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Prawn monosex populations as biocontrol agents for snail vectors of fish parasites

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ABSTRACT

An unresolved problem in aquaculture ponds is the susceptibility of freshwater fish to parasitic diseases, such as those caused by *Centrocestus* trematodes, which are transmitted by snails of the Thiaridae family. Two species of this family are common in *Tilapia* aquaculture ponds in Israel, the endemic *Melanoides tuberculata* and the invasive *Thiara scabra*, both being hosts of various disease-causing parasitic trematodes. Promising biocontrol agents are freshwater prawns of the genus *Macrobrachium*, which are known to be voracious predators of freshwater snails. Prawns in contrast to fish, occupy a benthic niche in the aquaculture pond and may thus be expected to prey on disease-carrying snails, which burrow into the pond bottoms. Prawns also bring the added advantage of constituting a high-value side-product to the pond-produced biomass without the need for additional feed. Following recent biotechnological advances in monosex prawn production, non-reproducing monosex prawn populations are now commercially available for testing as ecologically safe biocontrol agents in aquaculture. Laboratory predation experiments examining the ability of monosex *Macrobrachium rosenbergii* prawns to prey on *Melanoides tuberculata* and *T. scabra* snails showed that even a single prawn weighing > 4 g is capable of exterminating dozens of snail hatchlings per day and hence of preventing hatchling recruitment in both snail species. Large prawns exhibited significantly superior predation abilities with respect to large snails of both species, with *Melanoides* snails being more susceptible to predation than *Thiara* snails. This study is the first to integrate laboratory studies with field observations on the utility of monosex all-female *Macrobrachium rosenbergii* prawns as biocontrol agents of damaging snails in fish ponds. These prawns were found to be effective biocontrol agents in *Tilapia* growout aquaculture ponds in terms of reduction in both snail abundance and rates of fish infection with the snail-borne parasites. The promising results of this biocontrol method call for further study aimed at optimizing the biocontrol power and profit of monosex prawn populations in polyculture with fish as part of wider and more comprehensive ecological risk assessment studies.

1. Introduction

Aquaculture, including the finfish industry, is one of the fastest growing animal production sectors worldwide (Zhou 2017), with *Tilapia* being the second most widely cultured fish globally. Almost 6 million metric tons of *Tilapia* are produced worldwide annually, with the industry showing an average annual growth rate of 10% over the last 20 years (FAO 2017). This trend in *Tilapia* aquaculture has driven the sector towards more intensive aquaculture systems with increasingly higher stocking densities, which renders the fish particularly vulnerable to pathogens (Garcia et al. 2013). One such pathogen that has surprisingly attracted relatively little attention in the scientific literature is the fish gill trematode *Centrocestus formosanus* (Digenea: Heterophyidae). This parasitic worm requires three hosts to complete its life-cycle—a snail and a fish as its intermediate hosts and a vertebrate (mammal or bird) as its definitive host (Chen 1948). In the fish, the trematode infects the gills and forms metacercariae that impair respiratory efficiency, which restricts growth and may lead to a decrease in survival rates (Arguedas Cortes et al. 2010). If an infected fish is consumed by a vertebrate (a bird in most cases), the worm matures in

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the vertebrate small intestine, where it produces eggs that are released in the feces. Any eggs released into a water body will hatch and infect the first intermediate host, for example, the freshwater snail *Melanoides tuberculata*. This parthenogenetic snail is endemic to the Middle East, but in recent years has become an invasive species in various countries worldwide, taking its parasitic trematodes with it (Pinto et al. 2018; Pointier and McCullough 1989). Indeed, *Melanoides tuberculata* serves as an intermediate host for 37 species of parasitic trematodes that infect not only fish and birds, but also mammals, including humans (Chai et al. 2013; Pinto and De Melo 2011). In both cultured and wild fish populations, including economically important and endangered fish species, infection with *C. formosanus* is associated with severe pathology (Fleming et al. 2011; Gjurcevic et al. 2007; Mitchell et al. 2002). Since no treatment for infected fish is currently available, most efforts to control the parasitic trematode and its mollusk host rely on the use of non-sustainable chemical molluscicides (Francis-Floyd et al. 1997; Mitchell et al. 2007), which may harm non-targeted species. Alternative snail control programs include the mechanical removal of snails from the pond bottom. However, this method is expensive and is only partially successful, since industrial aquaculture facilities are not isolated from the surrounding environment where snails are abundant and can easily repopulate the ponds (Leighton et al. 2000). Biological control of the snail vectors is considered the most environmentally safe method for exterminating the trematodes, but it is also not hazard free. The most serious risk in the introduction of biocontrol agents is the establishment of permanent invasive populations of the biocontrol agent in the new environment. Such an invasion could result in displacement of native species, interbreeding with local species, and the introduction of new pathogens (Ehlers 2011; Howarth 2001). There is also a risk that the biocontrol species will affect non-targeted organisms and biodiversity.

For the biocontrol of snails in aquaculture ponds, the black carp *Mylopharyngodon piceus* is a possible candidate. This species is an effective predator of snails, but it is not a suitable biocontrol species for intensive aquaculture facilities, since it competes with the farmed fish for food and the availability of carp fingerlings is not always reliable (Ben-Ami and Heller 2001). Moreover, species of carp, like many other fish species, are also susceptible to infection with *C. formosanus*, and, therefore, their efficiency as biocontrol agents is far from ideal (Slootweg 1995). Alternative candidates – those used in the current study – are freshwater prawns of the genus *Macrobrachium*, which are known to be voracious predators of freshwater snails.

In our study area, the Valley of Springs, Israel, *C. formosanus* was first detected more than three decades ago in the Sea of Galilee (Farstey 1986), but it has become a serious pest in commercial aquaculture ponds only in the past 15 years. In this area, in addition to the endemic *Melanoides tuberculata*, two species of invasive Thiaridae snails, *Thiara scabra* and *Tarebia granifera*, act as intermediate hosts for parasitic trematodes, whose definite hosts include fish, birds and various vertebrates (including intestinal flukes in humans). The target species for the current study were *Melanoides tuberculata* and *T. scabra*, which are the most common species found in *Tilapia* aquaculture ponds in our study area (Ben-Ami 2006; Chontananarth and Wongsawad 2010; Heller et al. 2014; Jayawardena et al. 2011). In this study, the freshwater prawn *Macrobrachium rosenbergii* was tested as a putative biocontrol agent for the commercial *Tilapia* ponds of the Valley of Springs. The rationale for the choice of *Macrobrachium rosenbergii* was twofold. Since prawns and fish occupy different niches in the pond, the species has already been used in co-culture with *Tilapia* [as means of intensifying food production systems (Garcia-Pérez et al. 2000; Tidwell et al. 2010; Uddin et al. 2007)]. Also, recent biotechnology solutions for the production of non-breeding *Macrobrachium rosenbergii* monosex populations – both all-female and all-male (Levy et al. 2017; Ventura et al. 2012) – has laid down the foundation for the exploitation of these prawns as environmentally safe and sustainable biocontrol agents against damaging freshwater snails (Savaya-Alkalay et al., 2018a, 2018b). Moreover, prawns of the *Macrobrachium* genus are not susceptible to fish-gill trematode infection and are voracious predators of freshwater snails (Lee et al. 1982; Roberts and Kuris 1990). Several laboratory studies have demonstrated that *Macrobrachium* prawns eliminate snail hatchlings (Savaya-Alkalay et al., 2018a, 2018b; Sokolow et al. 2014), but a controlled field experiment is needed to demonstrate that the prawns exhibit similar predation patterns under field conditions. In the present study, prawn predation efficiencies were examined under laboratory conditions and, for the first time, in a field study that included sampling of both snails and fish.

2. Materials and methods

2.1. Laboratory experiments

2.1.1. Animals

The *Macrobrachium rosenbergii* prawns used for the laboratory experiments were supplied by Colors Ltd., Hatzeva, Israel (all-male prawns) and by Northern Prawn Ltd., Dan, Israel (all-female prawns). Both all-males and all-females population are normal males and females with respect to their genotype and phenotype (Levy et al. 2017; Ventura et al. 2012). Only naive prawns that had never fed on any snail species were used in the predation experiments. Prawns were fed three times a week (every other day) with shrimp pellets (Raanan Fish Feed, I.Z. Miluot, Israel, 40% protein) and once a week with frozen food (*Artemia* and bloodworms, Ocean Nutrition Ltd., CA, USA). Snails were collected from the aquaculture ponds at Kfar Ruppin (N 32.452889, E 35.555352) during the 2017 and 2018 fish growout seasons. Both prawns and snails were maintained in 100-L plastic tanks with submerged biological and mechanical filters to maintain water quality. An air condition system maintained the water temperature at 27 ± 3 °C.

2.1.2. Proof of concept experiment

To determine the utility of conducting a comprehensive biocontrol study, a pilot proof-of-concept experiment was first performed by introducing a large male (84 g) *Macrobrachium rosenbergii* prawn into a tank containing 10 mature *T. scabra* snails. The tank was monitored daily until all the snails had been consumed (10 days). The conditions were maintained as detailed above.

2.1.3. Male and female prawn predation on Melanoides tuberculata snails

This experiment was conducted with *Macrobrachium rosenbergii* prawns of both sexes to determine whether there is any difference in the predation efficiencies between male and female prawns. The experiment included 4 replicates of 5 treatment groups, which differed in prawn weights (details are given in Table 1), where each 100-L tank was stocked with 10 mature *Melanoides tuberculata* snails as the starting population. The weights of prawns and snails are presented in Table 2. The experiment was conducted in two repetitions, each time for 8 weeks. Snail survival was monitored weekly, and large (starting population) and small (recruited hatchlings) snails were counted separately.

Table 1
Experimental setup for the *Melanoides tuberculata* predation experiment.

Males		Females	
Control	No prawns	Control	No prawns
PLs	3 post-larvae prawns	PLs	3 post-larvae prawns
Small (~4.5 g)	1 prawn	Small (~5 g)	1 prawn
Medium (~14 g)	1 prawn	Medium (~14 g)	1 prawn
Large (~28 g)	1 prawn	Large (~28 g)	1 prawn

Table 2
Prawn and snail weights (g) in the laboratory prawn predation experiments.

	Prawn size group				Snail species	
	PL	Small	Medium	Large	*M. tuberculata*	*T. scabra*
M. tuberculata	0.18 ± 0.03	4.9 ± 0.5	14.4 ± 0.8	28.05 ± 1.4	2.09 ± 0.01	–
T. scabra	0.12 ± 0.04	6.4 ± 0.5	14.8 ± 1.1	24.4 ± 1.1	–	4.63 ± 0.01
Both snail species	0.49 ± 0.02	5.1 ± 0.3	15.1 ± 1.1	32.4 ± 4.5	1.73 ± 0.01	2.73 ± 0.02

PL - post larvae. 'Prawn weight' indicates weight of an individual animal. 'Snail weight' indicates the weight of 10 mature snails. Values in the Table are means ± SE.

2.1.4. Prawn predation on T. scabra snails

Since no difference in predation performance was found between male and female prawns, the experiment to investigate predation on *T. scabra* was conducted with female *Macrobrachium rosenbergii* prawns. The experimental design was similar to that described above. The weights of prawns and snails for this experiment are presented in Table 2.

2.1.5. Predation preference for snail species

Since both *Melanoides tuberculata* and *T. scabra* snails are found in the fish ponds in our study area, an experiment was designed to examine whether any predation preference on the part of the prawns for one species over the other existed. The design of the experiment was the same as that above with the exception that in each tank the starting population comprised 5 mature *Melanoides tuberculata* snails and 5 mature *T. scabra* snails. Experimental procedures and data collection were also similar to those in the above experiments. The weights of prawns and snails for the predation preference experiment are presented in Table 2.

2.2. Field experiment

2.2.1. Animals and aquaculture facilities

The field experiment took place between July and October 2018. All-female *M. rosenbergii* prawns were supplied by Northern Prawn Ltd., and hybrid tilapia (*Oreochromis niloticus* X *O. aureus*) were produced by the Maoz-Haim Aquaculture Farm. In order to maximize the predation efficiency and since prawns are size-selective with respect to their predation over snails (Savaya-Alkalay et al., 2018a, 2018b), two size classes of prawns were used for the field experiment. Prawn post larvae (PL; ~22,000 at ~0.075 g) and medium-sized prawns (450 specimens at ~20 g) were stocked in each of three commercial aquaculture ponds at Kfar Ruppin (treatment ponds). Three additional ponds stocked only with fish fingerlings served as the control. Information regarding pond sizes and density of animals is presented in Table S1. All ponds were treated with commercial copper sulfate (25 kg per 1000 m^2) as a molluscicide one week before stocking to ensure an equal starting point with respect to the pond snail population.

2.2.2. Snail survey

Snails were collected from same four collection points (fixed with the 'Mapit GIS' GPS application) in each of the ponds immediately before the beginning of the experiment and after the ponds had been harvested. The collection was performed with a 25 × 25 (cm) sieve, where all materials in the sieve (snails, mud, stones, etc.) were collected for future analyses. Snails were washed and graded into four size classes (mm width): 0–2, 2–4, 4–6, 6+. After the size separation, the snails were stocked into water tanks for 24 h to detect vitality: Living snails climbed the tank walls, while dead snails remained on the bottom.

2.2.3. Fish infection

At the end of the season (October 2018), 10 fish were sampled from each pond for inspection and analysis of parasitic infection. Fish were anesthetized on ice for 10 min, and the first gill arch on each side of the fish was dissected out and observed under a microscope. Metacercarial counts were performed, and the average number of metacercariae per fish was determined for statistical analyses.

2.3. Statistical analyses

2.3.1. Laboratory experiments

In all three experiments (2.1.3, 2.1.4, 2.1.5), the combined effect of prawn size and sex (2.1.3) on the number of snails (both size and species) was tested using a set of repeated-measures ANOVA analyses. Since prawns belonging to the PL group had demonstrated non-consistent effects on the number of snails consumed in all experiments, the relation between PL prawn biomass (grams) and the number of small and large snails consumed in all experiments was analyzed using a set of simple linear regression models.

2.3.2. Field experiment

The effects of the prawns on the total biomass of snails of both species and on the proportion of live snails (live snails/total number of snails) under field conditions were tested using a similar set of repeated-measures ANOVAs. In addition, the effect of the prawns on the average number of parasites detected in fish gills under field conditions was tested using nested ANOVA, with prawn treatment as the whole plot factor (prawn treated ponds vs. no-prawn control ponds) and the experimental pond nested within the prawn treatment.

Whenever the data violated the assumptions of normality and homogeneity, count data were square-root transformed ($y = \sqrt{x + 0.5}$), biomass data were log transformed and proportions data were transformed using an angular transformation ($y = \arcsin\sqrt{x}$), prior to all analyses. In addition, in all cases (due to violation of the sphericity assumption for univariate tests) within subject, variation was examined using a set of multivariate tests. Significant differences between groups were examined post-hoc using the Tukey HSD test. Differences between specific prawn size treatments were tested using specific pair-wise a-priori contrasts. All analyses were performed using STATISTICA v.12 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Laboratory experiments

3.1.1. Proof-of-concept

Fig. 1 shows *T. scabra* snails at five different times during the proof-of-concept experiment, which demonstrated that by day 10 all the snails had died. This encouraging result has led us to study the predation efficiencies of *Macrobrachium rosenbergii* prawns on the two species of Thiaridae snails, as described below.

3.1.2. Prawn predation on Melanoides tuberculata

3.1.2.1. Effect of prawn sex. Overall, there was no significant effect of prawn sex on the number of snails consumed (repeated-measures ANOVA: $F_{1,33} = 0.15$, $p = .694$). Moreover, none of the interactions between prawn sex and any of the other within-subject factors were significant (see Table S3 for the complete repeated measures ANOVA results).

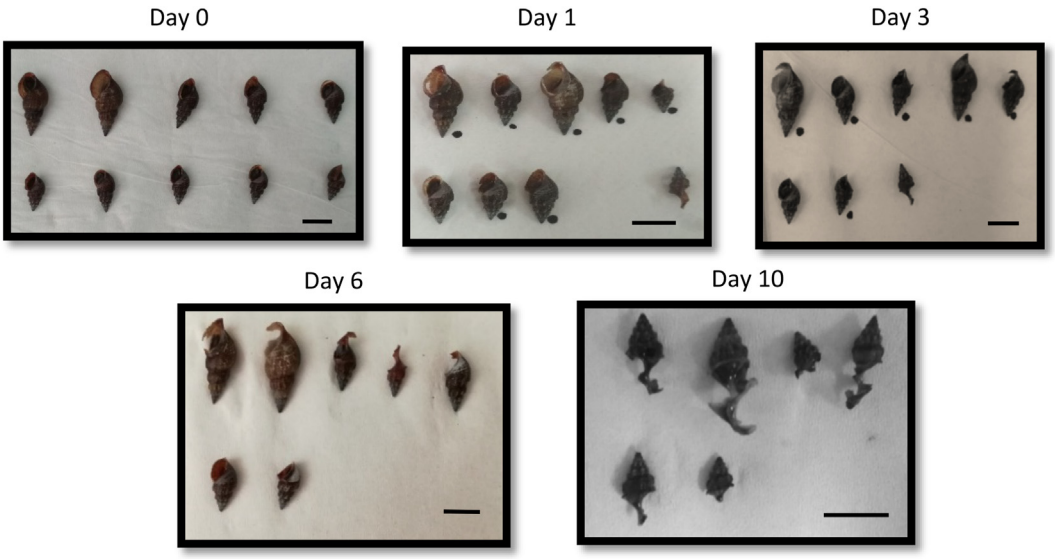

Fig. 1. Predation proof of concept: 10 mature *Thiara scabra* snails in the presence of a large (84 g) prawn. Live snails are positioned with the opperculum faced upwards, and partially damaged snails are marked with a black dot. Ten days after the experiment began, all the snails had been preyed upon by the prawn. Bar represents 1 cm.

3.1.2.2. Effect of prawn size. The statistical analysis revealed significant differences in the number of snails consumed by prawns of various sizes (repeated-measures ANOVA: $F_{4,33} = 79.06$, $p < .001$). Notably, there was a significant interaction between prawn size and snail size (multivariate tests for repeated-measures ANOVA: $F_{4,20} = 132.44$, $p < .001$; see Table S4 for the complete multivariate test for repeated measures ANOVA results). As shown in Fig. 2, PL prawns significantly reduced the number of small snails in the two experimental replicates (experiment 2.1.3; Fig. 2C; a-priori contrasts control vs. PL: $t_{78} = -4.62$, $p < .001$). Similarly, the small-sized prawns further reduced the number of small snails (Fig. 2C; a-priori contrasts PL vs. small: $t_{78} = -9.45$, $p < .001$) in both experimental replicates. In addition, there were no significant differences in the consumption and consequent elimination of all the small snails by prawns of small, medium and large sizes (a-priori contrasts for small vs. medium vs. large: $t_{78} = -2.43$, $p = .020$) in the two experimental replicates (Fig. 2C). The PL and small-size groups did not significantly reduce the number of large snails compared to the control in the two experimental replicates (Fig. 2A; a-priori contrasts control vs. PL and small: $t_{78} = 0.01$, $p = .98$). In contrast, both medium- and large-sized prawns significantly reduced the number of large snails in both experimental replicates (a-priori contrasts control PL and small vs. medium and large: $t_{78} = -5.29$, $p < .001$), with large prawns consuming the highest number of large snails in both experimental replicates (Fig. 2A; a-priori contrasts medium vs. large: $t_{78} = -2.84$, $p = .007$).

3.1.3. Prawn predation on T. scabra

3.1.3.1. Effect of prawn size. The statistical analysis revealed significant differences in the number of snails consumed by prawns of various sizes (repeated-measures ANOVA: $F_{4,33} = 49.21$, $p < .001$), but this effect was inconsistent for small- vs. large-sized snails (prawn size × snail size interaction: $F_{4,15} = 51.88$, $p < .001$). Specifically, prawns of all sizes did not significantly reduce the number of large snails in comparison to the no-prawn control (Fig. 2B). However, prawns of small, medium and large sizes eliminated all the small snails by the end of the experiment (Fig. 2D). Remarkably, there were no significant differences in the consumption of small snails by prawns of small, medium and large sizes (a-priori contrasts small vs. medium and large: $t_{78} = -0.03$, $p = .972$). In addition, prawns of the PL group did not reduce the number of small snails in comparison to the control (Fig. 2D; a-priori contrasts control vs. PL: $t_{78} = -1.65$, $p = .119$).

3.1.4. Prawn predation preference between the two snail species

3.1.4.1. Small snails (hatchlings). Prawns of all sizes significantly reduced the number of small snails (repeated-measures ANOVA: $F4{,}15 = 5.23$, $p < .001$). Specifically, prawns of small, medium and large sizes completely eliminated all the small snails by the end of the experiment.

3.1.4.2. Large snails. Prawns preferred *T. scabra* to *Melanoides tuberculata* snails (2.5 ± 0.10 and 3.93 ± 0.21; mean number of surviving individuals ± SE, respectively. Repeated-measures ANOVA: $F_{7,9} = 3.77$, $p = .034$). Full results for surviving snails are presented in Table S3.

3.1.5. Post larvae predation abilities

Due to the availability of the PL at the times of the various experiments, the PL used in three laboratory experiments were of different sizes (Table S1). While in the *Melanoides tuberculata* predation experiment the PL (~0.18 g) had a significant effect on snail hatchlings, in the *T. scabra* experiment the PL group (~0.12 g) did not differ from the control group (Fig. 2D). In the predation preference experiment (*Melanoides tuberculata* vs *T. scabra*), the PL were larger than in the other two experiments (~0.49 g) and, therefore, almost completely exterminated the snail hatchlings compared to the control group (Fig. 3; 4.75 ± 2.25 and 96 ± 17 snails alive in the PL and control group, respectively). Full results of surviving snails in this experiment are presented in Table S3.

For the three experiments taken together, there was a positive linear relation between PL weight and consumption of both small and large snails (linear regression: $F_{1,14} = 5.26$, $p = .03$, $R^2 = 0.273$ and $F_{1,14} = 4.74$, $p = .04$, $R^2 = 0.253$ for small and large snails, respectively).

3.2. Field experiment

3.2.1. Snails

In general, prawns have significantly reduced the total biomass of snails of all species and size classes ($F_{1,4} = 8.45$, $p = .043$). Similarly, prawns have significantly reduced the proportion of living snails ($F_{1,4} = 25.35$, $p = .007$). Moreover, there was a significant interaction

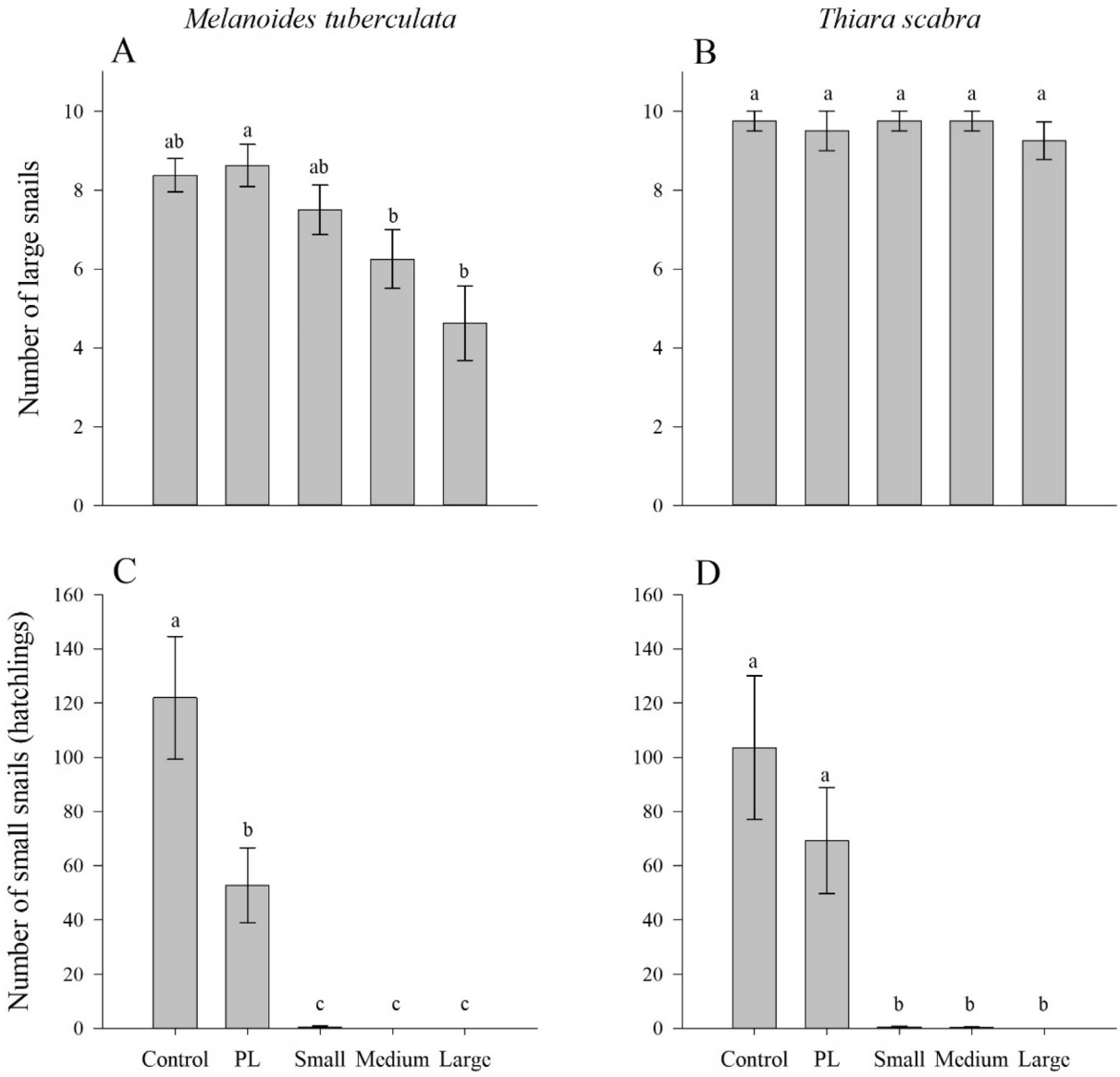

Fig. 2. Predation of snails by prawns under laboratory conditions. (A) & (B) Number of surviving large snails (starting population) by the end of the experiment for *Melanoides tuberculata* and *Thiara scabra*, respectively. (C) & (D) Number of small (hatchling) *M. tuberculata* and *T. scabra* snails by the end of the experiment. Bars represent the means ± SE.

between treatment and size class (Fig. 4B; $F_{3,2} = 22.73$, $p = .042$). Specifically, prawns have eliminated all living snails of the two smallest size classes [size 1(0–2 mm): $t_{78} = -11.02$, $p < .001$, size 2(2–4 mm): $t_{78} = -7.38$, $p = .001$]. Also, prawns have significantly reduced the proportion of living snails in the third size class [size 3(4–6 mm):$t_{78} = -3.19$, $p = .033$], but had no significant effect over snails of the largest size class [size 4(6+ mm):$t_{78} = -1.33$, $p = .253$].

3.2.2. Fish

Although there was a great variability in parasite load between the different experimental ponds (within each treatment: nested ANOVA-experimental pond (treatment)), there were significant differences between the no-prawn controls and the prawn-treated ponds in the average number of parasites per fish (nested ANOVA- treatment: $F_{4,54} = 8.36$, $p = .044$) at the end of the growout season (Fig. 5A).

4. Discussion

Previous predation experiments have shown that *Macrobrachium rosenbergii* prawns are voracious predators of freshwater snails under laboratory conditions (Lee et al. 1982; Savaya-Alkalay et al., 2018a, 2018b; Sokolow et al. 2014). To our knowledge, the present study is the first to demonstrate that prawns significantly reduced snail recruitment under both laboratory and field conditions. Moreover, we have extended the array of damaging snails that can potentially be controlled by freshwater prawns from the Planorbidae (genera *Bulinus* and *Biomphalaria*) and Ampullariidae genus *Pomacea*) families to include members of the Thiaridae family (genera *Thiara* and *Melanoides*).

The proof-of-concept experiment on the predation abilities of *Macrobrachium rosenbergii* on *T. scabra* snails established the rationale for a more extensive study of the predation efficiencies of *Macrobrachium rosenbergii* prawns on Thiaridae snails. In previous studies it was found that the predation efficiencies of prawns, similarly to those of other crustaceans, are size selective; namely, the prawns start

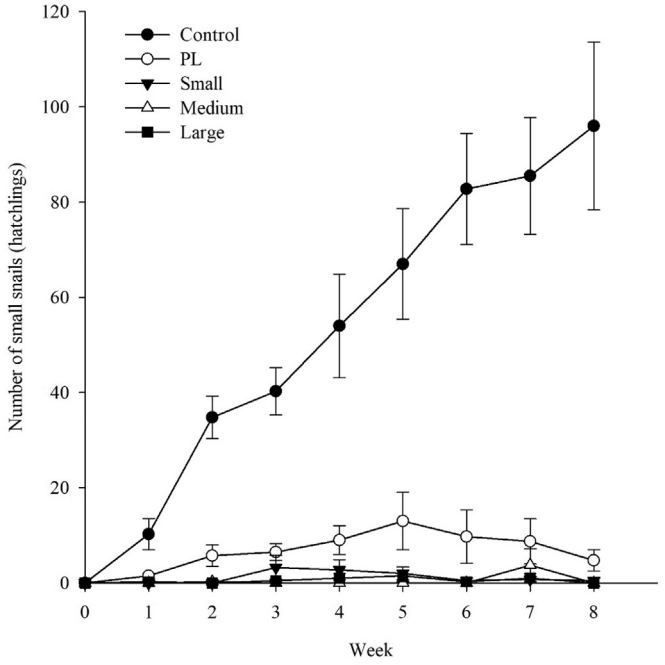

Fig. 3. Number of small snail (hatchlings) in the predation preference experiment. Bars represent means ± SE.

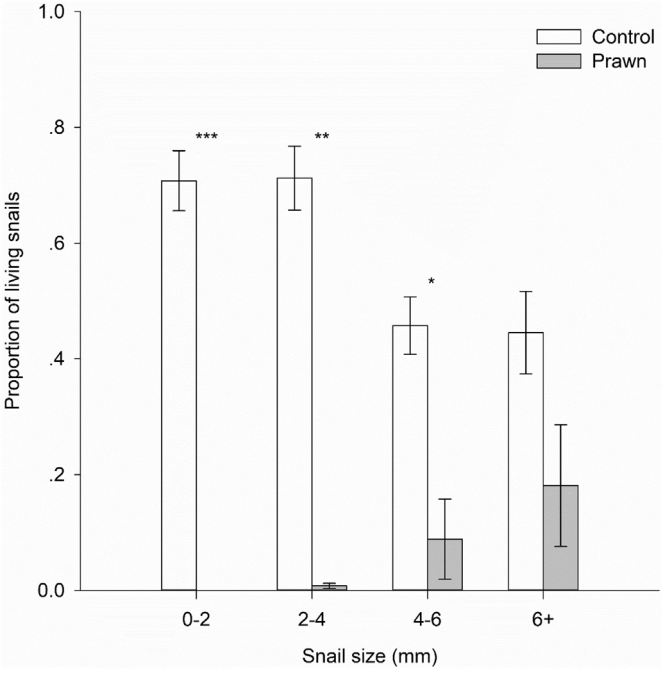

Fig. 4. Snails collected from fish ponds at the end of the experiment. Proportion of live snails out of all snails presented in (A). Bars represent means ± SE. Asterisks represent significant differences between groups (P < .05, P < .01 and P < .001 for *, **, *** respectively).

their predation activity with the smallest prey items (snail hatchlings or eggs) and later continue to prey upon larger snails (Savaya-Alkalay et al., 2018a, 2018b; Torres et al. 2012). Therefore, the complete elimination of snail hatchlings by mature prawns in this study was somewhat predictable. Importantly, under certain experimental conditions, the PLs used in this study were as successful predators of snail hatchlings as mature prawns were. However, whereas the larger PL completely eliminated the snail hatchlings, the smaller PL were less

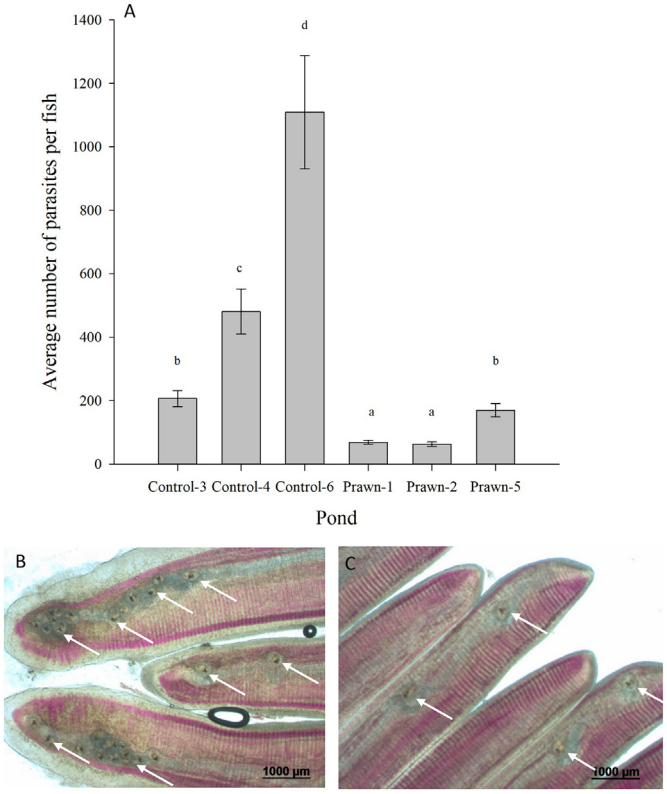

Fig. 5. Parasite infection in the fish. (A) Average number of parasites per fish in the different ponds. (B) & (C) Gills of representative infected fish from ponds 6 (control) and 5 (treatment), respectively. White arrows indicate metacercariae. Bars represent means ± SE. Letters represent significant differences between groups (P < .05).

efficient predators. Therefore, the current findings that prawns weighing above 0.45 g were as efficient as the mature prawns, with respect to elimination of snail hatchlings, is remarkable.

Similarly to the laboratory-based predation results, prawns in the field significantly reduced both the biomass and the proportions of live snails in all the 'treatment' fish ponds. As expected, the prawns had a more marked effect on the proportion of live snails in the two smallest size classes. Complete eradication of snail hatchings is expected to constrain the recruitment of the snail population for the following generations (Chesson 1998). Such a reduction in snail recruitment would decrease the loads of mature snails that are potential hosts for the parasite, and hence decrease the abundance of parasites in the water body and on the fish gills (Thien et al. 2015). Moreover, preferential predation on trematode-infected snails (over healthy non-infected snails) might further decrease parasitic loads in the ponds (Swartz et al. 2015).

While snail biomass and vitality are important indicators of biocontrol efficiency (Roberts and Kuris 1990; Sokolow et al. 2014), the final goal of the suggested strategy is to decrease infection in fish. Therefore, the most important and encouraging result of this study is the reduction in the level of infection in the fish in the treatment ponds. The microscopic examination of fish gills revealed a significant difference between the treatment and control groups at the end of the growout season. This significant reduction in infection rates indicates that the prawns' positive effect on fish health can be observed under aquaculture conditions even after only one season of intervention.

To the best of our knowledge, the agent most commonly used as a molluscicide in aquaculture ponds is copper sulfate, which is applied prior to the introduction of fish to the pond. However, use of this chemical is associated with a number of drawbacks in addition to its lack of complete efficacy as a molluscicide. In addition to its toxicity to

snails, copper sulfate is toxic to algae, fungi, various aquatic organisms, mammals (Ezeonyejiaku et al. 2011) and is especially toxic to fish in high temperatures (Wise et al. 2006). Since Thiridae snails can burrow into the mud and 'isolate' themselves from the environment by sealing their operculum, and since their reproductive strategy is extremely efficient (parthenogenetic and viviparous), it would be extremely difficult to remove them completely from any environment that they have invaded. Therefore, our results are of major importance to the aquaculture industry, since the introduction of snail-preying prawns to aquaculture ponds could not only improve fish health, but also decrease the use of chemical molluscicides, thereby reducing their negative impact on the environment (Herbeck and Unger 2013) and ensuring healthier ecosystems. Moreover, from an economic point of view, the added value of using prawns as biocontrol agents should be similar to the expected income from polyculture of prawns with various fish species (Ferdous Alam and Murshed-e-Jahan 2008; Tidwell et al. 2010). Furthermore, a recent bio-economic model (Hoover et al. 2019) presents a promising scenario for aquaculture-biocontrol combined effort and now that monosex prawn populations have become commercially available, their use as biocontrol agents can be considered environmentally safe (Molcho et al. 2020; Savaya, 2019).

The most remarkable aspect of the current study is that it is the first application of the proposed biocontrol technique beyond the laboratory in commercial aquaculture ponds. Since tilapia–prawn polyculture is a common practice (Garcia-Pérez et al. 2000; Molcho et al. 2020; Tidwell et al. 2010; Uddin et al. 2007), the present study focused on the effect of the prawns on snail abundances and on the intensity of fish parasite infection rather than polyculture performance, economic contribution and ecological considerations. Further research is required to optimize the efficiency and economic value of the proposed biocontrol method, such as: optimal prawn size, optimal prawn and fish stocking densities, and additional fish species that could benefit from such a solution. Comprehensive economic analyses and ecological risk assessments are required for distinct target areas and culture systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.735016.

References

Arguedas Cortes, D., Dolz, G., Romero Zuniga, J.J., Jimenez Rocha, A.E., Leon Alan, D., 2010. *Centrocestus formosanus* (Opisthorchiida: Heterophyidae) as a cause of death in gray tilapia fry *Oreochromis niloticus* (Perciforme: Cichlidae) in the dry Pacific of Costa Rica. Rev. Biol. Trop. 58, 1453–1465.

Ben-Ami, F., 2006. First report of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae) from Israel. Nautilus 120, 156–161.

Ben-Ami, F., Heller, J., 2001. Biological control of aquatic pest snails by the black carp *Mylopharyngodon piceus*. Biol. Control 22, 131–138.

Chai, J.Y., Sohn, W.M., Yong, T.S., Eom, K.S., Min, D.Y., Lee, M.Y., Lim, H., Insisiengmay, B., Phommasack, B., Rim, H.J., 2013. *Centrocestus formosanus* (Heterophyidae): human infections and the infection source in Lao Pdr. J. Parasitol. 99, 531–536.

Chen, H., 1948. Some early larval stages of *Centrocestus formosamu* (Nishigori, 1924). Lingnan Sci. J. 22, 93–104.

Chesson, P., 1998. Recruitment limitation: a theoretical perspective. Aust. J. Ecol. 23, 234–240.

Chontananarth, T., Wongsawad, C., 2010. Prevalence of *Haplorchis taichui* in field-collected snails: a molecular approach. Korean J. Parasitol. 48, 343.

Ehlers, R.-U., 2011. Regulation of biological control agents and the EU policy support action REBECA. In: Regulation of Biological Control Agents. Springer, pp. 3–23.

Ezeonyejiaku, C., Obiakor, M., Ezenwelu, C., 2011. Toxicity of copper sulphate and behavioral locomotor response of tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) species. Online J. Anim. Feed Res. 1, 130–134.

FAO, 2017. Fisheries Statistical Database, Global Aquaculture Productuion (Fisheries Global Information System, Online Query).

Farstey, V., 1986. Infection of the Snail *Melanoides tuberculata* (Müller 1774) and of Cichlid Fish in Lake Kinneret, by *Centrocestus sp*.(Heterophyidae) and Other Trematodes. MSc Thesis. Hebrew University of Jerusalem (Hebrew, English Abstract).

Ferdous Alam, M., Murshed-e-Jahan, K., 2008. Resource allocation efficiency of the prawn-carp farmers of Bangladesh. Aquac. Econ. Manag. 12, 188–206.

Fleming, B.P., Huffman, D.G., Bonner, T.H., Brandt, T.M., 2011. Metacercarial distribution of *Centrocestus formosanus* among fish hosts in the Guadalupe River drainage of Texas. J. Aquat. Anim. Health 23, 117–124.

Francis-Floyd, R., Gildea, J., Reed, P., Klinger, R., 1997. Use of Bayluscide (Bayer 73) for snail control in fish ponds. J. Aquat. Anim. Health 9, 41–48.

Garcia, F., Romera, D.M., Gozi, K.S., Onaka, E.M., Fonseca, F.S., Schalch, S.H.C., Candeira, P.G., Guerra, L.O.M., Carmo, F.J., Carneiro, D.J., Martins, M.I.E.G., Portella, M.C., 2013. Stocking density of Nile tilapia in cages placed in a hydroelectric reservoir. Aquaculture 410, 51–56.

Garcia-Pérez, A., Alston, D.E., Cortés-Maldonado, R., 2000. Growth, survival, yield, and size distributions of freshwater prawn *Macrobrachium rosenbergii* and tilapia *Oreochromis niloticus* in polyculture and monoculture systems in Puerto Rico. J. World Aquacult. Soc. 31, 446–451.

Gjurcevic, E., Petrinec, Z., Kozaric, Z., Kuzir, S., Kantura, V.G., Vucemilo, M., Dzaja, P., 2007. Metacercariae of *Centrocestus formosanus* in goldfish (Carassius auratus L.) imported into Croatia. Helminthologia 44, 214–216.

Heller, J., Dolev, A., Zohary, T., Gal, G., 2014. Invasion dynamics of the snail *Pseudoplotia scabra* in Lake Kinneret. Biol. Invasions 16, 7–12.

Herbeck, L.S., Unger, D., 2013. Pond aquaculture effluents traced along back-reef waters by standard water quality parameters, δ15N in suspended matter and phytoplankton bioassays. Mar. Ecol. Prog. Ser. 478, 71–86.

Hoover, C.M., Sokolow, S.H., Kemp, J., Sanchirico, J.N., Lund, A.J., Jones, I.J., Higginson, T., Riveau, G., Savaya, A., Coyle, S., Wood, C.L., Micheli, F., Casagrandi, R., Mari, L., Gatto, M., Rinaldo, A., Perez-Saez, J., Rohr, J.R., Sagi, A., Remais, J.V., De Leo, G.A., 2019. Modelled effects of prawn aquaculture on poverty alleviation and schistosomiasis control. Nat. Sustain. 2, 611–620.

Howarth, F.G., 2001. Environmental issues concerning the importation of non-indigenous biological control agents. In: Lockwood, J.A., Howarth, F.G., Purcell, M.F. (Eds.), Balancing Nature: Assessing the Impact of Importing Non-Native Biological Control Agents (An International Perspective). Thomas Say Pubs. in Entomologypp. 70–99.

Jayawardena, U., Rajakaruna, R., Amerasinghe, P., 2011. Cercariae of trematodes in freshwater snails in three climatic zones in Sri Lanka. Ceylon J. Sci. (Biol. Sci.) 39.

Lee, P.G., Rodrick, G.E., Sodeman Jr., W.A., Blake, N.J., 1982. The giant Malaysian prawn, *Macrobrachium rosenbergii*, a potental predator for controlling the spread of schistosome vector snails in fish ponds. Aquaculture 28, 293–301.

Leighton, B.J., Zervos, S., Webster, J.M., 2000. Ecological factors in schistosome transmission, and an environmentally benign method for controlling snails in a recreational lake with a record of schistosome dermatitis. Parasitol. Int. 49, 9–17.

Levy, T., Rosen, O., Eilam, B., Azulay, D., Zohar, I., Aflalo, E.D., Benet, A., Naor, A., Shechter, A., Sagi, A., 2017. All-female monosex culture in the freshwater prawn *Macrobrachium rosenbergii*–a comparative large-scale field study. Aquaculture 479, 857–862.

Mitchell, A.J., Goodwin, A.E., Salmon, M.J., Brandt, T.M., 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. N. Am. J. Aquac. 64, 55–59.

Mitchell, A.J., Hobbs, M.S., Brandt, T.M., 2007. The effect of chemical treatments on red-rim melania *Melanoides tuberculata*, an exotic aquatic snail that serves as a vector of trematodes to fish and other species in the USA. N. Am. J. Fish Manag. 27, 1287–1293.

Molcho, J., Levy, T., Benet, A., Naor, A., Savaya, A., Manor, R., Abramov, A., Aflalo, E.D., Shechter, A., Sagi, A., 2020. Three generations of prawns without the Z chromosome: viable WW *Macrobrachium rosenbergii* all-female populations in polyculture with *Oreochromis niloticus*. Aquaculture 515, 734531.

Pinto, H.A., De Melo, A.L., 2011. A checklist of trematodes (Platyhelminthes) transmitted by *Melanoides tuberculata* (Mollusca: Thiaridae). Zootaxa 2799, 15–28.

Pinto, H.A., Goncalves, N.Q., Lopez-Hernandez, D., Pulido-Murillo, E.A., Melo, A.L., 2018. The life cycle of a zoonotic parasite reassessed: experimental infection of *Melanoides tuberculata* (Mollusca: Thiaridae) with *Centrocestus formosanus* (Trematoda: Heterophyidae). PLoS One 13.

Pointier, J.-P., McCullough, F., 1989. Biological control of the snail hosts of *Schistosoma*

mansoni in the Caribbean area using *Thiara spp*. Acta Trop. 46, 147–155.
Roberts, J.K., Kuris, A.M., 1990. Predation and control of laboratory populations of the snail *Biomphalaria glabrata* by the freshwater prawn *Macrobrachium rosenbergii*. Ann. Trop. Med. Parasitol. 84, 401–412.
Savaya, A., 2019. Monosex Prawn Population as Biocontrol Agents over Damaging Snails, Life-Science. Ph.D. Thesis. Ben-Gurion University of the Negev.
Savaya-Alkalay, A., Demba-Ndao, P., Jouanard, N., Diane, N., Aflalo, D.E., Barki, A., Sagi, A., 2018. Exploitation of reproductive barriers between *Macrobrachium* species for responsible aquaculture and biocontrol of schistosomiasis in West-Africa. Aquac. Environ. Interact. 10, 487–499.
Savaya-Alkalay, A., Ovadia, O., Barki, A., Sagi, A., 2018b. Size-selective predation by all-male prawns: implications for sustainable biocontrol of snail invasions. Biol. Invasions 20, 137–149.
Slootweg, R., 1995. Snail control by fish: an explanation for its failure. Naga ICLARM Q. 18, 16–19.
Sokolow, S.H., Lafferty, K.D., Kuris, A.M., 2014. Regulation of laboratory populations of snails (*Biomphalaria and Bulinus spp*.) by river prawns, *Macrobrachium spp*. (Decapoda, Palaemonidae): implications for control of schistosomiasis. Acta Trop. 132C, 64–67.
Swartz, S.J., De Leo, G.A., Wood, C.L., Sokolow, S.H., 2015. Infection with schistosome parasites in snails leads to increased predation by prawns: implications for human schistosomiasis control. J. Exp. Biol. 218, 3962–3967.
Thien, P., Madsen, H., Nga, H., Dalsgaard, A., Murrell, K., 2015. Effect of pond water depth on snail populations and fish-borne zoonotic trematode transmission in juvenile giant gourami (*Osphronemus goramy*) aquaculture nurseries. Parasitol. Int. 64, 522–526.
Tidwell, J.H., Coyle, S.D., Bright, L.A., 2010. Polyculture of nile tilapia, *Oreochromis niloticus*, either confined in cages or unconfined in freshwater prawn, *Macrobrachium rosenbergii*, ponds. J. World Aquacult. Soc. 41, 616–625.
Torres, M.V., Giri, F., Williner, V., 2012. Size selective predation on an invasive bivalve, *Limnoperna fortunei* (Mytilidae), by a freshwater crab, *Zilchiopsis collastinensis* (Trichodactylidae). J. Crustac. Biol. 32, 698–710.
Uddin, M.S., Rahman, S.S., Azim, M.E., Wahab, M.A., Verdegem, M.J., Verreth, J.A., 2007. Effects of stocking density on production and economics of Nile tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems. Aquac. Res. 38, 1759–1769.
Ventura, T., Manor, R., Aflalo, E., Weil, S., Rosen, O., Sagi, A., 2012. Timing sexual differentiation: full functional sex reversal achieved through silencing of a single insulin-like gene in the prawn, *Macrobrachium rosenbergii*. Biol. Reprod. 86, 6.
Wise, D.J., Mischke, C.C., Greenway, T., Byars, T.S., Mitchell, A.J., 2006. Uniform application of copper sulfate as a potential treatment for controlling snail populations in channel catfish production ponds. N. Am. J. Aquac. 68, 364–368.
Zhou, X., 2017. An overview of recently published global aquaculture statistics. FAO Aquac. Newslett. 6.