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Efficacy of Giant River Prawn *Macrobrachium rosenbergii* in Controlling the Invasive Snail *Pomacea canaliculata*: Implications for Ecological Farming

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Abstract: The invasive golden apple snail *Pomacea canaliculata* has a strong reproductive capacity and has rapidly spread in Asian countries. Current control methods include physical, chemical, and biological approaches, but there has been limited research on the control of *P. canaliculata* in its different life stages. This study assessed the effectiveness of using giant river prawns *Macrobrachium rosenbergii* in controlling juveniles of *P. canaliculata* through a controlled indoor experiment. The density, size, and dispersal range of recently hatched juvenile snails were significantly lower among those kept with prawns than those kept without prawns, indicating a control effect of *M. rosenbergii* at least on *P. canaliculata* juveniles. Furthermore, the study speculates on the potential application of *M. rosenbergii* in the context of a rice–prawn symbiotic system of ecological farming to control invasive *P. canaliculata*. In terms of effectiveness and safety, its application might lead to a win-win situation for both rice-farm profits and the ecological benefits of invasive species control.

Keywords: apple snail; aquaponics; biocontrol; invasive species; Malaysian freshwater prawn

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1. Introduction

The golden apple snail *Pomacea canaliculate* (family Ampullariidae in the order Mesogastropoda) is native to tropical and subtropical regions of South America, including the Plata and Amazon river basins [1,2]. Introduced in China in the 1980s for edible snail farming, the effort was later abandoned because of the snail's poor taste [1–3]. However, owing to the species' strong reproductive capacity and adaptability, *P. canaliculate* quickly spread to freshwater environments, such as rice paddies and water chestnut fields [1,3–5], leading to explosive invasions in multiple provinces in China, Vietnam, the Philippines and Thailand [6–8]. Its rapid population growth and extensive plant consumption have caused wide-ranging impacts on local biodiversity through competition with native species [6], and the species has been listed among the top 100 of the world's worst invasive alien species [9]. New species of *Pomacea* are continuously being identified using molecular methods; thus, apart from population expansions, the genus *Pomacea* is relatively speciose [10].

Currently, the control methods for *P. canaliculate* mainly include physical, chemical, and biological methods [4]. Physical control involves picking up, trapping, or intercepting snails and their eggs. This process is simple and environmentally friendly but requires substantial manpower and materials with associated financial costs and shows slow results [6,11]. Chemical control is the most commonly used method, employing molluscicides such as formulations of niclosamide, triclosan, and piperonyl butoxide [12]. This approach has several advantages, such as quick effect, broad applicability, and an evident control effect, but it also incurs high costs and poses potential toxicity to aquatic organisms, eventually affecting ecosystem functionality [13]. Some researchers have explored molluscicides derived from plants to attract and kill *P. canaliculate*, such as extracts from the perennial plant

Ipomoea cairica [14–17]. However, the mechanism of action of plant-based molluscicides is not well studied, and the effective molluscicidal components are difficult to identify and quantify [16,18,19].

Biological control, which utilizes inter-species interactions, offers advantages such as durability, high efficiency, environmental friendliness, and relatively low costs [20], making this an innovative approach for *Pomacea* management [21,22]. One such method is to control the abundance of vegetation that species of *Pomacea* prefer, which has been proven effective [23]. Furthermore, researchers found that 26 out of 46 freshwater animals investigated were capable of preying on *P. canaliculate* juveniles [24]. Currently, bio-control of various ampullariid species has employed the common shelducks *Tadorna tadorna* [24,25], Chinese soft-shelled turtle *Pelodiscus sinensis* [22], common carp *Cyprinus carpio* [26], and black carp *Mylopharyngodon piceus* [26], with relatively good control effects. Nevertheless, the life cycle of *P. canaliculate* consists of three stages: eggs, juveniles, and adults, with a total lifespan of 2–5 years [27]; with high egg-production rates, one female snail can reproduce up to 300,000 juveniles annually, and the juveniles can reach sexual maturity within 3–4 months, leading to overlapping generations [28]. Most existing biological control research on these snails has focused on the clearance and control of adults while neglecting their extremely strong reproductive ability, which results in rapid population recovery [21,22,24,25,29]. Therefore, the entire life cycle of *P. canaliculate* juveniles needs to be considered part of the control effort, but there are few reports on the biological control of juvenile snails.

Based on its rapid growth and development, a broad diet, large size, and delicious meat, the Malaysian freshwater prawn *Macrobrachium rosenbergii* (family Palaemonidae in the order Decapoda) has become an important species in Asian shrimp aquaculture industry, supporting agricultural development in many regions [30,31]. Introduced in China in 1976, the artificial rearing of prawn larvae became successful in subsequent years, leading to widespread farming across provinces and cities [32]. Although the red swamp crayfish *Procambarus clarkii* has been the main species used in the traditional rice–prawn symbiotic system of ecological farming [33,34], in recent years, factors such as degraded germplasm quality, insufficient seed supply, and weak industry systems have affected farming profits. Instead, *M. rosenbergii* has gradually become a new option in this model of ecological farming owing to its rapid growth, short farming cycle, stable market price, and suitability for rearing during high temperatures in summer [35,36].

In Bangladesh and China, some animal–rice farmers now culture snails for use as feed for prawn [37]; furthermore, snail shells in the diet of juvenile prawns maybe beneficial for their growth [38]. Considering the characteristics of *P. canaliculate* invasions and the current trend to promote and develop rice–prawn symbiotic ecological farming techniques as well as the suitability of *M. rosenbergii* growth and farming in regions known to have these snail invasions [36,39,40], this study investigated the effect of *M. rosenbergii* as a predator on the survival and growth of *P. canaliculate* juveniles under controlled laboratory conditions, thereby exploring the potential to use this prawn species to control the snails in rice paddies.

2. Materials and Methods

2.1. Experimental Materials

Pomacea canaliculata eggs and subadults were collected from rice fields and pond aquaculture systems in Maoming, Guangdong, China. For the egg collection, freshly laid egg masses were chosen from the same day's oviposition. For the collection of subadults, individuals with a shell height of 2–3 cm were selected. Once a sufficient quantity of snail egg masses and subadults were collected, they were transported to the laboratory for use in the experiment.

Prior to the experiment, the collected subadult snails were acclimated to the laboratory environment by housing them in plastic tanks measuring 0.6 m × 0.4 m × 0.4 m (length × width × height) for 3 days. The tanks were covered with fine wire mesh to prevent the snails from escaping. The snails were fed fresh lettuce as food once daily and provided with dechlorinated, aerated water, with the water periodically replaced to maintain the cleanliness of the tanks. Following acclimation, individuals demonstrating good vitality were selected for the experiment.

Juvenile *M. rosenbergii* for the study were sourced from Jiangsu Shufeng Prawn Breeding Co. Ltd, Gaoyou, China, and then reared in experimental aquaculture ponds at Huzhou University. Individuals displaying normal vitality and consistent size (~8 cm in total length) were chosen for the experiment.

2.2. Experimental Design

The experimental design of the indoor trials consisted of two snail size treatments (subadult snails vs. recently hatched juvenile snails) × two shrimp treatments (snail with shrimp vs. snail without shrimps) × 3 replicates and a snail-absent control × 3 replicates. Four treatment groups plus the control group were established as follows:

a. Subadult snails reared with shrimps: Each experimental tank was stocked with 40 *P. canaliculate* subadults (shell width of ~2 cm) along with 8 *M. rosenbergii*; to mitigate against damage caused by aggressive interactions, only female shrimps were selected;
b. Subadult snails reared without shrimps: Each experimental tank was stocked with 40 *P. canaliculate* subadults (shell width of ~2 cm) and without the addition of *M. rosenbergii*;
c. Recently hatched juvenile snails reared with shrimps: 10 egg masses (~20 g each) of *P. canaliculate* were evenly distributed on a wire mesh (2 mm aperture, which allowed newly hatched juveniles to pass through but prevented the passage of subadults) positioned at the top of each tank, and tank was stocked with 8 *M. rosenbergii*;
d. Recently hatched juvenile snails reared without shrimp: Similar to the above treatment, 10 egg masses (~20 g each) of *P. canaliculate* were evenly distributed on the wire mesh on the top of each tank but without the presence of *M. rosenbergii*;
e. Snail-absent control with only shrimps: The experimental tank contained only 8 *M. rosenbergii*.

Each treatment was replicated three times, resulting in a total of 15 experimental tanks (0.6 m length × 0.4 m width× 0.4 m height).

The experimental tanks were supplied with air tubes for continuous aeration. Specialized pellets formulated for *M. rosenbergii*, based on their daily feeding requirements in routine aquaculture, were added to the tanks. Additionally, an ample quantity of fresh lettuce was provided to nourish the subadult snails. Each evening, any remaining food residue was cleared from the tanks.

2.3. Data Collection and Analysis

To measure snail population density, three random areas of 10 × 10 cm were selected in each tank to count the number of snails. To measure snail size, randomly selected snails from each experimental tanks were measured for shell width using a vernier caliper. To calculate the distribution probability of juvenile and subadult snails in the experimental tanks, the bottom of each tank was divided into 24 areas of 10 × 10 cm each, and the number of areas with snails present was counted, which was divided by 24, thereby assessing the impact of *M. rosenbergii* on the extent of area where snails were active.

The egg masses began hatching on day 11 of the experiment and finished hatching by day 18. The population density of juvenile snails was measured across 6 days of the experiment, from day 12 to day 17. Snail shell width was measured on days 12, 14, and 17. The distribution probability of snails was also measured for 6 days, from day 12 to day 17.

The body length of *M. rosenbergii* was determined by briefly removing each prawn from the tank and measuring it with vernier calipers.

To establish the treatment with subadult snails, synchronization with the treatment groups with egg masses was maintained by introducing subadult snail into the experimental tanks on day 11, which corresponded to the start of egg hatching. The population density of subadult snails was measured for 6 days, on days 12 to 17. Shell width of subadult snails was measured on the 12th, 14th, and 17th days. The distribution probability of subadult snails in the experimental tanks was also measured for 6 days, from days 12 to 17. The body length of *M. rosenbergii* was measured on days 1, 12, and 18 of the experiment.

Two-way analysis of variance and *t*-tests were employed to assess differences between treatments with and without *M. rosenbergii* as well as differences between treatments with the two snail sizes. The data were analyzed using STATISTICA 13, and graphical representations were created using Adobe Illustrator.

3. Results

3.1. Impact of M. rosenbergii on the Quantity of P. canaliculata

The density of newly hatched juvenile snails was significantly lower in the presence of shrimp ("shrimp-present" group) than in the absence of shrimp ("shrimp-absent" group) (Figure 1). The mean density of recently hatched juvenile snails in the shrimp-present group was 3.15 ± 0.89 ind./dm^2, whereas in the shrimp-absent group, it was 29.31 ± 2.60 ind./dm^2, and the difference was significant (t = 9.519, $p < 0.001$).

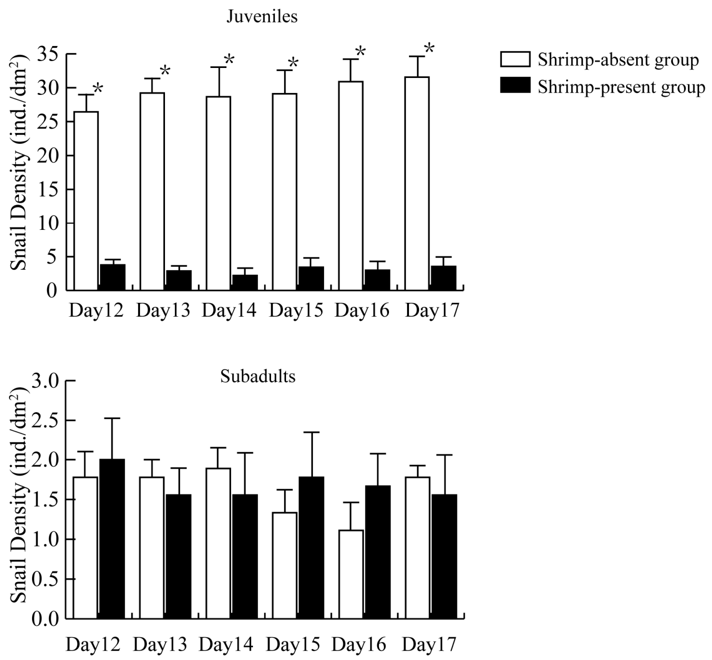

Figure 1. The density of recently hatched juvenile snails versus subadult snails in the shrimp-present and shrimp-absent groups. The asterisk (*) represents a significant difference ($p < 0.05$) between shrimp-absent group and shrimp-present group.

As the days since hatching progressed, the density of recently hatched juvenile snails in the shrimp-absent group exhibited a slightly increasing trend, although differences across the 6 days measured were not significant ($p > 0.05$). In contrast, the density of recently hatched juvenile snails in the shrimp-present group remained consistently low across the days measured.

The density of subadult snails showed no significant differences across days of the experiment in the shrimp-present group as compared with in the shrimp-absent group (Figure 1), with the former group largely maintaining the initial density of subadults set in the experiment.

Macrobrachium rosenbergii can directly ingest smaller juvenile snails, whereas they employ their chelae to shred the shells of larger juveniles before ingesting them. Subadult snail shells that had been damaged from attacks by *M. rosenbergii* were also noted throughout the experiment.

3.2. *Size of P. canaliculata Surviving in the Presence of M. rosenbergii*

Beyond the first observation, the shell width of recently hatched juvenile snails was significantly smaller in the shrimp-present group than in the shrimp-absent group (Figure 2). The average shell height of juvenile snails in the shrimp-present group was 0.036 ± 0.006 cm over 6 days, whereas it was 0.114 ± 0.008 cm in the shrimp-absent group, showing a significant difference between the two groups (t = 8.082, p < 0.001).

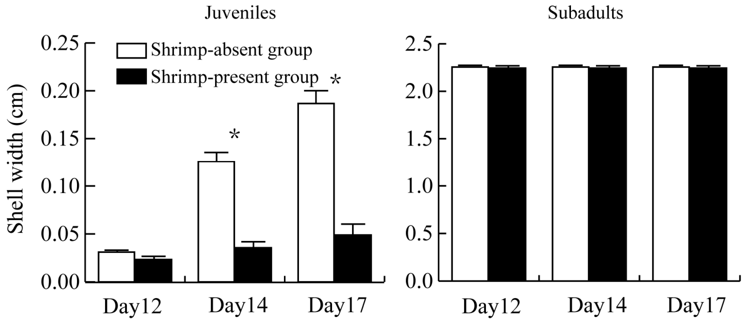

Figure 2. Shell width of recently hatched juvenile snails versus subadult snails in the shrimp-present and shrimp-absent groups. The asterisk (*) represents a significant difference (p < 0.05) between shrimp-absent group and shrimp-present group.

Shell width of recently hatched juvenile snails increased significantly (p < 0.001) as days since hatching increased in both the shrimp-present and shrimp-absent groups, but width growth was greater in the shrimp-absent group.

Shell width of subadult snails did not differ significantly between the shrimp-present and shrimp-absent groups, and there was minimal change in shell width observed over the 6-day observation period.

3.3. *Influence of M. rosenbergii on the Activity of P. canaliculata*

The distribution probability for recently hatched juvenile snails was significantly lower in the shrimp-present group than in the shrimp-absent group (Figure 3). The average distribution probability of juvenile snails in the shrimp-present group over the 6-day observation period was 0.106 ± 0.016, while in the shrimp-absent group, it was 0.899 ± 0.007, and the difference between groups was significant (t = 47.032, p < 0.001).

As the days since hatching increased, the distribution probability for newly hatched juvenile snails gradually decreased in the shrimp-absent group (p < 0.05), indicating a trend of gradual aggregation. The distribution probability for recently hatched juvenile in the shrimp-present group remained consistently low, with no clear pattern observed across the days of the experiment. Recently hatched juveniles in the shrimp-absent group were observed to beg more evenly distributed in the experimental tank, while the juveniles in the shrimp-present group were concentrated mainly at the edges.

Overall, the distribution probability for subadult snails was likewise significantly lower in the shrimp-present group than in the shrimp-absent group (Figure 3). The average distribution probability for subadult snails over six days was 0.625 ± 0.018 in the shrimp-present group and 0.906 ± 0.006 in the shrimp-absent group, revealing a significant difference between the two groups (t = 14.851, p < 0.001).

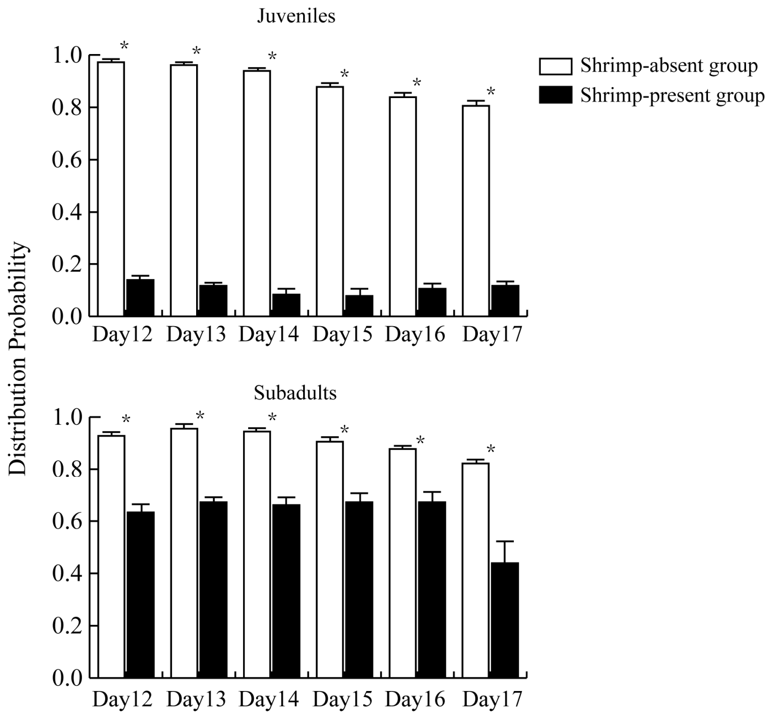

Figure 3. Distribution probability for recently hatched juvenile snails and subadult snails in the shrimp-present and shrimp-absent groups. The asterisk (*) represents a significant difference ($p < 0.05$) between shrimp-absent group and shrimp-present group.

The Tukey HSD test results indicated no significant difference in the average distribution probability between the shrimp-absent groups of recently hatched juvenile and subadult snails ($p = 0.984$). However, there was a significant difference in the average distribution probability between shrimp-present groups of hatched juveniles and subadult ($p < 0.001$).

3.4. M. rosenbergii Survival and Size Changes and Estimation of their Control Efficiency on P. canaliculata

A comparison of the change in size of the *M. rosenbergii* among the treatment groups and the controls (Table 1) showed no significant differences during the experiment, and all prawns survived. Therefore, variations in the individual sizes of the *M. rosenbergii* throughout the experiment could not have influenced the *P. canaliculata* that they were reared with. The *M. rosenbergii* exhibited slight growth during the 18-day experiment, with no significant differences in growth among groups, and the magnitude of size change was minor.

Based on the results, a preliminary assessment of the control efficiency of *M. rosenbergii* on *P. canaliculata* could be made. Accordingly, the control efficiency of *M. rosenbergii* based on the population density of newly hatched juveniles of *P. canaliculata* was estimated to be 89.26%, with an inhibitory effect on juveniles' growth based on the shell width of surviving snails reaching 68.61%, and inhibition of the extent of area where juvenile snails were active was estimated as 88.16% (Table 2). However, *M. rosenbergii* had no significant impact on the density and growth of subadults of *P. canaliculata* ($p > 0.05$), and inhibition of the active area of the subadult reached 30.98% (Table 2).

Table 1. The change in size of *Macrobrachium rosenbergii* during the rearing experiment with *Pomacea canaliculata*.

	Mean Prawn Size		
	Prawns with Juvenile Snails	**Prawns with Subadult Snails**	**Controls (Prawns without Snails)**
Number of *M. rosenbergii* at the start of the experiment	8	8	8
Number of *M. rosenbergii* at the end of the experiment	8	8	8
Average length of *M. rosenbergii* (cm)	8.30 ± 0.01	8.29 ± 0.01	8.28 ± 0.01
Length of *M. rosenbergii* on day 1 (cm)	8.23 ± 0.01	8.23 ± 0.01	8.25 ± 0.01
Length of *M. rosenbergii* on day 12 (cm)	8.31 ± 0.05	8.30 ± 0.02	8.28 ± 0.02
Length of *M. rosenbergii* on day 18 (cm)	8.36 ± 0.03	8.35 ± 0.02	8.31 ± 0.02

Table 2. Control efficiency * of *Macrobrachium rosenbergii* on *Pomacea canaliculata* in the laboratory experiment, based on snail density (number), snail growth (shell width), and extent of the snails' active area.

	Number	**Shell Width**	**Range of Activity**
Juvenile	89.26%	68.61%	88.16%
Subadult	−4.60%	0.49%	30.98%

* Control efficiency = (Mean value in shrimp-absent snail treatment—Mean value in shrimp-present snail treatment)/Mean value in control group (shrimp only).

4. Discussion

4.1. Effectiveness of M. rosenbergii in Controlling P. canaliculata

The results of this experiment demonstrate that *M. rosenbergii* will prey on newly hatched juveniles of *P. canaliculata*, significantly repressing the population of juvenile snail snails (Figure 1). Similarly, Roberts et al. [41] found that *M. rosenbergii* will prey on the freshwater snail *Biomphalaria glabrata*.

The presence of *M. rosenbergii* slowed the growth of individual *P. canaliculata* juveniles in the experimental tanks (Figure 2). This could be attributed to reduced opportunities for the juvenile snails to feed because of the presence of predation by the prawns. Differential feeding preferences exhibited by *M. rosenbergii* for various sizes of snails results in them selecting juvenile snails [42].

Additionally, the presence of *M. rosenbergii* limited the distribution of *P. canaliculata* juveniles in the experimental tanks (Figure 3). The consumption of juvenile snails by *M. rosenbergii* would accordingly result in less area occupied by surviving snails [43]. However, juvenile snails might exhibit active avoidance behavior, but this requires further investigation. These findings demonstrate the potential value of using *M. rosenbergii* in controlling invasive *P. canaliculata*, managing both the scale of *P. canaliculata* reproduction and the spread of juveniles.

Notably, in our experiment, *M. rosenbergii* had almost no impact on subadults of *P. canaliculata* with a shell height greater than 2 cm (Table 2). Thus, it can be predicted that the inhibitory effect of *M. rosenbergii* will be limited for populations of larger-sized adults. This result is partly attributable to the small size of *M. rosenbergii* used in the experiment and partly because the shells of subadult or adult *P. canaliculata* are relatively hard, making it difficult for many predators to break them [21,44,45]. Therefore, when using cultured *M. rosenbergii* for controlling *P. canaliculata* invasion, additional measures like manual removal of adult snails may be needed to improve the control effect.

4.2. Potential Application of M. rosenbergii for Controlling P. canaliculata Invasion

Currently, some studies have conducted experiments on controlling *P. canaliculata* using symbiotic systems of ecological farming, such as rice–duck and rice–fish schemes, primarily by employing natural predators of the invasive species [46,47]. Studies that utilized the ecological farming model of raising *Tadorna tadorna* in rice fields to control *P. canaliculata* found that co-cultivating ducks with rice could also reduce the overwintering and residual snail population in rice fields [48,49]. The co-cultivation of ducks primarily takes advantage of their preference for feeding on juvenile snails, thereby reducing the snail population. However, during the early stages of plant growth, ducks may consume tender plant shoots, necessitating their timely removal from the rice paddies [50]. Moreover, the high cost and extensive labor required for duck farming, along with the need to strictly control the number of ducks released to avoid water pollution [51], limits the applicability of using rice–duck co-cultivation to control agricultural *P. canaliculata*.

Black carp are carnivorous fish that feed on snail meat. Researchers have found that stocking black carp can be an effective method for controlling *P. canaliculata* in water chestnut fields. However, to achieve better control of *P. canaliculata*, larger-sized black carp and a sufficient water depth that allows their movement are required [52], which imposes significant limitations on the widespread promotion of black carp for controlling agricultural invasions of *P. canaliculata*.

In contrast, *M. rosenbergii* can be farmed in a wide range of areas and has modest feed requirements, making it suitable for aquaculture. Moreover, *P. canaliculata* provides a rich and convenient source of biological material for *M. rosenbergii* to effectively prey on as a dietary supplement beyond mere feed, thereby achieving biocontrol. A previous study found that a higher percentage of *P. canaliculata* in the feed formula was more beneficial for the molting and growth of *M. rosenbergii* [53].

4.3. Potential Risks of Using M. rosenbergii for Biocontrol of P. canaliculata

Although some natural predators can exert some control over *P. canaliculata*, large-scale promotion of these predators poses certain biosecurity risks. Regarding the red swamp crayfish *Procambarus clarkii*, for example, its omnivory threatens native biodiversity, and its burrowing behavior can disrupt eco-structural integrity, with risks for using it as a biocontrol agent [54]. In comparison, the use of *M. rosenbergii* to control *P. canaliculata* poses a lower safety risk. First, although *M. rosenbergii* is also an introduced species in China, it is a tropical shrimp with a preferred temperature range of 25–30 °C and a minimum limit of 14 °C [55]. Below 18 °C, it exhibits reduced activity, decreased feeding, slowed growth, and increased mortality, meaning it is unable to survive the winter in most parts of China and Japan [55,56]. Second, *M. rosenbergii* requires seawater for breeding, as it cannot complete its life history and reproduction in freshwater [32,55]. Therefore, it is difficult to establish a reproductive population in pure freshwater environments where *P. canaliculata* is active, making invasion virtually impossible. Furthermore, *M. rosenbergii* is a benthic species capable of swimming only short distances, making it unable to disperse over great distances [55,56]. Recent experiments have employed all-male prawn juveniles for biocontrol purposes, thereby mitigating the risks of its reproduction [42]. Thus, it appears that the use of *M. rosenbergii,* especially its sex-biased seeding [42], to control *P. canaliculata* invasion would have minimal impact on local biodiversity.

Currently, the deployment of *M. rosenbergii* for controlling invasive *P. canaliculata* in Asian rice paddies appears to be a prudent approach. However, considering the varying potential risks across different regions in Asia, separate strategies for its application should be considered. In Southeast Asia, where *M. rosenbergii* is a native species that does not pose a risk of biological invasion, its promotion for controlling *P. canaliculata* in rice paddy systems is highly feasible. However, in China and Japan, where *M. rosenbergii* is an introduced aquaculture species, there is a risk of its population expansion. Although the current capacity of *M. rosenbergii* for wild reproduction and dissemination is limited in these regions, precautionary measures against potential invasiveness are necessary when advocating its utilization for *P. canaliculata* control.

We recommend the following measures when considering *M. rosenbergii* for biocontrol:

- Consider its deployment in isolated water bodies, artificial wetlands, or rice paddies, avoiding introduction into natural water habitats;
- Employ physiologically stable *M. rosenbergii* juveniles for release to minimize the introduction of individuals with high variability. The utilization of sex-biased seedings of *M. rosenbergii* might to ensure biological security. Simultaneously, the potential for hybridization between *M. rosenbergii* and indigenous freshwater prawn species should also be investigated to prevent hybridization-induced population dispersion [57];
- Capture *M. rosenbergii* from the release areas during autumn and winter, subjecting them to dry ponds or sun-drying treatments.

Simultaneously, in East Asian countries in general, the use of locally adapted shrimp species with similar physiological traits and dietary habits to manage *P. canaliculata* reproduction and expansion is also worth investigating to further mitigate ecological risks associated with the use of a non-native species. For instance, in China, the indigenous East Asian river prawn *M. nipponense* shares similarities with *M. rosenbergii* and therefore merits research into its feasibility and effectiveness as a biocontrol agent against *P. canaliculata*.

5. Conclusions

This study demonstrated the moderate biocontrol effect of *M. rosenbergii* on *P. canaliculata* juveniles under laboratory conditions. Subsequent research could be conducted on a pilot scale, such as in rice fields or constructed wetland ecosystems, to further elucidate the value of using *M. rosenbergii* to control *P. canaliculata*. Given the uncertainties in profit of *P. clarkii* caused by fluctuations in the market price, seasonal diseases, and ecological risks for rice paddy farming as well as in rice–fish and rice–shrimp co-cultivation, introducing *M. rosenbergii* into fields affected by a rampant *P. canaliculata* invasion might be an effective and safe choice. On one hand, such application could optimize an aquaculture species and significantly improve economic returns in agricultural fields; however, the choice could help control these highly invasive snails, inhibiting their reproduction and spread, thus effectively reducing their harmful impact on agricultural fields, constructed wetlands, and other ecosystems, thereby producing positive ecological benefits.

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Data Availability Statement: All data, models, or code generated or used during the study are available from the corresponding author by request.

Conflicts of Interest: The authors declare no conflict of interest.

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