

Temperature affects predation of schistosome-competent snails by a novel invader, the marbled crayfish *Procambarus virginalis*

Author

Faiad, Sara M, Williams, Maureen A, Goodman, Maurice, Sokolow, Susanne, Olden, Julian D, Mitchell, Kaitlyn, Andriantsoa, Ranja, Gordon Jones, Julia Patricia, Andriamaro, Luciano, Ravoniarimbinina, Pascaline, Rasamy, Jeanne, Ravelomanana, Tsilavina, Ravelotafita, Salohy, Ravo, Ranaivosolo, Rabinowitz, Peter, et al.

Published

2023

Journal Title

PLoS ONE

Version

Version of Record (VoR)

DOI

[10.1371/journal.pone.0290615](https://doi.org/10.1371/journal.pone.0290615)

Rights statement

© 2023 Faiad et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Downloaded from

<https://hdl.handle.net/10072/433425>

Griffith Research Online

<https://research-repository.griffith.edu.au>

RESEARCH ARTICLE

Temperature affects predation of schistosome-competent snails by a novel invader, the marbled crayfish *Procambarus virginalis*

Sara M. Faiad¹, Maureen A. Williams^{1,2}, Maurice Goodman³, Susanne Sokolow^{3,4}, Julian D. Olden¹, Kaitlyn Mitchell³, Ranja Andriantsoa⁵, Julia Patricia Gordon Jones⁶, Luciano Andriamaro⁵, Pascaline Ravoniarimbina⁵, Jeanne Rasamy^{5,7}, Tsilavina Ravelomanana^{5,7}, Salohy Ravelotafita⁷, Ranaivosolo Ravo⁷, Peter Rabinowitz^{8,9}, Giulio A. De Leo^{3,10}, Chelsea L. Wood^{1*}

1 School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, United States of America, **2** Department of Biology, McDaniel College, Westminster, MD, United States of America, **3** Hopkins Marine Station, Dept. of Oceans and of Earth System Science, Doerr School of Sustainability, Stanford University, Stanford, CA, United States of America, **4** Marine Science Institute, University of California, Santa Barbara, Santa Barbara, CA, United States of America, **5** Réseau International Schistosomiase Environnement Aménagement et Lutte (RISEAL) Madagascar, Madagascar, **6** School of Natural Science, Bangor University, Bangor, Gwynedd, United Kingdom, **7** Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar, **8** Department of Environmental/Occupational Health Sciences, Global Health, University of Washington, Seattle, WA, United States of America, **9** Center for One Health Research (COHR), University of Washington, Seattle, WA, United States of America, **10** Woods Institute for the Environment, Stanford University, Stanford, CA, United States of America

* chelwood@uw.edu



OPEN ACCESS

Citation: Faiad SM, Williams MA, Goodman M, Sokolow S, Olden JD, Mitchell K, et al. (2023) Temperature affects predation of schistosome-competent snails by a novel invader, the marbled crayfish *Procambarus virginalis*. PLoS ONE 18(9): e0290615. <https://doi.org/10.1371/journal.pone.0290615>

Editor: Giorgio Mancinelli, Università del Salento, ITALY

Received: September 16, 2022

Accepted: August 12, 2023

Published: September 13, 2023

Copyright: © 2023 Faiad et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The data that support the findings of this study are openly available in the Dryad Digital Repository: DOI [10.5061/dryad.m905qfv71](https://doi.org/10.5061/dryad.m905qfv71). The code used to produce the statistical results reported herein is openly available via GitHub at https://github.com/wood-lab/Faiad_et_al_2023_PLoS_One.

Funding: SMF was supported by a SAFS Scholarship from the School of Aquatic and Fishery Sciences at the University of Washington, a

Abstract

The human burden of environmentally transmitted infectious diseases can depend strongly on ecological factors, including the presence or absence of natural enemies. The marbled crayfish (*Procambarus virginalis*) is a novel invasive species that can tolerate a wide range of ecological conditions and colonize diverse habitats. Marbled crayfish first appeared in Madagascar in 2005 and quickly spread across the country, overlapping with the distribution of freshwater snails that serve as the intermediate host of schistosomiasis—a parasitic disease of poverty with human prevalence ranging up to 94% in Madagascar. It has been hypothesized that the marbled crayfish may serve as a predator of schistosome-competent snails in areas where native predators cannot and yet no systematic study to date has been conducted to estimate its predation rate on snails. Here, we experimentally assessed marbled crayfish consumption of uninfected and infected schistosome-competent snails (*Biomphalaria glabrata* and *Bulinus truncatus*) across a range of temperatures, reflective of the habitat range of the marbled crayfish in Madagascar. We found that the relationship between crayfish consumption and temperature is unimodal with a peak at ~27.5°C. Per-capita consumption increased with body size and was not affected either by snail species or their infectious status. We detected a possible satiation effect, i.e., a small but significant reduction in per-capita consumption rate over the 72-hour duration of the predation experiment. Our results suggest that ecological parameters, such as temperature and crayfish

Graduate School Boeing International Fellowship from the University of Washington, a foundry 10 Marine Science Research Grant, and by the Future Rivers program at the University of Washington as part of a National Science Foundation (NSF) National Research Traineeship award (DGE 1922004). CLW was supported by a Pilot Research Grant from the UW Population Health Initiative and UW's EarthLab, which provided additional support to SMF, as well as the Belmont collaborative Forum on Climate, Environment and Health and the National Science Foundation grant ICER-2024383. GADL was supported by the Belmont collaborative Forum on Climate, Environment and Health and the National Science Foundation grant ICER-2024383 and DEB – 2011179. JPGJ's involvement was funded by the UKRI Global Challenges Research Fund award made available by the Higher Education Funding Council for Wales to Bangor University (W19/36HE). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

weight, influence rates of consumption and, in turn, the potential impact of the marbled crayfish invasion on snail host populations.

Introduction

The human burden of environmentally transmitted infectious diseases depends strongly on ecological factors [1], such as the presence of natural enemies [2]. As antagonistic interactors that regulate the transmission of infectious disease, natural enemies can potentially include predators, competitors, or parasites of reservoir hosts or of the disease agent itself [2, 3]. However, evidence for disease transmission regulation by natural enemies is sparse, and generalities regarding the influence of these antagonists remain elusive [4, 5]. This knowledge gap is of particular concern, not just for understanding the potential role of natural enemies in controlling and eliminating infectious disease, but also because non-native species are becoming established in new regions at unprecedented rates [6]. Non-native species have long been recognized as having profound effects on ecosystem services [7–9]. Like native enemies, invasive species influence the abundance and distribution of their prey and competitors [10, 11], with important implications for transmission of environmentally transmitted infectious diseases—a phenomenon that has remained largely unexplored.

One such invader, with a rapidly expanding range and the potential to influence disease transmission, is the parthenogenetic marbled crayfish (*Procambarus virginalis*)—the only clonal decapod ever described [12]. The marbled crayfish is believed to have arisen from a non-clonal ancestor in the American Cambaridae family and is phylogenetically similar to *Procambarus fallax* [12]. First appearing in the German aquarium trade in the mid-1990s, this novel invader quickly spread across Europe [13]. Following its introduction to Madagascar in 2005, the marbled crayfish began to spread across the country [13–15], overlapping with the distribution of freshwater snails (*Biomphalaria pfeifferi* and *Bulinus* spp.) that serve as the intermediate hosts of schistosomiasis (*Schistosoma mansoni* and *S. haematobium*, respectively [16]).

Schistosomiasis is a parasitic disease endemic to tropical and subtropical regions [17]. Freshwater snails (largely from three genera: *Biomphalaria*, *Bulinus*, and *Oncomelania*) [18] play an obligate role in the transmission of *Schistosoma* spp. worms. Snails serve as intermediate hosts in the *Schistosoma* life cycle, incubating and shedding infectious *Schistosoma* cercariae (free-swimming, larval worms) into streams, ponds, and lakes. These cercariae go on to penetrate the skin of humans bathing, wading, washing laundry, or otherwise in contact with contaminated freshwaters. Over 250 million people are infected with schistosomiasis globally, totaling to 3.31 million disability-adjusted life years annually [19]. Though schistosomiasis infections rarely result in mortality, the disabilities associated with the disease are severe and burdensome, falling second in both disability-associated burden and prevalence only to intestinal nematode infections [19]. Notably, these estimates do not consider the economic and social impacts of disease, though it is widely understood that the compromised health of community members—a result of untreated schistosomiasis infections—reduces the opportunities for social and economic development [20–22]. Despite its impacts on human morbidity and mortality, schistosomiasis remains prevalent, disproportionately impacting poor and marginalized communities, particularly those that lack the public infrastructure needed for access to safe drinking water, adequate sanitation, and healthcare [23].

The World Health Organization (WHO) estimated that in 2019 over 235 million people were at risk of schistosomiasis infection and required preventative treatment, with 90% of

those individuals living in Africa [24]. The standard treatment—an oral dose of the anthelmintic praziquantel—is considered effective in treating adult parasites (although there is rising concern of selection for resistance [25]); however, at this time, fewer than half of those in need of treatment ($105,420,110 / 235,378,761 = 44.8\%$) receive it [24]. Mass drug administration has also fallen short due to high rates of re-infection, as individuals are often repeatedly exposed to contaminated water sources [26–30] and treatment is ineffective against juvenile worms [31–33]. In response, the WHO recognizes snail control as essential to reducing *Schistosoma* spp. transmission and recommends the implementation of snail control strategies, in combination with mass drug administration, to control and eliminate schistosomiasis [34]. Historically, snail control has been accomplished with chemical molluscicides and habitat modification, such as vegetation removal, draining wetlands, cementing canals, and alterations to water flow [35]. Alternatively, snail populations can be reduced by abundant populations of predators (e.g., crustaceans, birds, and fish) or competitors (e.g., other snail species). Indeed, natural enemies show some promise in the reduction of human schistosomiasis [36–39]. For example, the abundance of molluscivorous cichlids (*Trematocranus placodon*) was negatively correlated with intermediate snail host abundance, and temporal declines in fish abundance were associated with increases in schistosomiasis [39]. In the Senegal River Basin—the epicenter of the world’s largest intestinal schistosomiasis epidemic—areas previously inhabited by and now hypothesized to be absent of native river prawns experienced greater increases in schistosomiasis infections compared to regions falling outside of the prawns’ range [40], and localized reintroductions significantly reduced snail population numbers and, in turn, human disease prevalence [38].

While some introductions of non-native enemies in previous disease control efforts have resulted in unanticipated negative consequences [2], introduced species have also been used with success to reduce the abundance of zoonotic reservoirs of disease and of human disease burden [10, 41, 42]—representing a potential benefit of non-native species [43]. Regarding schistosomiasis, the abundance of the invasive red swamp crayfish (*Procambarus clarkii*) was significantly correlated with the disappearance of snails in Egyptian irrigation channels [41], and an established population of *P. clarkii* reduced snail abundance in Kenya [42]. Local schoolchildren were significantly less likely to become infected with *Schistosoma haematobium* where crayfish were present than in their absence—though environmental conditions influenced the crayfish’s impact on snail populations and local schistosomiasis prevalence and intensity [42].

Prey–predator relationships between gastropods and freshwater crayfish—both native and non-native—are well-documented [44], and numerous field and experimental studies highlight the regulatory impact of crayfish on snail populations [45–51]. Regulation of gastropod abundances by freshwater crayfish occurs through a combination of consumptive and non-consumptive mechanisms, though it remains unclear which mechanism drives this widespread pattern [45, 52]. Snail populations decline in the presence of crayfish as a function of predation (i.e., a consumptive effect). On the other hand, the presence of predators, including crayfish, can indirectly impact snail populations if their presence results in changes in snail physiology (e.g., growth rate) [53–56], morphology [e.g., shell thickness, 57; shape, reviewed in 44], or behavior [e.g., habitat use, 54, 58, 59; feeding rate, 54] that, in some cases, decrease fitness [57]. Snail anti-predator behaviors include change in habitat use, reduced feeding rate, crawling up onto vertical substrates, and moving above the waterline (also known as “water quitting”)—all of which have been observed when snails are exposed to a crustacean predator [54–56, 59; additional behavioral responses to predators reviewed in 44].

Snails exposed to trematode infections, including *Schistosoma* spp., exhibit behavioral differences compared to uninfected conspecifics, which may alter predator risk and the rate at which snails are consumed by a predator [60–63]. Snails exposed to and presumably infected

by *Schistosoma* move more slowly and less frequently than do uninfected conspecifics, suggesting that infected snails should be at greater risk for predation [60]. When exposed to simulated predation cues—a caged riverine prawn, *Macrobrachium vollehovienii*, paired with crushed snail conspecifics—infected snails also showed a diminished anti-predator response compared to uninfected snails (where the anti-predator response is defined as “water quitting,” along with an aversion to open water and a preference for hiding under sheltered areas within the tank) [60]. Indeed, prawns preferentially consume *Bi. glabrata* snails exposed to *S. mansoni* and *Bu. truncatus* snails exposed to *S. haematobium* [60]. Differences in the consumption rate of uninfected and infected snails could alter the regulatory impact of crayfish on snail populations and, perhaps, human disease burden. Schistosomiasis control efforts may be enhanced by snail predators, if infected snails are consumed preferentially; however, intervention efforts may be hindered should predators avoid consuming infected snail hosts [60].

Like most crayfish [reviewed in 44], marbled crayfish are omnivores, said to eat “almost anything” [64]. However, plant material and snails seem to be their preferred food items [64]. Indeed, Andriantsoa et al. [65] anecdotally observed that native snails were absent from sites inhabited by this invader, suggesting that predation was not only occurring but that crayfish presence might reduce snail abundance to zero. Predation was later confirmed in a laboratory setting [65], highlighting the species’ potential to serve as a biological control of snails in Madagascar—something that is urgently needed in a country where prevalence in some villages can range up to 94% [66, 67].

The traits that make the marbled crayfish a particularly successful (and worrisome) invader may also make the species a formidable predator of Madagascar’s native snails, including schistosome-competent snails. Madagascar is diverse in habitat and climate [65]. Marbled crayfish can tolerate a wide range of ecological conditions and colonize diverse habitats [65], including areas outside of the limited geographic range of native crayfish species (Parastacidae; *Astacoides*) [68, 69]. The invader has been found in rice fields irrigated by thermal water reaching temperatures as high as 37°C, in 20°C river habitats [65] and has withstood temperatures as low as 5°C in a laboratory setting, though survivorship drastically declines at this extreme temperature [70]. Not only is this thermal plasticity and habitat diversity a feat for a clonal species, it also suggests that the marbled crayfish may be able to serve as a snail predator in habitats where other, endemically occurring snail predators cannot occur—perhaps, a collateral benefit of an otherwise destructive invasive species. However, rates of crayfish mortality, growth, and consumption vary greatly across temperatures [70–72]. Rates generally increase simultaneously with temperature until a thermal optimum is reached, beyond which performance declines [73]. The temperature at which this thermal limit occurs varies considerably among crayfish species [74–76]. Marbled crayfish consumption (of carrots and worms [*Tubifex tubifex*]) ceases below 10°C [70], while the upper thermal limit remains unknown. Likewise, the thermal optimum of consumption, or the temperature at which consumption peaks, also remains unknown for marbled crayfish. As such, temperature plays a critical role in our understanding of the marbled crayfish’s potential as a biological control agent of snail intermediate hosts in Madagascar, as well as other regions in which the marbled crayfish has invaded and schistosomiasis is endemic.

This study identifies the conditions under which marbled crayfish prey on schistosome-competent snails. We addressed the following questions: i) Does temperature affect crayfish feeding rates on schistosome-competent snails and, if yes, is the relationship between temperature and feeding rate increasing, decreasing, or unimodal with a peak at intermediate temperatures? ii) Does the rate of crayfish consumption differ between snail species and between infected and uninfected snails across temperatures?, and iii) Does crayfish body size mediate the response of feeding rate to temperature?

We experimentally assessed marbled crayfish consumption of uninfected versus infected snails across a range of temperatures, reflective of the environmental conditions across the habitat range of the marbled crayfish in Madagascar. We hypothesized that the relationship between temperature and feeding rate would be unimodal with a peak at intermediate temperatures. Additionally, we hypothesized that snail infection status would influence the rate of consumption, as behavioral and physiological differences between uninfected and infected snails may make animals of differing infection status more or less accessible, detectable, or desirable to predators. Finally, we hypothesized that temperature may mediate the influence of weight on consumption, because temperature influences large-bodied organisms differently than those that are smaller in size [77]. Overall, the results from the present study enhance our understanding of the biotic and abiotic factors that impact the rate at which a recent invader, the marbled crayfish (*Procambarus virginalis*), consume schistosome-competent snails.

Materials & methods

Animal husbandry

Marbled crayfish were reared in freshwater aquaria filled with artificial pond water [78]. Crayfish tanks varied in size (3.72 L, 11.7 L, or 81.3 L), depending on the age, size, and rearing density of the crayfish. Crayfish, prior to becoming subjects in experiments, were typically housed with between two and four conspecifics. Juvenile crayfish were regularly removed from adult husbandry tanks and either relocated to a smaller tank (11.7 L) without adults or euthanized. Once included in the experiment, crayfish were housed individually in 11.7-L tanks. Husbandry tanks were held at room temperature (~25°C), whereas the temperature of experimental tanks was controlled and monitored (see below). All crayfish were regularly fed frozen carrots, except during experimental trials. Marbled crayfish (*Procambarus virginalis*) were obtained through private sellers on Etsy (<https://www.etsy.com/>) and Aquabid (<https://www.aquabid.com/>). Permission to import and house marbled crayfish for use in this study was provided by the State of Washington's Department of Fish and Wildlife (Shellfish Import Permit No. 22–3020).

Snails were reared in freshwater aquaria (either 3.72- or 11.7-L tanks, depending on the density of snails), filled with artificial pond water [78]. Tanks underwent 100% water changes one to two times per week [79]. Snails were regularly fed romaine lettuce, which was refreshed during the bi-weekly water changes. All *Biomphalaria glabrata* (M-line, naive and exposed to *S. mansoni* strain PR-1) and *Bulinus truncatus* (Egypt, naive and exposed *S. haematobium* strain Egyptian) snails were provided by the NIAID Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD, USA) through NIH-NIAID Contract HHSN272201700014I for distribution through BRI Resources.

Experiments

Our methods largely replicated previous experimental predation trials between crustaceans and *Schistosoma*-competent mollusks [80]. Briefly, one marbled crayfish (*Procambarus virginalis*) was held in combination with a set density ($n = 12$) of either *Bi. glabrata* or *Bu. truncatus* snails in a 11.7-L tank. An "average" size class of snails (6–10 mm shell length for *Bi. glabrata*; 5–10 mm shell length for *Bu. truncatus*) was used. Crayfish length and weight were measured prior to the start of each experimental period. Crayfish varied in weight between 1.54 g and 14.44 g with an average \pm SE of 6.62 ± 0.117 g.

The total duration of each experimental period was 72 hours, with observations and snail replacement taking place every 12 hours. Each experimental period consisted of 7 total time points (0, 12, 24, 36, 48, 60, 72), in which each 12-hour increment constituted a "trial," for a

total of 6 trials per experimental period. At the conclusion of each trial, the number of snails above the water line, the number of snails on the lettuce (described below), the number of snails inside and under the shelter/hiding, and the number of snails in open water were counted and summed to reflect the total number of snails remaining in the tank. Additionally, the total number of empty, intact shells and the total number of dead snails were recorded at the conclusion of each trial. Shattered shell pieces were not included in empty shell counts, as it was too difficult to determine how many broken pieces constituted a singular shell. For each trial, we derived the total number of snails missing and presumably consumed as follows: the initial number of snails at each trial ($n = 12$), minus the number of snails remaining, minus the number of snails dead but not consumed. The number of snails consumed and the number of dead snails were totaled to determine the total number of snails to be replaced/added to the experimental tank. All counts were repeated and confirmed by a second observer. At the conclusion of each trial, dead snails and empty, intact shells were removed, snail density ($n = 12$) was reset, and the number of snails replaced/added was recorded. At the conclusion of the 72-hour experimental period, any remaining snails were removed from experiment tanks and returned to temperature-acclimated holding tanks. Crayfish remained in their tanks, allowing us to control for individual crayfish identity in analyses.

Crayfish and snails were provided with food throughout the duration of the experimental period. Specifically, at the beginning of the experimental period (time point “0”), a piece of romaine lettuce was added to each experimental tank to serve as a food source for snails. Additionally, one invertebrate pellet was placed into each of the tanks, including control tanks, to serve as an alternative source of food for crayfish. This reflected our assumption that crayfish are omnivorous, and are not limited to eating only snails in their natural habitats. Each experimental tank also contained a piece of PVC pipe, which served as a shelter for the crayfish and snails.

We observed crayfish consumption rate across five temperature conditions—15, 20, 25, 30, and 35°C. This range reflects the diverse temperatures at which marbled crayfish have been found in Madagascar (20°C to 37°C) [65]. Though marbled crayfish can survive in temperatures as low as 5°C for extended periods of time, previous experiments have suggested that consumption ceases below 10°C [70]. However, consumption has been observed at 15°C, and therefore, this may reflect the lower thermal limit of crayfish feeding behavior. Animals underwent a temperature acclimation period, in which the water temperature changed 1–1.5°C/day until the desired temperature was reached. Animals were then held at the experimental temperature for at least 12 hours prior to the start of the experiment. Given that 15°C and 35°C would near the thermal limits of both the crayfish and snails [60, 65, 70, 81, 82], we included control tanks, from which crayfish were absent, to exclude the effect of temperature-associated snail death and ensure that snail mortality accurately reflected crayfish consumption.

We were interested in the influence of snail infection status on crayfish consumption rates and, therefore, varied snail infection status between experimental tanks. Each individual crayfish was held either with all “exposed” or “naive” (hereafter, “uninfected”) snails of one of two species included in the present study: *Biomphalaria glabrata* and *Bulinus truncatus*. Exposed snails were held at room temperature (~25°C) for ~30 days post-exposure (exposure date provided by the reagent provider, BRI) to allow infections to adequately mature [83] before being used in experiments. Following the post-exposure period, exposed snails were assumed to be infected and will be referred to as such hereafter.

We were limited by the availability of infected *Bu. truncatus* and *Bi. glabrata* snails (Table 1, Fig 1). As such, the first round of experiments included only uninfected snails. Round 1 of experiments began on 14 June 2021 and concluded on 13 August 2021. In Round 1 of experiments, we conducted seven (72-hour long) predation experiments for each set temperature (15, 20, 25, 30, and 35°C) for a total of 42 (12-hour long) trials (observations) per temperature,

Table 1. Summary tables displaying the observed proportion of snails consumed by crayfish, unavailable for crayfish consumption, or dead by causes other than crayfish consumption.

a.						
temperature	consumed		unavailable		dead	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
15°C	106	0.15 (0.17)	95	0.09 (0.16)	174	0.03 (0.07)
20°C	109	0.30 (0.25)	95	0.13 (0.16)	181	0.02 (0.06)
25°C	108	0.33 (0.27)	95	0.11 (0.15)	178	0.02 (0.06)
30°C	110	0.38 (0.26)	95	0.11 (0.14)	181	0.02 (0.05)
35°C	107	0.23 (0.27)	92	0.15 (0.18)	176	0.01 (0.04)
b.						
status	consumed		unavailable		dead	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
infected	119	0.33 (0.25)	233	0.13 (0.17)	233	0.01 (0.04)
uninfected	421	0.27 (0.26)	239	0.11 (0.15)	657	0.02 (0.06)
c.						
time	consumed		unavailable		dead	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
12 hrs.	86	0.31 (0.27)	75	0.10 (0.14)	142	0.02 (0.06)
24 hrs.	88	0.36 (0.31)	79	0.11 (0.14)	147	0.02 (0.06)
36 hrs.	87	0.23 (0.21)	79	0.13 (0.17)	144	0.02 (0.06)
48 hrs.	86	0.31 (0.28)	79	0.09 (0.13)	144	0.01 (0.04)
60 hrs.	95	0.19 (0.21)	80	0.13 (0.17)	155	0.03 (0.05)
72 hrs.	98	0.28 (0.24)	80	0.14 (0.20)	158	0.02 (0.07)
d.						
species	consumed		unavailable		dead	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
<i>Bi. glabrata</i>	268	0.27 (0.24)	236	0.10 (0.16)	449	0.01 (0.04)
<i>Bu. truncatus</i>	272	0.29 (0.27)	236	0.14 (0.15)	441	0.03 (0.07)

Proportions are grouped by (a) temperature treatments, (b) infection status, (c) trial time blocks, and (d) snail species. Because not all data were used to inform consumption estimates, and separate control trials were conducted without crayfish to assess snail predator-avoidance behavior, sample sizes differ among data used to evaluate consumption, predator avoidance, and other mortality. Proportions are given as a mean \pm SD.

<https://doi.org/10.1371/journal.pone.0290615.t001>

with one crayfish individual held in combination with either uninfected *Bi. glabrata* or uninfected *Bu. truncatus* snails. Round 1 also included two (72-hour long) experiments in control tanks with snails and no crayfish for each set temperature for uninfected *Bi. glabrata* and *Bu. truncatus*, for a total of 14 (12-hour long) trials for each temperature for each species in control conditions Round 2 of experiments, which included both uninfected and infected snails, began on 25 October 2021 and concluded on 17 December 2021. In Round 2 of experiments, two 72-hour experiments, for a total of 14 (12-hour long) trials for each set temperature (15, 20, 25, 30, and 35°C) were conducted in both the experimental and control conditions for both uninfected and infected *Bi. glabrata* and *Bu. truncatus* snails. Crayfish individuals used in Round 1 of experiments were also used in Round 2, barring mortality.

Analysis

We were interested in the influence of temperature, snail species, and snail infection status on the rate at which crayfish consumed snails. We assumed that crayfish body size (measured as weight in grams) would also influence consumption, because larger animals should consume more and may respond differently to temperature compared to smaller animals. Over the course of the experiment, we encountered instances when there were too many snails in a tank (i.e., more snails than the set density of 12). This may have occurred if observers overlooked a hiding snail. When this occurred, the trial in which too many snails were observed and the

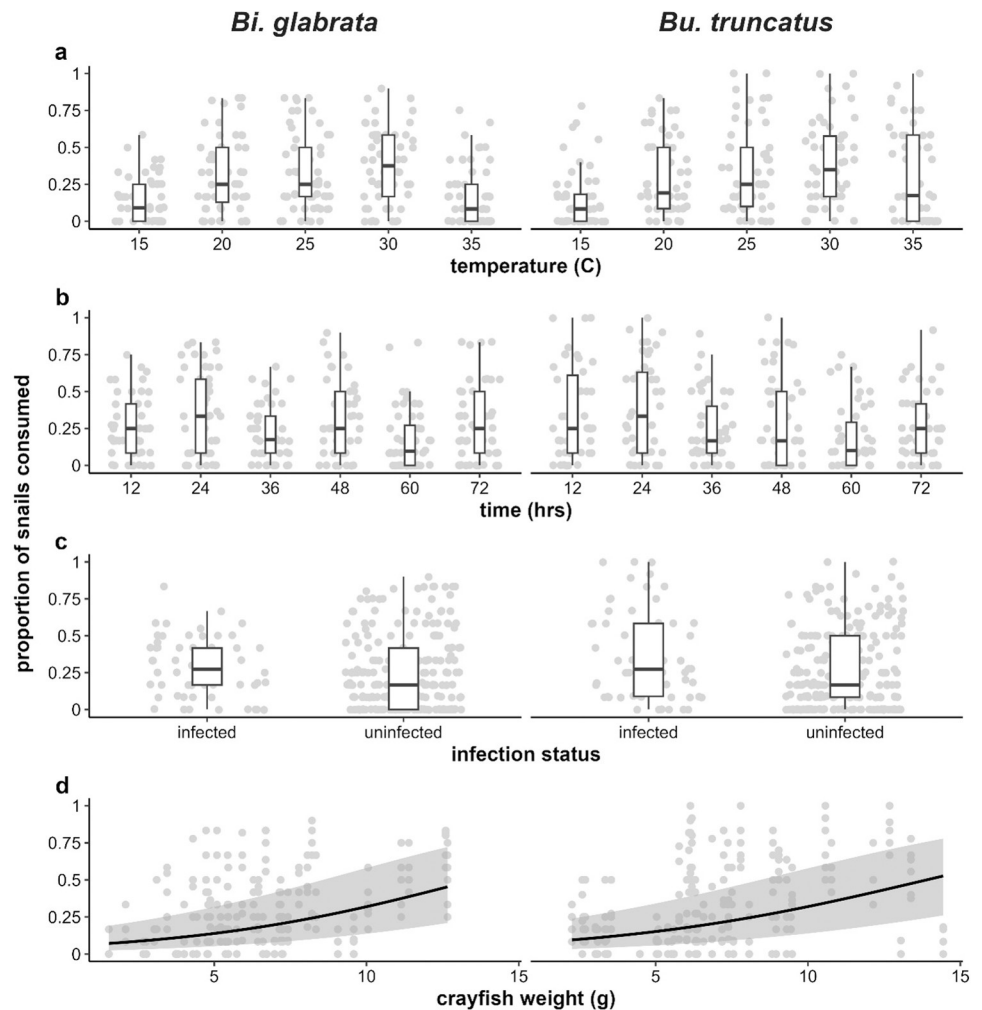


Fig 1. Bivariate plots displaying the proportion of snails consumed in each trial as jittered points with (a-c) overlaid boxplots for treatment variables, and (d) an overlaid regression curve (\pm standard error) for crayfish weight. Columns display data for each snail species, with rows displaying consumption across (a) temperature, (b) time block, (c) snail infection status, and (d) crayfish weight.

<https://doi.org/10.1371/journal.pone.0290615.g001>

previous trial were removed from analyses, resulting in the exclusion of 52 data points out of a total of 960 (5%). In addition, 16 instances of crayfish molting (the trial the molt was observed, as well as the previous and following trials) were excluded from analyses, as crayfish are known to cease feeding during molting [84]. Control tanks did not have crayfish ($n = 350$ observations) and were used to estimate background snail mortality. Therefore, only data from experimental trials ($n = 542$) were included in analyses of crayfish consumption. All analyses were run in R version 4.3.1 (2023-06-16) [85].

We analyzed crayfish consumption rates using a generalized linear mixed model (GLMM) implemented in the “glmmTMB” package in R [85, 86]. As each tank was stocked with 12 snails at the start of each trial, we used a binomial likelihood with a logit link function such that for the number of snails eaten y_i in each trial i :

$$y_i \sim \text{Binomial}(p_i, N_{s,i} - N_{d,i})$$

$$\text{logit}(p_i) = \alpha + \beta X_i + \epsilon_{c,i} + \epsilon_{r,i} + \epsilon_{cr,i}$$

where p_i as the expected probability of consumption per-snail, $N_{s,i} = 12$ is the initial snail density, $N_{d,i}$ is the number of snails which died but were not consumed, α is an intercept, and β is the vector of coefficients corresponding to the matrix of predictors X_i . We chose a binomial model with a logit link because it (1) intrinsically accounts for the fact that the response y_i is bounded between 0 and 12, and (2) accounts for the sigmoidal mean–variance relationship which is typical of binary outcome (e.g., consumed / not consumed) data; neither of these conditions are met by a Gaussian or Poisson likelihood [87]. As fixed effects, we fit snail species (2 levels), infection status (2 levels), temperature (5 levels: 15, 20, . . . 35°C), trial time (6 levels: 12, 24, . . . 72 hours), and all second- and third-order interactions among these predictors, as well as crayfish weight and the interaction between temperature and crayfish weight (Fig 1). To account for repeated observations of the same crayfish over several weeks of the experiment, we fit random intercepts for each crayfish ($\epsilon_{c,i}$) and experimental run ($\epsilon_{r,i}$), as well as an interaction between them to allow crayfish intercepts to vary across runs ($\epsilon_{cr,i}$). We used Wald χ^2 tests to conduct null hypothesis tests of main effects and interactions at the $\alpha = 0.05$ level [88]. Following significant main effects or interactions, we conducted post-hoc pairwise comparisons of the estimated marginal effects using the R package “emmeans”, adjusting p-values using the Tukey method [89], and using compact letters displays to aid interpretation [90].

In line with our experimental design, temperature and time were coded as categorical predictors, but the model estimates displayed an apparent unimodal effect of temperature and a negative, monotonic effect of time, so we conducted further tests to assess the continuous effects of these variables. For temperature, we fit a generalized additive mixed model (GAMM) using the R package “mgcv”, with the same response distribution and random effects structure as the above model, but including as fixed effects only a temperature spline and weight. We used this model to visualize continuous thermal consumption curves at 3, 7, and 11 g (approximately the 10% quantile, mean, and 90% quantiles of observed crayfish weights, respectively). To assess the linear effect of time on per capita consumption rate implied by the estimates from the main model, we computed post-hoc polynomial contrasts, extracting the linear trend component and standard error following a multivariate normal distribution around the estimated marginal means and variance-covariance matrix (on the log-odds scale) for each time point [91, 92].

Though snail density was reset prior to the start of each trial, snails could have moved to various positions within the tank (i.e., above the water line, under the shelter/hiding) where they would be inaccessible to crayfish consumption during the trial. Anti-predatory behavior may be related to infection status, as infected snails are expected to move more slowly and less frequently than uninfected snails [60]. Therefore, we fit a model to assess the number of unavailable snails (the number above the water line or under shelter/hiding) as a function of snail species, snail infection status (uninfected or infected), and condition (control or experimental). As in the main model, we used a binomial likelihood and logit link and included all second- and third-order interactions among fixed effects. As crayfish were absent from the control trials, we used only random week effects in this model. We included only data from experimental round 2 ($n = 472$), as infected snails were not examined in round 1.

Lastly, we assessed whether snails died by causes other than predation in the control tanks, and whether there were differences in background mortality between the control and treatment tanks. We employed a binomial GLMM with a logit link, regressing the number of dead snails in each trial on the same set of fixed and random effects used for the unavailable snails model, but including data from both experimental runs ($n = 890$). For both models, we evaluated main effects and interactions using Wald tests and conducted Tukey-adjusted pairwise comparisons as above.

Results

Snail consumption was significantly associated with temperature ($\chi^2(4) = 29.1$, $p < 0.001$), time point ($\chi^2(5) = 97.1$, $p < 0.001$), and crayfish weight ($\chi^2(1) = 18.9$, $p < 0.001$), but not with infection status ($\chi^2(1) = 0.42$, $p = 0.52$) or snail species ($\chi^2(1) = 0.02$, $p = 0.88$) (Fig 1). No second- or third-order interactions among the main effects were significant (S1 Table). Temperature displayed an apparent unimodal effect on consumption, with probability of consumption at the 30°C treatment ($\mu = 0.37$, 95% CI = [0.24, 0.52]) significantly greater than at both the 15°C ($\mu = 0.10$, 95% CI = [0.05, 0.18], $p = 0.002$) and 35°C treatments ($\mu = 0.13$, 95% CI = [0.07, 0.23], $p = 0.02$, Fig 2). GAMM estimates suggest that consumption peaks between the 25 and 30°C treatments, at approximately 27.2°C.

While larger crayfish consumed more snails across temperature treatments, the absence of a significant interaction between temperature and crayfish weight ($\chi^2(4) = 1.02$, $p = 0.91$) suggests that the shape and maxima of crayfish thermal consumption curves does not vary among crayfish of different sizes. Estimates of snail consumption probability across time points are consistent with a ~9% decrease in the odds snail consumption every 12 hours, indicative of a small but significant satiation effect ($e^{\beta \times 12} = 0.91$, $t(432) = -4.2$, $p < 0.001$, Fig 3).

The proportion of snails unavailable to prawns did not generally differ between infected and uninfected snails ($\chi^2(1) = 3.5$, $p = 0.06$, S2 Table). We did, however, find a significantly higher proportion of unavailable snails in experimental ($\mu = 0.17$, 95% CI = [0.14, 0.20]) than in the control ($\mu = 0.04$, 95% CI = [0.03, 0.05]) treatments ($\chi^2(1) = 90.6$, $p < 0.001$), supporting the hypothesis of a pro-active anti-predatory behavior. We also estimate significantly more unavailable *Bu. truncatus* ($\mu = 0.13$, 95% CI = [0.10, 0.15]) than *Bi. glabrata* ($\mu = 0.06$, 95% CI = [0.04, 0.07]) snails ($\chi^2(1) = 9.1$, $p = 0.003$). There was a significant interaction between experimental condition (control vs treatment) and species ($\chi^2(1) = 66.14$, $p < 0.001$), with more unavailable *Bu. truncatus* than *Bi. glabrata* in control tanks (Fig 4). There was also a significant interaction between condition and infection status ($\chi^2(1) = 66.14$, $p < 0.001$), which appears to be driven by a significantly higher proportion of infected ($\mu = 0.20$, 95% CI = [0.16,

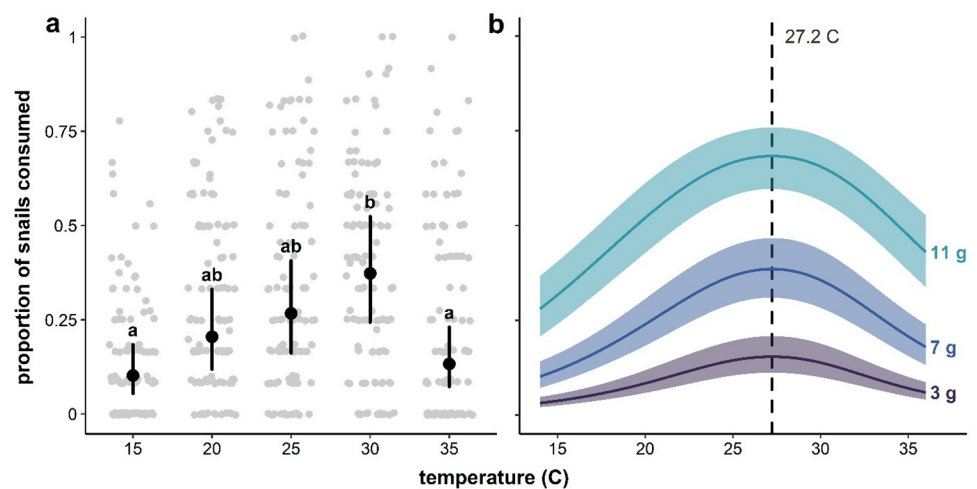


Fig 2. (a) Estimated marginal means and confidence intervals for probability of snail consumption at 15, 20, 25, 30, and 35°C, superimposed on jittered points corresponding to observed snail consumption proportions. (b) Continuous temperature curves (and 95% confidence bands) for 3, 7, and 11g individuals (approximately the 10% quantile, mean, and 90% quantile of crayfish weights). Estimates that do not share a letter are significantly different. Both discrete (GLMM estimated) and continuous (GAMM estimated) effects are consistent with a unimodal effect of temperature on consumption, with GAMM curves suggesting a peak around 27.2°C.

<https://doi.org/10.1371/journal.pone.0290615.g002>

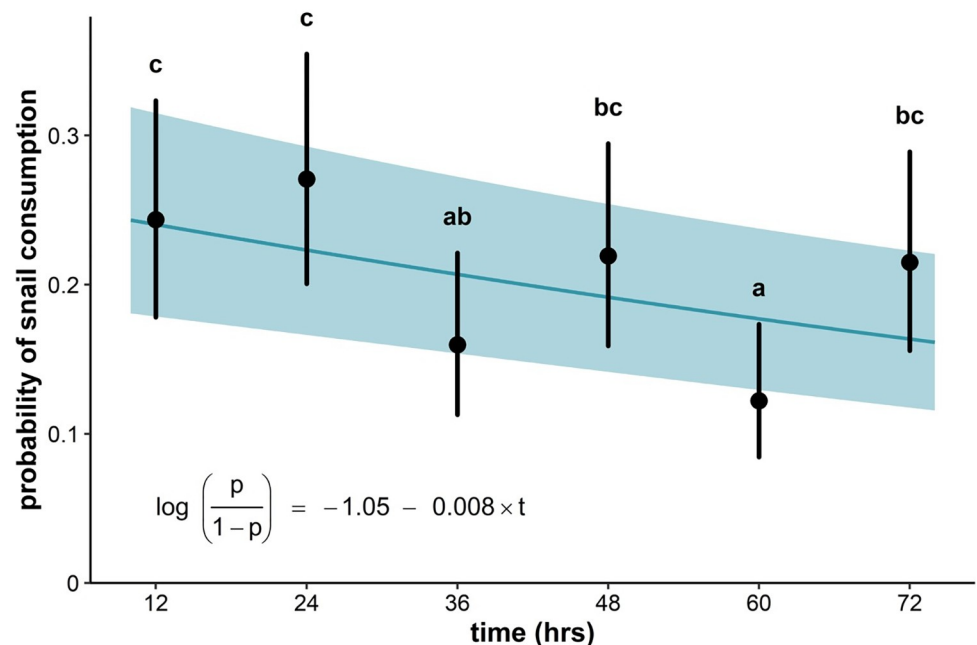


Fig 3. Estimated marginal means and confidence intervals for probability of snail consumption at trial time points (12 to 72 hrs at 12 hr intervals), superimposed on the linear time trend (and 95% confidence band). Estimates that do not share a letter are significantly different. Results are consistent with a small but significant satiation effect. The linear trend is consistent with a ~9% decrease in the odds of snail consumption every 12 hours, indicative of a small but significant satiation effect ($e^{-0.008 \times 12} = 0.91$, $t(432) = -4.2$, $p < 0.001$, $r = -0.49$).

<https://doi.org/10.1371/journal.pone.0290615.g003>

0.24]) than uninfected ($\mu = 0.13$, 95% CI = [0.10, 0.16]) *Bu. truncatus* in the experimental tanks ($p = 0.009$) with no other significant differences between uninfected and infected snails (Fig 4).

Minimal snail mortality occurred in control conditions. Of 350 total 12-hour long control trials, there were only 20 instances (6% of trials) in which the number of snails remaining in the tank was less than the original number of 12. Specifically, mortality was $0.2\% \pm 0.1\%$ (SE) for *Bu. truncatus* and $0.5\% \pm 0.1\%$ for *Bi. glabrata* in control tanks. Conversely, mortality in experimental tanks was significantly higher than in control tanks both for *Bu. truncatus* ($4.6\% \pm 0.5\%$, Wald test $t(881) = -5.17$, $p < 0.001$, S3 Table) and for *Bi. glabrata* ($1.7\% \pm 0.3\%$, $t(881) = -3.06$, $p = 0.012$), corresponding to a nearly 50-fold reduction in projected life expectancy for *Bu. truncatus* and a nearly 7-times reduction in projected life expectancy for *Bi. glabrata* under laboratory predation conditions (S1 Fig).

Discussion

In this set of experiments, the rate at which marbled crayfish consumed schistosome-competent snails was significantly influenced by temperature and crayfish weight. Per-capita crayfish consumption was shown to be a unimodal function of water temperature that peaked at intermediate temperatures between 26 and 29°C. Consumption, however, was not influenced by other factors, such as snail species and snail infection status and there was no interaction between temperature and weight. Crayfish consumed fewer snails at the low and high temperature extremes (15, 20, and 35°C, respectively) compared to moderate temperatures (25 and 30°C). In our experiment, 15°C and 20°C occurred on the rising slope of the crayfish's thermal consumption curve and 35°C on the falling slope. Crayfish consumption generally increased

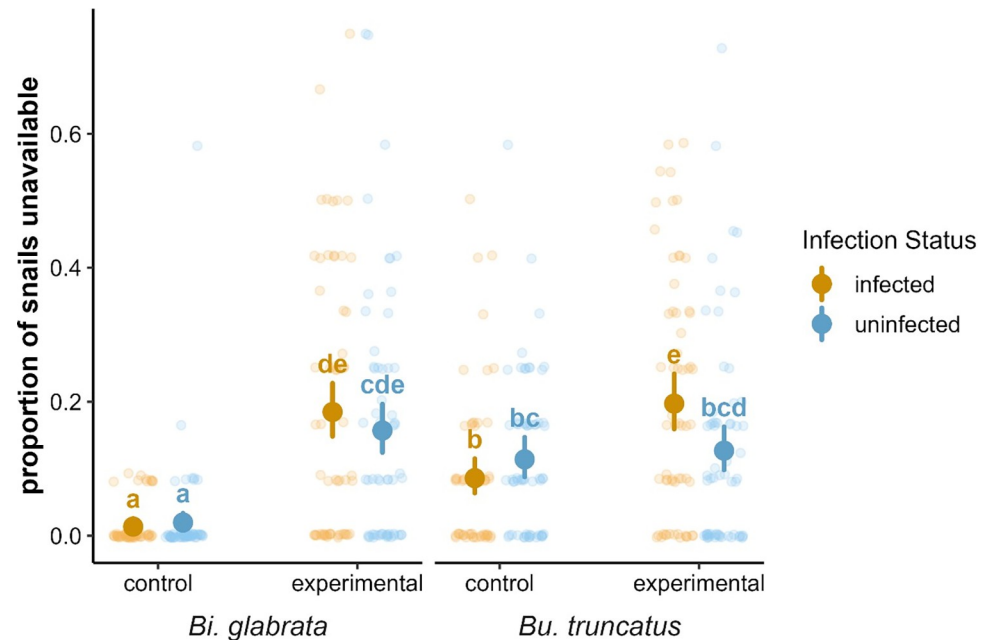


Fig 4. Estimated marginal means and confidence intervals for the proportion of unavailable snails across species, infection status, and experimental condition, superimposed on jittered points corresponding to observed proportions of unavailable snails in trials. Estimates that do not share a letter are significantly different.

<https://doi.org/10.1371/journal.pone.0290615.g004>

with increasing temperature until $\sim 27.5^{\circ}\text{C}$, at which point consumption declined. Though a similar trend emerges in previous studies, thermal optima, or the temperature at which the rate of consumption is maximized, varies considerably amongst crayfish species [74–76]. Taken together, our results demonstrate that, under laboratory conditions, marbled crayfish are voracious predators of schistosome-competent snails and that this regulatory effect is modulated by climatic conditions. These results provide a strong foundation for future investigation of the potential of marbled crayfish to regulate human disease burden by reducing intermediate host snails. Field studies are warranted to determine the regulatory effect of the invasive marbled crayfish on snail populations—and, in turn, human schistosomiasis burdens—across a broad thermal range, including habitats falling outside of the range of naturally occurring snail predators. Future studies should also investigate the relationship between marbled crayfish presence and human schistosomiasis burden, which cannot be explicitly examined within the scope of this work.

In general, larger crayfish consumed a greater number of snails across all temperatures. This pattern is consistent with previous findings for other crustacean species, in which large crayfish (*P. clarkii*) and prawns (*Macrobrachium* spp.) consumed significantly more schistosome-competent snails than smaller conspecifics [80, 93, 94]. However, marbled crayfish consumed fewer *Bi. glabrata* and *Bu. truncatus* snails, on average, than did prawns of similar size (extra small to medium-sized prawns, ranging from < 1 g to 10 g; consumption rate = 4.67 snails/12 hours).

Our results suggest that abiotic (temperature) and biotic (crayfish weight) factors influence rates of consumption and, in turn, the potential regulatory control of the marbled crayfish on snail host populations. However, interpretation of the present findings must be contextualized within the natural ecosystems in which *Schistosoma* transmission takes place, and therefore, we must bear in mind that crayfish are just one player impacted by temperature. Temperature plays an important, if not *the* most important, role in the distribution of schistosome-

competent snails and human schistosomiasis at the large geographical scale [95, reviewed in 96, 97]. Snail hatching, growth, fecundity, and survivorship are also substantially impacted by temperature [81, 82 reviewed in 96, 97]. In an experiment investigating the influence of temperature on the biology of schistosome-competent snails, Sturrock and Sturrock [82] found that *Bi. glabrata* snails held at 25°C and 30°C hatched two weeks earlier than conspecifics held at 20°C. Egg hatching was not observed in 35°C aquaria, and hatchlings transferred to these tanks did not survive past two weeks. Following hatching, growth rate increased with increasing temperature (20–30°C), and snails in the 25°C and 30°C tanks reached sexual maturity and began laying eggs eight weeks earlier than snails in 20°C tanks. The number of eggs laid per two-week period varied considerably across temperature conditions, with snails laying six to eight times as many eggs in the 25°C condition compared to other conditions. Survivorship was also highest in 25°C tanks [82]. Likewise, temperature impacts the penetration and subsequent development of *Schistosoma* miracidia (measured as the number of daughter sporocysts) [98] in snail intermediate hosts, as well as the production of free-swimming cercariae [99], cercarial emergence [100], and survival [101]. Miracidial infection in snails increased with increasing temperature (ranging from 10°C to 40°C at 3°C intervals), as did the number of daughter sporocysts produced within a snail host to a point (production decreased at 40°C) [98]. Cercarial production increases between 15°C and 31°C [99]. As snail metabolic activity, energy, and vitality (e.g., fecundity, survival, and mortality rate) increases with rising temperatures (to a point), so does cercarial production with the snail intermediate host [100, 101, reviewed in 96]. In general, cercariae survival decreases as the water temperature increases, with exponential increases in mortality occurring at temperatures below 15°C and greater than 35°C [101]. Taken together, the results of our study demonstrate that the optimum temperature for crayfish consumption coincides well with that of snail egg production and hatching [82]. When crayfish consumption of intermediate snail hosts is expected to be hindered by low or high temperatures (i.e., the thermal limit), as demonstrated by the present study, cercarial production and survival—and, in turn, the risk of schistosomiasis transmission and infection—is also expected to be lower [99, 101].

An important finding was that neither snail species nor snail infection status were significantly associated with rates of marbled crayfish consumption. Swartz and colleagues [60] postulated that schistosomiasis control efforts may be enhanced by snail predators, if infected snails are consumed preferentially. On the other hand, intervention efforts may be hindered should predators avoid consuming infected snail hosts. Prawns (*Macrobrachium vollehenovonii*) preferentially consume *Bi. glabrata* snails exposed to *S. mansoni* and *Bu. truncatus* snails exposed to *S. haematobium* [60], but we found that marbled crayfish did not preferentially consume infected snails. One possible explanation is that *Schistosoma* spp. infections were not given adequate time to develop initially or that exposure to differing temperatures resulted in variations in the rate at which infections developed [102], making the difference between uninfected and infected snails negligible. While prawns are native to the sub-Saharan riverine system, the marbled crayfish evolved only recently [12–15] from a progenitor species (*Procambarus fallax*) that has a distribution that does not overlap with schistosome-infected snails [103, 104]. As such, marbled crayfish have not co-evolved with schistosome-competent snails, meaning that snails, regardless of infection status, may not have yet developed anti-predator behavior to this unfamiliar predator. Alternatively, the marbled crayfish, as a recently evolved invader, may also not be able to detect physiological differences between uninfected and infected snails that may otherwise make infected animals more detectable or desirable to predators [105]. Therefore, differences in predator preference between infected and uninfected snails may be diminished in the interaction between marbled crayfish and snails. The present

study may have also lacked statistical power, hindering our ability to detect an effect of snail infection status on crayfish consumption rates across time points.

We postulated that crayfish consumption of uninfected and infected snails would be influenced by the number of snails available to be consumed, which could differ depending on snail infection status (as described above) [60]. We observed more unavailable snails in experimental trials compared to control trials, indicating that both *Bi. glabrata* and *Bu. truncatus* snails respond to the presence of marbled crayfish with enhanced anti-predator behavior. Snail availability, however, did not generally differ between infected and uninfected snails in either the experimental or control conditions. This suggests that both *Bu. truncatus* and *Bi. glabrata* snails, regardless of infection status, engage in anti-predator behaviors when exposed to a marbled crayfish individual in the experimental condition. The fact that there were no systematic differences in the number of unavailable snails between the infected and uninfected conditions suggests that the lack of an overall effect of infection status on crayfish consumption rates probably does not arise from opposing effects of preference and accessibility (i.e., our experiments do not support the hypothesis that crayfish prefer infected snails but infected snails are harder to get, or vice versa). These findings contrast those of Swartz and colleagues [60], who found that snails infected with *Schistosoma* spp. exhibit a diminished anti-predator response compared to uninfected snails when exposed to simulated predation cues—an unexpected outcome given that the two studies used genetically identical strains of snails and parasites. However, in the present experiment, snail behavior and crayfish consumption are confounded in the experimental treatment; that is, the difference between the number of unavailable snails in control versus experimental treatments could be due either to differences in snail behavior (i.e., more snails are choosing habitats that make them unavailable in the experimental condition compared to the control condition) or crayfish consumption (i.e., there are fewer unavailable snails because crayfish have eaten snails in the predation experiments). It is therefore possible that, in the absence of crayfish consumption (by caging crayfish in the experimental tanks and preventing them to predate on snails), we might have observed a greater number of unavailable snails in the experimental treatment, and this would have revealed more marked expression of anti-predator behavior among uninfected snails compared to infected snails, consistent with Swartz et al. [60]. Future studies should monitor uninfected and infected snail behavior in a simulated predator condition (described by [60]), where the marbled crayfish is present but unable to access and consume the snails and consumption is simulated through the addition of crushed conspecifics.

As with any experimental study, several caveats are worth noting. Behavioral differences are known to arise between aquarium- and naturally reared marbled crayfish; aquarium-reared individuals tend to be more active and aggressive [105], suggesting that laboratory consumption rates may not reflect the rates occurring in nature. However, previous field observations in Madagascar found that native snails were absent from areas in which the marbled crayfish had established [65], providing supportive (although not conclusive) field evidence that this invader may prey upon snails in nature. Previous studies have also demonstrated links between the abundance of schistosome-competent snails and human infection burdens [38, 42, 60, 80, 106]. Though this is promising evidence in support of the crayfish's potential to serve as a biological control of snail intermediate hosts, our results demonstrate that ecological parameters, such as crayfish weight and temperature, could influence the relationship between invasive enemies, snails, and human schistosomiasis burden. For example, based on peak rates of consumption from the experiment, crayfish in lower (<25°C) and higher (>30°C) water temperatures may be less likely to reduce snail populations. Anyway, no sustained transmission of schistosomiasis seems to occur when average temperature in the warmest quarter exceeds ca. 31–32°C [97], so other ecological processes concur to limit transmission risk at high

temperature and reduction of crayfish consumption rate in the upper thermal range of the distribution won't be relevant. On the other hand, per-capita crayfish consumption rate might be significantly lower than at peak in the Madagascar highland where mean annual temperature is around 25°C or below. Also, our experiments showed that per capita consumption rate in the 6 consecutive 12-hour long trials of the predation experiments slightly decreased with time, a possible indication of satiation, thus slightly reducing the effectiveness of predation control by marbled crayfish. Jointly with the observation that snails exhibited a clear anti-predatory behavior and actively searched for predation refugia, there is the possibility that in natural wildlife conditions, predation rate might be lower than estimated in our laboratory experiments.

While invasion of alien species should be avoided by any means, we observe that, according to the results of this laboratory experiment and field evidence of a reduction of snail abundance where the crayfish invaded, the presence of the marbled crayfish may provide an unexpected co-benefit to the people of Madagascar: crayfish-driven reductions in burdens of human schistosomiasis [38, 41, 42]—something that is urgently needed in a country where prevalence in some villages can range up to 94% [66, 67]. However, this effect needs to be weighed against other considerations as the country leadership grapples with all the ecological and social impacts of a significant number of invasive species [107] and, specifically, of the marbled crayfish. Several studies have clearly documented that the invasion of the Louisiana crayfish *Procambarus clarkii* can have dramatic impacts on native invertebrate fauna [108, 109]. Introduced marbled crayfish directly threaten Madagascar's native aquatic wildlife [65]—a pressing concern given approximately 90% of plant species, 36% of birds, 90% of mammals, 96% of reptiles, 33% of fish and 86% of macroinvertebrates are endemic to the island [110].

On the other hand, natural predators of the obligate host snail of schistosome parasites may be playing an important role in the diet of children [111, 112], serving as an opportunistic and rapidly renewable nutritional source in a country where ~50% of children experience stunted growth due to lack of sufficient dietary protein [113]. Non-native species have long been recognized as having profound effects on ecosystem services [7–9] through their influence on the abundance and distribution of their prey and competitors [10, reviewed in 11]. However, empirical evidence of such consequences does not exist for the vast majority of non-native introductions [114–116]. Non-native species may, in some regards, have negligible and even positive impacts on ecosystems [43, 117], and careful, balanced evaluations of all the benefits and disservices associated with species introductions are needed to better inform management strategies [43, 117–119]. Drawing upon examples of non-native crayfish, the impact of a non-native species on ecosystems may vary considerably and is probably species-specific and context-dependent [120–123]—thus limiting our ability to make broad predictions regarding the multi-faceted impacts of invasive species on biodiversity and ecosystem services, including the regulation of disease agents. In general, existing evidence of the positive impact of natural enemies on human diseases is exceedingly rare, as are empirical investigations linking species interactions within the environment to outcomes of human disease [reviewed in 2].

Our study shows unequivocally that crayfish are voracious predators of schistosome-competent snails and that temperature modulates consumption rates. Whether crayfish are able to reduce schistosomiasis prevalence in the human population by controlling snail abundance at the transmission sites has yet to be determined. Field studies are needed to determine whether the snail consumption documented here will translate into impacts on snail populations and human schistosomiasis burden. However, the possibility remains that reductions in schistosomiasis transmission might be a silver lining to the invasion of *Procambarus virginalis* in Madagascar. Before any proactive attempt to deploy non-native species for schistosomiasis control, future studies will need to carefully quantify the co-occurring ecological impacts and potential

benefits for nutrition and reduction in schistosomiasis transmission risk of nature-based solutions for disease control. Until then, it is imperative that the invasive crayfish must not be introduced to freshwaters beyond its current range. The present work lays the foundation for future exploration of the potential role of marbled crayfish in the regulation of schistosome-competent snails and draws attention to the untapped potential for non-native species to regulate disease.

Supporting information

S1 Fig. Estimated means and confidence intervals for snail mortality across species experimental condition. Estimates that do not share a letter are significantly different.

(TIF)

S1 Table. Wald tests for main effects and interactions for binomial GLMM fit to the number of consumed snails in experimental trials. Asterisks indicate significant predictors.

(DOCX)

S2 Table. Wald tests for main effects and interactions for binomial GLMM fit to the number of unavailable snails in control and experimental trials. Asterisks indicate significant predictors.

(DOCX)

S3 Table. Wald tests for main effects and interactions for binomial GLMM fit to the number of dead snails in control and experimental trials. Asterisks indicate significant predictors.

(DOCX)

Acknowledgments

B. glabrata (M-line) and *B. truncatus* (Egypt) provided by the NIAID Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD, USA) through NIH-NIAID Contract HHSN272201700014I for distribution through BEI Resources. We thank Dr. Cheryl Logan (California State University, Monterey Bay) for her assistance with calculating thermal performance curves. We would also like to thank Allie Bilson, Irissa Danke, Madeline Domico, Henry Feldman, Genevieve Kent, Samantha Kuhn, Ana Olsen, Michelle Munoz, and Ryan Pittsinger for assisting in data collection.

Disclaimer: Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Author Contributions

Conceptualization: Sara M. Faiad, Maureen A. Williams, Susanne Sokolow, Julian D. Olden, Kaitlyn Mitchell, Ranja Andriantsoa, Julia Patricia Gordon Jones, Luciano Andriamaro, Pascaline Ravoniarimbina, Jeanne Rasamy, Tsilavina Ravelomanana, Salohy Ravelotafita, Ranaivosolo Ravo, Peter Rabinowitz, Giulio A. De Leo, Chelsea L. Wood.

Data curation: Sara M. Faiad, Maureen A. Williams, Maurice Goodman.

Formal analysis: Sara M. Faiad, Maureen A. Williams, Maurice Goodman, Susanne Sokolow, Julian D. Olden, Kaitlyn Mitchell, Giulio A. De Leo, Chelsea L. Wood.

Funding acquisition: Sara M. Faiad, Susanne Sokolow, Julia Patricia Gordon Jones, Luciano Andriamaro, Pascaline Ravoniarimbina, Jeanne Rasamy, Tsilavina Ravelomanana, Peter Rabinowitz, Chelsea L. Wood.

Investigation: Sara M. Faiad, Maureen A. Williams.

Methodology: Sara M. Faiad, Maureen A. Williams, Susanne Sokolow, Julian D. Olden, Ranja Andriantsoa, Giulio A. De Leo, Chelsea L. Wood.

Project administration: Sara M. Faiad.

Resources: Sara M. Faiad, Chelsea L. Wood.

Supervision: Chelsea L. Wood.

Visualization: Sara M. Faiad, Maureen A. Williams.

Writing – original draft: Sara M. Faiad.

Writing – review & editing: Sara M. Faiad, Maureen A. Williams, Maurice Goodman, Susanne Sokolow, Julian D. Olden, Kaitlyn Mitchell, Ranja Andriantsoa, Julia Patricia Gordon Jones, Luciano Andriamaro, Pascaline Ravoniarimbina, Jeanne Rasamy, Tsilavina Ravelomanana, Salohy Ravelotafita, Ranaivosolo Ravo, Peter Rabinowitz, Giulio A. De Leo, Chelsea L. Wood.

References

1. Horwitz P, Wilcox BA. Parasites, ecosystems and sustainability: an ecological and complex systems perspective. *International Journal for Parasitology*. 2005; 35:725–32. <https://doi.org/10.1016/j.ijpara.2005.03.002> PMID: 15925596
2. Jones IJ, Sokolow SH, De Leo GA. Three reasons why expanded use of natural enemy solutions may offer sustainable control of human infections. *People and Nature*. 2022; 4:32–43. <https://doi.org/10.1002/pan3.10264> PMID: 35450207
3. Lopez LK, Duffy MA. Mechanisms by which predators mediate host–parasite interactions in aquatic systems. *Trends in Parasitology*. 2021; 37:890–906. <https://doi.org/10.1016/j.pt.2021.06.006> PMID: 34281798
4. Duffy MA, Hall SR, Tessier AJ, Huebner M. Selective predators and their parasitized prey: are epidemics in zooplankton under top-down control? *Limnology and Oceanography*. 2005; 50:412–20.
5. Lugassy L, Amdouni-Boursier L, Alout H, Berrebi R, Boète C, Boué F, et al. What evidence exists on the impact of specific ecosystem components and functions on infectious diseases? A systematic map. *Environmental Evidence*. 2021; 10:11.
6. Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, et al. No saturation in the accumulation of alien species worldwide. *Nature Communications*. 2017; 8:14435. <https://doi.org/10.1038/ncomms14435> PMID: 28198420
7. IPBES. The global assessment report on biodiversity and ecosystem services: summary for policymakers [Internet]. Bonn: IPBES secretariat; 2019[cited 5 Aug 2022]. Available from: https://www.researchgate.net/publication/348845184_Summary_for_policymakers_of_the_global_assessment_report_on_biodiversity_and_ecosystem_services_of_the_Intergovernmental_Science-Policy_Platform_on_Biodiversity_and_Ecosystem_Services
8. Lockwood JL, Hoopes MF, Marchetti MP. *Invasion Ecology*. 2nd ed. Hoboken: Wiley-Blackwell; 2013.
9. Lodge DM, Simonin PW, Burgiel SW, Keller RP, Bossenbroek JM, Jerde CL, et al. Risk analysis and bioeconomics of invasive species to inform policy and management. *Annual Review of Environment and Resources*. 2016; 41:453–88.
10. Gherardi F, Acquistapace P. Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology*. 2007; 52:1249–59.
11. David P, Thebault E, Anneville O, Duyck PF, Chapuis E, Loeuille N. Impacts of invasive species on food webs: a review of empirical data. *Advances in Ecological Research*. 2017; 56:1–60.

12. Gutkenst J, Maiakovska O, Hanna K, Provataris P, Horn H, et al. Phylogeographic reconstruction of the marbled crayfish origin. *Communications Biology*. 2021; 4:1096. <https://doi.org/10.1038/s42003-021-02609-w> PMID: 34535758
13. Gutekunst J, Andriantsoa R, Falckenhayn C, Hanna K, Stein W, Rasamy J, et al. Clonal genome evolution and rapid invasive spread of the marbled crayfish. *Nature Ecology & Evolution*. 2018; 2:567–73. <https://doi.org/10.1038/s41559-018-0467-9> PMID: 29403072
14. Jones JPG, Rasamy JR, Harvey A, Toon A, Oidtmann B, Randrianarison MH, et al. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions*. 2009; 11:1475–82.
15. Kawai T, Scholtz G, Morioka S, Ramanamandimby F, Lukhaup C, Hanamura Y. Parthenogenetic alien crayfish (Decapoda: Cambaridae) spreading in Madagascar. *Journal of Crustacean Biology*. 2009; 29:562–7.
16. Global Atlas of Helminth Infections (GAHI) [Internet]. London: Global Atlas of Helminth Infections; c2022 [cited 5 Aug 2022]. Distribution of schistosomiasis survey data in Madagascar; [about 4 screens]. Available from: <https://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-madagascar>
17. World Health Organization [Internet]. Geneva: World Health Organization (WHO); c2022 [cited 5 Aug 2022]. Schistosomiasis; [about 8 screens]. Available from: <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>
18. Centers for Disease Control and Prevention (CDC) [Internet]. Atlanta: U.S. Department of Health & Human Services; c2019 [cited 5 Aug 2022]. Parasites—Schistosomiasis; [about 5 screens]. Available from: <https://www.cdc.gov/parasites/schistosomiasis/biology.html>
19. Hotez PJ, Alvarado M, Basáñez MG, Bolliger I, Bourne R, Boussinesq M, et al. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical Diseases*. 2014; 8:e2865. <https://doi.org/10.1371/journal.pntd.0002865> PMID: 25058013
20. Bonds MH, Keenan DC, Rohani P, Sachs JD. Poverty trap formed by the ecology of infectious diseases. *Proc. R. Soc. B*. 2010; 277:1185–92. <https://doi.org/10.1098/rspb.2009.1778> PMID: 20007179
21. Parker M. Re-assessing disability: the impact of schistosomal infection on daily activities among women in Gezira Province, Sudan. *Social Science & Medicine*. 1992; 35:877–90. [https://doi.org/10.1016/0277-9536\(92\)90102-v](https://doi.org/10.1016/0277-9536(92)90102-v) PMID: 1411688
22. Tanner M. Evaluation of public-health impact of schistosomiasis. *Tropical Medicine and Parasitology*. 1989; 40:143–8. PMID: 2505373
23. World Health Organization [Internet]. Geneva: World Health Organization (WHO); c2022 [cited 5 Aug 2022]. Neglected tropical diseases; [about 3 screens]. Available from: <https://www.who.int/news-room/questions-and-answers/item/neglected-tropical-diseases>
24. World Health Organization [Internet]. Geneva: World Health Organization (WHO); c2022 [cited 12 Sept 2022]. Preventive Chemotherapy (PC) Data Porta; [about 5 screens]. Available from: <https://www.who.int/data/preventive-chemotherapy>
25. Cioli D, Pica-Mattoccia L, Basso A, Guidi A. Schistosomiasis control: praziquantel forever? *Molecular and Biochemical Parasitology*. 2014; 195:23–9. <https://doi.org/10.1016/j.molbiopara.2014.06.002> PMID: 24955523
26. Chandiwana SK, Woolhouse ME, Bradley M. Factors affecting the intensity of reinfection with *Schistosoma haematobium* following treatment with praziquantel. *Parasitology*. 1991; 102:73–83. <https://doi.org/10.1017/s0031182000060364> PMID: 1903882
27. Garba A, Lamine MS, Barkiré N, Djibo A, Sofo B, Gouvras AN, et al. Efficacy and safety of two closely spaced doses of praziquantel against *Schistosoma haematobium* and *S. mansoni* and re-infection patterns in school-aged children in Niger. *Acta Tropica*. 2013; 128:334–44.
28. Moloney NA, Hinchcliffe P, Webbe G. Loss of resistance to reinfection with *Schistosoma japonicum* in mice after treatment with praziquantel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1987; 81:247–54.
29. N'Goran EK, Utzinger J, N'Guessan AN, Müller I, Zambé K, Lohourignon KL, et al. Reinfection with *Schistosoma haematobium* following school-based chemotherapy with praziquantel in four highly endemic villages in Côte d'Ivoire. *Tropical Medicine & International Health*. 2001; 6:817–25.
30. Webster BL, Diaw OT, Seye MM, Faye DS, Stothard JR, Sousa-Figueiredo JC, et al. Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Tropica*. 2013; 128:292–302. <https://doi.org/10.1016/j.actatropica.2012.09.010> PMID: 23022016

31. Gönner R, Andrews P. Praziquantel, a new broad-spectrum antischistosomal agent. *Zeitschrift für Parasitenkunde*. 1977; 52:129–50.
32. Pica-Mattoccia L, Cioli D. Sex- and stage-related sensitivity of *Schistosoma mansoni* to in vivo and in vitro praziquantel treatment. *International Journal for Parasitology*. 2004; 34:527–33.
33. Sabah AA, Fletcher C, Webbe G, Doenhoff MJ. *Schistosoma mansoni*: chemotherapy of infections of different ages. *Experimental Parasitology*. 1986; 61:294–303.
34. World Health Organization [Internet]. Geneva: World Health Organization (WHO); c2022 [cited 12 Sept 2022]. News / WHO launches new guideline for the control and elimination of human schistosomiasis; [about 5 screens]. Available from: <https://www.who.int/news/item/22-02-2022-who-launches-new-guideline-for-the-control-and-elimination-of-human-schistosomiasis>
35. Sokolow SH, Wood CL, Jones IJ, Lafferty KD, Kuris AM, Hsieh MH, et al. To reduce the global burden of human schistosomiasis, use “old fashioned” snail control. *Trends in Parasitology*. 2018; 34:23–40. <https://doi.org/10.1016/j.pt.2017.10.002> PMID: 29126819
36. Diakit  NR N'Zi KG, Ouattara M, Coulibaly JT, Saric J, Yao PK, et al. Association of riverine prawns and intermediate host snails and correlation with human schistosomiasis in two river systems in south-eastern C te d'Ivoire. *Parasitology*. 2018; 145:1792–800.
37. Halstead NT, Hoover CM, Arakala A, Civitello DJ, De Leo GA, Gambhir M, et al. Agrochemicals increase risk of human schistosomiasis by supporting higher densities of intermediate hosts. *Nature Communications*. 2018; 9:837. <https://doi.org/10.1038/s41467-018-03189-w> PMID: 29483531
38. Sokolow SH, Huttinger E, Jouanard N, Hsieh MH, Lafferty KD, Kuris AM, et al. Reduced transmission of human schistosomiasis after restoration of a native river prawn that preys on the snail intermediate host. *PNAS*. 2015; 112:9650–5. <https://doi.org/10.1073/pnas.1502651112> PMID: 26195752
39. Stauffer JR, Madsen H, McKaye K, Konings A, Bloch P, Ferreri CP, et al. Schistosomiasis in Lake Malawi: relationship of fish and intermediate host density to prevalence of human infection. *EcoHealth*. 2006; 3:22–27.
40. Sokolow SH, Jones IJ, Jocque M, La D, Cords O, Knight A, et al. Nearly 400 million people are at higher risk of *schistosomiasis* because dams block the migration of snail-eating river prawns. *Phil. Trans. R. Soc. B*. 2017; 372:20160127.
41. Khalil MT, Sleem SH. Can the freshwater crayfish eradicate schistosomiasis in Egypt and Africa? *The Journal of American Science*. 2011; 7:457–62.
42. Mkoji GM, Hofkin BV, Kuris AM, Stewart-Oaten A, Mungai BN, Kihara JH, et al. Impact of the crayfish *Procambarus clarkii* on *Schistosoma haematobium* transmission in Kenya. *Am. J. of Trop. Med. and Hyg.* 1999; 61:751–9. <https://doi.org/10.4269/ajtmh.1999.61.751> PMID: 10586907
43. Schlaepfer MA, Sax DF, Olden JD. The potential conservation value of non-native species. *Conservation Biology*. 2011; 25:428–37. <https://doi.org/10.1111/j.1523-1739.2010.01646.x> PMID: 21342267
44. Covich AP. Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia*. 2010; 653:191–215.
45. Dorn NJ. Consumptive effects of crayfish limit snail populations. *Freshwater Science*. 2013; 32:1298–308.
46. Dorn NJ, Wojdak JM. The role of omnivorous crayfish in littoral communities. *Oecologia*. 2004; 140:150–9. <https://doi.org/10.1007/s00442-004-1548-9> PMID: 15064944
47. Johnson PT, Olden JD, Solomon CT, Vander Zanden MJ. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia*. 2009; 159:161–70. <https://doi.org/10.1007/s00442-008-1176-x> PMID: 18941789
48. Klose K, Cooper SD. Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities. *Freshwater Biology*. 2012; 57:526–40.
49. Kreps TA, Baldrige AK, Lodge DM. The impact of an invasive predator (*Orconectes rusticus*) on freshwater snail communities: insights on habitat-specific effects from a multilake long-term study. *Canadian Journal of Fisheries and Aquatic Sciences*. 2012; 69:1164–73.
50. Lodge DM, Kershner MW, Alois JE, Covich AP. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology*. 1994; 75:1265–81.
51. Nystr m PER, Br nmark C, Graneli W. Patterns in benthic food webs: a role for omnivorous crayfish? *Freshwater Biology*. 1996; 36:631–46.
52. Preisser EL, Bolnick DI, Benard MF. Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology*. 2005; 86:501–9.
53. Crowl TA, and Covich AP. Predator-induced life-history shifts in a freshwater snail. *Science*. 1990; 247:949–51. <https://doi.org/10.1126/science.247.4945.949> PMID: 17776452

54. Lewis DB. Trade-offs between growth and survival: responses of freshwater snails to predacious crayfish. *Ecology*. 2001; 82:758–65.
55. Turner AM. Non-lethal effects of predators on prey growth rates depend on prey density and nutrient additions. *Oikos*. 2004; 104:561–9.
56. Turner AM, Montgomery SL. Spatial and temporal scales of predator avoidance: experiments with fish and snails. *Ecology*. 2003; 84:616–22.
57. Hoverman JT, Auld JR, Relyea RA. Putting prey back together again: Integrating predator-induced behavior, morphology, and life history. *Oecologia*. 2005; 144:481–91. <https://doi.org/10.1007/s00442-005-0082-8> PMID: 15891826
58. Covich AP, Crowl TA, Alexander JE Jr, Vaughn CC. Predator-avoidance responses in freshwater decapod-gastropod interactions mediated by chemical stimuli. *Journal of the North American Benthological Society*. 1994; 13:283–90.
59. Turner AM, Fetterolf SA, Bernot RJ. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia*. 1999; 118:242–7. <https://doi.org/10.1007/s004420050724> PMID: 28307700
60. Swartz SJ, De Leo GA, Wood CL, Sokolow SH. Infection with schistosome parasites in snails leads to increased predation by prawns: implications for human schistosomiasis control. *The Journal of Experimental Biology*. 2015; 218:3962–7. <https://doi.org/10.1242/jeb.129221> PMID: 26677260
61. Bernot RJ. Trematode infection alters the antipredator behavior of a pulmonate snail. *Journal of the North American Benthological Society*. 2003; 22:241–8.
62. Levri EP. Perceived predation risk, parasitism, and the foraging behavior of a freshwater snail (*Potamopyrgus antipodarum*). *Canadian Journal of Zoology*. 1998; 76:1878–84.
63. Levri EP. Parasite-induced change in host behavior of a freshwater snail: parasitic manipulation or byproduct of infection? *Behavioral Ecology*. 1999; 10:234–41.
64. Vogt G, Tolley L, Scholtz G. Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. *Journal of Morphology*. 2004; 261:286–311. <https://doi.org/10.1002/jmor.10250> PMID: 15281058
65. Andriantsoa R, Tönges S, Panteleit J, Theissinger K, Carneiro VC, Rasamy J, et al. Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. *BMC Ecology*. 2019; 19:8. <https://doi.org/10.1186/s12898-019-0224-1> PMID: 30727994
66. Rasoamananjahaja CF, Rahetilahy AM, Ranjatoarivony B, Dhanani N, Andriamaro L, Andrianarisoa SH, et al. Baseline prevalence and intensity of schistosomiasis at sentinel sites in Madagascar: informing a national control strategy. *Parasites & Vectors*. 2016; 9:50. <https://doi.org/10.1186/s13071-016-1337-4> PMID: 26822783
67. Spencer SA, Penney JM, Russell HJ, Howe AP, Linder C, Rakotomampianina AL, et al. High burden of *Schistosoma mansoni* infection in school-aged children in Marolambo District, Madagascar. *Parasites & Vectors*. 2017; 10:307.
68. Jones JPG, Andriahajaina FB, Hockley NJ, Crandall KA, Ravoahangimalala OR. The ecology and conservation status of Madagascar's endemic freshwater crayfish (Parastacidae; Astacoides). *Freshwater Biology*. 2007; 52:1820–33.
69. Toon A, Pérez-Losada M, Schweitzer CE, Feldmann RM, Carlson M, Crandall KA. Gondwanan radiation of the southern hemisphere crayfishes (Decapoda: Parastacidae): evidence from fossils and molecules. *Journal of Biogeography*. 2010; 37:2275–90.
70. Veselý L, Buřič M, Kouba A. Hardy exotics species in temperate zone: can “warm water” crayfish invaders establish regardless of low temperatures? *Scientific Reports*. 2015; 5:16340. <https://doi.org/10.1038/srep16340> PMID: 26572317
71. Jimenez SA, Faulkes Z. Establishment and care of a colony of parthenogenetic marbled crayfish, Marmorkrebs. *Invertebrate Rearing*. 2010; 1:10–8.
72. Seitz R, Vilpoux K, Hopp U, Harzsch S, Maier G. Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. *Journal of Experimental Zoology*. 2005; 303:393–405. <https://doi.org/10.1002/jez.a.143> PMID: 15828010
73. Angilletta MJ. *Thermal adaptation: a theoretical and empirical synthesis*. Oxford: Oxford University Press; 2009.
74. Rodríguez Valido CA, Johnson MF, Dugdale SJ, Cutts V, Fell HG, Higgins EA, et al. Thermal sensitivity of feeding and burrowing activity of an invasive crayfish in UK waters. *Ecohydrology*. 2021; 14:e2258.
75. Seals C, Eversole AG, Tomasso JR, Petrosky BR. Effects of temperature on feeding activity of the White River crayfish *Procambarus acutus acutus*. *Journal of the World Aquaculture Society*. 1997; 28:133–41.

76. Whitley GW, Rabeni CF. Maximum daily consumption and respiration rates at four temperatures for five species of crayfish from Missouri, USA (Decapoda, *Orconectes* spp.). *Crustaceana*. 2002; 75:1119–32.
77. Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. Toward a metabolic theory of ecology. *Ecology*. 2004; 85:1771–89.
78. Biomedical Research Institute (BRI) [Internet]. Rockville: Biomedical Research Institute; c2022 [cited 4 Aug 2022]. Artificial pond water for the cultivation of *Biomphalaria glabrata*, *Bulinus truncatus* and *Oncomelania hupensis*; [about 2 screens]. Available from: <https://www.afbr-bri.org/schistosomiasis/standard-operating-procedures/artificial-pond-water/>
79. Biomedical Research Institute (BRI) [Internet]. Rockville: Biomedical Research Institute; c2022 [cited 4 Aug 2022]. Changing containers of snails [about 2 screens]. Available from: <https://www.afbr-bri.org/schistosomiasis/standard-operating-procedures/changing-containers-of-snails/>
80. Sokolow SH, Lafferty KD, Kuris AM. Regulation of laboratory populations of snails (*Biomphalaria* and *Bulinus* spp.) by river prawns, *Macrobrachium* spp. (decapoda, palaemonidae): implications for control of schistosomiasis. *Acta Tropica*. 2014; 132:64–74. <https://doi.org/10.1016/j.actatropica.2013.12.013> PMID: 24388955
81. Joubert PH, Pretorius SJ, De Kock KN, Van Eeden JA. Survival of *Bulinus africanus* (Krauss), *Bulinus globosus* (Morelet) and *Biomphalaria pfeifferi* (Krauss) at constant high temperatures. *African Zoology*. 1986; 21:85–8.
82. Sturrock RF, Sturrock BM. The influence of temperature on the biology of *Biomphalaria glabrata* (Say), intermediate host of *Schistosoma mansoni* on St. Lucia, West Indies. *Annals of Tropical Medicine and Parasitology*. 1972; 66:385–390.
83. Lewis FA, Stirewalt MA, Souza CP, Gazzinelli G. Large-scale laboratory maintenance of *Schistosoma mansoni*, with observations on three schistosome/snail host combinations. *The Journal of Parasitology*. 1986; 72:813–29.
84. Huner JV, Barr JE. Red swamp crawfish: Biology and exploitation. Louisiana Sea Grant College Program; 1991.
85. R Core Team. R: a language and environment for statistical computing. Version 4.0.4 [software]. R Foundation for Statistical Computing. 2021 [cited 5 Aug 2022]. Available from: <https://www.R-project.org/>
86. Brooks M. E., Kristensen K., Benthem K. J. van, Magnusson A., Berg C. W., Nielsen A., et al. (2017). GlimmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, 9(2), 378–400. <https://doi.org/10.32614/RJ-2017-066>
87. Bolker B. M. (2008). Ecological models and data in R. In *Ecological Models and Data in R*. Princeton University Press.
88. Fox J., & Weisberg S. (2019). *An R Companion to Applied Regression* (Third). Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
89. Lenth R. V. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. <https://CRAN.R-project.org/package=emmeans>
90. Piepho H.-P. (2004). An algorithm for a letter-based representation of all-pairwise comparisons. *Journal of Computational and Graphical Statistics*, 13(2), 456–466.
91. Anderson RL and Houseman EE. 1942. Tables of orthogonal values extended to N = 104. *Agricultural Experiment Station Iowa State College of Agriculture and Mechanic Arts Research Bulletin* 297: 695–772.
92. Howell D. 2010. *Statistical Methods for Psychology*. London, UK: Wadsworth Publishing Company Inc.
93. Roberts JK, Kuris AM. Predation and control of laboratory populations of the snail *Biomphalaria glabrata* by the freshwater prawn *Macrobrachium rosenbergii*. *Annals of Tropical Medicine & Parasitology*. 1990; 84:401–12.
94. Sulieman Y, Pengsakul TT, Guo Y, Huang SQ, Peng WX. Laboratory and semi-field evaluation on the biological control of *Oncomelania hupensis* snail (Gastropoda: Pomatiopsidae), the intermediate host of *Schistosoma japonicum*, using *Procambarus clarkii* crayfish (Crustacea: Cambaridae). *Egyptian Journal of Biological Pest Control*. 2013; 23:215–220.
95. De Leo GA, Stensgaard AS, Sokolow SH, N'Goran EK, Chamberlin AJ, Yang GJ, et al. Schistosomiasis and climate change. *BMJ*. 2020; 371:m4324.
96. Adekiya TA, Aruleba RT, Oyinloye BE, Okosun KO, Kappo AP. The effect of climate change and the snail-schistosome cycle in transmission and bio-control of schistosomiasis in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health*. 2020; 17:181.

97. Stensgaard A-S, Vounatsou P, Sengupta ME, and Utzinger J. Schistosomes, snails and climate change: Current trends and future expectations. *Review Acta Trop.* 2019; 190:257–268. <https://doi.org/10.1016/j.actatropica.2018.09.013> PMID: 30261186
98. Upatham ES. The effect of water temperature on the penetration and development of St. Lucian *Schistosoma mansoni* miracidia in local *Biomphalaria glabrata*. *Southeast Asian J. Trop. Med. Public Health.* 1973; 4:367–70.
99. McCreesh N, Booth M. The effect of simulating different intermediate host snail species on the link between water temperature and schistosomiasis risk. *PloS one.* 2014; 9:e87892. <https://doi.org/10.1371/journal.pone.0087892> PMID: 24988377
100. Poulin R. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology.* 2006; 132:143–51. <https://doi.org/10.1017/S0031182005008693> PMID: 16393363
101. Lawson JR, Wilson RA. The survival of the cercariae of *Schistosoma mansoni* in relation to water temperature and glycogen utilization. *Parasitology.* 1980; 81:337–48.
102. Mangal TD, Paterson S, Fenton A. Predicting the impact of long-term temperature changes on the epidemiology and control of schistosomiasis: a mechanistic model. *PLoS one.* 2008; 3:e1438. <https://doi.org/10.1371/journal.pone.0001438> PMID: 18197249
103. Hendrix AN, Loftus WF. Distribution and relative abundance of the crayfishes *Procambarus alleni* (Faxon) and *P. fallax* (Hagen) in southern Florida. *Wetlands.* 2000; 20:194–9.
104. Bernot RJ. Parasite–host elemental content and the effects of a parasite on host-consumer-driven nutrient recycling. *Freshwater Science.* 2013; 32:299–308.
105. Linzmaier SM, Goebel LS, Ruland F, Jeschke JM. Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. *Ecosphere.* 2018; 9:e02385.
106. Wood CL, Sokolow SH, Jones IJ, Chamberlin AJ, Lafferty KD, Kuris AM, et al. Precision mapping of snail habitat provides a powerful indicator of human schistosomiasis transmission. *PNAS.* 2019; 116:23182–91. <https://doi.org/10.1073/pnas.1903698116> PMID: 31659025
107. Kull CA, Tassin J, and Carriere SM. Approaching invasive species in Madagascar. *Madagascar Conservation and Development* 2015; 9:60–70.
108. Gherardi F. Invasive crayfish and freshwater fishes of the world. *Rev. sci. tech. Off. int. Epiz.* 2010; 29:241–54. <https://doi.org/10.20506/rst.29.2.1973> PMID: 20919580
109. Oficialdegui FJ, Sanchez MI, and Clavero M. One century away from home: how the red swamp crayfish took over the world. *Rev. Fish Biol. Fisher.* 2020; 30:121–135.
110. Critical Ecosystems Partnership Fund. Ecosystem Profile: Madagascar and the Indian Ocean Islands [Internet]. Madagascar: Conservation International; 2014 [cited 5 Aug 2022]. Available from: https://www.cepf.net/sites/default/files/ecosystemprofile_madagascar_en.pdf
111. Susanne SH, Hutterer E, Jouanard N, Hsieh MH, Lafferty KD, Kuris AM, et al. 2015. Reduced transmission of human schistosomiasis after restoration of a native river prawn that preys on the snail intermediate host. *PNAS* 112:9650–9655.
112. Hoover CM, Sokolow SH, Kemp J, Sanchirico JN, Lund AJ, Jones IJ, et al. 2019. Modelled effects of prawn aquaculture on poverty alleviation and schistosomiasis control. *Nature Sustainability* 2, 611–20. <https://doi.org/10.1038/s41893-019-0301-7> PMID: 33313425
113. United States Agency for International Development (USAID). Madagascar: Nutrition profile [Internet]. United States Agency for International Development (USAID); 2018 [cited 2022 Aug 4]. Available from: https://www.usaid.gov/sites/default/files/documents/tagged_Madagascar-Nutrition-Profile_17_Aug.pdf
114. Kulhanek SA, Ricciardi A, Leung B. Is invasion history a useful tool for predicting the impacts of the world's worst aquatic invasive species? *Ecological Applications.* 2011; 21:189–202. <https://doi.org/10.1890/09-1452.1> PMID: 21516897
115. Larson BM, Kueffer C. Managing invasive species amidst high uncertainty and novelty. *Trends in Ecology & Evolution.* 2013; 28:255–6. <https://doi.org/10.1016/j.tree.2013.01.013> PMID: 23434389
116. Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, et al. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution.* 2013; 28:58–66.
117. Schlaepfer MA, Sax DF, Olden JD. Toward a more balanced view of non-native species. *Conservation Biology.* 2012; 26:1156–8. <https://doi.org/10.1111/j.1523-1739.2012.01948.x> PMID: 23082954
118. Sagoff M. Do non-native species threaten the natural environment? *Journal of Agricultural and Environmental Ethics.* 2005; 18:215–36.

119. Vitule JRS, Freire CA, Vazquez DP, Nuñez MA, Simberloffs D. Revisiting the potential conservation value of non-native species. *Conservation Biology*. 2012; 26:1153–5. <https://doi.org/10.1111/j.1523-1739.2012.01950.x> PMID: 23083005
120. Chucholl F, Chucholl C. Differences in the functional responses of four invasive and one native crayfish species suggest invader-specific ecological impacts. *Freshwater Biology*. 2021; 66:2051–63.
121. Dunoyer L, Dijoux L, Bollache L, Lagrue C. Effects of crayfish on leaf litter breakdown and shredder prey: are native and introduced species functionally redundant? *Biological Invasions*. 2014; 16:1545–55.
122. Jackson MC, Jones T, Milligan M, Sheath D, Taylor J, Ellis A, et al. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology*. 2014; 59:1123–35.
123. Twardochleb LA, Olden JD, Larson ER. A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*. 2013; 32:1367–82.