ISSN: 1412-4718, eISSN: 2581-138x

Yudistira DH · Gunawan FA · Arif SA · Sato S

Role and key species of freshwater snails in enhancing plant growth performance of duckweeds (*Spirodela polyrhiza*) in rice paddy fields

Abstract. Freshwater snails are widely distributed in the rice field ecosystem. Freshwater snails' feeding and metabolic activities can potentially increase the complexity of freshwater ecosystems, particularly through the nutrient cycle. *Cipangopaludina chinensis*, *Physa acuta,* and *Semisulcospira libertina* are commonly found in Japan's paddy field ecosystems. *Spirodela polyrhiza* is expected to support biodiversity, improve soil fertility, and reduce greenhouse gas emissions in paddy field ecosystems, while providing food and shelter for the snails. In this study, we investigated the effects of these three snail species on the growth of *Spirodela polyrhiza*. The results showed that the presence of *P. acuta* or *C. chinensis* could promote the growth of *S. polyrhiza* compared with the presence of *S. libertina*, a mix, and the control. The relative growth rate for *S. polyrhiza* in the presence of *P. acuta*, *C. chinensis* + *P. acuta,* and *C. chinesis* was 1.10 ± 0.39, 1.10 ± 0.39, and 1.06 ± 0.31 fronds/day, respectively. In the *S. libertina* treatment, the number of *S. polyrhiza* fronds decreased as the snails consumed the plants. The number of fronds on the last day of treatment was 1.10 ± 1.10 fronds with a relative growth rate of 0.004 ± 0.030 fronds/day, whereas for the combination of *C. chinensis* + *S. polyrhiza* it was 4.80 ± 3.32 and 8.70 ± 3.61 fronds, respectively, with relative growth rate 0.13 ± 0.05 and 0.275 ± 0.06 fronds/day, respectively. Interestingly, these interspecific interactions increased *S. polyrhiza* performance, as indicated by greater frond length, root length, and frond chlorophyll content. This study highlights that interspecific interactions create complexity in the paddy field ecosystem, providing good conditions for biodiversity and indirectly supporting rice production.

Keywords: Biodiversity · Duckweed · Ecosystem engineering · Natural farming · Nutrient cycling

Submitted: 22 January 2025, Accepted: 18 March 2025, Published: 30 April 2025
DOI: https://doi.org/10.24198/kultivasi.v24i1.61084

Yudistira DH[1]* · Gunawan FA[2] · Arif SA[2] · Sato S[3]
[1] United Graduate School of Agricultural Science, Iwate University, Iwate Prefecture, Japan
[2] Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Indonesia
[3] Faculty of Agriculture, Yamagata University, Yamagata Prefecture, Japan
*Correspondence: aryayudistira1809@gmail.com

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Freshwater snails are known to graze on macrophytes in the aquatic ecosystem. This activity enhances ecosystem stability and promotes coexistence in shallow aquatic systems (Lv et al., 2022), while also releasing nutrients into the environment after snails consume macrophytes. The nutrients released by snails can be beneficial for macrophyte growth. According to Pinowska (2002), the average phosphorus and nitrogen release rate by *Lymnaea* (Galba) *turricula* were 24.20±11.70 µg PO_4-P and 48.90±29.60 µg NH_4-N g^{-1} snail FW d^{-1}. Studies have observed that the presence of grazing snails strongly reduced nitrogen uptake and biomass of algae (Koleszár et al., 2021). According to Koleszár et al. (2021), in the absence of grazing freshwater snails, the *Ceratophyllum*-epiphyton complex lowered nitrogen and phosphorus concentrations in the medium more rapidly and exhibited higher pH levels compared to when snails were present. The results of research conducted by Jo et al. (2020) showed that over six years, using freshwater snails increases soil nutrients such as available nitrogen (N) and available phosphorus (P) compared to soil without freshwater snails. Additionally, applying freshwater snails increased soil pH in sodic soils. Also, these freshwater snails can serve as a biocontrol agent for reducing sodium adsorption, improving soil physical properties, and increasing infiltration rate (Jo & Pak, 2019). This finding is important for the development of organic farming with freshwater snails. From this perspective, freshwater snails are not harmful to the ecosystem. However, only a few studies have addressed the importance of interactions between freshwater snails in the ecosystem. These interactions can be divided into interspecific or intraspecific. Such interactions increase ecosystem diversity and can enhance ecosystem services such as nutrient cycling and decomposition of organic matter (Wilson et al., 2008).

Interactions occur not only between freshwater snail species but also with aquatic plants. In the rice field ecosystem, aquatic plants can play both beneficial and detrimental roles, since some species may become invasive or act as weeds (Bablee et al., 2024). Studies have shown that the relationship between freshwater snails and aquatic plants can be mutualistic. *Radix swinhoei* can increase the growth of submerged macrophytes by consuming epiphytes from the plant surface. As a result, not only do the plants grow better, but the biomass of freshwater snails also increases (Zhi et al., 2020). In this study, three species of freshwater snails were selected to examine the interspecific interaction on the growth performance of *S. polyrhiza* as a model plant. Duckweed, *S. polyrhiza*, is widely distributed in various freshwater ecosystems, mainly in rice field, and has several important roles, such as reducing the greenhouse effect of subtropical paddy fields (Wang et al., 2015), increasing yield as green manure (Yao et al., 2017), and increasing biodiversity (Fahmi et al., 2021). Additionally, duckweed provides favorable conditions for freshwater snails as shelters or food resources (Nagai et al., 1979). Certain species of freshwater snails commonly found in rice fields, such as *Cipangopaludina chinensis* and *Semisulcospira libertina* are native to Japan, and *Physa acuta* is an invasive species. All three species are widely distributed throughout Japan.

Some researchers have noted that *C. chinensis* plays a positive role in paddy plants and exerts indirect effects on the abundance of organisms in the terrestrial ecosystem (Dewi et al., 2017). Additionally, *C. chinensis* can affect the abundance of terrestrial organisms and may influence microbial communities directly or indirectly (Olden et al., 2013). In organic farming, the use of freshwater snails like *C. chinensis*, can play a significant role in enhancing nutrient cycling (Ernest et al., 2024; Kurniawan et al., 2018) and fostering ecosystem balance (Dewi et al., 2017; Kurniawan et al., 2018). Their ability to interact with aquatic plants, decompose organic matter, and regulate nutrient availability makes them valuable contributors to sustainable agricultural practices (Panteleit et al., 2018). For example, the grazing activity of these snails can help control unwanted aquatic weeds while simultaneously releasing nutrients that promote plant growth (Jong-Song et al., 2018). Additionally, nutrients excreted by these snails have been observed to increase rice plant performance (Kurniawan et al., 2018).

Based on these considerations, we hypothesize that *C. chinensis* is a key species in paddy field. The snail used in this experiment included *C. chinensis* because it is a native snail originating from various regions in Japanalong with *S. libertina*. In addition to these two snails, this experiment used *P. acuta* as an invasive freshwater snail in Japan. These three freshwater snails are not categorized as pests because they do not cause severe damage.

Materials and Methods

Preparation of Plants. Duckweeds were collected from a rice field in the Takasaka area of Tsuruoka, Yamagata Prefecture, Japan. After collection, the duckweeds were separated based on their species before being brought to the Applied Zoology Laboratory, Yamagata University. The experiment used *Spirodela polyrhiza* as the main duckweed species. Duckweeds were kept in 3-liter boxes filled with paddy soil and tap water without any additional nutrients. These boxes were set up in a rearing room within the laboratory, providing controlled environmental conditions for growth before they were used in experiments.

Collection of Animals. In this experiment, *C. chinensis*, *S. libertine*, and *P. acuta* were used. The first two are native snails originating from various regions in Japan, while *P. actuta* is an invasive freshwater snail in Japan. These three species were collected from the Yutagawa area, Tsuruoka, Yamagata Prefecture, Japan. Snails were maintained in 3.0 L boxes, and each species was put into a separate box. The boxes were filled with paddy field soil for snails to feed on. Snails were reared for one week before they were used in the experiment, allowing them to adapt to the laboratory environment.

Experimental Mesocosm Setup. The mesocosm experiment was conducted using a 535 mL plastic cup filled with tap water and 100 g of paddy soil. The paddy soil was collected from paddy fields at Yamagata University, Takasaka, Tsuruoka City, then dried and sieved to remove debris before use. The tap water was aged for 24 hours to reduce chromium levels before being added to the mesocosm.

After adding the water, a single frond of duckweed was introduced into each mesocosm and allowed to acclimate for 24 hours. Following this acclimation period, one individual snail from the designated species was introduced into each mesocosm. The setup was then placed in an incubator (Sanyo MIR 253) set to a temperature of 25 ± 1°C and humidity of 75%. The light cycle was controlled at 12 hours light : 12 hours dark (L:D 12:12 h) to simulate natural conditions. This experiment included four different treatments, with each treatment replicated ten times (Table 1).

Table 1. Design of treatment

Treatment (Code)	Description	Replication
C. chinensis (CC)	*C. chinensis* (width: 20.27±0.59cm) + *S. polyrhiza*	10
P. acuta (PA)	*P. acuta* (width: 1.02±0.02 cm) + *S. polyrhiza*	10
S. libertina (SL)	*S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
C. chinensis + *P. Acuta* (CCPA)	*C. chinensis* (width: 20.27±0.59cm) + *P. acuta* (width: 1.02±0.02 cm) + *S. polyrhiza*	10
S. Libertina + *C. chinensis* (SLCC)	*C. chinensis* (width: 20.27±0.59cm) + *S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
P. acuta + *S. Libertina* (PASL)	*P. acuta* (width: 1.02±0.02 cm) + *S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
Control (C)	*S. polyrhiza*	10

Data Collection and Statistical Analysis. The number of duckweed fronds was counted every four days using a ruler. Every week, water pH data were recorded using a handheld Meyer (Marfied Eco pH Device). At the end of the treatment period, we measured biometric parameters of duckweeds, including root length, frond length, and fronds chlorophyll, using a handheld SPAD meter (Konica Minolta).

The relative growth rate (RGR) of *S. polyrhiza* was calculated using the formula:

$$RGR = (Xt - Xo)/dt$$

where Xt and Xo are the final and initial frond numbers, respectively, and dt is the duration of observation (days). To assess significant differences in duckweed growth, relative growth rate, pH, biometrics parameters, and freshwater snail survival, all data were tested for normality by the Shapiro-Wilk test and for homogeneity of variance by Bartlett's test. Biometric data were analysed using a one-way ANOVA followed by Tukey's HSD post-hoc test. Duckweed growth, RGR, pH, and snail survival data were analyzed with a Kruskal-Wallis test followed by the Kruskal-Nemenyi post-hoc test. All statistical analyses were performed in R (Version 4.0.3).

Results and Discussion

Effect of Freshwater Snail Interspecific Interaction on Plant Growth Performance. Over the past several decades, duckweed species have been studied for applications such as animal feed (Cruz-Velásquez et al., 2014; Mwale & Gwaze, 2013; Sońta et al., 2018), green manure for rice plants (Yao et al., 2017), and wastewater treatments (Chen et al., 2018; James, 2016). The growth of *S. polyrhiza* can be influenced by various factors such as nutrients in the aquatic environment, temperature, light intensity, and plant-animal interactions in the ecosystem (Fahmi et al., 2021; Jin et al., 2021; Strzałek & Kufel, 2021). One interesting finding is that the presence of freshwater snails and interspecific interaction between species can promote the growth of *S. polyrhiza*.

The results showed that *S. polyrhiza* grew well in the presence of *C. chinensis*, *P. acuta,* and their interaction (X^2=39.06, df=6, P > 0.05). In Figure 1, the *P. acuta* treatment had a growth rate of 1.28 fronds per day, followed by the interspecific combination *P. acuta* and *C. chinensis,* which showed a growth rate of 1.27, and then *C. chinensis* alone at 1.24 fronds per day. These three treatments were significantly different from the control (X^2=39.063, df=6, P < 0.05). These findings suggest that both freshwater snail species can enhance nutrient availability, consistent with several reports indicating that freshwater releases nutrients into the water, thereby promoting plant growth. Previous research showed that the presence of *B. aeruginosa* affected TN and TP content in the water (Mo et al., 2017). Some research also suggested *B. aeruginosa* could promote biomass and influence C, N, and P stoichiometry of submerged macrophytes like *Vallisneria natans* and *Hydrilla verticillata* (Li et al., 2019). According to Li et al. (2009), *Radix swinhoei* can release nutrients at a rate of approximately 0.66 µg NH_4^-N and 49.77 x 10-4µg PO_4^-Pmg−1 d−1 after consumption submerged plant. Adults of *Lymnaea turricula* can release 29.5µg NH_4^-N and 18.9µg PO_4^-Pmg^{-1} d^{-1} even without consuming any plant material (Pinowska, 2002). Taken together, previous literature shows that nutrient release rates can differ among species and their activities. However, our study emphasizes the snail's effect on plant growth.

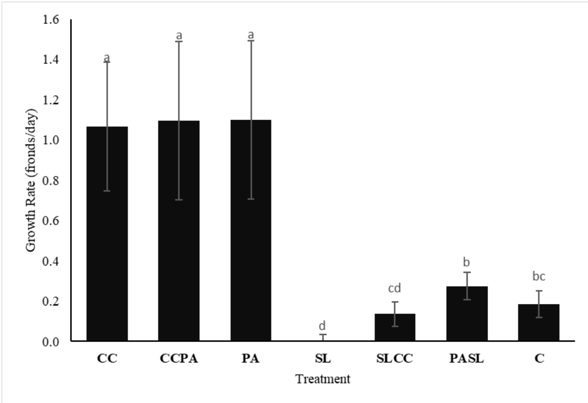

Figure 1. Relative growth rate of *S. polyrhiza* plant. CC= *C. chinensis*, CCPA= *C. chinensis* + *P. acuta*, PA= *P. acuta*, SL= *S. libertina*, SLCC= *S. libertina* + *C. chinensis*, PASL = *S. libertina* + *P. acuta*, C= control. Bars represent means ± standard errors. Different letters above bars indicate significant differences (P < 0.05) according to the Kruskal-Wallis test followed by the Kruskal-Nemenyi post-hoc test

The treatment with *S. libertina* showed the lowest mean frond count, reaching day 28 at 1.10 ± 1.10, which was significantly different from the control (X^2=39.063, df=6, P < 0.05). As shown in Table 2, there is a clear trend of decreasing fronds in *S. libertina* treatment compared to the mixture of *S. libertina* with *C. chinensis* or *P. acuta*. Interestingly, all treatments involving *P. acuta* and *C. chinensis* appeared to increase the number of fronds. The mean fronds when *S. libertina* was mixed with *P. acuta* were 8.70 ± 0.61, and when mixed with *C. chinensis* it reached 4.80 ± 3.32. These finding suggest that even a single individual snail can promote *S. polyrhiza* growth, and varying snail densities may not significantly affect its growth rate. This observation aligns with Li et al. (2019), who reported that different snail densities did not significantly influence the growth of *E. nuttallii* under low-nutrient conditions. According to these results, freshwater snail species could be a key factor in *S. polyrhiza* growth.

The growth of *S. polyrhiza has* been shown to benefit the performance of rice plants. When *S. polyrhiza* is present, it can reduce the density of weeds by 97% and increase yield by 23% (Wang et al., 2022). An abundance of duckweed during the early stage of rice plant growth can suppress weeds through competition for nutrients and light, as duckweed floats on the water surface (Yao et al., 2017). Moreover, high duckweed coverage decreased the pH value and water temperature while enhancing the SPAD value of rice plants (Jing et al., 2024). The rapid growth of duckweed can also be facilitated by the presence of snails (Ernest et al., 2024). Indeed, Ernest et al. (2024) found that the duckweed numbers increased more in the presence of snails compared to treatments without snails over a two-year experiment.

The treatment with *S. libertina* resulted in the lowest average frond count, with a mean of 1.10 ± 1.10 on day 28, which was significantly different from the control (X^2 = 39.063, df = 6, $P < 0.05$). The duckweed was consumed by *S. libertina* during the experiment. However, there is still a lack of information regarding the ability of *S. libertina* to consume duckweed. As shown in Table 2, there is a clear trend of decreasing frond numbers in the *S. libertina* treatment compared to treatments where *S. libertina* was mixed with *C. chinensis* and *P. acuta*. Interestingly, all treatments involving *P. acuta* and *C. chinensis* showed a potential increase in frond numbers. The mean frond counts for *S. libertina* mixed with *P. acuta* were 8.70 ± 0.61, while the mixture with *C. chinensis* resulted in a mean frond count of 4.80 ± 3.32.

Overall, our study indicates that the presence of certain freshwater snail species can be a key factor in promoting *S. polyrhiza* growth. These findings are consistent with Li et al. (2019), who found that different varying snail densities did not significantly affect *E. nuttallii* growth in low-nutrient environments, suggesting that snail-mediated effects are not strictly density dependent.

After 28 days of the growth experiment, we measured various plant biometric parameters. The results, presented in Table 3, show that *S. libertina* increased frond surface area compared to the control, though this difference was not statistically significant. Figure 2 shows that the number of fronds in the single-species *S. libertina* treatment was almost the same as control, while *C. chinensis* and *P. acuta* treatment showed a higher abundance of fronds. However, there were no significant effects on root length or chlorophyll content, which contrasts somewhat with the earlier observations of *S. libertina* reducing frond numbers. The combination of *C. chinensis* and *P. acuta* resulted in the longest fronds, with an average length of 0.60 ± 0.04 cm, nearly double that of the control. In terms of root length and frond chlorophyll content, *P. acuta* showed the highest average, followed closely by *C. chinensis*. The excretion of nitrogen and phosphorus by snails is known to enhance plant biomass (Edgar et al., 2022). Due to the rapid growth of the plants, intraspecific competition increased, leading to longer root growth (Jin et al., 2021). In this experiment, the combination of *C. chinensis* and *P. acuta* also promoted rapid biomass accumulation, likely due to phosphorus contributions, as noted by Yang et al. (2020). Furthermore, Jong-Song et al. (2018) found that saline rice fields supplemented with freshwater snails and half the usual fertilizer application produced yields comparable to those achieved with conventional chemical fertilizers. These findings suggest that freshwater snails can enhance plant growth even in challenging environments.

Table 2. Effect of freshwater snail presence on *S. polyrhiza* fronds growth

Treatment	Number of Fronds (fronds/day)						
	4	8	12	16	20	24	28
CC	1.70±0.21 a	2.70±0.67 ab	4.80±1.27 ab	7.90±2.62 a	16.00±5.73 a	22.00±7.40 a	30.90±5.41 a
CCPA	1.60±0.22 ab	2.10±0.38 ab	3.40±0.67 ab	6.30±1.81 a	14.50±5.21 a	20.60±6.35 a	31.70±5.50 a
PA	1.50±0.27 abcd	2.70±0.50 ab	4.90±1.20 ab	7.30±2.15 a	13.60±3.67 a	19.40±3.86 a	31.80±5.42 a
SL	0.80±0.20 d	0.80±0.29 c	0.30±0.30 d	0.30±0.30 c	0.20±0.20 d	0.60±0.60 d	1.10±1.10 d
SLCC	1.00±0.26 bcd	1.10±0.55 c	1.30±0.60 cd	1.70±0.70 bc	2.60±1.71 cd	3.00±2.05 cd	4.80±3.32 cd
SLPA	1.40±0.27 abc	3.70±0.63 a	5.00±0.87 a	6.40±1.28 a	7.00±1.82 ab	7.30±2.25 b	8.70±0.61 b
C	1.00±0.00 cd	1.50±0.17 bc	2.20±0.50 bc	2.60±1.28 b	2.80±0.29 bc	4.40±0.48 bc	6.20±0.89 bc

Note: Values are shown as means (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences according to the Kruskal-Wallis test (P < 0.05), followed by the Kruskal-Nemenyi post-hoc test.

Effect of Interspecific Interaction on Water pH. According to the data, the range of pH values in treatments with freshwater snail ranged between 5.32 and 5.82 (Table 5). This range seems not to have affected the growth of *S. polyrhiza*. However, interactions among *P. acuta* and *S. libertina* produced the lowest average pH value, whereas the presence of *C. chinensis* alone or in combination was associated with higher pH.

The observed pH conditions did not affect *S. polyrhiza* growth, as this species can grow in a wide pH range (3.0-10.0) (Mclay, 1976). Other researchers have noted that pH 6-8 does not influence duckweed growth but can increase the total ammonia (Caicedo et al., 2000). The average water pH may also influence freshwater snails. Chiu et al. (2002) found that *C. chinensis* survives in water pH ranging from 4.0 to 9.0, but its shell size is affected by pH. Cretini and Galloway (2024) reported that lower water pH conditions reduce shell size. Under acidic conditions, freshwater snails showed minimal shell length growth and increased shell erosion. Water pH condition is influencing the availability of calcium, which is crucial for shell formation. Lower water pH conditions can reduce calcium uptake, further inhibiting shell growth and strength (Glass & Darby, 2009; Grosell & Brix, 2009).

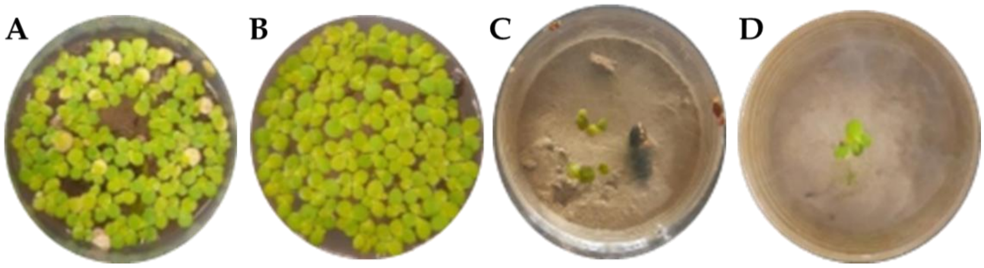

Figure 2. Effect of different freshwater snail species on the growth of *S. polyrhiza*. (A) *C. Chinensis*, (B) *P. Acuta*, (C) *S. Libertina*, and (D) Control

Table 3. Effect of freshwater snail presence on the final plant biomass of *S. polyrhiza*

Treatment	Biomass		
	Fronds Length (cm)	Root Length (cm)	Chlorophyll
CC	0.51±0.02 b	2.58±0.09 b	24.34±0.91 bc
CCPA	0.60±0.04 a	2.34±0.09 b	27.08±1.39 a
PA	0.46±0.02 bc	3.52±0.08 a	27.44±1.28 a
SL	0.42±0.02 bc	1.07±0.24 c	22.46±1.16 bc
SLCC	0.53±0.04 b	1.35±0.07 c	22.28±1.77 bc
SLPA	0.51±0.03 b	1.52±0.16 c	27.16±1.54 a
C	0.38±0.02 c	1.16±0.23 c	19.01±1.34 c

Note: Values are presented as mean (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences (P<0.05) according to Tukey's post-hoc test.

Table 4. Effects of Interspecific Interaction on Water pH

Treatment	Water pH (Week-)			
	1	2	3	4
CC	6.29±0.16 ab	5.95±0.08 ab	6.06±0.14 ab	5.82±0.09 ab
CCPA	6.13±0.12 ab	5.78±0.05 c	5.82±0.15 b	5.65±0.06 bc
PA	6.20±0.11 ab	5.57±0.07 d	5.50±0.05 c	5.48±0.05 cd
SL	6.41±0.18 a	6.01±0.06 a	6.04±0.08 ab	6.12±0.18 a
SLCC	6.24±0.10 b	5.93±0.04 ab	6.14±0.10 a	6.09±0.19 ab
SLPA	5.50±0.11 c	5.07±0.06 e	5.59±0.08 c	5.32±0.06 d
C	6.18±0.03 ab	5.87±0.02 bc	5.82±0.05b	5.45±0.06 d

Note: Values are presented as mean (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences according to Kruskal-Wallis test (P < 0.05), followed by Kruskal-Nemenyi post-hoc test.

Freshwater Snail Survivorship and Interaction. Interspecific interactions play a key role in snail survival. The data show that the mortality of *S. libertina* alone in single-species treatments was six times higher than in the *C. chinensis* + *P. acuta* treatment. The percentage of mortality for *S. libertina* was 60%, but decreased when *P. acuta* was present. *Physa acuta* survived the experiment in all interspecific treatments. As shown in Table 2, *S. libertina* alone consumed *S. polyrhiza* fronds, leaving only 1.10 ± 1.10 fronds and exhibiting higher mortality, whereas *S. libertina* mixed with *C. chinensis* or *P. acuta* supported greater frond number, 4.80 ± 3.32 and 8.70 ± 0.61, respectively, and lower snail mortality. When *S. libertina* died under conditions where shell-attached algae grew rapidly in the mesocosm, it also inhibited the growth of *S. polyrhiza*.

Table 5. Freshwater snail mortality (%)

Species	Mortality Percentage (%)		
	C. chinensis	***P. acuta***	***S. libertina***
C. chinensis	10.00±10.00 bc	10.00±10.00 bc	20.00±13.33 bc
P. acuta	0.00±0.00 c	10.00±10.00 bc	0.00±0.00 c
S.libertina	0.00±0.00 c	30.00±15.28 b	60.00±16.33 a

Note: Values are presented as means (± standard error). Different letters within the the same row indicate significant differences (P<0.05) according to Tukey's post-hoc test

The increased mortality rate was likely caused by algae attached to the *S. libertina* shell and by the still water condition within mesocosms. The presence of other freshwater snails, such as *P. acuta*, could help reduce *S. libertina* mortality by half relative to the single-species treatment (Table 4), as it consumed algae attached to the *S. libertina* shell. Meanwhile, the presence of *C. chinensis* was important for *S. libertina* survival, as *C. chinensis* harbored shell-attached algae that *S. libertina* could feed on, reflecting *S. libertina*'s phytophagous diet as a food resource compared with *P. acuta,* which did not contain algae on the shell. *Semisulcospira libertina* in nature is a phytophagous diet (Antonio et al., 2010). Additionally, *C. chinensis* could help the growth of *S. libertina*'s shell by grazing on it. Algae rich in omega-6 fatty acids, commonly found in shell-attached biofilms, were a food source for *C. chinensis* after it fed on another snail's shell (Fujibayashi et al., 2016). This experiment revealed two key interactions among freshwater snail species and their effects on plant growth.

Conclusion

The presence of *P. acuta* resulted in the highest growth rate of *S. polyrhiza* (1.28 fronds/day), followed by the combination of *P. acuta* and *C. chinensis* (1.27 fronds/day). While *S. libertina* caused the lowest growth rate (1.1 fronds/day) due to high mortality and algae accumulation on shells, the presence of *C. chinensis* also positively influenced growth (1.24 fronds/day). The combination of *C. chinensis* and *P. acuta* achieved the best results for frond length (0.60 cm), while *P. acuta* alone had the highest root length (3.52 cm) and chlorophyll content (27.44). Treatments with *S. libertina* in combination with other snails improved survival and mitigated algae growth. Water pH was slightly affected but remained within a range suitable for *S. polyrhiza* growth.

Acknowledgments

We express our gratitude to the Agriculture Ecology Laboratory, Faculty of Agriculture, Yamagata University, for providing moral and material support throughout the research process.

References

Antonio ES, Kasai A, Ueno M, Kurikawa Y, Tsuchiya K, Toyohara H, Ishihi Y, Yokoyama H, Yamashita Y. 2010. Consumption of terrestrial organic matter by estuarine molluscs determined by analysis of their stable isotopes and cellulase activity. Estuarine, Coastal and Shelf Science, 86(3): 401–407. https://doi.org/10.1016/j.ecss.2009.05.010

Bablee AL, Bashar A, Alam MM, Hasan NA, Haque MM, Hansen LH, Jørgensen NOG. 2024. Identification of aquatic plant species suitable for growing in integrated multi-trophic aquaculture systems in Southwest Bangladesh. Sustainability, 16(24): 11113. https://doi.org/10.3390/su162411113

Caicedo JR, Van Der Steen NP, Arce O, Gijzen HJ. 2000. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Water Research, 34(15): 3829–3835. https://doi.org/10.1016/S0043-1354(00)00128-7

Chen G, Fang Y, Huang J, Zhao Y, Li Q, Lai F, Xu Y, Tian X, He K, Jin Y, Tan L, Zhao H. 2018. Duckweed systems for eutrophic water purification through converting wastewater nutrients to high-starch biomass: Comparative evaluation of three different genera (*Spirodela polyrhiza*, *Lemna minor* and *Landoltia punctata*) in monoculture or polyculture. RSC Advances, 8(32): 17927–17937. https://doi.org/10.1039/c8ra01856a

Chiu YW, Chen HC, Lee SC, Chen CA. 2002. Morphometric analysis of shell and operculum variations in the viviparid snail, Cipangopaludina chinensis (Mollusca: Gastropoda), in Taiwan. Zoological Studies-Taipei-, 41(3): 321-331.

Cretini, CP, Galloway KA. 2024. Acidic Apple Snails: Effects of climate change on the mechanical properties of an invasive gastropod. Integrative and Comparative Biology, 64(2): 270–278. https://doi.org/10.1093/icb/icae013

Cruz-Velásquez Y, Kijora C, Vergara-Hernández W, Schulz C. 2014. Effect of fermented aquatic macrophytes supplementation on growth performance, feed efficiency and digestibility of Nile Tilapia (*Oreochromis niloticus*) juveniles fed low fishmeal diets. Livestock Research for Rural Development, 27(9): 1-8.

Dewi VK, Sato S, Yasuda H. 2017. Effects of a mud snail *Cipangopaludina chinensis laeta* (Architaenioglossa: Viviparidae) on the abundance of terrestrial arthropods through rice plant development in a paddy field. Applied Entomology and Zoology, 52(1): 97–106. https://doi.org/10.1007/s13355-016-0458-8

Edgar MR, Hanington PC, Lu R, Proctor H, Zurawell R, Kimmel N, Poesch MS. 2022. The first documented occurrence and life history characteristics of the chinese mystery snail, *Cipangopaludina chinensis* (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada. Bioinvasions Records, 11(2): 449-460. https://doi.org/10.3391/bir.2022.11.2.18

Ernest NM, Yudistira DH, Eware MS, Sato S. 2024. The effects of the density of the mud snail *Cipangopaludina chinensis* (Martens)(Architaenioglossa: Viviparidae) on the development of rice plant. Asian Journal of Agriculture, 8(2): 79-87. https://10.13057/asianjagric/g080201

Fahmi IZ, Putra NS, Indarti S, Sato S. 2021. Preference of the waterlily aphid, *Rhopalosiphum nymphaeae* (Hemiptera: Aphididae) on four hostplants. Jurnal Perlindungan Tanaman Indonesia, 25(1): 21-27.

Fujibayashi M, Sakamaki T, Shin W, Nishimura O. 2016. Food utilization of shell-attached algae contributes to the growth of host mud snail, *Bellamya chinensis*: Evidence from fatty acid biomarkers and carbon stable isotope analysis. Limnologica, 57: 66–72. https://doi.org/10.1016/j.limno.2015.11.006

Glass N, Darby P. 2009. The effect of calcium and pH on Florida apple snail, *Pomacea paludosa* (Gastropoda: Ampullariidae), shell growth and crush weight. Aquatic Ecology, 43: 1085-1093. https://doi.org/10.1007/s10452-008-9226-3

Grosell M, Brix K. 2009. High net calcium uptake explains the hypersensitivity of the freshwater pulmonate snail, *Lymnaea stagnalis*, to chronic lead exposure. Aquatic toxicology, 91(4): 302-11. https://doi.org/10.1016/j.aquatox.2008.10.012

Jin Y, Zhang Q, Zhang L, Lei N, Chen J, Xue W, Yu F. 2021. Distinct responses of frond and root to increasing nutrient availability in a floating clonal plant. PLOS ONE, 16(10): 1-12. https://doi.org/10.1371/journal.pone.0258253

Jing L, Wang X, Zhao Y, Li F, Su Y, Cai Y, Zhao F, Dong G, Yang L, Wang Y. 2024. Impact of duckweed (*Lemna minor* L.) growing in paddy fields on rice yield and its underlying causes. *Agronomy*, 14(4): 726.

Jong-Song J, Song-Ho P, Song-Ok C. 2018. Possibility and effects of using Ampullaria tischbeini (Dohrn) snail in saline paddy field. Organic Agriculture, 8(4): 335-347.

Jo JS, Pak S. 2019. The use of the freshwater snail *Ampullaria tischbeini* (Dohrn) as a biological control agent for remediation of salt-affected soil. Archives of Agronomy and Soil Science, 65(12): 1677 - 1687.

https://doi.org/10.1080/03650340.2019.1575508

Jo JS, Pak SH, Ri CH, Cha SO, Kim GR, Ri CI, Kim IS. 2020. Changes in soil properties for 6 years of using the freshwater snail (*Ampullaria tischbeini Dohrn*) in a reclaimed sodic soil. Journal of Soils and Sediments, 20(2): 1018–1025. https://doi.org/10.1007/s11368-019-02472-7

Koleszár G, Nagy Z, Peeters ETHM, Borics G, Várbíró G, Birk S, Szabó S. 2021. The role of epiphytic algae and grazing snails in stable states of submerged and of free-floating plants. Ecosystems, 25: 1371-1383. https://doi.org/10.1007/s10021-021-00721-w

Kurniawan AH, Sato S, Yasuda, H. 2018. Effects of ambient temperature and the mud snail Cipangopaludina chinensis laeta (Architaenioglossa: Viviparidae) on performance of rice plants. *Applied entomology and zoology*, *53*, pp.137-141.

Li KY, Liu ZW, Hu YH, Yang HW. 2009. Snail herbivory on submerged macrophytes and nutrient release: Implications for macrophyte management. Ecological Engineering, 35(11): 1664–1667. https://doi.org/10.1016/j.ecoleng.2008.05.009

Li W, Fu H, Li Y, Nie W, Yuan G, Gao G, Fan H, Zhong J. 2019. Effects of nutrient enrichment and *Bellamya aeruginosa* (Reeve) presence on three submerged macrophytes. Hydrobiologia, 833(1): 95–105. https://doi.org/10.1007/s10750-019-3884-0

Lv T, Guan X, Fan S, Han C, Gao Z, Liu C. 2022. Snail communities increase submerged macrophyte growth by grazing epiphytic algae and phytoplankton in a mesocosm experiment. Ecology and Evolution, 12(2): 1-12. https://doi.org/10.1002/ece3.8615

Mclay CL. 1976. The effect of pH on the population growth of three species of duckweed: *Spirodela oligorrhiza*, *Lemna minor* and *Wolffia arrhiza*. Freshwater Biology, 6(2): 125–136. https://doi.org/10.1111/j.1365-2427.1976.tb01596.x

Mo S, Zhang X, Tang Y, Liu Z, Kettridge N. 2017. Effects of snails, submerged plants and their coexistence on eutrophication in aquatic ecosystems. Knowledge and Management of Aquatic Ecosystems, 418: 1-7 https://doi.org/10.1051/kmae/2017034

Mwale M, Gwaze FR. 2013. Characteristics of duckweed and its potential as feed source for chickens reared for meat production: A review. Scientific Research and Essays, 8(18): 689–697. https://doi.org/10.5897/SREX12.003

Nagai S, Yamamoto H, Ishii K, Otsuka M, Kobayashi M. 1979. Rearing and population growth of freshwater snail, *Semisulcospira libertina* in the Laboratory. Venus (Journal of the Malacological Society of Japan), 38(1): 25–34. https://www.jstage.jst.go.jp/article/venusjjm/38/1/38_KJ00004342764/_article

Olden JD, Ray L, Mims MC, Horner-Devine MC. 2013. Filtration rates of the non-native Chinese mystery snail (*Bellamya chinensis*) and potential impacts on microbial communities. Limnetica, 32(1): 107–120.

Panteleit J, Horgan FG, Türke M, Schmidt A, Schädler M, Bacht M, Brandl R, Hotes S. 2018. Effects of detritivorous invertebrates on the decomposition of rice straw: evidence from a microcosm experiment. Paddy and Water Environment, 16: 279-286.

Pinowska A. 2002. Effects of snail grazing and nutrient release on growth of the macrophytes *Ceratophyllum demersum* and *Elodea canadensis* and the filamentous green alga *Cladophora sp.* Hydrobiologia, 479(1991): 83–94. https://doi.org/10.1023/A:1021070616130

Sońta M, Rekiel A, Batorska M. 2018. Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture - A review. Annals of Animal Science, 19(2): 257–271. https://doi.org/10.2478/aoas-2018-0048

Strzałek M, Kufel L. 2021. Light intensity drives different growth strategies in two duckweed species: *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden. *PeerJ*, 9: 1-14. https://doi.org/10.7717/peerj.12698.

Wang C, Li S, Lai DYF, Wang W, Ma Y. 2015. The effect of floating vegetation on CH_4 and N_2O emissions from subtropical paddy fields in China. Paddy and Water Environment, 13: 425–431. https://doi.org/10.1007/s10333-014-0459-6

Wang F, Wang S, Xu S, Shen J, Cao L, Sha Z, Chu Q. 2022. A non-chemical weed control strategy, introducing duckweed into the paddy field. *Pest Management Science*, 78(8): 3654-3663.

Jurnal Kultivasi Vol. 24 (1) April 2025
ISSN: 1412-4718, eISSN: 2581-138x

Wilson AL, Watts RJ, Stevens MM. 2008. Effects of different management regimes on aquatic macroinvertebrate diversity in Australian rice fields. Ecological Research, 23(3): 565–572. https://doi.org/10.1007/s11284-007-0410-z

Yang Y, Zhang J, Liu L, Wang G, Chen M, Zhang Y, Tang X. 2020. Experimental study on phosphorus release from sediment with fresh-water snail (*Bellamya aeruginosa*) bioturbation in eutrophic lakes. J Soils Sediments, 20(5): 2526-2536. DOI: 10.1007/s11368-020-02614-2.

Yao Y, Zhang M, Tian Y, Zhao M, Zhang B, Zhao M, Zeng K, Yin B. 2017. Duckweed (*Spirodela polyrhiza*) as green manure for increasing yield and reducing nitrogen loss in rice production. Field Crops Research, 214: 273–282. https://doi.org/10.1016/j.fcr.2017.09.021

Zhi Y, Liu Y, Li W, Cao Y. 2020. Responses of four submerged macrophytes to freshwater snail density (*Radix swinhoe*i) under clear-water conditions: A mesocosm study. Ecology and Evolution, 10(14): 7644–7653. https://doi.org/10.1002/ece3.6489