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Effects of Environmental Factors on the Uptake Rates of Dissolved Nitrogen by a Salt-water Green Alga (*Oocystis borgei* Snow)

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Abstract Uptake rates for dissolved nitrogen (DN) by a marine alga (*Oocystis borgei*) were examined in a ^{15}N tracer experiment. Maximal uptake rates for all forms of DN were observed at temperatures between 25 and 30°C and at algal concentrations between 3.22×10^8 and 4.78×10^8 cell L^{-1}. Light intensity required to achieve the maximal uptake rate was 45 μmol m^{-2} s^{-1} for dissolved inorganic nitrogen (DIN = NO_3^-, NO_2^-, NH_4^+) and methionine, and 126 μmol m^{-2} s^{-1} for urea. Salinity required to achieve the maximal uptake rate was 12.85 ppt for DIN, 19.89 ppt for urea and 26.2 ppt for methionine.

Keywords Dissolved nitrogen · Environmental factors · *Oocystis borgei* · Uptake rate

Deterioration of water quality in intensive shrimp culture ponds leads to poor growth and low production of the cultivated animals. Excessive feeding and animal wastes often result in the accumulation of ammonia and nitrite which are toxic to aquatic organisms (Racotta and Hernández-Herrera 2000). Methods for the removal of dissolved inorganic nitrogen (DIN) in the aquaculture system have been sought to improve water quality (Roselien et al. 2007). One approach is to use selected planktonic microalgae which are capable of dominating the plankton community in the aquaculture ponds, actively assimilating DIN and providing dissolved oxygen to improve water quality. For example, Joel (1976) reported 95 % of DIN from wastewater influent was removed by algal assimilation. Benjamas et al. (2003) used a cyanobacterium (*Spirulina platensis*) to reduce DIN from shrimp culture tanks. They found high nitrate concentrations (16–18 mg L^{-1}) with the absence of *S. platensis*. Semicontinuous harvest of *S. platensis* reduced nitrate to 4 mg L^{-1}, and ammonium and nitrite to 0.0–0.15 mg L^{-1}, respectively.

Previous studies have indicated that microalgae not only can utilize DIN (Dugdale and Dugdale 1965; Gu and Alexander 1993), but also utilize dissolved organic nitrogen (DON). Berman and Bronk (2003) demonstrated that many species of phytoplankton are capable of utilizing urea under laboratory conditions. Gilbert et al. (2006) found that when concentrations exceeded 1 μmol N L^{-1}, urea may become an important source of nitrogen for phytoplankton. Collos et al. (2007) compared the relative uptake rates of nitrate, nitrite, ammonium and urea, and found that urea is the major source of nitrogen that supported the growth of the dinoflagellate *Alexandrium catenella*. Phytoplankton also use small DON molecules, such as dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA) as their nitrogen source (Schell 1974).

Uptake rates for dissolved nitrogen are influenced by a number of environmental factors (Mallick and Rai 2007; Talbot et al. 2008). Gu and Alexander (1993) found that the uptake rate for NH_4^+-N, NO_3^--N, urea-N and N_2-N by *Anabaena flos-aquae* were closely related to algal biomass, water temperature and light level in a subarctic lake. Presing et al. (2001) indicated a positive relationship between the uptake rates for NH_4^+-N, urea-N and NO_3-N, temperature and light intensity in Lake Balaton. Algal uptake varied at different temperatures (Gu and Alexander 1993),

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or at the same temperature when grown with different sources of nitrogen (Dugdale and Dugdale 1965).

Oocystis borgei (Chlorophyta) is a unicellular alga that is widely distributed in organic-rich waters such as estuaries and aquaculture ponds. This alga often forms a stable population, and can tolerate various physical and chemical conditions in shrimp ponds (Huang and Wang 2002). Recent studies have indicated that a microalgal community dominated by *O. borgei* may help improve water quality and control the growth of shrimp disease-related bacteria (Huang and Wang 2002). There have been reports on the growth requirements by *O. borgei* (Huang and Wang 2002). However, environmental effects on the utilization of various nitrogen sources by this alga are currently lacking. This study was designed to understand the uptake rates of various forms of dissolved nitrogen by *O. borgei* under a selected range of water temperatures, salinities, light intensities and algal concentrations. Findings from this study may provide an ecological basis for the intensive culture of this alga in shrimp ponds for better water quality management.

Materials and Methods

Oocystis borgei was provided by the Aquatic Ecology and Aquaculture Environmental Research Lab, Guangdong Ocean University, China. The algae used in the uptake experiment were stocked in a series of 100 mL flasks with an identical concentration of 2.288×10^8 cell L^{-1}. We examined the uptake rates for three forms of DIN enriched with ^{15}N, namely nitrate as $NaNO_3$, nitrite as $NaNO_2$ and ammonium as NH_4Cl; and two forms of DON, namely urea as $(NH_2)_2CO$ and methionine (Met-N) as $HO_2CCH(NH_2)CH_2CH_2SCH_3$ (98 atom %, Sigma-Aldrich Corp., St. Louis, USA).

The experiment for the effects of water temperature on algal uptake rates was conducted at 6 temperatures, 10.0, 15.0, 20.0, 25.0, 30.0 and 35.0°C, with a constant light intensity of 39 μmol m^{-2} s^{-1} and salinity of 26.2 ppt, respectively. For the experiment on the effect of salinity on uptake rates, 6 salinities, 6.5, 12.9, 19.9, 26.2, 32.7 and 39.9 ppt were used with a constant water temperature and light intensity of 28.0°C and 39 μmol m^{-2} s^{-1}, respectively. For the effect of solar radiation, 6 light intensities, 18, 45, 72, 99, 126, and 153 μmol m^{-2} s^{-1} were used with a constant water temperature and salinity of 28.0°C and 26.2 ppt, respectively. To test the effect of different algal concentrations, 6 algal cell densities, 1.659×10^8, 3.22×10^8, 4.78×10^8, 6.35×10^8, 7.91×10^8 and 9.47×10^8 cells L^{-1} were used with a constant water temperature, light intensity and salinity at 28.0°C, 39.0 μmol m^{-2} s^{-1} and 26.2 ppt, respectively. All levels of treatments were run with triplicate flasks in an illuminated chamber.

Incubations were terminated after 4 h. The algal biomass was filtered onto precombusted (450°C) GF/C filters, which were dried at 60°C and loaded into tin capsules. All samples were shipped to Washington State University, USA, for stable nitrogen isotope analysis. The uptake rate (V, μg g^{-1} h^{-1}) was calculated following Dugdale and Wilkerson (1986):

$$V = \frac{^{15}N_s - {^{15}N_n}}{(^{15}N_{enr} - {^{15}N_n}) \times T} \times PON \quad (1)$$

where $^{15}N_s$ is the atom% of algae at the end of the uptake experiment; $^{15}N_n$ is the natural abundance of ^{15}N (0.0036765); $^{15}N_{enr}$ is the atom% of ^{15}N at the beginning of the experiment; T is the experiment time (h) and PON is the total organic nitrogen content (μg g^{-1}) of the culture medium. Statistical tests were made using SPSS (Version 15, SPSS Inc, Chicago, USA). Differences or similarities on uptake rates were examined using Analysis of Variance (ANOVA) followed by Duncan's multiple comparisons.

Results and Discussion

There were significant differences in uptake rates for all forms of DN in all treatments (ANOVA, all $p < 0.05$). Post hoc analysis of multiple comparisons all showed significant differences among levels of each treatment although not all uptake rates were statistically different between all levels within a treatment.

Temperature affects enzymatic activity, which in turn influences algal uptake rates for nutrients. Many algae have an optimal range of water temperature for their growth. Within this range, uptake rates increase as the temperature increases (Wang et al. 2001). In this study, the uptake rates of all forms of DN by *O. borgei* were the lowest at a water temperature of 10°C, and increased as water temperature increased (Fig. 1). At 25°C, the uptake rate for Met-N reached the highest level. However, the maximal uptake rates for NO_3^--N, NO_2^--N, NH_4^+-N and urea-N were observed at 30°C. Uptake rates decreased when temperature was >30°C (Fig. 1). It is not known why the highest uptake rate for Met-N occurred at a lower water temperature than for the other forms of DN. Nevertheless, this range of water temperatures (25–30°C) for highest uptake rates is typical for the subtropical shrimp ponds. This suggests that *O. borgeiis* is a good candidate for the removal of DN in the aquaculture systems from the subtropical region.

Light intensity, as the sole source of radiation energy for algal cells, is one of the most important factors controlling nitrogen uptake (Hanisak and Harlin 1978). Light plays

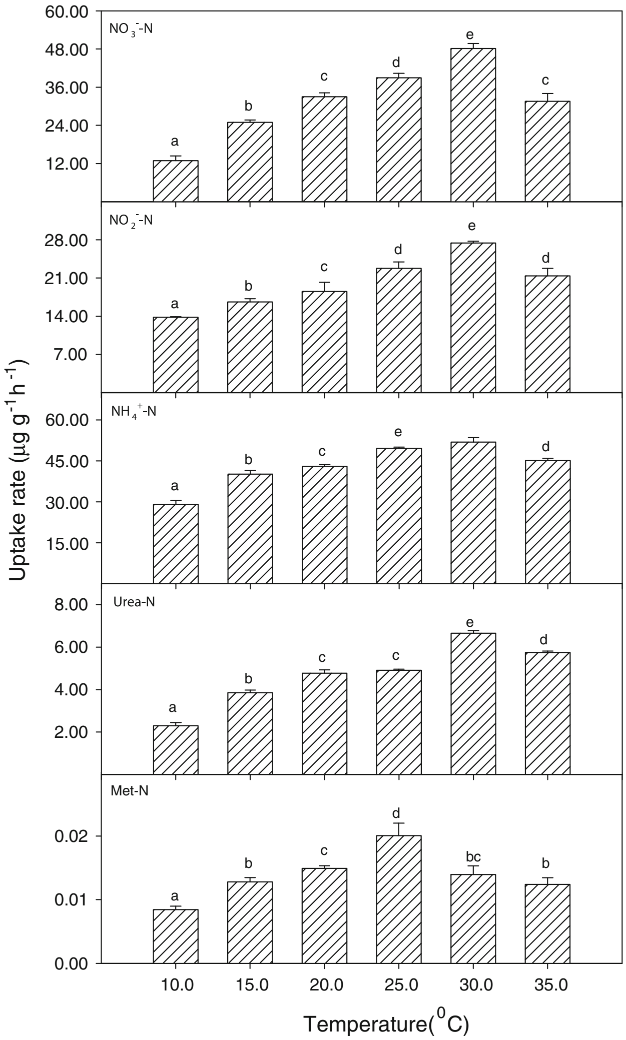

Fig. 1 Uptake rates of NO_3^--N, NO_2^--N, NH_4^+-N, urea-N and Met-N by *O. borgei* at 6 temperature levels. *Different letters* indicate significant differences between treatments ($p < 0.05$)

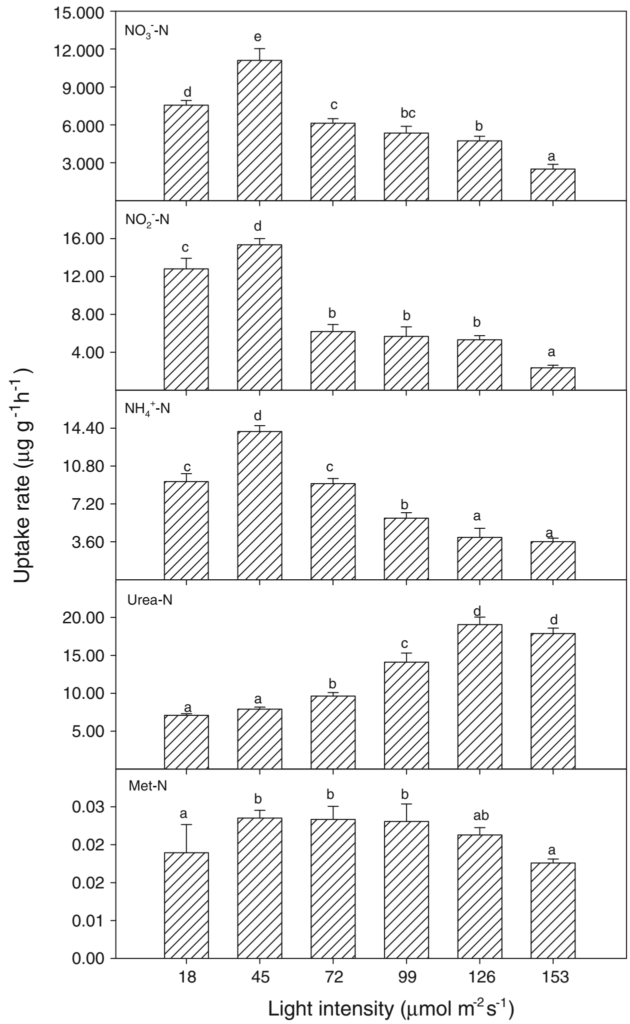

Fig. 2 Uptake rates of NO_3^--N, NO_2^--N, NH_4^+-N, urea-N and Met-N by *O. borgei* at 6 light levels. *Different letters* indicate significant differences between treatments ($p < 0.05$)

important roles in the synthesis of photosynthetic pigments and enzymes. Light also indirectly influences algal nutrient uptake by accelerating active transfer of alkaline phosphatase and by providing a carbon backbone for the synthesis of amino acids and protein (Harris 1978). Gu and Alexander (1993) indicated a linear relationship between light intensity and urea uptake rates by *A. flos-aquae*. A similar finding was observed in marine phytoplankton by Presing et al. (2001). Results from this experiment showed increases in DN uptake rate with increases in light intensity, but the uptake rate for all forms of DN decreased when light intensity reached 153 μmol m^{-2} s^{-1} (Fig. 2). The light intensity required to obtain the highest uptake rate for DIN by *O. borgei* was 45 μmol m^{-2} s^{-1}, while the highest uptake for urea-N was found at a light intensity of 126 μmol m^{-2} s^{-1}. This indicates that more energy is required to assimilate organic nitrogen than DIN.

The uptake rates of all forms of DN at different salinities increased initially and then decreased (Fig. 3). Lowest uptake rates for NO_2^--N, urea-N and Met-N were found at a salinity of 6.49 ppt and the highest uptake rates for all DIN forms were found at 12.85 ppt. The highest uptake rate for urea-N and Met-N were found at salinity of 19.89 and 26.2 ppt, respectively. At salinity of 39.9 ppt, algal uptake rates for NO_3^--N and NH_4^+-N dropped to the lowest level (Fig. 3). Salinity affects algal uptake rates through the changes in osmosis; both low and high salinity may pose detrimental conditions for algal cells. Sodium ions participate in algal photosynthesis, nutrient uptake, and acid balance (Talbot and de la Noüe 2010). There are different requirements for salinity in different algae. For example, optimal salinities are 14–23 ppt for *Skeletonema costatum* (Tonis and Serge 2003), 25–31 ppt for *Prorocentrum dantatum* (Chen et al. 2005) and 25 ppt for

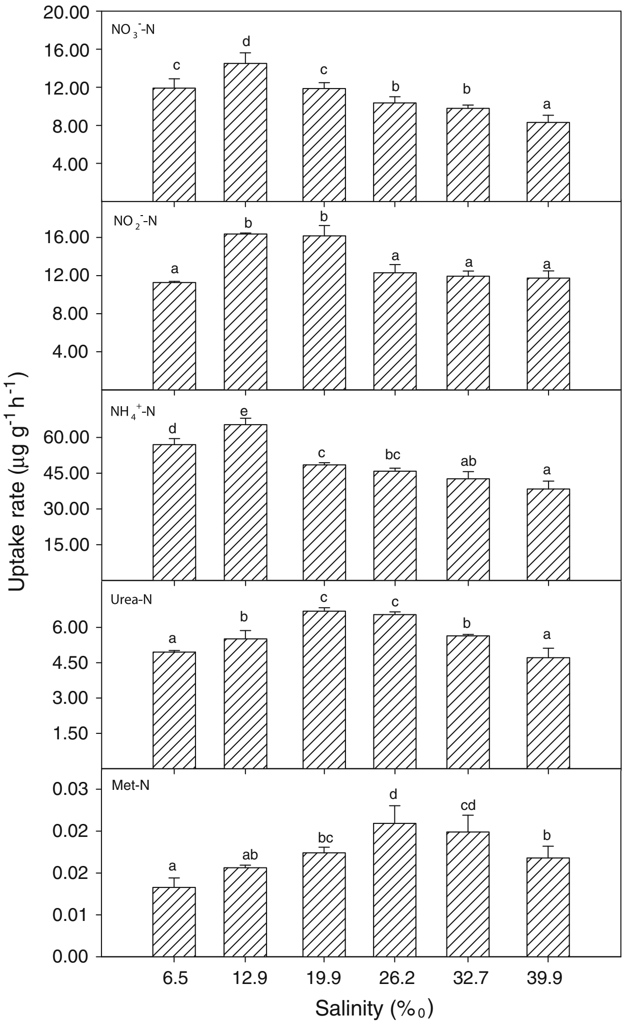

Fig. 3 Uptake rates of NO_3^--N, NO_2^--N, NH_4^+-N, urea-N and Met-N by *O. borgei* at 6 salinity levels. *Different letters* indicate significant differences between treatments ($p < 0.05$)

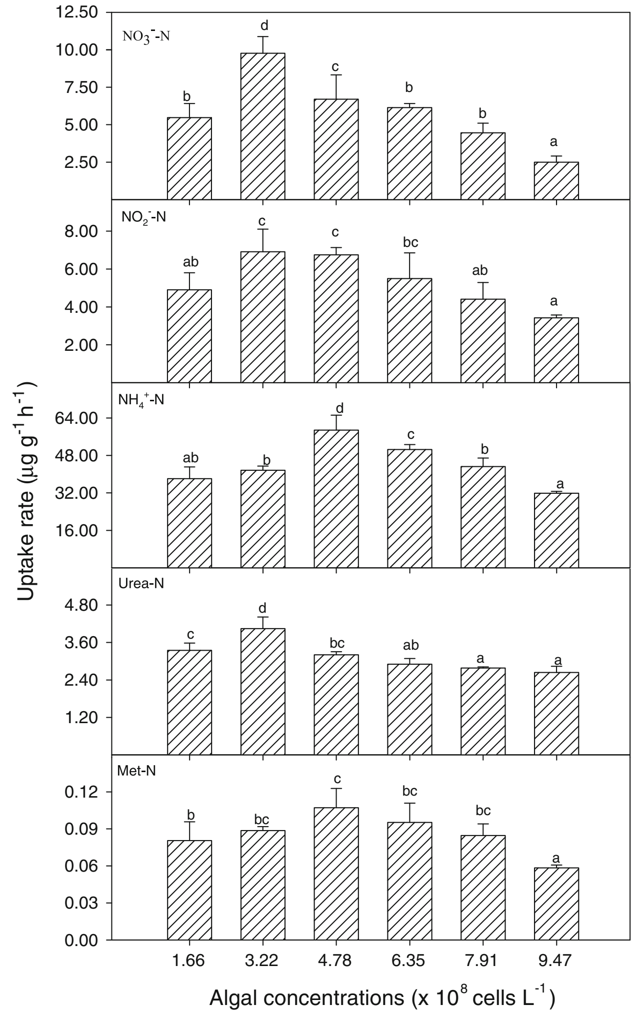

Fig. 4 Uptake rates of NO_3^--N, NO_2^--N, NH_4^+-N, urea-N and Met-N by *O. borgei* at 6 algal concentrations. *Different letters* indicate significant differences between treatments ($p < 0.05$)

Chaetoceros curvisetus whose growth is inhibited when salinity reaches 30 ppt (Mao et al. 2007). Recent research has indicated that the utilization rate for nitrogenous nutrients by *Ulvalactuca* is greatly affected by salinity (Lartigue et al. 2003).

Uptake rates for NO_3^--N, NO_2^--N and urea-N were the highest at an algal concentration of 3.22×10^8 cell L^{-1} (Fig. 4). The highest uptake rates for NH_4^+-N and Met-N were found at a higher algal concentration (4.78×10^8 cells L^{-1}) than other forms of DN. The lowest uptake rates for all forms of DN were found at the highest algal concentration (9.47×10^8 cells L^{-1}) used in this experiment. Mallick and Rai (2007) found the highest removal rate for pollutants at an algal dry weight of 0.1 g L^{-1}. Uptake rate will be limited by nutrient concentration when algal concentrations reach high levels. Furthermore, at high algal concentrations, self shading and resource competition result in high mortality and low growth rate and subsequently low algal uptake rate. High uptake rates for all forms of DN were observed at algal densities between 3.22×10^8 and 4.78×10^8 cells L^{-1}.

In summary, this study using a stable isotope tracer (^{15}N) revealed different responses of algal uptake rates for various forms of DN to a wide range of water temperatures, light intensities, salinities and algal concentrations which are commonly found in the subtropical coastal marine waters. Our finding on the high uptake rates for DIN by *O. borgei* has the potential to add insightful information on the use of planktonic algae to remove toxic nitrogenous contaminants and improve water quality in intensive aquaculture systems.

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