Under what conditions can a novel invader (the marbled crayfish, *Procambarus fallax f. virginalis*) exert predation pressure on schistosome-competent snails?

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Abstract

Under what conditions can a novel invader (the marbled crayfish, *Procambarus fallax f. virginalis*) exert predation pressure on schistosome-competent snails?

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The human burden of environmentally transmitted infectious diseases can depend strongly on ecosystem factors, including the presence or absence of natural enemies. Like natural enemies, non-native species influence the abundance and distribution of their prey and competitors, yet the impact of these invaders on the transmission of diseases remains largely unexplored. One environmentally transmitted infectious disease with potential to be influenced by natural enemies is schistosomiasis, a parasitic disease infecting over 200 million people across South America, Asia, and Africa. The marbled crayfish (*Procambarus fallax f. virginalis*) 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 *Schistosoma* spp. worms. Marbled crayfish can tolerate a wide range of ecological conditions, colonize diverse habitats, and may serve as an enemy of schistosome-competent snails in areas where natural predators cannot– something that is urgently needed in a country where schistosomiasis prevalence in some villages can range up to 94%. Here, I 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. Crayfish consumption was significantly influenced by crayfish weight, as well as the interaction between weight and temperature. Compared to small crayfish, large crayfish experienced a greater increase in consumption at moderate temperatures (25 and 30°C) relative to extreme temperatures (15, 20, and 35°C). The temperature at which the rate of consumption was highest (i.e., the thermal optimum) was estimated to be 31.1°C for small crayfish (<3.90 g), 27.7°C for medium crayfish (3.91 – 6.62 g), and 28.7°C for large crayfish (> 6.3 g). Neither snail species nor snail infection status were significantly associated with consumption. My results suggest that ecological parameters, such as temperature and crayfish weight, influence rates of consumption and, in turn, the potential regulatory impact of the marbled crayfish on snail host populations.

INTRODUCTION

The human burden of environmentally transmitted infectious diseases can depend strongly on ecosystem factors (Horwitz and Wilcox, 2005), including the presence or absence of natural enemies (Jones et al., 2022). Antagonistic interactors that regulate the transmission of infectious disease, natural enemies can include predators, competitors, or parasites of reservoir hosts or of the disease agent itself (Jones et al., 2022). However, research on natural enemies is sparse, and generalities regarding the influence of these antagonists remain elusive (Duffy et al., 2005; Lugassy et al., 2021). This is of particular concern, not just for the untapped potential of natural enemies in the control and elimination of infectious disease, but because non-native species are establishing in new regions at exponentially increasing rates (Seebens et al., 2017). Like natural enemies, invasive species, through direct and indirect effects, influence the abundance and distribution of their prey and competitors (Gherardi, 2007; reviewed in David et al., 2017), yet the impact of these invaders on the transmission of environmentally transmitted infectious diseases remains largely unexplored. Some introductions of non-native enemies in previous disease control efforts have resulted in unanticipated negative consequences (reviewed in Jones et al., 2022); however, introduced species have also been used with success to reduce the abundance of zoonotic reservoirs of disease and of human disease burden (Khalil and Sleem, 2011; Mkoji et al., 1999; reviewed in Gherardi, 2007). This begs the question: *Can non-native species exert regulatory effects on environmentally transmitted infectious diseases, as natural enemies might?*

Billions of people are at-risk for endemic, emerging, and re-emerging infectious diseases, with disease burden and suffering falling disproportionately on those living in poverty (Lozano et al., 2012; WHO Commission on Macroeconomics and Health & World Health Organization, 2001). Clinical interventions alone (e.g., mass drug administration) may not adequately address environmentally transmitted diseases (Sokolow et al., 2016), but the efficacy of these control efforts may improve when paired with interventions that target environmental reservoirs (Garchitorena et al., 2017). One environmentally transmitted infectious disease with potential to be influenced by natural enemies is schistosomiasis. Schistosomiasis is a parasitic disease endemic to tropical and subtropical regions (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, Accessed 28 July 2022). Over 200 million people are infected with schistosomiasis across South America, Asia, and Africa (Steinmann et al., 2006; Hotez and Kamath, 2009). The total number of individuals infected with and vulnerable to schistosomiasis (i.e., the global burden of schistosomiasis) falls second only to that of malaria (Steinmann et al., 2006). Freshwater snails (largely from three genera: *Biomphalaria*, *Bulinus,* and *Oncomelania*; https://www.cdc.gov/parasites/schistosomiasis/biology.html) 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 dishes, or otherwise in contact with contaminated freshwaters. 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 (https://www.who.int/news-room/questions-and-answers/item/neglected-tropical-diseases, Accessed 28 July 2022). This creates a “poverty trap”, as the compromised health of community members–a result of untreated schistosomiasis infections–reduces the opportunities for social and economic development (Bonds et al., 2010; Parker, 1992; Tanner, 1989).

In 2019, the World Health Organization (WHO) estimated that 236.6 million people were at-risk of schistosomiasis infection and required preventative treatment, with 90% of those individuals living within Africa (Steinmann et al., 2006; https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, Accessed 28 July 2022). The standard treatment, an oral dose of the anthelmintic praziquantel, is effective at treating adult worm infections; however, in 2019, fewer than half of those in need of treatment (44.5%) received it (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis, Accessed 28 July 2022). Mass drug administration has also fallen short due to high rates of re-infection, as individuals are often repeatedly exposed to contaminated water sources (Chandiwana et al., 1991; Garba et al., 2013; Moloney et al., 1987; N’Goran et al., 2001; Webster et al., 2013) and treatment is ineffective against juvenile worms (Gönnert and Andrews, 1977; Pica-Mattoccia and Cioli, 2004; Sabah et al., 1986). In response, the WHO recently endorsed snail control combined with mass drug administration as the most effective strategy for reducing schistosomiasis prevalence (Sokolow et al., 2016). 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

(Sokolow et al., 2018). Alternatively, snail populations can be reduced by predators (e.g., crustaceans, birds, and fish) and competitors (e.g., other snail species). Indeed, natural enemies show some promise in the reduction of human schistosomiasis (Diakité et al., 2018; Halstead et al., 2018; Sokolow et al., 2015; Stauffer et al., 2006). For example, schistosomiasis transmission was not detected in the regions surrounding Lake Malawi–an African great lake with shorelines touching Malawi, Mozambique, and Tanzania–prior to 1985. As a result of overfishing, molluscivorous cichlids (*Trematocranus placodon*) declined, coinciding with increases in schistosomiasis prevalence (Stauffer et al., 1997). In a 2003 follow-up investigation, Stauffer and colleagues found that temporal declines in fish abundance coincided with increases in schistosomiasis (Stauffer et al., 2006).

The relationships among snails, their natural enemies, and human schistosomiasis is also demonstrated in the Senegal River Basin, the epicenter of the world's largest intestinal schistosomiasis epidemic. Following completion of the Diama Dam in 1986, the region experienced an increase in urogenital schistosomiasis (*S. haematobium*) infections, along with the introduction and spread of intestinal schistosomiasis (*S. mansoni*; Southgate, 1997; Sow et al., 2002). One hypothesis suggests that dam construction disrupted native river prawn (*Macrobrachium* spp.) migration routes (Sokolow et al., 2014; Sokolow et al., 2015; Sokolow et al., 2017; Swartz et al., 2015), resulting in above-dam prawn population declines (Savaya Alkalay et al., 2014). In the absence of their natural predator, snail populations may have increased, thereby increasing transmission of schistosomiasis to people (Sokolow et al., 2014; Sokolow et al., 2015; Swartz et al., 2015). Indeed, a study comparing schistosomiasis infections before and after the construction of 14 large dams in sub-Saharan Africa found that, following dam construction, increases in schistosomiasis prevalence were greater within areas previously inhabited by prawns compared to those outside of the prawns' range (Sokolow et al., 2017). Furthermore, localized reintroduction of native river prawns significantly reduced snail population numbers and, in turn, human disease prevalence (Sokolow et al., 2015). Snail host and riverine prawn densities were also negatively correlated in Côte d'Ivoire, though there was no relationship between these densities and human schistosomiasis burden (Diakité et al., 2018). Finally, agrochemical exposure can indirectly increase infected snail (*Biomphalaria glabrata*) densities, through chemically mediated mortality of predators (crayfish, *Procambarus alleni*, and giant water bugs, *Belostoma flumineum*; Halstead et al., 2018).

Clearly there are some cases in which natural enemies are powerful agents in the control of environmentally transmitted parasites. However, in an increasingly invaded world (Seebens et al., 2017), it is imperative that ecologists understand the ways in which non-native species may also influence ecosystem dynamics, including the transmission of infectious disease. Like natural enemies, non-native species influence the abundance and distribution of their prey and competitors (Gherardi, 2007; reviewed in David et al., 2017), highlighting the potential role of non-native species in disease regulation. Though some introductions of non-native enemies in previous disease control efforts have resulted in unanticipated negative consequences (reviewed in Jones et al., 2022), introduced species have also been used with success to reduce snail intermediate host populations, and even human schistosomiasis burden. The abundance of red swamp crayfish (*Procambarus clarkii*) was significantly correlated with snail disappearance in Egyptian irrigation channels (Khalil and Sleem, 2011). Snails diminished and "sometimes complete[ly] disappear[ed]" following the introduction of *P. clarkii*, while snail densities did not decline in areas from which *P. clarkii* were absent (Khalil and Sleem, 2011). Similarly, an established population of *P. clarkii* reduced snail abundance in Kenya, and 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 (Mkoji et al., 1999).

Indeed, the prey–predator relationship between gastropods and freshwater crayfish—both native and non-native— is well-documented (reviewed in Covich, 2010), and numerous field and experimental studies highlight the regulatory impact of crayfish on snail populations (Dorn, 2013; Dorn and Wojdak, 2004; Johnson et al., 2009; Klose and Cooper, 2012; Kreps et al., 2012; Lodge et al., 1994; Nyström et al., 1996). Regulation occurs through a combination of consumptive and nonconsumptive mechanisms, though it remains unclear which mechanism drives this widespread pattern (Dorn, 2013; Preisser et al., 2005). 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, Crowl and Covich, 1990; Lewis, 2001; Turner, 2004; Turner and Montgomery, 2003), morphology (e.g., shell thickness, Hoverman et al., 2005; and shape, reviewed in Covich, 2010), or behavior (e.g., habitat use, Covich et al., 1994; Lewis, 2001; Turner et al., 1999; and feeding rate, Lewis, 2001) that, in some cases, decrease fitness (Hoverman et al., 2005). 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 crayfish predator (Lewis, 2001; Turner, 2004; Turner et al., 1999; Turner and Montgomery, 2003; additional behavioral responses to predators reviewed in Covich, 2010). Behavioral, physiological, and morphological alterations reflect the indirect, nonconsumptive effects of predators on snail populations.

Furthermore, 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 (Bernot, 2003; Levri, 1998, 1999; Swartz et al., 2015). Snails exposed to and presumably infected by *Schistosoma* move more slowly and less frequently than do uninfected conspecifics, which suggests that infected snails should be at greater risk for predation (Swartz et al., 2015). When exposed to simulated predation cues—a caged riverine prawn, *Macrobrachium vollenhovenii*, paired with crushed snail conspecifics—infected snails also show 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; Swartz et al., 2015). Indeed, prawns preferentially consume *Bi. glabrata* snails exposed to *S. mansoni* and *Bu. truncatus* snails exposed to *S. haematobium* (Swartz et al., 2015). Though differences in snail behavior appear to be the driving force behind the preferential consumption of infected snails, other mechanisms may be at play. For example, physiological differences between uninfected and infected snails may make infected animals more or less detectable or desirable to predators (Bernot, 2013). 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 (Swartz et al., 2015).

The marbled crayfish (*Procambarus fallax f. virginalis*) first appeared in the German aquarium trade in the mid-1990s (Scholtz et al., 2003). This unique species reproduces unisexually (i.e., via parthenogenesis), making it the only clonal decapod ever described (Scholtz et al., 2003). 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* (Scholtz et al., 2003). Following its introduction to Madagascar in 2005, the marbled crayfish quickly spread across the country (Gutekunst et al., 2018; Jones et al., 2009, Kawai et al., 2009), overlapping with the distribution of freshwater snails (*Biomphalaria pfeifferi* and *Bulinus spp.)* that serve as the intermediate host of *Schistosoma mansoni* and *S. haematobium*, respectively (https://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-madagascar, Accessed 28 July 2022). Like most crayfish (reviewed in Covich, 2010), marbled crayfish are omnivores, said to eat "almost anything" (Vogt et al., 2004). However, plant material and snails seem to be their preferred food items (Vogt et al., 2004). Indeed, Andriantsoa et al. (2019) 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 (Andriantsoa et al., 2019), 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% (Rasoamanamihaja et al., 2016; Spencer et al., 2017).

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 (Andriantsoa et al., 2019). Marbled crayfish can tolerate a wide range of ecological conditions and colonize diverse habitats (Andriantsoa et al., 2019), including areas outside of the geographic range of native crayfish species (Parastacidae*; Astacoides*; Jones et al., 2007; Toon et al., 2010). This invader has been found in rice fields irrigated by thermal water, which reached temperatures as high as 37°C, as well as in 20°C river habitats (Andriantsoa et al., 2019). 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, naturally occurring snail predators cannot occur–perhaps, a collateral benefit of an otherwise destructive invasive species.

Though marbled crayfish have been shown to withstand a range of thermal conditions, rates of crayfish mortality, growth, and consumption vary greatly across temperatures (Jimenez and Faulkes, 2010; Seitz et al., 2005; Veselý et al., 2015). In a series of laboratory experiments, marbled crayfish mortality was lowest at 20°C, compared to lower (15°C) and higher (25°C and 30°C) temperatures (Jimenez and Faulkes, 2010; Seitz et al., 2005). Under optimal light exposure, 80% of crayfish in 20°C waters survived until the end of the 200-day experiment period, whereas 50% of crayfish died between 50 and 80 days when held at 15°C (Jimenez and Faulkes, 2010; Seitz et al., 2005). Crayfish held at 15°C also grew 10.5 mm less than those held in optimal conditions, which were determined to occur between 20°C and 25°C (Jimenez and Faulkes, 2010; Seitz et al., 2005). Additional laboratory studies indicate that marbled crayfish can withstand temperatures as low as 5°C for extended periods of time, though survivorship drastically declines at this extreme temperature (Veselý et al., 2015). Ectotherms, including crayfish, tend to follow a consistent pattern in response to temperature; performance increases with increasing temperature until a thermal optimum is reached, beyond which point performance declines (Angilletta, 2009). Indeed, crayfish consumption rates adhere to this pattern, though the temperature at which this thermal limit occurs varies considerably among crayfish species (Rodríguez Valido et al., 2021; Seals et al., 1997; Whitledge and Rabeni, 2002). Marbled crayfish consumption ceases below 10°C (Veselý et al., 2015), 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 and for the role of non-natural enemies in a world that is becoming increasingly invaded.

I aimed to address the following questions: i) **Do crayfish feeding rates on schistosome-competent snails differ among temperatures across a temperature range,** ii) **Does the rate of crayfish consumption differ between infected and uninfected snails across temperatures, and** iii) **Does crayfish body size mediate the response of feeding rate to temperature?** My objective was to test whether and under what conditions marbled crayfish prey on schistosome-competent snails. I experimentally assessed marbled crayfish consumption of uninfected versus infected snails across a range of temperatures, reflective of the habitat range of the marbled crayfish in Madagascar. I hypothesized that the rate of consumption would differ across temperature extremes, with consumption decreasing at the lower and upper bounds of the thermal range tested. Following previous studies, which found that marbled crayfish cease feeding below 10°C (Veselý et al., 2015), I predicted that consumption would diminish at the low temperature extreme (15°C). I also predicted that consumption would stabilize or diminish above 35°C, as this nears 37°C, the highest recorded temperature that marbled crayfish have been found to inhabit in Malagasy waters (Andriantsoa et al., 2019). Optimal consumption was predicted to occur between 20°C and 30°C. Additionally, I 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. Behavioral differences between uninfected and infected snails may alter the position of snails within the tank and, consequently, the number of snails available to be consumed. Infected snails move more slowly and less frequently than uninfected conspecifics and show a muted expression of anti-predator behavior (Swartz et al., 2015). This suggests that infected snails should be at greater risk of predation (Swartz et al., 2015). Indeed, prawns preferentially consume *Bi. glabrata* snails exposed to *S. mansoni* and *Bu. truncatus* snails exposed to *S. haematobium* (Swartz et al., 2015). Alternatively, physiological drivers, though unable to be detected within the present study design, may alter the detectability, desirability, and consumption rate of infected and uninfected snails. With differences in detectability and desirability in mind, I also considered that the rate at which crayfish consume snails across the 72-hour experimental period (described below) could differ between infected and uninfected snails. For example, crayfish may be better able to detect or exhibit a preference for snails of one infection status compared to the other. Crayfish may then consume infected snails quickly and become satiated more rapidly compared to uninfected conspecifics, or vice versa. Therefore, time could mask the effect of snail infection status on consumption, if the same number of infected and uninfected snails are, ultimately, consumed. Taken together, I predicted that snail infection status, regardless of snail species, would significantly influence rate of consumption. Identification of the mechanism driving differences in consumption rate, should they be observed, is beyond the scope of the present work. Finally, I hypothesized that temperature may mediate the influence of weight on consumption, because temperature influences large-bodied organisms differently compared to those smaller in size (Brown et al., 2004). Overall, the results from the present study will enhance our understanding of the conditions under which the novel invader (the marbled crayfish, *Procambarus fallax f. virginalis*) can exert predation pressure on schistosome-competent snails.

METHODS

Obtaining animals

Marbled crayfish (*Procambarus fallax f. virginalis)* were obtained through private sellers on Etsy (https://www.etsy.com/) and Aquabid (https://www.aquabid.com/). These animals–asexual reproducers that have been shown to be adaptable to varying environmental conditions and plastic in their preferred habits (Andriantsoa et al., 2019; Jimenez and Faulkes, 2010; Seitz et al., 2005)–have high invasive potential and therefore represent a potential threat to environments where they have not yet been introduced. 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).

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.

General Care

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 (Collins et al., 2021). Tanks underwent 100% water changes one to two times per week (Lewis and Barnes, 2021). Snails were regularly fed romaine lettuce, which was refreshed during the bi-weekly water changes.

Crayfish were also reared in freshwater aquaria filled with artificial pond water (Collins et al., 2021). 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. Adult 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 (so that the lab population size would remain under the limit of 40 animals specified by a Washington Department of Fish and Wildlife Shellfish Import Permit). 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.

Treatments

Rounds

I was limited by my ability to obtain "exposed" (hereafter, "infected") *Bu. truncatus* and *Bi. glabrata* snails. As such, the first round of experiments included only "naive", or uninfected snails. Round 1 of experiments began on 14 June 2021 and concluded on 13 August 2021. Round 2 of experiments, which included both uninfected and infected snails, began on 25 October 2021 and concluded on 17 December 2021.

Temperature

I 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; Andriantsoa et al., 2019). Though marbled crayfish can survive in temperatures as low as 5°C for extended periods of time, consumption ceases below 10°C (Veselý et al., 2015). 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 held at the experimental temperature for at least 12 hours prior to the start of the experiment.

Infected vs. Uninfected Snails

Additionally, I was interested in the influence of snail infection status on crayfish consumption rates, as river prawns *(Macrobrachium vollenhovenii*) show a preference for consuming infected (versus uninfected) snails during predation experiments (Swartz et al., 2015). I varied snail infection status between experimental tanks. Each individual crayfish was held either with all infected or uninfected snails of one of two species included in the present study: *Biomphalaria glabrata* and *Bulinus truncatus*. Exposed snails were held for ~3 weeks post-exposure (exposure date provided by the reagent provider, BRI) before being used in experiments to allow infections to adequately mature (Lewis et al., 1986).

Experimental vs. Control Conditions

I anticipated that 15°C and 35°C would be near the thermal limits of both the crayfish and snails based on previous studies (Andriantsoa et al., 2019; Joubert et al., 1986; Sturrock and Sturrock, 1972; Swartz et al., 2015; Veselý et al., 2015). As such, I wanted to ensure that snail mortality accurately reflected crayfish consumption and not temperature-associated snail death. To do this, I included control tanks, from which crayfish were absent. Experimental tanks held one crayfish in combination with snails.

Experiments

My experiments largely replicated the methods of Sokolow et al. (2014). Briefly, one marbled crayfish (*Procambarus fallax f. 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.

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, 26, 48, 60, 72), in which each 12-hour increment constituted a “trial.” 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. 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. These totals were used to determine the total number of snails consumed (12, the set density of snails, minus the number of snails remaining minus the number of dead snails equals the number of missing and presumably consumed snails). 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.

To best replicate natural conditions, crayfish and snails were provided with food throughout the duration of the experimental period. 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 my assumption that crayfish would not be limited to snails or starving when encountering snails in natural habitats.

In Round 1 of experiments, a total of 42 trials per experimental temperature (15, 20, 25, 30, and 35°C) were conducted in the experimental condition, in which one crayfish individual was held in combination with either uninfected *Bi. glabrata* or uninfected *Bu. truncatus* snails (Fig. 1). Round 1 also included 14 control trials per experimental temperature for uninfected *Bi. glabrata* and *Bu. truncatus* (Fig. 1). In Round 2 of experiments, a total of 14 trials per experimental 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 (Fig.1).

Analysis

I was interested in the influence of temperature, snail species, and snail infection status on the rate at which crayfish consumed snails. I 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. Time point “0” was not included in analyses, as this represented the start of the 72-hour experimental period and, as such, consumption could not yet have occurred. Over the course of the experiment, I also 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 and, thus, assumed this snail to have been consumed at a previous time point. This would have resulted in miscalculations of the total number of snails remaining, as well as the total number of snails to be added into the tank during replacement. When this occurred, the trial in which too many snails were observed and the previous trial were removed from analyses, resulting in the exclusion of 52 erroneous data points out of a total of 960 (5.4%). In addition, 16 total instances of 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 (Huner and Barr, 1991). As mentioned above, crayfish were absent from, and therefore could not consume snails, in control tanks ($n = 350$). As such, only data from experimental trials ($n = 542$) were included in analyses of crayfish consumption. I analyzed crayfish consumption rates with a linear mixed-effects model using the *lmer()* function in the lme4 package (Bates et al., 2020). Fixed effects included snail species, snail infection status, crayfish weight, temperature, and the interaction between crayfish weight and temperature. Random effects are explained below.

Crayfish individuals were used repeatedly throughout both rounds of experimentation, and I assumed that crayfish would become larger over time. Therefore, I compared the weight of crayfish in the first and second rounds of experiments. Crayfish weight differed significantly between Round 1 (mass ± SE = 5.70 g ± 0.163) and Round 2 (mass ± SE = 7.80 g ± 0.132; $t_{536.1} = -9.993$, $p < 0.0001$). I controlled statistically for crayfish body size to account for this difference, by including crayfish weight as a fixed effect. Furthermore, I added a nested random intercept of trial date nested within experiment round to control for additional variation associated with the dates on which experiments and rounds were run. This random effect should account for temporal autocorrelation in the dataset, as well as residual effects of crayfish residency time in the lab other than crayfish weight.

Individual crayfish consumption was observed over the course of the 72-hour experimental period, with rates of consumption being calculated for each time point, except time point “0” (described above). I assumed that crayfish would become satiated over time, meaning that crayfish may consume fewer snails in later time points. To account for the similarity of consumption rates within individual crayfish and across similar time points, crayfish identity nested within time point (12, 24, 36, 48, 60, or 72 hours) was also included as a random intercept.

I was not only interested in the rate at which marbled crayfish consume schistosome-competent snails, but also the temperature at which the highest rate of consumption occurs (i.e., the thermal optimum). Once again, I assumed that crayfish body size (measured as weight in grams) would influence consumption, which may also be mediated by the interaction between body size and temperature. As such, the thermal optimum of consumption was calculated for three size classes of crayfish: small (<3.90 g), medium (3.91 – 6.62 g), and large (> 6.3 g). Size class criteria were determined following my initial analysis of the data and reflect the weight series automatically generated by the *ggpredict()* function in the ggeffects package (Lüdecke 2018), which was used to depict the rate of consumption as a function of temperature and crayfish weight–an interaction that the aforementioned analyses found to have a significant effect on consumption. Individual crayfish weight was collected at the beginning of each experimental period and extrapolated to all trials within that experimental period. Individual crayfish could then be represented in multiple size classes, as experiments continued and crayfish became larger over time. As such, consumption data was subset according to crayfish weight at the time of experiments. For each weight class of crayfish, I calculated the average rate of consumption of snails (the total number of snails consumed per 12-hour trial)–regardless of species and infection status, as the aforementioned analyses also found that these two variables did not significantly influence crayfish consumption–for each temperature (15, 20, 25, 30, and 35°C). Thermal performance curves were fitted to all model formulations available within the rTPC package (Padfield et al., 2021) that would converge with the dataset. I first checked the model fits visually, disregarding any obvious misfits. AIC scores were then generated using the *AIC()* function in rTPC package (Padfield et al., 2021) and used to compare the relative fit of each model. After determining the model that best fits the data, the temperature at which optimum consumption occurs was estimated using the *get_topt()* function in the rTPC package (Padfield et al., 2021); estimates should be accurate up to 0.001°C (Padfield et al., 2021).

I performed two additional analyses to address hypotheses about whether and how crayfish consumption rate varied between infected and uninfected snails. In the second analysis, I adapted the original model to address the hypothesis that the rate at which crayfish consume snails across the 72-hour experimental period could differ between infected and uninfected snails. For example, crayfish may consume infected snails quickly and become satiated more rapidly in the "infected" treatment than in the "uninfected" treatment, but consume the same number of infected versus uninfected snails over the entire 72-hour experimental period. To explore this possibility, I conducted an additional analysis of crayfish consumption rates using a linear mixed-effects model in which snail species, snail infection status, crayfish weight, experimental temperature, time point (12, 24, 36, 48, 60, and 72 hours), the interaction between crayfish weight and temperature, and the interaction between snail infection status and time point were included as fixed effects. As in the model described above, random effects included trial date nested within experiment round and crayfish identity. Analyses were performed using the *lmer()* function in the lme4 package (Bates et al., 2020).

The third analysis addressed the hypothesis that crayfish consumption rates may have been influenced by the number of snails available to be consumed. Though snail density (n=12) was reset at the conclusion 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 and, therefore, unavailable for consumption. The number of unavailable snails may have varied with snail infection status, as infected snails move more slowly and less frequently than uninfected snails (Swartz et al., 2015). Likewise, when exposed to caged prawns and crushed conspecifics, infected snails show a muted expression of anti-predator behavior (Swartz et al., 2015). Therefore, I compared the number of unavailable snails (unavailable snails = the number of snails above the water line + the number of snails under the shelter/hiding) as a function of snail species, snail infection status (uninfected or infected), and condition (control or experimental). To do so, I used a linear mixed-effects model in which the number of unavailable snails was the response variable and snail infection status, condition, snail species, and the interaction between snail infection status, condition, and snail species were included as fixed effects. Trial date was included as a random effect. A comparison between uninfected and infected snails could not be performed for Round 1, as infected snails were not included in Round 1 of experiments. As such, only data from Round 2 of experiments were used for this analysis (n = 472). Analyses were performed using the *lmer()* function in the lme4 package (Bates et al., 2020).

To verify that snail loss and mortality were the result of crayfish consumption and not the product of some other process (e.g., natural mortality, snail escape), I included a control treatment in each experiment. Crayfish were absent from all control tanks. I performed a one-sample t-test to compare the number of remaining snails within control tanks to 12, the set density of snails.

All analyses were run in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

RESULTS

After removing data for crayfish that molted and erroneous data points, a total of 542 trials were included in the analysis. Crayfish varied in weight between 1.54 g and 14.44 g with an average ± SE of 6.62 ± 0.117 g. On average, during a 12-hour trial period, crayfish consumed 1.6 snails at 15°C, 3.5 snails at 20°C, 3.8 snails at 25°C, 4.4 snails at 30°C, and 2.7 snails at 35°C. Crayfish consumption was significantly influenced by crayfish weight (estimate for the effect of weight ± SE = +0.4181 ± 0.1031, t = 4.056, df = 361.6, $p < 0.0001$; Table 1).

Crayfish weight modulated the response of consumption to temperature. The interaction between crayfish weight and temperature was significant at 25°C (estimate for the effect of the interaction ± SE = +0.4204 ± 0. 0.1279, t = 3.288, df = 481.0, p = 0.0011; Table 1) and 30°C (estimate for the effect of the interaction ± SE = +0.2916 ± 0.1110, t = 2.430, df = 477.3, p = 0.0155; Table 1; Figs 2, S1). Compared to small crayfish, large crayfish experienced a greater increase in consumption at moderate temperatures (25 and 30°C) relative to extreme temperatures (15, 20, and 35°C). Neither snail species (*Bi. glabrata* versus *Bu. truncatus;* Table 1) nor snail infection status (infected versus uninfected; Table 1; Figs 2, S1) were significantly associated with consumption.

Consumption data was subset according to crayfish weight at the time of experiments, resulting in a total of 98 small crayfish (<3.90 g, n = 7) trials, 208 medium crayfish (3.91 – 6.62 g, n = 10) trials, and 236 large crayfish (> 6.3 g, n = 12) trials to be included in the generation of thermal performance curves, in which “n” reflects the total number of individual crayfish that were included in each weight class. The temperature at which the rate of consumption was highest (i.e., the thermal optimum) was estimated to be 31.1°C for small crayfish, 27.7°C for medium crayfish, and 28.7°C for large crayfish (Figs S4, S5, and S6, respectively); estimates should be accurate up to 0.001°C (Padfield et al., 2021). Small and large crayfish consumption data were fit to the *beta_2012* model (Table S1, Figs S4 and S6, respectively), whereas medium crayfish data were fit using the *quadratic_2008* model (Table S1, Fig. S5).

The second analysis found that crayfish consumption was not significantly influenced by the interaction between time point and snail infection status (estimate for the effect of the interaction ± SE = -0.0008 ± 0.0121, t = -0.068, df = 512.4, p = 0.9458; Table S2, Figs S2, S3).

In the third analysis, the three-way interaction among snail infection status, condition, and snail species was not significant (p = 0.2022; Table 2 and Fig. 3). The interaction between snail infection status and condition was also not significant (p = 0.3343), nor was the interaction between snail infection status and snail species (p = 0.5548) or the interaction between condition and snail species (p = 0.0817; Table 2 and Fig. 3). The main effect of snail infection status was not significant (p = 0.8067; Table 2 and Fig. 3). However, the number of unavailable snails was significantly greater in the experimental compared to the control condition (average ± SE: control = 0.74 ± 0.08, experimental = 2.0 ± 0.14; estimate for the main effect of condition ± SE = +2.099 ± 0.3186, t = 6.588, df = 443.1, p < 0.0001; Table 2 and Fig. 3) and among *Bu. truncatus* compared to *Bi. glabrata* snails (average ± SE: *Bu. truncatus* = 1.6 ± 0.12, *Bi. glabrata* = 1.2 ± 0.13; estimate for the main effect of snail species ± SE = +0.9170 ± 0.3228, t = 2.841, df = 444.9, p = 0.0047; Table 2 and Fig. 3).

Minimal snail loss occurred in control conditions. Of 350 total control trials, there were only 20 instances (5.7% of trials) in which the number of snails remaining (the total number of living snails above the water line, on the lettuce, inside and under the shelter/hiding, and in open water) was less than the set density of 12. Though results from a one-sample t-test were significant (t_{349}= -4.498, p < 0.0001), the mean number of snails remaining in control tanks ± SE was 11.94 ± 0.02.

DISCUSSION

The rate at which marbled crayfish consumed schistosome-competent snails was significantly influenced by crayfish weight, as well as the interaction between weight and temperature. For all crayfish weight classes, optimum consumption occurred between 27 and 32°C. Consumption, however, was not influenced by other ecological parameters, such as snail species and snail infection status.

The present experiments established that marbled crayfish are predators of schistosome-competent snails, in agreement with Andriantsoa et al. (2019) and the numerous field and experimental studies highlighting the regulatory impact of crayfish on snail populations (Dorn, 2013; Dorn and Wojdak, 2004; Johnson et al., 2009; Klose and Cooper, 2012; Kreps et al., 2012; Lodge et al., 1994; Nyström et al., 1996). On average, crayfish consumed 3.2 snails per 12 hours (± SE = 0.1288139; equivalent to the 4 snails/15 hours [= 3.2 snails/12 hours] observed by Andriantsoa et al., 2019), though factors such as crayfish body weight and temperature influenced feeding rates.

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 prawns (*Macrobrachium* spp.) consumed significantly more schistosome-competent snails than small prawns (Roberts and Kuris, 1990; Sokolow et al., 2014). However, crayfish consumed fewer 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). More recently established populations are likely to be made up of smaller crayfish, which – according to the results of the present study – would have a lesser impact on snail abundances. As a newly established population grows, both in crayfish density and size, the impact on snail populations may increase. Therefore, established populations of older and larger crayfish individuals may have a greater impact on snail abundance and, in turn, human schistosomiasis burden.

Consumption was not only influenced by crayfish weight, but also by temperature. Collectively, crayfish consumed fewer snails at the low and high temperature extremes (15 and 35°C, respectively) compared to moderate temperatures (20, 25, and 30°C). In my experiment, 15°C occurred on the rising slope of the crayfish’s thermal consumption curve and 35°C on the falling slope. Crayfish consumption generally increased with increasing temperature until ~27 to 32°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 peaks, varies considerably amongst crayfish species (Rodríguez Valido et al., 2021; Seals et al., 1997; Whitledge and Rabeni, 2002). In the present study, the thermal optimum was estimated to be 31.1°C for small crayfish, 27.7°C for medium crayfish, and 28.7°C for large crayfish. Furthermore, I found that consumption was mediated by a significant interaction between temperature and crayfish weight. Compared to small crayfish, large crayfish experienced a greater increase in consumption at moderate temperatures (25 and 30°C) relative to extreme temperatures (15, 20, and 35°C). Applied to the natural system (i.e., the freshwaters of Madagascar), larger crayfish may have the greatest per capita impact on snail abundances in habitats ranging in temperature from 25°C to 30°C. On the other hand, rates of consumption for small crayfish–though likely to be lower compared to that of larger crayfish–would be expected to vary much less, regardless of habitat temperature. My results suggest that ecological parameters, such as temperature and crayfish weight, influence rates of consumption and, in turn, the potential regulatory impact of the marbled crayfish on snail host populations.

Neither snail species nor snail infection status were significantly associated with marbled crayfish consumption of snails. Swartz and colleagues (2015) 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 volenhovenii*) preferentially consume *Bi. glabrata* snails exposed to *S. mansoni* and *Bu. truncatus* snails exposed to *S. haematobium* (Swartz et al., 2015), but I found that marbled crayfish did not preferentially consume infected snails. Alternatively, I considered that either infected or uninfected snails may be consumed in greater numbers at earlier time points, satiating the crayfish earlier in the 72-hour experimental period, but leading to the same total amount of consumption for infected and uninfected snails over the 72 total hours. Time point (in hours) could then mask the effect of snail infection status on consumption. The second analysis found that crayfish consumption was not significantly influenced by the interaction between time point and snail infection status. This result is unexpected, as infected snails move more slowly and less frequently than uninfected conspecifics, suggesting that infected snails should be at greater risk for predation (Swartz et al., 2015). While prawns are native to the sub-Saharan riverine system, the marbled crayfish evolved only recently (Gutekunst et al., 2018; Jones et al., 2009; Kawai et al., 2009; Scholtz et al., 2003) from a progenitor species (*Procambarus fallax*) that does not overlap with schistosome-infected snails (Hendrix and Loftus, 2000; Hobbs, 1942). 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 (Bernot, 2013). Therefore, differences in predator preference between infected and uninfected snails may be diminished in the interaction between marbled crayfish and snails.

On the other hand, I posited that crayfish consumption of uninfected and infected snails may be influenced by the number of snails available to be consumed, which could differ depending on snail infection status (as described above; Swartz et al., 2015). I 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 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 are 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., it is not the case that crayfish prefer infected snails but infected snails are harder to get, or vice versa). These findings contrast those of Swartz and colleagues (2015), who found that snails infected with *Schistosoma* spp. exhibit a diminished anti-predator response compared to uninfected snails when exposed to simulated predation cues, which is surprising, given that the two studies used genetically identical strains of snails and parasites. However, in my 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 experimental condition). It is therefore possible that, in the absence of crayfish consumption, 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. (2015). Future studies should monitor uninfected and infected snail behavior in a simulated predator condition (described by Swartz et al., 2015), where the marbled crayfish is present but unable to access and consume the snails and consumption is simulated through the addition of crushed conspecifics.

Several caveats should be borne in mind when interpreting these results. First, behavioral differences are known to arise between aquarium- and naturally reared marbled crayfish; aquarium-reared individuals tend to be more active and aggressive (Linzmaier et al., 2018), suggesting that laboratory consumption rates may not reflect the rates occurring in natural ecosystems. Second, previous field observations in Madagascar found that native snails were absent from areas in which the marbled crayfish had established (Andriantsoa et al., 2019), suggesting 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 (Mkoji et al., 1999; Sokolow et al., 2014; Sokolow et al., 2015; Swartz et al., 2015; Wood et al., 2019). Though this is promising evidence in support of the crayfish's potential to serve as a biological control of snail intermediate hosts, my 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, the present study found that the temperature at which the rate of consumption was highest (i.e., the thermal optimum) was estimated to be 31.1°C for small crayfish, 27.7°C for medium crayfish, and 28.7°C for large crayfish. Therefore, in lower (<27.7°C) or higher (>31.1°C) temperature environments, crayfish may be less likely to reduce snail populations.

However, it's important to bear in mind that crayfish are just one player impacted by temperature. While other factors, such as snail habitat suitability (Stensgaard et al., 2016) and precipitation (De Leo et al., 2020; reviewed in Adekiya et al., 2019 and Stensgaard et al., 2019) influence the distribution of schistosome-competent snails and human schistosomiasis, temperature plays an important, if not *the* most important, role (De Leo et al., 2020; reviewed in Adekiya et al., 2019 and Stensgaard et al., 2019). Snail hatching, growth, fecundity, and survivorship are also substantially impacted by temperature (Joubert et al., 1986; Sturrock and Sturrock, 1972; reviewed in Adekiya et al., 2019 and Stensgaard et al., 2019). In a 32-week experiment investigating the influence of temperature on the biology of schistosome-competent snails, Sturrock and Sturrock (1972) 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 (Sturrock and Sturrock, 1972). Likewise, temperature impacts the penetration and subsequent development of *Schistosoma* miracidia (measured as the number of daughter sporocysts, Upatham, 1973) in snail intermediate hosts, as well as the production of free-swimming cercariae (McCreesh and Booth, 2014), cercarial emergence (Poulin, 2006), and survival (Lawson and Wilson, 1980). 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, Upatham, 1973). Cercarial production increases between 15°C and 31°C (McCreesh and Booth, 2014). 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 (Mangal et al., 2008; Poulin, 2006; reviewed in Adekiya et al., 2019). In general, cercariae survival decreases as the water temperature increases, with exponential increases in mortality occurring at temperature below 15°C and greater than 35 °C (Lawson and Wilson, 1980). To understand the efficacy of marbled crayfish predators as regulatory agents of snail intermediate hosts and human schistosomiasis burden, we must also understand the ways in which temperature influences schistosome-competent snails and *Schistosoma* spp. worms at each life stage.

Field data are needed to quantify the actual consumption rates occurring in nature and to understand how these rates may shift with variations in crayfish weight and habitat temperature.

This work would be especially valuable in determining the regulatory effect of the invasive marbled crayfish on snails—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 must also investigate the relationship between marbled crayfish presence and human schistosomiasis burden, which cannot be explicitly examined within the scope of this work.

The introduced marbled crayfish directly threatens Madagascar's native aquatic wildlife (Andriantsoa et al., 2019)–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 (Critical Ecosystems Partnership Fund, 2014). The crayfish may also endanger the agricultural production of rice – a diet staple for Malagasy people, who face extreme poverty (Anastacio et al., 2005). On the other hand, the invasive crayfish may be playing an important role in the diet of children, 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 (USAID, 2021). Finally, these voracious snail predators could reduce the abundance of snail disease reservoirs, thereby reducing transmission of schistosomiasis to people (Khalil and Sleem, 2011; Mkoji et al., 1999; Sokolow et al., 2015). The introduction of the marbled crayfish may provide an unexpected benefit to the people of Madagascar: crayfish-driven reductions in burdens of human schistosomiasis– something that is urgently needed in a country where prevalence in some villages can range up to 94% (Rasoamanamihaja et al., 2016, Spencer et al., 2017). However, this benefit needs to be contextualized among the many other ecological and socio-ecological impacts of the crayfish; these various cross-currents must be quantified before any management action is warranted.

Non-native species have long been recognized as having profound effects on ecosystem services (IPBES, 2019; Lockwood et al., 2013; Lodge et al., 2016) through their influence on the abundance and distribution of their prey and competitors (Gherardi, 2007; reviewed in David et al., 2017). However, empirical evidence of such consequences does not exist for the vast majority of non-native introductions (Kulhanek et al., 2011; Larson et al., 2013; Simberloff et al., 2013). Non-native species may, in some regards, have negligible and even positive impacts on ecosystems (Schlaepfer et al., 2011, 2012), and careful, balanced evaluations of all the benefits and disservices associated with species introductions are needed to better inform management strategies (Sagoff, 2005; Schlaepfer et al., 2011, 2012; Vitule et al., 2012). 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 (Churcholl and Churcholl, 2021; Dunoyer et al., 2014; Jackson et al., 2014; Twardochleb et al., 2013)—thus limiting our ability to make broad predictions regarding the impact of invasive species on ecosystem services, including the regulation of disease agents. Likewise, existing evidence of the impact of natural enemies is exceedingly rare, as are empirical investigations linking species interactions within the environment to outcomes of human disease (reviewed in Jones et al., 2022). 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.

Natural enemies–predators, competitors, or parasites of reservoir hosts or of the disease agent itself –have the potential to regulate environmentally transmitted infectious diseases, such as schistosomiasis (Diakité et al., 2018; Halstead et al., 2018; Jones et al., 2022; Sokolow et al., 2015; Stauffer et al., 2006). As non-native species establish in new regions at exponentially increasing rates (Seebens et al., 2017), transmission regulation roles once held by native species could be assumed by invaders. Though introductions of non-native enemies in previous disease control efforts resulted in detrimental consequences (reviewed in Jones et al., 2022), introduced species have had success in reducing snail intermediate host populations, and even human schistosomiasis burden (Khalil and Sleem, 2011; Mkoji et al., 1999). In Madagascar, marbled crayfish have been observed in diverse habitats with a wide range of temperatures (Andriantsoa et al., 2019), including areas in which native crayfish species (Parastacidae*; Astacoides*) are unable to thrive (Jones et al., 2007; Toon et al., 2010). This invader has been shown to be a snail predator under laboratory conditions and may serve as an enemy of these intermediate snails hosts in areas where natural predators cannot. Might reductions in schistosomiasis transmission be a silver lining to the invasion of *Procambarus fallax f. virginalis* in Madagascar? Field studies are needed to determine whether the snail consumption documented here will translate into impacts on snail populations and human schistosomiasis burden.

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Competing interests

No competing interests declared.

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TABLES & FIGURES

Table 1: Results from linear mixed-effects model for rate of consumption (total number of snails consumed/12 hours), n = 542. Analyses were performed using the *lmer* function in the lme4 package (Bates et al., 2020). Significant p values are indicated with *

	parameter estimate	**standard error**	**df**	**t-value**	**p-value**
Intercept	-1.647278	0.908014	136.473403	-1.814	0.07185
Snail species	0.003547	0.213944	476.883267	0.017	0.98678
Infection status	0.080879	0.288214	421.543862	0.281	0.77914
weight	0.418126	0.103098	361.573899	4.056	6.13e-05***
temp20	0.641834	0.911030	476.290483	0.705	0.48146
temp25	-0.233143	0.907956	477.216990	-0.257	0.79746
temp30	1.254595	0.855428	474.989130	1.467	0.14314
temp35	0.226118	0.852375	474.267526	0.265	0.79091
weight:temp20	0.187898	0.123356	477.495535	1.523	0.12837
weight:temp25	0.420396	0.127858	481.005696	3.288	0.00108**
weight:temp30	0.291625	0.119989	477.278942	2.430	0.01545*
weight:temp35	0.160131	0.117065	473.747311	1.368	0.17200

Table 2: Results from the third analysis, a linear mixed-effects model for the number of unavailable snails (unavailable snails = the number of snails above the water line + the number of snails under the shelter/hiding) according to snail infection status (uninfected or infected), condition (control or experimental), snail species (*Bu. truncatus* or *Bi. glabrata*) and their interaction, n = 472. Analyses were performed using the *lmer()* function in the lme4 package (Bates et al., 2020). Significant p values are indicated with *

	parameter estimate	**standard error**	**df**	**t-value**	**p-value**
Intercept	0.17173	0.22651	373.07499	0.758	0.44883
Infection status[uninfected]	0.07768	0.31727	442.77491	0.245	0.80669
condition[experimental]	2.09890	0.31859	443.14461	6.588	1.27e-10***
snail species[*Bu. truncatus*]	0.91696	0.32279	444.86220	2.841	0.00471**
infection status:condition	-0.43361	0.44865	442.02298	-0.966	0.33432
infection status:snail species	0.26638	0.45071	443.14077	0.591	0.55481
condition:snail species	-0.78817	0.45164	443.26586	-1.745	0.08166
infection status:condition:snail species	-0.81045	0.63460	442.21722	-1.277	0.20224

Figure 1:

<u>Legend</u>	🟨 Control Uninfected (no crayfish)	🟦 Control Infected (no crayfish)	🟩 Experimental Uninfected (+ 1 crayfish)	🟧 Experimental Infected (+1 crayfish)	n = # of trials/ treatment

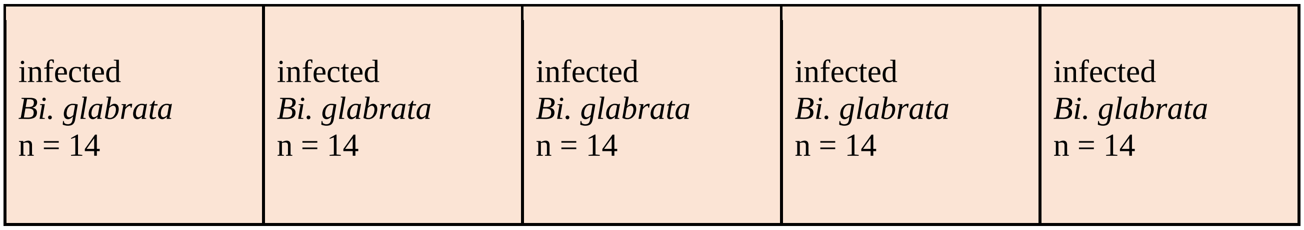
-

Round 1 (14 June 2021 to 13 August 2021)				
15°C	***20°C***	***25°C***	***30°C***	***35°C***
🟨 uninfected *Bu. truncatus* n = 14	🟨 uninfected *Bu. truncatus* n = 14	🟨 uninfected *Bu. truncatus* n = 14	🟨 uninfected *Bu. truncatus* n = 14	🟨 uninfected *Bu. truncatus* n = 14
🟨 uninfected *Bi. glabrata* n = 14	🟨 uninfected *Bi. glabrata* n = 14	🟨 uninfected *Bi. glabrata* n = 14	🟨 uninfected *Bi. glabrata* n = 14	🟨 uninfected *Bi. glabrata* n = 14
🟩 uninfected *Bu. truncatus* n = 42	🟩 uninfected *Bu. truncatus* n = 42	🟩 uninfected *Bu. truncatus* n = 42	🟩 uninfected *Bu. truncatus* n = 42	🟩 uninfected *Bu. truncatus* n = 42
🟩 uninfected *Bi. glabrata* n = 42	🟩 uninfected *Bi. glabrata* n = 42	🟩 uninfected *Bi. glabrata* n = 42	🟩 uninfected *Bi. glabrata* n = 42	🟩 uninfected *Bi. glabrata* n = 42

-

Round 2 (25 October 2021 to 17 December 2021)

15°C	**20°C**	**25°C**	**30°C**	**35°C**
uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14
uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14
uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14	uninfected *Bu. truncatus* n = 14
uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14	uninfected *Bi. glabrata* n = 14
infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14
infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14
infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14	infected *Bu. truncatus* n = 14



infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14	infected *Bi. glabrata* n = 14

Figure 1: Experimental design of the present study. One marbled crayfish (*Procambarus fallax f. virginalis*) was held in combination with a set density (n =12) of either *Biomphalaria glabrata* or *Bulinus truncatus* snails in a 11.7-L tank. In this figure, n refers to the number of trials conducted for each treatment. Note that the physical location of all tanks and conditions on lab shelving was randomized.

Figure 2:

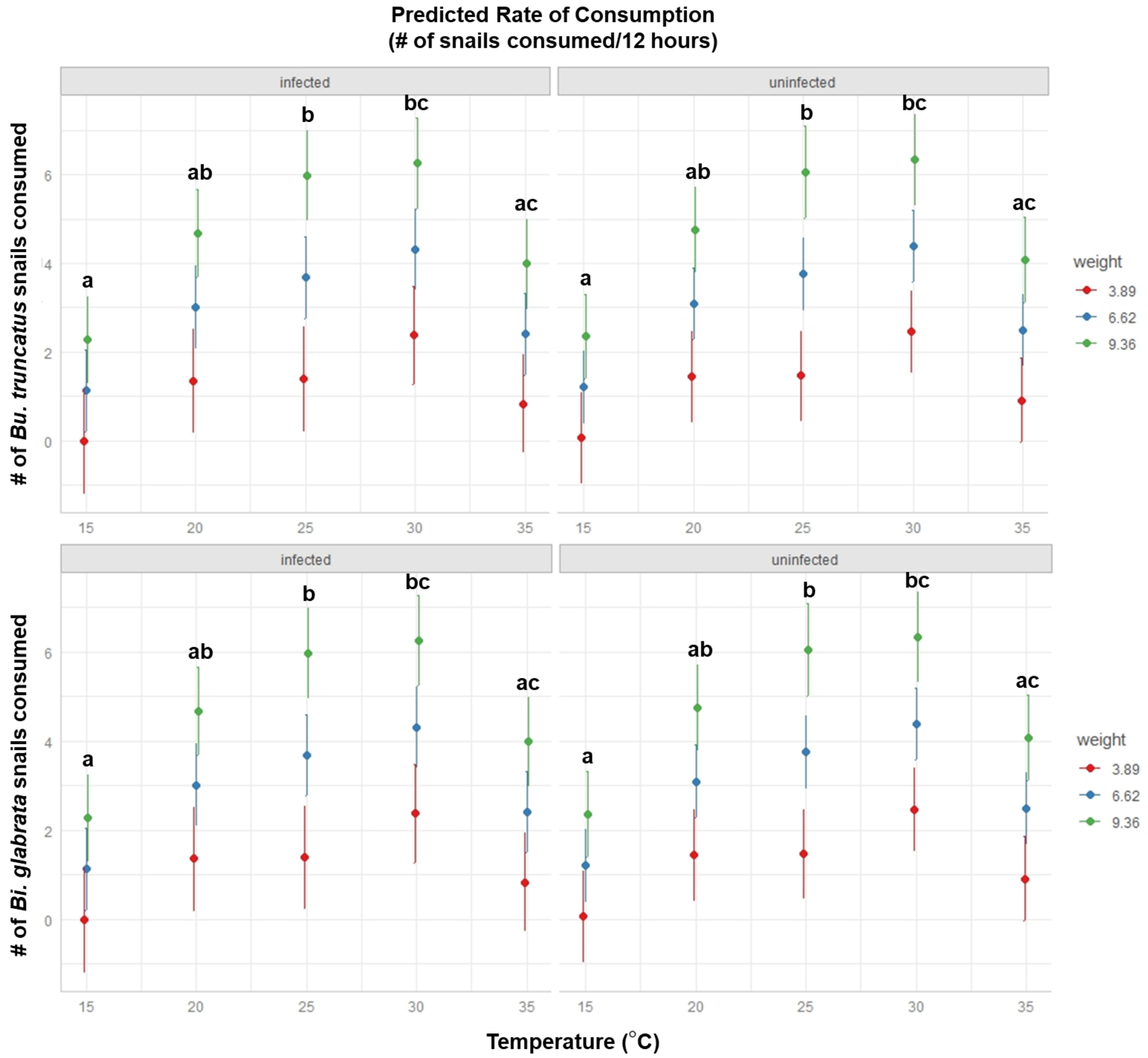


Figure 2: Predicted rate of consumption of *Bu. truncatus* and *Bi. glabrata* snails (# of snails consumed/12 hours) as a function of crayfish weight and snail infection status (infected or uninfected). A linear mixed-effects model for rate of consumption (total number of snails consumed/12 hours) found that crayfish consumption was significantly influenced by crayfish weight ($p < 0.0001$) and crayfish weight modulated the response of consumption to temperature. Letters indicate temperature treatments that differ from one another with respect to the interaction between crayfish weight and temperature; that is, temperature treatments that share no letters are those where the interaction between weight and temperature is significant, or where large-bodied and small-bodied crayfish differ in their response to temperature. Compared to small crayfish, large crayfish experienced a greater increase in consumption at moderate temperatures (25 and 30°C) relative to extreme temperatures (15, 20, and 35°C). Figure 2 was created using the *ggpredict()* function in the ggeffects package (Lüdecke, 2018) in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure 3:

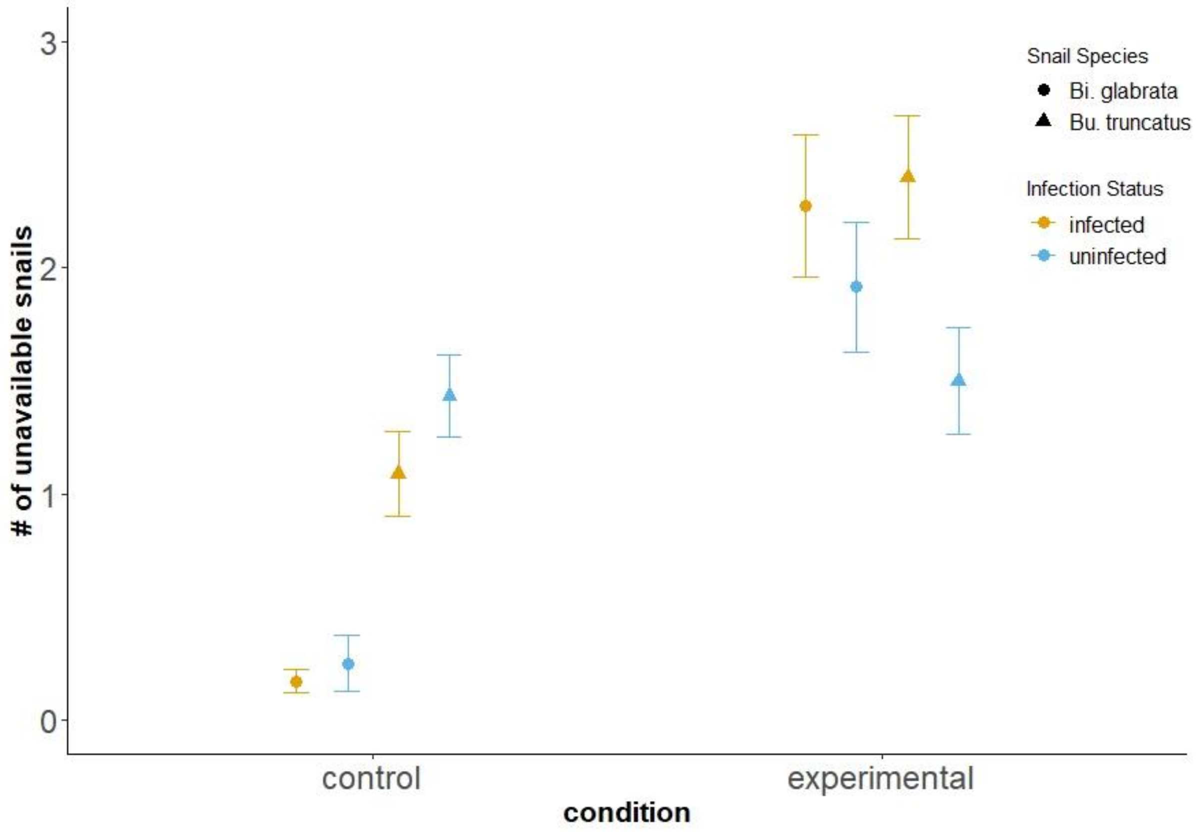


Figure 3: The number of unavailable uninfected and infected snails in each condition (control or experimental). The third linear mixed effects model found that the three-way interaction between snail infection status, condition, and snail species was not significant (p = 0.2022). Likewise, none of the two-way interactions (between snail infection status and condition, snail infection status and snail species, and condition and snail species) were significant (p = 0.3343, p = 0.5548, and p = 0.0817, respectively). The effect of snail infection status (uninfected or infected) was not significant (p = 0.8067); however, the number of unavailable snails was significantly influenced by condition (p < 0.0001) and snail species (p = 0.0047). There tended to be more unavailable snails in experimental trials compared to control trials, and among *Bu. truncatus* snails compared to *Bi. glabrata* snails. This suggests that both *Bu. truncatus* and *Bi. glabrata* snails, regardless of infection status, engage in anti-predator behaviors when exposed to predation cues in the experimental condition. Furthermore, some behavioral differences may exist between *Bu. truncatus* and *Bi. glabrata* snails and that these differences may influence crayfish consumption. However, the number of unavailable snails and crayfish consumption are confounded and unable to undergo further discrimination in the present study.

SUPPLEMENTARY INFORMATION

Table S1: AIC scores used to compare the relative fit of each model available in the rTPC package (Padfield et al., 2021) that would converge with my dataset. Thermal performance curves were fitted for three size classes of crayfish: small (<3.90 g), medium (3.91 – 6.62 g), and large (> 6.3 g).

crayfish size class	**model name**	**AIC value**
small	beta_2012	8.417
	kamykowski_1985	8.573
	briere2_1999	9.659
	lrf_1991	10.11
	lactin2_1995	10.13
	thomas_2012	10.38
	ratkowsky_1983	10.53
	oneill_1972	10.94
	spain_1982	10.94
	hinshelwood_1947	11.03
	joehnk_2008	11.37
	quadratic_2008	11.42
	modifiedgaussian_2006	11.49
	pawar_2018	11.49
	thomas_2017	11.67
	weibull_1995	12.23
	gaussian_1987	12.39
	flinn_1991	13.53
	sharpeschoolfull_1981	20.42
medium	quadratic_2008	11.19

	gaussian_1987	11.53
	flinn_1991	11.89
	lrf_1991	12.46
	modifiedgaussian_2006	12.61
	beta_2012	12.62
	oneill_1972	12.67
	pawar_2018	12.73
	lactin2_1995	12.80
	spain_1982	12.82
	briere2_1999	12.83
	hinshelwood_1947	12.84
	thomas_2012	12.84
	ratkowsky_1983	13.14
	kamykowski_1985	13.18
	weibull_1995	13.25
	joehnk_2008	14.16
	thomas_2017	14.41
	sharpeschoolfull_1981	21.04
large	beta_2012	-342.3
	joehnk_2008	-308.3
	thomas_2017	-304.9
	thomas_2012	-21.49
	briere2_1999	-15.37
	kamykowski_1985	-15.12
	lrf_1991	-10.24
	ratkowsky_1983	-6.073

	weibull_1995	0.5039
	oneill_1972	1.153
	gaussian_1987	2.642
	modifiedgaussian_2006	4.458
	pawar_2018	5.907
	flinn_1991	6.826
	quadratic_2008	9.960
	lactin2_1995	11.35
	spain_1982	13.19
	hinshelwood_1947	21.46
	sharpeschoolfull_1981	30.00

Table S2: Results from the second analysis, an adapted linear mixed-effects model for rate of consumption (total number of snails consumed/12 hours), n = 542. Analyses were performed using the *lmer()* function in the lme4 package (Bates et al., 2020) in R version 4.0.4 (2020-02-15) (R Core Team, 2021). Significant p values are indicated with *

	parameter estimate	**standard error**	**df**	**t-value**	**p-value**
Intercept	-1.090	1.0120	196.3	-1.078	0.28229
Snail species	0.0017	0.2137	521.4	0.008	0.99383
Infection status	0.1150	0.5884	526.6	0.195	0.84514
time	-0.0123	0.0108	512.1	-1.145	0.25265
weight	0.4163	0.1029	377.4	4.045	6.34e-05***
temp20	0.6592	0.9100	514.3	0.724	0.46913
temp25	-0.2076	0.9069	514.8	-0.229	0.81905
temp30	1.2780	0.8546	514.4	1.495	0.13542
temp35	0.1806	0.8518	514.7	0.212	0.83215
Infection status:time	-0.0008	0.0121	512.4	-0.068	0.94580
weight:temp20	0.1828	0.1232	515.6	1.483	0.13870
weight:temp25	0.4119	0.1278	518.3	3.224	0.00134**
weight:temp30	0.2861	0.1199	517.4	2.386	0.01738*
weight:temp35	0.1657	0.1170	514.1	1.416	0.15732

Figure S1:

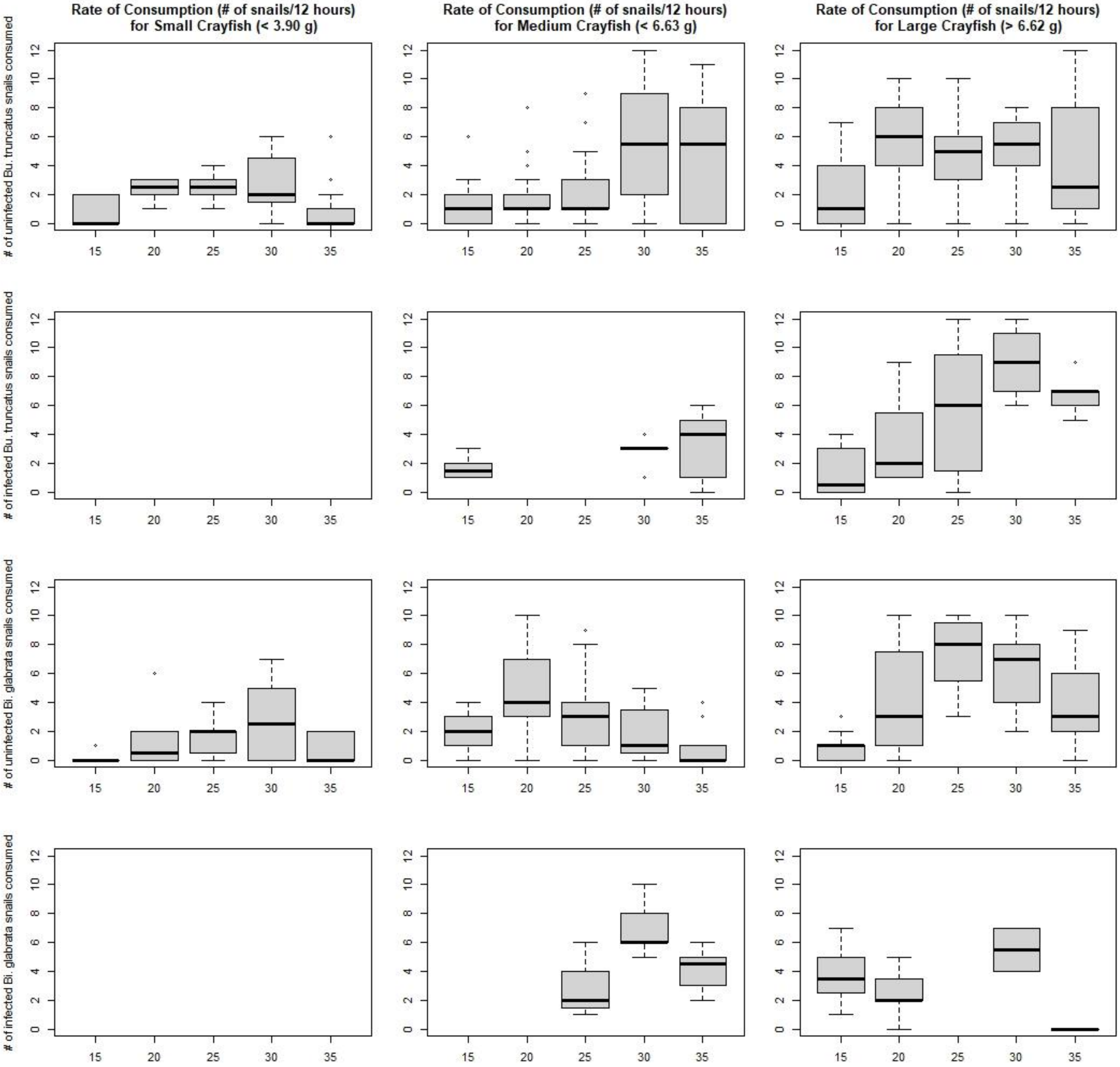


Figure S1: Raw data for the rate of consumption of *Bu. truncatus* and *Bi. glabrata* snails (# of snails consumed/12 hours) as a function of crayfish weight class and snail infection status (infected or uninfected). A linear mixed-effects model for rate of consumption (# of snails consumed/12 hours) found that crayfish consumption was significantly influenced by crayfish weight ($p < 0.0001$) and crayfish weight modulated the response of consumption to temperature. Compared to small crayfish, large crayfish experienced a greater increase in consumption at moderate temperatures (25 and 30°C; $p = 0.0011$ and $p = 0.0155$, respectively) relative to extreme temperatures (15, 20, and 35°C). I was not able to conduct consumption trials for small crayfish (< 3.90 g) consuming infected *Bu. truncatus* and *Bi. glabrata* snails. Plots were created using the *par* and *plot* functions in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure S2:

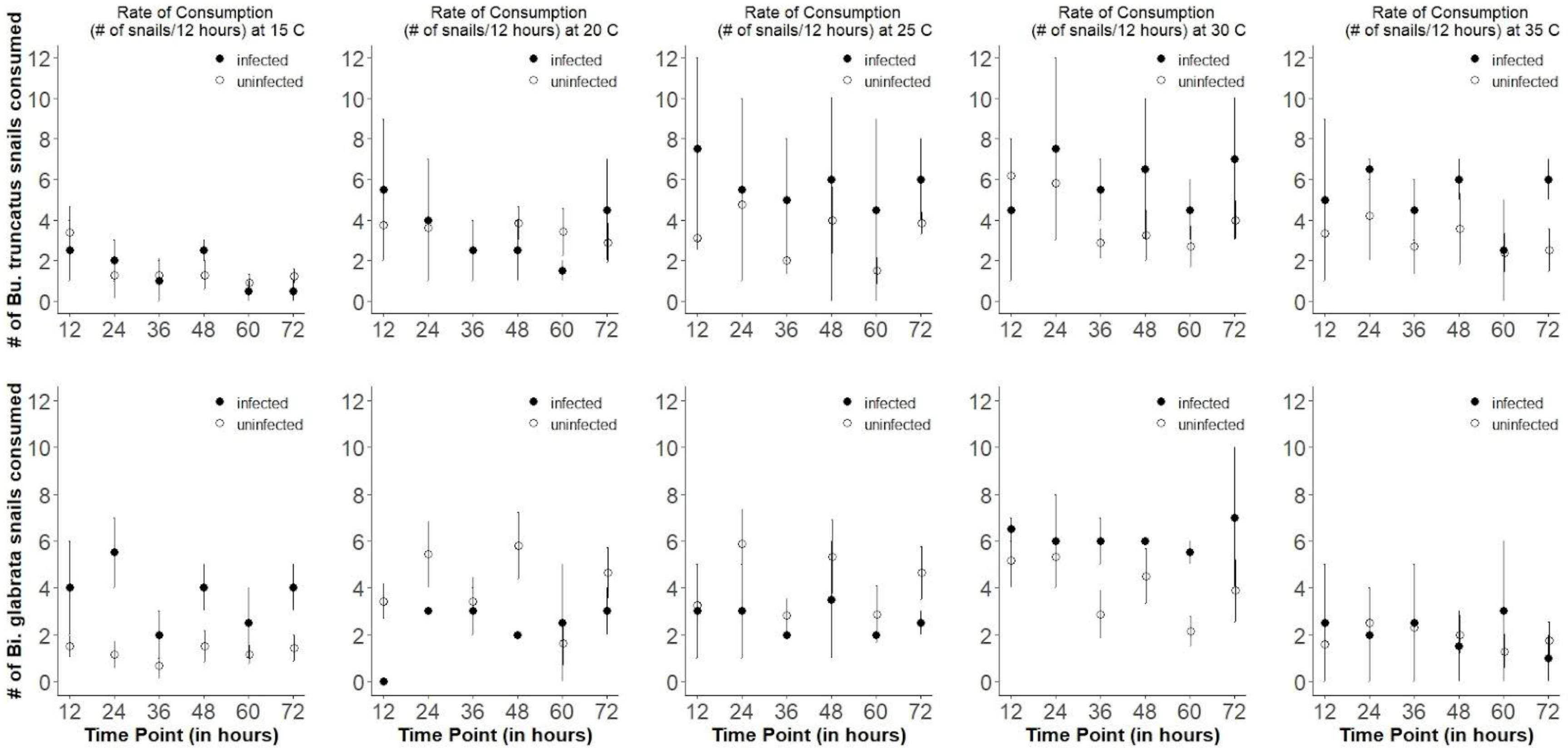


Figure S2: Raw data for the rate of consumption of *Bu. truncatus* and *Bi. glabrata* snails (# of snails consumed/12 hours) across the seven trials (0, 12, 24, 26, 48, 60, 72 hours) that constituted an experimental period for all temperatures (15, 20, 25, 30, and 35°C. The second analysis, in which time point was included as a fixed effect rather than a random effect, found that crayfish consumption was not significantly influenced by the interaction between time and snail infection status (p = 0.9458). Plots were created using the functions *ggplot* and *grid.arrange* in the packages ggplot2 (Wickham, 2016) and gridExtra (Auguie, 2017), respectively, in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure S3:

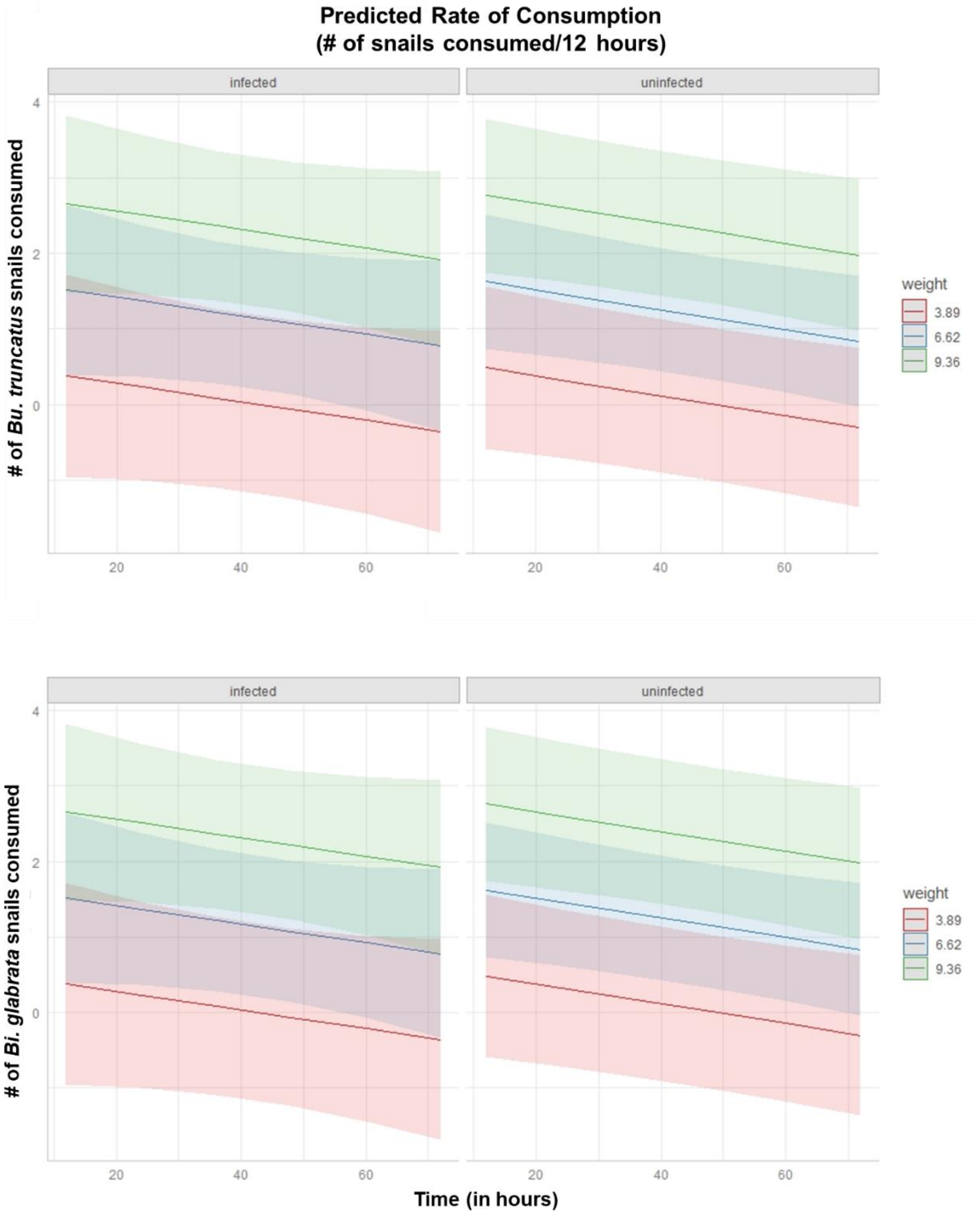


Figure S3: Predicted rate of consumption of *Bu. truncatus* and *Bi. glabrata* snails (# of snails consumed/12 hours) across time (in hours), as a function of crayfish weight and snail infection status (infected or uninfected), n = 542. The second analysis, in which time point was included as a fixed effect rather than a random effect, found that crayfish consumption was not significantly influenced by the interaction between time and snail infection status (p = 0.9458). Figure S3 was created using the *ggpredict* function in the ggeffects package (Lüdecke, 2018) in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure S4:

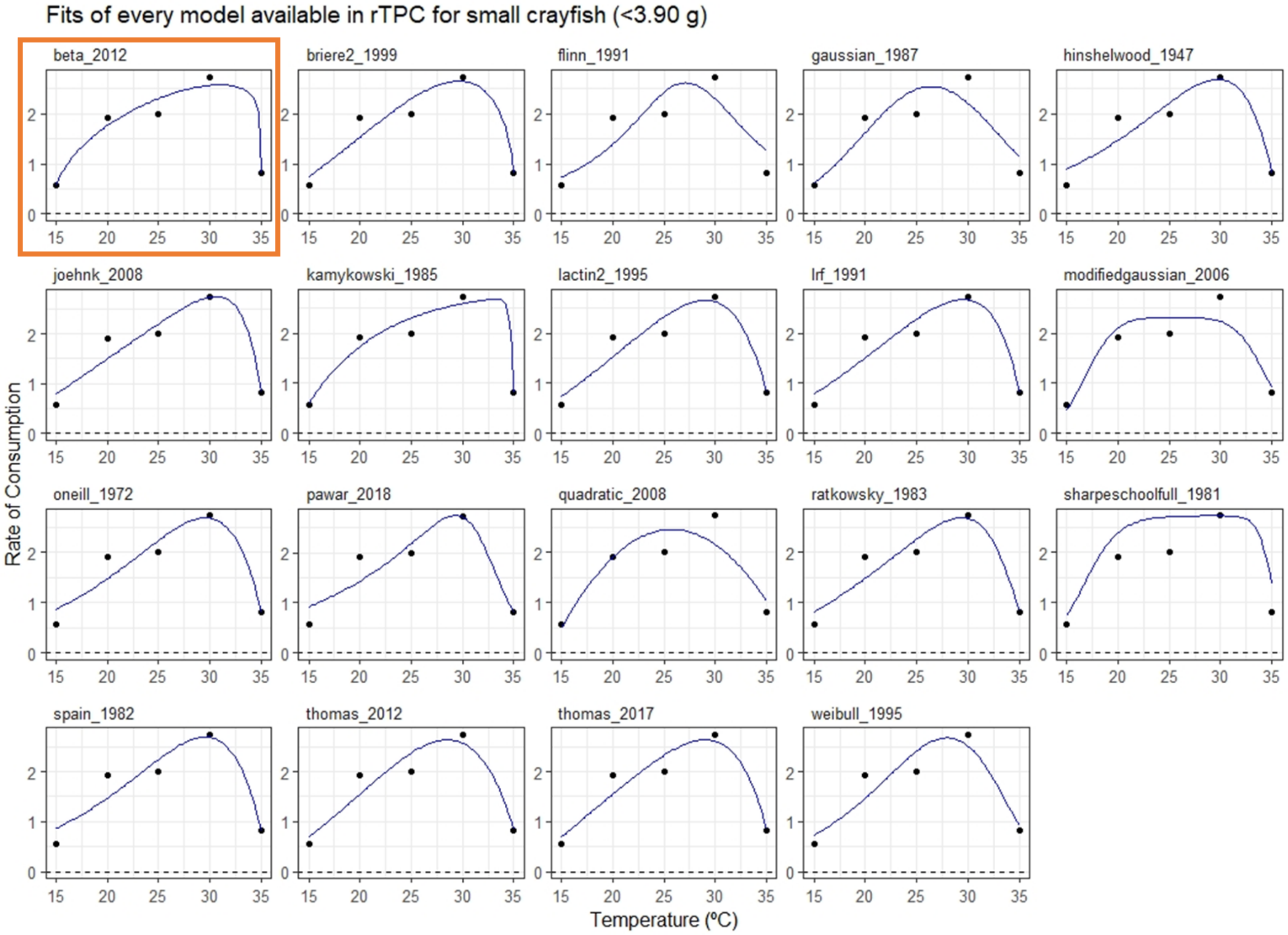


Figure S4: Thermal performance curves for small crayfish (<3.90 g) were fitted to all model formulations available within the rTPC package (Padfield et al., 2021) that would converge with the dataset. Model fits were first checked visually, and any obvious misfits disregarded. AIC scores were then generated using the *AIC()* function in rTPC package (Padfield et al., 2021) and used to compare the relative fit of each model. Small crayfish consumption data were best supported by the *beta_2012* model. The temperature at which the rate of consumption was highest (i.e., the thermal optimum) was calculated using the *get_topt()* function in the rTPC package (Padfield et al., 2021) and estimated to be 31.1°C for small crayfish. Figure S4 was created using the *ggplot* function in the ggplot2 package (Wickham, 2016) in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure S5:

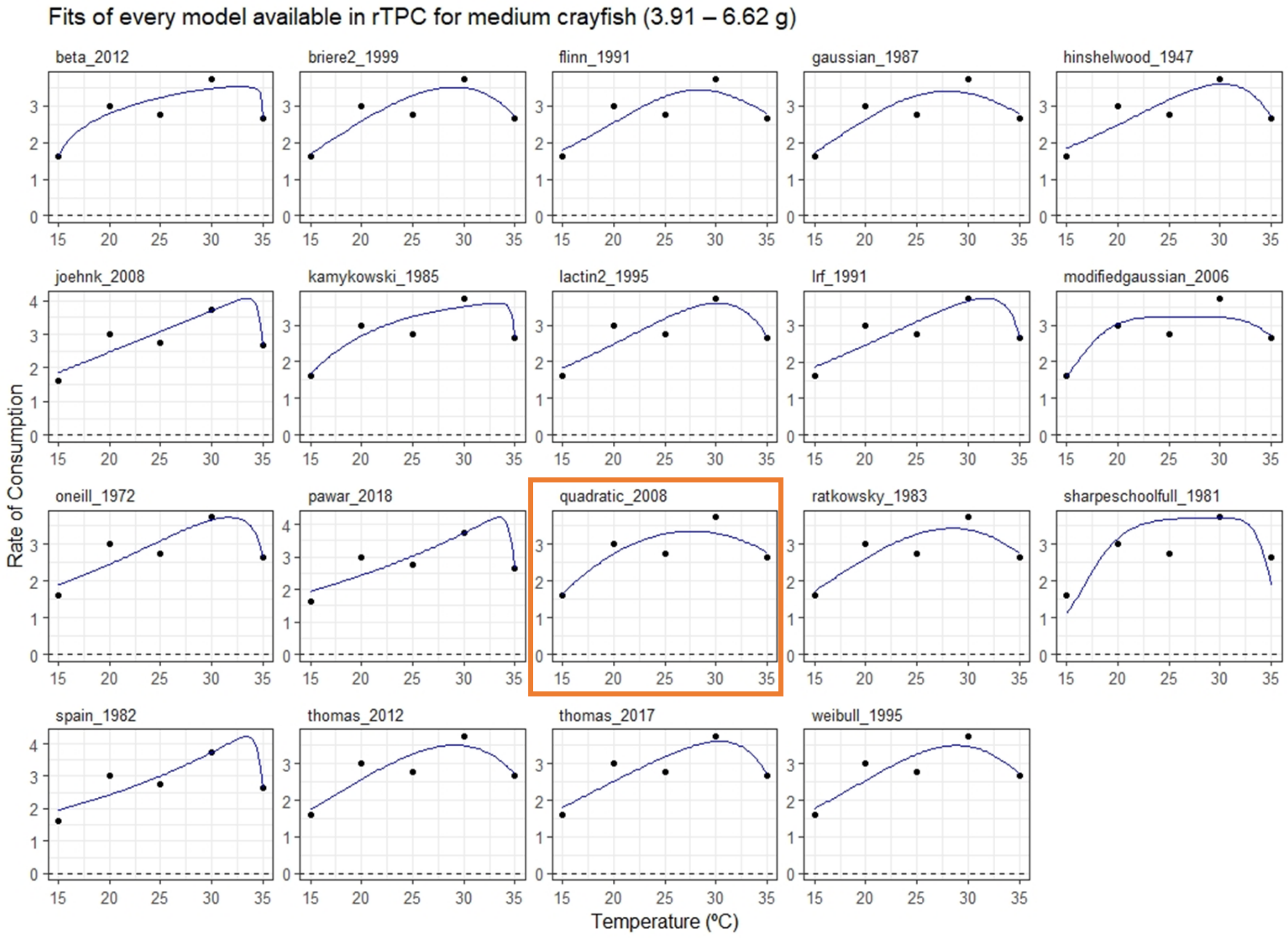


Figure S5: Thermal performance curves for medium crayfish (3.91 – 6.62 g) were fitted to all model formulations available within the rTPC package (Padfield et al., 2021) that would converge with the dataset. Model fits were first checked visually, and any obvious misfits disregarded. AIC scores were then generated using the *AIC()* function in rTPC package (Padfield et al., 2021) and used to compare the relative fit of each model. Medium crayfish consumption data were best supported by the *quadratic_2008* model. The temperature at which the rate of consumption was highest (i.e., the thermal optimum) was calculated using the *get_topt()* function in the rTPC package (Padfield et al., 2021) and estimated to be 27.7°C for medium crayfish. Figure S5 was created using the *ggplot* function in the ggplot2 package (Wickham, 2016) in R version 4.0.4 (2020-02-15) (R Core Team, 2021).

Figure S6:

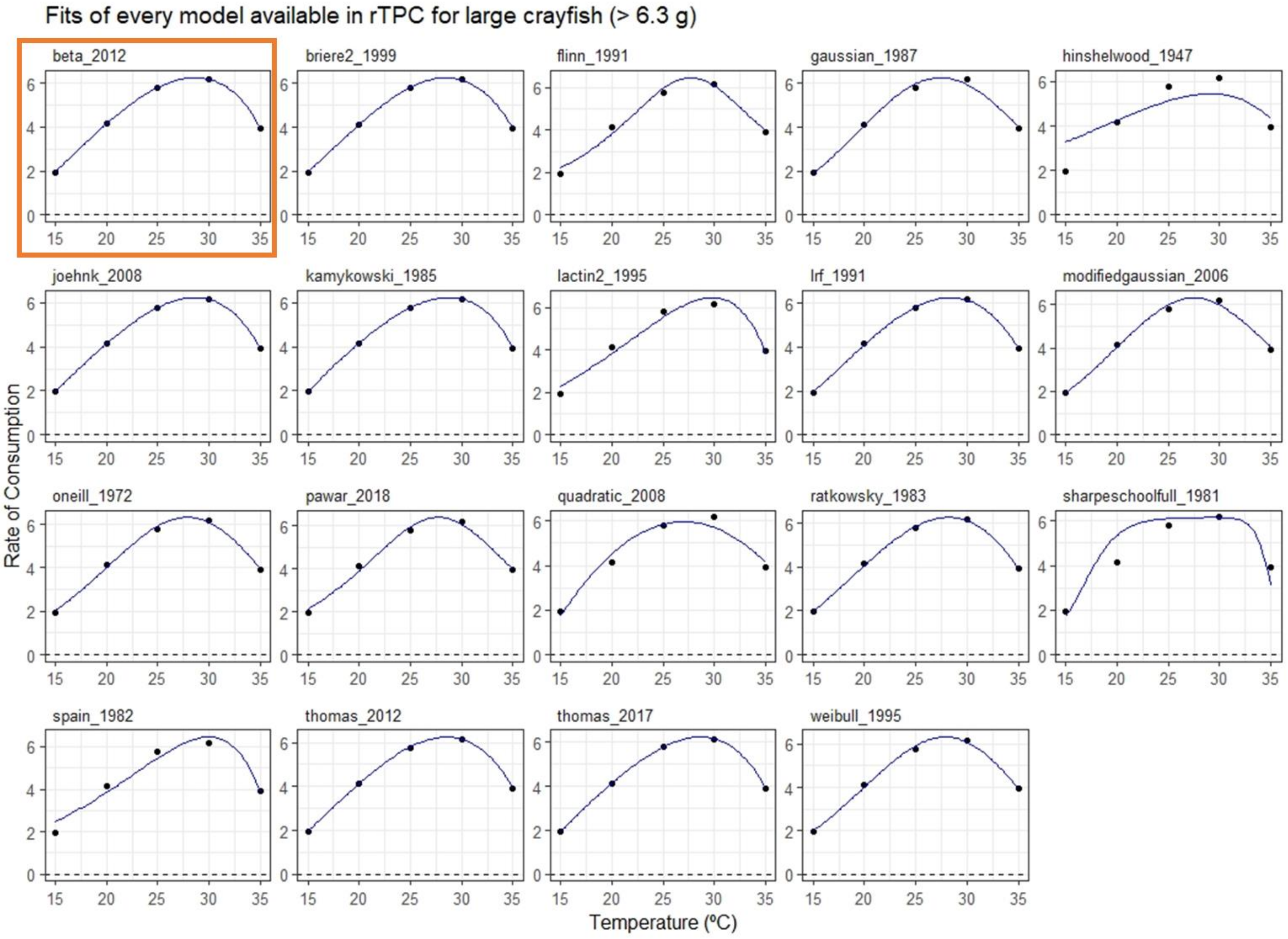


Figure S6: Thermal performance curves for large crayfish (> 6.3 g) were fitted to all model formulations available within the rTPC package (Padfield et al., 2021) that would converge with the dataset. Model fits were first checked visually, and any obvious misfits disregarded. AIC scores were then generated using the *AIC()* function in rTPC package (Padfield et al., 2021) and used to compare the relative fit of each model. Large crayfish consumption data were best supported by the *beta_2012* model. The temperature at which the rate of consumption was highest (i.e., the thermal optimum) was calculated using the *get_topt()* function in the rTPC package (Padfield et al., 2021) and estimated to be 28.7°C for large crayfish. Figure S6 was created using the *ggplot* function in the ggplot2 package (Wickham, 2016) in R version 4.0.4 (2020-02-15) (R Core Team, 2021).