isochrysis* sp., the internal pools of urea were smaller in cells grown on urea than on $NO_3^-$, although this difference was not significant (Table 2, ANOVA, Tukey HSD, $p < 0.05$). Also, with the exception of *S. major*, intracellular urea concentrations were higher for cells grown on urea than on $NH_4^+$. In the dinoflagellates grown on urea, urea made up 9.9 to 42% of total cellular N, but only made up <1.5% in the other species grown on urea (Table 2). Intracellular $NH_4^+$ contributed from 1.51 to 54% of total cellular N in all 5 species.

## Urease activity

The dinoflagellates *Prorocentrum minimum* and *Karlodinium veneficum* had significantly higher $V_{max\text{-}cell}$ and $V_{max\text{-}vol}$ rates than the other 3 species (1-way ANOVA, $p < 0.05$; Figs. 1 & 2, Table 3). The next highest $V_{max\text{-}cell}$ rates were seen in the dinoflagellate *Heterocapsa triquetra*, followed by the cryptophyte *Storeatula major*, and lastly the haptophyte *Isochrysis* sp. *K. veneficum* had significantly higher rates of $V_{max\text{-}cell}$ when grown on $NO_3^-$ or urea than when grown

Table 2. Intracellular $NH_4^+$ and urea concentrations per cell, N content of cells, and contribution of $NH_4^+$ and urea to total cellular N content (±SD) of phytoplankton grown on different N sources. Full species names as in Table 1. n: number of replicate cultures

| Phytoplankton species | n | Growth substrate | $NH_4^+$ (mM-N) | % of total cellular N | Urea (mM-N) | % of total cellular N | Total cellular N (pg-at N $cell^{-1}$) | Cell volume ($\mu m^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| **Dinoflagellates** | | | | | | | | |
| *P. minimum* | 3 | $NO_3^-$ | 233 ± 124 | 15.3 ± 5.80 | 36.3 ± 33.0 | 2.92 ± 2.84 | 2.02 ± 0.38 | 1436 |
| | | $NH_4^+$ | 314 ± 138 | 25.2 ± 8.86 | 12.4 ± 16.0 | 0.98 ± 1.20 | 1.76 ± 0.24 | 1436 |
| | | Urea | 280 ± 46.8 | 25.4 ± 4.98 | 110 ± 18.0 | 9.92 ± 1.60 | 1.59 ± 0.08 | 1436 |
| *K. veneficum* | 3 | $NO_3^-$ | 503 ± 148 | 45.0 ± 9.62 | 21.6 ± 30.6 | 2.54 ± 2.20 | 1.49 ± 0.22 | 1345 |
| | | $NH_4^+$ | 477 ± 71.1 | 53.8 ± 4.99 | 33.7 ± 6.15 | 2.76 ± 2.32 | 1.19 ± 0.13 | 1345 |
| | | Urea | 246 ± 74.7 | 39.0 ± 9.65 | 280 ± 53.0 | 42.0 ± 8.98 | 0.84 ± 0.07 | 1345 |
| *H. triquetra* | 2 | $NO_3^-$ | 86.3 ± 52.0 | 30.9 ± 13.9 | 2.40 ± 3.39 | 0.79 ± 1.12 | 0.35 ± 0.06 | 1293 |
| | | $NH_4^+$ | 79.5 ± 6.66 | 23.1 ± 4.90 | 13.6 ± 10.5 | 3.55 ± 1.92 | 0.46 ± 0.13 | 1293 |
| | | Urea | 19.3 ± 0.12 | 7.87 ± 1.63 | 80.6 ± 0.79 | 32.8 ± 6.67 | 0.33 ± 0.07 | 1293 |
| **Cryptophyte** | | | | | | | | |
| *S. major* | 3 | $NO_3^-$ | 5.41 ± 1.10 | 2.33 ± 0.47 | 6.07 ± 4.91 | 2.47 ± 1.83 | 0.85 ± 0.09 | 3635 |
| | | $NH_4^+$ | 5.48 ± 0.57 | 3.44 ± 0.29 | 5.82 ± 2.90 | 3.62 ± 1.72 | 0.58 ± 0.06 | 3635 |
| | | Urea | 6.61 ± 1.11 | 4.46 ± 0.76 | 2.18 ± 0.62 | 1.47 ± 0.42 | 0.54 ± 0.01 | 3635 |
| **Haptophyte** | | | | | | | | |
| *Isochrysis* sp. | 2 | $NO_3^-$ | 27.9 ± 2.82 | 3.15 ± 0.46 | 8.66 ± 6.87 | 0.96 ± 0.73 | 0.14 ± 0.01 | 167.8 |
| | | $NH_4^+$ | 19.3 ± 10.2 | 2.44 ± 1.24 | 1.64 ± 2.29 | 0.20 ± 0.29 | 0.13 ± 0.00 | 167.8 |
| | | Urea | 10.5 ± 1.28 | 1.51 ± 0.25 | 4.94 ± 4.08 | 0.72 ± 0.62 | 0.12 ± 0.01 | 167.8 |

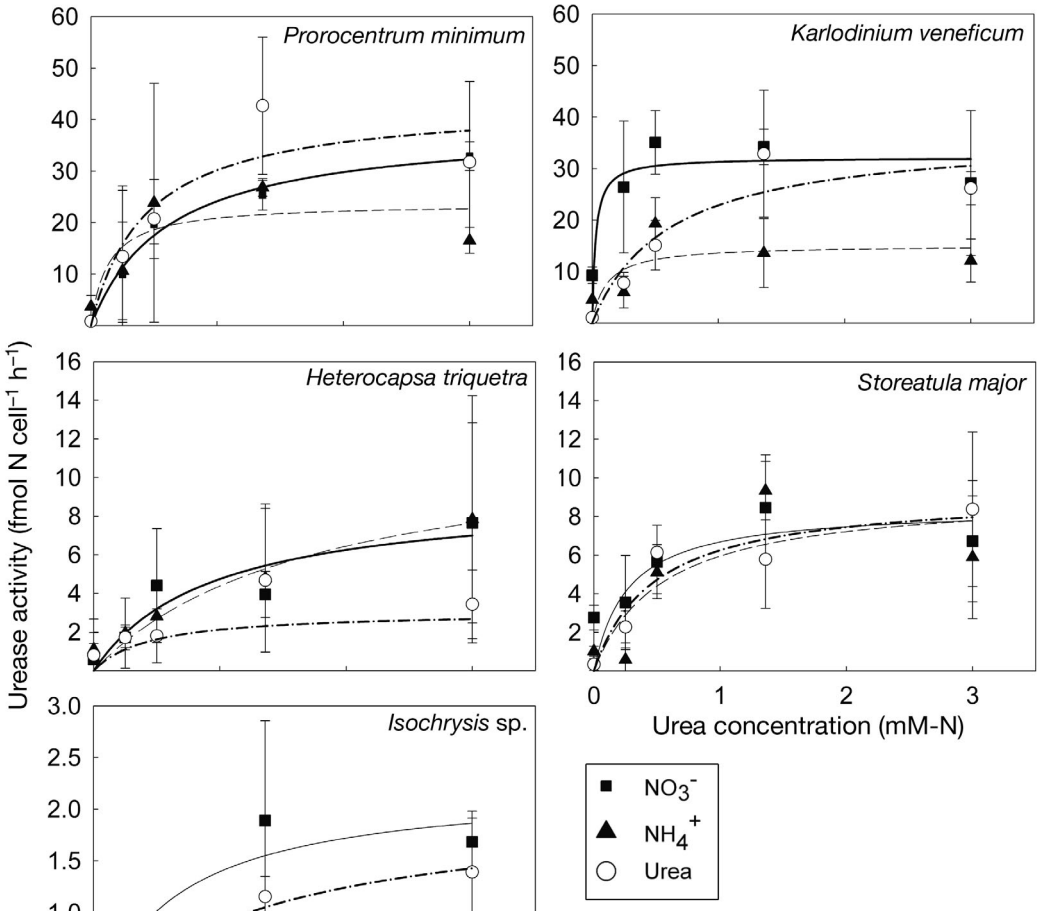


Fig. 1. Kinetic curves of urease activity per cell in 5 phytoplankton species as a function of urea concentration. Each line represents data from cultures grown on a different N substrate. The kinetic parameters for each relationship are reported in Table 3

Table 3. Average urease kinetic parameters (±SD), specific urease activity normalized on a per cell basis ($V_{max\text{-}cell}$, fmol N cell$^{-1}$ h$^{-1}$) and half-saturation constant for enzyme activity ($K_m$, mM N), for 5 phytoplankton species (see Table 1 for full names). Data represent mean (±SD) for all replicates. The correlation coefficient ($r^2$) represents the best fit to a non-linear model, and the p-value shows whether the fit is significant. All measurements were conducted during mid- to late exponential growth phase

| Phytoplankton species | n | $NO_3^-$ | | | | $NH_4^+$ | | | | Urea | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_{max\text{-}cell}$ | $K_m$ | $r^2$ | p | $V_{max\text{-}cell}$ | $K_m$ | $r^2$ | p | $V_{max\text{-}cell}$ | $K_m$ | $r^2$ | p |
| **Dinoflagellates** | | | | | | | | | | | | | |
| *P. minimum* | 3 | 38.9 ± 3.28 | 0.61 ± 0.15 | 0.98 | <0.01 | 23.7 ± 6.47 | 0.14 ± 208 | 0.64 | 0.10 | 43.4 ± 10.1 | 0.44 ± 0.33 | 0.86 | 0.02 |
| *K. veneficum* | 3 | 32.1 ± 5.93 | 0.26 ± 0.93 | 0.64 | 0.11 | 15.1 ± 5.38 | 0.11 ± 0.25 | 0.34 | 0.30 | 36.7 ± 9.31 | 0.60 ± 0.45 | 0.87 | 0.02 |
| *H. triquetra* | 2 | 9.14 ± 2.82 | 0.92 ± 0.72 | 0.85 | 0.03 | 12.1 ± 3.23 | 1.77 ± 0.96 | 0.94 | <0.01 | 4.71 ± 1.44 | 0.46 ± 0.46 | 0.70 | 0.08 |
| **Cryptophyte** | | | | | | | | | | | | | |
| *S. major* | 3 | 8.46 ± 2.26 | 0.27 ± 0.28 | 0.49 | 0.19 | 9.23 ± 3.90 | 0.55 ± 0.70 | 0.66 | 0.10 | 9.22 ± 1.77 | 0.48 ± 0.29 | 0.90 | 0.01 |
| **Haptophyte** | | | | | | | | | | | | | |
| *Isochrysis* sp. | 2 | 2.24 ± 0.42 | 608 ± 337 | 0.92 | 0.01 | 1.15 ± 0.19 | 867 ± 381 | 0.95 | <0.01 | 2.03 ± 0.44 | 1269 ± 624 | 0.95 | <0.01 |

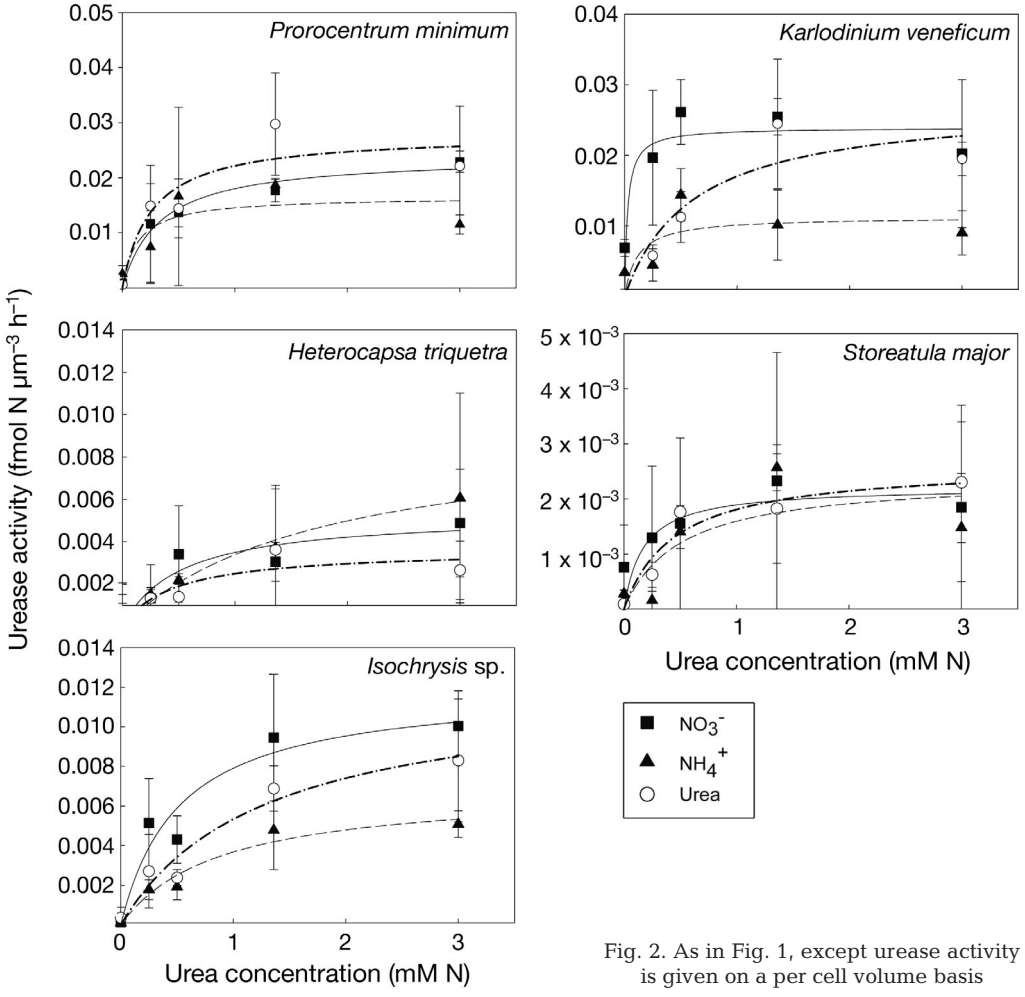


Fig. 2. As in Fig. 1, except urease activity is given on a per cell volume basis

on $NH_4^+$ (1-way ANOVA, Tukey HSD, $p < 0.01$). The same was true for *P. minimum*, but not as significant (1-way ANOVA, Tukey HSD, $p = 0.23$). There were no significant differences in $V_{max\text{-}cell}$ among N sources in the other 3 species (1-way ANOVA, Tukey HSD, $p > 0.43$).

The patterns of $V_{max\text{-}vol}$ rates among species differed somewhat from those of $V_{max\text{-}cell}$. As above, *Karlodinium veneficum* and *Prorocentrum minimum* had the highest rates of $V_{max\text{-}vol}$; however, *Storeatula major* had the lowest $V_{max\text{-}vol}$ (Fig. 2). *K. veneficum* had significantly higher average $V_{max\text{-}vol}$ than *Heterocapsa triquetra*, *S. major*, and *Isochrysis* sp. (1-way ANOVA, Tukey HSD, $p < 0.05$). Both *P. minimum* and *K. veneficum* had higher $V_{max\text{-}vol}$ when grown on urea or $NO_3^-$ than when grown on $NH_4^+$.

Overall differences in $K_m$ between species were not significant (1-way ANOVA, $p > 0.05$), but significant differences among N sources were seen in 1 species (Table 3): *Karlodinium veneficum* had significantly lower $K_m$ when grown on $NO_3^-$ and $NH_4^+$ than when grown on urea (1-way ANOVA, Tukey HSD, $p < 0.01$).

## DISCUSSION

Among the 5 phytoplankton species studied, the 3 dinoflagellates exhibited the highest rates of urease activity and the largest intracellular pools of $NH_4^+$ and urea. Three factors may have contributed to these differences. First, the dinoflagellate cultures, when grown on all N sources, had some residual N in the media when urease activity was assessed, compared to *Storeatula major* and *Isochrysis* sp., for which the cultures had no residual N in the media for all growth N sources. Thus, methodological differences may have contributed to the differences in urease activity or in the sizes of the internal pools. However, this is unlikely to be the case for urease activity, as a previous study of urease regulation in the dinoflagellate *Alexandrium fundyense* showed that urease activity increased when the cells were N starved (Dyhrman & Anderson 2003). Thus, it is likely that the rates of urease activity for the dinoflagellates were conservative.

Differences in cell size may also have contributed to differences in internal N pools. The cryptophyte *Storeatula major* had the largest cell size, but smaller $NH_4^+$ and urea pools despite having a total intracellular N content comparable to that of the smaller dinoflagellates (Table 2). The haptophyte *Isochrysis* sp. had the smallest cell size and the smallest urea pools, but not the smallest $NH_4^+$ pools. Regardless of cell size, intracellular urea concentrations of the species studied here were comparable to concentrations found in other species, which range from non-detectable to 15 mM N in the diatoms *Phaeodactylum tricornutum* and *Thalassiosira gravida* and the green alga *Chlorella fusca* (Wheeler 1983). The diatom *T. weissflogii* (0.42 ± 0.08 mM N) had significantly lower urea concentrations compared to the dinoflagellate *Prorocentrum minimum* (2.71 ± 0.21 mM N) and the pelagophyte *Aureococcus anophagefferens* (4.65 ± 0.31 mM N) when grown on urea (Fan et al. 2003). When grown on urea, all 3 dinoflagellates had higher intracellular urea concentrations than did the chlorophyte and haptophyte. Within the dinoflagellates, one of the larger species (*Karlodinium veneficum*) had the largest intracellular urea pools, while the smallest (*Heterocapsa triquetra*) had the smallest intracellular urea pools. Also among the dinoflagellates, rates of urease activity, either per cell or per cell volume, also showed a decreasing progression from *K. veneficum* or *P. minimum* to *H. triquetra*.

In the dinoflagellates, the intracellular urea concentrations were greater than $K_m$, suggesting that *in vivo* urease activity rates were near to maximal in those species. This was not the case for the cryptophyte and haptophpyte. $K_m$ is an intrinsic property of the enzyme and hypothetically should not vary within a species grown on different N sources. However, estimates of $K_m$ made during this study (Table 3) were highly variable even within species, which likely reflects the sensitivity of calculated $K_m$ values to the number and distribution of urea concentrations assayed above and below $K_m$. Nevertheless, our values were consistent with previously reported values for phytoplankton urease $K_m$, which range from 0.12 to 0.46 mM N (Syrett & Leftley 1976, Palinska et al. 2000, Fan et al. 2003).

Differences in internal N pools and urease activity may also reflect a difference in internal regulation by N substrate between species and species groups. The latter is most easily explored in those species for which internal N pools were large, i.e. the dinoflagellates. If a phytoplankton species has similar $V_{max}$ rates when grown on different N sources, urease may not be regulated by physiological factors (e.g. N source or growth status) and therefore may be expressed constitutively. Many bacteria and cyanobacterial ureases are tightly regulated by the N regulatory system (e.g. control gene A, ntcA; Flores & Herrero 2005). However, there is some evidence for regulation in dinoflagellates by N substrates. *Alexandrium fundyense* had higher rates of urease activity in a urea-grown culture, compared to cultures grown in $NH_4^+$ and $NO_3^-$ (Dyhrman & Anderson 2003). Urease activity in *A. fundyense* was also induced by N-starvation (Dyhrman & Anderson 2003). In our study, both *Prorocentrum minimum* and *Karlodinium veneficum* had higher urease activity rates in urea and $NO_3^-$ grown cultures compared to $NH_4^+$ grown cultures as indicated by the rates of $V_{max\text{-}cell}$. Overall, these 2 dinoflagellates, as well as *A. fundye*nse previously studied, appear to have reduced rates of urease activity when grown on $NH_4^+$ compared to other N sources. Ureases in these species appear to be repressed by $NH_4^+$, or, alternatively, up-regulated by urea and/or $NO_3^-$.

Other enzymes that are involved with N acquisition and assimilation in phytoplankton appear to be regulated by N sources or other physiological factors (Berges & Mulholland 2008). Nitrate reductase (NR) is induced by the presence of $NO_3^-$, but repressed by $NH_4^+$ in diatoms (Berges 1997, Parker & Armbrust 2005) and chlorophytes (Song & Ward 2004), and does not appear to be repressed by $NH_4^+$ in dinoflagellates (Berges 1997). There are early indications that variations in the N-terminus of the NR gene may explain differences in regulation of NR among diatom species (Allen et al. 2005). In the green alga *Dunaliella primolecta*, glutamine synthetase (GS) activity is inhibited by increasing $NH_4^+$ concentrations *in vivo*, but not *in vitro* (Seguineau et al. 1989).

As for NR and GS, urease activity in phytoplankton may differ in regulation within or among taxonomic groups. In a clone of the diatom *Thalassiosira weissflogii*, urease activity ($V_{max}$) was the same regardless of growth N source (Peers et al. 2000), but was down-regulated in another clone when grown on $NO_3^-$ (Fan et al. 2003, Lomas 2004). Another indication that phytoplankton ureases are more likely to be regulated than expressed constitutively is that rates of urease activity among phytoplankton grown on urea varied considerably, with dinoflagellates and cyanobacteria having a higher urease activity on a per cell or per cell volume basis, respectively (Solomon 2006).

The results from past studies and this study suggest that dinoflagellates may have a more efficient mechanism for developing large intracellular urea pools, either through surface membrane transport proteins or via the urea cycle, and are able to retain urea within the cell in vacuoles, much like for $NO_3^-$ in diatoms (e.g. Eppley & Coatsworth 1968). All dinoflagellate species studied here had intracellular urea concentrations similar to or greater than estimated urease $K_m$. Therefore, as long as the intracellular urea pool is available to the

enzyme, urease should operate near $V_{max}$ *in vivo.* The difference in rates of urease activity among the 5 phytoplankton species, and within the dinoflagellates, may be because the regulation of urease occurs by a combination of different physiological factors, not just the quantity and quality of N in intracellular pools or growth medium.

Many dinoflagellates are recognized to form harmful blooms, and there is a growing body of literature that suggests that these species may proliferate in environments in which urea is a common N form. The proportion that urea contributes to total N uptake rates is correlated with the percent of the phytoplankton assemblage that is composed of dinoflagellates in some locations, such as in the subtropical waters of Moreton Bay, Australia (Glibert et al. 2006). Urea pulses can also be followed by development of toxic dinoflagellates, which was observed with *Alexandrium catenella* in Thau lagoon, southern France (Collos et al. 2007). The reasons why these harmful dinoflagellates may be better competitors for urea may at least in part be explained by biochemical differences in regulation of urease activity between dinoflagellates and other phytoplankton taxonomic groups.

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