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The effects of the density of the mud snail *Cipangopaludina chinensis* (Martens) (Architaenioglossa: Viviparidae) on the development of rice plant

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Abstract. *Ernest NM, Yudistira DH, Samy-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 J Agric 8: 79-87.* This study investigated the influence of different mud snails, *Cipangopaludina chinensis* (Martens) (Architaenioglossa: Viviparidae), and densities on rice plant growth in various environments. The numbers of snails per plant were 0, 2, 5, 7 and 0, 1, 4, 8, respectively. The best density was selected from the first experiment and used in a second investigation outside, which lasted until harvest. In the laboratory, 0 and 5 snails positively impacted the height, while 1, 4, and 8 snails boosted the growth and chlorophyll content. In the greenhouse, 2, 5, and 7 improved the height of rice plants; 0, 5, and 7 snails impacted the number of leaves, then 5 and 7 raised the chlorophyll content. Outside, 2, 5, and 7 snails increased plant height, whereas five snails improved the number of leaves, and 5 and 7 snails enhanced the number of tillers. Subsequently, a density of five snails with four rice seedlings per hill was applied based on the highest average performance criteria recorded across all three test conditions. In 2022, the snail treatment significantly impacted chlorophyll content, plant height, and slight weeding activity. Additionally, during 2023, snails increased the number of tillers, panicles, yields, and the abundance of duckweed and algae. The soil's carbon-nitrogen ratio levels had no significant difference between the two years. As for the survival of animals, notable variations were observed. The findings of this study suggested that fluctuations in the mud snail population benefited rice plant development, as did the occurrence of duckweed and algae, which depend on the surrounding conditions.

Keywords: Density, mud snail, rice plant performance, test conditions

INTRODUCTION

Chemical fertilizers containing nitrogen and phosphorous are used excessively to promote the growing demand for rice, resulting in environmental pollution (Kong et al. 2014; Drechsel et al. 2015). Among some listed adverse effects, the over-application of chemical fertilizer has increased soil deterioration and contamination (Amundson et al. 2015; Steffen et al. 2015). Furthermore, overusing chemical fertilizers and pesticides is a risk and a threat to sustainable agriculture (Byrareddy et al. 2019). In addition, agrochemical inputs negatively affect aquatic ecosystems and groundwater, causing eutrophication and biodiversity loss (Zhang et al. 2018; Albou et al. 2024). It has also been demonstrated that animals in rice fields are vulnerable to human activities like tillage chemicals and agricultural inputs (Ko et al. 2021; Choi et al. 2022). Although they are exposed to the destruction of their habitat and pollution, many aquatic and terrestrial animal species inhabit the rice fields, playing a crucial role in wetland ecosystems (Luo et al. 2014; Dinh et al. 2020). However, several studies support the protection and preservation of biodiversity in wetland rice production because ecological services can increase yields and lower the need for chemical inputs (Dermiyati and Ainin 2014; Propper et al. 2024). For example, it has been demonstrated that earthworms can improve soil fertility and consequently boost yield (Zhang et al. 2022a). Among the organisms that inhabit paddy fields, each group has functions like pollinators, natural enemies, and parasitoids for a good balance (Rahim et al. 2022). In aquaculture, the findings demonstrated that crayfish decrease methane and nitrous oxide emissions (Hu et al. 2022).

Mud snails, *Cipangopaludina chinensis* (J.E.Gray, 1833) or (*Bellamya chinensis*) are aquatic species that inhabit rivers and rice fields. They feed on organic matter, algae, and microorganisms and can also increase nitrogen and phosphorus in aquatic ecosystems (Kingsbury et al. 2021). Studies have shown that mud snails can improve rice plants' growth performance by producing excreta in favorable conditions, which will decompose and release nutrients to the rice plants (Kurniawan et al. 2018). Recently, studies have shown increased rice production, with 25 specimens per two square meters in density. Understanding the effects of mud snail density on rice plant performance is necessary, as they can increase plant biomass and indirectly link or interact with the increase of other species in paddy fields (Dewi et al. 2017). Throughout this research, we focused on the best density of mud snails, which is ideal for rice production. However, the first experiment focused on the vegetative period, which is essential and predictive for better rice production.

The experiment was conducted in three different environmental conditions: in the laboratory using a growth chamber, greenhouse, and outside with various densities of mud snails in several containers, assuming that the snails' excrement would supply nutrients and enhance the performance of the rice plants. We carried out observations and comparisons based on factors such as height, number of leaves, tillers, and SPAD (Soils Plant Analysis Development) value to determine the optimal density of snails per rice plant. We experimented multifactorial, hypothesizing that rice plants will perform at one of the following densities: (0, 2, 5, and 7) and (0, 1, 4, 8) mud snails per plant during the vegetative stage. According to the three tests in the vegetative phase with several densities in the first experiment, five snails per rice responded to the best criteria among the three test conditions. Subsequently, the rice plants were associated with four rice seedlings per hill outside in the enormous containers with two treatments: snail and no snail. The experiment was conducted until harvest for the final evaluation, considering factors such as rice plant performance, yield, and plant biomass. In addition, we considered the soil's carbon and nitrogen ratio (C/N ratio), the existence of weeds, and other unexpected situations, such as the emergence of algae and duckweed.

MATERIALS AND METHODS

First experiment

The study occurred from 15 February to 21 May 2022 at Faculty of Agriculture, Yamagata University, Tsuruoka Campus, Japan, in the Animal Ecology Laboratory, Greenhouse, and outside.

The soil

The soil was collected from the Takasaka field (38°41'49.7"N 139°49'07.7"E) on the university farm in November 2021 and used for the whole experiment. After being firstly dried in the greenhouse, it was dried a second time in the oven (DX301; Yamato) at 110°C for 24 hours to decrease weed pests and pathogens that could infect the rice during the experiment. The soil was sieved with a 5 mm mesh and weighed, and 700 g of soil was transferred to each container at a 6cm depth. The sizes of plastic containers that served at miniatures paddy fields during the experiment were (L x W x H) 14 x 6 x 16 (cm).

The water

A half liter of tap water was filled to a height of 2 to 3cm in the beginning because of the height of the young rice plant (14-15 cm) for better observation. Two weeks later, 1-1.5 liters were added to a height of 7-9 cm with a daily addition of 100 mL, then 200-250 mL from the third week until the fifth and last because of evapotranspiration and water consumption by the rice.

*The mud snails (*Cipangopaludina chinensis*)*

We collected approximately 1000 snails at 38°45'32.5"N 139°48'51.6"E in Araikyoda Tsuruoka City, Yamagata Prefecture. They were stored in 2 containers (L x W x H) measuring 90 x 60 x 20 (cm) with 20 kg of soil collected from the Takasaka field at the university farm, combined with 40 liters of water, and kept in the greenhouse. To measure the snails' shell size, we randomly sampled 100/1000 individuals, and an average of 22.5±0.3 mm was obtained. After the calibration, we removed the algae from the snails, cleaned them, and stored them in larger containers measuring 40 cm long, 28 cm wide, and 23 cm high, filled with 1 kg of soil and 10 liters of water. Therefore, 324 snails were used in the three tested environments.

The rice

The seed used for this experiment was taken from the laboratory stock of the Sasanishiki rice variety kept in a plastic bag harvested from the farming campaign in 2021.

The rice seedlings were made with 25 g of seed for 15 days in an incubator (Sanyo incubator, MIR-253) at 25°C before the beginning of each experiment in a (Lx W x H) 30 x 23 x 5 (cm) tray with 500 g of soil sterilized in the oven (DX301; Yamato) at 110°C for 24 h. The rice plants used varied in height from 13 to 14 cm. We transplanted one rice plant per container.

The treatments consisted of randomly chosen groups of 0, 2, 5, 7 and 0, 1, 4, 8 snails in each container, combined with one rice plant and 1 liter of water. The tap water filled per container was a half-liter with a height of 6 to 7 cm at the beginning because of the size of the young rice plant (13-14 cm) for good observation, and two weeks later, 1-1.5 liters were added with a height of 7-9 cm with a daily addition of 100 mL, then 200-250 mL from the third week until the fifth and last because of evapotranspiration and water consumption by the rice.

The growth chamber (BIOTRON)

From 15 February to 22 March 2022, for the combination (0, 1, 4, and 8 snails/rice plant) and from 15 April to 20 May 2022 for the second set (0, 2, 5 and 7/rice plant) with (N=4) in the laboratory of animal ecology (38°44'08" N, 139°49' 39"E) at 401 m asl. It was set at day/night at a temperature of 25°C/20°C and 50%/75% relative humidity during the day from 6 a.m. to 6 p.m. with 11,000-12,000 Lux light intensity measured with a light meter (LM-8,000/ No Q754248).

The greenhouse

The experiment in the greenhouse and outside was conducted during the same period from 16 April to 21 May 2022 (38º44'12" N 139º49'40"E) 517 at masl. The temperature ranged from 25 to 40°C, and relative humidity was measured from 20 to 40% with a thermo-hygrometer (TANITA; TT-580). The light intensity was more than 20.000 Lux. The treatments and replication densities were the same as in the growth chamber.

Outside

At (38°44'11" N 139°49'40"E) 517 at masl near the greenhouse. The temperature ranged from 22 to 26°C. Relative humidity ranges from 60-80%. The rainfall was 100 to 200mm (Japan Meteorological Agency 2022; 2023). The treatments and replication densities were the same as in the growth chamber.

Monitoring

The data were recorded at 5-day intervals, and the leaf nitrogen content was determined using a SPAD meter (Konica Minolta SPAD-502 plus chlorophyll meter). The rice plants' height was assessed using a 100-cm ruler, while the quantity of leaves and tillers was counted manually. We did not record the deaths of animals, and juveniles were removed daily for a better assessment. They were released in the canal because the males or females were not identified during the calibration. Additionally, the eggs of frogs and tadpoles were removed outside the experiment.

Second experiment (Containers in the field)

The soil was collected from the Takasaka field (38°41'49.7"N 139°49'07.7"E) at the university farm in early April 2022, sieved with a 5 mm mesh and weighed using a digital scale (UX6200H; Shimadzu). 30kg of soil were transferred into each container sizing 90 x 60 x 20 (L X W X H cm) to reach a height of 5 cm, stored in the greenhouse, and ready for use. Then, containers were transported to the field (38° 4411.1 N" 139°49'44.2" E) and filled with 30 liters of water at the end of May 2022. Next, 100 g of soil was collected from each container before transplanting and after harvest in years 1 and 2 for C/N ratio analysis with an automatic, highly sensitive NC Analyzer (Sumigraph, NC-220F) and aspartic acid as a reagent.

*The mud snails (*Cipangopaludina chinensis*)*

We selected 500 from the remaining individuals, with an average of 22.5±0.5 mm shell height obtained and stored in larger containers measuring 90 x 60 x 20 (L X W X H cm) filled with 5 kg of soil and 20 liters of water. Therefore, 300 mud snails were used for the experiment and 200 for replacing the dead.

The rice is transplanted at a 30 x 16 cm distance in a paddy field with 3 to 4 plants per hill. This is equivalent to 20-21 hills per square meter based on my observations and visits to several farms, and the density of mud snails in paddy fields in Japan varies from 0.25-30 individuals per square meter (Nakanishi et al. (2014). Following this, the ratio of snails/rice could be 1, 1.5, or 2 snails per hill of rice plant. In the second experiment, we used two treatments: 50 snails per container of 0.54 $m^{2.}$ According to the three tests in the vegetative phase with several densities in the first experiment, 5 snails per rice plant density presented the best results among the three test conditions. Subsequently, 5 snails were associated with four rice seedlings per hill outside in the most oversized containers for the snail treatment and no snail treatment (N=6); this corresponds to 100 individuals per square meter (Karatayev et al. 2009).

Rice plants

On 28 May 2022, in year 1, and 19 May 2023, in year 2, from the Takasaka farm, we collected a 38-day-old Sasanishiki rice variety and 10 hills, with 4 plants each transplanted per container, at 30 cm between rows and 15 cm between hills at 10 cm from the edge. Therefore, 12 containers were placed in the field, 6 without snails and 6 with snails, for an average of 22.5±0.3 mm in shell height, with algae attached to their shells and calibrating with a manual caliper.

Monitoring

The snail observations were made daily to control and limit the possible contamination of the experiment with the decomposition of dead snails, which can increase the nitrogen in the experiment (Yanygina 2021). The dead animal was removed constantly and gradually replaced by others who were kept for such cases or eventuality. Additionally, the juveniles of snails, the eggs of frogs, and tadpoles were removed for better assessment.

We biweekly measured the plant's height using a one-meter steel ruler; the tillers and leaves were manually counted. We determined the leaf nitrogen content. The water temperature was measured with a digital waterproof thermometer CT-285WP. To evaluate the weeding by snails, we collected the floating plants removed by them due to their self-burial and scrawling activities that were only present in their containers. Then, the quantity of weeds removed manually was compared to those from snails' activities. The algae were collected manually. The weeds, algae and duckweed were subjected to oven drying at 70°C for 24 hours using an oven model (DX301-Yamato). This process allowed us to determine the dry weight of the plant biomass. Conversely, the rice plants were air-dried in a greenhouse after the harvest in September each year. The rice was manually harvested with a tiny grass sickle when the grain moisture content was at 20%, as determined by a moisture meter (Kett; Code: 5100-No BH 188 90). The dry weight of all plant samples was measured using a digital balance model (AUW220D-Shimadzu). Engraining was performed manually using a metal comb, while hulling was done using an electric huller (Otake, FC2K- No 1780582).

Statistical analysis

The comparisons were conducted using Fisher's one-way ANOVA to determine if there were statistical differences between the means of two or more unrelated treatments, using Tukey's HSD method for post hoc analysis. Additionally, it was checked using polynomial regression for better confidence in determining the optimal density. In the first experiment, GLM determined the effect of temperatures in different locations on plant performance. We compared Snail and No-snail treatments in the second experiment using the Welch two-sample t-test, except for the weeding activity, which was analyzed using the same method as in the first experiment, and the algae in the first year using the Kruskal Wallis test because of the non-normality of the data. We used R Version 4.0.2 to perform all statistical analysis methods at $P<0.05$ for the significance level.

RESULTS AND DISCUSSION

First experiment

The effects of temperature

The research analyzed with GLM the interaction between the effects of temperature and density in the different locations (laboratory at 25.2±0.1°C; greenhouse at 34.8±0.4°C and outside at 24.1±0.5°C) on various plant growth metrics: height, number of leaves, SPAD (chlorophyll content), and tillers. For the temperature effect, the quadratic model shows that temperature significantly impacts height, SPAD, and number of tillers (all $p < 0.01$),, with high R-squared values indicating a good fit for height (0.9584) and SPAD (0.3984), but a lower fit for tillers (0.2391). However, the number of leaves is not significantly affected by temperature, showing low R-squared (0.08147) and high p-values ($p>0.1$).

Regarding snail density and location, density positively affects all plant metrics with significant ($p<0.05$ for height, leaves, SPAD, and tillers). The location significantly decreased the height and SPAD of rice plants in the greenhouse and outside, conversely, in the laboratory. However, the location did not significantly affect the number of leaves; the number of tillers was significantly higher outside than in the laboratory and greenhouse.

Temperature and snail density significantly influence plant growth, with temperature having a notable quadratic effect and density positively correlating with plant performance. The laboratory, followed by the greenhouse, significantly impacted height and SPAD while the tillers were enhanced outside, and no notable variations were observed in the number of leaves. These results suggest optimizing environmental conditions and snail density can enhance rice plant performance.

Assessment of the rice plant's performance with the densities of 0, 2, 5, and 7 snails in the growth chamber, greenhouse, and outside during its growing phase

This research examined the performance of rice plants in three different situations. Within the growth chamber, the height of the plants exhibited noteworthy variations with T0 and T5, as shown by the statistical analysis (df=3, F=136 5.0492, P=0.0172). The output showed that the number of leaves (df=3, F=1.8161, P=0.1978), the number of tillers (df=3, F=1, P=0.4262), and the SPAD value (df=3, F=1.1713, P=0.3613) were not statistically significant. The greenhouse experiment revealed a variation in plant height in T2, T5, and T7 (df=3, F=3.8759, P=0.0377), as well as in leaf number in T5 and T7 (df=3, F=4, P=0.0345) and SPAD values (df=3, F=10.499, P=0.0011). The number of tillers (df=3, F=3.0857, P=0.0680) showed no differences. In the outdoor experiment, T2, T5, and T7 showed significant differences in the height of plants (df=3, F=4.9697, P=0.0181) in T2, T5, and T7, the number of leaves (df=3, F=10.037, P=0.0013) in T5, and the number of tillers in T5 and T7 (df=3, F=143 8.5385, P=0.0026). However, the SPAD value showed no notable variation (df=3, F=3.1763, P=0.0634) (Table 1).

Furthermore, the polynomial regression showed the following results in the height, leaves, tillers, and SPAD values in growth chambers, greenhouses, and outside 35 Days After Transplantation (DAT). The snail densities (0, 2, 5, and 7 per rice plant) in the growth chamber presented no significant differences in height among different snail densities, with the highest mean of performances observed at a density of 5 snails per plant. However, the number of leaves, tillers, and SPAD values did not vary significantly across different snail densities in this environment. Based on the regression model showed there is no relationship between the densities and the plant performance (df=13, F=1.22, p=0.32, R^2=0.02). Although there were no significant differences in height caused by the snail densities in the greenhouse, the number of leaves, tillers, and SPAD values varied greatly, with the highest average SPAD value seen at a density of 5 snails per plant. A regression linear model gave a perfect fit to the calculation between the density of snails and SPAD value with the equation $Y = 45.45 + 0.83x$. In the outside environment, there were significant differences in height, number of leaves, and tillers among different snail densities, with the highest mean values observed at a density of 5 snails per rice plant. In contrast, SPAD values did not vary significantly. The regression analysis was done for the outside experiment with good remarkable value. The plant height showed the quadratic equation as $Y = 37.82+1.06x-0.12x^2$. From this, the optimum density of the snail for plant height is 4.27 snails/rice plant or about 4 to 5 snails/plant (df=13, F=4.02, p=0.04, R^2=0.28). Meanwhile, the leaves and tiller numbers' quadratic regression showed $Y = 21.86 + 4.37x-0.51x^2$ and $Y = 8.84 + 1.50x-0.16x^2$, respectively. The optimum density for increasing the number of leaves is 4.28 snails/plot and 4.62 snails/plant or equally about 4 to 5 snails/plant, respectively (number of leaves, df=13, F=9.05, p=0.003, R^2=0.51; Tillers number, df=13, F=6.84, p=0.009, R^2=0.43).

Assessment of the rice plant's performance with the densities of 0, 1, 4, and 8 snails in the growth chamber, greenhouse, and outside during its growing phase

The rice plant performance showed the following results: In the growth chamber, (the plant height df=3, F=4.5156, P=0.02432) and (SPAD value df=3, F=5.8256, P=0.010764) in T1, T4, and T8 showed significant differences. The number of leaves (df=3, F=0.30733, P=0.81967); the number of tillers (df=3, F=1.913, P=0.18134) showed no different. In the greenhouse, all the treatments; the plant height (df=3, F=1.1263, P=0.37729); the number of leaves (df=3, F=0.32997, P=0.80385); the SPAD value (df=3, F=1.4017, P=0.29018); and the tillers (df=3, F=0.35065, P=0.78947) no change among. The experiment outside resulted in no significant differences; the plant height (df=3, F=0.47953, P=0.70252; the number of leaves df=3, F=0.19364, P=0.8987; the number of tillers df=3, F=0.37681, P=0.77141; the SPAD value df=3, F=1.9117, P=0.18155) (Table 2).

In addition, using the polynomial regression to assess snail densities (0, 1, 4, and 8 snails per plant) in the growth chamber showed significant differences in height were observed among different snail densities, with the highest mean height observed at a density of 4 snails per plant. A regression analysis showed a significant relationship between density and plant height. The quadratic equation of the plant height showed $Y = 61.48+2.22x-0.24x^2$. Based on the equation, the optimum height was at 4.62 snails/plant or about five snails/plant (df=13, F=7.24, p=0.007, R^2=0.45). However, there were no significant differences in the number of leaves, tillers, and SPAD values across different snail densities in this environment. There were no significant differences in height, number of leaves, tillers, or SPAD values among different snail densities in the greenhouse and outdoor environments. These findings highlight the complex interplay between snail density and plant performance across different environmental conditions. In conclusion, maintaining the moderate density of about 4 to 5 snails/container per plant showed the optimum result for the plant performance.

The outputs based on Fisher's one-way ANOVA using Tukey's HSD method for post hoc analysis demonstrated that the densities of 0, 1, 4, 5, and 8 snails in the growth chamber showed significant height and SPAD value results. In the greenhouse, densities of 2, 5, and 7 positively influenced the height of rice plants; 0, 5, and 7 impacted the number of leaves, and 5 and 7 the SPAD value. Outside, 2, 5, and 7 snails increased plant height, while five snails increased the number of leaves, and the combination of 5 and 7 snails with rice resulted in a notable variation in tiller. Based on the analysis, five snails have shown a higher influence on the rice plants. In addition, the polynomial regression analysis indicated similar results with a density of 4-5 snails per rice plant. Conclusively, we adopted five snails as the optimal density, which was applied in the second experiment.

Second experiment

Assessment of the rice plants' performance with the density of 5 snails until harvest

In 2022, no significant variations were observed in (the SPAD value t=-1.3255, df=9.8637, p=0.2149; the plant height t=-0.99095, df=9.2101, p-value=0.347; the number of tillers t=-1.4428, df=9.2006, p=160 0.1822) in June. In July (SPAD value, t=-2.9796, df=9.9785, p=0.01385; the plant height, t=-2.4317, df=9.9295, p=0.03551), was different while (the number of panicles, t=-0.77594, df=9.3791, p=0.4569; the total plant biomass, t=-0.58501, df=8.8466, p=0.5732) were not significantly different in the first year. In 2023 (June, the plant height t=-2.9421, df=6.7117, p=0.02271; the SPAD value t=-5.6872, df=9.7616, p=0.000221; the number of tillers t=-3.0069, df=7.8192, p=0.01734); July, the plant height t=-3.7185, df=7.707, p=0.006288; the number of tillers t=-2.6173, df=6.4153, p=0.03736; and the number of panicles t=-4.6393, df=9.602, p=0.001028; the total plant biomass (t=-4.128, df=8.5915, p=0.002836) were all significantly different; in addition, the treatment with snails recorded higher values compared to no snails (Table 3).

The initial and final C/N ratio

During 2022 and 2023, the initial and final means of carbon, nitrogen, and C/N ratio levels did not differ significantly between the treatments. In the first year, (carbon t=0.21832, df=9.5299, p=0.8318; nitrogen t=-0.22169, df=9.9991, p=0.829; C/N ratio t=0.70443, df=7.8958, p=0.5014). In the second year, carbon (t=-0.012488, df=9.9705, p=0.9903); nitrogen (t=- 173 0.34835, df=10, p=0.7348); and C/N ratio (t=1.7343, df=8.4919, p=0.1189). However, we observed a slight increase in total carbon and nitrogen in the first year of the snail treatment compared to no snails, with a decrease in the second year. The carbon-nitrogen ratio (C/N) ratio remained slightly low in snail treatments compared to no snail during the two years (Table 4).

The collected quantity of algae and duckweed

In the first and the second years, the algae and duckweed were in great quantity in snail treatment compared to no snails and showed much difference. For 2022, (algae x^2=18.529, df=1, p=1.674e-05; duckweed t=-3.832, df=9.1481, p-value=0.003896) and 2023 (algae t=7.4407, df=5.0249, p=0.0006765; duckweed t=-3.1129, df=5.0349, p=0.02622) (Table 5).

The yield of rice

In 2022, the finding showed that snail treatment led to a higher mean yield but had no difference statistically (rice with shell t=-1.0186, df=9.2037, p 184=0.334; hulled rice t = -0.48547, df=8.9457, p=0.639). Both treatments decreased, while snail treatments maintained higher yields, which were significantly different (rice with shell t = - 6.9208, df=6.2305, p=0.0003822; hulled rice t = - 5.7498, df=6.4811, p=0.0009195) (Figure 1).

The weeding activity by snails compared to manual in 2022

In the first year, there was no significant difference between snail and manual weeding in May (df=2, F=0.806, P=0.465). However, at the beginning of June (df=2, F=65.86, P=3.73e-08) and the end of June (df=2, F=4.431, P=0.0308), significant differences were observed in the presence of mud snails. In 2023, excess algae limited the observation of wedding activity (Figure 2).

The survival of snails

During 2022 survival, the number of dead individuals was low (t=14.432, df=7.3529, p=1.163e-06). It increased in 2023 with a significant difference (t=-6.9845, df=10, p=3.786e-05) (Figure 3).

Table 1. The mean of height, leaves, tillers and spad in the growth chamber, greenhouse and outside 35 DAT (Days After Transplantation) for Density 1

Environment	Snails density	Height	Leaves	Tillers	SPAD
Growth chamber	T0	62.75±1.43ab	25±1.68 a	9±0.40a	49±1.58a
	T2	61.75±1.25b	26.25±1.43a	9.5±0.28a	51.45±0.75a
	T5	67.25±0.75a	28.75±1.37a	9.75±0.47a	51.7±1.31a
	T7	62.25±0.94b	23.5±2.02a	8.5±0.86a	51.57±0.93a
Greenhouse	T0	42±0.91b	22.25±1.88ab	8.75±0.62a	45.6±0.88b
	T2	42.75±1.25ab	20.75±1.93b	8±0.70a	46.67±0.92b
	T5	45.75±0.47a	25.5±2.10ab	10±0.70a	50.25±0.87a
	T7	44.25±0.47ab	28.5±0.5a	10.25±0.25a	51±0.48a
Outside	T0	38.12±0.65b	22.75±1.37b	9.25±0.47b	45.15±0.45a
	T2	38.75±0.32ab	26.5±1.32b	10.25±0.47b	46.3±0.59a
	T5	40.75±0.59a	33±1.47a	13.25±0.85a	47.3±0.70a
	T7	38.87±0.37ab	26.5±1.19b	11±0.40 ab	47.42±0.58a

Note: The same letters after means within the same column indicate a non-significance difference determined by Tukey's HSD post-hoc Test, Pr>Fc: $P < 0.05$

Table 2. Mean of height, leaves, tillers and spad in the growth chamber, greenhouse and outside 35 DAT (Days After Transplantation) for Density2

Environment	Snails density	Height	Leaves	Tillers	SPAD
Growth chamber	0	61.62±0.55b	30.25±1.79a	10.25±0.75a	51.±0.38b
	1	63.25±1.43ab	32.5±1.84a	12.25±0.25a	51.72±0.49ab
	4	66.5±0.86 a	32.25±2.68a	12.25±0.85a	53.62±1.04ab
	8	63.37±0.74ab	30.5±1.93a	11.25±0.75a	54.2±0.33a
Greenhouse	0	42.5±0.64a	24.25±1.37a	9.25±0.47a	46.95±0.88a
	1	42±0.91a	23.25±0.85a	9.25±0.47a	47.85±0.56a
	4	43±0.70a	23.5±1.04a	9.25±0.47a	48.52±1.54a
	8	43.75±0.47a	22.25±2.13a	8.5±0.95a	49.62±0.39a
Outside	0	38.87±0.77a	26±1.87a	10.75±1.18	45.2±1.06a
	1	39.5±0.73a	27±2.1a	12±0.70a	45.8±0.56a
	4	39.37±0.47a	25.25±2.01a	11.25±0.47a	47.87±1.09a
	8	38.5±0.64a	27.5±2.95a	11.5±0.86a	47.87±1.18a

Note: The same letters after means within the same column indicate a non-significance difference determined by Tukey's HSD post-hoc Test, Pr>Fc: $P < 0.05$

Table 3. Mean of SPAD value, plant height, tiller number, and rice plant biomass in the containers with and without snails in 2022 and 2023

Year	Treatments	SPAD value		Plant height (cm)		Tiller number per hill		Number of panicles	Dry biomass (g)
	Month	June	July	June	July	June	July		
2022	No Snails	42.6±086a	27.61±0.53b	29.08±0.86a	46.1±1.19b	18.9±0.87 a	20.6±0.91a	192.5±10.7a	201.61±16.6a
	Snails	44.1± 076a	29.8±0.50a	29.5±0.63a	47.86±1.3a	20.46±0.64a	21.8±0.71a	203±8.24 a	213.45±11.4a
2023	No Snails	28.15±0.57b	26.7±0.08b	28.16±0.34b	45.96±0.3b	9.58±0.41 b	10.41±058b	77.16±3.87b	104.56±6.49b
	Snails	32.45±0.49a	28.36±011a	29.28±0.14a	47.26±0.16a	11.01±0.23a	12.05±0.22a	105.6±4.76a	136.53±4.22a

Note: The same letters after means within the same column indicate a non-significance difference determined by the Welch Two Sample t-test: $P<0.05$

Table 4. The mean (±SE) of carbon, nitrogen and C/N ratio in the containers with and without Snails 2022 and 2023

Year	Treatments	Initial			Final		
		Carbon	Nitrogen	C/N Ratio	Carbon	Nitrogen	C/N Ratio
2022	No Snails	1.43±0.08a	0.123±0.02a	11.68±0.61a	1.37±0.03a	0.146±0.003a	9.37±0.22a
	Snails	1.44±0.02a	0.121±0.003a	11.99±0.47a	1.35±0.04a	0.147±0.003a	9.19±0.12a
2023	No Snails	1.59±017a	0.124±0.01a	12.88±0.27a	1.424±0.12a	0.11±0.009a	12.77±0.22a
	Snails	1.85±013a	0.146±0.01a	12.76±0.22a	1.426±0.11a	0.11±0.009a	12.31±0.14a

Note: The same letters after means within the same column indicate a non-significance difference determined by the Welch Two Sample t-test: $P<0.05$

Table 5. The mean value of algae, duckweed and weeds per container

Year	Treatments	Algae (g)	Duckweed (g)
2022	No snails	0±0b	0.50±0.29b
	Snails	6.76±4.33a	2.45±0.41a
2023	No snails	0.83±0.35b	0.133±0.06b
	Snails	53.51±7.06a	3.65±1.12a

Note: The same letters after means within the same column indicate a non-significance difference determined by the Welch Two Sample t-test: $P < 0.05$

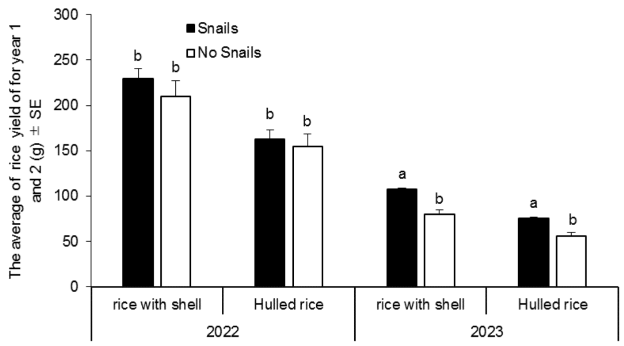

Figure 1. The mean of rice yield per container. Means (± S.E.) showing the same letter are insignificant P<0.05

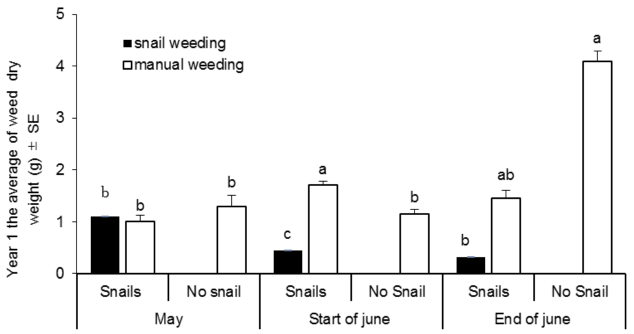

Figure 2. The average of the dry weight of weeds from mud snails and manual weeding activity in 2022. Means (± S.E.) showing the same letter are insignificant P<0.05

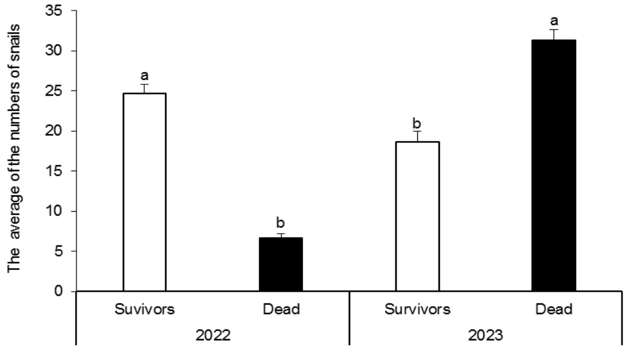

Figure. 3 The Survival of snails in 2022 and 2023. Means (± S.E.) showing the same letter are insignificant P<0.05 using the Welch Two Sample t-test

Discussion

This study demonstrated that variations in the number of mud snails and temperature differences in the three environments influence rice growth. These confirm the previous study, which showed that temperature influences mud snail excreta production and that both influence rice plant biomass, as found by Kurniawan et al. (2018). The growth chamber in the laboratory, with an average temperature of 25.2°C, presented an excellent performance in rice plants' height and SPAD value with the two groups of densities compared to the greenhouse and outside. The results are similar to those presented by Sato (1972), who indicated that rice development in the growth chamber is higher than usual. Furthermore, Pasuquin et al. (2013) conducted experiments to evaluate several rice cultivars in a controlled environment (growth chamber), and the findings demonstrated a greater augmentation in biomass at a temperature of 25°C.

The results obtained in the greenhouse at an average temperature of 34.8°C presented low height and SPAD value compared to the growth chamber but higher outside at an average temperature of 24°C like those of Hussain et al. (2019), who suggested that an increase in temperature will intensify the photosynthesis of the rice and enhance its biomass. In addition, a temperature of 35°C increases the rice plant biomass, according to Dubey et al. (2019). During our experiment, the temperature within the greenhouse was consistently greater than in the other two test circumstances, resulting in height and SPAD value compared to outside. This aligns with Wang and Hijmans (2019), who discovered that rice exhibits adaptability to elevated temperatures and shifting environmental conditions, leading to enhanced plant productivity. Rising temperatures may boost the growth of biomass in rice plants.

The height of the plant was the shortest and showed the lowest SPAD value. Yoshida (1973) researched temperature effects on rice in the growth phases, finding that rice produced the most leaves and tillers at a minimum of 22°C and a maximum of 25-31°C during the optimal period of 3 to 5 weeks. This study is consistent with the treatment results with five snails outside with a temperature average of 24.1°C, obtaining a significant and largest average number of leaves and tillers compared to the growth chamber and greenhouse. Likewise, Zhou et al. (2021) conducted a study to assess the influence of temperature on rice growth and productivity. Research has shown that the optimal outcome may be predicted at 24.1°C. This finding supports the observation in our study, with a higher number of tillers and leaves recorded within a temperature range of 22 to 26°C outside.

The results suggest that snail excreta, in general, can increase plant height, tillers, number of leaves, and SPAD value, thereby boosting rice plant biomass. Outside, the number of tillers and leaves increased, decreasing in the growth chamber and greenhouse, where the plants were taller with higher chlorophyll content. According to Burnett et al. (2018), the activities of mud snails decreased gradually with the rising temperature. Additionally, it was demonstrated that the increase could lower the snail's excrement production, reducing the supply of nutrients derived from the excreta and consequently affecting rice performance, as Kurniawan et al. (2018) reported.

The higher densities of snails influenced the performance of rice plants. These results could be justified by Zhang et al. (2022b) and Zhou et al. (2023), who suggested that a high density of snails can decompose organic matter and increase the levels of nitrogen and phosphorus, which will enhance the expansion of the plant in the aquatic environment. Compared to the other treatments, the density of five snails demonstrated at least two or more plant performance criteria in all the locations.

This study showed that the density of five mud snails per hill of four rice plants increases the growth and yield of rice. Mud snails increased the incidence and quantity of duckweed and algae. The snail treatment enhanced the SPAD values and the number of tillers in the first year. Snails boosted all the factors related to the rice plant development in the second year. These align with Olden et al. (2013) and Edgar et al. (2022), who suggested that the snails' excreta provide nitrogen and phosphorus and can increase plant biomass; this can justify the higher quantity of algae and duckweed collected in the snail treatments. Yang et al. (2020) found that mud snails can release a significant quantity of phosphorus, which is also essential for growing plants. Furthermore, Hou et al. (2021) proved that mud snails could break down organic materials and release nutrients necessary for plants' growth.

The carbon and nitrogen concentrations were insignificant, possibly due to competition between algae, duckweed, weed, and rice plants, which uptake the highest quantity of nutrients provided by snail excreta decomposition in containers with limited space and a reserve or stock of nutrients. The carbon and nitrogen levels increased slightly, and the ratio of carbon to nitrogen decreased in treatments involving snails compared to the no-snail treatments in 2022, indicating an improvement in soil quality. This observation insinuates that snail excreta enrich soil organic matter and significantly enhance microbial communities. A lower C/N ratio suggests that materials in the soil decompose more rapidly, improving fertility, as Benites and Ashburner (2003) and Zhou et al. (2019) indicated. In the second year of 2023, the carbon and nitrogen in the soil decreased in both treatments. However, the carbon-nitrogen ratio remained low with the activities of snails, which was the opposite without snail activities in no snail treatment. Wibowo and Kasno (2021) justify this by indicating that a decrease in carbon in the soil leads to a diminution of nitrogen, resulting in low soil fertility, which also explains the low rice yield in 2023.

The first experiment demonstrated the highest rice plant performance, with a density of five snails, probably due to a higher rate of excreta released. However, Kurniawan et al. (2018) found that temperature primarily influenced the processes. In the first and second years of the final experiment, rice plant development, duckweed, and algae increased, all influenced by mud snails. According to Dewi et al. (2017), the excrement released by mud snails can increase the availability of nutrients that influence plant biomass.

A slight weeding activity of mud snails was observed in 2022, which can be justified by the fact that they were scrolling and moving in self-burial movements, and these uproot young plants, which later floated on the water. This activity was not observable in 2023 due to the abundance of algae. The findings align with Iwai et al. (2018), who suggested a reduction of weeds (plant biomass) with the bioturbation of snails in the paddies field.

Regarding the survival of snail individuals, the highest number of deaths during the experiment could be caused by the water temperature. In the natural environment of the snail, *C. chinensis laeta* ideal temperature is 21.4°C and 21.5°C ±0.5°C according to Collas et al. (2017). The high temperature of 30°C in the water decreases the immune system and makes snails more susceptible to infections, as indicated by Seppälä and Jokela (2011). Additionally, mud snails cannot tolerate temperatures of 30°C and above, according to Karatayev et al. (2009). These may justify the high rate of death we recorded in our study during July and August 2022, temperatures between 27 and 29°C in 2023 and 30-31°C in the water. Another factor could be that the water was stagnant in the containers without flow and recycled only by the rainfall, which is a very different condition from their natural habitat.

In conclusion, the study showed that raising the density of mud snails enhances rice plant performance and the biomass of algae and duckweed, all of which are influenced by environmental conditions. This study occurred in a laboratory, a greenhouse, and outside using containers. Research on different densities in the paddy field environment is necessary to better understand the role and importance of mud snails.

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