Journal of
Plant Ecology

Research Article

Responses of morphological and physiological traits to herbivory by snails of three invasive and native submerged plants

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Handling Editor: Yu-Long Feng

Received: 10 June 2021, **First Decision:** 9 July 2021, **Accepted:** 26 August 2021, **Online Publication:** 15 September 2021

Abstract

The submerged plant species Carolina fanwort (*Cabomba caroliniana*) has become a dominant invasive aquatic plant in the Lake Taihu Basin (LTB) in China. Introduced species may escape their original specialist enemies and encounter fewer enemies in their new environment. They were assumed to have suffered less herbivory than native species as they are relatively unpalatable (the enemy release hypothesis [ERH]). The objective of this study was to compare the responses of *C. caroliniana* with those of co-occurring native species to herbivory from native herbivores. We conducted a mesocosm experiment to record the responses of *C. caroliniana* and two commonly co-occurring native submerged plant counterparts, water thyme (*Hydrilla verticillata*) and Eurasian watermilfoil (*Myriophyllum spicatum*), to herbivory by two native generalist gastropod snails, *Radix swinhoei* and *Sinotaia quadrata*. Plant morphological traits (total biomass, shoot/root [S/R] biomass ratio and relative growth rate [RGR]) and physiological traits (leaf total nonstructural carbohydrate [TNC], lignin, and cellulose) were recorded. The snail *S. quadrata* rarely influenced the plant traits of the three submerged plants. With the increasing numbers of *R. swinhoei* treatments, most of the plant traits of *H. verticillata* and *M. spicatum* changed, while those of *C. caroliniana* showed a relatively stable fluctuation. This result indicates that *C. caroliniana* is more resistant to herbivory by the snail *R. swinhoei*, which is consistent with the ERH hypothesis. This finding indicates that herbivorous snail species contributes to the invasion of *C. caroliniana*, which potentially alters the species composition of submerged plants in the plant community.

Keywords aquatic plant, biological invasion, *Cabomba caroliniana*, enemy release hypothesis, Lake Taihu Basin, plant–herbivore interactions

3种入侵和本地沉水植物形态和生理性状对螺类牧食的响应

摘要：沉水植物水盾草(*Cabomba caroliniana*)已成为中国太湖流域的优势入侵水生植物。与外来物种的原产地环境相比，引入地新环境中存在的专食性天敌较少。外来物种可能会逃避其原产地环境中的天敌牧食，又因为它们的适口性相对较差，从而导致在引入地外来物种通常比本地物种遭受的牧食者影

响更低(天敌逃逸假说)。本研究的目的是比较水盾草与共生的本地沉水植物对本地牧食者的响应。我们进行了一个中宇宙尺度实验，研究了水盾草和两种共生的本地沉水植物黑藻(*Hydrilla verticillata*)和穗花狐尾藻(*Myriophyllum spicatum*)对两种本地广食性腹足纲螺类萝卜螺(*Radix swinhoei*)和环棱螺(*Sinotaia quadrata*)的牧食响应。记录了它们的形态性状指标(总生物量、冠根比和相对生长率)和生理性状指标(叶片总非结构性碳、木质素和纤维素)。研究结果表明，环棱螺对3种沉水植物性状指标的影响较少。随着本地广食性螺类萝卜螺数量的增加，黑藻和穗花狐尾藻大部分植物性状发生了改变，而水盾草的植物性状表现出相对稳定的趋势。水盾草对萝卜螺的牧食更具抵抗力，这与天敌逃逸假说的假设一致。这一发现说明牧食性螺类促进了水盾草的入侵，这可能会改变沉水植物群落中的物种组成。

关键词：水生植物，生物入侵，水盾草(*Cabomba caroliniana*)，天敌逃逸假说，太湖流域，植物-牧食者关系

INTRODUCTION

Plant invasions have been listed as one of the most severe threats to freshwater ecosystems globally (Hussner *et al.* 2017). Compared with terrestrial ecosystems, freshwater ecosystems are more vulnerable to invasion (Francis 2012; Thomaz *et al.* 2015). Widespread import and export trade has resulted in numerous plants, especially aquatic species, being introduced into China without risk assessments (Zhan *et al.* 2017). Over the past few decades, ecologists have been uncertain about why certain plants have become widespread and are challenging to eliminate, and the mechanisms of successful plant invasion in natural habitats remain controversial (van Kleunen *et al.* 2010a; Zedler and Kercher 2004). Many hypotheses have been proposed; however, it seems that there is no universal explanation for successful invasions (Dawson *et al.* 2012; Mitchell *et al.* 2006; van Kleunen *et al.* 2010b).

The enemy release hypothesis (ERH) indicates that when a novel species is introduced to a new habitat that lacks specialist herbivores from its original surroundings, it suffers less from the effects of resident enemies than the native species because the introduced species are relatively unpalatable, allowing them to grow, reproduce and spread successfully (Colautti *et al.* 2004; Keane and Crawley 2002). Invasive plants are assumed to be less affected by natural enemies (Chun *et al.* 2010; Xiong *et al.* 2008). This assumption dates back to Darwin, which is far earlier than the founding date of invasive biology (Hufbauer and Torchin 2008). Some successful biocontrol cases have highlighted the importance of introducing specialist herbivores and have thus provided indirect evidence for the ERH (Abrantes *et al.* 2012; Legaspi and Legaspi 2008; Lu *et al.* 2010).

Previous studies have shown that the widespread invasive plants water hyacinth (*Eichhornia crassipes*) and alligator weed (*Alternanthera philoxeroides*) have caused severe effects in freshwater ecosystems in China (Wang *et al.* 2017; Wu *et al.* 2017; Yu *et al.* 2019). Recently, a newly introduced invasive plant, Carolina fanwort (*Cabomba caroliniana*), has become prevalent in an environmentally sensitive area, the Lake Taihu Basin (LTB), and has garnered considerable concern (Liu *et al.* 2018a; Zou *et al.* 2012). Originating from the southern United States and South America, the plant was introduced to China in the late 1980s and listed as an invasive species in the fourth patch of '*The List of Alien Invasive Species in China's Natural Ecosystem*' in China. An investigation showed that *C. caroliniana* had become a dominant species in the LTB, and the rapid spread of this plant species may indicate that it has not yet reached its distribution limit (Huang *et al.* 2020). Concerns have increased because this plant leads to higher water quality deterioration than native plants, which may increase the outbreak of phytoplankton (Lu *et al.* 2018). The plant hosts has no specialized herbivores but only generalist herbivores (Schooler *et al.* 2006; Wilson *et al.* 2007). However, it is not a preferred food of grass carp (*Ctenopharyngodon idella*), and there are no reports of herbivory by mammals, birds or other vertebrates of the plant, and little is known about insect herbivores of the plant (Wilson *et al.* 2007). *Cabomba caroliniana* was a palatable food to the snail, *Pomacea canaliculata*, and the crayfish, *Procambarus clarkii*; however, a considerable reduction in its palatability was noticed when it was grazed upon by two generalist herbivores (Morrison and Hay 2011). Studies (for instance, Liu *et al.* 2018a and Zou *et al.* 2012) on this plant species have been performed in China, although its invasion mechanism and reason for its fast spread remain ambiguous. To the best of our knowledge, there have been no previous studies on the responses to herbivore grazing among *C. caroliniana* and co-occurring native plants.

As two major groups of aquatic biotas in freshwater ecosystems, submerged plants and snails have a close coevolutionary relationship and profoundly impact one another (Dodds and Whiles 2019; Mormul *et al.* 2018). Herbivorous snails must overcome the extensive variety of plant physical and chemical defenses that have arisen due to evolution (Molles 2015). The leaf total nonstructural carbohydrate (TNC), lignin and cellulose are the most common structural materials that defend the herbivory from herbivores (Baraza *et al.* 2007; Lambers and Oliveira 2019; Tomasella *et al.* 2020). The physical structure and chemical composition of plants affect their palatability as food, and snails tend to choose foods with better palatability because low-palatability food affects their growth and reproduction (Ohta *et al.* 2011; Tibbets *et al.* 2010). In this study, the responses of *C. caroliniana* and two common co-occurring submerged native plants, the Eurasian watermilfoil (*Myriophyllum spicatum*) and water thyme (*Hydrilla verticillata*), to the two native herbivorous snail species *Radix swinhoei* and *Sinotaia quadrata* were recorded over a mesocosm experiment. This study aimed to clarify the following: (i) the responses of plant morphological and physiological traits to herbivory by snails of three invasive and native submerged plants and (ii) whether *C. caroliniana* suffers less damage from native herbivores than native species and it also aims to determine the possible means of invasion by the plant.

MATERIALS AND METHODS

Study area

Lake Taihu is the third-largest freshwater lake in China, with an area of approximately 2338 km^2 and a mean depth of 1.9 m, and it has been studied considerably as a model shallow lake (Qin *et al.* 2007). This lake lies downstream region of the Yangtze River, one of the most developed areas in China (Qin *et al.* 2009; Taihu Basin Authority [TBA] 2019). Although the harvest of aquatic plants has been banned, the loss of aquatic plant vegetation has still been observed in this area (Liu *et al.* 2018b; Zhang *et al.* 2018). The mesocosm experiment was conducted in the field at the Taihu Laboratory for Lake Ecosystem Research (TLLER), which is on the shore side of the Lake Taihu in Binhu District, Wuxi City, Jiangsu Province.

Plant and snail materials

Three submerged plant species were included in our experiment. The native plant *H. verticillata* is a dominant submerged plant in the LTB that is extensively applied to ecological restoration efforts in this area due to its fast growth rate and high adaptation to eutrophication (Zhang *et al.* 2013). The other native plant, *M. spicatum*, has a global distribution and is detected in most lakes in the LTB (Wu *et al.* 2015; Xie *et al.* 2013). Having American origins, *C. caroliniana* has been a dominant invasive submerged species in the LTB (Huang *et al.* 2020; Zou *et al.* 2012). Young shoots of the three plants were collected from a pond (31.4221° N, 120.2154° E) with infrequent human interference near Lake Taihu in June 2018. They were preincubated outdoors in TLLER with sediment from the lake for a month before the experiment was initiated.

The two snail species, *R. swinhoei* and *S. quadrata*, were selected as grazers in our experiment due to their high abundance in the same pond described above, and they are also the most common snail species in the LTB (Li *et al.* 2009; Ye *et al.* 2020). The snails were collected from the pond and cultivated in a 500-L polyethylene (PE) bin (covered with sunshade net at noon) at TLLER for 1 month. The water temperature fluctuation in the bin was consistent with that of the lake water, in the range of 18–25 °C. We followed the guidelines of the *Chinese Laboratory Animal Management Regulations (2017)* throughout the experiment; all snails were released into their natural habitat after the experiment.

Experimental design

The experiment was performed in 120-L cylindrical PE buckets. Sediment from the Meiliang Bay of Lake Taihu was collected, sieved (with a 40-mesh sieve), and added to the buckets to a depth of 20 cm (as measured from the bottom of the bucket). The density of the sediment was 1.47 ± 0.02 g/cm^3, and the concentrations of N and P were 1.19 ± 0.04 and 0.14 ± 0.01 mg/g (means ± SD), respectively. To prevent disturbance from raindrops, lids were placed on the buckets when rain was forecasted, and they were removed after rain. Two factors were included in the experiment: the plant species and the snail density. We conducted a field investigation at the end of July 2018 in natural habitats in the LTB. In the 1 × 1 m^2 randomly placed quadrats, the range of abundance of *R. swinhoei* was 94 to 176 ind./m^2, with an average density of 126 ind./m^2, and the range of abundance of *S. quadrata* was 15–103 ind./m^2, with an average density of 75 ind./m^2. The same snail densities were calculated according to the area of the experimental bucket (basal area = 0.16 m^2). These two densities were employed as medium snail densities (126 ind./m^2 × 0.16 m^2 = 20 ind. of *R. swinhoei* and 75 ind./m^2 × 0.16 m^2 = 12 ind. of *S. quadrata*) in the experiment (Fig. 1). The two snail densities were employed as factors (without snails: control group [CK]; low snail density R10: 10 ind./bucket of *R. swinhoei* and B6: 6 ind./bucket of *S. quadrata*; medium snail density R20: 20 ind./bucket of *R. swinhoei* and B12: 12 ind./bucket of *S. quadrata*; and high snail density: R40: 40 ind./bucket of *R. swinhoei* and B24: 24 ind./bucket of *S. quadrata* in the treatment groups (Fig. 1). Each herbivory group was replicated five times (n = 5), and a total of 35 buckets were used.

The experiment began on 10 August 2018. At the beginning of the experiment, the lake water was poured into the buckets to 40 cm (as measured from the bottom of the bucket), and one shoot from each of the three plant species was cultivated in mixed culture for one week (Fig. 1). We had conducted another experiment from 20 June to 4 August 2018 in TLLER. One individual plant of each of the three plants was selected and cultivated in the same bucket with the same experimental setup in the experiment. The results showed that the interspecific competition among the three plant assemblages is rather low (Huang *et al.* 2021). Water from Lake Taihu was used during the experiment, and the environmental parameters of the lake water were total nitrogen = 0.65 ± 0.12 mg/L, total phosphorus = 0.046 ± 0.002 mg/L, pH = 7.92 ± 0.21, and conductivity (Cond) = 429 ± 39 μS/cm (means ± SD). Shoots measuring 20–25 cm long for all three plants were selected, washed carefully with tap water to remove any attached algae, and planted in the buckets. Five shoots were randomly selected, and their initial total biomass was recorded (*H. verticillata*: 0.718 ± 0.027 g, *M. spicatum*: 0.746 ± 0.020 g, and *C. caroliniana* 0.641 ± 0.017 g; means ± SD). After 1 week, all of the shoots had survived and adapted to the new environment (Gu *et al.* 2018; Huang *et al.* 2018; Li *et al.* 2016). The water depth in each bucket was then increased to 75 cm (measured from the bottom of the bucket). Medium-sized snails (fresh weight of *R. swinhoei* is 1.221 ± 0.205 g, and that of *S. quadrata* is 1.528 ± 0.141 g; means ± SD) were

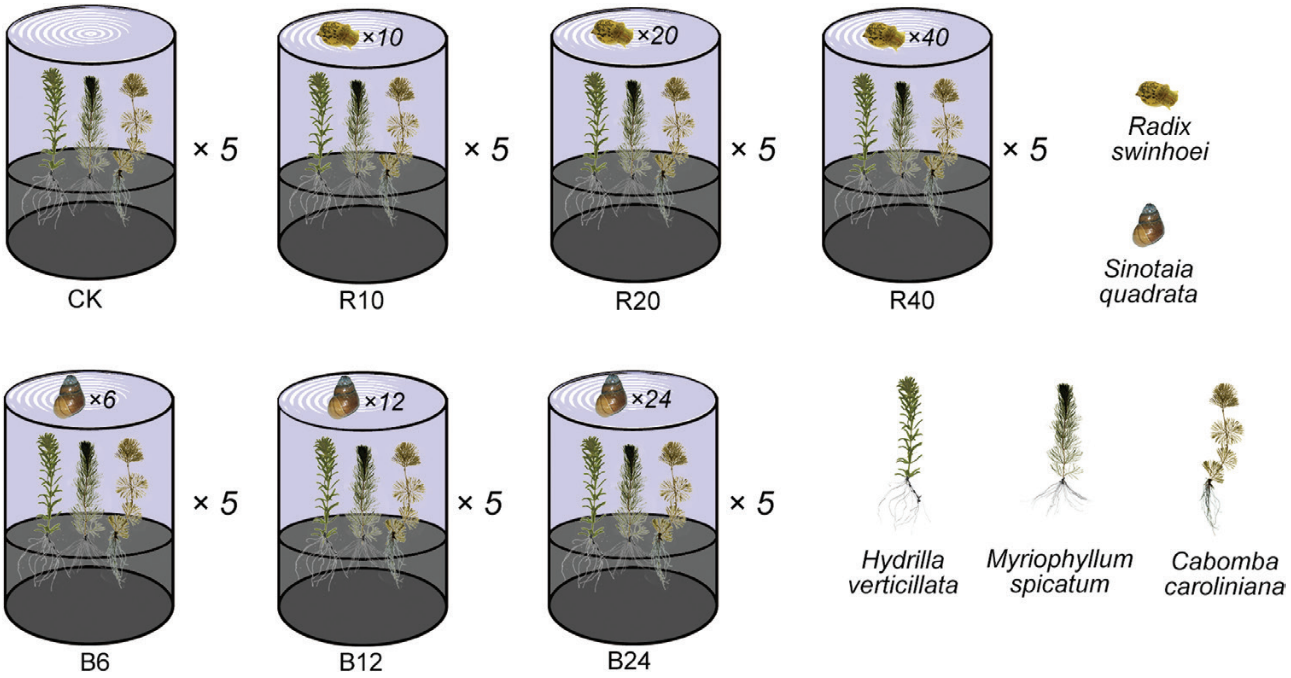

Figure 1: Design of the mesocosm experiment. Two factors were established: the plant species (*Hydrilla verticillata*, *Myriophyllum spicatum* and *Cabomba caroliniana*) and the snail (*Radix swinhoei* and *Sinotaia quadrata*) density. One shoot of each of the three plant species was transplanted into all the mesocosms. In the control (CK) group, no snails were added, while to other groups, different numbers of snails were added according to their densities in the natural habitat (low snail density: R10 and B6; medium snail density: R20 and B12; and high snail density: R40 and B24).

Table 1: The linear mixed model analysis of the responses of morphological and physiological traits of three submerged plants (native *Hydrilla verticillata* and *Myriophyllum spicatum*, and invasive *Cabomba caroliniana*) to herbivory by the snail *Radix swinhoei*

	Plant species (PS)		Snail numbers (SN)		PS × SN	
	F	*P*	*F*	*P*	*F*	*P*
Total biomass	16.668	**<0.001**	31.605	**<0.001**	7.344	**<0.001**
S/R biomass ratio	4.543	**0.016**	4.026	**0.012**	1.680	0.146
RGR	26.033	**<0.001**	35.559	**<0.001**	8.976	**<0.001**
Leaf TNC	3.770	**0.030**	43.011	**<0.001**	17.108	**<0.001**
Leaf lignin	1.348	0.269	2.044	0.120	1.387	0.239
Leaf cellulose	46.767	**<0.001**	5.842	**0.002**	3.203	**0.010**

Significant values ($P < 0.05$) are highlighted in bold. The plant species and snail numbers of *R. swinhoei* were used as fixed factors, and bucket was used as a random factor in the linear mixed model analysis. Abbreviations: RGR = relative growth rate, S/R = shoot/root, TNC = total nonstructural carbohydrate.

chosen and then starved for three days before the experiment began. The two snail species were then placed inside the buckets (Fig. 1).

After 1 month, the leaves of *M. spicatum* and *H. verticillata* subjected to the R20 and R40 treatments were strongly inhibited, which we infer was due to the snails influencing the morphological and physiological traits of the plants, and we ended the experiment and harvested the plants. The root and shoot of each plant were separated and oven-dried at 60 °C to a constant weight. The shoot/root (S/R) biomass ratio and relative growth rate (RGR) were calculated as follows:

$$\text{S/R biomass ratio} = \text{shoot dry mass/root dry mass} \quad (1)$$

$$\text{RGR (mg g}^{-1}\text{ day}^{-1}) = (\ln r_2 - \ln r_1)/(t_2 - t_1) \quad (2)$$

where r_1 is the initial dry plant mass at time t_1; r_2 is the dry plant mass at harvest time t_2; and $t_2 - t_1$ is the experimental duration. In this study, $t_2 - t_1 = 31$ d.

The leaf TNC, lignin, and cellulose contents were measured with a UV-Vis 2450 spectrophotometer (Shimadzu, Tokyo, Japan). We did not record the development of phytoplankton or periphyton biomass; however, we found no algal outbreaks and very little periphyton development in the mesocosms during the experimental period.

Data analysis

All the data were tested to determine whether they satisfied the assumption of having a normal distribution (using the Shapiro–Wilk test) and the homoscedasticity of variances (using Levene's test). If the data did not satisfy the two assumptions, a $\log(x + 1)$ transformation was applied. Linear mixed model analysis was performed, with plant species and numbers of the two types of snails as fixed factors and the bucket as a random factor. One-way ANOVA was applied to test for differences in plant traits during the experiment. The outcomes were considered significant at $P < 0.05$. All statistical analyses were conducted using SPSS Statistics 26 (IBM Corp., Armonk, NY, USA).

RESULTS

Except for the physiological trait leaf lignin, significant differences ($P < 0.05$) in other plant traits, namely, total biomass, S/R biomass ratio, RGR, leaf TNC and cellulose, were found between the native *H. verticillata* and *M. spicatum* and invasive *C. caroliniana* (Table 1). Similar results were also found in the morphological trait comparison; specifically, except for S/R biomass ratio, significant differences ($P < 0.05$) were found in the other plant traits, namely, total biomass, RGR, leaf TNC, lignin and cellulose, in the three plants indicated (Table 2).

Between the two native generalist herbivores, the different snail numbers of *R. swinhoei* had a significant effect ($P < 0.05$) on all the plant traits except for the leaf lignin of the three plants (Table 1). In contrast, the snail numbers of *S. quadrata* did not have a significant influence ($P > 0.05$) on any of the plant traits in the three plant species (Table 2).

Except for S/R biomass ratio and leaf lignin, the interactions between plant species and snail numbers of *R. swinhoei* with other plant traits, namely, total biomass, RGR, leaf TNC and cellulose, were not

Table 2: The linear mixed model analysis of the responses of morphological and physiological traits of three submerged plants (native *Hydrilla verticillata* and *Myriophyllum spicatum*, and invasive *Cabomba caroliniana*) to herbivory by the snail *Sinotaia quadrata*

	Plant species (PS)		**Snail numbers (SN)**		**PS × SN**	
	F	***P***	***F***	***P***	***F***	***P***
Total biomass	151.486	**<0.001**	1.435	0.124	0.164	0.170
S/R biomass ratio	1.699	0.119	0.116	0.192	0.105	0.200
RGR	83.599	**<0.001**	1.443	0.124	0.149	0.181
Leaf TNC	107.539	**<0.001**	2.781	0.105	4.196	0.100
Leaf lignin	54.081	**<0.001**	2.567	0.107	1.423	0.123
Leaf cellulose	105.400	**<0.001**	0.128	0.943	0.343	0.910

Significant values ($P < 0.05$) are highlighted in bold. The plant species and snail numbers of *S. quadrata* were used as fixed factors, and bucket was used as a random factor in the linear mixed model analysis. Abbreviations: RGR = relative growth rate, S/R = shoot/root, TNC = total nonstructural carbohydrate.

significant ($P > 0.05$). The interactions between plant species and snail numbers of *S. quadrata* were not significant for any plant traits ($P > 0.05$).

The plant traits of the three plant species, *H. verticillata*, *M. spicatum* and *C. caroliniana*, did not show much fluctuation in any of the treatments of the snail *S. quadrata*, which indicates that the snail did not show an apparent preference or have a dramatic influence on most of the plant traits in the three species (Figs 2–4). In contrast, *R. swinhoei* had a leading role in this impact on the plants (Figs 2–4). The snail had a high preference for *H. verticillata* and *M. spicatum* over *C. caroliniana* in the experiment (Figs 2–4).

The total biomass, S/R biomass ratio and RGR of *H. verticillata* and *M. spicatum* showed similar significant decreases ($P < 0.05$) with an increase in the density of *R. swinhoei* (Figs 2 and 3). However, for *C. caroliniana*, the effect of herbivory by *R. swinhoei* did not lead to a significant difference (Figs 2 and 3).

The leaf TNC contents of *H. verticillata* and *M. spicatum* showed a significant decrease ($P < 0.05$) with an increase in the density of *R. swinhoei* (Fig. 4a). However, for *C. caroliniana*, the effect of herbivory by *R. swinhoei* did not show a significant difference (Fig. 4a).

The leaf lignin and cellulose contents of *H. verticillata* and *M. spicatum* showed a significant increase ($P < 0.05$) with an increase in the density of *R. swinhoei* (Fig. 4b and c). However, *C. caroliniana* showed the highest and most stable values for leaf lignin and cellulose contents across all treatments (Fig. 4b and c), which indicated that the two snail species rarely grazed it.

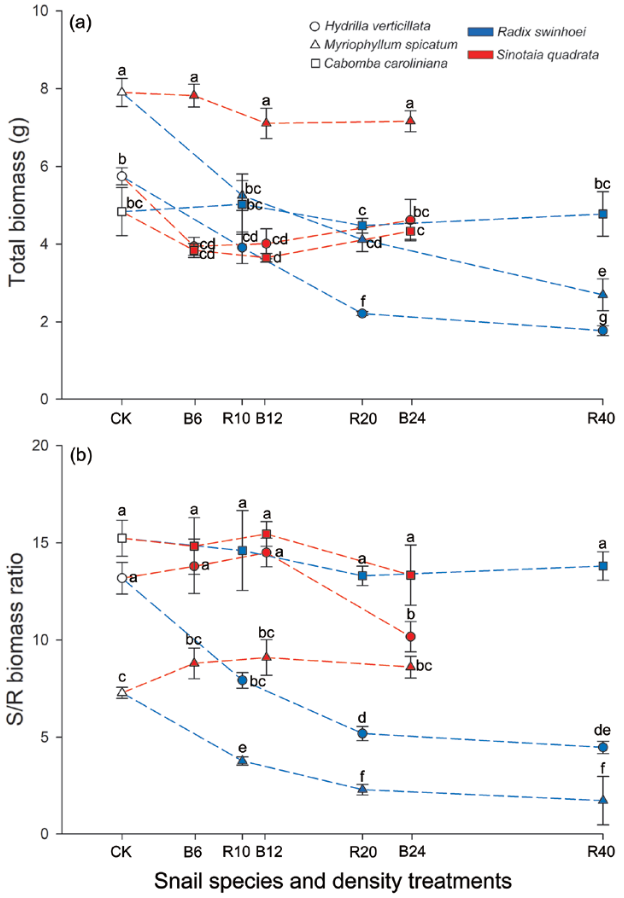

Figure 2: The (**a**) total biomass and (**b**) shoot/root (S/R) biomass ratio of *Hydrilla verticillata*, *Myriophyllum spicatum*, and *Cabomba caroliniana* in the experiment. Open symbol: control (CK) groups; filled symbol: snail treatment groups. The values are means ± SE (n = 5). Different lowercase letters indicate significant differences between treatments (P = 0.05).

DISCUSSION

Low palatability may facilitate the successful invasiveness of *C. caroliniana*

Our study demonstrates that the snail *S. quadrata* rarely influences the plant traits of the three submerged plants, the native *H. verticillata* and *M. spicatum*, and invasive *C. caroliniana*. The plant traits of *C. caroliniana* showed a relatively stable fluctuation in response to the different *R. swinhoei* treatments, which indicates that it was more resistant than the other plant species to herbivory by this native generalist. Under herbivore pressure, invasive *C. caroliniana* suffered minor damage from the native generalist herbivores in this study, corresponding to the ERH.

It is generally believed that invasive plants have more advantageous traits (e.g. a higher RGR or morphological plasticity) than native plants, which has enhanced the ability of invasive species to compete with native plants (life-history [LH] hypothesis) (Richards *et al.* 2006; van Kleunen *et al.* 2010b). A global consensus showed that faster-growing species (as indicated by a higher RGR) tend to be the most invasive (Dawson *et al.* 2011). For the invasive plant *C. caroliniana* addressed in this study, we did not observe this type of trend over native plants (Fig. 3), which is not consistent with the LH. A previous study also showed that *C. caroliniana* did not show advantageous root traits or much higher plasticity index values than *M. spicatum* (Huang *et al.* 2018). However, we determined that the RGR of *C. caroliniana* maintained a stable tendency compared with that of *M. spicatum* or *H. verticillata* when subjected to herbivore stress (Fig. 3).

Submerged plants and filamentous algae are primary producers in freshwater ecosystems. Petruzzella *et al.* (2020) showed that the herbivorous

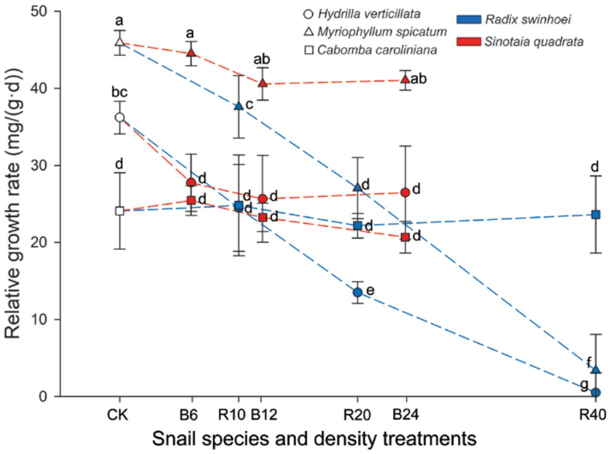

Figure 3: The RGR of *Hydrilla verticillata*, *Myriophyllum spicatum* and *Cabomba caroliniana* in the experiment. Open symbol: control (CK) groups; filled symbol: snail treatment groups. The values are means ± SE (n = 5). Different lowercase letters indicate significant differences between treatments (P = 0.05).

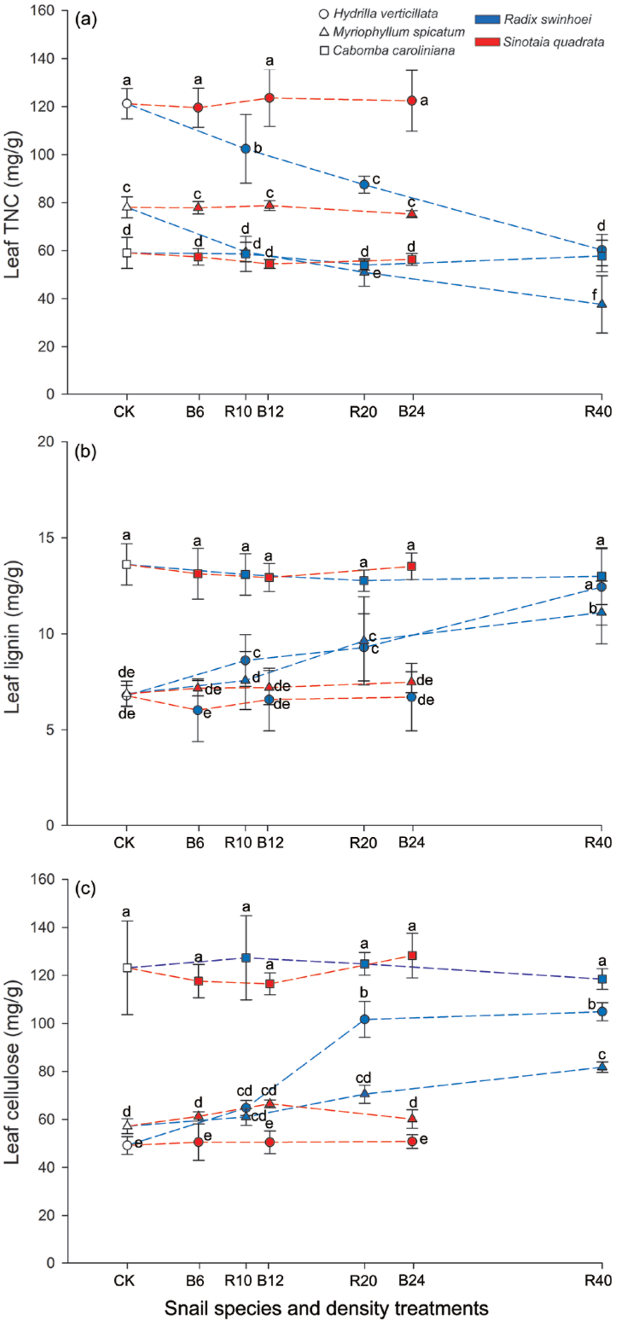

Figure 4: The (**a**) leaf TNC, (**b**) leaf lignin and (**c**) leaf cellulose content of *Hydrilla verticillata*, *Myriophyllum spicatum* and *Cabomba caroliniana* in the herbivory experiment. Open symbol: control (CK) groups; filled symbol: snail treatment groups. The values are means ± SE (n = 5). Different lowercase letters indicate significant differences between treatments (P = 0.05).

snail, *Lymnaea stagnalis*, fed mainly on filamentous algae that had indirectly positive effects on submerged plants, which contradicts our study. The reason may be because of the snail distinction between the two studies. As one of the dominant snail species in the downstream reaches of the Yangtze River Basin, previous studies have shown that *R. swinhoei* can scratch epiphytic algae, which promotes the growth of submerged plants, and it can directly graze submerged plants, especially when the density of this snail species is high and other foods are inadequate or unpalatable (Cao *et al.* 2014; Li *et al.* 2009; Mormul *et al.* 2018). In this study, we found that this snail did not have significant effects on *C. caroliniana*. This result is consistent with the results from a previous comparative study, which shows that native plants are more palatable to *R. swinhoei* than introduced plants that grow in Liangzi Lake in the midstream reaches of the Yangtze River (Xiong *et al.* 2008). A previous study also showed that *R. swinhoei* has a low preference for *M. spicatum* with high tannin content (Li *et al.* 2004). A relatively low leaf TNC content and high lignin and cellulose (both are low nutritive structural compounds of plant cell walls) contents indicate that a plant has low digestibility and palatability (Baraza *et al.* 2007; Lambers and Oliveira 2019), which may explain why *C. caroliniana* was rarely grazed by snails when the three plant species grew together in this study.

Herbivorous snails have a regulatory effect on the growth of submerged plants, which may affect plant community structure

A meta-analysis of 123 native animal exclusion experiments in natural terrestrial ecosystems showed that herbivores significantly reduced plant abundance, biomass, survival and reproduction, which indicates that herbivores are essential in regulating the production and diversity of plant communities (Jia *et al.* 2018). Ali and Soltan (2006) discovered that snails had an inhibitory effect on the abundance of *M. spicatum*; we also observed a similar tendency in this plant in this study. In freshwater ecosystems, meta-analysis results demonstrated that biotic resistance was driven by consumption rather than competition (Alofs and Jackson 2014). A previous study showed that *C. caroliniana* did not perform better or have greater competitive ability than native submerged plants (Huang *et al.* 2021). In our study, we demonstrated that herbivorous snails have an inhibitory effect on the growth of the native plants *H. verticillata* and *M. spicatum* but a neutral effect on the invasive plant *C. caroliniana*, which may affect the community structure of submerged plants. *Hydrilla verticillata* showed relatively low resistance to herbivory by the native snails in this study. However, once it is introduced outside China, it may become invasive if it can escape herbivorous grazing by native Chinese snails. Further studies consisting of common-garden conditions in both native and invading areas of native and invasive species are essential.

Submerged plants have a central role in maintaining or restoring the clear water state of shallow lakes (Liu *et al.* 2018b). With the rapid increase in the economy and population in the LTB, habitat degradation has been extensively recognized (Zhang *et al.* 2018). We face a paradox in these severe environmental problems that make it unsuitable to survive for native aquatic plants, and only introduced invasive plants will grow. *Cabomba caroliniana* has been successfully used to reduce or slow the transformation of a clear water state to a turbid water state, but its potential invasiveness may be far-reaching. Furthermore, the effect of herbivorous snails on the species abundance and composition of native and introduced submerged plants remains to be investigated.

CONCLUSIONS

We conducted a mesocosm experiment to record the responses of the invasive submerged plant species *C. caroliniana* and two normally co-occurring native counterparts, *H. verticillata* and *M. spicatum*, to herbivory by two widespread native snails. The results demonstrated that the snail *S. quadrata* did not have a noticeable herbivory effect on any of the three plant species. The snail *R. swinhoei* has inhibitory effects on *H. verticillata* and *M. spicatum* but a neutral effect on *C. caroliniana*, facilitating the successful invasion of *C. caroliniana* and may affect the plant community structure. Our findings indicate that herbivorous snail species are contributing to the invasion of *C. caroliniana*. However, it is not certain that the successful invasion of *C. caroliniana* is caused by its release from its original enemies in the introduced habitats. Our study provides direct evidence that invasive plants are more resistant than native plants to herbivory by generalist herbivores in freshwater ecosystems.

Funding

This work was supported by the National Science Foundation of China (NSFC) (31930074, 41877415 and 32001157), the Science and Technology Service

Network Initiative (KFJ-STS-QYZD-156) of the Chinese Academy of Sciences (CAS), and the Project of the Young Scientist Group (2021NIGLAS-CJH01) of Nanjing Institute of Geography and Limnology (NIGLAS), CAS.

Conflict of interest statement. The authors declare that they have no conflict of interest.

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