Trophic impacts of invasive alien crayfishes under climate warming

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Abstract

Climate change is facilitating the spread of warm-adapted species into temperate regions. Predictive risk assessments are needed to determine and prioritize high-impact invasions, but key ecological information on the performance of potential invaders is often lacking. In aquatic ecosystems, introduced crayfishes can cause dramatic changes in community composition, food webs, and ecosystem function. Two species that are considered emerging invasion threats to the Great Lakes basin are the red swamp crayfish (*Procambarus clarkii*), which has a global invasion history, and the marbled crayfish (*Procambarus virginalis*), a popular pet species that reproduces parthenogenetically.

My research applied an experimental design that quantified and compared per capita effects of *P. clarkii* and *P. virginalis* by testing their functional responses—the rate of resource consumption in relation to resource availability—at two temperatures, 18°C and 26°C, reflecting current and projected mean maximum summer surface temperatures, respectively, for the nearshore Great Lakes. In one set of experiments, I tested the effect of reproductive status on the feeding behaviour of *P. virginalis*. I found that gravid state significantly reduced feeding rates at both 18°C and 26°C, and temperature had an effect only on the length and frequency of gravid stages. In a second set of experiments, I tested the effects of temperature on consumption of animal and plant resources, including situations when an alternative food source was present (permitting. resource switching). In these experiments, *P. clarkii* consumed more animal prey and macrophytes than *P. virginalis* at either temperature. During resource-switching experiments, *P. virginalis* at 18°C and *P. clarkii* at 26°C had higher consumption rates of animal prey, and *P. virginalis* at 18°C exhibited higher consumption rates of macrophytes than *P. clarkii* at 18°C. Collectively, these experiments revealed the complexity of the effects of temperature, phenology, resource availability and species identity on consumer-resource interactions.

Résumé

Le changement climatique facilite la propagation d'espèces tropicales vers les régions tempérées. Des prévisions de risques sont nécessaires pour déterminer et donner la priorité aux invasions à fort impact, mais les informations écologiques essentielles sur les performances des envahisseurs potentiels font souvent défaut. Dans les écosystèmes aquatiques, les écrevisses introduites peuvent provoquer des changements spectaculaires dans la composition des communautés, les réseaux alimentaires et le fonctionnement des écosystèmes. Deux espèces considérées comme des menaces d'invasion émergentes dans le bassin des Grands Lacs sont l'écrevisse de Louisiane (*Procambarus clarkii*), qui a une zone d'invasion mondiale, et l'écrevisse marbrée (*Procambarus virginalis*), une espèce populaire pour les aquariophiles, qui se reproduit parthénogénétiquement.

Pour mes recherches j'ai utilisé un protocole expérimental qui m'a permis de quantifier et comparer les effets par capita de *P. clarkii* et de *P. virginalis* en testant leurs réponses fonctionnelles - le taux de consommation des ressources par rapport à leur disponibilité - à deux températures différentes, 18°C et 26°C, qui reflètent les températures de surface estivales maximales moyennes actuelles ainsi que prévisionnelles pour le littoral des Grands Lacs. Dans une première série d'expériences, j'ai testé l'effet du statut reproductif sur le comportement de consommation de *P. virginalis*. J'ai trouvé que l'état gravide réduisait de façon significative les taux d'alimentation à 18°C et 26°C, et que la température avait un effet uniquement sur la longueur et la fréquence des états gravides. Dans une deuxième série d'expériences, j'ai testé les effets de la température sur la consommation de ressources animales et végétales, y compris dans des situations où une source alternative de nourriture était présente (i.e. transition des ressources). Dans ces expériences, *P. clarkii* a consommé plus de proies animales et de macrophytes que *P. virginalis* quelle que soit la température. Pendant les expériences de transition entre les ressources, *P. virginalis* à 18°C et *P. clarkii* à 26°C ont eu des taux de consommation de proies animales plus élevés, et *P. virginalis* à 18°C a présenté des taux de consommation de macrophytes plus élevés que *P. clarkii* à 18°C. Collectivement, ces expériences ont révélé la complexité des effets de la température, de la phénologie, de la disponibilité des ressources et des espèces sur les interactions consommateur-ressource.

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Contribution of Authors

This thesis was completed under the supervision of Prof. Anthony Ricciardi at McGill University, who will be a co-author on the submitted manuscripts. Experiments in Chapter 1 were led by me with the help of Jennifer Pham who will also be a co-author on the submitted manuscript. Statistical analysis of both chapters, and experiments of Chapter 2 were completed by me. Prof. Ricciardi provided instrumental feedback and editing of my manuscripts. This being the case, and reflecting the intention to submit for publication, plural pronouns are used throughout the thesis.

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General Introduction

The problem of biological invasions in freshwater ecosystems

Biological invasion describes the process by which an introduced organism establishes a sustainable population in a region beyond its historic native range, often as a result of human activities (Ricciardi 2013). Such species are termed ‘non-native’, ‘nonindigenous’, or ‘alien’. The term ‘invasive’ is applied to those non-native species that spread and establish exceptionally well, and often dominate or impact recipient communities.

Invasive species are a major driver of global biodiversity loss and ecosystem alteration (Clavero and García-Berthou 2005; Bellard et al. 2016). In freshwater ecosystems, invasive species are one of several stressors causing high rates of species loss (Ricciardi and Rasmussen 1999; Dextrase and Mandrak 2006; Dudgeon et al. 2006). The impact of invasion on freshwater biodiversity may be worsened by the geographic isolation of many watersheds. Like islands and other insular habitats, freshwater ecosystems contain biota that are ecologically naïve and thus more sensitive to the effects of a broad suite of alien species introduced through human activities such as pet releases, bait bucket dumping, ballast water discharge, and sport fish stocking (Ricciardi and MacIsaac 2011). Dramatic changes in community composition and ecosystem function from invasions by novel predators (Zaret and Paine 1973; Goldschmidt et al. 1993; Nyström et al. 2001; Tronstad et al. 2010) and suspension feeding bivalves (Fera et al. 2017) have been well studied. By comparison, there has been far less research on the impacts of generalist omnivores on freshwater food webs (Jackson et al. 2017; but see e.g., Dorn and Wojdak 2004)

Climate warming and the Environmental Matching Hypothesis

Climate change has important repercussions for biological invasions in freshwater ecosystems. Warmer water temperatures, reduced ice cover duration, and modified stream flow patterns are among the transformative effects of climate change on lakes, rivers, and other water bodies (Poff et al. 2002). For native species, these drastic changes could become additional stressors that push them further towards extirpation or extinction (Rahel and Olden 2008). For invasive species, these changes could bring conditions closer to those to which they are adapted and thus facilitate their success and impact. Cold intolerance is a significant barrier for the establishment and propagation of invasive species in high temperate regions (Rahel and Olden 2008; Hulme 2017). Exposure to water temperatures beyond the optimal range of a species can limit growth (Seitz et al. 2005), feeding rates (Iacarella et al. 2015), and fecundity (Peruzza et al. 2015). By lifting thermal barriers, elevated temperatures thus allowing warm-adapted non-native species to establish self-sustaining populations (Hulme 2017) and expand their range poleward into regions where they would previously be excluded by the cold climate (Rahel and Olden 2008). The impacts of invaders, particularly ectotherms, are also dependent on temperature regimes (Iacarella et al. 2015; Daugaard et al. 2019). The Environmental Matching Hypothesis (Ricciardi et al. 2013; Iacarella et al. 2015) predicts that an invader’s per capita effect increases when environmental conditions are closer to those that are physiologically optimal for population growth. For many warm-adapted species in the pet trade, their introduction under less severe temperature constraints may allow them to more exploit resources more efficiently and compete with natives more effectively (Cadi and Joly 2004; McCard et al. 2021).

Pet trade as major pathway to invasion

The global exotic pet trade is increasingly lucrative while the number of affected species continues to expand. Over the past decade billions of plants and animals a year were traded (Gippet and Bertelsmeier 2021). This industry is the source of some high-impact invaders (Lockwood et al. 2019). Of particular concern, the pet industry may favour species that are predisposed to becoming invasive, as traits associated with invasiveness (e.g., generalist habitat requirements, broad tolerance, rapid reproduction) favor easy cultivation and commercial success (Gippet and Bertelsmeier 2021).

The magnitude of the aquatic pet trade and its contribution to biological invasions is difficult to estimate, especially with the emergence of online commerce (Faulkes 2018; Olden et al. 2021). However, the legal global ornamental fish market has been estimated to be worth USD$4.2–9.5 billion a year, a figure that made higher by the addition of the illegal ornamental aquatic trade (King 2019). Risk assessments of living organisms in trade have been underused relative to the magnitude of the invasion threat posed (Howeth et al. 2015). With respect to popular aquarium species, there is a need for predictive ecological information to develop risk assessments and aid prioritization and prevention.

Invasive alien crayfishes

Popular in the aquatic pet trade, crayfish are typically the largest and longest-lived invertebrates in temperate freshwater environments (Gherardi 2007; Faulkes 2015). They are generalist and opportunistic omnivores that can act as keystone consumers in both lentic and lotic waters (Gherardi 2006), and they can adapt their diet to available resources in new environments by quickly learning to exploit novel food items (Tricarico and Aquiloni 2016). In their introduced ranges around the world, they have caused substantial changes to invertebrate, amphibian, and fish communities (Dorn and Wojdak 2004; Tricarico and Aquiloni 2016). For example, Dorn and Wojdak (2004) compared ponds that were invaded by crayfish to uninvaded ponds and found that crayfish invasion reduced populations of fish, amphibians, and gastropods, and caused declines in the biomass and diversity of macrophytes. These and other cases demonstrate that invasive crayfishes can impact species at all trophic levels (Table 1).

Invasive crayfish also tend to be more aggressive than their native counterparts and can displace native crayfishes and benthic fishes from shelters, thus leaving these native species more vulnerable to predation (Gherardi 2007; Tricarico and Aquiloni 2016; Church et al. 2017). In addition, invasive crayfish have broad socioeconomic impacts; they can consume terrestrial crops to such an extent that they are considered agricultural pests (Tricarico and Aquiloni 2016). Further, their burrowing activities increase the erosion of riverbanks to the point of collapse and disturb agricultural fields to the detriment of crop yields (Correia and Ferreira 1995; Loureiro et al. 2015; Sanders et al. 2021).

Crayfishes also functionally act as “shredders”—animals that feed on coarse particulate organic matter in such a way as to expedite its microbial breakdown (Cummins et al. 1989). Their feeding activities generate fine organic particles of less than 1 mm in diameter consisting primarily of feces as well as vegetal fragments too small to be ingested by many macroscopic animals (Cummins et al. 1989; Benfield 2007). Reduced macrophyte biomass and diversity caused by invasive crayfishes can have ramifying effects on the invaded community (Rodríguez et al. 2003; Dorn and Wojdak 2004). Macrophytes mediate light transparency, water flow, water temperature, and sediment and organic matter retention (Carpenter and Lodge 1986), and their loss can promote a change in stable state to a turbid water system (Rodríguez et al. 2003).

Overconsumption of macrophytes can also increase plant decomposition and thus affect nutrient cycling by transforming a previously gradual release of energy into a rapid pulse (Cummins et al. 1989; Benfield 2007). Clearly, by altering fundamental habitat characteristics and processes, invasive crayfish can re-engineer entire lentic ecosystems.

***Procambarus* spp. as invasion threats**

The Great Lakes–St. Lawrence River system contains at least four invasive and two expanding crayfish species, of which the rusty crayfish (*Faxonius rusticus*) is the most widespread and locally abundant, outcompeting over a dozen native species (Hamr 2010; Peters et al. 2014; Hossie and Hamr 2022). Of particular interest as invasion threats are crayfishes in the genus *Procambarus*, which are warm-adapted omnivorous species with optimal temperature ranges for growth ranging between 20°C and 30°C (Aiken and Waddy 1992). Two of these congeneric species pose an emerging threat to the Great Lakes: the red swamp crayfish (*Procambarus clarkii*) and the marbled crayfish (*Procambarus virginalis*) (Hamr 2021).

Native to the southern United States, *P. clarkii* began invading the lower Great Lakes since at least 1967 (Peters et al. 2014). Elsewhere, it has become a dominant crayfish throughout Europe and has established non-native populations on all continents except Antarctica and Australia (Loureiro et al. 2015; Oficialdegui et al. 2019, 2020). Due to its recent population expansions and wide use as live bait, there has been growing concern for its invasion risk more locally in North America (Egly et al. 2019). The recorded impacts of *P. clarkii* are numerous and affect more than freshwater environments (Dörr et al. 2020). Through its burrowing behaviour, *P. clarkii* can re-engineer physical habitat (Correia and Ferreira 1995; Souty-Grosset et al. 2016). Its generalist feeding activities can cause rapid depletions of invertebrate prey (Correia 2002) and significant losses in macrophyte abundance (Carreira et al. 2014). The loss of macrophytes can cause clearwater lakes can become turbid and trigger declines in abundances of macroinvertebrates, amphibians, and waterfowl (Rodríguez et al. 2005). Overgrazing can cause damage to rice agriculture (Grigarick and Way 1982; Anastácio and Marques 1997; Anastácio et al. 2000).

In contrast, *P. virginalis* has no confirmed invasive populations in North America[1] but has an invasive range in Asia (Kawai and Takahata 2010), Africa (Jones et al. 2009), and in Europe where it has recently undergone a dramatic expansion (Ercoli et al. 2019; Grandjean et al. 2021; Sanna et al. 2021; Scheers et al. 2021). It was first discovered in the German aquarium trade as the parthenogenetic triploid lineage of the American Slough crayfish *Procambarus fallax* (Scholtz et al. 2003), a mutation thought to have arisen in captive populations[2]. While *P. virginalis* was once considered to be solely the parthenogenetic form of *P. fallax* owing to their morphological similarity and shared mitochondrial DNA (Martin et al. 2010), the two closely related species have important differences in ecology, life history, and genetics; furthermore, they are reproductively incompatible (Scholtz et al. 2003; Lyko 2017; Vogt et al. 2019). Consequently, *P. virginalis* is now considered to be a new species with no native range. It has few recorded impacts to date. Some analyses suggest that *P. virginalis* is a generalist feeder like *P. clarkii* (Lipták et al. 2019; Linzmaier et al. 2020; Muuga 2021). In its invasive range in Poland, it may have important impacts on amphibians (Maciaszek et al. 2022). In Germany, it has been shown rely heavily on arthropod prey (Linzmaier et al. 2020). In the Slovak Republic, it

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[1] There has been an unconfirmed sighting of *P. virginalis* from the Toronto area (https://www.inaturalist.org/observations/98794697). Accessed on 20 January 2023.

[2] According to an unpublished report, asexual female individuals of *P. fallax* have recently been identified in a wild Florida population. Lyko, F. 2022 Origin and propagation of the marbled crayfish. Abstracts of the 23rd Congress of the International Association of Astacology, Hluboka nad Vltavou, Czech Republic, June 2022.

was found to feed most importantly on algae and detritus (Lipták et al. 2019). Seemingly, like many other crayfishes, it has a generalist opportunistic diet (Gherardi 2006).

Consumer-resource dynamics as a predictor of impact

High-impact invasive species have the ability to rapidly exploit resources and can produce direct and indirect impacts on communities and food webs (Correia 2002; Carreira et al. 2014; Jackson et al. 2017). Invasive crayfishes in particular, have caused strong ecological impacts through their exploitation of resources (Table 1). Resource consumption rates tend to be higher in high-impact invaders than in trophically-similar invader and native species (Morrison and Hay 2011; Dick et al. 2013, 2014). One method of assessing potential trophic impacts is to compare the classical functional response of consumers (i.e., their experimentally derived rate of resource consumption in relation to resource availability) across different environmental contexts and different species (Dick et al. 2014; Iacarella et al. 2015). The shape of the response typically fits one of three models: a linear relationship between prey consumption and prey density (Type I), characteristic of suspension feeders; an asymptotic (Type II) relationship, which is deemed to be destabilizing to prey populations because low densities of prey cannot escape predation; and a sigmoidal (Type III) response is stabilizing for prey populations, because it is defined by the presence of a prey refugium at low prey densities (Figure 1). Further ecological information is provided by individual parameters such as attack rate, handling time, and maximum feeding (Pritchard et al. 2017). Higher attack rates increase prey consumption at low prey densities, and lower handling times increase maximum feeding rates; both these parameters have been correlated with observed trophic impacts of invaders in the field (Dick et al. 2014). Interspecific and interpopulation comparisons of functional response parameters can thus yield insight into an invader's potential impact on the abundance and diversity of species at lower trophic levels. It also offers valuable predictive information for species that do not yet have well-documented invasion histories (Dick et al. 2013).

Though extrapolating complex field impacts from small-scale lab experiments remains an issue, this method provides insight into how various biotic and abiotic factors may govern the magnitude of an invader's impacts (Médoc et al. 2018; Cerato et al. 2019; Grimm et al. 2020). Comparative functional responses give us at least a first approximation of an invader's potential future impacts and can also be further improved by combining maximum feeding rates with a proxy measure of an invader's abundance—e.g., lifespan, fecundity, or lifetime fecundity (Relative Impact Potential; Dickey et al. 2018, 2020, 2021), or by combining the calculated attack rates and handling times to make a more informative predictor of impact than maximum feeding rates alone (Functional Response Ratio; Cuthbert et al. 2019). The flexibility of this method asserts its value for risk assessment.

Research objectives

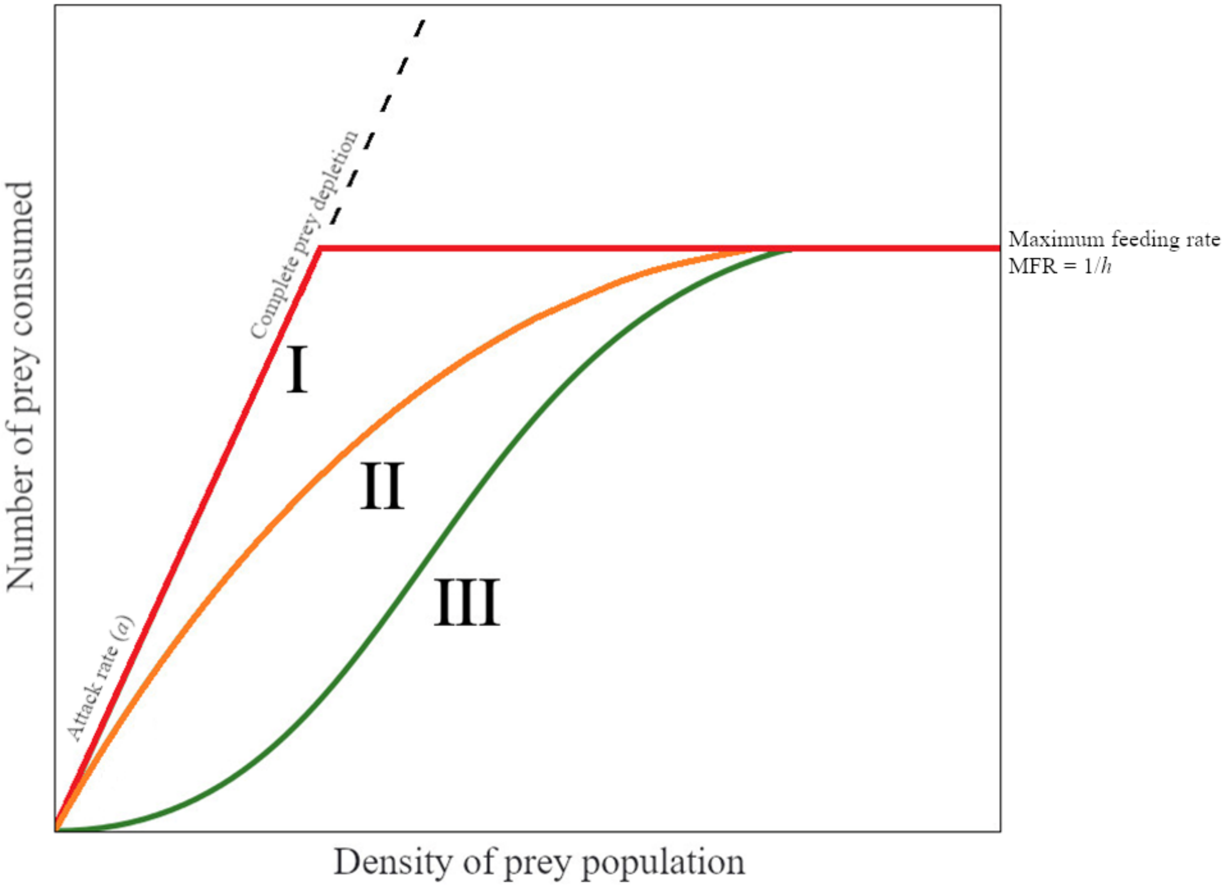
The goal of this research was to determine how different biotic and abiotic contexts affect resource-consumer dynamics and ultimately determine the impacts of crayfish invasions. Here, the rate of resource consumption was measured during a series of temperature-controlled lab experiments on the red swamp crayfish (*Procambarus clarkii*) and marbled crayfish (*Procambarus virginalis*). My objectives were to determine 1) the effect of temperature on the trade-off between reproductive ability and resource consumption; and 2) the effect of temperature, resource type, and resource availability on *per capita* effects. The results are organised into two chapters describing studies that apply the comparative functional response approach in their design.

Table 0.1. Examples of documented impacts of invasive alien crayfishes attributable to their resource use.

Impact	Mechanism	Direction	Species	Comments
Biodiversity change	Predation	Negative	*Pacifastacus leniusculus*	Altered community composition and reduced taxon richness (Ruokonen et al. 2014)
			Faxonius rusticus	Significant decreases in snail populations (Wilson et al. 2004)
			Procambarus clarkii	Loss of newts due to consumption of egg masses and larvae (Gamradt and Kats 1996)
			Faxonius virilis	Reduced fish recruitment from egg predation (Dorn and Wojdak 2004)
Biodiversity change	Herbivory	Negative	*Faxonius virilis*	Loss of macroalgae and the filamentous green metaphyton (Dorn and Wojdak 2004)
	Herbivory	Negative	*Faxonius rusticus*	Loss of macrophytes (Lodge et al. 1994)

Biodiversity change			*Procambarus clarkii*	Consumption of terrestrial plants (Grey and Jackson 2012)
Habitat alteration	Predation	Negative	*Faxonius virilis*	Predation potentially indirectly reduced peak dissolved oxygen (Dorn and Wojdak 2004)
Habitat alteration	Herbivory	Negative	*Procambarus clarkii*	Shift from clear to turbid phase in a lake due to macrophyte consumption (Rodríguez et al. 2003)
Food web alteration	Herbivory	Positive	*Faxonius virilis*	Increase in blue-green algae due to consumption of other types (Dorn and Wojdak 2004)
			Faxonius virilis	Increased plankton populations (Dorn and Wojdak 2004)

Figure 0.1. Typical functional responses that characterize resource consumption by animals.



Chapter 1 – Influence of reproductive state and temperature on the functional response of the marbled crayfish, Procambarus virginalis

Abstract

Predictive information on the trophic impacts of an invasive consumer can be gained by experimentally measuring its functional response—the rate of prey consumption in relation to available prey density. However, a common gap in such information is how functional response varies with the phenology and reproductive state of the consumer. Here, we tested the effect of reproductive state and temperature on the functional response of the marbled crayfish (*Procambarus virginalis*), a parthenogenetic species that is globally popular in the pet trade and considered to be an invasion threat to the North American Great Lakes. We applied two thermal treatments representing the ambient and projected maximum summer surface temperatures, respectively, for nearshore areas of the lower Great Lakes. We found that gravid individuals continued to feed, but their rate of consumption of benthic invertebrate prey (chironomid larvae) was dramatically reduced across prey densities. Furthermore, contrary to functional response studies on non-gravid animals, their attack rate and prey handling times did not differ with temperature. Warming reduced the maximum feeding rates of nongravid individuals, while increasing the frequency and duration of gravid states. Our findings underscore the need for quantifying variation in per capita effects across reproductive cycles and temperatures, to better inform invasive species risk assessments.

Introduction

The impact of a non-native species population is a function of the abundance and per capita effects of individuals in its invaded range (Parker et al. 1999; Dick et al. 2017b). Myriad direct and indirect ecological impacts have been linked to the invader's per capita rates of resource consumption when such resources (e.g. food, water, habitat space) are limiting (Correia 2002; Ricciardi et al. 2013; Dick et al. 2013; Carreira et al. 2014). Rates of resource consumption by high-impact invaders tend to be higher than for low-impact invaders and functionally similar native species (Morrison and Hay 2011; Dick et al. 2013). High-impact invaders also tend to have higher reproductive rates (e.g. Marchetti et al. 2004; Keller et al. 2007; Ricciardi et al. 2013) and can thus attain abundances that amplify their per capita effects on native species (Bradley et al. 2019).

Resource consumption efficiency can be compared across taxa, populations, locations, and environmental conditions (such as temperature), using experimental measurements of an invader's functional response—its rate of resource consumption in relation to resource availability (e.g., prey density). Functional response experiments have been shown to be useful for predicting, to some extent, invasion success and ecological impacts (Dick et al. 2013, 2014, 2017a; Jackson et al. 2017; but see Landi et al. 2022). Comparisons of functional responses yield quantitative data on the per capita effects of potential or emerging invaders, and thus can inform risk assessments that otherwise must rely upon data from well studied populations (e.g., Chucholl 2016; Egly et al. 2019). Two key parameters of the functional response have significance for understanding consumer-resource interactions: attack rate and prey handling time. Higher attack rates increase predation pressure at low prey densities, whereas longer handling times reduce maximum feeding rates (Pritchard et al. 2017b). The shape of the response curve is also informative. For example, a Type III response is a sigmoidal relationship defined by reduced predation (a prey refuge) at low prey densities and therefore drives a stabilizing predator-prey interaction. In contrast, a Type II response is defined by a hyperbolic increase in the amount of prey consumed with increasing prey density until an asymptote is reached, such that a specialist predator can drive a prey population to extinction (Dick et al. 2013). In comparisons of consumer species and environmental contexts, a higher maximum feeding rate by an introduced consumer has been linked to greater impacts on prey populations in the field (Dick et al. 2013, 2014; Mofu et al. 2019). Thus, functional responses can yield predictive information on the direct and indirect effects of introduced consumers on communities and food webs.

Procambarus virginalis: an emerging invasive species threat

The most popular crayfish in the North American pet trade is the marbled crayfish (*Procambarus virginalis*), also known as ‘marmorkrebs’ (Faulkes 2015; Chucholl and Wendler 2017). The species apparently originated as a novel genotype in the German aquarium trade and gained attention as the only known obligate parthenogenetic crayfish (Scholtz et al. 2003). This reproductive trait is thought to have arisen from a mutation in captive populations of the American Slough crayfish *Procambarus fallax*, making *P. virginalis* a triploid descendant now generally regarded as a distinct species (Scholtz et al. 2003; Vogt et al. 2015, 2019; Lyko 2017) because it is reproductively incompatible with its ancestor; therefore, it has no defined native range. Following numerous pet trade escapes, its invasive range spans three continents: Europe, Asia and Africa (Nonnis Marzano et al. 2009; Chucholl et al. 2012; Ercoli et al. 2019; Grandjean et al. 2021; Scheers et al. 2021). This fast growing, early maturing and highly fecund species (Seitz et al. 2005; Kouba et al. 2021) has not yet established a population in North America, in spite of being commercially available. In recent years, management efforts have been made to prevent introductions in the Great Lakes region; for example, the province of Ontario and the state of Michigan have prohibited possession and sale of *P. virginalis*. However, given its high popularity in the pet trade (Faulkes 2015, 2018), it seems likely that the species will become established in North America.

To date, ecological impacts of *P. virginalis* are scarcely documented (Jones et al. 2009; Faulkes et al. 2012; Lipták et al. 2019). Recent efforts have been made to predict its potential effects in comparison with native species (Chucholl and Chucholl 2021). The parthenogenetic ability of *P. virginalis* contributes to its impact risk, as ecologically disruptive invaders often possess traits that confer an ability to attain high local population densities (Marchetti et al. 2004; Keller et al. 2007; Ricciardi et al. 2013). It can produce a 40% higher mean number of pleopodal eggs than equal-sized red swamp crayfish (*Procambarus clarkii*), a congeneric species that has an extensive invasion history (Vogt 2021). Such a remarkable fecundity and self-cloning ability will increase the risks of introduction and establishment, as hobbyists' aquaria quickly become overrun and a single individual could be sufficient to trigger exponential population growth (Chucholl et al. 2012).

Though efforts have been made to incorporate proxies of abundance into comparative functional response analyses (Dick et al. 2017b; Dickey et al. 2018, 2020), the effect of higher reproductive ability on consumption rates has been largely ignored—despite observed variation of performance across reproductive cycles in introduced species (Little 1976; Gutiérrez-Yurrita and Montes 1999). Crayfish are known to reduce or cease feeding when females are gravid (Little 1976; Linzmaier and Jeschke 2020), and for this reason egg-bearing females are typically omitted from functional response experiments (Linzmaier and Jeschke 2020). However, for a species with largely unconstrained reproductive ability, the exclusion of gravid females can lead to inaccurate risk assessment.

Climate warming is expected to alter trophic interactions and the stability of prey populations in aquatic systems (Iacarella et al. 2015; Daugaard et al. 2019; Mofu et al. 2019; Avlijaš et al. 2022). As a parthenogenetic species, *P. virginalis* is a valuable model organism for studying the effect of reproductive state on resource consumption under different thermal regimes. Here, we conducted experiments in the laboratory to quantify 1) differences in the functional response of gravid and non-gravid female crayfish, and 2) how functional response and gravid state are affected by an increase in temperature projected for the nearshore Great Lakes under climate warming.

Methods

Animal provenance and care

Procambarus virginalis individuals were obtained from a local pet breeder and kept for the duration of the experiment in temperature-controlled chambers. As crayfish are territorial and aggressive (Tricarico and Aquiloni 2016), individuals were kept in their own holding tanks (27.9 × 16.8 × 13.7 cm) supplied with a shelter (~10 cm segment of PVC pipe) and air stones. Each crayfish was fed two Wardley shrimp pellets every two days. Between the months of May to October, the gravid state of each individual was assessed daily. To maintain water quality, 75% water replacement was done at the end of every week (Linzmaier and Jeschke 2020). As crayfish are habitually nocturnal (Larson and Olden 2016), they were kept on a reversal of their usual 12:12 day:night, with lighting provided from 20h00 to 08h00, to ensure peak activity during experiments.

Crayfish were kept at 18°C for two weeks while they acclimated to the laboratory environment. For those individuals that were intended to be used in a high-temperature treatment (26°C, see below), tank temperature was increased by 1°C per day until 26°C was reached, then the crayfish were left to acclimate to this higher temperature for 10 days (Whitledge and Rabeni 2002).

Comparative functional response experiments

Experiments were run at 18°C and 26°C, chosen to represent the current and projected mean maximum nearshore surface water temperatures, respectively, in lakes Erie and Ontario for the period 2070–2100 (Trumpickas et al. 2009, 2015). Non-gravid crayfish were only used if they had neither molted nor been gravid for a minimum of one week prior. Gravid crayfish were tested at any point during egg development until the appendages and eyespots became visible and the egg was no longer easy to delineate (King 1993; Reynolds 2002). Experiments were run two days after their last feeding, to standardize hunger levels in individuals (Grimm et al. 2020; Chicatun 2021), after which non-gravid and gravid crayfish were moved from their everyday tanks to experimental tanks one hour prior to experiments (Chicatun 2021).

Experimental crayfish were randomly offered different densities of bloodworms (*Chironomus* larvae). Bloodworms were kept frozen until needed and then thawed immediately before being introduced to the crayfish tanks. Frozen bloodworms allowed for easily stored, promptly available, large numbers of prey items of a standard size, which are readily consumed by crayfish in feeding trials (Guo et al. 2017; Laverty et al. 2017; Chicatun 2021). Although a reduction in handling time can be expected from the use of non-mobile prey, it does not affect our functional response comparisons across treatments. Experimental densities of bloodworms (n=5, 15, 25, 50, 100, 140, 160, and 180) were selected based on previous work conducted in our lab (Chicatun 2021).

Experiments were run for three hours in the dark, after which the crayfish were removed from the tank and the remaining prey were counted (South et al. 2019; Linzmaier and Jeschke 2020; Madzivanzira et al. 2021). This procedure was replicated eight times for each prey density within each treatment. Trials were completed in a randomised order. Re-used experimental crayfish were given five days of recovery prior to their use in a new trial (Rosewarne et al. 2016; South et al. 2019; Chicatun 2021). Although the same animals were used more than once, re-used individuals were always exposed to different densities.

Statistical Analyses

Statistical analyses were conducted using R (version 4.1.2) and the FRAIR package (Pritchard et al. 2017a, b). For each treatment, we selected a model using three different methods to distinguish the functional response type. We first used Juliano's method (*frair_test* function of the FRAIR package), which tests for the evidence of Type II or Type III responses (Pritchard et al. 2017b). Note that Type I functional response responses are exclusive to filter feeders (Jeschke et al. 2004) and are not relevant to our study. The second method fitted a generalized functional response model using the *frair_fit* function while allowing a scaling exponent (q) to vary; q is equal to 0 in Type II responses and is different from 0 in Type III responses (Pritchard et al. 2017b). The third method involved visual inspection of the proportion of prey consumed as a function of the initial prey density (Alexander et al. 2012). If the weighted regression declines, it is identified as a Type II response; whereas if it is sigmoidal (i.e., characterized by an initial increase, followed by a decline) it is a Type III response (Pritchard et al. 2017b; Grimm et al. 2020).

The selected model was fitted to the data with the *frair_fit* function of the FRAIR package, which uses maximum likelihood estimation (Pritchard et al. 2017b). Type II curves were fitted with Rogers' 'random predator equation' for non-replaced prey, whereas Type III curves were fitted with Hassell's equation (Alexander et al. 2012; Grimm et al. 2020). From these fitted model equations attack rate (a) and handling time (h) were extracted.

Fitted functional responses were then compared using two methods. The first method is the *frair_compare* function, but it can only be used if the curves are of the same model type. The second method relies on bootstrapping the fits using *frair_boot* to generate 95% confidence intervals (CI) of each functional response (n = 999) and associated parameters such as attack rate and handling time (Iacarella et al. 2015; South et al. 2019; Grimm et al. 2020; Linzmaier and Jeschke 2020). These 95% CI of the individual functional responses can then be used to visually assess graphed functional responses. The degree to which the CIs overlap determines statistical difference (South et al. 2019; Grimm et al. 2020; Linzmaier and Jeschke 2020). With all functional responses graphed, we compared how crayfish responses change with temperature or gravid state.

To measure differences in total days spent gravid and total clutch number, a generalized linear model was tested on data collected during daily care assessments. Days spent gravid included the number of days in which crayfish were brooding eggs or young, out of a possible 184 days. Total clutch number was defined as the number of clutches attempted within the recorded time frame. In the generalized linear model, total days spent gravid and total clutch number were the response variables and temperature was the explanatory variable. Days spent gravid per clutch, defined as the number of consecutive days a crayfish spent brooding eggs or young, was modelled with a generalized linear mixed model using the nlme package (Bates et al. 2015; Pinheiro et al. 2022). Crayfish could have multiple clutches within the given time frame, so we modelled days spent gravid per clutch in relation to a fixed effect of temperature and crayfish ID as a random effect (Zuur et al. 2009).

Results

It is clear from both the results of *frair_compare* and the bootstrapped 95% confidence intervals that gravid individuals consumed substantially less prey than non-gravid individuals, regardless of temperature (Figure 1, Table 1). Our *frair_compare* results indicate that, when gravid, *P. virginalis* attack rates decrease and their handling times increase (Tables 1 & 2). The maximum feeding rate of gravid versus nongravid females was reduced by 93% at 18°C and by 85% at 26°C (Figure 1). Response type did not change with reproductive state or temperature treatment. All curves fitted in this experiment were found to be Type II responses (Figure 1).

The total number of days, from May to October, that individuals spent in the gravid state did not differ with temperature (Table 3; $p>0.05$). However, individuals at 26°C spent significantly less time gravid per clutch and had significantly more clutches than individuals at 18°C (Table 3; $p<0.05$).

Although the higher temperature treatment in our study is close to the temperature at which the growth rate of *P. virginalis* in the lab is maximal (25°C; Seitz et al. 2005), and functional responses are hypothesized to be higher at temperatures approaching the thermal growth optimum of the consumer (Iacarella et al. 2015), maximum feeding rates of non-gravid individuals were reduced at 26°C compared with 18°C (Figure 1).

Discussion

Effects of reproductive state on feeding behaviour

Our results revealed the extent to which gravidness affects the functional response of *P. virginalis* at different temperatures. Reduced feeding during gravid stages has been shown for multiple crayfish species to occur shortly after the female has deposited her eggs and it continues through a feedback loop of brooding pheromones until the larvae disperse (Little 1976; Reynolds 2002; Mathews 2011). During this time, female metabolism slows down (Gutiérrez-Yurrita and Montes 1999; Aquiloni and Gherardi 2008). Significant differences in diet choices were found between juvenile and adult red swamp crayfish *Procambarus clarkii* (Correia and Anastácio 2007; Carreira et al. 2017), and reduced feeding behaviour during reproductive cycles was observed in *P. clarkii* and several *Faxonius* species (Little 1976; Mathews 2011). However, to our knowledge, differences in crayfish feeding during critical life history stages have not been previously examined in a functional response experiment.

Though the maximum feeding rates of gravid individuals in our experiment were significantly higher than zero, functional response experiments to date have tested only male or non-gravid female crayfish, based on the assumption that feeding during the gravid state is insignificant (Linzmaier and Jeschke 2020; Chucholl and Chucholl 2021). Given the capacity for every individual within a population of *P. virginalis* to become gravid at some point in their lifespan, changes to the functional response induced by this state should be quantified and explicitly considered in impact metrics (Dick et al. 2017b; Dickey et al. 2020). Where such information is lacking, per capita effects—and ultimately field impacts—could be underestimated where population densities are high. For example, based on the observed differences in the maximum feeding rates of gravid versus nongravid females in our experiment, we estimate that the feeding rate of a non-gravid individual is matched by 5.5 gravid individuals at 18°C and 13.9 gravid individuals at 26°C (Tables 1 & 2).

Effects of temperature

We speculate that for *P. virginalis* and other species whose invasion risk has been linked to parthenogenesis there exists a trade-off between two key traits of invasion success: reproductive ability and the exploitation of resources necessary to sustain an incipient population (Parker et al. 1999; Ricciardi et al. 2013; Dick et al. 2017b). A similar balance exists between somatic growth and resource consumption, as feeding is also reduced by molting during ecdysis (Reynolds 2002). At least for *P. virginalis* in the laboratory, the relationship between reproduction and consumption seems unaffected by temperature. Contrary to studies showing that warming strongly affects consumer feeding rates and can shift functional responses to destabilizing Type II relationships (Iacarella et al. 2015; Daugaard et al. 2019; Mofu et al. 2019; Avlijaš et al. 2022), maximum feeding rates and response type of gravid crayfish did not differ between 18°C and 26°C (Table 2; Figure 1). From May to October, individuals spent the same total number of days in a gravid state at higher temperatures (Table 3). This was unexpected, as warmer temperatures typically provoke higher energetic demands (Croll and Watts 2004; Carreira et al. 2017) and thus reproductive output at sub-optimal high temperatures should be lower. There is an accelerating effect of temperature on hatch time whereby individuals at 26°C spent less time gravid per clutch (Table 3), but this may also be linked to a lower larval survival rate (King 1993; Aydın and Dilek 2004). The number of clutches, however, also increases with temperature. Individuals had more clutches at 26°C than at 18°C (Table 3), which could balance against the mortality caused by shorter hatch times; temperature might then cause no overall reduction of fecundity.

It is worth noting that our experiments tested a cultivated population kept at both 18°C and 26°C for longer periods than they would experience with seasonal variation in the wild. For this reason, the frequency of reproductive events in our population could differ from wild populations, which have thus far been observed to spawn 1–3 times per year (Ercoli et al. 2019; Vogt et al. 2019; Vogt 2021).

Conclusions

These findings, and those of some recent studies (Dalal et al. 2021; Haubrock et al. 2020), underscore the value of comparing per capita effects across phenological and ontogenetic stages of invasive animals to better inform risk assessment. In a study comparing functional responses of female *Gammarus pulex* amphipods that were either non-ovigerous or had embryonic broods, Dalal et al (2020) found that females carrying immature-stage embryonic broods had significantly higher attack rates than those with mature-stage embryonic broods. Thus, functional response experiments that account for changes in the reproductive cycle of a consumer can reveal more nuanced information concerning trophic interactions.

Our observations show that temperature contexts affect not only the consumption rate of *P. virginalis* but also their fecundity (Figure 1; Table 2 & 3). Quantifying these responses under different environmental conditions allows us to better characterize variation in per capita effects as well as the potential impact of an established population as a whole (Iacarella et al. 2015; Dickey et al. 2020). In the present study, we tested aquarium-reared individuals of *P. virginalis*, because the species is most likely to invade the Great Lakes region and other water bodies through pet release. We recommend that similar tests be done on self-sustaining *P. virginalis* populations in the wild. Ultimately, knowledge of climatic and other environmental influences on crayfish reproductive phenology and behaviour could be applied to invasive species management (see Gutiérrez-Yurrita and Montes 1999).

Tables

Table 1.1. Fitted coefficients for each gravid and non-gravid functional responses at both temperatures.

Treatment	Type	First-order		*a*	*h*	1/*h*
Gravid 18ºC	II	-0.0098	***	0.28 ***	0.14 ***	7.19
Normal 18ºC	II	-0.0082	***	0.89 ***	0.010 ***	100
Gravid 26ºC	II	-0.012	***	0.32 ***	0.13 ***	7.81
Normal 26ºC	II	-0.017	***	1.50 ***	0.023 ***	43

Asterisks denote significant p values (*** < 0.001)

Table 1.2. Results from *frair_compare* difference tests of the attack rates (*a*) and handling time (*h*) between the fitted gravid and non-gravid functional responses at both temperatures.

Fit 1	Fit 2	Parameter	Estimate	Std. Error	p-value	
Gravid 18ºC	Gravid 26ºC	Δa	-0.04	0.055	0.46	
		Δh	0.01	0.011	0.28	
Normal 18ºC	Gravid 18ºC	Δa	0.61	0.059	< 0.001	***
		Δh	-0.13	0.0085	< 0.001	***
Normal 26ºC	Gravid 26ºC	Δa	1.19	0.01	< 0.001	***
		Δh	-0.1	0.0068	< 0.001	***
Normal 18ºC	Normal 26ºC	Δa	-0.62	0.10	< 0.001	***
		Δh	-0.013	0.001	< 0.001	***

Asterisks denote significant p values (*** < 0.001)

Table 1.3. Results from GLMs and GLMM on gravid *P. virginalis* at both 18ºC and 26ºC.

Measurement	Mean Gravid 18ºC	Mean Gravid 26ºC	p-value
Total days out of 184	47.21	54.27	0.47
Number of clutches	1.07	2.09	0.0008 **
Days gravid per clutch	49.84	26.9	<0.0001 ***

Asterisks denote significant p values (*** < 0.001; ** < 0.01)

Figures

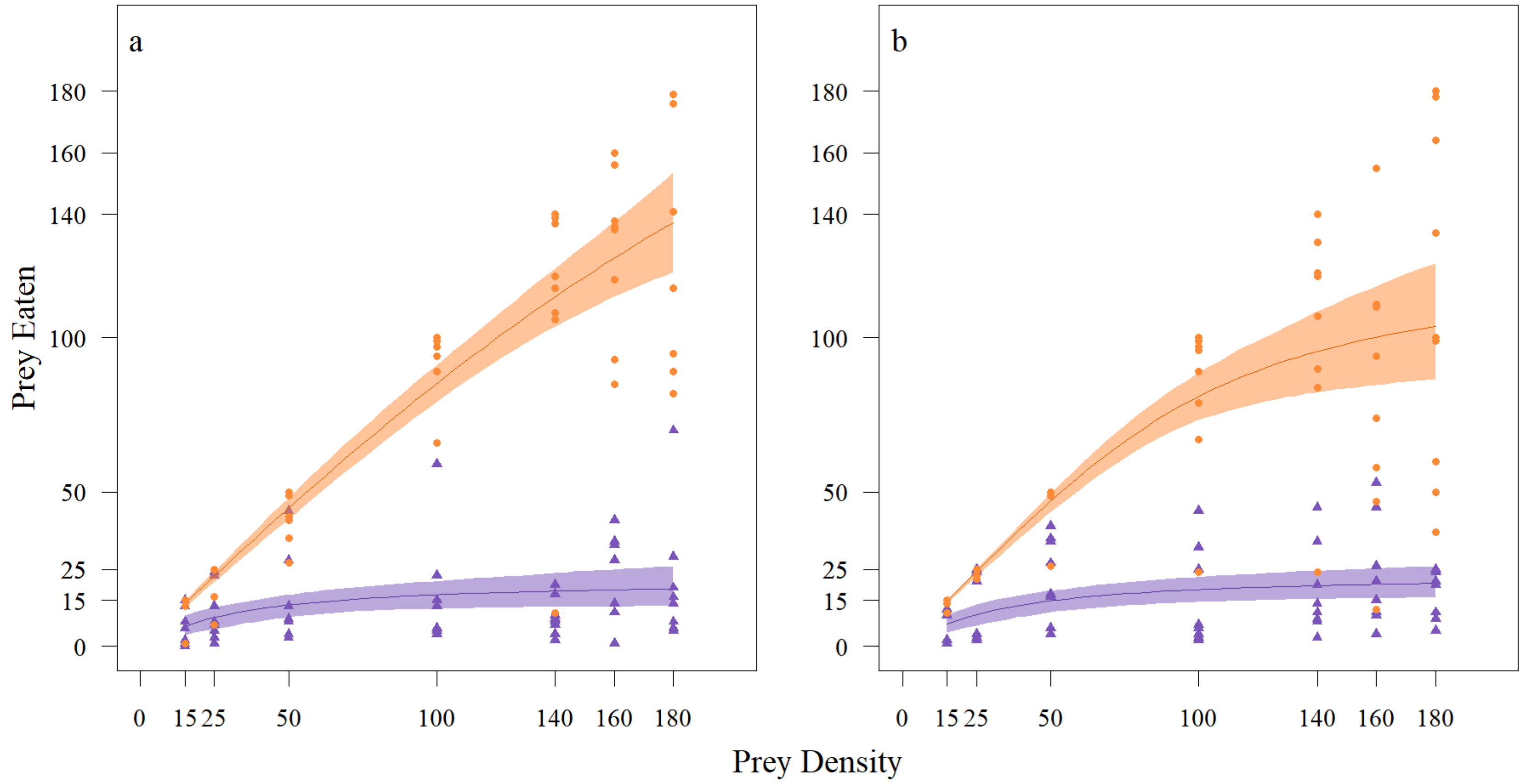


Figure 1.1. Functional responses with bootstrapped 95% confidence intervals (shaded regions) for gravid (purple triangles) and non-gravid (orange circles) *P. virginalis* at 18ºC (a) and 26ºC (b). Lines represent the best fit model for each population (Type II).

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Linking statement

Biotic and abiotic context dependencies influence the per capita effects and field impacts of introduced species and are thus a major challenge for risk assessment (Ricciardi et al. 2013). Notably, climate change can exacerbate the impacts of warm-adapted non-native species by shifting thermal conditions toward their physiological optima (Environmental Matching Hypothesis; Ricciardi et al. 2013; Iacarella et al. 2015). The role of the physical environment in contributing to variation in per capita effects can be tested by incorporating specific abiotic context dependencies into experimental designs; hence the inclusion of contrasting temperatures in the studies described in the previous and forthcoming chapters.

The previous chapter studied the influence of a key life history trait on the per capita effect of an invasive crayfish. I found that the functional response of *P. virginalis* is greatly influenced by its reproductive status, which provided a more nuanced view of the presumed advantage of frequent parthenogenetic reproduction. Overall, I showed how controlled tests of behaviour and phenological characteristics could contribute to a more comprehensive understanding of invasive species performance.

In the next chapter, I describe a more detailed study that compares the feeding behaviour of *P. virginalis* with a congeneric invasive species, in which both species are presented with varying amounts of different prey types. For this study, I adapted a method to quantify and compare consumption of plants as well as resource-switching between plant and animal material.

Chapter 2 – Climate warming brings added complexity to the trophic impacts of invasive alien crayfishes

Abstract

The red swamp crayfish (*Procambarus clarkii*) and the marbled crayfish (*Procambarus virginalis*) are congeneric invasive species that could have impacts on biodiversity and food webs if they become established in the Great Lakes. In this experiment, we tested the functional responses of these omnivores to bloodworms, macrophytes, and mixed (resource-switching) diets, at two different temperatures, to gain predictive information on their trophic impacts under present (18°C) and future (26°C) climate scenarios. During bloodworm- and macrophyte-only experiments, red swamp crayfish exhibited higher rates of bloodworm and macrophyte consumption than marbled crayfish at either temperature. During resource-switching experiments, marbled crayfish exhibited higher consumption of bloodworms and macrophytes than red swamp crayfish. Overall, these results suggest that the per capita trophic effects of these species are dependent on ambient temperature and resource availability.

Introduction

Invasive omnivores such as crayfish can alter communities and food webs through their consumptive effects on the population size, biomass, and taxonomic composition of benthic invertebrates, macrophytes, periphyton, and detritus (Correia 2002; Carreira et al. 2014; Jackson et al. 2017). A key ecological trait associated with the most disruptive invasive species is their high resource consumption efficiency (Dick et al. 2013). Species that can rapidly acquire, transform or consume resources can attain high local abundance and outcompete resident species for shared resources (Morrison and Hay 2011; Ricciardi et al. 2013). A high rate of resource consumption can alter trophic linkages and thus can have ramifying effects on communities (Simon and Townsend 2003).

Resource consumption and other per capita effects of invading species vary with local biotic and abiotic conditions (Ricciardi et al. 2013; Dalal et al. 2021; Dickey et al. 2021). As environmental conditions approach their physiological optima, invaders are predicted to have greater impacts on communities and food webs (Environmental Matching Hypothesis; Ricciardi et al. 2013; Iacarella et al. 2015). In aquatic environments in particular, temperature differences across time and space can cause context-dependent variation in the colonization success and impact of an invader (Iacarella et al. 2015; Madzivanzira et al. 2021). Climate warming is thus altering invasion risk, most notably by reducing temperature constraints for warm adapted non-native species, allowing them to establish, exploit resources more efficiently, and compete better with native species (Rahel and Olden 2008; Hulme 2017).

Crayfishes (Procambarus spp.) as invasive consumers

Crayfishes are keystone consumers in aquatic environments (Gherardi 2006). As opportunistic omnivores, the impacts of their feeding activities are wide ranging and can transmit across trophic levels (Olsen et al. 1991). They can adapt quickly to novel prey (Tricarico and Aquiloni 2016) and reduce invertebrate, amphibian, and fish populations in their invasive ranges (Dorn and Wojdak 2004; Tricarico and Aquiloni 2016).

As a functional feeding group, crayfishes are important "shredders" (sensu Cummins et al. 1989)—feeding on coarse particulate organic matter and expediting its microbial breakdown (Correia 2002; Alcorlo et al. 2004; Lipták et al. 2019; Linzmaier et al. 2020). Thus, they can have direct impacts on the composition and abundance of aquatic macrophytes (Smart et al. 2002; Carreira et al. 2014). For example, *P. clarkii* has been known to cause the disappearance of native floating-leaved and submerged plants in Lake Naivasha (Smart et al. 2002). This greater consumption of macrophytes causes changes in stable states, with clear macrophyte-dominant lakes becoming turbid (Rodríguez et al. 2003). The reduction in macrophyte biomass can have cascading negative effects on amphibians, other invertebrates, and herbivorous waterfowl (Rodríguez et al. 2005).

The ability of crayfish to switch between animal prey and plant resources could generate potentially contrasting *per capita* effects on different levels of the food web. This density-dependent switch has a stabilizing effect on the food web as low prey populations are relieved from predation (Lalonde et al. 1999). In the case of invasive omnivores, however, vegetation could sustain them while prey numbers are low, maintaining predation pressure and leading to enhanced invasiveness (Eubanks 2005).

Prey-switching in comparative functional responses

Feeding efficiency of potential invaders under different environmental contexts can be quantified and compared using their functional response—the rate of resource consumption in relation to resource availability (Landi et al. 2022). Two major parameters are attack rate (*a*) and handling time (h), from which the maximum feeding rate ($1/h$) can be derived and compared among consumer species as an indicator of voracity. Combinations of these parameters can reveal consumer tendencies that have predictive value (Cuthbert et al. 2019). Furthermore, the fitted model corresponds to one of a set of generalized response types that indicate the potential per capita effects of a consumer on a prey population or food resource. Type II (hyperbolic) response is deemed to be destabilizing to prey populations, where feeding pressure remains high even when prey densities are low. A Type III (sigmoidal) response is stabilizing, reflecting a refugium against predation at low prey densities (Dick et al. 2013). Comparative functional responses have been shown to predict trophic impacts of invaders in the field (Dick et al. 2013).

Functional response experiments of generalist predators have long implied that it is best to model prey-switching through Type III curves (Real 1977; Murdoch 1969; Van Baalen et al. 2001; Tschanz et al. 2007). However, functional response experiments usually only measure this effect as it pertains to two different prey items (Cuthbert et al. 2018; McCard et al. 2021). Experiments that measure switching between animal prey and plants only measure the consumption of one as it pertains to the presence or absence of the other (Médoc et al. 2018).

Recognizing that crayfish are good model organisms for studying the impacts of invasive omnivores on freshwater ecosystems (Granados et al. 2019), we conducted a series of feeding experiments that measured and compared the *per capita* effects of crayfish on macrophytes and bloodworms.

Methods

Animal and macrophyte collection and care

P. virginalis and *P. clarkii* populations were sourced from local pet breeders, with circa 24 and 30 adult individuals acquired for *P. clarkii* and *P. virginalis*, respectively. Thus, the individuals were not adapted to wild conditions and were not sized-matched, owing to stock availabilities (Appendix 1). However, as these species would likely be introduced into the Great Lakes region through pet release (Chucholl and Wendler 2017), the sizes used here are representative of potentially released individuals.

For the duration of the experiment, crayfish were held in a climate-controlled phytotron chamber. As crayfish are habitually nocturnal (Larson and Olden 2016), they were also kept on a reversal of their usual 12:12 day:night cycle, with light provided during the night from 20:00 to 08:00, to ensure peak activity during experiments. As they are territorial and aggressive (Tricarico and Aquiloni 2016), individuals were kept in separate holding tanks and provided with a shelter (a PVC cylinder) to reduce stress. Tanks were aerated using aquarium pumps air stones. Each crayfish was fed two Wardley shrimp pellets every two days. To maintain water quality, 75% water changes were done once a week (Linzmaier and Jeschke 2020). Crayfish were initially kept at 18°C for two weeks while they acclimated to the laboratory environment (Whitledge and Rabeni 2002). For the warm temperature treatment, temperature was increased by 1°C per day until it reached 26ºC, then crayfish were left to acclimate to this new temperature for 1 month and kept at this temperature for the entirety of the experiment.

Macrophytes used in these experiments were Eurasian watermilfoil (*Myriophyllum spicatum*) collected from an established non-native population in a Quebec lake (Lac Hertel, Mont St-Hilaire QC; 45°32'39.8"N 73°08'47.8"W) and from an artificial boating canal (Parc

Jean-Drapeau, Montreal; 45°30'28.3"N 73°31'36.1"W). After being brought into the lab, the macrophytes were stripped of their leaves, blotted dry, cut into individual segments of 5cm, and frozen for future use; a pilot study indicated that crayfish readily fed upon thawed macrophyte segments.

Comparative functional response experiments

Functional response experiments were conducted at two different temperatures: 18°C, which is within the range of current mean maximum nearshore surface water temperatures in the lower Great Lakes, and 26°C, within the range of mean maximum surface water temperatures projected for lakes Erie and Ontario in the coming decades (Trumpickas et al. 2009, 2015).

Crayfish were randomly chosen out of those that had neither molted nor were gravid for a minimum of 1 week prior, owing to reduced feeding rates of gravid females (Chapter 1) or of individuals during ecdysis (Linzmaier and Jeschke 2020). Crayfish were size-matched between temperatures, but not between trials (Appendix 1). Experiments were run 2 days after the crayfish were last fed, to standardize hunger levels in individuals (Grimm et al. 2020; Chicatun 2021). The chosen crayfish were moved from their holding tanks to experimental tanks 1h prior to experiments (Grimm et al. 2020).

In the first set of trials testing crayfish consumption of animal prey, crayfish were offered varying densities of bloodworms, i.e. *Chironomus* sp. (Chironomidae) larvae. Bloodworms were stored in a freezer and were thawed prior to being introduced to experimental tanks. Using previously frozen bloodworms allows for large numbers of promptly available prey items that are readily consumed by crayfish (Carreira et al. 2017; Guo et al. 2017; Laverty et al. 2017). Densities of 5, 15, 25, 50, 100, 140, 160, 180 and 300 bloodworms were selected for use in these trials, based on pilot studies and previous work (Chicatun 2021). The maximum density (300) was provided only to *P. clarkii* as our pilot studies showed that they would consume significantly more than *P. virginalis*. Experimental crayfish were allowed to feed for 3 hours in the dark, after which, they were removed from the tank and the remaining prey were counted (South et al. 2019; Linzmaier and Jeschke 2020; Madzivanzira et al. 2021). This procedure was replicated 8 times for each prey density. Trials were completed in a randomised order; animals were used more than once, but always at different densities. Re-used experimental crayfish were also given five days of recovery prior to their use in a new trial (Rosewarne et al. 2016; South et al. 2019; Chicatun 2021).

During our second set of trials crayfish were only given macrophytes. These macrophyte-only experiments were run identically to bloodworm-only experiments with respect to design, temperatures, and replicate numbers. Crayfish were provided with defrosted macrophyte segments of varying densities (1, 2, 3, 4, 5, 6, 8, and 10) as a food resource. Macrophyte stalks were defrosted, blotted dry and weighed, prior to being introduced to the experimental tanks. Crayfish were left to consume the macrophyte segments for 3hrs; after which all remaining fragments were removed, measured, blotted dry and weighed again.

Following these trials, resource-switching experiments were conducted. Using the same protocols as above, crayfish were offered the same varying densities of macrophyte segments or bloodworms as a focal resource type, as well as a consistent amount of the alternative resource type (either 140 bloodworms or 5 macrophyte segments). These alternative densities were chosen based on the results of pilot studies. Remaining food material was collected and counted or blotted dry and weighed. *P. virginalis* were not tested at 26°C, owing to their high mortality at this temperature.

Statistical analysis of functional responses

Statistical analyses were conducted using R (version 4.1.2) and the FRAIR package (Pritchard et al. 2017*a, b*). Analysis followed the same steps elaborated in Chapter 1: model selection, fitting, and comparison. At first, we used Juliano's method (*frair_test*), fitted a generalized model, and visually inspected the proportion of prey eaten. These tests yielded contradictory results, so Juliano's method was applied as the primary test, following previous studies that similarly favoured it (e.g., Guo et al. 2017; Dalal et al. 2021; Chucholl and Chucholl 2021). When Juliano's method failed for *P. clarkii* at 26°C during macrophyte-focused resource-switching trials, we fitted a generalized model to determine the most appropriate curve type. Data were then fit with a model of the selected curve type for each treatment using *frair_fit* to determine attack rate (*a*) and handling time (*h*), and then compared to one another both by 1) *frair_compare* and 2) bootstrapping the fits using *frair_boot* to generate 95% confidence intervals (CI) of each functional response.

Analysis of macrophyte feeding propensity

Several macrophyte feeding trials resulted in no consumption of macrophyte segments (zero count) and these were dropped from our analyses to avoid an underestimation of the maximum feeding rate. A logit model was used to compare the binomial measure of untouched macrophytes to consumed macrophytes. For macrophyte-only experiments where we had data for all 4 treatments (*P. virginalis* and *P. clarkii* at both temperatures), consumption was modelled in relation to fixed effects of initial macrophyte density, species, temperature, and the interaction between these last two treatments, as well as the random effect of crayfish individual to compensate for the difference between individual crayfish. For macrophyte-focused resource-switching trials, in which we had 3 treatments (due to high *P. virginalis* mortality at 26°C during this set of trials), consumption was modelled in relation to initial macrophyte density, species and temperature as fixed effects and crayfish individual as a random effect.

Functional Response Ratio

From functional response parameters, we calculated the Functional Response Ratio (FRR)—a metric that was developed to compare the per capita effects of consumers and has been shown to distinguish invasive from native species (Cuthbert et al. 2019). It is calculated as follows:

$$FRR = \frac{a}{h}$$

wherein *a* is the attack rate of the consumer, and *h* is the handling time. FRR allows for better impact predictions as it balances the information given by both parameters in situations where they may be contradictory (Cuthbert et al. 2019).

Results

All curves fitted from the bloodworm-only and macrophyte-only trials were Type II responses, clearly indicated by the negative first order term (Figures 2.1 & 2.2; Tables 2.1 & 2.3). During macrophyte-focused resource-switching trials at 26°C, *P. clarkii* exhibited a Type III response. All other treatments in resource-switching trials elicited Type II responses (Figures 2.3 & 2.4; Table 2.5).

Bloodworm-only functional response results

Our results suggest that *P. clarkii* could outcompete *P. virginalis* for bloodworms (Figure 2.1). At 18°C, *P. clarkii* had higher attack rates and lower handling times than *P. virginalis*. At

26°C, despite having lower attack rates, *P. clarkii* had lower handling times than *P. virginalis* resulting in a higher maximum feeding rate (Tables 2.1 & 2.2).

Elevated temperature had a greater effect on *P. virginalis,* which had both higher handling times and higher attack rates at 26°C than at 18°C. In contrast, *P. clarkii* had no difference in handling times at both temperatures; only its attack rates were higher at 18°C (Tables 2.1 & 2.2).

The FRR indicated that *P. clarkii* would likely have a higher impact on bloodworms at both temperatures than *P. virginalis*. Based on this metric, the strongest trophic impacts would be exerted by *P. clarkii* at 18°C (FRR = 127.58), followed by *P. clarkii* at 26°C (FRR = 94.67), *P. virginalis* at 26°C (FRR = 60.48), and *P. virginalis* at 18°C (FRR = 36.70: Table 2.1).

Macrophyte-only functional response

Attack rates did not differ between species at either temperature. However, *P. clarkii* consumed macrophytes at a greater rate than *P. virginalis,* owing to lower handling times at both temperatures (Tables 2.3 & 2.4).

Unlike in bloodworm-only trials, temperature had no obvious effect on *P. virginalis* attack rates or handling times (Tables 2.3 & 2.4). The results of our logit model, however, showed that *P. virginalis* was more likely to consume macrophytes at 26°C than at 18°C (p = 0.016). The interaction term between species and temperature was also significant (p = 0.016), indicating that the temperature effect differed between species. Propensity to consume macrophytes by *P. clarkii* was the same at both temperatures (p = 0.28). There was no difference in handling times between the two temperatures, but at 18°C *P. clarkii* had higher attack rates than at 26°C (Tables 2.3 & 2.4).

The FRR comparison suggested that the greatest consumptive impacts on macrophytes would be from *P. clarkii* at 18°C (FRR = 10.87), followed by *P. clarkii* at 26°C (FRR = 3.90), and then *P. virginalis* at either temperature (FRR = 2.71; Table 2.3).

Resource-switching functional response

The two species had differing responses to bloodworms in the presence and absence of macrophytes. At 18°C, *P. virginalis* exhibited higher attack rates and lower handling times than *P. clarkii*, whereas *P. clarkii* consumed more bloodworms at 26°C than at 18°C, with higher attack rates and lower handling times (Tables 2.5 & 2.6). Thus, our bloodworm-focused resource-switching trials suggested that *P. virginalis* at 18°C will have the strongest consumptive impact on bloodworms (FRR = 121.20), followed by *P. clarkii* at 26°C (FRR = 77.22) and at 18°C (FRR = 15.53; Table 2.5).

In macrophyte-focused resource-switching trials, *P.clarkii* exhibited a Type III response at 26°C, making it impossible to perform a *frair_compare* comparison with 18°C (Table 2.7). A visual interpretation shows that maximum feeding rates, and thus handling times, overlap (Figure 2.4). Our logit model showed *P. clarkii* was just as likely to consume macrophytes at 26°C as at 18°C (p = 0.85). The comparison of *P. virginalis* and *P. clarkii* at 18°C found no difference between attack rates, but *P. clarkii* handling times were higher (Table 2.6). The logit model suggested that at 18°C *P. virginalis* would consume macrophytes just as often as *P. clarkii* (p = 0.098), and initial macrophyte density had no effect on whether macrophytes were consumed (p = 0.077). As FRRs can only be calculated for Type II curves, we do not have a value for *P. clarkii* in macrophyte-focused resource-switching trials. FRRs, then, were similar between *P. virginalis* and *P. clarkii* at 18°C, with *P. virginalis* having a slightly higher FRR (3.29) than *P. clarkii* (2.82; Table 2.5).

When comparing resource-switching trials to those without alternative resources, attack rates were higher when a resource was given alone without an alternative, for both species and temperatures. Resource-switching curves had lower handling times, except at 18°C for *P. clarkii*, which had higher handling times in trials with only one resource (Table 2.6).

Discussion

Effect of temperature on consumption

Assuming the published temperature ranges for *Procambarus* species can predict greater impacts on resources (Ricciardi et al. 2013; Iacarella et al. 2015), consumption rates of both species should have been highest at the 26°C treatment; but this was not the case for either species. During both bloodworm- and macrophyte-only trials, maximum feeding rates of *P. clarkii* did not differ between temperatures. During bloodworm-only trials, *P. virginalis* appeared to have a higher maximum feeding rate at 18°C than at 26°C (Tables 2.1 & 2.2). In macrophyte-only trials there was no difference in functional responses between temperatures, though *P. virginalis* showed a propensity to feed on macrophytes more often at 26°C.

These feeding results appear to contradict the Environmental Matching Hypothesis, as neither temperature corresponds with a maximized feeding rate for *P. clarkii* and only attack rates followed expected trends. Also observed by other authors, functional responses can vary across conspecific populations, but attack rates responded more strongly to temperature than maximum feeding rates (Chicatun 2021). This is consistent with Englund et al. (2011) as attack rates are more strongly linked to temperature than maximum feeding rates. A stronger relationship is observed in crustaceans whose handling times tend to be negatively affected by temperature (Uiterwaal and DeLong 2020); reported optimal temperature ranges of species may not be useful to predict maximum feeding rates of crayfish functional response outcomes.

In a similar study by Carreira et al. (2017) studying the effect of resource type on the performance of *P. clarkii* during simulated heat waves, they found that animal prey diets reduced survival and growth rates of crayfish under higher temperatures while crayfish increased macrophyte consumption to meet higher energetic demands under warmer temperatures. They found a positive effect of a mixed or macrophyte-only diet on the survival of adults. This suggested resource switching feeding behaviour could be strongly linked to temperature. In this study we observed a similar temperature response in *P. virginalis* which reduced consumption of bloodworms at 26ºC; however, *P. clarkii* did not show this pattern

Our population of *P. clarkii* sustained their consumption rate of both bloodworms and macrophytes regardless of temperature. This result cannot be explained by optimal temperature growth ranges, nor by differences in resource preference from energetic demands of high temperatures. Acute increases in temperature typically provoke increases in metabolism and energetic demands (Hochachka and Somero 2014), but metabolic performance is also mediated by acclimation time. Nyboer and Chapman (2017) found that a longer acclimation period (of 3 weeks) reduces standard metabolic rate and maximum metabolic rate, while increasing critical thermal maxima in tropical fish. This phenomenon has not yet been studied in crayfish, but as we used a long pre-trial acclimation period compared to Carreira et al. (2017) this may have altered our differential response between 18ºC and 26ºC.

Effect of resource availability on consumption

In resource-switching trials, not all *P. clarkii* and *P. virginalis* treatments had a Type III functional response (Table 2.5). When considering a model selection method to determine this, we were guided by other authors who adopted Juliano’s method as being the most appropriate (Guo et al. 2017; Chucholl and Chucholl 2021; Dalal et al. 2021). Despite issues delineating between curve types for *P. clarkii* at 26ºC, we found all other resource-switching responses to be Type II. We note that a different conclusion would have arisen had we depended upon a different selection method. We will leave it to other researchers to decide whether model selection should be made by comparing test methods or based on one particular test deemed to be most robust or informative.

The Type II responses were unexpected, given that Type III responses typify resource switching (Real 1977; Murdoch 1969; Van Baalen et al. 2001; Tschanz et al. 2007). For macrophyte-focused trials, this may be explained by the near complete consumption of the alternate density of bloodworms, reducing the confounding effect this has on crayfish consumption of macrophytes across densities. For bloodworm-focused trials, it could be due to a strong preference of *P. clarkii* and *P. virginalis* for bloodworms even at small prey densities. According to other studies, *P. clarkii* readily consume animal prey species, but are more selective concerning macrophytes (Gherardi and Barbaresi 2007). Though the crayfish readily consume Eurasian watermilfoil (*Myriophyllum spicatum*) they might not prefer it over bloodworms.

Our results highlight the complexity that resource-switching adds to functional responses and trophic impact predictions. For *P. clarkii* at 26ºC and *P. virginalis*, consumption rates were significantly higher during resource-switching trials, perhaps due to the size difference of individuals between trials. For *P. clarkii* at 18ºC, however, we found that maximum feeding rates of bloodworms and macrophytes during resource-switching trials were lower, despite their larger size. The inclusion of an alternate resource could significantly reduce maximum feeding rate of the focal resource for *P. clarkii*, indicating perhaps a greater omnivorous ability. This effect must be considered when deriving impact predictions from functional response experiments, as the inclusion of alternate resources may change which species has a higher maximum feeding rate or FRR.

Avenues for future work

Omnivory can complicate interpretation of functional response experiments. Plant-based functional responses should be considered when designing experiments intended to predict the trophic impacts of omnivorous predators. Our results add evidence that resource preference could influence the outcomes of relative impact predictions between invasive species, especially as climate warms. The effect of temperature acclimation time on metabolic performance, and how this may impact resource preference and impacts, requires further investigation. Though heat waves may have strong impacts on food preferences, temperature adaptation may lead to contradictory results. Interpretations of feeding experiments for risk assessment must give consideration of how temperature exposure and acclimation affect resource switching, particularly for emerging invasion threats like *P. virginalis*, whose feeding preferences and functional responses remain understudied.

Tables

Table 2.1. Fitted coefficients and curve types for each *P. virginalis* and *P. clarkii* functional responses at both temperatures for bloodworms.

Fit	Type	First-order		*a*		*h*		1/*h*	*a*/*h*
P. virginalis 18ºC	II	-0.011	***	0.73	***	0.020	***	50	36.70
P. virginalis 26ºC	II	-0.017	***	1.51	***	0.025	***	40	60.48
P. clarkii 18ºC	II	-0.012	***	1.53	***	0.012	***	83.33	127.58
P. clarkii 26ºC	II	-0.0073	***	1.14	***	0.012	***	83.33	94.67

Asterisks denote significant p-values (*** < 0.001)

Table 2.2. Results from *frair_compare*, a difference test of the attack rates (*a*) and handling time (*h*) between the fitted *P. virginalis* and *P. clarkii* functional responses at both temperatures for bloodworms.

Fit 1	Fit 2	Parameter	Estimate	Std. Error	p-value	
P. virginalis 18ºC	*P. virginalis* 26ºC	Δ*a*	-0.78	0.10	< 0.001	***
		Δ*h*	-0.0052	0.0011	< 0.001	***
P. clarkii 18ºC	*P. clarkii* 26ºC	Δ*a*	0.39	0.074	< 0.001	***
		Δ*h*	0.00033	0.00053	0.54	
P. virginalis 18ºC	*P. clarkii* 18ºC	Δ*a*	-0.80	0.068	< 0.001	***
		Δ*h*	0.0075	0.00096	< 0.001	***
P. virginalis 26ºC	*P. clarkii* 26ºC	Δ*a*	0.38	0.11	0.00041	***
		Δ*h*	0.013	0.00078	< 0.001	***

Asterisks denote significant p-values (*** < 0.001)

Table 2.3. Fitted coefficients and curve types for each *P. virginalis* and *P. clarkii* functional responses at both temperatures for macrophytes.

Fit	Type	First-order		*a*		*h*		1/*h*	*a*/*h*
P. virginalis 18ºC	II	-0.043	***	0.87	**.**	0.32	***	3.13	2.71
P. virginalis 26ºC	II	-0.035	***	0.62	***	0.23	***	4.41	2.71
P. clarkii 18ºC	II	-0.070	***	1.56	***	0.14	***	6.99	10.87
P. clarkii 26ºC	II	-0.041	***	0.61	***	0.16	***	6.37	3.90

Asterisks denote significant p-values (*** < 0.001; **.** < 0.1)

Table 2.4. Results from *frair_compare*, a difference test of the attack rates (*a*) and handling time (*h*) between the fitted *P. virginalis* and *P. clarkii* functional responses at both temperatures for macrophytes.

Fit 1	Fit 2	Parameter	Estimate	Std. Error	p-value	
P. virginalis 18ºC	*P. virginalis* 26ºC	Δa	0.19	0.42	0.66	
		Δh	0.088	0.047	0.059	**.**
P. clarkii 18ºC	*P. clarkii* 26ºC	Δa	0.94	0.27	0.00054	***
		Δh	-0.014	0.020	0.50	
P. virginalis 18ºC	*P. clarkii* 18ºC	Δa	-0.69	0.52	0.19	
		Δh	0.18	0.042	2e-05	***
P. virginalis 26ºC	*P. clarkii* 26ºC	Δa	0.0016	0.19	0.99	
		Δh	0.070	0.030	0.022	*

Asterisks denote significant p-values (*** < 0.001; * < 0.05; **.** < 0.1)

Table 2.5. Fitted coefficients and curve types for each *P. virginalis* and *P. clarkii* functional responses at both temperatures.

A. Bloodworm-focused resource-switching

Fit	Type	First-order	*a*	*h*	1/*h*	*a*/*h*
P. virginalis 18ºC	II	-0.0029 ***	0.61 ***	0.005 ***	200	121.20
P. clarkii 18ºC	II	-0.0036 ***	0.30 ***	0.019 ***	52.63	15.53
P. clarkii 26ºC	II	-0.0052 ***	0.70 ***	0.009 ***	111.11	77.22

B. Macrophyte-focused resource-switching

Fit	Type	First-order	*a*	*h*	1/*h*	*a*/*h*
P. virginalis 18ºC	II	-0.028 ***	0.50 ***	0.15 ***	6.62	3.29
P. clarkii 18ºC	II	-0.043 ***	0.64 ***	0.23 ***	4.42	2.82
P. clarkii 26ºC	III	NA	NA	0.23	4.33	NA

Asterisks denote significant p-values (*** < 0.001)

Table 2.6. Results from *frair_compare*, a difference test of the attack rates (*a*) and handling time (*h*) between the fitted *P. virginalis* and *P. clarkii* functional responses at both temperatures. *P. clarkii* at 26ºC is omitted from *frair_compare* due to the difference in model type.

A. Bloodworm-focused resource-switching

Fit 1	Fit 2	Parameter	Estimate	Std. Error	p-value	
P. clarkii 18ºC	*P. clarkii* 26ºC	Δa	-0.40	0.029	< 0.001	***
		Δh	0.009	0.001	< 0.001	***
P. virginalis 18ºC	*P. clarkii* 18ºC	Δa	0.31	0.025	< 0.001	***
		Δh	-0.013	0.001	< 0.001	***

B. Macrophyte-focused resource-switching

Fit 1	Fit 2	Parameter	Estimate	Std. Error	p-value	
P. virginalis 18ºC	*P. clarkii* 18ºC	Δa	-0.14	0.20	0.49	
		Δh	-0.075	0.035	0.033	*

Asterisk denotes a significant p-value (*** < 0.001; * < 0.05)

Table 2.7. Results from *frair_compare*, a difference test of the attack rates (*a*) and handling time (*h*) between the fitted *P. virginalis* and *P. clarkii* functional responses at both temperatures comparing, resource only curves and resource-switching curves. *P. virginalis* at 26ºC and *P. clarkii* at 26ºC macrophyte comparisons are omitted from due to high mortality and difference in model type respectively.

	Resource type	Parameter	Estimate	Std. Error	p-value	
P. virginalis 18ºC	Bloodworms	Δa	0.13	0.043	0.0031	**
		Δh	0.015	0.0010	< 0.001	***
	Macrophytes	Δa	0.37	0.47	0.43	
		Δh	0.17	0.047	0.00029	***
P. clarkii 18ºC	Bloodworms	Δa	1.24	0.058	< 0.001	***
		Δh	-0.0062	0.0014	0.00001	***
	Macrophytes	Δa	0.92	0.30	0.0025	**
		Δh	-0.083	0.029	0.0038	**
P. clarkii 26ºC	Bloodworms	Δa	0.44	0.054	< 0.001	***
		Δh	0.0028	0.00063	0.00001	***

AppendixI. Carapace length ± SE of every group of crayfish used in these experiments.

Trial	*P. virginalis*		*P. clarkii*	
	18C	26C	18C	26C
Bloodworm-only	29.29 ± 0.26	29.17 ± 0.24	34.91 ± 0.23	34.97 ± 0.29
Macrophyte-only	29.40 ± 0.28	29.97 ± 0.22	35.88 ± 0.34	34.71 ± 0.25
Bloodworm-focused Switching	34.09 ± 0.40		37.73 ± 0.38	38.31 ± 0.33
Macrophyte-focused Switching	34.63 ± 0.40		38.22 ± 0.36	38.50 ± 0.36

Appendix II. Results from our model selection methods.

Trial	Species	Temp (°C)	Juliano's method 1st order term	q±SE	Lowest AICc value	Visual inspection
Bloodworm-only	*P. virginalis*	18	-0.011 ***	0.53 ± 0.10 ***	Type III	Type II
Bloodworm-only	*P. virginalis*	26	-0.017 ***	0.27 ± 0.11 *	Type III	Type II
Bloodworm-only	*P. clarkii*	18	-0.012 ***	0.32 ± 0.06 ***	Type III	Type II
Bloodworm-only	*P. clarkii*	26	-0.0073 ***	-0.42 ± 0.01 ***	Type II	Type II
Macrophyte-only	*P. virginalis*	18	-0.043 ***	-0.81 ± 0.55	Type II	Type II
Macrophyte-only	*P. virginalis*	26	-0.035 ***	-0.77 ± 0.39 .	Type II	Type II
Macrophyte-only	*P. clarkii*	18	-0.069 ***	0.26 ± 0.42	Type II	Type II
Macrophyte-only	*P. clarkii*	26	-0.041 ***	2.10 ± 0.86 *	Type III	Type III
Bloodworm-Focused	*P. virginalis*	18	-0.0029 ***	0.29 ± 0.052 ***	Type III	Type III
Bloodworm-Focused	*P. clarkii*	18	-0.0036 ***	0.057 ± 0.083	Type II	Type II
Bloodworm-Focused	*P. clarkii*	26	-0.0052 ***	0.37 ± 0.052 ***	Type III	Type III
Macrophyte-Focused	*P. virginalis*	18	-0.028 ***	-0.64 ± NA NA	Type II	Type II
Macrophyte- Focused	*P. clarkii*	18	-0.043 ***	1.85 ± NA NA	Type III	Type II
Macrophyte-Focused	*P. clarkii*	26	NA	1.43 ± 0.86 .	Type III	Type III

Figures

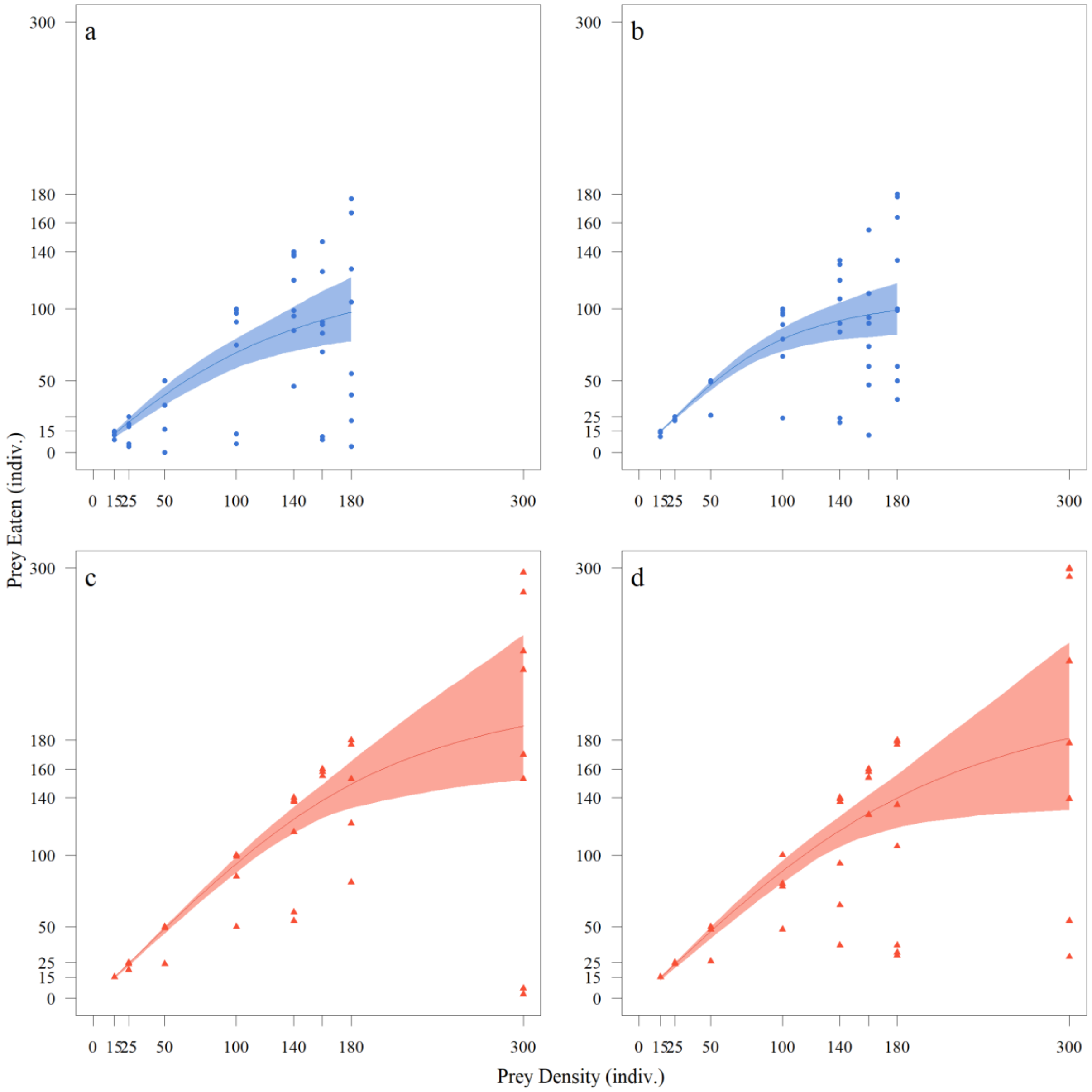

Figure 2.1. Functional responses with bootstrapped 95% confidence intervals (shaded regions) for *P. virginalis* crayfish (blue circles) at 18ºC (a) and 26ºC (b), and *P. clarkii* (red triangles) at 18ºC (c) and 26ºC (d), when presented with bloodworms. Lines represent the best fit model (Type II) for each population.

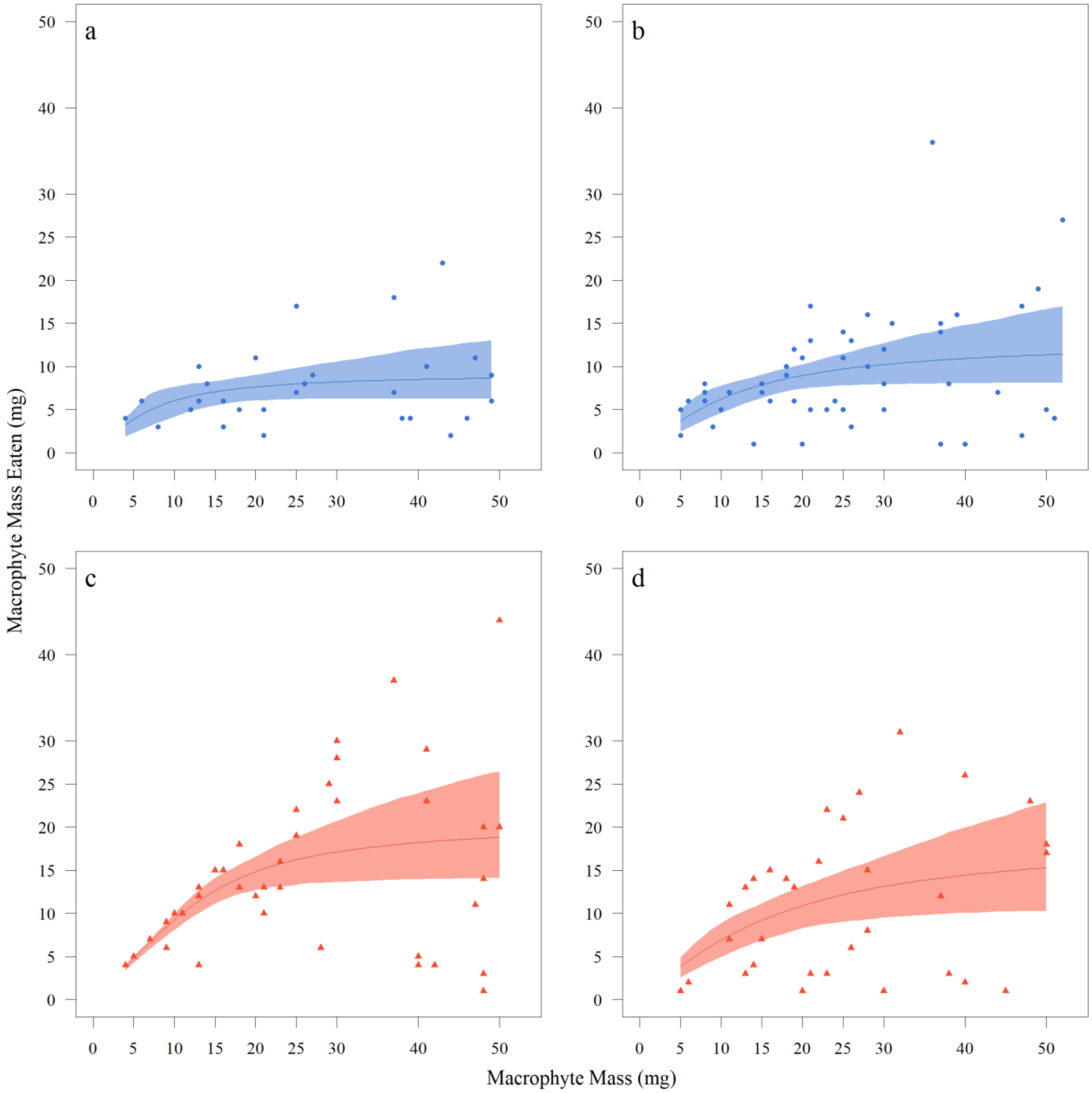

Figure 2.2. Functional responses with bootstrapped 95% confidence intervals (shaded regions) *P. virginalis* crayfish (blue circles) at 18ºC (a) and 26ºC (b), and *P. clarkii* (red triangles) at 18ºC (c) and 26ºC (d), when presented with macrophytes. Lines represent the best fit model (Type II) for each population.

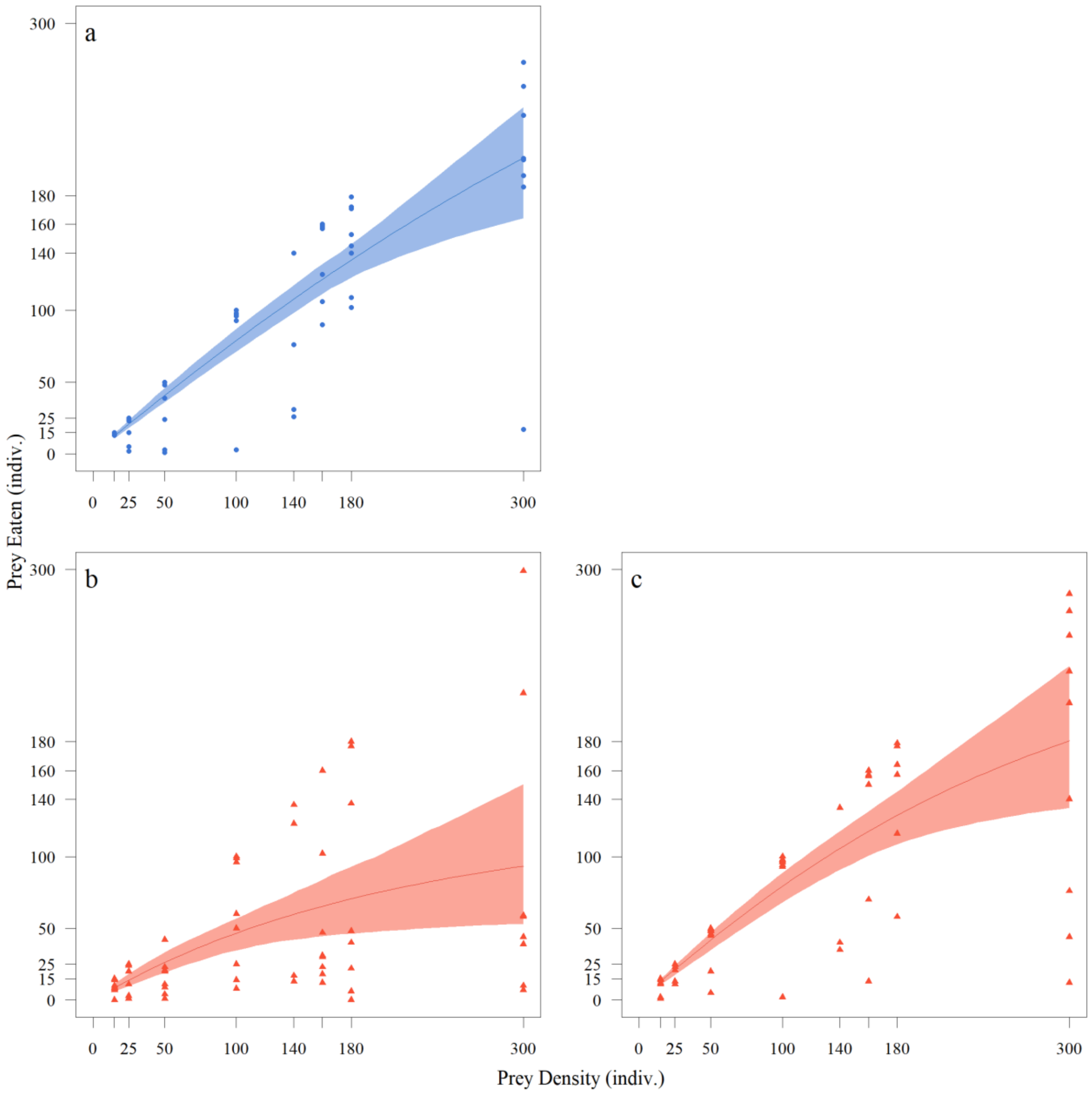

Figure 2.3. Functional responses with bootstrapped 95% confidence intervals (shaded regions) for *P. virginalis* crayfish (blue circles) at 18ºC (a) and 26ºC (b), and *P. clarkii* (red triangles) at 18ºC (b) and 26ºC (c), when presented with bloodworms and a stable amount of macrophytes. Lines represent the best fit model (Type II) for each population.

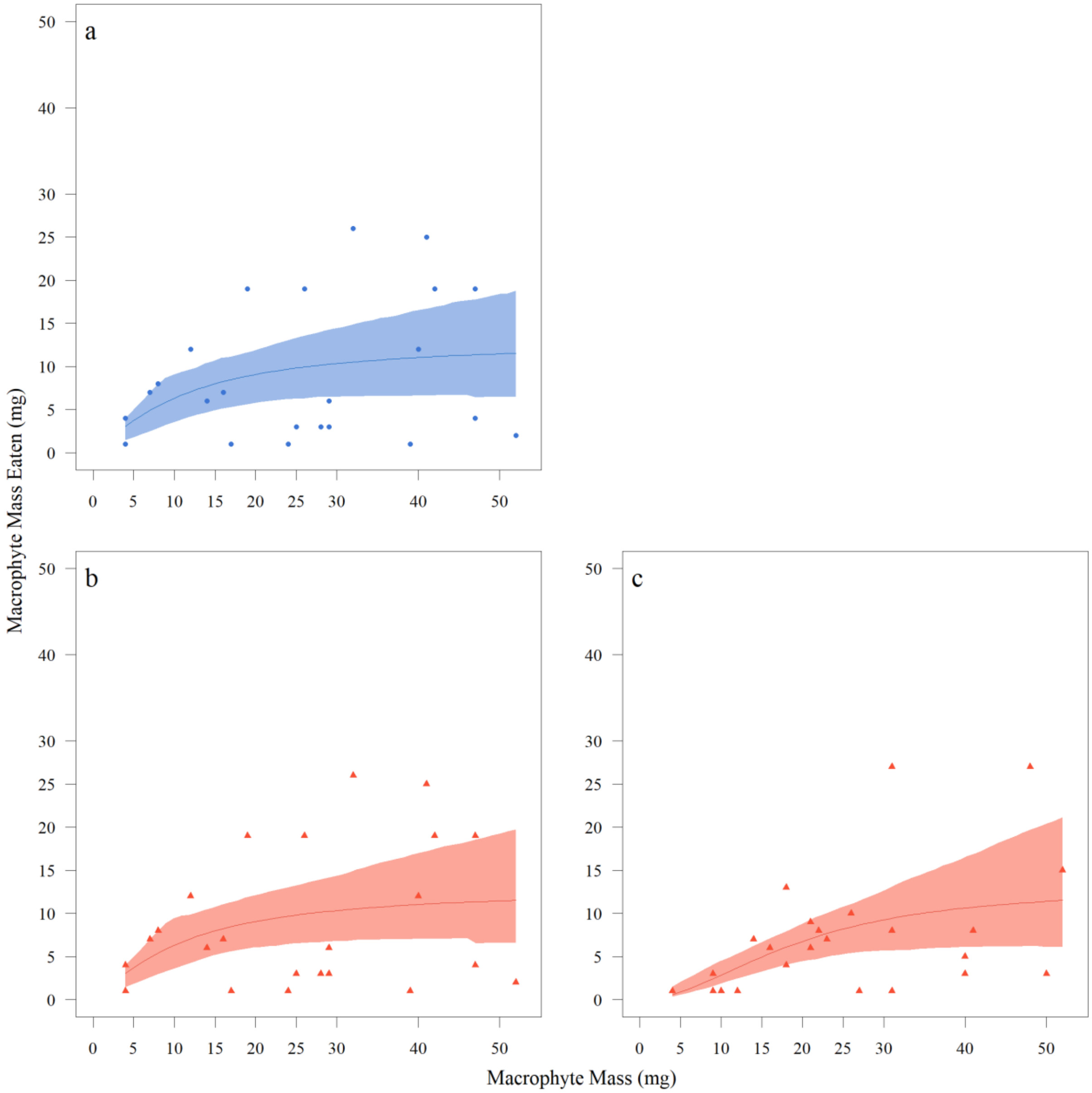

Figure 2.4. Functional responses with bootstrapped 95% confidence intervals (shaded regions) for *P. virginalis* crayfish (blue circles) at 18ºC (a) and 26ºC (b), and *P. clarkii* (red triangles) at 18ºC (b) and 26ºC (c), when presented with macrophytes and a stable amount of bloodworms. Lines represent the best fit model (Type II or Type III) for each population.

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Discussion & Final Conclusions

Summary and implications of findings

The outcome of a biological invasion is highly context dependent, owing to the influence of biotic and abiotic factors (Ricciardi et al. 2013; Dick et al. 2014; Iacarella et al. 2015). The experiments presented here have revealed that temperature, reproductive state, species identity, and resource availability have a strong, potentially complex influence on the performance of invasive omnivores. Through the use of comparative functional responses and associated metrics, I was able to explain how a combination of these factors can mediate the per capita trophic effects of two congeneric crayfishes. The results suggest the explanatory value of this experimental approach when complexity is added to the methodology.

Temperature had an effect on length and frequency of gravid states but not on the trade-off between reproductive ability and resource consumption. Resource consumption remained low during crayfish gravid states regardless of temperature, but gravid states were shorter and more frequent at higher temperatures (Chapter 1). Omission of these sorts of trade-offs could lead to erroneous estimates of the field impacts of invasive species. The reduction in the maximum feeding rate of *P. virginalis* in its gravid state was 93% at 18°C and 85% at 26°C. Thus, the consumption rate of one non-gravid individual is matched by 5.6 gravid females at 18°C and 13.9 gravid females at 26°C. Though this species is unlikely to be as reproductively active throughout the year in a temporally heterogeneous environment, gravid state could still have a significant impact on the *per capita* effects of an introduced population.

Comprehensive impact prediction would need to account for consumption reduction, probable abundances in the wild, and frequency of gravidness in wild populations. For a species like *P. virginalis*, this will be challenging. Crayfish abundances in the wild are hard to measure accurately (Larson and Olden 2016) and field studies on *P. virginalis* behaviour and remain sparse. Though reproductive cycles are well studied in sexual crayfish species (Reynolds 2002), more research still needs to be done on the reproductive cycle of *P. virginalis* gravid states, particularly in the wild. In the absence of information of this type, the inclusion of phenological traits in comparative functional responses is imperative to improve the explanatory value of invader impacts.

Gravid states are an extreme example of phenology affecting behaviour. Other phenological traits, however, affect behaviour in more subtle ways. As crayfish go through ontogenetic shifts they experience shifts in diet (Correia 2003; Carreira et al. 2017). For example, juvenile *P. clarkii* are more likely to consume animal prey than adults (Gutiérrez-Yurrita et al. 1998). If only one life stage is used in functional response trials, this shift has the potential to lead to underestimates of impacts.

The omission of macrophyte consumption from functional response trials causes similar problems. Not all crayfishes are macrophyte shredders as some prefer algae, detritus, or other forms of plant matter (Momot 1995), but functional response experiments with solely animal prey may lead misrepresentative impact predictions for plant communities. For example, macrophyte-only functional response trials showed that *P. virginalis* consistently consumed less macrophytes than *P. clarkii*. Though this relationship remained the same across temperatures, had bloodworm-only trial results been used as a substitute for *P. virginalis*' impacts on macrophytes, it would have been assumed that an increase in temperature followed a decrease in consumption. Instead, there was no difference in macrophyte consumption by *P. virginalis* at either temperature. Including different resources, therefore, is important to unravelling the interaction effects of resource type and temperature on the per capita effect of omnivorous aquatic invaders.

These differences in consumption also highlight a need for resource switching in functional response experiments. *P. clarkii* experienced a switch, having higher maximum rate in bloodworm- and macrophyte-only trials to having lower maximum feeding rates than *P. virginalis* in resource switching trials. Thus, in the presence of both resource types, *P. virginalis* may have greater impacts than *P. clarkii* on macrophytes and animal prey. The relatively simple resource switching design reveals more nuanced responses hinting at a broader complexity that prey choice offers in the field.

Complexity of resource switching is only worsened by climate warming shown by contradictory results to the literature. In bloodworm- and macrophyte-only trials, *P. virginalis* experienced a decline in feeding efficiency at 26°C. *P. clarkii*, on the other hand, showed a resilience to temperature indicating a greater ability to adapt to climate change. This would contradict information on crayfishes' ability to adapt to climate change. According to a study by Hossain et al. (2018), 87% of crayfish species could be highly sensitive to climate change. In bloodworm-focused resource switching trials, *P. clarkii* saw a higher consumption at 26°C than 18°C showing that in the presence of macrophytes *P. clarkii* we can distinguish a higher impact on animal prey. This is in complete contradiction to the findings of Carreira et al. (2017) that found a negative effect of heat waves on animal prey consumption in *P. clarkii* even in the presence of plant material. We believe this contradiction comes from a much longer acclimation time of *P. clarkii* in this study. Species and populations with varied optimal temperatures ranges and acclimation histories can react significantly differently to temperature changes (Gherardi et al. 2013; Chicatun 2021). Still, of the studies on crayfish diet choices, very few have considered the effect of temperature.

Advancing the comparative functional response approach

The problems of extrapolating complex interactions in the wild from small lab-based experiments are obvious. They do not enlighten us on competition outcomes between species nor of indirect impacts of invasive species (Dick et al. 2017). When used appropriately, however, comparative functional responses are an important tool to explore consumer-resource dynamics and impact outcomes for invaders that have limited invasive ranges. It has also repeatedly been shown to predict some field impacts of aquatic invaders (Dick et al. 2013, 2014; Iacarella et al. 2015), and remains an effective tool to inform decision makers.

Yet subsequent functional response experiments can still be improved upon. For example, the continued inclusion of phenologies for species with important life history stages and traits, and of resource availabilities and resource switching for a diverse array of omnivorous species. In light of multiple authors finding differences in functional response rates of conspecific populations (Grimm et al. 2020; Chicatun 2021), the importance of population provenance should also be tested. Cultivated populations, from aquaculture or the pet trade, will have different selective pressures and acclimation histories than established populations in the wild.

Our results also implied a complicated relationship between functional responses and temperature. To clarify this relationship functional responses should be tested across a range of temperatures to determine if response is truly unimodal across a temperature range and where that mode may lie (see Uiterwaal and DeLong 2020). For the purpose of this study, I should have used a range of temperatures that included the projected means under climate warming instead of just two temperatures. The temperature optima for these crayfishes would have more likely been found across such a range.

In fact, the next generation of functional response experiments should incorporate different forms of temperature variations, especially in resource switching experiments. Short term trends, like temperature peaks and heat waves, have already been shown to effect consumer-resource dynamics (Carreira et al. 2017), and should be compared to the effect long term climate trends, such as different acclimation histories and seasonal temperature variations. Varying exposure and acclimation times, and including temperature variations and ranges would help untangle the complex threads of variables interacting with temperature and affecting functional responses.

Finally, future applications of the comparative functional response approach need to carefully justify the methods used to select model type. Many issues came up during the selection of model fits. Different selection methods provide different models to interpret. As the curve type determines the stabilizing or de-stabilizing effect of the consumer-resource interaction, the proper selection of model is critical to effective interpretation of results.

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