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Abstract

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Human schistosomiasis is a globally impactful parasitic disease that is transmitted through freshwater snails. Efforts to reduce the density of these intermediate host snails have historically improved human health outcomes, but the use of toxic pesticides can harm ecosystems. As a consequence, there is strong interest in predatory biocontrol, which uses native predators, such as prawns or fish, to consume snails and disrupt transmission. By consuming prey that are intermediate hosts to parasites, predators can limit parasite transmission and human prevalence. In the schistosome-host system, prawns have been identified as a sustainable biocontrol for the intermediate hosts, *Biomphalaria* and *Bulinus* snails, but environmental changes occurring in endemic transmission sites, such as plant invasions, could affect prawn-based biocontrol of snails. Through a series of trials, predation rate of treatment groups with water hyacinths, invasive plants commonly found in endemic areas with schistosomiasis, and without the water hyacinth plant. The predation rates were measured as liters of snail habitat cleared by the 1 prawn per day. The rates were 10.7 (95% CI: 9.27, 12.3) for the experimental treatment with water hyacinths and 11.1 (95% CI: 9.66, 12.8) for without treatment, demonstrating no change due to the treatment group. The lack of statistically significant differences between experimental groups results in a finding that is deeply biologically significant: the presence of invasive water hyacinths would not impede the use of prawns as a means of biocontrol to control the population of schistosome-susceptible

snails in the environmental context that the experiments were tested within. Although water hyacinth may be problematic due to it being an invasive plant, it is unlikely to disrupt efforts to promote prawn-based control of snails and schistosomes in areas that where schistosomiasis is endemic.

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Introduction

Predators can influence disease dynamics in their prey by reducing prey densities or shortening the infectious period of the prey (1). Both of these mechanisms suggest that predators generally reduce transmission of parasites in populations of their prey (2). Therefore, identifying conditions that promote either large population sizes of predators or high per capita predation rates has been proposed as a novel strategy to control vectors or intermediate hosts for human pathogens, such as mosquitos or snails (3, 4).

One challenge in the field of predatory biocontrol is that local environmental conditions can influence the physiology, behavior, and ecology of predators and prey in ways that might reduce the efficacy of predators. In general, the environment can affect predators' abilities to detect, reach, and successfully capture prey. This effect could be particularly pronounced for plants, reef-building organisms such as corals and oysters, and other ecosystems engineers that drastically alter the three dimensional structure of habitats that they come to dominate (5). Invasive species that act as ecosystem engineers may be disproportionately likely to influence predator-prey interactions, since, by definition, they alter habitat structure, attain high densities, and displace other native species (6). If invasive species alter the efficacy of predatory biocontrol on human disease vectors or intermediate hosts, then controlling these invasions could help or hinder efforts to improve human health.

Here I tested whether the presence of a widespread and problematic invasive plant altered the predatory biocontrol potential of giant river prawns, *Macrobrachium vollenhovenii*, on freshwater snails that serve as the intermediate hosts of human schistosomes. Human schistosomes, several species of parasitic blood flukes (worms) that develop through a juvenile life stage in freshwater snails and mature and reproduce as separate-sex adult worms inside humans, are the causative agents of schistosomiasis (7). There are three main schistosome species that cause illness in humans, *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*. Over 200 million people are infected with schistosomes and another 700 million people are at risk of infection in 76 developing countries, impacting the health of many people worldwide (8). As a result, human schistosomiasis is the second most impactful neglected tropical disease (NTD) globally (7). Schistosomiasis results in pathological effects ranging from liver fibrosis to bladder dysfunction (8). In more severe, chronic cases, patients may develop portal hypertension, hepatosplenomegaly, and even hepatic failure (8). In addition, the current drug available for mono-therapy, praziquantel, has been used for multiple decades, so drug resistance is an emerging risk (2, 9). Given these challenges, the translocation, conservation, and reintroduction of predators of snails has been periodically proposed to reduce snail densities and therefore transmission potential (4, 10, 11).

Schistosomes have a complex life cycle in which they are excreted from humans as eggs and hatch into miracidia upon contact with freshwater (7) (Fig 1). They use cilia to actively find and penetrate certain species of compatible snail intermediate hosts. Once infected, snails can produce hundreds or thousands of cercariae, which are the free-swimming life stages that infect humans by burrowing through skin (7). Once inside a human, worms mature into adults and sexually reproduce, resulting in eggs 4-6 weeks after initial infection (7). Many of the pathological effects of schistosomiasis is caused by the buildup of these eggs and the resulting chronic inflammatory reaction (12). As the life cycle of the schistosomes use snails as an intermediate host, transmission of the parasites to humans require there to be infected snails in the environment.

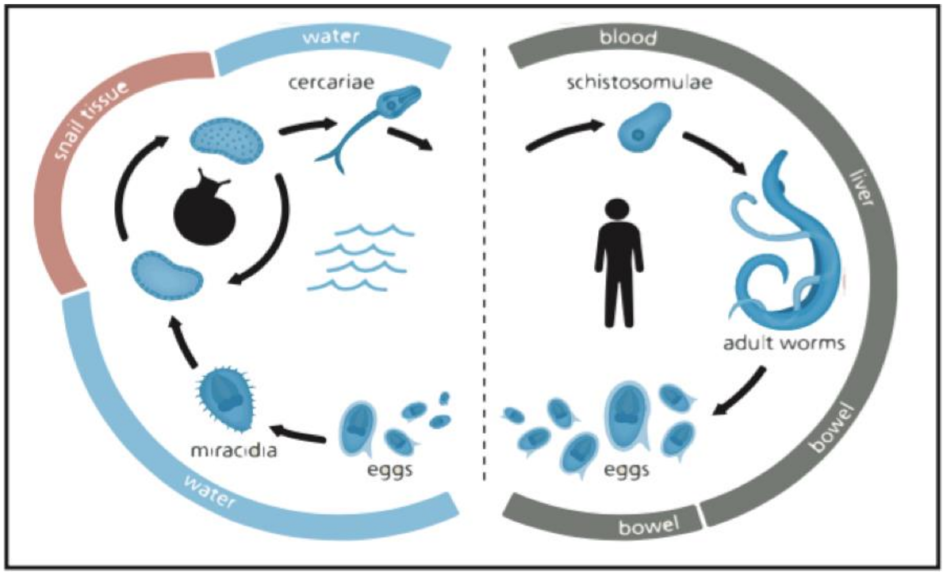

Figure 1: The complex life-cycle of schistosomes that utilize specific snails as intermediate hosts. They then infect humans as their definitive hosts and are excreted from humans as eggs.

Since snails are obligate intermediate hosts for schistosomes, their abundance is related to human risk of infection. In fact, one major strategy to disrupt schistosome transmission is “snail control,” reducing the abundance of snails, especially through the use of pesticides, such as niclosamide. However, natural factors, such as predators or competitors, can also decrease snail abundance, and approaches based on biocontrol may be preferable because they do not involve the use of broadly toxic chemicals to aquatic environments (4).

Schistosome-transmitting snails, such as *Biomphalaria spp.* are vulnerable to predators, such as fish, prawns, crayfish, waterbugs, and dragonflies, so there has been a recent resurgence in interest in predatory biocontrol on snails in order to control the transmission of schistosomes (13). Freshwater prawns have particularly been suspected to be useful biocontrols for schistosomiasis-susceptible snails since they are effective predators of this intermediate host (14). However, earlier attempts at using prawns as biocontrols have found strong effects in the laboratory and little to no effect in field trials, suggesting that typical laboratory tests of snail predation have limited ecological relevance. One reason for this could be the simplified environmental contexts used in laboratory studies. For example, snails associate with many species of plants, which they use as habitat, food source, and as refuge from predators, which would have varying effects on predator-prey interactions.

Macrobrachium vollenhovenii prawns have been used in multiple studies because they share similar habitats and are native to large endemic areas of schistosomiasis in Africa (13). These prawns preferably prey on the intermediate host snails, hatchings, and eggs in their natural habitats, suggesting the possibility of prawn use in other areas as well (13). Ecological theory suggests that these river prawns act as “intraguild predators” of the schistosome by consuming both the intermediate host snails, acting as a competitor to the schistosome, as well as preying upon the larval worm parasites (4).

It has been suspected that in the Senegal River Basin, cases of schistosomiasis increased due to environmental changes caused by the development of the Diama Dam (14). This construction disrupted the natural predator of *Schistosoma*-susceptible snails, *M. vollenhovenii* prawns, by blocking the path to their normal breeding sites which prevented them from reproducing at a normal rate (14). However, the direct correlation between the decrease in prawn numbers and the increase of schistosomiasis infection cases has not yet been established and needs to be studied further because other ecological factors besides the construction of the dam may have had an effect (14). Therefore, the impact of the environment must be better understood to best utilize tools such as biocontrols in the form of predators.

Invasive plants commonly impact water bodies in schistosome endemic areas (15), and therefore, they could impact predation rates on snails. One particular plant that invades areas commonly affected by schistosomes is water hyacinth, *Eichhornia crassipes*, which is suspected to have an effect on the intermediate host snails, *Biomphalaria sudanica* and *B. choanomphala*, of *Schistosoma mansoni* in Lake Victoria in East Africa (15). One study found that the growing density of the water hyacinths was positively correlated with the increasing abundance of *Biomphalaria sudanica* snails (15). However, due to the complex effects that hyacinths can have on the ecosystem, such as proliferating uncontrollably, more studies are required to understand the potential mechanisms behind this change.

Water hyacinth changes the three-dimensional structure of underwater habitats in ways that I hypothesize could influence the efficacy of predators of snails. On one hand, the snails might be able to utilize the plants by hiding in the roots or climbing up the stem to escape the sediment level, to avoid being consumed by the prawns, thus reducing the strength of snail control by prawns. On the other hand, the prawns could also utilize the roots of the plants to climb up to reach more snails or as a hiding place to camouflage itself in order to better prey on the snails, increasing predation rate. Therefore, I conducted a short-term predation experiment to test these alternative hypotheses.

Methods

I obtained *Biomphalaria glabrata* from the NIAID Schistosomiasis Resource Center, and they were fed every other day with a mixture of chicken feed and agar. They were kept in HHCOMBO artificial lake water (16). I procured *Macrobrachium vollenhovenii* prawns (Aquaculture of Texas, USA) and maintained eight prawns individually with continuous aeration in 15 L square plastic containers filled with 10 L HHCOMBO in an incubator at 26 degrees Celcius and 99% humidity. I fed the prawns daily - every prawn received on average 3 pieces of Purina Aquamax Fingerling 300 fish food per day. I cleaned the containers once every two weeks by emptying out the water and cleaning away remaining algae growth and salt buildup as well as completely replacing HHCOMBO media.

Figure 2: Experimental set-up of the containers, with the controls on the left and the environmental factor (water hyacinth) on the right, with air supply connected.

I conducted three predation trials using individual prawns, snails, and hyacinth plants. Each day in between the experimental trials when snails were not provided as a food source, I fed the prawns using the fish food. In Trial 1, I added one medium water hyacinth plant to each of four of the eight containers, leaving four as controls (Fig. 1). I then added 15 snails per container and measured the number of snails alive after 24 hours. In Trial 2, I added 10 snails and assessed survival at 6 hours. Trials 1 and 2 resulted in all of the snails being consumed, so they were used as pilot studies in order to find the optimal conditions for snail density and time duration to be used in Trial 3 in order to detect a difference between the treatments. In Trial 3, I used a split-plot design, in which I ran two subtrials, switching the presence or absence of hyacinth for each prawn between the two subtrials. In these subtrials, I added 30 snails and assessed survival at 23 ± 1 hours. The experimental design was altered in order to observe some snail survival (0% survival in Trials 1 and 2) and take into account the identity of the prawn as a random factor to control for variation among individual prawn predation rates. I analyzed snail survival with a generalized linear mixed model, with plant treatment as a fixed effect and prawn identity as a random factor.

I also calculated per capita predation rates for each prawn by assuming that these predators exhibit a type 1, or linear, functional response, which is equivalent to assuming a constant attack rate (17). Assuming a constant attack rate, or equivalently a constant snail death rate, implies a type II survivorship curve, with the probability of any given snail remaining surviving to time *t*, S(*t*) equal to:

$$S(t) = e^{-fPt}$$

Where *f* is the per capita predation rate of prawns on snails, with units L t^{-1} of habitat cleared of snails per prawn, and *P* is prawn density (here, *P* = 0.1 prawns L^{-1}). The equation assumes that there is not enough snail density to satiate the prawns in the volume of the experimental tanks presented. Using this equation and the observed values of the snails alive at the start and end of the subtrials, I estimated the predation rate (along with the 95% confidence interal) with and without hyacinth using maximum likelihood estimation and the binomial error distribution, which is appropriate for modeling observed counts of successes (survival) vs. failures (deaths) from trials with known quantities of snails (18).

Results

In Trials 1 and 2, the prawns consumed every snail offered. Therefore, I will focus on the results of Trial 3. The average percentage of snails eaten per day was calculated as approximately 74.1% (± 30.5%) consumed in containers with plants and 75.2% (± 30.4%) of snails consumed in containers without plants. On average, snails consumed ~74.7% of the snails offered in both subtrials of Trial 3. Using the percentages of snails consumed in the presence and absence of water hyacinth, the predation rates (f), measured as liters of snail habitat cleared by the prawns per day. The f-values were 10.7 (9.27, 12.3) for the experimental treatment with water hyacinths and 11.1 (9.66, 12.8) for without treatment (Fig. 2). The generalized linear mixed model found no significant effect of plant presence on snail predation ($p = 0.74$). Similarly, the maximum likelihood analysis found no differences in predation rates between the two treatments. The error bars for both treatment groups overlap almost completely, which corresponds to the predation rate not differing (Fig. 2).

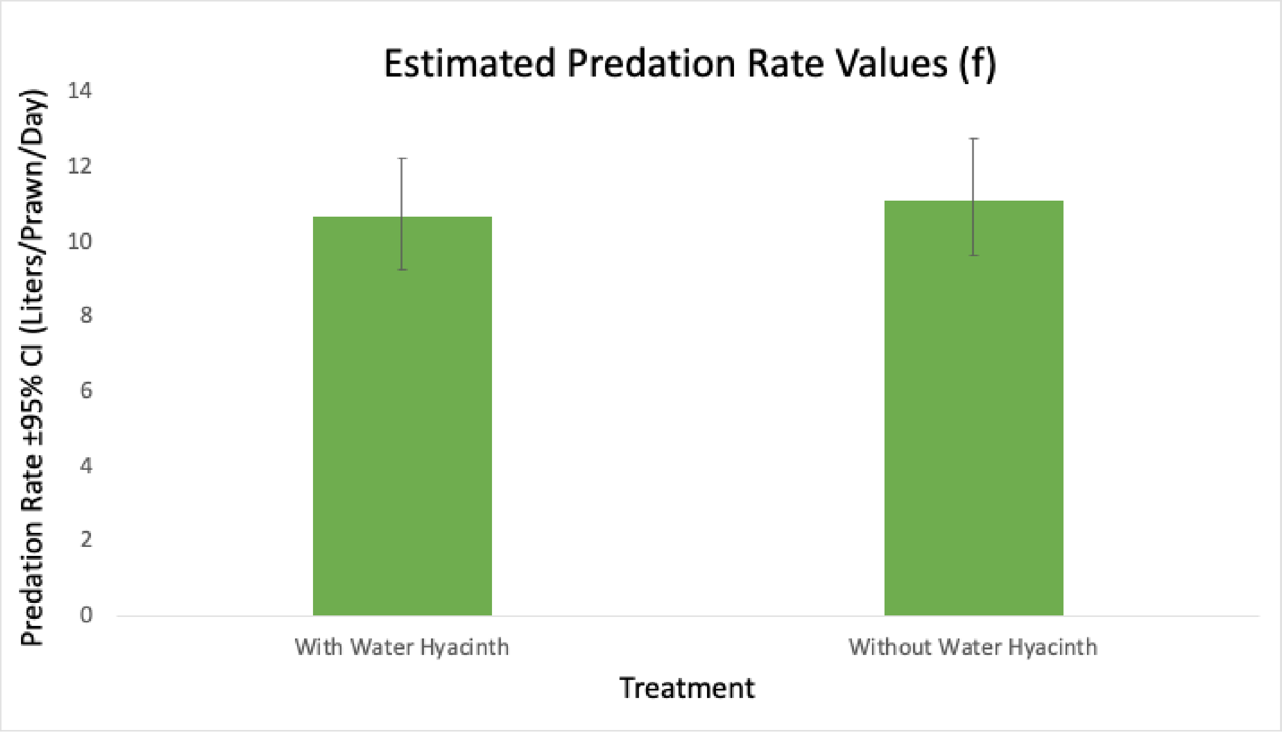

Figure 3: The estimated predation rate values (f) measured as volume (liters) of water cleared of snails per day with 95% confidence intervals containers with and without water hyacinth.

Conclusion

Predators play an important role in every ecosystem, and they can influence disease dynamics in their prey. By consuming prey that are intermediate hosts to parasites, predators can be an important factor in limiting parasite transmission and prevalence, which can be critical if prey serve as vectors or intermediate hosts of human parasites. In the schistosome-host system, prawns have been identified as a sustainable biocontrol for the intermediate hosts, *Biomphalaria* and *Bulinus* snails (4). However, whether environmental changes occurring in endemic transmission sites, such as plant invasions (15), could affect prawn-based biocontrol of snails has never been tested.

I hypothesized that invasive water hyacinth could affect predation rates on snails because water hyacinth could either provide a refuge for snails to avoid prawns or provide a scaffold over which prawns could climb and better locate snails. However, I found that predation rate of *Biomphalaria glabrata* snails by prawns was not affected by the environmental change of the addition of water hyacinth. This information can contribute to the simultaneous management of hyacinth and schistosomes in areas impacted by both because it means that hyacinth removal, which may be desired to improve water quality and access, is unlikely to decrease predatory biocontrol.

In this study, the lack of statistical significance between experimental groups results in a finding that is deeply biologically significant: the presence of invasive water hyacinths would not impede the use of prawns as a means of biocontrol to control the population of schistosome-susceptible snails in the environmental context that I tested within. The feeding rate/death rate of the snails is on average 10.9 liters per prawn per day. These prawns could be used as a method of snail control to control the level of infections by schistosomes even in the presence of the water hyacinths. These results are important in better understanding the epidemiological and ecological consequences of environmental change, in the form of invasive plant species, in relation to parasite-host interactions of the intermediate snail hosts. This knowledge impacts the way that predators can be used as factor to manipulate and decrease schistosome parasites in areas with the hyacinths. One potential conclusion is that eliminating hyacinth, which is problematic for other reasons, such as disrupting the other parts of the natural ecosystem due to being and invasive and novel species in that area, is unlikely to disrupt efforts to promote prawn-based control of snails and schistosomes.

Future Directions

There are still many open questions regarding the influence of water plants on the snail-schistosome system. Future work should further investigate the role of plant species other than water hyacinth. Water hyacinth has extensive and dense roots, which could be used as a climbing surface by snails or predators. Other invasive plants in schistosome endemic sites, such as water lettuce, duckweed, and azolla have less extensive, sparse roots. Thus, differences in physical structure could lead to different effects on predation. The specific structural components that influence prawn predation could be examined by introducing synthetic plants in the lab set-up with various structures, changing parts such as the root densities and lengths. In addition to measuring predation over time trials, direct observation of snail and prawns interacting could lend insight into how these species interact with the unique structural components, such as root length and density, of the treatment groups.

For example, I could investigate the behavioral interactions that snails or predators have in the presence of various plants or structural features. I would use treatment groups in which the prawn would be caged to observe how the snails interact with the plants in the presence of the predator but without being consumed so that they would be able to continuously interact with the environment for a prolonged amount of time. In other groups, I would add uncaged prawns that could interact with the plants along with the snails. These treatments would allow for better understanding of how snails interact with the structures of their environment when predators are present. This would be a way to rule out other components besides the physical elements, such as biological makeup, when comparing the effects of the environment on schistosome-host relationships. Overall, understanding the impacts of environmental factors, such as invasive plants, on schistosome transmission to the intermediate snail hosts is an important step towards informing public health workers' decisions and gaining fundamental understanding of the ecology of the schistosome-host system.

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