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# NOTES

# Growth and Nitrate-Nitrogen Uptake by the Cyanobacterium *Lyngbya wollei*

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## INTRODUCTION

Over the past 30 years, the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont), family Oscillatoriaceae, has become increasingly common in ponds, lakes, and reservoirs of the southeastern United States (Beer et al. 1986; Speziale et al. 1988, 1991; Speziale and Dyke 1992). Dense mats of this organism can restrict navigation and recreational uses, and decomposing mats can produce offensive odors. Phlips et al. (1992) found summer blooms of *L. wollei* in Lake Okeechobee, and Cowell and Botts (1994) reported massive mats in Kings Bay, the headwaters of Crystal River, Florida. More recently, the Southwest Florida Water Management District (SWFWMD, pers. comm.) has found smaller, principally benthic mats in Rainbow River, an Outstanding Florida Water.

Although *L. wollei* is known to be a nitrogen fixer (Phlips et al. 1992), blooms in Florida commonly occur in locations with high $NO_3^-$-N concentrations like Lake Okeechobee and Crystal River. Thus the increased growth in Rainbow River may be attributable to an increase in nitrate concentrations of ground-water discharging from the springs (Jones et al. 1996); principle anthropogenic sources of nitrate to the aquifer in regions around Rainbow River were inorganic fertilizer (59%) and wastes from horses and cattle (41%). Current levels of $NO_3^-$-N in Rainbow River are high (~1 mg $L^{-1}$) compared to other spring-fed rivers in central Florida (SWFWMD, pers. comm.). Two questions therefore were posed: 1) will further increases in nitrogen levels in the Rainbow River produce additional increases of cyanobacterial biomass; and 2) will the biomass diminish if nitrogen concentrations are reduced? This study measured growth under continuous flow of varying nitrate-nitrogen concentrations and uptake of nitrate-nitrogen under static conditions.

## MATERIALS AND METHODS

Rainbow River is a small (~7.5 km length), spring-fed river in Marion County, FL (29°07'N, 82°26'W) that flows into the Withlacoochee River at Dunnellon, FL. *Lyngbya wollei* mats were collected from the headwater spring at Rainbow River State Park (in June of 2000 and 2001 for growth and uptake experiments, respectively), placed in plastic bags, and stored in a cooler for return to the laboratory.

Debris and algal epiphytes were removed from the *L. wollei* mats by washing, picking debris with forceps, and using sonication (60 Hz); periods of sonication were 10 min. Filaments were examined for epiphytes (principally diatoms) and structural damage on a Leitz-Labovert inverted microscope; numbers of visible epiphytes were counted on 2 cm lengths of 10 randomly selected filaments from a *Lyngbya* mat. Significantly fewer epiphytes (t tests, $p < 0.05$) were present on filaments after being washed and sonicated in deionized water than before washing and sonication. The healthy, cleaned filaments were placed in 100 by 80 mm storage dishes (Corning 3250 100) usually containing water from Juniper Springs that has a low nitrogen content. Sixty-two mg $L^{-1}$ of $CaCl_2$ was added to the Juniper Springs water to maintain osmotic balance and to approximate the Ca content of water from Rainbow River.

### Growth Rates

Cleaned filaments were pre-incubated in Juniper Springs water (to pre-acclimate them to low nitrogen conditions) for 2 to 3 days at 23 C under 56 µmol photons $m^{-2}$ $s^{-1}$ of light for 12 h each day. Six nitrogen concentrations (0.07, 0.3, 0.6, 0.9, 1.2, and 1.5 mg $L^{-1}$) were prepared by adding $NaNO_3$ to Juniper Springs water with 62 mg $L^{-1}$ of additional $CaCl_2$. Four tufts (small mats, ranging from 10.2 to 12.7 mg wet wt) of the pre-incubated *L. wollei* were placed in a 400-mL flask filled with the appropriate nutrient concentration; each nutrient concentration was replicated three times (a total of 18 flasks). Because *Lyngbya* filaments can become detached from tufts or entangled within other free-floating tufts, each flask was divided into four compartments by plastic strips (to separate individual tufts) with small open spaces at the bottom that allowed the nutrient solution to flow into each compartment. A Watson-Marlow multichannel cartridge pump, connected by tubing to 40 L carboys of the test nitrate concentrations, provided 960 mL $d^{-1}$ at continuous flow to the bottom of each flask. Nitex plankton netting (100 µm mesh) was placed over the compartments in each flask to prevent loss of filaments in the discharge from the flasks. Fluorescent lights above the flasks provided 37 to 45 µmol photons $m^{-2}$ $s^{-1}$ to the filaments for 12 h each day. One randomly selected tuft was removed from each flask at 4-day intervals, dried in an oven for 24 h at 45 C, and weighed on an Ohaus electron-

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ic balance (±0.01 mg); the fourth tuft was an extra in case of manipulative problems. Reference tufts from the experimental mats were used to determine the initial (day 0) dry weight and to calculate a regression equation for the relationship between wet and dry weights. Dry weight data are presented as mean mg dry wt per tuft.

### Uptake Rates

Static uptake experiments were conducted in July 2002 using tufts of *L. wollei* filaments that were washed, sonicated, and placed in Juniper Springs water with 62 mg $L^{-1}$ of $CaCl_2$ added. The filaments were pre-incubated for 6 days, and the water was changed every 3 days. The day before the experiment, the Juniper Springs water was enriched with 0.5 mg $L^{-1}$ of $NO_3^-$-N to activate nitrate reductase in the cells (Shaner and Boyer 1976; Beevers and Hageman 1980). At the start of the experiment, five nitrate concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg $L^{-1}$) were prepared with Juniper Springs water and analyzed with a Beckman DU 520 spectrophotometer to develop a standard curve for nitrate-nitrogen concentration; absorbance was measured at 220 nm (ultraviolet spectrophotometric screening method 4500-$NO_3^-$ B; Clesceri et al. 1989). The uptake experiment was conducted in 125 mL Erlenmeyer flasks containing 50 mL of $NO_3^-$-N solution and approximately 32 mg dry wt of *L. wollei*. The flasks were placed in an incubator at 21.4 C for 24 h under 40 to 50 µmol photons $m^{-2}$ $s^{-1}$. Uptake was measured using changes in nitrate-nitrogen concentrations of the water, before and after incubation.

### Statistical Analyses

Data were statistically analyzed using one-way (for individual dates) or repeated measures ANOVAs and conservative multiple comparison tests (the Bonferroni adjustment or Tukey's HSD). Normality and homogeneity of variances were ascertained using the Kolmogorov-Smirnov and the Levene median tests, respectively (Fox et al. 1995). Log or square root transformations sometimes were necessary to achieve normality, homogeneity of variances, or both.

## RESULTS AND DISCUSSION

Because the growth and uptake experiments required low nitrogen concentrations, Juniper Springs water was used instead of Rainbow Spring water for washing, sonicating and testing. The two springs differed in $NO_3^-$-N (0.082 and 0.962 mg $L^{-1}$, respectively) and $CaCO_3$ (42 and 104 mg $L^{-1}$) but not in $NH_4^+$, $PO_4^{3-}$, $Fe^{2 \text{ or } 3+}$ or $SO_4^{3-}$ (SWFWMD, pers. comm.). Growth was compared for *L. wollei* in Rainbow River water (at ambient $NO_3^-$-N concentration) and Juniper Springs water that was adjusted to 1 mg $L^{-1}$ $NO_3^-$-N. One-half of the Juniper Springs cultures were augmented with 62 mg $L^{-1}$ $CaCl_2$ to approximate that of Rainbow River water and the other half were not augmented. Three replicates of each of these cultures were incubated for 12 days, and then dry weights were determined. The *Lyngbya* grew in all three treatments (ranging from 1.87- to 2.05-fold), but a one-way ANOVA showed no difference ($p = 0.34$) between the three treatments. This suggested that the low nitrate concentration of the ambient Juniper Springs water was responsible for the low growth rates observed in a preliminary experiment. Because the Juniper Springs water with no added $CaCl_2$ produced a lower increase in mean biomass (27.8 mg dry wt) than Juniper Springs water with $CaCl_2$ (30.8 mg dry wt), augmentation of Juniper Springs water with $CaCl_2$ was used in subsequent experiments.

### Growth Experiment

The biomass of *L. wollei* (dry wt) increased slowly over the first 4 days and then rapidly (1.6- to 2.5-fold) over the next 8 days (Figure 1a) at all $NO_3^-$-N concentrations. By day 12, dry weight in all concentrations greater than 0.3 mg $L^{-1}$ $NO_3^-$-N ranged from 73.4 to 80.0 mg per tuft, whereas it was only 48.4 and 57.3 mg per tuft respectively in the 0.07 and 0.3 mg $L^{-1}$ $NO_3^-$-N concentrations. A repeated measures ANOVA, covering the whole experiment, showed that days were significantly different ($p < 0.001$) and that the $NO_3^-$-N concentrations differed ($p < 0.045$). Tukey's pairwise comparison of biomass differences on specific dates showed no significant differences ($p > 0.05$) within days 0, 4 and 8, but by day 12, biomass in the four highest $NO_3^-$-N concentrations (1.5, 1.2, 0.9 and 0.6 mg $L^{-1}$) differed significantly ($p < 0.05$) from 0.3 and 0.07 mg $L^{-1}$.

Growth rates (mg dry wt $d^{-1}$) during this experiment were greatest for all nitrogen concentrations between days 4 and 8 (Figure 1b). However, the increases were appreciably greater in the 0.6 through 1.5 mg $L^{-1}$ $NO_3^-$-N concentrations than in the 0.3 and 0.07 mg $L^{-1}$ concentrations. On day 8, growth rates for the two lower $NO_3^-$-N concentrations were 2.6 and 2.4 mg dry wt $d^{-1}$ whereas growth rates at the higher concentrations ranged from 4.2 to 5.9 mg dry wt $d^{-1}$. From days 8 to 12 growth rates increased slightly (0.3 to 1.5 mg dry wt $d^{-1}$) in the 0.3, 0.6, and 0.9 mg $L^{-1}$ $NO_3^-$-N concentrations but remained almost constant (deviating <0.2 mg dry wt $d^{-1}$) in the 1.5, 1.2 and 0.07 mg $L^{-1}$ $NO_3^-$-N concentrations. This suggested that the *L. wollei* was approaching saturation for growth at the higher $NO_3^-$-N concentrations.

### Nitrate Uptake Experiment

In the static experiment, nitrogen uptake increased steadily with $NO_3^-$-N concentration of the medium, ranging from 0.99 to 4.81 mg $g^{-1}$ dry wt $h^{-1}$ (Figure 1c). The rate of uptake increased 3.5-fold between 0.5- and 1.0 mg $L^{-1}$ $NO_3^-$-N, and between 1.0- and 1.5 mg $L^{-1}$ the increase was 1.3-fold. At higher concentrations (2.0 and 2.5 mg $L^{-1}$) uptake increased only 1.1-fold. This experiment suggested that under static conditions *L. wollei* was approaching nitrate-nitrogen saturation at a concentration near 2.5 mg $L^{-1}$.

In response to the two questions posed, our short-term data suggest that an increase in the $NO_3^-$-N concentrations of Rainbow River should not produce a significant increase in the biomass of *L. wollei*. The filaments are already showing maximal growth at concentrations between 0.6 and 1.5 mg $L^{-1}$ $NO_3^-$-N, as shown by statistically similar results for biomass and growth rates. Unfortunately it also appears that the biomass will not diminish with as much as a 2-fold reduction in

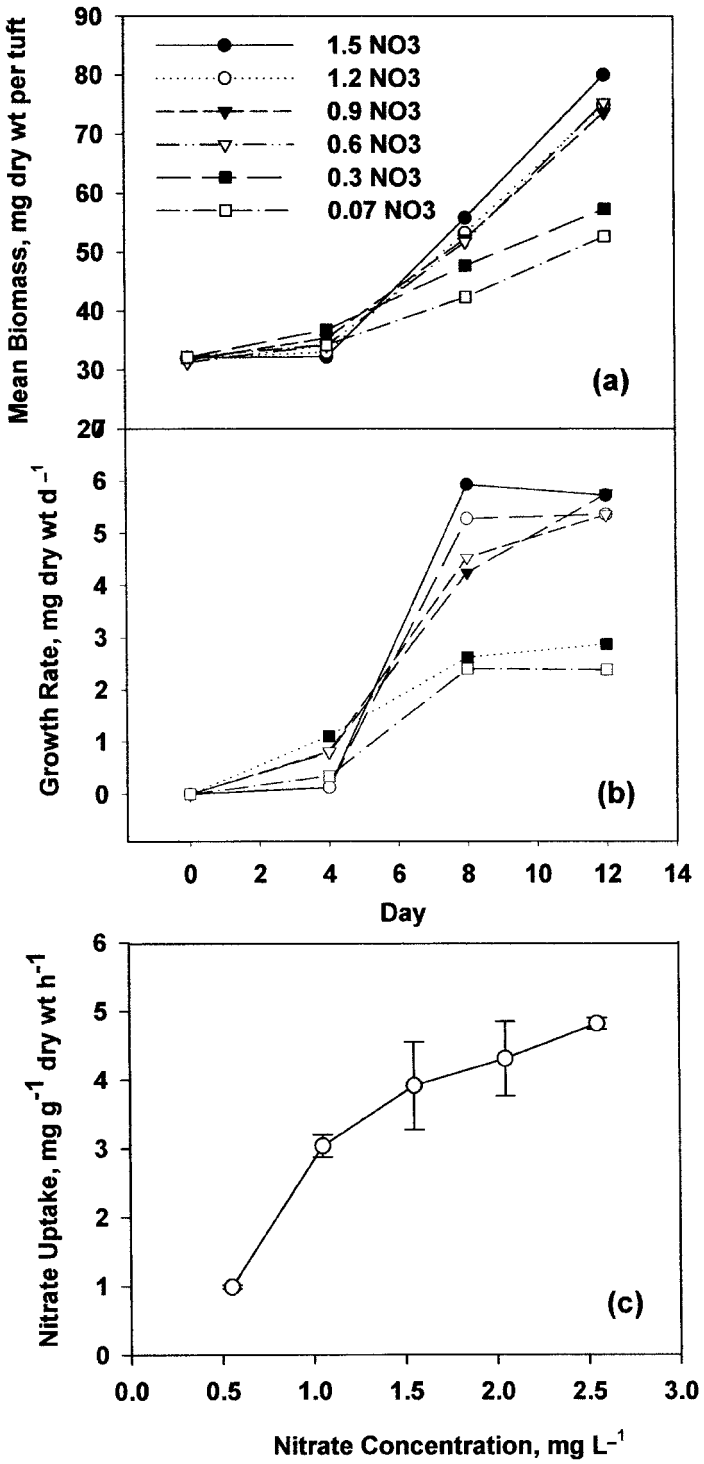


Figure 1. Biomass (a), growth rates (b) and nitrate-nitrogen uptake (c) of *Lyngbya wollei* from Rainbow River, Florida. Biomass and growth rates were 12 day, continuous flow experiments at 6 $NO_3^-$-N concentrations; symbols are means of three samples. Biomass standard errors ranged from <0.1 to 10 mg. Uptake values are means ±1 SE with three replicates.

ambient $NO_3^-$-N levels since growth rates and biomass only were significantly lower at 0.3 and 0.07 mg $L^{-1}$. Apparently, a reduction to <0.3 mg $L^{-1}$ would be needed and this may not be possible because of long-term nitrogen entrainment in the ground-water system.

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