Bellamya aeruginosa (Reeve) promote the growth of Elodea nuttallii (Planch.) H. St John in high nutrient environment

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\textbf{ABSTRACT}

\textit{Elodea nuttallii} (Planch.) H. St John is an invasive alien submerged macrophyte, which grows very fast and dominates in many Chinese waters. \textit{Bellamya aeruginosa} (Reeve) is a widely distributed benthonic organism in China. This snail species rarely grazes submerged macrophytes and may promote their growth by feeding on epiphyte and phytoplankton. The above two species can commonly be found together in nature waters and their interaction may promote the growth of both species, which could disturb the ecological balance. In this paper, effects of different densities of \textit{B. aeruginosa} at two different nutrient stages on water quality and the growth of \textit{E. nuttallii} were studied. The results showed that the growth rates (GRs) of \textit{E. nuttallii} were not significantly affected by different \textit{B. aeruginosa} densities in the low nutrient (LN) stage. However, in the high nutrient (HN) stage, the GRs of the aboveground parts of \textit{E. nuttallii} in the high density (HD) groups were considerably higher than the control (CK) and low density (LD) groups. The water chlorophyll (Chl) and nitrate-nitrogen (NO\textsubscript{3}-N) contents increased substantially with increasing \textit{B. aeruginosa} density in the LN stage, while the Chl and NO\textsubscript{3}-N contents in the LD groups were significantly higher than in the HD and CK groups in the HN stage. The results of this paper indicated that \textit{B. aeruginosa} could promote the growth of \textit{E. nuttallii} by reducing the Chl contents in the water in high-nutrient environment rather than in low-nutrient environment, which highlighted that \textit{B. aeruginosa} may strengthen the invisibility of \textit{E. nuttallii} in eutrophic water caused by human activities.
**Introduction**

Freshwater snails and submerged macrophytes are both important biological groups in aquatic ecosystem and relate well in many ways (Brönmark and Bronmark 1985; Sheldon 1987; Zhu et al. 2013). Submerged macrophytes are primary producers in aquatic ecosystems. They help to maintain the biodiversity and functions of aquatic ecosystems, mediate the biogeochemical cycles of water nutrients, and maintain clear water state (Carpenter and Lodge 1986; Scheffer et al. 1992). Submerged macrophytes provide habitat and spawning sites for freshwater snails and can improve their living condition. Freshwater snails can filter phytoplankton in the water and scrape algae attached on submerged macrophytes, which may alleviate the intensity of competition for light and nutrient. The metabolism of freshwater snails have an impact on many physical-chemical indexes of water and sediment, which may change the growth of submerged macrophytes or their community structure (Pieczyńska 2003; Li et al. 2009; Cao et al., 2014).

In this study, we chose the freshwater snail *Bellamya aeruginosa* (Reeve) and the submerged macrophyte *Elodea nuttallii* (Planch.) H. St John as our research species. *B. aeruginosa* is an important benthonic organism, with a wide distribution in waters of Southeast Asia, India and Africa, especially, in waters of Yangtze River basin, Yellow River basin and Yungui Plateau in China (Cai et al. 2016; Li et al. 2016). It has high genetic diversity and often coexists with aquatic plants (Gu et al. 2015). This snail species rarely grazes submerged macrophytes and may promote their growth by feeding on the nutrient and light competitor, epiphyte algae and phytoplankton (Zheng et al. 2011; Zhu et al. 2013). *E. nuttallii* was chosen because it is an invasive alien species for Chinese water bodies, which grow very fast and dominate in many waters (Xu et al. 2007). These two species can commonly be found together in nature waters and their interaction may promote the growth of both species, which could disturb the ecological balance.

This study investigated effects of the coexistence of *B. aeruginosa* and *E. nuttallii* on water quality and the growth of *E. nuttallii* at two different nutrient levels. The aim of this study is to clarify how *B. aeruginosa* affects the growth of *E. nuttallii* and the water quality. We hypothesized that: (a) *B. aeruginosa* can promote the growth of *E. nuttallii* and the water quality; (b) the growth of *E. nuttallii* with *B. aeruginosa* presence was better than that of *E. nuttallii* without *B. aeruginosa* presence when the water was nutrient enriched.

**Materials and methods**

*Experimental design*

The experiment was performed in 12 glass tanks (length × width × height: 0.6 × 0.5 × 1.0 m) at the Poyang Lake Model Experimental Research Base in Gongqing city, Jiangxi Province, China. The glass tanks were put in a large greenhouse (180 × 110 × 21 m) whose roof was made of steel frame with large panes of glass, which was very helpful to control light intensity and temperature. The seedlings of *E. nuttallii* were collected from the aquatic plant nursery in the research base. Healthy macrophytes without branches were selected, which were cut at 15 cm from the top of the branch. The initial fresh and dry weights of *E. nuttallii* were 0.51 ± 0.06 and 0.055 ± 0.008 g, respectively. The sediment was collected from the Poyang Lake near the base and mixed evenly after removing the stones and debris. The organic matter, total nitrogen and phosphorus contents in the sediment was 5.22%, 1.35 mg/g and 0.91 mg/g, respectively.
The sediment was placed into a plastic rectangular tray (19.5 × 13.5 × 5 cm) to a depth of 4 cm. Eight seedlings of *E. nuttallii*, which were washed carefully, were planted into each tray at a depth of 3 cm. Six trays containing the planted macrophytes were then placed into the glass tanks. Air aerated tap water was slowly added to the tanks to a height of 0.8 m (120 L water). The 12 glass tanks were divided into three groups with different densities of *B. aeruginosa*: the control group (CK, no snails), the low-density snail group (low density (LD), 30 snails, density of 125/m³) and the high-density snail group (high density (HD), 90 snails, density of 375/m³). Each group had four replicates.

The experiment was divided into two stages. The first stage was the low nutrient (LN) stage during which no additional nutrients were added, and the main source of nutrients in the system was the sediment. Water quality parameters were measured every 10 d, and this stage lasted for 90 d. After 90 d, three trays of *E. nuttallii* were collected from each glass tank in order to measure the growth of the macrophytes, and the remaining three trays of *E. nuttallii* in each tank were used for the second stage of the experiment.

The second stage was the high nutrient (HN) stage. During this stage, ammonium nitrate and potassium dihydrogen phosphate solution were added into each glass tank, so that the initial total nitrogen (N) and phosphorous (P) contents in the water body were approximately 3.2 mg/L and 0.64 mg/L, respectively. Water quality parameters were then measured every 7 d, and this stage lasted for 28 d. At the end of the experiment, the *E. nuttallii* samples in the remaining three trays were collected from each glass tank for macrophyte growth determination.

**Water and macrophyte parameter determination**

During the experiment, water temperature (T), dissolved oxygen (DO), pH, conductivity (COND), oxidation-reduction potential (ORP) and chlorophyll (Chl) were measured using a portable multi-parameter water quality analyzer (HQ40D, Hach, USA). The ammonium nitrogen (NH₄-N), nitrate-nitrogen (NO₃-N) and orthophosphate phosphorus (PO₄-P) contents were measured in the laboratory using Chinese national standard methods (Huang 2000).

The macrophyte samples were carefully washed with distilled water at least three times and separated into aboveground and belowground parts. They were repeatedly dried with water-absorbing paper until no water dropped by hard shaking. The wet weight of aboveground and belowground biomass of the submerged macrophytes was measured using electronic scale. The growth rate (GR) of the submerged macrophytes was calculated by a formula: $GR = (M_2 - M_1)/dt$, where $M_1$ and $M_2$ are the initial wet weight and the post-harvest wet weight of submerged macrophytes, respectively, and $dt$ is the macrophyte growth time (days).

**Statistical analysis**

Data processing, analysis and plotting were completed using SPSS software (SPSS Inc., USA). The effects of different densities of *B. aeruginosa* on the GRs of aboveground and belowground parts of *E. nuttallii* were investigated by the one-way analysis of variance method (one-way ANOVA). Post hoc multiple comparisons among treatments were performed using Tukey HSD tests. All data were tested for normality and homogeneity before performing ANOVAs. Data were transformed (Sqrt(x), square(x), artan(x), reciprocal(x) or/and log10(x)) to obtain normality and/or homogeneity if they did not meet the
basic assumptions. The threshold for significance differences among groups was at the level of $p < 0.05$.

**Results**

**The growth of *E. nuttallii***

In the LN stage, the GRs of the aboveground and belowground parts of *E. nuttallii* in the CK group averaged at $0.032 \pm 0.002$ d$^{-1}$ and $0.015 \pm 0.002$ d$^{-1}$, respectively. There were no significant differences ($p > 0.05$) for the GRs of the aboveground and belowground parts of *E. nuttallii* among CK, LD and HD groups. In the HN stage, the GRs of the aboveground parts of *E. nuttallii* in the HD group (averaged at $0.195 \pm 0.028$ d$^{-1}$) were significantly higher ($p < 0.05$) than the CK and LD groups (averaged at $0.156 \pm 0.011$ d$^{-1}$ and $0.123 \pm 0.020$ d$^{-1}$, respectively). However, the GRs of the belowground parts were negative with the HD group significantly lower ($p < 0.05$) than that of the CK group (Figure 1).

**Dynamic changes of the water quality parameters**

In the LN stage, the COND in the CK group was relatively stable as time progressed but exhibited an upward trend in the LD and HD groups, with the HD group always being higher than the LD group. In the HN stage, with the exception of Day 111, the COND values of the three groups were in the order of HD > LD > CK and showed a downward trend. The pH values of the CK, LD and HD groups showed an increasing trend and stabilized over time in both the LN stage and HN stage. The water ORP values of the three groups showed an initial downward trend, and then stabilized with time (Figure 2).

The Chl contents in the water body exhibited a downward trend after an initial increase in both the LN and HN stages. In the LN stage (with the exception of Day 60), the Chl contents in the water body of the three groups were in the order of HD > LD > CK. In the HN stage, the Chl contents of the LD group were generally higher than the HD and CK groups. The water DO of the three groups at both nutrient stages showed a decreasing trend with time following an initial increase (Figure 2).

The NO$_3$-N contents in the water showed a decreasing trend in both the LN and HN stages and were in the order of HD > LD > CK in the LN stage and in the order of LD > HD > CK in the HN stage. The changes in the NH$_4^-$ contents were not apparent in the LN stage but showed a decreasing trend in the HN stage.
The PO₄-P in the water remained at a low level during the LN stage and decreased with increasing time during the HN stage, and the PO₄-P contents in the CK group were always higher than in the LD and HD groups. The changes of the NO₃-N, NH₄-N, and PO₄-P contents altered the N:P in the water. In the LN stage, the N:P was stable over time, although a certain degree of fluctuation was observed, while in the HN stage, the N:P in the water increased with increasing snail density (Figure 3).

Figure 2. Changes of water parameters (a) COND, (b) pH, (c) ORP, (d) Chl and (e) DO with time at different nutrient stages among experimental treatments.
In the HN stage, the NH₄-N and PO₄-P removal rates of each group were between 88 and 97%, and the NO₃-N removal rates were between 30 and 60%. Among them, the NO₃-N removal rate of the LD group was significantly higher than the CK and HD groups ($p < 0.05$); the NH₄-N removal rate of the CK group was significantly higher than the LD group and HD group ($p < 0.05$); and the PO₄-P removal rate increased with increasing snail density.

**Discussion**

A large number of studies have assessed the effects of freshwater snails on the growth of submerged macrophytes, but with no consistent conclusions. This is primarily because the interaction between snails and submerged macrophytes is influenced by numerous factors, such as the species and density of the snails, the species and growth period of the submerged macrophytes, and the nutrient level of the water bodies (Underwood 1991; Pinowska 2002; Li et al. 2009). This study was different from other studies in that the submerged macrophyte was planted with sediment while most of others without
sediments (Underwood 1991; Pinowska 2002; Li et al. 2009). The results of this study demonstrated that different densities of *B. aeruginosa* did not significantly influence the growth of the aboveground and belowground parts of *E. nuttallii* in the LN stage. Conversely, during the HN stage, the high *B. aeruginosa* density substantially promoted the growth of the aboveground parts of *E. nuttallii*, indicating that the metabolism of *B. aeruginosa* may not be the primary factor affecting the growth of *E. nuttallii* in the present study. Submersed macrophytes can absorb nutrient from both water and sediment dependent on the nutrient concentrations and relative availabilities in sediment vs. water column (Rattray et al. 1991; Madsen and Cedergreen 2002; Cao et al. 2011). In the LN stage of this study, sediment may be the primary source of nutrient for *E. nuttallii*. However, during the HN stage, the Chl content in the HD group was significantly lower than the CK and LD groups, suggesting that *B. aeruginosa* at a HD can promote the growth of *E. nuttallii* by inhibiting algal growth.

The COND of the water was most significantly affected by *B. aeruginosa*. In the LN and HN stages, the COND of the water body increased with increasing *B. aeruginosa* density, suggesting that *B. aeruginosa* can increase the ion content of the water. However, the parameters which resulted in the changes of COND cannot be determined from this experiment and need to be further explored.

In this experiment, the interactive effects of *B. aeruginosa* and *E. nuttallii* led to high ammonia nitrogen and orthophosphate phosphorus removal rates, and a low nitrate-nitrogen removal rate. This may be because ammonia nitrogen and orthophosphate phosphorus are the main nutrient sources for the growth of *E. nuttallii*. When both ammonia nitrogen and nitrate-nitrogen are present, submerged macrophytes preferentially utilize ammonia nitrogen as the N source (Cao et al. 2011). The study of Zhu et al. (2013) demonstrated that the existence of *B. aeruginosa* can significantly reduce the N:P of water bodies. However, in the present study, the coexistence of *B. aeruginosa* and *E. nuttallii* increased the N:P of the water body, which may be attributed to the absorption of a large amount of P in the water by *E. nuttallii*. In addition, the N and P in the sediment could also be absorbed by submerged macrophyte, which could potentially change the pH and redox potential of the sediment and water, and thus the N:P of the water, which need further studies.

**Disclosure Statement**

No potential conflict of interest was reported by the authors.

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