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^{15}N isotope fractionation in an aquatic food chain: *Bellamya aeruginosa* (Reeve) as an algal control agent

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Abstract

^{15}N isotope tracer techniques and ecological modeling were adopted to investigate the fractionation of nitrogen, its uptake and transformation in algae and snail (*Bellamya aeruginosa* Reeve). Different algal species were found to differ in their uptake of nitrogen isotopes. *Microcystis aeruginisa* Kütz. demonstrated the greatest ^{15}N accumulation capacity, with the natural variation in isotopic ratio (δ^{15}N) and the isotope fractionation factor (ε, ‰) being the highest among the species investigated. The transformation and utilization of ^{15}N by snails differed depending on the specific algae consumed (highest for *Chlorella pyrenoidosa* Chick., lowest for *M. aeruginisa*). When snails was seeded in the experimental pond, the algae population structure changed significantly, and total algal biomass as well as the concentration of all nitrogen species decreased, causing an increase in water transparency. A model, incorporating several chemical and biological parameters, was developed to predict algal biomass in an aquatic system when snails was present. The data collected during this investigation indicated that the gastropods such as snails could significantly impact biological community and water quality of small water bodies, suggesting a role for biological control of noxious algal blooms associated with eutrophication.

Key words: ^{15}N fractionation; algal bloom; *Bellamya aeruginosa*; ecological modeling; eutrophication
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Introduction

Eutrophication continues to be a problem for many freshwater lakes and ponds, which bring to algal blooms. Populations of some species are likely to decline in response to changes in water chemistry, as well as food type and availability, while other populations may expand. Certain animals have been identified as potentially beneficial in situations of highly elevated nutrient loading. Biomanipulation is an important method to control water blooms, and there were many reports on feeding silver carp and other algal-eater (Heerdt and Hootsmans, 2007; Lu et al., 2002). Similarly, snails have been found to lessen the effects of eutrophication in some systems (Chase and Knight, 2006). Snails have been identified as a cornerstone species in freshwater ecosystems, playing an important role in control community composition and transformation of algal biomass (Loman, 2001). Suppression of algal blooms has been correlated with the presence of snails (Stelzer and Lamberti, 2002). However, the ecological mechanisms by which snails influence algae abundance and composition, nitrogen circulation and transformation, are unclear.

Separation of nitrogen isotopes through fractionation can be used in studies of eutrophication and nutrient cycling in freshwater (Xu et al., 2008) and, in particular, marine environments (Tamelander et al., 2006). Nitrogen isotopic fractionation of marine microalgae is frequently employed in the investigation of the marine biogeochemical cycling of nitrogen (Altabet, 2006). Recent studies have reported the use of a stable nitrogen isotope to investigate marine eutrophication (Cole et al., 2004). However, the use of ^{15}N isotope is less common in studies of eutrophication and freshwater algae (Townsend et al., 2007). In the present study, we used a ^{15}N isotope tracer technique to investigate the effects of the freshwater snail, *Bellamya aeruginosa* Reeve, on nitrogen fractionation and transformation of algae and established an ecological model reflecting how this gastropod control eutrophication.

1 Materials and methods

1.1 Experimental setup

1.1.1 Pond experiment

The ecological pond experiment was performed at the

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experimental station in Jiangsu Academy of Agricultural Sciences. Cement ponds (12 m × 5 m × 2 m) were set up in the tidal flats and filled with 75 m^3 of water collected from the Doulong River (Dafen, Jiangsu Province, China). The initial two treatments were no snail as control and with snails; three replicate ponds were studied in each treatment (total of six ponds). *B. aeruginosa* specimens were collected by a net from the bed of the Doulong River. The collected snails were washed with tap water to remove any algae attached to the shell. The snails (the average size of about (30 ± 0.55) mm and weight of (2.8 ± 0.12) g were placed in unchlorinated tap water for one week without feeding to eliminate gut contents. Five kilograms of *B. aeruginosa* were placed in each pond of the treatments. Diammonium phosphate was added to the water to achieve initial concentrations of total nitrogen (10.88 mg/L), ammonium nitrogen (6.35 mg/L) and total phosphate (1.24 mg/L). Samples of *B. aeruginosa* were collected weekly for eight weeks.

Algal species in the control ponds were isolated under microscope, and cultured in ^{15}N culture medium. The experiment was designed as nitrogen-limiting with an N:P ratio of 4:1 in the culture medium. The labeled-N with 10.38% $(^{15}NH_4)_2$-SO_4 (Shanghai Research Institute of Chemical Industry, China) was used.

1.1.2 Aquarium experiment

The isotope-labeling experiments with three replicates were carried out in an aquarium (70 cm × 50 cm × 25 cm) for 15 days. The five treatments were: (1) labeled *Scenedesmus arcuatus* Lag; (2) labeled *Chlorella pyrenoidosa* Chick.; (3) labeled *Microcystis aeruginisa* Kütz.; (4) labeled mixed algae (*C. pyrenoidosa*, *Chaetophora elegans* Roth, *S. arcuatus*, and *M. aeruginisa*); (5) labeled *M. aeruginisa* and non-labeled *C. pyrenoidosa*. Each aquarium with 40 L tap water was aerated to maintain adequate dissolved oxygen level and decrease chlorine. One hundred snails (2.8–3.2 g each) were placed into each aquarium. The labeled algae from the ponds were centrifuged to concentrate the cells and washed three times with tap water. The cleaned algal cells were used to prepare 500 mL of culture medium (nominal cell density of 8×10^8/L). The concentrated algal cells were added to the test aquarium twice a day as food for the snails, and the water was aerated and agitated to keep the cells suspended.

1.2 Sampling and measurements

Snail samples in aquarium experiment were collected for analysis once every 24 hr. The samples were placed in distilled water for 72 hr to empty the guts of food and dried at 40°C to remove the shell. The soft tissue of snail was acid-digested, total nitrogen was determined using ^{15}N mass spectrographic analysis (Shearer and Kohl, 1986).

Water samples extracted with siphon from snail aquarium were analyzed for particulate nitrogen (PN) and other nutrients. A known volume of water was filtered using a Whatman® GF/F filter at 450°C. Membrane samples were used to detect PN and $\delta^{15}N_P$, and filtrate was used to measure dissolved nutrients. PN was measured using a CHN automatic analysis detector (NA1500 type, Fisons, Britain).

The density of algae and zooplankton were determined microscopically. Total nitrogen, total phosphate, COD_{Mn} and water transparency were regularly measured (MEP-PRC, 2006).

1.3 ^{15}N parameters and fractionation model determination

It has been assumed that the isotopic state of nitrogen absorbed by algae was fixed (Altabet, 2006); reaction rate constants for ^{14}N and ^{15}N are ^{14}K and ^{15}K, respectively. The $^{15}K/^{14}K$ ratio is defined as α, and is expressed by the following Eq. (1):

$$a = \frac{^{15}K}{^{14}K} = \frac{\mathrm{d}^{15}N_S}{^{15}N_S} \Big/ \frac{\mathrm{d}^{14}N_S}{^{14}N_S} \tag{1}$$

where, the subscript S represents the concentration of dissolved nitrogen.

It is assumed that f represents the percentage of N that is not absorbed. Because ^{15}N is present in nature in only very small quantities, ^{14}N being the more common isotope, $f \approx {}^{14}N_S/{}^{14}N_{S,0}$, can be integrated into Eq. (1), thus yielding:

$$a\ln f = \ln\frac{^{15}N_S}{^{15}N_{S,0}} \tag{2}$$

The isotope ratios of R, the "delta" notation employed for other stable isotope systems, δ^{15}N and δ^{15}N‰ are defined as:

$$R = {}^{15}N/{}^{14}N \approx {}^{15}N/\left({}^{14}N + {}^{15}N\right) \tag{3}$$

$$\delta^{15}N = \left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}} \tag{4}$$

$$^{15}N‰ = \delta^{15}N \times 1000 \tag{5}$$

Current literature typically refers to ε values which can be defined as:

$$\varepsilon = 10^3(a-1) = \frac{\delta^{15}N_S - \delta^{15}N_{S,0}}{\ln f} \tag{6}$$

The mass balance of ^{15}N can be represented by Eq. (4):

$$f\delta^{15}N_S + (1-f)\,\delta^{15}N_P = \delta^{15}N_{S,0} \tag{7}$$

where, the subscript P represents the ^{15}N absorbed by microalgae. $\delta^{15}N_S$ can be simplified to:

$$\delta^{15}N_P = \delta^{15}N_{S,0} - \varepsilon\frac{f\ln f}{1-f} \tag{8}$$

To quantify nutrient transfer from different algae to snails, the ^{15}Np accumulation and transfer rate were determined as follows:

$$^{15}\text{N accumulation} = \text{total nitrogen content} \times E_A\% \tag{9}$$

$$\text{Transfer rate of } {}^{15}N = \frac{\text{(the accumulation of } {}^{15}N \text{ / } {}^{15}N \text{ from any given diet)}}{\text{the accumulation of } {}^{15}N \text{ / } {}^{15}N \text{ from algae}} \times 100\% \tag{10}$$

In order to provide a quantitative representation of the algae removal by *B. aeruginosa*, the built the model with the following variables: treatment water volume, circle rate, weight of the snails, removal rate, nutrient concentration and respiratory rate (Li et al., 2004).

$$\frac{dP_d}{dt} = (G_{P_d} - K \times T - G_{Z_1} \times \frac{Z_1}{P_d} - G_{Z_2} \times \frac{Z_2}{P_d} - d) \times P_d - R_d \times C_r \times P_d \quad (11)$$

$$G_{P_d} = (u_0 \times K_1 \times T)(C_{IN} \times (K_{IN} + C_{IN}))(C_{IP} \times (K_{IP} + C_{IP})) \quad (12)$$

where, P_d is biomass of the aquatic algae; G_{P_d} is algae ratio growth rate; K is algae respiratory rate; T is water temperature; G_{Z_1} is zooplankton ratio grow rate; Z_1 is zooplankton density; G_{Z_2} is excretion rate of *B. aeruginosa*; Z_2 is weight of *B. aeruginosa*; d is algae degradation rate; R_d is algae removal rate by *B. aeruginosa*; C_r is circulation rate; u_0 is the highest excretion rate ratio; K_1 is the algae multiplying thermal constant; C_{IN} is inorganic nitrogen concentration; C_{IP} is inorganic phosphate concentration; K_{IN} is nitrogen saturation constant; K_{IP} is phosphate saturation constant.

1.4 Statistic analysis

One-way ANOVA, *t*-test and Duncan's new multiple range method were adopted for statistic analysis.

2 Results

2.1 Effects of *B. aeruginosa* on water quality

The presence of snails had a significant effect on several water quality parameters (Table 1). Algal biomass decreased in the treatment containing snails, as did all of the nitrogen parameters and COD. Although the nitrite concentration decreased from 0.5 to 0.09 mg/L, the difference was not significant. Water transparency improved in treatment containing snails, as evidenced by a significant increase in diaphaneity, which rose from a mean of 23 cm in the control to 77 cm.

2.2 Utilization and fractionation of nitrogen in different algae

There was a significant difference in $\delta^{15}N_P$ values among different types of algae. The general trend in $\delta^{15}N_P$ values was *M. aeruginisa* > *S. arcuatus* > *C. pyrenoidosa*. The $\delta^{15}N$ value for *M. aeruginisa* was significantly higher than that for the mixed group, *S. arcuatus* and *C. pyrenoidosa*, but not significantly different from *C. pyrenoidosa* and *M. aeruginisa* combined group (Table 2). The $\delta^{15}N$ value for *M. aeruginisa* was significantly different from *C. pyrenoidosa*, but not from the other algal groups (Table 2). The ^{15}N absorption curves, however, were similar for *M. aeruginisa*, *C. pyrenoidosa* and *S. arcuatus* (Fig. 1a). $\delta^{15}N_P$ was low early in the growth period, but as dissolved nitrogen consumption rose, thereby did $\delta^{15}N_P$, reaching an exponential growth phase at about 7–11 day, and a plateau at 11–13 day. The maximum $\delta^{15}N_P$ values for *M. aeruginisa*, *Chlorella* and *S. arcuatus* were 9.66%, 7.60% and 6.58%, respectively (Fig. 1a).

The nitrogen isotope microalgae fraction ε can be obtained from the slope (Table 2). ε was the highest in *M. aeruginisa*, followed by *C. pyrenoidosa* and then *S. obliques*. Analysis of the data indicated that the differences in ε and $\delta^{15}N_P$ among the different species of algae might be associated with the rate at which different nitrogen forms were transported across cell membranes.

2.3 Transfer and accumulation of ^{15}N in different algae ingested by *B. aeruginosa*

^{15}N was detected in *B. aeruginosa* on the first day of feeding and the amount present in the snails increased over time (Fig. 2b). $\delta^{15}N_P$ in the snails reached a maximum of 4.38‰ on day 10 by *C. pyrenoidosa*. Although the concentration dropped on day 11, it did not decrease in a monotonic fashion, remaining elevated but with fluctuating levels. It is likely that $\delta^{15}N_P$ rose until a saturation point was reached, and then began an up-down cycle, but always remaining below the maximum value reached earlier. δ^{15}Np achieved the first peak on day 3 by *M. aeruginisa*, declined to 0.27‰ on day 7, and then began to increase again, reaching a maximum of 2.19‰ on day

Table 1 Characterization of water in group 1 (control with no snail) and group 2 (with *B. aeruginosa*)

Treatment pond	Algal biomass (mg/L)	TN (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	COD_{Mn} (mg/L)	Diaphaneity (cm)
No snail	91.08 ± 3.90	6.7 ± 1.56	1.99 ± 0.31	0.74 ± 0.21	0.5 ± 0.44	34.2 ± 3.32	23 ± 5.94
With snail	19.41 ± 1.12**	3.41 ± 0.54**	0.98 ± 0.13**	0. 40 ± 0.22*	0.09 ± 0.20	7.3 ± 2.14**	77 ± 6.78**

Data are expressed as mean ± SE (n = 5); *$P \leqslant 0.05$; ** $P < 0.01$ based on t-test.

Table 2 $\delta^{15}N_p$, ε, accumulation and utility rate of ^{15}N in different algae treatments

	Labeled alga treatment				
	S. arcuatus	*M. aeruginisa*	*C. pyrenoidosa*	*M. aeruginisa* and non-labeled *C. pyrenoidosa*	Mixed algae
$\delta^{15}N_P$ (‰)	3.19 ± 0.22 c	4.92 ± 0.34 a	3.96 ± 0.21 b	4.90 ± 0.32 ab	4.70 ± 0.31 b
ε (‰)	4.37 ± 1.33 c	8.45 ± 1.22 a	6.56 ± 1.43 b	8.43 ± 1.23 ab	5.43 ± 1.05 b
^{15}N accumulation (mg)	0.65 ± 0.27 b	0.44 ± 0.24 b	1.14 ± 0.46 a	0.41 ± 0.25 b	0.89 ± 0.37 ab
^{15}N transfer (%)	5.78 ± 0.35 b	4.22 ± 0.32 b	6.01 ± 0.34 a	4.24 ± 0.37 b	5.27 ± 0.26 ab

Values are expressed as mean ± SE (n = 5).
Data with different letters within a column are significantly different ($P < 0.05$) based on Duncan's new multiple range method.

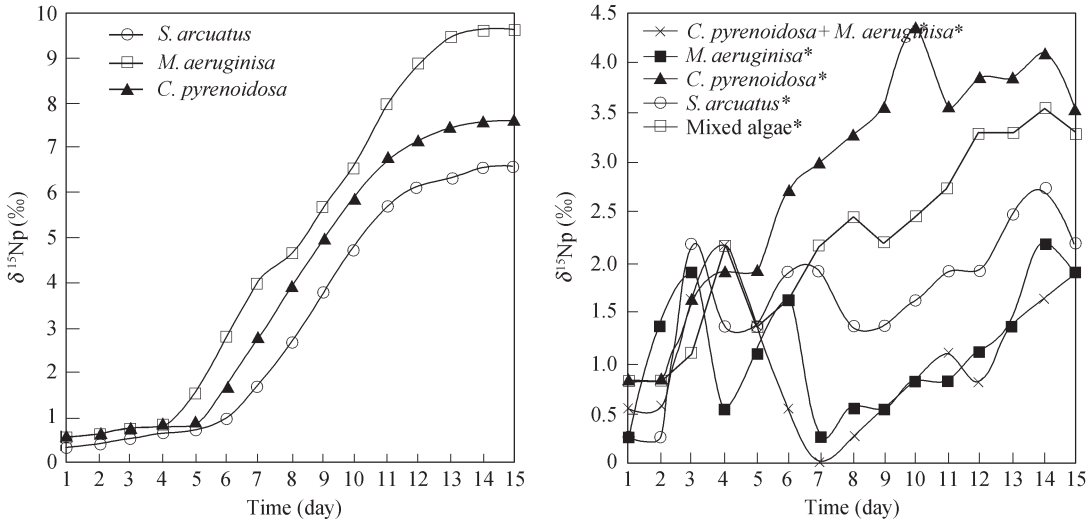

Fig. 1 Absorption curve of ^{15}N in different algae (a) and ^{15}N changes in *B. aeruginosa* after feeding different algae (b). *: Labled ^{15}N.

14, which was less than the peak achieved by other algae. ^{15}N accumulation reached a maximum on day 4, and then declined to 0 on day 7, when *B. aeruginosa* ingested the mixture of *C. pyrenoidosa* and *M. aeruginisa*. This demonstrated that all of the ^{15}N that had been taken up was subsequently excreted. *B. aeruginosa* was not highly selective with regard to food, although ingestion of *M. aeruginisa* appeared to be secondary to the non-blue green algae, such as *C. pyrenoidosa*.

The concentrations of ^{15}N in *B. aeruginosa* varied over time and fluctuated with algal taxon. Although ^{15}N accumulation in the snails rose and fell over the experimental period, the general trend was upward and levels tended to stabilize after 11 to 14 days (Fig. 2a). This pattern suggests equilibration, where the amount of ^{15}N excreted was equivalent to that taken up. The highest value (1.74 mg) was for *C. pyrenoidosa*, which was reached after 10 days. The ^{15}N absorption curve from *M. aeruginisa* was similar, at least initially, to the *M. aeruginisa* and *C. pyrenoidosa* mixed group, but accumulation in the latter hit 0 after 7 days and then increased gradually until the end of the test.

The transfer rate of ^{15}N from algae to *B. aeruginosa* demonstrated temporal shifts (Fig. 2b). The metastasis rate was high for the first 7 days but then decreased. Different algae produced different peak ^{15}N transfer rates. The peak ^{15}N transfer rate for the mixture of algae was 8.17% after one day, which was higher than in all other treatments. Statistical analysis indicated that the high transfer rate was consistent with the general characteristics of this species.

2.4 Model and validation of algae reductions by *B. aeruginosa*

The mean biomass of treatment group 2, 19.41 mg/L was significantly ($P < 0.01$) less than in the control group 1 (91.08 mg/L), representing a 78.69% reduction in algal biomass (Table 1). In the control group the blue-green algae multiplied dramatically and formed a bloom of *M. aeruginisa*. In contrast, water clarity in the treatment containing the snails remained high, with no evidence

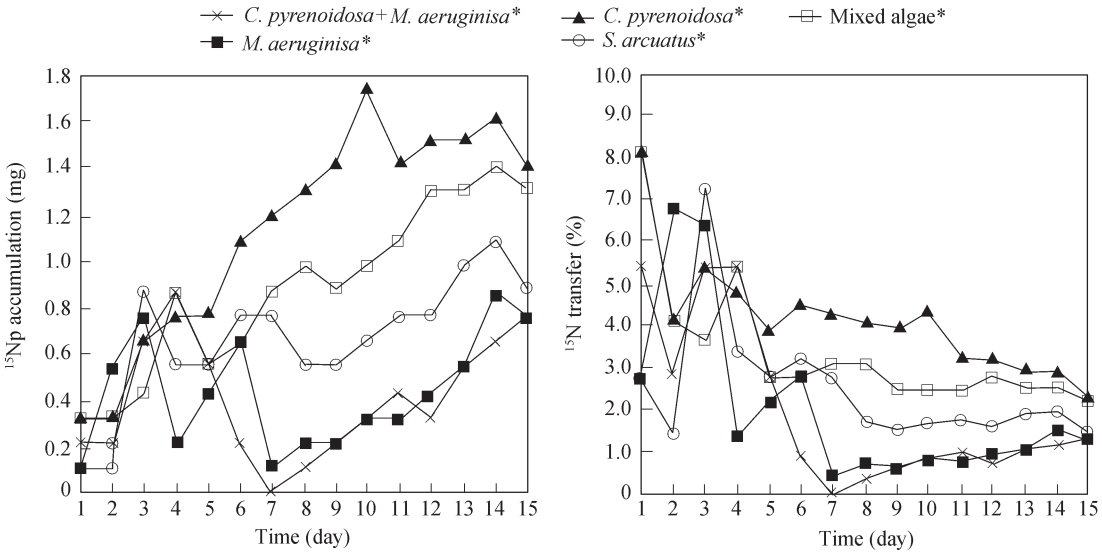

Fig. 2 ^{15}Np accumulation of different algae in *B. aeruginosa* (a) and transfer rate of ^{15}N from algae to *B. aeruginosa* (b). *: Labled ^{15}N.

Table 3 Predicted and measured algal biomass after 51 days of growth with different starting cell concentrations

Pond No.	1	2	3	4	5	6	7	8
Initial	15.33	35.65	66.47	75.56	90.78	122.67	135.67	150.84
Predicted	5.11	8.57	18.54	29.00	68.54	88.67	92.20	109.87
Measured	5.21	8.44	17.9	27.87	65.33	100.21	112.32	132.56
Removal (%)	66.01	76.33	73.07	63.12	28.03	18.31	17.21	12.12
Deviation (%)	1.92	–1.54	–3.58	–4.05	–4.91	11.52	17.91	17.12

of excessive blue-green growth. However, *Chaetophora elegans* Roth and *Stigeoclonium elongatum* (Has.) Kütz. density did increase in the treatment containing snails.

Using data from different time intervals in the model (model (11) and (12)) and running scenarios, the algal biomass was simulated and compared to the actual biomass. Except for unexpected variations in July, which were believed to be associated with higher than expected rainfall, the simulated biomass values correlated well with measured concentrations.

In order to identify the range over which model predictions would be valid, 5 kg of *B. aeruginosa* were placed into ponds with different amounts of algae. Initial biomass ranged from 15.33 to 150.84 mg/L in eight ponds (Table 3). After 51 days, algal biomass decreased in all treatments, with reductions ranging from 12.12% to 76.33%. The decrease percentage in algal biomass was higher when the initial biomass was lower. This change would be expected as that a lower initial concentration would impede the ability of algae to replace cells lost through ingestion. The model did a good job of predicting algal biomass, particularly when initial cell concentrations were lower. Through No. 5 pond, the predicted value deviated from the actual value by less than 5%, which is considered valid for the purposes of this evaluation. In ponds No. 6, No. 7 and No. 8, with initial cell concentrations of 122.67, 135.67 and 150.84 mg/L, respectively, deviations exceeded 11%, making the model predictions invalid.

3 Discussion

3.1 Fractionation of nitrogen and comparative uptake by different algae

The ε (‰) of *M. aeruginisa* was significantly higher than that of other algae, which may explain why this cyanobacterium can become dominant in eutrophic waters. The similarity early in the study (Han et al., 2006) suggests that the presence of the cyanobacteria may have been influencing ingestion by the snail, although that influence may have lessened with time.

Differences in nitrogen uptake indicate that *C. pyrenoidosa* was the most easily ingested by *B. aeruginosa*, while *M. aeruginisa* was the most difficult to ingest. *B. aeruginosa* selected *C. pyrenoidosa* and either ingested fewer, or could not process, *M. aeruginisa* cells. The lower ingestion rate or avoidance of *M. aeroginosa* cells by *B. aeruginosa* is reflective of what has been observed in both cladocera and fish (Han et al., 2006). The toxic microcystins produced by the blue-green *M. aeruginisa* may be an important factor in its avoidance by snails and other primary consumers.

3.2 Impact of *B. aeruginosa* on the aquatic ecosystem

The presence of *B. aeruginosa* significantly affected the composition, density and biomass of algae. The biomass of *C. elegans* and *S. elongatum*. increased in the pond containing *B. aeruginosa*. The result was different from sails eat filamentous algae species (Andrea et al., 2007). This increase may be due to the presence of a net which was hung in the water to provide colonization surfaces for the filamentous algae.

While the waste products of the snails include nitrogen, our results were similar to that concluded by Pinowska (2002), in that the ingestion of algae did not increase nutrient concentrations. Hence, long-term snail cultivation may cause a general decrease in aquatic nitrogen availability. Snails are usually considered scrapers (Andrea et al., 2007), ingesting attached algae and associated organic matter off hard surfaces. Our results, however, suggest that snails may also adopt filtering habits, particularly when phytoplankton density is high enough.

The rate at which *B. aeruginosa* ingests algae is affected by numerous factors including, but not limited to, filtering rate, excretion rate, primary biomass of algae and algal growth rate. The model used to demonstrate the impacts of *B. aeruginosa* on the algal populations and associated trophic status indicate that certain alterations are occurring within the aquatic systems, specifically a decrease in the density of phytoplankton populations as well as a drop in the concentrations of nitrogen and phosphate, which are usually the major nutrients that drive primary production. There is a definite quantitative relationship between the algal biomass and the snail biomass that is needed to control algae growth. An increase in the number of *B. aeruginosa* will result in concurrent reduction in algal biomass, up to a certain level or carrying capacity of snails, above which other biotic and abiotic factors become increasingly important, and ecosystem stability is threatened.

Acknowledgments

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