EVALUATING AND PREDICTING IMPACTS OF AUSTRALIAN REDCLAW CRAYFISH CHERAX QUADRICARINATUS AND LOUISIANA RED SWAMP CRAYFISH PROCAMBARUS CLARKII INVASIONS

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GENERAL ABSTRACT

Freshwater crayfish have become one of the most widely introduced freshwater taxa globally. Crayfish introductions have not spared the African continent, which, apart from Madagascar, is naturally devoid of native freshwater crayfish. These introductions are of concern because invasive crayfish have generally been shown to cause strong impacts on recipient ecosystems outside Africa. This study therefore set out to compile up-to-date information regarding crayfish introductions in Africa, their pathways and distributions and to improve our understanding of the nature and magnitude of their environmental impacts. A systematic review revealed that nine crayfish species have been introduced into the continent with five of those, *Astacus astacus*, *Cherax quadricarinatus*, *Faxonius limosus*, *Procambarus clarkii* and *Procambarus virginalis*, having established naturalised populations in fourteen African countries (Chapter 2). The main driver of these crayfish introductions was to provide socio-economic benefits but there is limited evidence of success. The thesis further documents attempts made to address crayfish knowledge gaps in Africa, including standardisation of *C. quadricarinatus* sampling gear (Chapter 3), determination of the distribution of *C. quadricarinatus* in the recently invaded Upper Zambezi Basin (Chapter 4), and predicting ecological and socioeconomic impacts of two crayfish species that are spreading rapidly in Southern Africa, namely, *C. quadricarinatus* and *P. clarkii* (Chapters 5 – 8). To standardise *C. quadricarinatus* sampling methods in Africa, two methods used in Southern Africa were compared and the Promar® collapsible trap baited with dry pellets was recommended as the best approach for *C. quadricarinatus* abundance studies due to the high catch per unit effort (CPUE), probability of capture and the suitability of dry dog food as a standard bait. This standard approach was used for crayfish surveys in the Zambezi Basin to comprehensively determine the spread and establishment of *C. quadricarinatus* across the basin. The

establishment of *C. quadricarinatus* in the Barotse Floodplain, Upper Zambezi Floodplains freshwater ecoregion was confirmed. Although the probability of capture and catch per unit effort (CPUE) of *C. quadricarinatus* in the Barotse floodplain were similar to that of older invasions in the basin (Lake Kariba and Kafue River), morphometric differences among *C. quadricarinatus* populations sampled from these invaded regions were detected. Although crayfish were not detected in other regions, for example, the Okavango Floodplains ecoregions, *C. quadricarinatus* have the potential to spread at a downstream and upstream rate of 49 and 12 km·year^{-1}, impacting native biota therein. To evaluate the potential for ecological impacts, the consumer-resource dynamics of *C. quadricarinatus* and *P. clarkii* were described in comparison to a native trophic analogue, the freshwater crab of the Potamonautes genus, preying on various native taxa ubiquitous to African aquatic and terrestrial ecosystems. The use of functional response (FR) and consumption experiments in this study showed the potential impacts of the two crayfish species on native resources and on resources that support livelihoods in invaded ecosystems. This in most cases was a result of their high attack parameter, which also resulted in high functional responses ratios (FRRs) compared to native crabs. The crayfish FR, FRRs and consumption rates were mostly enhanced under the high temperature treatments. The FRs and consumption results were then combined with the field biomasses of crayfish and crabs to calculate the relative impact potential (RIP) to successfully predict the degree of impact caused by crayfish species relative to crabs. Crayfish species consistently displayed RIP scores > 1 relative to the native crab irrespective of region, which were higher for the summer than the winter season, suggesting greater impact of the invaders compared to the native species. This study further estimated the socioeconomic losses due to catch spoilage by *C. quadricarinatus* which are up to 1500 t per year in the invaded Kafue River Basin, which translates to an annual income loss of US$ 2 million. Information provided in this study is vital for conservation management and to compel policymakers to develop

appropriate conservation management tools within regulatory frameworks, which could stop or minimise the spread of crayfish species and protect Africa from further losing aquatic biodiversity.

PREFACE

This thesis comprises seven data chapters which are presented as scientific papers, some of which have been published in peer-reviewed journals, with the remaining papers under review or in preparation. Included is a general introduction (Chapter 1) and a general discussion and conclusion (Chapter 9).

- Madzivanzira TC, South J, Wood LE, Nunes AL, Weyl OLF (2020) A review of freshwater crayfish introductions in Africa. *Reviews in Fisheries and Aquaculture*. https://doi.org/10.1080/23308249.2020.1802405.
- Madzivanzira TC, South J, Nhiwatiwa T, Weyl OLF (Accepted) Standardisation of Australian redclaw crayfish *Cherax quadricarinatus* sampling gear in southern Africa. *Water SA*.
- Madzivanzira TC, South J, Ellender BR, Chalmers R, Chisule G, Coppinger CR, Khaebeb HF, Jacobs FJ, Chomba M, Musando B, Mwale B, Nhiwatiwa T, Rennie CL, Richardson N, Weyl OLF (In Review) Distribution and establishment of the alien Australian redclaw crayfish, *Cherax quadricarinatus*, in the Zambezi Basin. *Aquatic Conservation: Marine and Freshwater Ecosystems*.
- Madzivanzira TC, South J, Weyl OLF (2021) Invasive crayfish outperform Potamonautid crabs at higher temperatures. *Freshwater Biology*, **00**: 1-4. doi: 10.1111/fwb.13691.
- Madzivanzira TC, South J, Weyl OLF (Awaiting submission to a journal) Predatory impacts of invasive crayfish in Africa are predicted by functional responses.
- Madzivanzira TC, South J, Weyl OLF (Awaiting submission to a journal) Potential impacts of invasive crayfish on aquatic macrophytes and artisanal fishery in Africa.
- Madzivanzira TC, South J, Weyl OLF (Awaiting submission to a journal) Relative impact potential of introduced crayfish species in Africa.

DECLARATION

I, Takudzwa Comfort Madzivanzira, hereby declare that this thesis submitted to the Department of Ichthyology and Fisheries Science, Rhodes University, is my original work and has not been previously submitted in any form to another university. I have not included ideas, phrases, passages or illustrations from another person's work without acknowledging their authorship.

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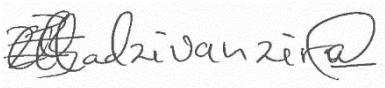
Signed: 

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I am grateful to the Department of Environmental Affairs (DEA) for issuing us with permits to sample, transport and keep a maximum 150 of each of the crayfish species (Permit Numbers: 50869181001115242, 50869181001120608 for *C. quadricarinatus* and 50869181001113030, 50869181002121045 for *P. clarkii*) and the Eastern Cape Department of Economic Development, Environmental Affairs and Tourism for issuing permits to sample crabs (Permit numbers: CRO 19/18CR and CRO 190 21/18CR). I am also grateful to Andre Hoffman from Mpumalanga Parks and Tourism Agency (MTPA) and Dr. Leon Barkhuizen from Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs

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DEDICATION

This thesis is dedicated to my late supervisor, and a great mentor, Professor Olaf Lawrence Friedrich Weyl.

Forever missed. May your dear soul rest in peace.

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CHAPTER 1

GENERAL INTRODUCTION

Freshwater ecosystems

Freshwater ecosystems (rivers, lakes, dams, and wetlands) are amongst the most biologically diverse per unit habitat volume on Earth, with more than 140 000 species (plants, fungi, vertebrates and invertebrates) compressed into just 2% of the world's surface area (Lehner and Döll 2004; Dudgeon et al. 2006; Strayer and Dudgeon 2010; Reid et al. 2019). They have inestimable economic, cultural, health, scientific and educational values (Albert et al. 2020).

The sustainable functioning of inland fisheries as provisional services is integral to achieving the United Nations' Sustainable Development Goals (SDGs) (https://www.un.org/sustainabledevelopment/). Ecosystem benefits associated with inland fisheries make a substantial contribution towards achieving: SDG 1 (No Poverty), SDG 2 (Zero Hunger), SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land) (Lynch et al. 2020). Despite the contribution of inland fish and fisheries in providing food for billions and livelihoods for millions of people globally, they tend to be underrepresented in water usage and policy decisions (Lynch et al. 2020).

Fish, and other associated products, are a key component of both diet and income for many communities (UNEP 2010). The provisional services are not only restricted to food, but include contributions to manufacturing and industry, technology, health care, and comprise elements of tools and weapons, apparel and jewellery, curios and souvenirs and musical instruments

(Olden et al. 2020). Beyond these provisional services, understanding the resident species of an ecological system, and how they are being exploited and threatened is vital in order to conserve rare species. It is important to develop sustainable use policies and management interventions for fishery species as well as to understand the ecological role that species play in a given habitat to keep the integrity of the ecosystem functioning (Brooks et al. 2016). Human demands on freshwater, (such as hydropower, agriculture, industry and domestic requirements), largely take precedence over conserving the ecological integrity of fish, fisheries, and the habitats that support them (Lynch et al. 2020).

Freshwater systems are consistently at higher risk of degradation than their marine and terrestrial counterparts (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006; Turak et al. 2017; Reid et al. 2019) which is resulting in the decline in abundance of many species (Grzybowski and Glińska-Lewczuk 2019). Anthropogenic stressors to freshwater ecosystems can be classified into twelve emerging threats, namely, changing climates, e-commerce and invasions, harmful algal blooms, infectious diseases, expanding hydropower, emerging contaminants, microplastic pollution, engineered nanomaterials, light and noise, freshwater salinisation, declining calcium and cumulative stressors (revised by Reid et al. 2019). In addition, the complex and synergistic interactions between these ecosystem stressors and freshwater biodiversity are increasingly compounded by the overarching threat of anthropogenically driven global climate change. Outcomes from these stressors include increasing temperatures and shifts in precipitation and river runoff, thereby increasing the difficulty in confidently predicting outcomes for biodiversity and consequential extinction risks (Brook et al. 2008; Reid et al. 2019).

Alarmingly, indicators are revealing rapid species declines and a large extinction risk in freshwater organisms (Reid et al. 2019). Freshwater species declined more steeply between 1970 and 2016 than either marine or terrestrial populations (WWF 2020). The Living Planet Index (LPI) for freshwater vertebrates declined by 84% relative to LPI declines of 38% and 36% for land and marine populations, respectively (WWF 2020). Although less comprehensively recorded, freshwater invertebrate populations are known to be declining faster than their terrestrial counterparts (Taylor et al. 2007; Clausnitzer et al. 2009; Cumberlidge et al. 2009) with 33% of aquatic invertebrates threatened with extinction, compared to 28% of terrestrial invertebrates (Sánchez-Bayoa and Wyckhuysb, 2019). However, in a recent study in the United States of America (USA), a time series analysis showed an overall lack of decline or increase of invertebrate communities, which was consistent across different feeding groups and was similar for relatively natural versus heavily disturbed sites (Crossley et al. 2020). In Europe, increasing trends in taxonomic richness and diversity of invertebrates across all recent time series analyses have been observed (Pilotto et al. 2020). It is therefore necessary to conduct these assessments regionally as the majority of these studies are being conducted in Europe and USA, leaving gaps in our knowledge regarding invertebrate population trends in other parts of the world, which may result in erroneous conclusions on a global scale.

Biological invasions

A biological invasion is a human mediated movement of species into a recipient novel environment where it is able to establish self-sustaining populations and spread, often resulting in detrimental effects to the native community (Mack et al. 2000; Richardson et al. 2010; Blackburn et al. 2011, 2019; Simberloff et al. 2013; McGeoch et al. 2016; Hulme et al. 2017;

Ricciardi et al. 2017). The spread of non-native/alien species are facilitated increasingly by human activities which break the geographical barriers to species distribution (Wilson et al. 2009; Richardson et al. 2010; Blackburn et al. 2011, 2014). Introduction pathways of alien species into a novel environment are often through intentional movements of species by humans for food, recreation, trade and other economic interests across the world (Nuñez et al. 2012) although unintentional introductions also occur through contamination or stowaway species (Minchin et al. 2013).

The invasion process consists of four stages, transport, colonisation, establishment and spread, each of which is crucial for a species to become invasive (Blackburn et al 2014). Not all introduced species become invasive, as some are not being capable of surviving and reproducing within the invaded ecosystem (Mack et al. 2000; Blackburn et al 2014). For the introduced species to become invasive, it has to physiologically adapt in the invaded ecosystem, overcome biotic resistance from native species, reproduce effectively enough to start spreading geographically and negatively impact the ecosystem(s) in the introduced range (Blackburn et al. 2015). The translocation of these invasive alien species IAS by humans overcomes the first barrier (geographic barrier) of the invasion process (Blackburn et al. 2011, 2015). Other barriers however, such as dam walls, weirs or even habitat suitability, prevent the unaided movement of IAS from their established ranges (Blackburn et al. 2011, 2015). Aquatic species in these cases are confined to the biogeographic island of the water body, and therefore invasiveness depends on the water body type and/or longitudinal and lateral connectivity to other nearby water bodies. As an example, the common carp *Cyprinus carpio* Linnaeus, 1758, is deemed invasive in a river basin while the same species in a small farm dam or contained pond is not (per EICAT classifications; Hawkins et al. (2015)). In the continuum of invasion phases, establishment of IAS stands at the interface between the initial arrival of propagules

and the integration of the invader into the recipient ecological community (Crooks and Rilov 2009). Introduced species are more likely to establish if more individuals are released (i.e., higher propagule pressure) and if they are released at multiple locations, or at more than one time (Blackburn et al. 2011, 2015). Once the IAS population establishes, the barriers that prevent their dispersal or spread need to be overcome for the IAS to expand its invasion range, where individuals must survive and reproduce. The more the IAS spreads, the more dissimilar from the introduction point the environment at these locations may be, and thus, a spreading population essentially faces multiple and sequential establishment events, under an ever-greater range of environmental conditions (Blackburn et al. 2011, 2015). Failure to pass any one of the barriers at any stage of the invasion results in failure of the species as an invader (Blackburn et al. 2011). Species known to be highly invasive elsewhere have failed to establish in several systems worldwide, including the topmouth gudgeon *Pseudorasbora parva* (Temminck and Schlegel 1846) which failed to establish in northeast London (Copp et al. 2007); two *Cherax* crayfish species which failed to establish in Southern Africa and Malaysia (Madzivanzira et al. 2020; Chapter 2), and the failure by the Largemouth bass *Micropterus salmoides* (Lacepède 1802) to establish in Lake Kariba in Zimbabwe (Marshall 2011).

Freshwater ecosystems have been recipients of thousands of species (https://nas.er.usgs.gov/News/News.aspx) translocated by humans outside their native ranges by an array of vectors such as ballast water, canals, deliberate introductions and releases from the pet trade and bait buckets (Kernan 2015). Ballast water from ships is a well-known transport vector of invasive aquatic species on regional and global scales (Ruiz et al. 2000; McGee et al. 2006; Keller et al. 2011; Bailey 2015). Large ships withdraw up to 20 million gallons of water at their loading ports, and in the process, take in biota, before disposing of them at their next destination (Ruiz et al. 2000; McGee et al. 2006; Keller et al. 2011; Bailey 2015). The

Laurentian Great Lakes basin has been invaded by at least 182 non-indigenous species and ~60% of these are Eurasian in origin (Ricciardi 2006), with negative impacts recorded almost immediately after their invasion (Bailey 2015). The construction of canals also facilitates the unaided movement of IAS from one region to another, for example the spread of *D. polymorpha* from the Ponto-Caspian Basin to the Atlantic and UK through the canal network (Keller et al. 2011). Many IAS have been intentionally introduced and have subsequently become widely dispersed (Faulkner et al. 2020). Legal and illegal introductions for aquaculture and sport fisheries in Southern Africa have resulted in the establishment of salmonids, centrarchids and tilapias in Southern Africa (Ellender et al. 2014) and crayfish species globally (Lodge et al. 2012; Madzivanzira et al. 2020; Chapter 2). Over 624 freshwater fish species have been introduced globally, mainly for aquaculture (51%), the pet trade (21%), sport fishing (12%) and fisheries (7%) (Gozlan 2008).

While it is still not clear as to whether the actual underlying processes can be identified with much confidence, the breadth and typicality of IAS environmental requirements or tolerances (climate matching) do seem to be the important determinants of establishment success and subsequent spread (Gaston and Blackburn 2000; Gaston 2003; Blackburn et al. 2015). Other factors, including the reproductive rate of IAS and the presence of enemies or mutualists can affect the establishment of IAS (Blackburn et al. 2011, 2015). Invasive alien species are characterised by high fecundity (e.g. invasive Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) (Zengeya et al. 2015), invasive crayfish (Lodge et al. 2012; Twardochleb et al. 2013)), voracious predation, phenotypic plasticity and generalist feeding behaviours (e.g. invasive crayfish (Gherardi 2007; Lodge et al. 2012; Twardochleb et al. 2013)), all traits typical of *r*-selected species, which makes them successful invaders.

The impacts of IAS can be diverse and widespread, resulting in both direct and indirect effects (Jeschke et al. 2014; Gallardo et al. 2015). A burgeoning number of IAS are deemed responsible for local and global extinctions of native species through various impact mechanisms. In freshwater systems, IAS can impact native species through predation, such as centrarchids of the genus *Micropterus*, which are apex predators that have already altered trophic structures and abundances of native biota globally (Weyl et al. 2010; Bezerra et al. 2019; Khosa et al. 2020). Invasive alien species can also impact native species through competition for resources, for example, the Louisiana red swamp crayfish, *Procambarus clarkii* (Girard 1852), which has caused the disappearance of a native crab in Lake Naivasha in Kenya (Foster and Harper 2007), and *O. niloticus* (Linnaeus 1758) which has almost replaced the native Kariba tilapia *O. mortimeri* (Trewavas 1966), the three spot tilapia *O. andersonii* (Trewavas 1966) and the greenhead tilapia *O. macrochir* (Boulenger 1912) in the Zambezi Basin (Ellender et al. 2014). *Oreochromis niloticus* has also caused extensive hybridisation and introgression with the native Mozambique tilapia *O. mossambicus* (Peters 1852) in the Limpopo River system, South Africa (Ellender et al. 2014). Concerns have also been raised about how IAS facilitate the spread of parasites and diseases. For example, the Asian tapeworm *Bothriocephalus acheilognathi* has made a shift from the common carp *Cyprinus carpio* Linnaeus 1758 to native fishes such as the *Labeobarbus* species in the South African freshwater systems (Ellender et al. 2014), and temnocephalan fauna have passed from *C. quadricarinatus* to Potamonautid crabs in the Zambezi Basin (Douthwaite et al. 2018). Invasive alien species also modify invaded ecosystems, resulting in cascading impacts to the invaded systems. Examples include *C. carpio*, which resuspend solids in the water column and increases turbidity as they search for food and expel non-food items (Weber and Brown 2009). The increased turbidity as a result of *C. carpio* invasion has numerous deleterious effects on freshwater systems, such as reduced light penetration, which affects primary productivity,

aquatic macrophyte growth, zooplankton filtering efficiency, and impaired foraging abilities of visual planktivorous and piscivorous fishes (Weber and Brown 2009). *Procambarus clarkii* also modifies its habitat through burrowing and the consumption of macrophytes, as recorded in Lake Naivasha, Kenya (Foster and Harper 2007). International goals have been implemented to stop or reduce these rising impacts, such as the Aichi Target 9 of the Convention on Biological Diversity, which commits signatories to control or eradicate priority IAS (Booy et al. 2020). However, this target has only been partially achieved and biological invasions remain a huge threat to biodiversity (Tickner et al. 2020). Despite conflicting views on the relative benefits and costs of a substantial proportion of IAS (Schwartz et al. 2012; Simberloff et al. 2013), many IAS are widely acknowledged to have severely negative effects on both ecology and economy (Blackburn et al. 2019).

Although the introduction of IAS is generally associated with various negative socio-economic impacts, their suitability for aquaculture and fishery enhancement is undeniable (Ellender et al. 2014). Several high-profile fisheries have been established in Africa based on the harvest of IAS, such as the Lake Tanganyika sardine *Limnothrissa miodon* (Boulenger 1906), in Lakes Kariba, Itezhi-tezhi and Cahora Bassa (Ellender et al. 2014). The fish community of Lake Naivasha, Kenya, presently comprises only introduced species, which have artificially created a commercial fishery that provides substantial societal benefits in a developing country with high poverty levels (Hickley et al. 2015). The international black bass (Centrarchids *Micropterus salmoides, M. dolomieu, M. puntulatus* and *M. floridanus*) tournaments in southern Africa also apply here; it includes teams from Mozambique, Namibia, South Africa, Swaziland, and Zimbabwe, and many anglers in the region are affiliated to the Bass Anglers Sportsman Society (BASS) in the U.S.A (Ellender et al. 2014). Other organisations in the region include the South African Freshwater Bank Angling Federation which primarily target

C. carpio, and the Cape Piscatorial Society (CPS) and Federation of South African Fly-Fishers (FOSAF) which targets *O. mykiss* and *S. trutta*. Despite high participation in these types of events and the perceived socioeconomic contribution of these alien invasive fishes to local and regional economies, the benefits of these alien species remain largely unquantified (Ellender et al. 2014; Hargrove et al. 2015). *Cyprinus carpio* is also the primary target of subsistence fishers in Lake Gariep (South Africa's largest impoundment) and provides a livelihood for more than 500 fishers (Ellender et al. 2009, 2010, 2014). Zimbabwe, Namibia and Botswana also have significant recreational fisheries specifically targeting introduced centrarchids or salmonids, however, information on participation is scant (Ellender et al. 2014). Conflicts arise from the risks posed by introducing or moving IAS into novel environments (Woodford et al. 2016). Stakeholders often debate the legislation put in place to control the introduction and spread of IAS as some recreational anglers believe that the introduction and spread should continue unhindered for their benefit at the expense of other associated socio-economic and ecological impacts to society (Ellender et al. 2014).

Public attention patterns differ among species groups and location but attention has been globally higher for climate change than for biological invasions (Jarić et al. 2020). Describing the negative effects of these IAS to public and policy makers is difficult, as ecological impacts are complex and require specific knowledge; if framed in an economic perspective the impacts become clearer, which can warrant preventative management action (Diagne et al. 2020a). Costs mostly arise from economic loss in the energy, agriculture, forestry and health sectors, diminished delivery of ecosystem services and the cost of controlling and eradicating IAS (Gallardo et al. 2019; Egoh et al. 2020; Diagne et al. 2020b; Courchamp et al. 2020). A comprehensive data compilation called InvaCost has been developed which describes the economic cost estimates associated with IAS (Diagne et al. 2020b). Despite useful platforms

such as InvaCost, debates continue in invasion science around the quantification of the costs associated with IAS (see Sagoff et al. 2020; Cuthbert et al. 2020). Discrepancies in methods and biases in reporting can cause erroneous judgements and conclusions to be formed, which negates knowledge progression, adversely affects public perceptions and attitudes and misleads policy makers regarding the true impact of IAS (Cuthbert et al. 2020). The negative costs of IAS per annum are currently estimated to be between €12.5 to 20 billion in Europe (Kettunen et al. 2009), US$ 120 billion in the United States of America (Pimentel et al. 2005), US$ 73.9 million in China (Xu et al. 2006), US$ 1 billion per year in South Africa (van Wilgen et al. 2020), and these are likely underestimated and will escalate with time (Bradshaw et al. 2016). The control or eradication of IAS can be expensive, and with numerous species and limited resources, decision-makers must carefully prioritise which IAS to manage and how (Booy et al. 2020). One approach that has been recommended is the Non-Native Risk Management (NNRM) scheme (Booy et al. 2017), which uses multiple criteria relevant to inform decision and policy makers (beyond solely monetary considerations) to score different aspects of IAS management, based on predefined invasion scenarios and strategies (Booy et al. 2020).

Invasive alien species are increasing globally (Ricciardi 2007; Gozlan 2008; Hulme et al. 2009; Seebens et al. 2017), to the extent that the biogeographic distinctiveness of different regions is becoming blurred (Capinha et al. 2015). Established IAS numbers per continent are predicted to increase from 2005 to 2050 by 36% (Seebens et al. 2020). Among individual taxonomic groups, invasive invertebrates are strongly projected to increase globally (Seebens et al. 2020). Ricciardi et al. (2017) identified the biotechnological, ecological, and socio-political issues which have a direct influence on the projected increases of the IAS. Given these globally wide-ranging impacts and mechanisms, limiting the spread of IAS is one of the most urgent conservation and resource management challenges of the 21st century (Faulkner et al. 2020;

Tickner et al 2020; Seebens et al. 2020; Madzivanzira et al. 2020). Thus, predictive developments for advanced warning of global shifts in invasion risks and opportunities is essential for developing strategies that can more effectively mitigate invasion threats (Ricciardi et al. 2017).

Freshwater crayfish

Some of the most successful aquatic IAS belong to the subphylum Crustacea (Devin et al. 2005). Within Europe 53% of freshwater IAS are crustaceans (Karatayev et al. 2009), with crayfish being the most prolific group of aquatic IAS (Ilhéu et al. 2007; Kouba et al. 2014). Humans have introduced crayfishes outside their native ranges, many of which have established populations that have subsequently spread widely (Hobbs and Lodge 2010). Freshwater crayfish species have socio-economic and ecological significance in several regions around the world, favouring their introductions into novel areas (Reynolds and Souty-Grosset 2012). Crayfish have been moved around the world for aquaculture, fisheries and as ornamental pets, or unintentionally, as unused bait or released by individuals as unwanted aquarium pets (Holdich 1999; Taugbøl and Skurdal 1999; Lodge et al. 2000; Gherardi 2006; Taylor et al. 2007; Lodge et al. 2012; Manfrin et al. 2019; Madzivanzira et al. 2020; Haubrock et al. 2021). Of the 692 described freshwater crayfish species (Crandall and De Grave 2017), 28 have established viable populations outside their native ranges (Gherardi 2010).

Freshwater crayfishes are a monophyletic group that dates back to the Triassic period (185–225 Mya) (Crandall et al. 2000; Crandall and Buhay 2008). Freshwater crayfish belong to the phylum Arthropoda, within the subphylum Crustacea and order Decapoda, infraorder Astacidea (Hobbs 1988) which includes three superfamilies, Astacoidea (Northern Hemisphere

crayfish), Nephropoidea (clawed lobsters) and Parastacoidea (Southern Hemisphere crayfish) (Crandall et al. 2000; Ahyong and O'Meally 2004; Meland and Willassen 2007; Crandall and De Grave 2017). The four families, Cambaridae, Cambaroididae, Cricoidoscelosidae and Astacidae make up Astacoidea (Crandall and De Grave 2017). The Cambaridae are distributed in North America east of the Rocky Mountains, north into southern Canada and south through Mexico and in Asia with over 420 species described (Crandall and Buhay 2007; Crandall and De Grave 2017). The Astacidae are distributed west of the Rocky Mountains (mainly in the Pacific North-West) and in Europe with 39 species described (Crandall and Buhay 2007; Crandall and De Grave 2017). At present, 692 described freshwater crayfish species are naturally distributed in all continents except mainland Africa and Antarctica (Huner and Lindqvist 1995; Crandall and Buhay 2008; Lodge et al. 2012; Crandall and De Grave 2017). Madagascar, however, has seven native crayfish with no living close relatives anywhere else on the entire planet (Boyko 2005; Ganzhorn et al. 2013). The taxonomy and identification of freshwater crayfish is described in Souty-Grosset and Fetzner Jr. (2016) and Crandall and De Grave (2017).

Crayfish are 10-legged, freshwater decapod crustaceans which resemble miniature lobsters (Nyström 2002; Jones et al. 2016). Their bodies consist of a cephalothorax (fused head and thorax) and an abdomen (Plate 1a and b). Large pincers or chelae and a powerful tail (abdomen) are distinguishing features. Colour patterns and texture on their body and claws can sometimes be used to distinguish between species (Nyström 2002; Jones et al. 2016). Crayfish inhabit the benthic zone of freshwater systems and prefers lentic or slow-moving waters (Nyström 2002; Crandall and Buhay 2008; Jones et al. 2016). Crayfish with strong burrowing traits (e.g. *P. clarkii*) are also found in terrestrial ecosystems. Crayfish are generalist omnivores and are considered opportunistic feeders, and commonly feed on aquatic plants, invertebrates,

decaying organic matter, fish eggs, and small fish (Nyström 2002; Crandall and Buhay 2008; Jones et al. 2016). Most crayfish species reproduce sexually, in open water during late spring or early summer (see *P. clarkii* and *C. quadricarinatus* sections in this Chapter, for reproduction processes). Other crayfish species reproduce by parthogenesis (e.g. marbled crayfish *Procambarus virginalis* Lyko 2017) (Jones et al. 2009; Souty-Grosset 2016). Most crayfish species are extremely hardy and can tolerate wide ranges of environmental variables. The tolerance for different temperatures differs between crayfish families, and Cambaridae are adapted to warmer climates, and tolerate higher temperatures than the northern species (Nyström 2002).

Introduced crayfishes negatively impact ecosystem services that involve a full range of well-studied mechanisms of ecological interactions with native species and humans (Hobbs and Lodge 2010). Due to their capability to integrate into the food web at many levels in invaded freshwater systems, and to subsist on the substantial energy reserves of the detrital pool, freshwater crayfish are good candidates for invading aquatic systems (Moyle and Light 1996). The threats posed by invasive freshwater crayfish are one of the greatest concerns for many freshwater ecologists (Peay et al. 2010; Furse and Coughran 2011; Coughran and Furse 2012; Leland et al. 2012). Several studies have focused on non-indigenous crayfish species (NICS) as predators of either native invertebrates or vertebrates, eggs and larvae (Gherardi 2007) with most showing lethal or sublethal effects on prey. One example is the impact of *P. clarkii* on invertebrates in Lake Naivasha, Kenya (Jackson et al. 2016). In addition, Signal crayfish *Pacifastacus leniusculus* (Dana 1852) were shown to negatively impact benthic fish in the Ouse River, England through predation (Guan and Wiles 1997). Recently, long term monitoring results showed that *P. leniusculus* has the capacity to cause the complete extirpation of some benthic fish species in the River Tees catchment, England (Galib et al. 2020). Freshwater

crayfish are also ecosystem engineers, and accelerate the rate of leaf-litter breakdown and the cycling of nutrients, as seen in the invasion by *P. leniusculus* in Europe freshwater systems (Doherty-Bone et al. 2018). Burrowing by several NICS such as *P. clarkii*, *P. leniusculus*, and the Yabby *Cherax destructor* Clark 1936 can be a problem in both aquatic and terrestrial systems (Gherardi 2007). The burrowing of *C. destructor* has been linked to damage to dam walls and irrigation canals (de Moor 2002). More frequently cited is the burrowing effect of *P. clarkii* on river banks (Huner 1977) and rice fields, which has affected agricultural productivity in Egypt and Morocco (Abdel-Kader 2016; Sara and El Moutaouaki 2019). Although not recorded as a burrowing species in its native habitat (Holdich 1999), *P. leniusculus* has caused considerable damage to river banks in the United Kingdom by burrowing (Sibley 2000). Introduced crayfish species also modify habitat by reducing macrophyte cover and abundance, which exposes juvenile fish and invertebrates to predation (Twardochleb et al. 2013). In Spain, extensive removal of macrophytes by *P. clarkii* is posited to have led to the local extinction of three plants, *Ceratophyllym demersum*, *Myriophyllum alterniflorum* and *Utricularia australis*, and associated snails *Lymnaea stagnalis* and *L. peregra* (Montes et al. 1993), although direct predation could have been the cause of the extinction of the two snail species (Alcorlo et al. 2004). Non indigenous crayfish species have the capacity to be a vector for the introduction of other invasive species, as they often transport invasive parasites and diseases (Lodge et al. 2012; Twardochleb et al. 2013; Gherardi 2007; Madzivanzira et al. 2020). There is extensive literature reporting that North American crayfish species carry the aetiological agent of the crayfish plague, *Aphanomyces astaci* Schikora (Oomycetes) (Gherardi 2007). A large number of European native crayfish populations have been decimated by the plague since the 1860s, leading to reduced production of the noble crayfish *Astacus astacus* (Linnaeus 1758) and the Danube crayfish *A. leptodactylus* (Eschscholtz 1823) by up to 90%, particularly in Germany, Scandinavia, Spain, and Turkey (Gherardi 2007). With the documented impacts of freshwater

crayfish globally, preventing their introduction, and the implementation of management programmes to control their populations is key (Lodge et al. 2012; Stebbing 2016).

Five NICS have been well studied globally, *P. clarkii*, Rusty crayfish *Faxonius rusticus* (Girard 1852), *P. leniusculus*, spiny-cheek crayfish *Faxonius limosus* (formerly *Orconectes limosus*) (Rafinesque 1817) and virile crayfish *Orconectes virilis* (Hagen 1870) (Twardochleb et al. 2013). Regardless of the original purpose of introduction, North American crayfish species generally become problematic after penetrating new localities outside their native ranges (Haubrock et al. 2021). These five species have the longest and most widespread history of crayfish invasion studies (Hobbs et al. 1989, Lodge et al. 2012), while other NICS have limited data (Twardochleb et al. 2013). Other emerging NICS include *C. quadricarinatus* and the Marbled crayfish/Marmorkrebs *Procambarus virginalis* (Lyko 2017; Jones et al. 2009; Andriantsoa et al. 2020; Madzivanzira et al. 2020). Although *C. quadricarinatus* is one of the world's most intensively studied freshwater NICS on captive biology, physiology, aquaculture and production, the wild ecology and invasion impacts remain poorly understood (Furse et al. 2015).

Given that the negative impacts exerted by NICS are severe, the Convention on Biological Diversity (CBD) approach, as complemented by the European Strategy, is viewed as an excellent framework to be followed to prevent the introduction of NICS and to eliminate the damage they inflict (Gherardi et al. 2011). Several diversified methods have been proposed or are used, alone or combined, for either eradication or the control of NICS, and fall into these categories: (1) mechanical removal, (2) physical methods, (3) biological control, (4) biocides, and (5) autocidal methods (Gherardi et al. 2011). Under the mechanical removal method, the invasive population of *F. rusticus* declined from 6500 to 206 after 6 weeks of continuous

trapping in the USA (Bills and Marking 1988) The physical method was attempted in 1995 in a trout farm dam in South Africa, whereby the water level was reduced and *P. clarkii* specimens were physically removed, however this process was unsuccessful, as Nunes et al. (2017b) sampled a *P. clarkii* specimen 22 years after the eradication programme. Natural predators have been considered for the control of NICS, for example, the European eel *Anguilla anguilla* (Linnaeus 1758) has been used to control of *P. clarkii* (Aquiloni et al. 2010), and increased fish predation, along with physical control, has been successfully applied against *F. rusticus* (Hein et al. 2007). Natural pathogens such as the *P. leniusculus* bacilliform virus (Longshaw et al. 2012) have also been considered as biological control agents against NICS (Stebbing et al. 2014). A synthetic pyrethroid biocide BETAMAX VET®, followed by draining of the ponds, was used for the successful eradication of *P. leniusculus* and the associated crayfish plague in the Dammane area, Norway (Sandodden and Johnsen 2010). Autocidal methods were used in the UK, using standard traps baited with gel permeated with water that had been preliminarily conditioned by mature *P. leniusculus* females (Stebbing et al. 2004). Efficacy of this method was, however, not proven, as control traps baited with food attracted a similar number of crayfish as the traps baited with sex pheromones (Stebbing et al. 2004). The mitigation and remediation options for invasive NICS described here have not been extensively explored, and have so far met with limited success (Gherardi et al. 2011; Manfrin et al. 2019). Unfortunately, no universal method exists as NICS are so diversified, and have populated various habitat types that no single method or universal solution is likely to be attainable (Freeman et al. 2010). Integrated management using a range of control and containment techniques to suit specific sites would likely yield the best results (Gherardi et al. 2011). Reducing the abundances of NICS using these methods can, however, also induce substantial changes in multiple phenotypic traits, which can constrain the recovery of basic ecosystem functions (such as benthic primary production, decomposition of organic matter) (Závorka et al. 2020).

Crayfish invasions in Africa

Mainland Africa is devoid of native crayfish species. This could be either due to evolutionary competition with freshwater crabs (Ortmann 1902; Lodge et al. 2012; Souty-Grosset and Fetzner Jr 2016) or simply that they did not evolve in Africa (Souty-Grosset and Fetzner Jr 2016). Madagascar, on the other hand, has seven endemic crayfish species of the Astacoides genus (Boyko et al. 2005; Souty-Grosset and Fetzner Jr 2016). Nine NICS have introduction histories into the African continent, namely, the noble crayfish *A. astacus*, smooth marron *Cherax cainii* Austin and Ryan 2002, *C. destructor*, *C. quadricarinatus*, *F. limosus*, *P. leniusculus*, *P. clarkii*, *P. virginalis* and the White River crayfish *Procambarus zonangulus* (Hobbs and Hobbs III 1990) (Madzivanzira et al. 2020; Chapter 2). In Africa, NICS were introduced for aquaculture, the pet trade and fisheries (Weyl et al. 2020). Escape from captivity, intentional releases and unaided spread have resulted in the establishment of five NICS (*A. astacus*, *C. quadricarinatus*, *F. limosus*, *P. clarkii* and *P. virginalis*) in African freshwater systems (Madzivanzira et al. 2020). The establishment of NICS in freshwater systems in the continent is concerning, considering the documented impacts of NICS in other regions. A comprehensive review of crayfish invasions in Africa, including more information on crayfish, is presented in Chapter 2.

The community assemblages of the invaded system can impede or facilitate establishment of NICS via biotic resistance (deRivera et al. 2005; Alofs and Jackson 2014) especially in African freshwater systems rich in biodiversity (Darwall et al. 2009, 2011). In African freshwater systems, freshwater crabs of the genus Potamonautes are trophically analogous to freshwater crayfish and are present in almost all African freshwater habitats (Cumberlidge and Daniels 2007). Potamonautid crabs provide essential nutrient cycling services (Hill and O'Keeffe 1992; Dobson 2004; Cumberlidge and Daniels 2007; Peer et al. 2015) and exhibit high degrees of

endemism and range restriction (Cumberlidge 2018; Wood et al. 2019) which makes them vulnerable to the impacts of habitat destruction and IAS introductions (Cumberlidge and Daniels 2007; Zeng and Yeo 2018). Both crabs and crayfish are polytrophic benthic omnivores, and are both direct predators and opportunistic scavengers (Hill and O'Keeffe 1992; Grey and Jackson 2012). Due to the trophic similarities of these two decapods, it is likely that crabs could either provide an important component of biotic resistance or be competitively excluded by crayfish invasions (see Elton 1958; Tilman 1999; Alofs and Jackson 2014; Dick et al. 2017a). In an attempt to deduce the mechanisms behind potential biotic resistance of freshwater crabs towards the two dominant invasive crayfish in Africa, South et al. (2020) compared the closing force and chelae morphology of the Cape river crab *Potamonautes perlatus* and two crayfish species: *P. clarkii* and *C. quadricarinatus*. These traits can be used as proxies for agonistic contest outcomes and prey handling capabilities. Female *P. perlatus* were shown to possess a significantly stronger maximum chela closing force than both sexes of *P. clarkii* and female *C. quadricarinatus*, but did not differ significantly from male *C. quadricarinatus*. While this shows some capacity for indigenous African freshwater crabs to offer biotic resistance, the closing force was significantly correlated with body mass in all species. Therefore, if crayfish attain a higher mass than crabs, they are likely to retain a competitive advantage, which may explain abundance patterns observed in the field (South et al. 2020). Freshwater crabs are understudied globally, although large deficits remain, particularly in African systems, as a result of low perceived relevance (regarding fisheries and ecology) as well as difficulties in sampling. Because of this, there are various uncertainties regarding taxonomy as well as basic biological data (tolerances, fecundity and habitat associations).

Study species

Two species, the Australian redclaw crayfish, *Cherax quadricarinatus* and the Louisiana red swamp crayfish, *Procambarus clarkii* are rapidly spreading in Southern Africa. For logistical purposes, these two NICS were selected for this study and taken to represent NICS in Africa.

Australian redclaw crayfish Cherax quadricarinatus

The Australian redclaw crayfish *Cherax quadricarinatus* (also known as red claw, red-claw, with or without the prefix 'Australian' or 'Queensland', tropical crayfish, tropical blue crayfish, redclaw yabby, and blue lobster) is a parastacid crayfish (Decapoda: Astacidea: Parastacidae) first described as *Astacus quadricarinatus* by the German zoologist Karl Eduard von Martens in 1868 (Haubrock et al. 2021). The natural distribution of *C. quadricarinatus* extends across the northern tip of Queensland and the Northern Territory of Australia into the lowland parts of southern Papua New Guinea (Riek 1969). In its native habitat, *C. quadricarinatus* populations are found in lentic systems such as lakes, dams, billabongs and rivers with low water velocity (Jones 1990). Within these water bodies *C. quadricarinatus* are bottom dwellers, and use rocks, submerged fallen logs, leaf matter and any other debris as refugia (Ruscoe 2002). *Cherax quadricarinatus* is not a strongly burrowing species but will occasionally excavate burrows to escape from desiccation (Jones 1990; Masser and Rouse 1997; Johnston et al. 2010). Like most freshwater crayfish species, *C. quadricarinatus* is an opportunistic and polytrophic omnivore which can feed on: a variety of plant matter from periphyton to aquatic macrophytes (Momot et al. 1978; Momot 1995), animal proteins, including invertebrates (Abrahamsson 1966), frog and fish eggs (Lodge et al. 1985); and carrion (Momot 1995).

Plate 1a. Male Australian redclaw crayfish *Cherax quadricarinatus* (Photo: Takudzwa C Madzivanzira taken from the Barotse floodplain, Upper Zambezi Ecoregion, Zambia).

The harsh physical environmental extremes of the native region of *C. quadricarinatus* means that they have evolved to be a robust crayfish species with broad climatic tolerances (Jones 1990). Its preferred water temperature ranges between 23 – 31°C, however, it tolerates a broad range between 11°C to as high as 35°C (Jones 1990). Adult *C. quadricarinatus* have been shown to tolerate pH between 6.5 to 9 and dissolved oxygen (DO) levels as low as 1.0 mg/L, although juveniles are more sensitive to low DO levels (Masser and Rouse 1997). *Cherax quadricarinatus* tolerates unionised ammonia concentrations of up to 1.0 mg/L and nitrite levels as high as 0.5 mg/L for short periods without noticeable adverse effects (Masser and Rouse 1997). It is a fast-growing crayfish species, reaching 50 to 90 g in a year, and has been

recorded to reach weights of 590 g (Wingfield 2002). The maximum lifespan of *C. quadricarinatus* is between 4 and 5 years and it can reach, on average, a mass of 300 g over that period (Jones 1990; Masser and Rouse 1997). Sexual maturity is reached at 6 to 9 months of age, and the species is capable of producing multiple clutches of offspring each year in the right climatic conditions (Jones 1990; Masser and Rouse 1997; Wingfield 2002). Mature male *C. quadricarinatus* develop a distinctive red or orange patch on the outside margin of the claws (Plate 1a). Individuals of a similar size to the specimen shown in Plate 1a without the reddish claw patch are usually females (Masser and Rouse 1997), however, the sex of an individual is best identified by examination of the genital openings on the underside of the cephalothorax at the base of the walking legs (Masser and Rouse 1997). Male crayfish have two short projections shaped like acorns on the bases of the last 4th pair of walking legs, whereas females have openings at the base of the 2nd pair of walking legs (McLay and van den Brink 2016). In this species, a variable proportion of intersex individuals can be found in which both female and male genital openings occur in different combinations (Medley and Rouse 1993; Sagi et al. 1996; Parnes et al. 2003; Barki et al. 2006). Intersex *C. quadricarinatus* are genetically female, but possess male traits with respect to fighting and they mate with receptive females (Parnes et al. 2003; Barki et al. 2006).

Reproduction in *C. quadricarinatus* will only occur when water temperature is above 23ºC and females produce between 300 and 800 eggs per brood, which they brood for 6 to 10 weeks (Jones 1990). The process of reproduction involves a mating stage (see Plate 1d for *P. clarkii*) whereby the male *C. quadricarinatus* attaches a spermatophore (whitish sack containing sperm) to the underside of the female between the third and fifth pair of legs (Masser and Rouse 1997; Barki and Karplus 1999). Within 24 hours the female releases eggs from the pore on the base of the third pair of walking legs, which are fertilised by the spermatophore upon release

(Jones 1990; Masser and Rouse 1997). Female *C. quadricarinatus*, like other crayfish species, attach the eggs to the swimmerets under their tail, which then remain attached throughout the incubation period (4 to 6 weeks). Females carrying eggs are called 'berried' or 'gravid' females (Plate 1b) and are easily recognised because they tightly curl their tail under the body for the first 3 weeks after spawning, and are also less active during this period. After hatching, the hatchlings remain attached to the females' swimmerets for about a week and then become independent from the mother (Jones 1990; Masser and Rouse 1997).

Plate 1b. Berried female Australian redclaw crayfish *Cherax quadricarinatus* with juvenile crayfish (Photo: Takudzwa C Madzivanzira taken from sugar cane field irrigation ponds, Inkomati Basin, Mpumalanga Province, South Africa).

The physical and biological traits of *C. quadricarinatus*, as described in this section, make it an excellent candidate for aquaculture (Jones 1990; Masser and Rouse 1997; Jones et al. 2000). Its texture and flavour compare favourably with commonly eaten marine crustaceans and, having a lobster-like appearance, it is positioned at the premium end of the crustacean market spectrum (Masser and Rouse 1997; Jones et al. 2000). In Australia *C. quadricarinatus* aquaculture is comparatively low, with only around 70 tons produced per annum and only 1985 tons were produced worldwide in 2018 (https://www.cabi.org/isc/datasheet/89135). In many of the introduced ranges, commercial aquaculture ventures have not proved to be profitable but they do contribute some economic profit through small scale ventures and subsistence fisheries (Lodge et al. 2012; Madzivanzira et al. 2020). In Jamaica, introduced *C. quadricarinatus* provides an important economic subsidy to fishers, particularly in years with poor harvest of the native *Machrobrachium* shrimp species (Pienkowski et al. 2015). Apart from food, *C. quadricarinatus* is also used for recreational fishing, research and ornamental purposes (Ahyong and Yeo 2007; Belle et al. 2011; Lodge et al. 2012). With such a wide range of utilities and its robust traits, the species has been translocated across Australia (Doupé et al. 2004; Leland et al. 2012) and to all continents except Antarctica (Lodge et al. 2012). *Cherax quadricarinatus* is the second most cultured and caught crayfish species globally and its importance in aquaculture and popularity in the aquarium trade have resulted in numerous species translocations, making *C. quadricarinatus* globally the second most cosmopolitan crayfish (Haubrock et al. 2021). As a consequence of escape from aquaculture facilities and the pet trade, plus illegal releases by recreational fishers, wild populations have established in a number of countries (Harlioğlu and Harlioğlu 2006, Lodge et al. 2012; Madzivanzira et al. 2020).

Louisiana red swamp crayfish Procambarus clarkii

The native range of *P. clarkii* extends from the South Central United States of America to Northern Mexico (Hobbs 1972) where wild populations have traditionally been harvested for human consumption (Hobbs and Lodge 2010). The native habitats for *P. clarkii* are seasonally dry and wet which permits the growth of vegetation that serves as food when the area is flooded (Huner and Barr 1991). When its habitat dries, *P. clarkii* burrows regardless of the time of the year. This makes *P. clarkii* an ecosystem engineer, as it constructs simple burrows consisting of a single opening, covered with a mud plug or chimney to reduce evaporation from the water's edge, and a tunnel widening to an enlarged terminal chamber (Huner and Barr 1991; Correia and Ferreira 1995). In periods of elevated temperatures or drought, these burrows can extend 0.4 – 1 m down the water table (Huner and Barr 1991). Burrow density is typically greatest in habitats with vegetation and fine sediments, and lowest in areas of gravel, sand or cobble with less vegetation (Correia and Ferreira 1995; Barbaresi et al. 2004). *Procambarus clarkii* exhibits considerable ecological plasticity, tolerating a range of environmental factors including temperature (provided water in burrows neither freezes nor exceeds 35°C (optimum range is 21 to 30°C)), pH (5.8–10), DO (as low as 3 mg/L) (Huner and Barr 1991), salinity of up to 35 ppt (Bissattini et al. 2015), and high pollution levels (Huner and Barr 1991). As with most crayfish, *P. clarkii* is an opportunistic omnivore, consuming plant material, detritus, animals and sediment (Huner and Barr 1991; Smart et al. 2002; Gherardi 2007; Gherardi and Barbaresi 2007). *Procambarus clarkii,* however, does show a persistent preference for plants and/or detritus, with plant consumption highest in summer and detritus feeding intense all year round (Correia 2002; Gherardi and Barbaresi 2007). Macroinvertebrates and fish are the predominant animal constituents of *P. clarkii* diet (Pérez-Bote 2004; Ilhéu et al. 2007).

Plate 1c. *Procambarus clarkii* collected from Mimosa Dam in Odendaalsrus, Free State Province, South Africa (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory).

Compared to *C. quadricarinatus*, the lifespan of *P. clarkii* (≤ 2 years) is shorter, but juveniles have high survival rates (Huner and Barr 1991). *Procambarus clarkii* is capable of spawning all year-round and some females can reproduce more than once per year. This species exhibits high fecundity: a 10 cm female can produce up to 500 eggs, whilst a smaller female can produce around 100 eggs (Huner and Barr 1991). The sexes, as with other crayfish species, (as described in the *C. quadricarinatus* section of this Chapter) are best identified by examination of the genital openings on the underside of the cephalothorax at the base of the walking legs (Masser and Rouse 1997). Reproduction in *P. clarkii* is regulated by pheromones perceived by receptors

located on the two antennae responsible for inter- and intraspecific recognition and behavioural modulation (Ameyaw-Akumfi and Hazlet 1975). The process of mating (Plate 1d) and reproduction is similar to that of *C. quadricarinatus* as well as other crayfish species. Berried female *P. clarkii* remain in their burrows as long as eight weeks and hatched craylings must moult twice before becoming self-sufficient (Hunter and Barr 1991).

Plate 1d. *Procambarus clarkii* mating in an experimental tank (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

Procambarus clarkii is regarded as the most cosmopolitan freshwater crayfish species and has been introduced to all continents except Antarctica and Oceania (Oficialdegui et al. 2019).

Aquaculture of *P. clarkii* is a multi-million-dollar industry in the USA, producing 72682 tons in 2018 (https://www.cabi.org/isc/datasheet/67878). Due to the voracity and omnivory of *P. clarkii*, it has been touted as a biocontrol agent for the snail vectors of *Schistosoma* sp. parasites which cause bilharzia as well as for the malaria vector *Anopheles* sp. mosquitoes (Madzivanzira et al. 2020). *Procambarus clarkii* is recognised as one of the world's worst invaders (Loureiro et al. 2015). In Europe, *P. clarkii* contributed to the dramatic decline of the native crayfish in the Astacidae family through direct competition and its transmission of *A. astaci* (García-Arberas et al. 2009; Gherardi 2007). Predatory impacts of *P. clarkii* have been recorded in the Iberian Peninsula (Cruz and Rebelo 2007), Sweden (Ilhéu et al. 2007), and Europe (Gherardi 2007). The burrowing behaviour of *P. clarkii* has increased bioturbation, altered water quality and increased nutrient release from sediment (Angeler et al. 2001) which induced cyanobacterial blooms (Yamamoto 2010). These effects were recorded in Las Tablas de Daimiel National Park, Spain (Angeler et al. 2001); Alentejo, Portugal (Geiger et al. 2005); and Japan (Yamamoto 2010). Intense herbivory by *P. clarkii* has been recorded in the Lake Chozas, Spain (Rodríguez et al. 2003); Lake Naivasha, Kenya (Smart et al. 2002); Lake Massaciuccoli, Italy (Gherardi et al. 1999); Lake Doccia, Italy (Gherardi and Acquistapace 2007); the Mediterranean wetlands (Geiger et al. 2005); and the Iberian peninsula (Cruz and Rebelo 2007), affecting the aquatic macrophyte species *Nymphoides peltata*, *Potamopeton crispus*, *Ultricularia australis* and *Potamogeton* sp. (Gherardi and Acquistapace 2007). Negative ecological impacts of *P. clarkii* have been well documented in the world with the exception of Africa.

Assessing distribution of NICS

Given the difficulty of controlling NICS once they are established, early detection and rapid response programs has become a critical element, particularly if eradication is the management

goal (Tobin et al. 2018; Faulkner et al. 2020). To obtain accurate population estimates, understand community structures, and monitor populations efficiently, effective sampling methods have to be implemented (Rabeni et al. 1997; Dorn et al. 2005). Although numerous sampling methods for crayfish populations are commonly used, most have serious biases and unknown efficiencies (Williams et al. 2014; Chadwick et al. 2020). Various passive and active sampling methods to capture and study crayfish have been employed for several decades (Williams et al. 2014). Methods include baited traps, hand netting/collecting, snorkelling or bankside above stream observation, visual assessments using SCUBA, electrofishing, and enclosure nets and seines (Haag et al. 2013; Williams et al. 2014; Larson and Olden 2016). Lentic or large lotic environments typically necessitate sampling by one of these approaches, with baited traps being the most commonly applied, although they are biased towards large adults and males over other members of the population (Brown and Brewis 1978; Capelli and Magnuson 1983). Recently, a novel 'triple drawdown' (TDD) method was developed to sample invasive populations of *P. leniusculus* in Northern England (Chadwick et al. 2020). This method revealed large numbers of juveniles which were not detectable using traditional methods. However, this method is only possible in smaller streams and with the correct technical infrastructure.

Baited traps to sample crayfish vary by design and dimensions between studies and countries, from the Swedish trappy commonly used in Europe to modified Gee minnow traps in North America to the baited hoop nets used in Australia (Larson and Olden 2016). A wide variety of gear have been used in crayfish surveys in Africa, such as Promar© collapsible traps (dimensions 61 cm × 46 cm × 20 cm; mesh size 10 mm) baited with dry dog food used for *C. quadricarinatus* sampling in South Africa (Nunes et al. 2017a), Swaziland (Nunes et al. 2017a), and the Upper and Middle Zambezi systems (Madzivanzira et al. 2020; Madzivanzira et al. In

Review; Chapter 4), opera traps baited with cooked maize meal (Marufu et al. 2014, 2018a, 2019) used for *C. quadricarinatus* sampling in Lake Kariba Zimbabwe (Marufu et al. 2014; 2018a; 2020), collapsible and cylindrical fladen traps (dimensions: 60 × 30 cm; mesh size 20 mm) baited with proprietary meatloaf for dogs used for *C. quadricarinatus* sampling in Kafue Zambia (Douthwaite et al. 2018); rectangular traps (dimensions 100 cm × 50 cm × 50 cm; mesh size 1 cm) (100 cm × 25 cm × 25 cm; mesh size 1 cm) (50 cm × 25 cm × 25 cm; mesh size 1 cm) baited with largemouth bass fish heads, Promar© collapsible traps (dimensions 61 cm × 46 cm × 20 cm; mesh size 1 cm) baited with dry dog food and pipe traps used for *P. clarkii* sampling in South Africa (Barkhuizen et al. Unpublished data), rectangular traps (dimensions 80 cm × 25 cm × 25 cm; mesh size 0.5 cm) baited with local fish or sardines (depending on availability) used for *P. clarkii* sampling in Morocco (Yahkoub et al. 2019), unspecified traps (dimensions 50 cm × 30 cm × 30 cm; mesh size 1 cm) baited with meat used for *P. clarkii* sampling in Kenya (Lowery and Mendes 1977), unspecified crayfish traps baited with pieces of freshwater fish (*Tilapia* sp., Nile perch or Largemouth bass) used for *P. clarkii* sampling in Kenya (Foster and Harper 2006), cylindrical traps (unspecified dimensions; mesh size 1.5 cm) baited with catfish used for *P. clarkii* sampling in Egypt (Ibrahim and Khalil 2009), and collapsible traps (dimensions 61 cm × 46 cm × 20 cm; mesh size 1 cm) baited with chicken meat used for *P. clarkii* sampling in Tunisia (Bouaoud et al. 2020).

Heterogeneous gears and sampling methods can result in disparate results in surveys seeking to estimate abundance or even the distribution of crayfish. For each NICS it is therefore not possible to incorporate data taken using different gears for comparison purposes unless the traps are standardised. Standardisation of sampling has been a critical component in the advancement of science, and most disciplines which inherently rely on field ecology, ranging from meteorology to water quality and chemistry monitoring, use data collection and reporting

techniques and protocols that are standardised between research groups and political jurisdictions (Larson and Olden 2016). Therefore, standard gear has to be agreed on for each species so that catch per unit effort (CPUEs) is comparable across the world.

Predicting impacts using functional responses and relative impact potential metrics

Freshwater crayfish invasions have been studied extensively around the world, but less so in Africa (see Madzivanzira et al. 2020; Chapter 2). This is of concern because crayfish invasions have generally been shown to result in strong impacts on recipient ecosystems (Lodge et al. 2012; Twardochleb et al. 2013). Given the absence of native crayfish on the African continent, negative impacts on native decapods, such as freshwater crabs from the Potamonautes genus, are likely to be strong (de Moor 2002; Nunes et al. 2016; Madzivanzira et al. 2020), as they have a very similar trophic position and have similar preferred habitats to crayfish, and are the most likely taxa to come into direct competition with introduced crayfish. Crabs are also likely to be more susceptible than other taxa to parasites that might be introduced in association with crayfish since the crayfish species have a greater resistance to the introduced parasites with which it has co-evolved with (de Moor 2002). Scientists and practitioners have been frustrated by a lack of predictive methodologies to reliably identify potentially damaging invaders and their likely magnitude of ecological impact (Laverty et al. 2017). Understanding the impact of IAS will promote progress toward a better understanding of the implications of biodiversity and ecosystems changes caused by IAS, which in turn will help to disentangle the aspects of scientific debates about IAS that are due to disparate definitions (Jeschke et al. 2014). This will therefore improve communication between scientists from different research disciplines and between scientists, managers, and policy makers (Dick et al. 2013, 2014, 2017a,b; Ricciardi et al. 2013; Jeschke et al. 2014). Predictive models are therefore needed to assess the ecological

impacts of IAS in the receiving ecosystem (Ricciardi and Rasmussen 1999; Ricciardi 2003; Dick et al. 2013).

A useful predictive tool in invasion biology is functional response (FR), which is a classical ecological phenomenon that describes the relationship between resource availability and consumption rate (Solomon 1949; Holling 1959, 1966). Functional response experiments have been used as a method of measuring and quantifying impacts of IAS across a range of trophic and taxonomic groups (see Uiterwaal et al. 2018; Functional Responses from Around the Globe in all Ecosystems FoRAGE database). The ability to incorporate abiotic dependencies (e.g. temperature and light regimes) in the FR approach makes it a robust and reliable tool to quantify impacts under environmental changes (Alexander et al. 2014; Iacarella et al. 2015; Dick et al. 2014; South et al. 2018; Dickey et al. 2018, 2020). Abiotic context dependencies must be incorporated in predator-prey model dynamics due to their ability to alter interaction strengths (Parker et al. 1999; Iacarella et al. 2015). Invasive alien species have been shown to exhibit higher per capita effects when compared to native analogous species (Dick et al. 2013, 2014; Alexander et al. 2014) and thus resources, such as native prey communities, are vulnerable to potential population depletion or extirpations (MacRae and Jackson 2001; Cucherousset and Olden 2011).

Solomon (1949) and Holling (1959) described three response curves. Type I is a linear response wherein handling parameter is not limited by the resource density (MacNeil et al. 1997; Jeschke et al. 2004) (See Fig. 1.1); Type II FR is characterised by a decelerating intake rate where the consumer is limited by behavioural and/or physiological processes, with such responses potentially leading to prey extirpation due to high proportional predation at low prey densities

(Juliano 2001) (See Fig. 1.1); Type III FR is sigmoidal, with low proportional intake rates at low prey densities (Holling 1959) which leads to low prey density refugia that can potentially stabilise prey populations (See Fig. 1.1). Holling (1959) created the original mathematical description of the Type II FR, known as Holling's disc equation. The mathematical function for Type III FR was simplified by Real (1977) using an analogy of allosteric enzymes and the Michaelis-Menton model. Type III FRs have typically been associated with predator learning (Dawkins 1971; Krebs 1974; Real 1977) and prey switching behaviours (Akre and Johnson 1979). There is some debate around the idea that Type III FRs can and do occur in a single predator–prey environment, as there is no need for switching behaviours to occur (Murdoch et al. 1975; Kalinkat et al. 2011).

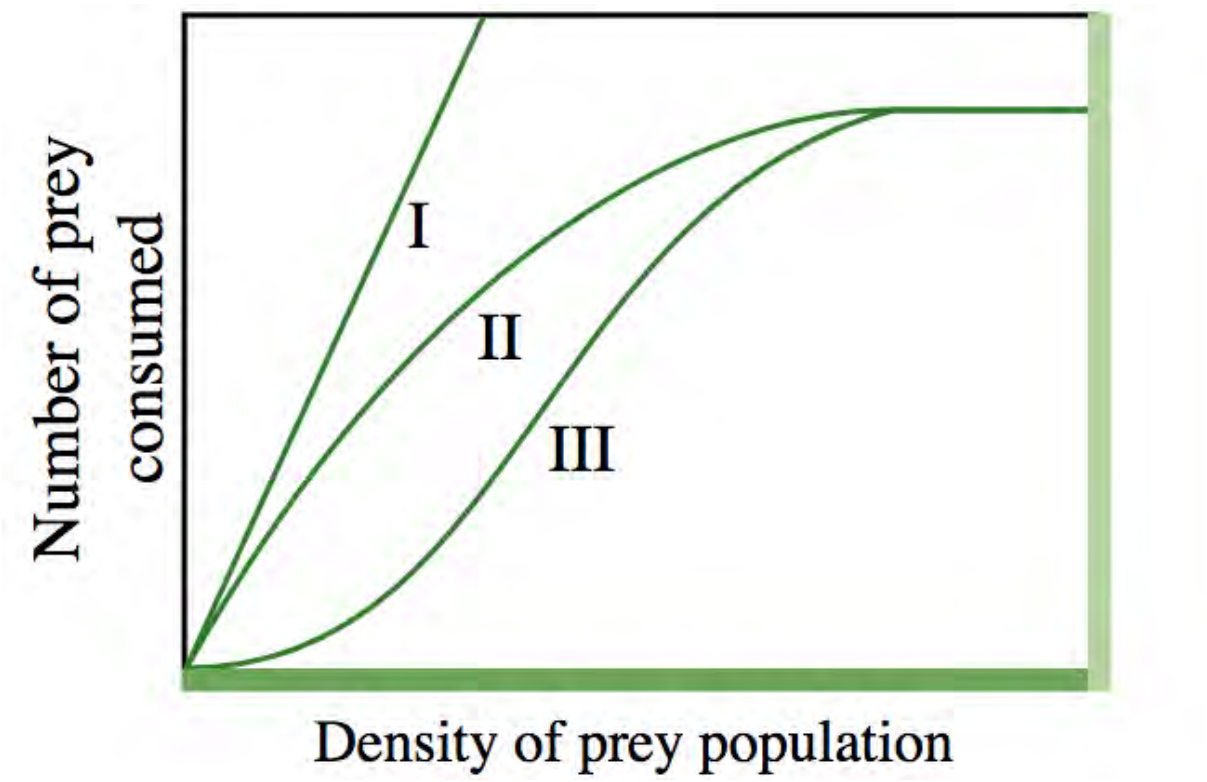

Figure 1.1. Graphs showing categorical functional response types (Type I, Type II, Type III).

The comparative functional response (CFR) approach allows for the explicit incorporation of context-dependent factors into experimental methodologies to predict high-risk invasion scenarios (Barrios-O'Neill et al. 2014a,b; Dick et al. 2014, Iacarella et al. 2015). A species with a significantly higher consumption rate than an analogous comparator species has the ability to acquire more resources, that is their interspecific interactions will be asymmetric (Britton et al. 2019). Unlike predation studies, which seek to directly measure the impact of an IAS in a particular location or on a particular species, the CFR approach uses simplified experimental conditions to generate relative (not absolute) parameters comparable across contexts (Howard et al. 2018). Studies using the CFR approach have consistently demonstrated that IAS, ranging from plants (Funk and Vitousek 2007) to invertebrates (Dick et al. 2013) and vertebrates (Alexander et al. 2014), consume available resources at a higher rate than their respective native analogous species (Howard et al. 2018). While these results support the general concept that successful IAS do well, in part, because they are more efficient at resource utilisation, context-dependent biotic interactions or abiotic conditions can cause variation in IAS FRs (Barrios-O'Neill et al. 2014a; Barrios-O'Neill et al. 2016; Paterson et al. 2015). Context dependencies as well as species-specific traits and species density were shown to play an important role when comparing the FR between populations occurring in their native and invaded ranges (Boets et al. 2019; Grimm et al. 2020).

One of the main developments of FR analysis is obtaining the most accurate parameter estimates, as these are the mechanistic components of the FR. The parameters are attack parameter (a), handling parameter (h), and maximum feeding rate estimate, defined as $1/hT$, wherein T is a constant fixed at 1 that represents the total time available for predation (Houck and Strauss 1985). Albeit T may be manipulated to produce parameters on different scales, but is usually fixed at 1 to represent experimental time. Therefore, 'h' is estimated as a fraction of

T, and in this case, represents several behaviours related to the searching, subduing, processing and digestion of prey items (Jeschke et al. 2002). Attack parameter is defined as the rate of potential detection and is expressed as a function of the number of encounters (Real 1977). To obtain the parameter estimates, the disc equation must be solved, but the disc equation relates explicitly to a predator searching at constant speed for randomly distributed prey items at 'a' constant 'h', with a constant prey density (Holling 1959; Houck and Strauss 1985). This becomes a logistical issue when trying to experimentally derive values for the FR of a species due to having to replace prey as they are consumed. This led to the development of the Random Predator Equation derived by Rogers (1972) and Royama (1970), which incorporates prey depletion into the model. To determine FR as Type II or Type III, the proportion of prey consumed to prey density for each population must first be fitted using a logistic regression with the package 'frair' (frair::frair_test). Because the logistic regressions generate negative first-order terms in all cases, indicative of Type II FR (Juliano 2001), the data must be fitted using the appropriate random predator equation, without prey replacement (Rogers 1972):

$$Ne = N_0(1 - exp\,(a(N_e h - T))) \quad (1)$$

where Ne is the number of prey eaten, N_0 is the initial density of prey, a is the attack parameter, h is the handling parameter and T is the total time available.

To compare the fitted FR curves among experimental treatments, the data is non-parametrically bootstrapped to produce 95% confidence intervals (CI) on the fit (Pritchard et al. 2017). Using this method, models between populations may be statistically compared by simply observing the overlap/lack of overlap, between model CIs (Pritchard et al. 2017). In addition, the *frair::frair_compare* function of the 'frair' R package (Bolker 2010; Pritchard et al. 2014) can be used to compare the differences in *a* and *h* estimates between populations, but is limited to

comparing those that are fit by the same model type, however this can be corrected by bootstrapping the estimates (Pritchard et al. 2017).

More recently, progress in developing the FR method towards predictive capacity has resulted in the combination of the parameters a and h to derive a Functional Response Ratio (FRR) (Cuthbert et al. 2019). This helps to elucidate potential intermediate impact where previously distinguishing differences between FR curves were problematic. The metric is centred in the assertion that high a values indicate high impact, while low h also implies high impact. Thus, the parameters are combined to create a ratio of a/h, higher values of which are found in high-impact invaders (Cuthbert et al. 2019). The FRR is calculated as:

$$FRR = a / h \quad (2)$$

where a is the attack parameter and h is the handling parameter derived from the FR curve.

The use of CFRs can be utilised further by altering the Parker-Lonsdale equation (Parker et al. 1999) wherein Impact (I) of an IAS can be quantified by Range (R), Abundance (AB) and per capita effect (E) (Equation 3).

$$I = R \, x \, AB \, x \, E \quad (3)$$

Dick et al. (2017b) developed the Impact Potential (IP) metric, which removes the range and makes IP the product of abundance (AB) and FR as a proxy for the per capita effect of a consumer (Equation 4).

$$IP = AB \, x \, FR \quad (4)$$

From this Equation 4, Dick et al. (2017b) proposed a Relative Impact Potential (RIP) metric which compares the impact of an invader to that of a native (Equation 4). When RIP is < 1 the IAS will have less impact than the native species and when RIP = 1 the IAS will have no impact above the native species; and when RIP > 1 there will be likely IAS impacts on prey populations. The (RIP) is calculated from:

$$RIP = \left(\frac{FR\ invader}{FR\ native}\right) \times \left(\frac{AB\ invader}{AB\ native}\right) \quad (5)$$

The RIP provides scientists and practitioners with a customisable, user-friendly and crucially powerful technique to inform IAS policy and management (Dickey et al. 2020). The RIP can further be used for other predictive elements which include (1) using propagule pressure to quantify the overall invasion risk, (2) assessing impact in the face of climate change by taking account of both changes associated with predator consumption rates and prey reproduction rates, (3) assessing biotic resistance against incoming IAS, (4) assessing the effect of evolution on IAS impacts, and (5) applying interspecific competition, changing the spatio-temporal patterns of invasion (Dickey et al. 2020).

Experiments to derive FR are informative but often are somewhat simplistic in the environments delivered and do not usually mimic the natural movement of prey between patches. These issues can be resolved by complimenting laboratory studies with field data (Smout and Linstrøm 2007; Dick et al. 2013; Barrios-O'Neill et al. 2014b) in order to further inform and corroborate predictions. It is also possible to observe FR in situ, which is more practical for larger species and species with complex needs. Documenting FRs in situ, however, can be misleading as predators are expected to aggregate in patches where prey is abundant, thus making it difficult to successfully document feeding rates in less abundant patches (Beauchamp 2009).

The merit of FR analysis is that it can be used to investigate ecological impact under numerous contextual influences, and combined with relevant field data on abundance or fecundity it could be subverted from being a predictor of biotic invasions to a general predictor of relative ecological impact by native species under climate change scenarios (Real 1979; Laverty et al. 2015; Dick et al. 2017a,b). This is investigated further in this study, as the FR of native predator species was assessed under different temperature conditions and resources (static and mobile) and combined with field data in order to understand the socioecological impacts of crayfish species.

Thesis outline

This study aims to: 1) provide a better understanding of the drivers and pathways that have facilitated crayfish introductions in Africa, 2) improve the understanding of the nature and magnitude of their associated impact by 3) providing a comprehensive base of knowledge on the invasion status of the Zambezi system with regards to current status, distribution, traits and areas at risk of invasion and 4) identify knowledge gaps to better prioritise their management and, ultimately, avoid further introductions. To this end, this thesis is divided into nine chapters, the first being the current introduction, followed by seven data chapters (Chapters 2 – 7) and a final discussion in Chapter 9. The seven data chapters explore the following:

- **Chapter 2**

  Literature from various sources was gathered, reviewed and synthesised to provide information on crayfish introductions, establishment, distribution and impacts in Africa, to identify future research needs on the continent. This chapter was published as: Madzivanzira TC, South J, Wood LE, Nunes AL, Weyl OLF (2020) A review of

freshwater crayfish introductions in continental Africa. *Review in Fisheries and Aquaculture*.

- **Chapter 3**

  Two methods that were used for *C. quadricarinatus* abundance studies in Southern Africa were compared to determine a standard sampling gear in Africa. This chapter is under review in the journal, Water SA:

  Madzivanzira TC, South J, Nhiwatiwa T, Weyl OLF (Accepted) Standardisation of Australian redclaw crayfish *Cherax quadricarinatus* sampling gear in southern Africa. *Water SA*.

- **Chapter 4**

  This chapter determined the invasion status and extent of invasion of *C. quadricarinatus* in the Zambezi Basin using a standard method depicted in Chapter 3. This chapter is under review in the journal, Aquatic Conservation: Marine and Freshwater Ecosystems:

  Madzivanzira TC, South J, Ellender BR, Chalmers R, Chisule G, Coppinger CR, Khaebeb HF, Jacobs FJ, Chomba M, Musando B, Mwale C, Nhiwatiwa T, Rennie CL, Richardson N, Weyl OLF (In Review) Distribution and establishment of the alien Australian redclaw crayfish, *Cherax quadricarinatus*, in the Zambezi Basin. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

- **Chapter 5**

  This chapter made the first attempt to quantify the potential impacts of *C. quadricarinatus* and *P. clarkii* in Africa. The functional responses of the two invasive crayfish species were compared with that of a native trophic analogue, the freshwater

crab *Potamonautes perlatus*, under two temperature treatments using catfish fry as prey. This chapter was published as:

Madzivanzira TC, South J, Weyl OLF (2021) Invasive crayfish outperform Potamonautid crabs at higher temperatures. *Freshwater Biology*, **00**: 1-4. doi: 10.1111/fwb.13691.

- **Chapter 6**

  This chapter evaluated the potential for ecological impacts using consumer-resource dynamics of the two established crayfish species, *C. quadricarinatus* and *P. clarkii* in comparison to the native trophic analogue, the freshwater crab *Potamonautes perlatus*, in preying upon various macroinvertebrate prey using comparative functional responses. This chapter will be submitted to a journal.

- **Chapter 7**

  This chapter evaluated the potential for socioecological impacts using consumption experiments of the two established crayfish species, *C. quadricarinatus* and *P. clarkii* in comparison to the native trophic analogue, the freshwater crab *Potamonautes perlatus*, on two static resources, macrophytes and dead fish. This chapter will be submitted to a journal.

- **Chapter 8**

  This chapter combined FR and consumption data from Chapters 5 – 7 with field abundance data to calculate the RIP of crayfish species relative to the native crabs. This chapter will be submitted to a journal.

CHAPTER 2

A REVIEW OF FRESHWATER CRAYFISH INTRODUCTIONS IN AFRICA

Abstract

This review summarises and analyses information on freshwater crayfish introductions in Africa. A total of 136 research papers and reports were found to be relevant. Forty-eight percent reported presence; 21% described negative impacts; 11% referred to potential socio-economic benefits; 9% evaluated control measures; 6% documented co-introduced parasites. Out of nine introduced crayfish species, five species *Astacus astacus*, *Cherax quadricarinatus*, *Faxonius limosus*, *Procambarus clarkii*, and *Procambarus virginalis* have established populations in the wild. *Astacus astacus* and *F. limosus* are present only in Morocco and *P. virginalis* is limited to Madagascar. *Cherax quadricarinatus* and *P. clarkii* have established populations in five and six countries, respectively. The main driver of crayfish introductions was to provide socio-economic benefits through aquaculture and fisheries development but there is limited evidence of success. Prevailing negative socio-economic impacts are linked to damage to agricultural water infrastructure, damage to fishing gear and declining fisheries performance. Ecological impacts pertain to direct and multi-trophic consumptive effects as well as indirect competitive effects primarily upon macro-invertebrates and potential spillover of parasites to other decapods. Research priorities are determining abundance, distribution and spread of crayfishes and assessing ecological impact to inform management decisions.

Introduction

Freshwater crayfishes include 692 described species that are naturally distributed in all continents except mainland Africa and Antarctica (Crandall and De Grave 2017). Freshwater crayfish have been introduced into all continents except Antarctica (Lodge et al. 2012). Intentional introductions have been for fisheries enhancement (Audenaerde 1994; Foster and Harper 2007; Nunes et al. 2017c), aquaculture (Lodge et al. 2012; Souty-Grosset and Fetzner 2016), and the pet trade (Lodge et al. 2012; Twardochleb et al. 2013; Souty-Grosset and Fetzner 2016). Unintentional introductions have occurred as stowaway propagules or via spread through interconnected waterways (Lodge et al. 2012).

Generally, freshwater crayfish species exhibit broad tolerances to a wide range of environmental conditions (Lodge et al. 2012), which, together with their large individual adult size, high fecundity and omnivorous trophic position (Hobbs and Lodge 2010), has facilitated their establishment and spread in a wide variety of freshwater habitats globally (Hobbs et al. 1989; Lodge et al. 2012; Twardochleb et al. 2013). Where present, freshwater crayfish tend to dominate invertebrate biomass (de Moor 2002; Geiger et al. 2005; Gherardi 2007) and, in their invasive range, they tend to exert an overall negative ecological impact on native biodiversity evidenced by reductions in the abundance of macrophytes, aquatic invertebrates, amphibians, and fishes (Twardochleb et al. 2013).

Mainland Africa is naturally devoid of freshwater crayfish species (Crandall and Buhay 2008; Lodge et al. 2012), in this region decapods in freshwater ecosystems are largely represented by potamonautid crabs (Wood et al. 2019). It is hypothesised that the absence of native crayfish in mainland Africa is either a result of evolutionary competition with freshwater crabs

(Ortmann 1902; Lodge et al. 2012; Souty-Grosset and Fetzner 2016) or that they never evolved there to begin with (Souty-Grosset and Fetzner 2016). In contrast, Madagascar has seven endemic crayfish species of the genus Astacoides which are phylogenetically distinct from all other currently described crayfish species (Boyko et al. 2005; Ganzhorn et al. 2013) and are endemic to an inland area of about 60 000 km^2 in the southeast highlands of Madagascar (Boyko et al. 2005). The presence of crayfish on Madagascar is likely due to the unique evolutionary dynamics and paleogeography of the island which has been separated from mainland Africa since the breakup of Gondwana in the Toarcian period around 160 million years ago (Biswas 2008).

There is evidence that nine crayfish species have been introduced into Africa: the noble crayfish *Astacus astacus* (Linnaeus 1758), smooth marron *Cherax cainii* Austin and Ryan 2002, yabby *Cherax destructor* Clark 1936, Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868), spiny cheek crayfish *Faxonius limosus* (Rafinesque 1817), American signal crayfish *Pacifastacus leniusculus* (Dana 1852), Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852), marbled crayfish *Procambarus virginalis* (Lyko 2017), and White River crayfish *Procambarus zonangulus* Hobbs and Hobbs 1990 (see https://www.cabi.org/isc/ and https://www.gbif.org/). Information on the introductions of these species, their status and distribution in different African countries, is scattered. In addition, given the evidence of ecological impacts on recipient ecosystems elsewhere (Lodge et al. 2012; Twardochleb et al. 2013), it is important that the consequences of these introductions are evaluated to assess the extent of their impacts and inform their management (Lodge et al. 2012).

This review summarises and analyses information on all crayfish introductions in Africa (including Madagascar and the island states), their pathways of introduction into each country and spread into the wild, as well as their current status, distribution, and reported impacts. This study compiles current information to better understand the reasons and pathways that have driven these introductions, improve the understanding of the nature and magnitude of the environmental impacts they have generated, and identify knowledge gaps to better prioritise their management and, ultimately, avoid further introductions.

Materials and methods

This systematic review was conducted following the guidelines for Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (see Moher et al. 2009), which include the establishment of a search protocol, data inclusion, data extraction, and analysis. The search protocol was undertaken using both the Institute of Scientific Information (ISI; Thomson Reuters) Web of Science online database and the SCOPUS database. These databases were used to search for English literature containing relevant information on crayfish species and introductions in Africa, published through to the end of 2019. For this, the search terms included the keyword combinations "crayfish"* AND "Africa."* With the aim of potentially retrieving additional relevant publications on this topic, given the number of lusophone and francophone countries in the region, the scope of the search protocol was enlarged to include articles published in Portuguese and French. In a similar manner to the English literature procedure, in March 2020 a search was carried out using the same terms, but in the two different languages ("lagostim" AND "Africa" in Portuguese, and "écrevisse" AND "Afrique" in French), in both ISI Web of Science and SCOPUS. Due to the extremely limited (or non-existent) number of results obtained for the searches in the two languages in these databases (see Results below), an additional search was undertaken in Google Scholar using the same

search terms. As Madagascar is the only country in Africa with native crayfishes, the literature considered in the analysis was for introduced crayfish species only.

The potential of each publication to contribute relevant information to this systematic review was subsequently examined. Publications that were clearly not related to the topic under study were excluded based on their title, abstract or after a careful reading of the entire text, if necessary (Pereira et al. 2019). Studies that were not possible to fully access online were requested from authors by e-mail. After examining all the articles selected by the inclusion criteria, the reference lists of all publications were checked for additional relevant articles and grey literature (Pereira et al. 2019). Those which were not found in previous searches were then included in the analysis. Grey literature is reported as "unpublished data".

After article examination, the countries where crayfishes were reported, the year reported, the crayfish species reported, the pathway of introduction/spread and the distribution in the country, where available, were recorded. The articles were further categorised according to the information generated by each study under the following headings: (1) introduction, distribution and spread; (2) co-introduced parasites; (3) impacts; (4) socio-economic benefits; (5) control. As many of the publications reviewed address several of these topics, a single article could be categorised under various headings.

Results

Literature search

Results presented herein are based on the total number of articles found in the ISI Web of Science and SCOPUS databases, and in Google Scholar for the Portuguese and French language searches, that matched the inclusion criteria (Pereira et al. 2019), as well as in the literature obtained from the reference lists of the selected publications. The search of English literature on the Web of Science database resulted in 420 articles, of which only 75 matched the inclusion criteria. The same search on the SCOPUS database resulted in 52 articles, all of which had already been retrieved from the ISI Web of Science search, hence, only results from the ISI Web of Science were used in this study. An additional 56 articles of interest, which were initially not found in any of the databases but were, however, sourced from the initial searched articles reference lists, were included in the final results.

Regarding the searches of literature in Portuguese and French, no results were found in the ISI Web of Science database. The search performed in SCOPUS yielded 12 results in Portuguese and nine results in French, but none of those were relevant for this study. The search performed using Google Scholar produced 425 results in Portuguese, none of which were of relevance to this review. The same search in French showed 3210 results, although, Google Scholar only displays the first 1000 search records which, in this case, were actually limited to 850 results. Out of these 850 results, only five articles were relevant for this study, and their reference lists did not generate any additional relevant articles. All the data used for the review is available on “https://doi.org/10.1080/23308249.2020.1802405”.

The earliest paper included in the present study was published in 1948 and described the introduction of crayfish species in Morocco. Since then, the number of papers studying invasive crayfish in continental Africa has been increasing, with a steady increase especially since 1988, and with most papers published in the period 2015–2019 (Figure 2.1). Forty-five percent of the papers reported on crayfish presence, distribution and spread; 6% reported on co-introduced parasites associated with their introductions; 11% of the articles assessed crayfish impacts; 31% referred to their socio-economic benefits; and 8% reported on crayfish control.

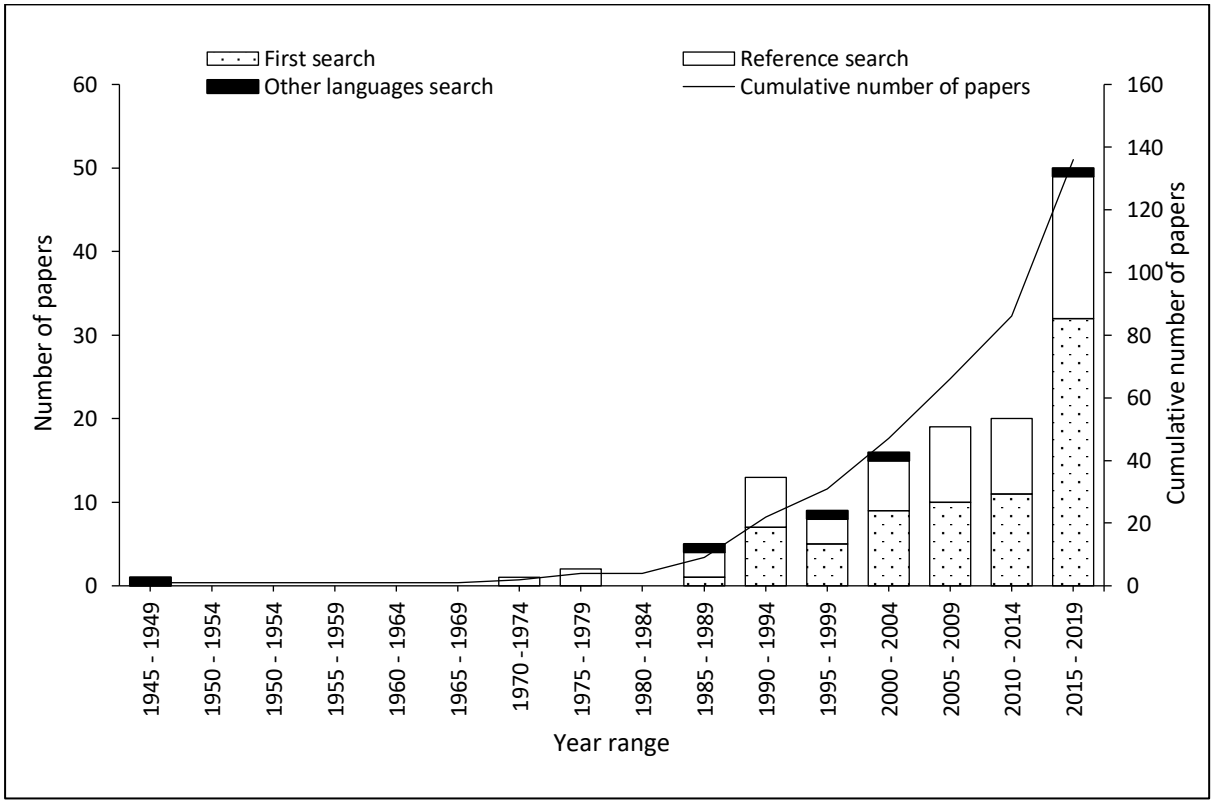

Figure 2.1. Number of papers (and cumulative number of papers) found in the English (first and references search) and other languages search (French and Portuguese) referring to crayfish introductions in continental Africa from 1948 to 2019.

Introduction, establishment, and spread

From the literature search, there is evidence for the introduction of nine crayfish species into Africa. These are three Australasian Parastacidae species, *Cherax quadricarinatus*, *Cherax cainii*, and *Cherax destructor*; five North American Cambaridae species, *Procambarus clarkii*, *Procambarus virginalis*, *Procambarus zonangulus*, *Pacifastacus leniusculus*, and *Faxonius limosus*; and a European Astacidae species, the European noble crayfish *Astacus astacus*. Of the nine introduced species, there is evidence for establishment in the wild of five species: *A. astacus*, *C. quadricarinatus*, *F. limosus*, *P. clarkii*, and *P. virginalis*. Details of these crayfish introductions are reviewed by country (in chronological order) below and are summarised in Tables 2.1 – 2.3. Internal translocations between African countries of *C. quadricarinatus* and *P. clarkii* are shown in Figures 2.2 and 2.3.

Table 2.1. *Astacus*, *Faxonius*, and *Pacifastacus* species introduced to continental Africa, their native range, country of introduction, year, purpose, and location where they were introduced, date, and pathway of observations in the wild, location, and their current status in each country.

Species/native range	Country	Introductions into the country			Wild populations			
		Year	Purpose	Location	Date reported	Pathway	Location	Status
Astacus astacus Noble crayfish/ Europe	Morocco	1914[a]	Fisheries	Jdida River	Failed	Release	Morocco	1914[a]
Faxonius limosus Spiny-cheek crayfish/ Eastern USA	Morocco	1937[b]	Fisheries	Lakes Roumi and Ifrah	Failed[b]			
		1940[c]	Fisheries	Lake Ifrah	1940s	Release	Lake Ifrah	Established in Lake Ifrah and several systems in the middle Atlas mountains[c]
		1948[d,e]	Fisheries	Lake Iffel	Late 1940s	Release	Lake Iffel	Established in Lake Iffel and several systems in the middle Atlas mountains[d,e]
Pacifastacus leniusculus American signal crayfish/ Northwestern USA	South Africa	1988[6]	Pet trade	Pet shops	No current records in captivity or wild[6]			

Sources: [a]Benabid and Khodari (2000); [b]Mouslih (1987); [c]Vivier (1948); [d]Melhaoui (Unpublished data); [e]Tabib (Unpublished data); [f]Nunes et al. (2017c)

Table 2.2. *Cherax* species introduced to continental Africa, their native range, country of introduction, year, purpose, and location where they were introduced, date, and pathway of observations in the wild, location, and their current status in each country.

Species/native range	Country	Introductions into the country			Wild populations			
		Year	Purpose	Location	Date reported	Pathway	Location	Status
Cherax cainii Smooth marron/ Australia	South Africa	1976 – 1985[a]	Aquaculture	Several farms in Western Cape, Free State, Eastern Cape, Kwazulu Natal and North West provinces	Only present in captivity at Smiling Valley Aquaculture farm[a]			
	Zambia	Early 1990s[b]	Aquaculture	Grubb Farm, Livingstone, upper Zambezi River	No current records in captivity or wild[b]			
Cherax destructor Yabby/ Australia	South Africa	1988[a]	Aquaculture	Kept at RAU, Randfontein farm in Gauteng Province and Gariep Dam Fisheries Station in Free State Province	No current records in captivity or wild[a]			
	Zambia	1992[b]	Aquaculture	Fish farm, Livingstone, upper Zambezi River	No current records in captivity or wild[c]			
Cherax quadricarinatus Australian redclaw crayfish/ Australia and Papua New Guinea	Morocco	2002[d]	Aquaculture	Fish farm in the Tangier region	Still in captivity for aquaculture[d]			
	Swaziland	Late 1990s[a,e]	Aquaculture	Farm next to Sand River Dam	2016	Escape	Sand River Dam	Established in the Sand River Dam and Mbuluzi River[a,e]
		Late 1990s[a,e]	Aquaculture	Farm near Manzini	2016	Escape	Usutu River	Established in the Usutu River[a,e]
	South Africa	[1]1988	Research	Zoology Department of the RAU	No records in captivity or wild establishments[a]			
		1980s[a]	Aquaculture	Farm in Western Cape	Farmer denied permits from South African Authorities to farm *C. quadricarinatus* and established farm in Swaziland[a]			
			Natural spread from Sand River Dam, Swaziland		2002	Unaided	Komati River	Established in the Inkomati Basin[a,e]
			Natural spread from Usutu River catchment, Swaziland		2016	Unaided	Usutu River	Present in Usutu River, Lake Nyamiti and Pongola River[a,e]

Zambia	1992[c]	Aquaculture	Miengwe Farm, Upper Kafue catchment	No current records in captivity or wild[e]			
	2001[c]	Aquaculture	Fish farm, Eastern end of the Kafue Flats	2001	Escape	Kafue River	Established in Kafue River and Lake Itezhi-tezhi[c]
	2001[c]	Aquaculture	Aquaculture cages at Siavonga, Lake Kariba	2002	Escape	Siavonga Lake Kariba	Established in Lake Kariba[c]
	2012[c]	Culture for consumption	Tank at Lealui in Mongu, Barotse flood plain	2014	Escape	Lealu Mongu	Established in the Barotse floodplain. Present in the Upper Zambezi River[c]
Zimbabwe		Unaided pathway from Siavonga Lake Kariba, Zambian side		2011	Unaided	Sanyati Basin, Lake Kariba	Established Lake Kariba, present in the Zambezi River downstream of Lake Kariba[f] Also present in Sebakwe Dam[g]
Mozambique		Unaided pathway from Usutu River catchment, Swaziland		2009	Unaided	Mbuluzi River	Established in Pequenos Libombos reservoir[h]
		Unaided pathway from the middle Zambezi system		2013	Unaided	Garganta basin, Lake Cahora Bassa	Present in Lake Cahora Bassa[c]
Namibia		Unaided pathway from Upper Zambezi		2016	Unaided	Zambezi River Katima Mulilo	Present in the Zambezi River in Katima Mulilo, Namibia[c]
Mauritius	Late 1980s	Aquaculture	Kept at a farm in Mauritius for aquaculture trials	No current records in captivity or wild[i]			

Sources: [a]Nunes et al. (2017c); [b]Mikkola (1996); [c]Douthwaite et al. (2018); [d]Fish Consulting Group (Unpublished data); [e]Nunes et al. (2017a); [f]Marufu et al. (2014); [g]AT. Chakandinakira (Department of Fisheries, Zimbabwe, pers. comm., 2019); [h]Chivambo et al. (2019); [i]New and Kutty (2010)

Table 2.3. *Procambarus* species introduced to continental Africa, their native range, country of introduction, year, purpose, and location where they were introduced, date, and pathway of observations in the wild, location, and their current status in each country.

Species/native range	Country	Introductions into the country			Wild populations			
		Year	Purpose	Location	Date reported	Pathway	Location	Status
Procambarus clarkii Louisiana red swamp crayfish/ Northern Mexico, and Southern and Southeastern USA	Egypt	1980[a]	Aquaculture	Fish farm in Manial Sheeha	1980s	Escape	Nile River	Established in the Nile River[a]
	Kenya	1966[b]	Fisheries and disease control	Solai and Subukia dams	Late 1960s	Release	Solai and Subukia dams	Established in Solai and Subukia dams and Eldoret river system[b]
		1970[b]	Fisheries	Lake Naivasha	1973	Release	Lake Naivasha	Established in Lake Naivasha and its tributaries[b]
	Morocco	Late 90s – early 2000s[c]	Fisheries	Nador canal, Merja Zerga	2010	Release	Nador canal, Merja Zerga	Established in the Merja Zerga, Merja Fouwarate and Nador Canal[c,d]
	Rwanda	Unknown	Biocontrol	Mukungwa Valley	2014	Release	Mukungwa Valley	Present in Mukungwa Valley[e]
	South Africa	1962 – 1987[f]	Pet trade	Pet shops in cities	No current records in captivity or wild[f]			
		1980s[f,g]	Fisheries	Driehoek Farm, Dullstroom Mpumalanga Province	1988	Release	Valleijspruit and Berry Dams	Present in Dullstroom farm ponds in the Crocodile River catchment[f,g]
		2016[h]	Sport and angling	Pet shops	2018	Release	Mimosa dam	Established in Mimosa Dam[h]
	Sudan	1975[i]	Unknown	Unknown systems in Sudan	No current records in captivity or wild[i]			
		Unaided pathway from Nile River, Egypt[j]			2019	Unaided	White Nile River	Present in the White Nile River[j]
	Tunisia	2014[k]	Sport and angling	Gombare Lake	2014	Release	Gombare Lake	Present in Gombare Lake, Lake Guitoune and Lebna dam[k]
	Uganda	1966[b]	Fisheries	Lake Bunyonyi	1966	Release	Lake Bunyonyi	Established in Lake Bunyonyi[b]

	Zambia	1979[l]	Aquaculture	Farm near Kitwe in Livingstone	No current records in captivity or wild[l,m]			
		1979[n]	Aquaculture	Farm in Livingstone	1995	Escape	Maramba River	Present in Maramba River[n]
Procambarus virginalis Marbled crayfish/Marmorkrebs/ No native range	Madagascar	2003[o]	Unknown	Ambohimangakely village	2003	Release	Ditches, fields, ponds in Ambohimangakely village	Present in several habitats from the central highlands to the east coast of Madagascar[o,p]
Procambarus zonangulus White River Crayfish/ South central USA	Egypt	1980[a]	Aquaculture	Fish farm in Manial Sheeha	1980s	Escape	Nile River	Got outcompeted by *P. clarkii*[a]

Sources: [a]Ibrahim and Khalil (2009); [b]Foster and Harper (2007); [c]Yahkoub et al. (2019); [d]Sara et al. (2019); [e]Seburanga et al. (Unpublished data); [f]Nunes et al. (2017c); [g]Nunes et al. (2017b); [h]Barkhuizen et al. (Unpublished data); [i]National Research Council (Unpublished data); [j]SEMA. Makawi (University of Khartoum, pers. comm. 2019); [k]Bouaoud et al. (2020); [l]Mikkola (1996); [m]Douthwaite et al. (2018); [n]Jones et al. (2009); [o]Andriantsoa et al. (2019)

Figure 2.2. Introduction routes of *Cherax quadricarinatus* from Australia to Africa and introduction locations within the continent. Dashed red lines show translocations within the African continent whilst the continuous red line shows introductions from outside the continent

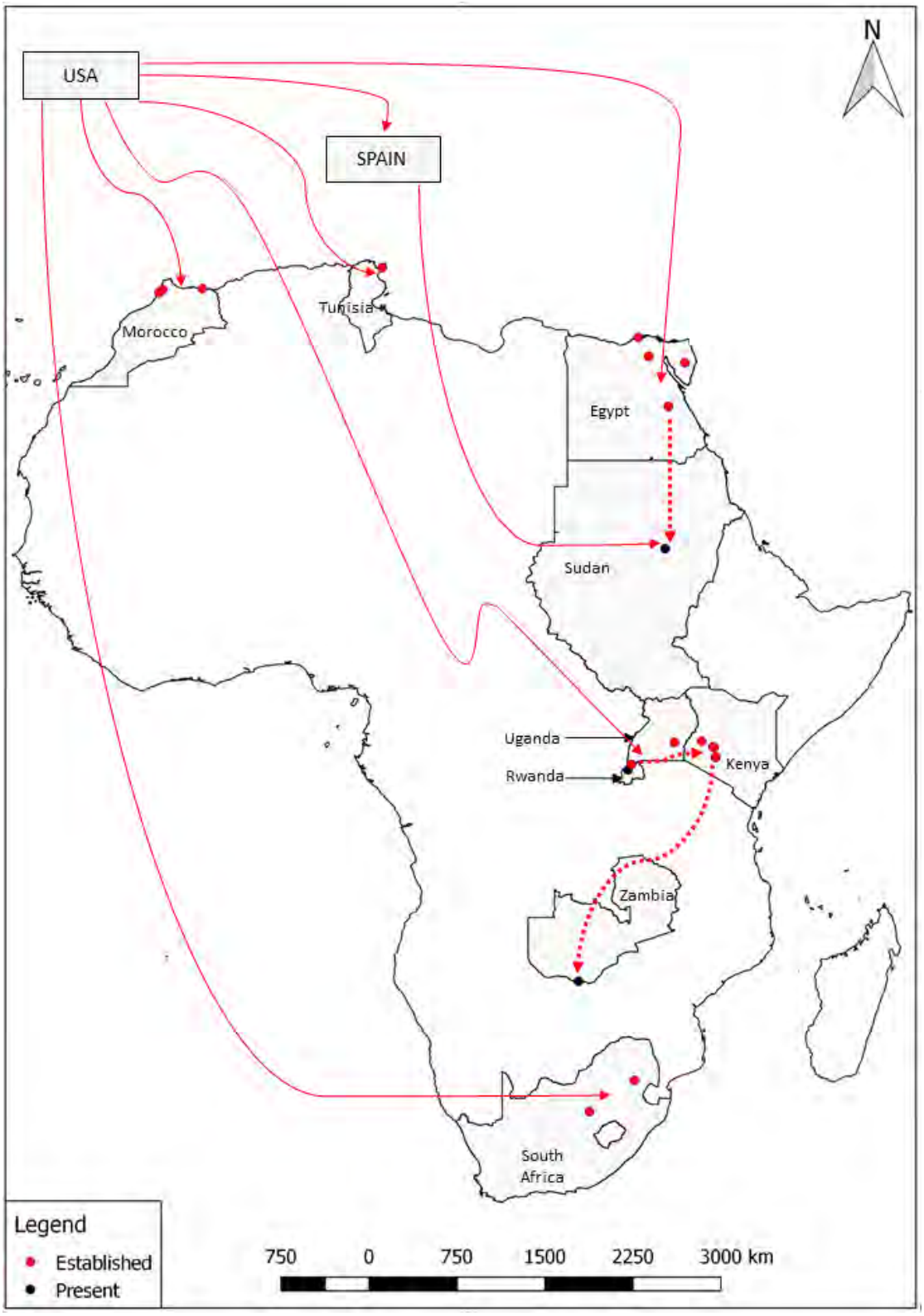

Figure 2.3. Introduction route of *Procambarus clarkii* from North America to Africa and introduction locations within the continent. Dashed red lines show translocations within the African continent whilst the continuous red line shows introductions from outside the continent

Morocco

Four freshwater crayfish species were introduced into Morocco for fisheries enhancement and for aquaculture (Tables 2.1 – 2.3). The first crayfish introduction into Morocco was *A. astacus* (Mouslih 1987; Benabid and Khodari 2000). This species was first introduced from France into the Ifrane province in 1914 as a personal initiative of Captain Belouin, Commander of the '21e Compagnie du '2e Étranger' and released into the Jdida River near Meknés (Mouslih 1987; Benabid and Khodari 2000). Although, this introduction appears to have failed, *A. astacus* were imported again in 1930 from the Paris region (France) and introduced into the Zerrouka and Tizguit rivers (Mouslih 1987). Establishment of *A. astacus* was successful in these rivers as well as in several associated impoundments (Mouslih 1987). Other subsequent introductions relate to the active production, stocking, and aquaculture of *A. astacus* by national agencies (Benabid and Khodari 2000). This includes the documented introduction of 2500 juveniles of the species from Germany in 1992 (Benabid and Khodari 2000). As a result, *A. astacus* is presently established in the Middle Atlas Mountains and parts of the Tizguit, Zerrouka, Sidi Mimoun, Ras-el-Ma, and Ben Smim River basins and associated impoundments (Chillasse et al. 2001; CABI 2020; Tabib Unpublished data).

In 2002, *C. quadricarinatus* was imported from Australia to a commercial fish farm in the Tangier region of Morocco (Fish Consulting Group Unpublished data). In 2014, this species was still in the experimental production phase and there is currently no evidence of Moroccan *C. quadricarinatus* being marketed, either nationally or internationally (Fish Consulting Group Unpublished data). No *C. quadricarinatus* have been reported in the wild in Morocco.

In 1937, *F. limosus* was introduced into lakes Roumi and Ifrah (Meknes region), but without success (Mouslih 1987). The first successful introduction was in 1940 into Lake Ifrah, where it soon established. In 1948, the Fishing Club of Fez introduced specimens from the Seine, in Paris, to Lake Iffel near the town of Fez (Vivier 1948). As a result of intentional introductions and natural spread, *F. limosus* is now widely established in Morocco, particularly at altitudes between 1000 and 2200 m, where it has successfully established in several natural lakes in the Middle Atlas region (Holdich and Black 2007; Melhaoui Unpublished data; Moroccan Farmer Unpublished data; Tabib Unpublished data).

Juvenile *P. clarkii* specimens were illegally introduced into the Nador Canal (Gharb region) and the Low Loukkos Marshes (Larache region) in the late 1990s or early 2000s by an owner of an eel farm in Kenitra (Yahkoub et al. 2019). Local fishermen first observed the species in the Nador Canal in early 2010(Yahkoub et al. 2019). Populations of *P. clarkii* established in both localities and the species is now widespread and abundant in the Gharb region where it has invaded tidal lagoons (e.g., Merja Zerga, Merja Fouwarate) and marshes (El Qoraychy et al. 2015; Sara and El Moutaouakil 2019; Yahkoub et al. 2019). Wetland sites on the Rmel plateau and Bas Loukous in the north-western coast of Morocco are also invaded by *P. clarkii* (Sara and El Moutaouakil 2019).

Egypt

Two crayfish species, *P. clarkii* and *P. zonangulus*, have been introduced into Egypt for aquaculture in the 1980s (Ibrahim and Khalil 2009) (Table 2.3).

Specimens of *P. clarkii* were introduced from the United States of America (USA) to a private fish farm at Giza governorate in Manial Sheeha, from here they accidentally escaped into the adjacent Nile River (Ibrahim et al. 1995). The species has been spreading rapidly throughout all aquatic ecosystems in Egypt since the 1980s. It is now established in aquatic systems from Giza to the whole Delta region in the north, and to Qena governorate in the south (Ibrahim et al. 1995; Hamdi 2011). In addition, *P. clarkii* has established populations in ditches in the Sinai desert due to connectivity facilitated by irrigation systems (Ibrahim and Khalil 2009; Khalil and Sleem 2011).

In the Nile River, *P. clarkii* accidentally escaped together with *P. zonangulus* (Ibrahim and Khalil 2009). For some time both species coexisted in mixed populations within many localities along the Nile River, although, *P. clarkii* was more abundant than *P. zonangulus* (Ibrahim and Khalil 2009). More recent surveys, however, suggest that *P. zonangulus* has disappeared completely from the Egyptian water bodies (Ibrahim and Khalil 2009).

Sudan

In 1975, *P. clarkii* was introduced to Sudan from Spain (National Research Council Unpublished data). This species also spread from the Nile River and is said to be present in the White Nile River close to Jebel Aulia Dam (SEMA Makawi, University of Khartoum Sudan pers. comm. 2019).

Uganda

The only crayfish species present in Uganda is *P. clarkii* (see Foster and Harper 2007). In 1966, *P. clarkii* imported from the USA was cultured at Fisheries Resources Research Institute/National Agricultural Research Organisation's ponds at Kajjansi near Entebbe and Lake Victoria (Lowery and Mendes 1977), where it was still present in 2006 (Foster and Harper 2007). The species is established in Lake Bunyonyi (Southwest Uganda), where it is exploited for the local restaurant trade. This species could be quite widespread in Ugandan freshwater systems, but under-recorded, and may even have colonised the periphery of Lake Victoria. Furthermore, anecdotal records suggest that *P. clarkii* may have established in the Kagera River, which enters Lake Victoria on the Uganda–Tanzania border (Foster and Harper 2007). Presently, *P. clarkii* is abundant in Lake Bunyonyi and around its catchment (Kabiza Wilderness Safaris pers. comm. 2019).

Kenya

The Kenyan Fisheries Department first introduced *P. clarkii* in 1966 from Uganda Fisheries Department Ponds at Kajansi near Entebbe, Uganda, with the intention to enhance commercial fisheries in dams and lakes in the area (Lowery and Mendes 1977; Oluoch 1990; Mikkola 1996). The secondary reason for introduction in Kenya was as a biocontrol agent for gastropod vectors of schistosomiasis (Hofkin et al. 1991). An unspecified number of *P. clarkii* were introduced to two dams located at Solai and Subukia, within the Rift Valley (Oluoch 1990). This species was also further introduced to various farm dams and ditches, as well as the streams and rivers draining these dams across Kenya, including the catchment area of the Nzoia River draining to Lake Victoria (Lowery and Mendes 1977). Since 1991, *P. clarkii* has also been recorded in abundance at Eldoret, on the Eldoret river system (Foster and Harper 2007).

Around 1970, approximately 300 *P. clarkii* were introduced into Lake Naivasha, near Marina Bay, from Subukia Dam (Oluoch 1990), to provide food for the introduced largemouth bass *Micropterus salmoides* (Foster and Harper 2007). In the immediate eastern basin of the lake, as well as in Marina Bay, *P. clarkii* established and thrived, reaching a density of three adult individuals per m^2 within three years (Lowery and Mendes 1977). In 1975, a commercial fishery was opened for *P. clarkii* in Lake Naivasha (Mikkola 1996) and by 1977, *P. clarkii* was prevalent throughout the lake (Oluoch 1990). Presently, *P. clarkii* is widely distributed throughout Lake Naivasha and its tributaries (Jackson et al. 2016).

Rwanda

The only recorded report of *P. clarkii* in Rwanda was in the Mukungwa Valley where it was introduced to control the invasive water hyacinth (Seburanga et al Unpublished data). There are no further details on its establishment and spread in Rwandan freshwater systems.

Swaziland

There are anecdotal reports that two *C. quadricarinatus* batches were introduced from Australia to Swaziland, one at a farm located near the Sand River Dam, close to the Komati River and the other to a farm near Manzini or Big Bend, in the Usutu River catchment (Nunes et al. 2017a). The farm located near the Sand River Dam was granted a permit in the late 1990s and successfully established. Crayfish subsequently escaped from captivity into the Sand River Dam and later spread via the Sand River into the Komati River, a transboundary river that flows through Swaziland and South Africa (de Moor 2002; de Moor 2004; Nunes et al. 2017a). The further detection of *C. quadricarinatus* in an outlet of Lake Nyamiti, in the Ndumo Game

Reserve (South Africa) in 2012, and the fact that in 2015 the species was established in the Usutu River close to Big Bend, implies that crayfish also escaped from the other aquaculture farm close to Manzini (in the Usutu River catchment) (Nunes et al. 2017a,c). This species has also colonised a large area of the Mbuluzi River and its tributary Mlawula (Nunes et al. 2017a).

South Africa

The literature on crayfish invasions in South Africa, from the first introductions up to 2017, are well summarised in Nunes et al. (2017c). Six crayfish species have been imported into South Africa: *P. clarkii*, *C. quadricarinatus*, *C. cainii*, *C. destructor*, *P. leniusculus*, and *Astacus* sp. (Tables 2.1 – 2.3). Pet trade in the 1980s resulted in the illegal importation of *P. clarkii*, *P. leniusculus* and *Astacus* sp. into South Africa (de Moor and Bruton 1988). Legal introductions brought *Cherax cainii* into South Africa for aquaculture initiatives, while *C. destructor* and *C. quadricarinatus* were imported for research purposes in 1988 (see Nunes et al. 2017c). There is evidence for the establishment of two species in the wild, *P. clarkii* and *C. quadricarinatus*.

In 1976, *C. cainii* was imported and introduced into South Africa by a private fish farmer in KwaZulu-Natal for aquaculture purposes, although, this venture was short-lived (de Moor and Bruton 1988). Several farmers developed an interest to venture into *C. cainii* farming and applied for permits, and several consignments of *C. cainii* were imported from Western Australia into South Africa (Mitchell and Kock 1988; van den Berg et al. 1990). Farming of *C. cainii* was somewhat unsuccessful and currently, only two farms are operational and located in the Eastern Cape Province (Nunes et al. 2017c). There are currently no reports of *C. cainii* in the wild (Nunes et al. 2017c), despite *C. cainii* individuals being detected in the Buffalo River

in the 1980s as a result of aquaculture escape (de Moor and Bruton 1988). *Cherax destructor* was introduced in 1988, together with *C. quadricarinatus*, from Australia into South Africa for experimental research (see Nunes et al. 2017c). While there are anecdotal records of *C. destructor* introductions by fishermen into several South African dams, there are no records of *C. destructor* in the wild in South Africa (Nunes et al. 2017c).

In 1988, *C. quadricarinatus* was imported into South Africa for research on its aquaculture potential at the Rand Afrikaans University (RAU, now University of Johannesburg) (see Nunes et al. 2017c). South Africa imposed biosecurity restrictions on importation and culturing of *C. quadricarinatus*, so instead a farmer established a *C. quadricarinatus* farm next to the Sand River Dam in neighbouring Swaziland, from where specimens later escaped and spread into South Africa (Nunes et al. 2017a,c). The first record of *C. quadricarinatus* in South African freshwater systems was in 2002, in the Komati River, Mpumalanga Province, close to the Swaziland border (see Nunes et al. 2017a). Recently, Nunes et al. (2017a) confirmed the widespread presence of *C. quadricarinatus* in the Komati River, and its presence in one of its tributaries, the Lomati River. This species was also confirmed to be present in the Crocodile River, although, the densities were low (Petersen et al. 2017). In 2012, *C. quadricarinatus* was detected in an outlet of Lake Nyamiti in the Ndumo Game Reserve (Du Preez and Smit 2013), probably as a result of its spread from the Usutu River in Swaziland (Nunes et al. 2017c). In 2013 the species was caught and sold in the villages next to the Ndumo Game Reserve (Coetzee et al. 2015), which suggests high abundances in the area (Nunes et al. 2017c).

Unconfirmed records of *P. clarkii* in South Africa are reported from 1962 and in the 1980s the species was illegally sold in pet shops (Nunes et al. 2017c). The first *P. clarkii* populations in

the wild were recorded in 1991 from two dams on a trout farm close to Dullstroom, in Mpumalanga (Schoonbee 1993). The attempt to eradicate these populations by reducing water level and physically removing specimens by hand and dipnets proved unsuccessful, as Nunes et al. (2017b) sampled a *P. clarkii* specimen 22 years later. In 2018, a second record of a wild established population of *P. clarkii* was discovered in Mimosa Dam, a small municipal dam in Odendaalsrus, Goldfields, Free State Province (L Barkhuizen, DESTEA pers. comm. 2018). This population is believed to have been introduced to provide stock for the pet trade (L Barkhuizen, DESTEA pers. comm 2018). Live *P. clarkii* specimens are also being illegally bred and sold as pets by aquarists and enterprising individuals in Port Alfred and Port Elizabeth in the Eastern Cape Province (J South, SAIAB, pers. comm. 2019).

Zambia

Four crayfish species were imported into Zambia for aquaculture: *P. clarkii*; *C. cainii*; *C. destructor*; and *C. quadricarinatus* (Audenaerde 1994). The first to be introduced was *P. clarkii* to a farm located in Livingstone in 1979 from Lake Naivasha, Kenya (Audenaerde 1994). Although, the farmer claimed that there were no *P. clarkii* escapees from his farm (see Douthwaite et al. 2018), a population of *P. clarkii* has been reported from the Maramba River, adjacent to the farm in Livingstone (Douthwaite et al. 2018). This species has been present in the river since 1995, although, never caught in large numbers (Douthwaite et al. 2018). Between 1979 and 1981, a batch of *P. clarkii* was also translocated from the farm in Livingstone to ponds near Kitwe, where cultivation failed, but there are reports that they escaped into the Kafue River (Audenaerde 1994). No established populations have however been reported from the Kafue catchment (Douthwaite et al. 2018). Another separate *P. clarkii* introduction was made to Siavonga on Lake Kariba, in 1979, but cultivation efforts there also

failed (Mikkola 1996) and there have been no reports of this species from Lake Kariba (Douthwaite et al. 2018; AT Chakandinakira, Department of Fisheries Zimbabwe pers. comm. 2019).

The three *Cherax* species were translocated from South Africa to Zambia in the early 1990s (Mikkola 1996), but only *C. quadricarinatus* has successfully established in the wild (see Douthwaite et al. 2018). Although, *C. quadricarinatus* individuals were initially taken to other sites, at unspecified times, the only record of establishment in the wild from these translocations is from Miengwe Farm on the upper Kafue catchment. Additionally, in 2001, *C. quadricarinatus* was imported from a farm that was closing down in Swaziland to a fish farm at the eastern end of the Kafue Flats, from where specimens escaped. The species has now spread to most parts of the Kafue catchment (Douthwaite et al. 2018).

Another batch of *C. quadricarinatus* imported from Swaziland were also transferred to aquaculture cages at Siavonga in Lake Kariba, from where they escaped into the lake in 2002 (Nakayama et al. 2010; Douthwaite et al. 2018). Besides this accidental escape into Lake Kariba, there are also anecdotal reports that *C. quadricarinatus* was intentionally introduced (Welz Unpublished data). Nakayama et al. (2010) showed that by 2008 *C. quadricarinatus* had established in the wild at Siavonga on the Zambian shore of the Sanyati Basin of Lake Kariba, a basin where the species is now present in high abundance (pers. obs. 2018).

In the upper Zambezi River, a new population was reported to be spreading on the Barotse floodplains after their introduction near Mongu (Nunes et al. 2016). This population arose from informal aquaculture escapes, as road construction workers had been culturing *C.*

quadricarinatus for their own consumption, which then escaped during the annual inundation of the floodplain (G Chisule, Ministry of Fisheries and Livestock Zambia pers. comm. 2019; M Chomba, WWF Zambia pers. comm. 2019). Individual *C. quadricarinatus* have subsequently been reported from sites 95 km upstream (Lukulu) of the introduction point in Lealui, Mongu and 100 km downstream (Senanga) from the introduction point. In addition, *C. quadricarinatus* was also reported from a major tributary, the Luanginga River 40 km from the introduction point (pers. obs. 2019).

Zimbabwe

Accidental and intentional introductions of *C. quadricarinatus* in Zambia led to spread of the species into Zimbabwe and consequently establishing in Zimbabwean side of Lake Kariba and the Zambezi River downstream of the dam wall. In 2011, the species was first reported on the Zimbabwean side of the Sanyati Basin of Lake Kariba (Marufu et al. 2014), an area where it is now established and abundant (Marufu et al. 2018a). This species has also been reported in the Nyaodza, Sanyati and Gache-Gache rivers, which feed into the Sanyati Basin of Lake Kariba (AT Chakandinakira, Department of Fisheries Zimbabwe pers. comm. 2019). This species is also present in Kanyemba on the Zambezi River below the Kariba Dam (AT Chakandinakira, Department of Fisheries Zimbabwe pers. comm. 2019). Populations of *C. quadricarinatus* have also been reported from Mazvikadei and Claw Dams in the middle Zambezi catchment (Douthwaite et al. 2018), Sebakwe Dam in the Midlands province of Zimbabwe, as well as the Save catchment in Gonarezhou National Park, Southwest of Zimbabwe (C Mungenge, Department of Fisheries Zimbabwe pers. comm. 2019).

Namibia

In 2016, specimens of *C. quadricarinatus* were first observed in the Zambezi River, Katima Mulilo (Douthwaite et al. 2018). It is possible that this is a result of dispersal from the *C. quadricarinatus* invasion in Mongu, Zambia.

Mozambique

The first observation of *C. quadricarinatus* was in the Pequenos Libombos reservoir, Maputo Province, southern Mozambique during late 2009, early 2010 (Chivambo et al. 2019). Assuming this was not the result of an exceptional translocation event, the species probably spread naturally from the Inkomati Basin in Swaziland through the Mbuluzi River until the Pequenos Libombos Dam (Nunes et al. 2017a; Chivambo et al. 2019). Another population of *C. quadricarinatus* was confirmed in the Garganta basin of Lake Cahora Bassa in 2015 (Douthwaite et al. 2018). The source of this population is most likely from upstream sources, which include Lake Kariba on the Zambezi River and the Kafue River in Zambia.

Tunisia

Between 2014 and 2015, *P. clarkii* was illegally introduced in the Gombare Lake catchment by aquarists (MW Bouaoud, University of Tunis El Mana Tunisia pers. comm. 2020). At present, there is evidence of the establishment of the species in Gombare Lake (Bouaoud et al. 2020). Dead specimens and burrows were also observed in Lake Guitoune and Lebna reservoir, which provides evidence of their presence in these localities (Bouaoud et al. 2020) (Table 2.3).

Madagascar

In 2003, *P. virginalis* was introduced to Madagascar in Ambohimangakely, a village 15 km from the capital city, Antananarivo by foreign contractors working on a road building project (Jones et al. 2009). Genetic sequencing indicates that the Malagasy *P. virginalis* population originated from a German stock (Gutekunst et al. 2018). The motivation for the introduction into Madagascar freshwater systems remains unknown (Jones et al. 2009). In 2003 vendors, fishermen and farmers in Ambohimangakely confirmed observing *P. virginalis* in drainage ditches, rice fields, brick pits, and fish ponds. In 2005, *P. virginalis* was observed being sold in markets close to Antananarivo (Jones et al. 2009). In 2017, this species had colonised lakes and rice fields in the central highlands, as well as swamps close to the east coastline covering a total area of about 100 000 km^2 (Gutekunst et al. 2018). The invasive ranges of *P. virginalis* in Madagascar includes overlap with the natural habitats of the native *Astacoides betsileoensis* (in Andragnaroa River, central Madagascar) and *Astacoides granulimanus* (in a channel connected to a rice field in Sahavondronina, North East of Madagascar) but not yet with that of the other five native *Astacoides* species with narrow distribution ranges (Andriantsoa et al. 2019).

Mauritius

Aquaculture trials to farm *C. quadricarinatus* were carried out in the late 1990s in Mauritius (Bhikajee Unpublished data). Due to production challenges, the aquaculture ventures failed despite heavy investment by both the government and the private sectors (de Lestang Unpublished data) and culture of *C. quadricarinatus* was discontinued (New and Kutty 2010). There are no reports of freshwater crayfish in the wild.

Co-introduced parasites and diseases

Invasive alien species (IAS) have the capacity to be a vector for the introduction of other invasive species, as they often transport invasive parasites and diseases (Hulme 2014). In South Africa, Mitchell and Kock (1988) reported the turbellarian *Temnosewellia chaeropsis* on *C. cainii* imported from Australia to commercial farms of South Africa. Avenant-Oldewage (1993) showed the potential for infection of native freshwater crabs (*Potamonautes warreni*) by non-native *T. chaeropsis* via vector *C. cainii*. In Lake Nyamiti South Africa, *C. quadricarinatus* sampled were infested with the non-native *Diceratocephala boschmai* (Du Preez and Smit 2013). Tavakol et al. (2016) reported three temnocephalans, *Craspedella pedum*, *D. boschmai*, and *Didymorchis* sp. on *C. quadricarinatus* from Laagwaterbrug and Masibekela dams in the Komati system, South Africa. In the Kafue flats in Zambia, Douthwaite et al. (2018) reported the presence of non-native temnocephalans, commonly found on *C. quadricarinatus*, on the crab *Potamonautes unispinus*. During recent surveys, temnocephalans were observed on crayfish individuals in the Barotse floodplain, Zambia and in Lake Kariba, Zimbabwe (pers. obs. 2019).

In the Nile River and its tributaries in Egypt, *P. clarkii* is a vector for the fungus *Trichosporon jirovecii*, which could potentially spread to other aquatic organisms (Abdallah et al. 2018). It is important to note that crayfish plague, caused by the fungus *Aphanomyces astaci* (Holdich 2003; Longshaw 2016) has not been reported from Africa, although, Foster and Harper (2006) hypothesised that this could have contributed to the disappearance of *Potamonautes loveni* stocks in Lake Naivasha, Kenya.

Ecological impacts

The impacts arising from crayfish introductions in Africa are summarised in Table 2.4. Of the five established crayfish species, there is only evidence for documented impacts of *C. quadricarinatus* and *P. clarkii*.

Table 2.4. Summary of documented impacts of crayfish species in continental Africa

Impact mechanism	*Cherax quadricarinatus*	*Procambarus clarkii*	*Procambarus virginalis*
Negative environmental effects			
Competition		●[1]	
Predation		●[1]	●[16]
Herbivory		●[2]	
Introduction of pathogens	●[10,11,14,]	●[1]	
Environmental modification		●[3,4,5]	●[16]
Negative socio-economic effects			
Destruction of dams, canals and levees		●[6]	
Destruction of fishing gear	●[9,15]	●[7,8]	
Predation on fish catches	●[9,15]	●[1]	
Impacts on agriculture		●[5]	●[16]
Positive socio-economic effects			
Establishment of fisheries	●[12,13]	●[1]	●[16,17]
Aquaculture	●[15]	●[16]	

● effect documented on African continent

Sources: [1]Foster and Harper (2006); [2]Hickley and Harper (2001); [3]Harper et al. (1990); [4]Gherardi et al. (2011); [5]Sara and El Moutaouaki (2019); [6] El-Gendy 2013; [7]Lowery and Mendes (1977); [8]Ibrahim and Khalil (2009); [9]Weyl et al. (2017); [10]Du Preez and Smit (2013); [11]Douthwaite et al. (2018); [12]Coetzee et al. (2015); [13]T.C. Madzivanzira, pers. obs. (2019); [14]Tavakol et al. (2016);[15]Nunes et al. (2016), [16]Andriantsoa et al. (2019); [17]Andriantsoa et al. (2020).

Impacts on macrophytes

In Lake Naivasha and its tributaries, the population expansion of *P. clarkii* in the 1970s coincided with the decline in the blue water lily Nymphaea caerulea and other floating-leaved and submerged macrophytes, as a result of direct consumption by crayfish (Hickley and Harper 2001; Harper and Mavuti 2004).

Impacts on other decapods

On mainland Africa, freshwater crabs are trophically analogous to crayfish and therefore are candidates to either offer biotic resistance or be highly susceptible to competition (Alofs and Jackson 2014). In the Malewa River in Kenya, for example, *Potamonautes loveni* was only recorded from sites where *P. clarkii* was absent, upstream of a weir, which likely acted as a migration barrier for *P. clarkii* (Foster and Harper 2006). In a separate study in Malewa River, Jackson et al. (2016) used a combination of field surveys and field experiments to examine the impacts of *P. clarkii* on native freshwater crabs. Growth rates of both species were reduced significantly in the presence of one another. Over a three year period, crab abundance declined at sites invaded by *P. clarkii*, with the species becoming extirpated at one locality (Jackson et al. 2016). In Lake Bunyonyi, Uganda, *P. clarkii* also appears to have negatively impacted abundances of the freshwater crab *Potamonautes mutandensis*, although, the specifics of this have not been studied (Cumberlidge 2018).

In an attempt to deduce mechanisms behind potential biotic resistance of freshwater crabs toward the two dominant invasive crayfish in southern Africa, South et al. (2020) compared the closing force and chelae morphology of the Cape river crab *Potamonautes perlatus* and the crayfish species *P. clarkii* and *C. quadricarinatus*. These traits can be used as proxies for prey handling capabilities and agonistic contests outcomes. Female *P. perlatus* showed a significantly stronger maximum chela closing force than female *C. quadricarinatus* and both sexes of *P. clarkii*, and did not differ significantly from male *C. quadricarinatus*. While this shows some capacity for native African freshwater crab species to offer biotic resistance, closing force was significantly correlated with body mass in all species. Therefore, if the crayfish attain a higher mass than the crabs, they are likely to retain a competitive advantage, which may explain abundance patterns seen in the field (South et al. 2020).

Impacts on invertebrates

Crayfishes have been assessed experimentally for biocontrol efficacy on disease vectors, the results of these can be considered as inferred ecological impacts on taxa in the wild. In Kenya and Egypt, *P. clarkii* effectively preys upon bulinid snails (Hofkin et al. 1991; Mkoji et al. 1999b), biomphalarid, bulinid, lymnaeid, melanoid, and helicid snails (Khalil and Sleem 2011). Similarly, *C. quadricarinatus* preys upon bulinid snails in Zambia (Monde et al. 2017). In Madagascar, freshwater biomphalarid snails were absent at locations colonised by *P. virginalis*, suggesting possible predation, which was confirmed in a laboratory experiment (Andriantsoa et al. 2019). In addition, under experimental conditions, *P. clarkii* has been shown to impact on abundance of anopheles mosquitoes in Kenya (Mkoji et al. 1999a) and the southern house mosquito in Egypt (Heikal et al. 2018). These examples infer that there is potential for both *P. clarkii* and *C. quadricarinatus* to exert pressure on freshwater invertebrate communities.

Impacts on food webs

Freshwater crayfish are among the most common omnivores in freshwater systems and are associated with substantial effects across multiple levels of freshwater food webs (Olsen et al. 1991; Dorn and Wojdak 2004; Nilsson et al. 2012; Twardochleb et al. 2013). Stable isotope analysis (SIA) has been used to document IAS conferred disruption to food webs and community structure, through comparing niche width of invaders relative to native species as well as nutrient transfer pathways across trophic levels (Bodey et al. 2011). In Africa, only three studies have investigated the diet of invasive crayfish using SIA: *P. clarkii* in Lake Naivasha (Grey and Jackson 2012), *P. clarkii* in Lake Naivasha tributaries (Jackson et al. 2016) and *C. quadricarinatus* in Lake Kariba (Marufu et al. 2018b). In Lake Naivasha, *P. clarkii* individuals exhibited considerable intraspecific isotopic variability, indicating a broad choice

in diet, ranging from submerged macrophytes, terrestrial plants, hippo dung matter, mixed detritus, chironomid larvae, and oligochaetes (Grey and Jackson 2012). In Lake Naivasha tributaries, Jackson et al. (2016) used SIA to determine the trophic niche width of *P. clarkii* and native crabs. Contrary to what was hypothesised, competition between the invasive *P. clarkii* and native crabs resulted in the reduction in the diet breadth of both species. In spite of this, performance in the native crabs was reduced in the presence of *P. clarkii*, as revealed by field experiments (Jackson et al. 2016). Marufu et al. (2018b) found that all *C. quadricarinatus* sizes were in the same trophic level. In Lake Kariba *C. quadricarinatus* diet comprised mostly macrophytes, followed by macroinvertebrates, detritus, and finally fish and crayfish (Marufu, et al. 2018b). Generally both small and large *C. quadricarinatus* consumed mainly macroinvertebrates and macrophytes, respectively, although, larger individuals consumed more fish (Marufu et al. 2018b).

Where introduced, crayfish have been incorporated into the diets of a variety of non-native and native predators. In Lake Naivasha, *P. clarkii* became incorporated into the diet of the introduced largemouth bass *Micropterus salmoides* (Hickley et al. 1994), common carp *Cyprinus carpio*, birds including herons, African fish eagle *Haliaeetus vocifer* and cormorants; and African clawless otter *Aonyx capensis* (Smart et al. 2002; Ogada et al. 2009). Local residents at Kantunta, Zambia reported seeing otters, reed cormorant *Phalacrocorax africanus*, hadada ibis *Bostrychia hagedash*, *H. vocifer*, marabou stork *Leptoptilos crumeniferus* eating *C. quadricarinatus* and finding them in the gut contents of the fish *Clarias gariepinus* and *Serranochromis* sp. in the area (Douthwaite et al. 2018). Remains of *C. quadricarinatus* have also been observed in the gut contents of *C. gariepinus*, *Oreochromis andersonii*, *Serranochromis macrocephalus*, *Synodontis macrostigma*, and *Mormyrus lacerda* from the Kafue flats, Zambia (Tyser and Douthwaite 2014). The pied kingfisher *Ceryle rudis* has been

seen eating juvenile crayfish on the middle Zambezi River (Douthwaite et al. 2018) and crayfish have been found inside the guts of largemouth bass caught at the Claw and Mazvikadei dams in Zimbabwe (Douthwaite et al. 2018). In Lake Kariba, Zimbabwe, results of stable isotope analyses highlighted that *C. quadricarinatus* was now becoming an important food source across all tigerfish size classes (Marufu et al. 2017). In Lake Kariba *C. quadricarinatus* was also found to be present in gut contents of *C. gariepinus* and *Heterobranchus longifilis* (AT Chakandinakira, Department of Fisheries Zimbabwe pers. comm. 2019). These SIA studies indicate the wide ecological niche that is occupied by crayfish in African environments and their increasing inclusion in the diets of higher level organisms. Actual assessments of impact on recipient communities have, however, not been investigated.

Socio-economic impacts

Positive socio-economic impacts

Crayfish introductions have almost always been intentional; hence, most introductions produce a potential positive impact on at least one ecosystem service (Lodge et al. 2012). With the exception of South Africa, where wild caught *P. clarkii* were illegally sold to the aquarium trade (L Barkhuizen, Free State DESTEA pers. comm. 2018) the intended use for the introduced crayfishes was for food. Kenya exported several hundred tonnes of live *P. clarkii* to Europe between 1975 and 1981 (Oficialdegui et al. 2019) when the industry collapsed as a result of a European Union imposed import ban due to a cholera outbreak in East Africa (Foster and Harper 2007). A resistance to consuming crayfish is reported from several countries. Foster and Harper (2007) reported that people residing near Lake Naivasha in Kenya did not eat crayfish and referred to them as “insects” or “red scorpions.” In Uganda, local people around Lake Bunyonyi do not eat crayfish because of its appearance (Foster and Harper 2007; Kabiza

Wilderness Safaris pers. comm. 2019) and in Egypt *P. clarkii* is called "the cockroach of the Nile" and is not widely consumed (Ibrahim and Khalil 2009). Despite this documented resistance to consuming freshwater crayfish at the local level, they are often on offer in restaurants which cater for a more cosmopolitan clientele in Kenya (Foster and Harper 2007), and Zambia and Mozambique (OLF Weyl SAIAB pers. comm. 2020). In Madagascar, where there is a culture of eating native crayfishes, *P. virginalis* is generally accepted as a source of dietary protein and it contributes positively to household economy and food security (Andriantsoa et al. 2019, 2020). There is little literature available on successful aquaculture for freshwater crayfish in Africa. Mikkola (1996) stated that few crayfish aquaculture projects that can be regarded as "successful" in the African continent (Mikkola 1996) and examples of failed crayfish farms are documented for Swaziland, South Africa (Nunes et al. 2017a,c), and Zambia (Nakayama et al. 2010; Douthwaite et al. 2018).

Negative socio-economic impacts

Crayfish have negatively impacted the livelihoods of people who rely on the fishing industry for subsistence and income in Africa. Crayfish impact directly upon fisheries by scavenging on and partially consuming fish caught in gillnets (Lowery and Mendes 1977; Weyl et al. 2017). In Lake Naivasha, Kenya, fishermen reported on how *P. clarkii* were spoiling their catches resulting from partial consumption of fish caught in gillnets (Lowery and Mendes 1977). Fishermen have also reported substantial damage to gill nets by *P. clarkii* in Lake Naivasha (Lowery and Mendes 1977) and in the Nile River, Egypt (Ibrahim and Khalil 2009). In the Kafue River, Zambia, Zambia, Weyl et al. (2017) reported observations that *C. quadricarinatus* predation on fish entangled in gill nets could result in losses of up to 30% of the catch, as well as considerable damage to the fishing gear. In Mozambique, predation by and competition with

C. quadricarinatus is hypothesised to have contributed to a decline in tilapia fisheries in Pequenos Libombos Reservoir (Chivambo et al. 2019). Many fishermen in Madagascar expressed that *P. virginalis* was destroying their fish catches as they perceive that crayfish can prey upon juvenile fishes (Andriantsoa et al. 2019).

In Egypt, *P. clarkii* damaged earth dams and irrigation canals (Ibrahim and Khalil 2009), as well as rice fields, through their burrowing activities (Abdel-Kader 2016). In the Gharb region of Morocco, *P. clarkii* excavated burrows that caused the loss of irrigation water beyond the reach of rice plant roots through accelerated infiltration (Sara and El Moutaouaki 2019). This caused an increase in the frequency of irrigation in rice fields close to the water source upstream, which then had knock on effects for other rice farmers downstream as a result of reduced water availability. In this region, burrowing activities by *P. clarkii* has caused a reduction in arable land area and an overall decline in rice yields (Sara and El Moutaouaki 2019). Around the catchment of Lake Bunyonyi in Uganda, farmers complained about *P. clarkii* making burrows in the fields, eating plant roots, spreading a fungus which destroys young crops and boring through earthen fish ponds causing leaks (Blanc Unpublished data). In Madagascar, *P. virginalis* populations have been shown to negatively impact rice farming due to burrowing activities, which dry up the rice fields and require farmers to regularly repair their banks and irrigation canal (Andriantsoa et al. 2020). Burrows made by *P. clarkii* have also been observed on the shores of Mimosa Dam in Free State Province, South Africa (Barkhuizen et al. Unpublished data).

Control

Compared to Europe and North America, little research has been done to address the collective impacts of crayfish introductions in the African continent, but based on their potential impacts, invasion history, and lack of evolutionary history in the region (other than in Madagascar), researchers advocate strongly against allowing for further introductions and spread of crayfish in freshwater systems (Mikkola 1996; de Moor 2002; Appleton et al. 2004; Lodge et al. 2005; Nunes et al. 2016; Nunes et al. 2017c; Weyl et al. 2017; Marshall 2019; South et al. 2020). Examples in this review have shown how difficult it has been to prevent crayfish introductions. Once crayfish establish, eradication is almost impossible and management is extremely difficult (Hobbs et al. 1989; Gherardi et al. 2011) and control measures include biological control, chemical application, and mechanical or physical removal (Gherardi et al. 2011; Manfrin et al. 2019).

In Africa, rice farmers in Egypt use the chemical Furadan 10G to control the expanding populations of *P. clarkii* as well as other pests, which are damaging their rice fields (Abdel-Kader 2016). Little information is however, available about the toxicity of Furadan 10G on other aquatic organisms (Alves et al. 2002; Abdel-Kader 2016). In an attempt to eradicate *P. clarkii* from the trout farm in South Africa, the water level in the dam where the species was present was reduced and crayfish were physically removed by hand or using dipnets (see Nunes et al. 2017c). This eradication program was unsuccessful, as 22 years later *P. clarkii* were still present in the dam (Nunes et al. 2017b). Burrowing species such as *P. clarkii* are unlikely to be controlled by such measures as draining of impoundments (de Moor 2002). Another eradication attempt, using a combination of methods which included intensive trapping and scoop netting, only reduced crayfish densities but did not result in eradication in Mimosa dam,

a small reservoir in the Free State province of South Africa (Barkhuizen et al. Unpublished data).

Monitoring

Given the difficulty of controlling crayfish populations once they are established, early detection and rapid response programs become a critical element, especially if eradication is the management goal (Tobin 2018; Faulkner et al. 2020). Key to this is the development of appropriate monitoring methods.

Trapping

Globally, baited traps are the most commonly applied method, although they are biased toward large adults and males over other members of the population (Brown and Brewis 1978; Capelli and Magnuson 1983). A variety of traps and baits have been used in crayfish research in Africa. These include collapsible traps baited with dry dog food, fish or chicken in South Africa (Nunes et al. 2017a), Swaziland (Nunes et al. 2017a) and Tunisia (Bouaoud et al. 2020); opera traps baited with cooked maize meal in Lake Kariba Zimbabwe (Marufu et al. 2014, 2018a); rectangular traps baited with fish in South Africa (Barkhuizen et al. Unpublished data) and Morocco (Yahkoub et al. 2019); and cylindrical traps baited with fish in Egypt (Ibrahim and Khalil 2009). As disparate gears and sampling methods can result in unequivocal results in estimates of population abundance and distribution of crayfish, developing a standard sampling method is critical to determining cohesive knowledge bases regarding crayfish distributions in Africa (Larson and Olden 2016). Therefore, research into developing feasible standardised field sampling approaches is an urgent requirement for further invasion assessments.

Citizen science

Citizen science can be a powerful tool in conservation as it encourages stakeholder engagement and understanding of the natural environment (Brossard et al. 2005; Seymour et al. 2020). Citizen science initiatives present an emerging opportunity for the collection of datasets on IAS, particularly in understudied regions such as Africa, where sampling effort and baseline information is often lacking. Numerous citizen science projects focused on IAS reflect its potential for data gathering on IAS while ensuring effective and high quality societal engagement (Novoa et al. 2018; Shackleton et al. 2019a,b). Involving local people in collecting data or even in conservation plans offers a good route to integrate public views and values concerning conservation actions that should be taken (Devictor et al. 2010; Seymour et al. 2020). It is, however, important to note that in the African context, poverty and a concomitant lack of access to equipment and network services is likely to be a considerable barrier to citizen science projects in rural areas. As a result, initiatives encouraging the public to report the presence of crayfish to local environmental authorities are more likely to gain traction. An example is an initiative that has been started between the South African Institute for Aquatic Biodiversity (SAIAB) and CABI to distribute posters and information to hotels and lodges across Namibia and Zambia to kick-start an early warning system for invasive freshwater crayfish.

eDNA

The application and advancement of molecular techniques are increasingly being recognised as emerging priorities for invasion science (Darling et al. 2017; Dehnen-Schmutz et al. 2018), presenting novel opportunities for crayfish monitoring. In particular, the environmental DNA (eDNA) approach is likely to become an important method for early detection of IAS

introductions, understanding trends in distribution, impacts on native biodiversity and ecosystems, and monitoring the effectiveness of management efforts, such as eradication attempts (Darling et al. 2017). Elsewhere, eDNA based methods have also been used to detect the presence of potential pathogens carried by invasive crayfish (Robinson et al. 2018; Wittwer et al. 2018). The relative merit of the application of species specific, compared to community based, eDNA methods will likely vary according to the management goal. Studies applying eDNA methods to the detection of crayfish currently remain fairly rare and largely focus on species specific detection methods (Geerts et al. 2018). Some successes in the detection of several species (*P. clarkii*, Geerts et al. 2018; *F. rusticus*, Dougherty et al. 2016; *P. leniusculus*, Harper et al. 2018; *P. virginalis*, Mauvisseau et al. 2019), suggest that eDNA analysis has the potential to become an efficient and reliable method for crayfish surveillance and management. To date, eDNA techniques have not been applied to crayfish detection in Africa. Further, it should be recognised that, although, much progress has been made toward standardisation and validation of eDNA methods, there are potential pitfalls linked to false positives and false negatives when assays are not fully validated, just as they can for many other ecological monitoring approaches (Ficetola et al. 2015). Consequently, the detection of crayfish in African freshwater systems using eDNA will require considerable field validation using traditional monitoring approaches such as trapping.

Conclusion

The most effective strategy in managing IAS is to prevent their arrival in the first place, and recent work involving risk analyses helps to refine estimates of the likely invasion pathways and the time at which the pathway is most likely to result in the successful establishment of an IAS (Tobin 2018; Faulkner et al. 2020). Nonetheless, countries which share land borders are only as secure as their neighbouring countries biosecurity legislation and implementation,

which is particularly concerning in the African context with regards to budget restrictions and large scale population flow throughout the region (Faulkner et al. 2020).

Examples in this review have shown how difficult it is to prevent crayfish introductions. In the event of a failure to prevent IAS from arriving, early detection and rapid response programs become a critical element, especially if eradication is the management goal (Robertson et al. 2020). Eradication becomes a less economically and biologically feasible option as the IAS occupies a larger area, the size of the invaded system increases, as well as reliability of methods used (Tobin et al. 2014). Although, crayfish eradication is notoriously difficult, if not impossible, improvements in traditional crayfish control methods and the emergence of novel control techniques also present a potential opportunity to increase the success of management attempts (see Manfrin et al. 2019 for a full review). Integrated approaches to control IAS are likely the most effective way forward (Stebbing et al. 2014), although, there have been few attempts at using an integrated approach to crayfish management in Africa, likely not only due to high costs and resource limitations but also lack of impact assessments.

Human attitudes and behaviours toward IAS are highly dependent on the socio-economic context, and this may result in management conflicts (Woodford et al. 2016; Zengeya et al. 2017). In Africa, positive socio-economic contributions of crayfish species are only documented for Kenya (Foster and Harper 2007) and Madagascar (Andriantsoa et al. 2019, 2020), however, local context will influence whether conflict over management interventions is likely to occur. For example, community resistance to management will likely be more pronounced in Madagascar than Kenya, since the invasive *P. virginalis* has been accepted as a key staple to local livelihoods in Madagascar, probably due to the occurrence of native crayfish

species in the country. Resistance from managing invasive crayfish is likely to be less pronounced in Southern African countries where there are few socio-economic benefits. There is a suspected relationship between greater levels of exposure to crayfish and the likelihood of acceptance into local people's livelihoods. Therein, if crayfish species become naturalised and utilized for economic gains then the possibility of management conflict is high as this will promote intentional propagation throughout freshwater systems. For progress to be made in addressing these potential conflicts, there is a need to establish a formal dialogue between regulators, crayfish users and conservationists as suggested by Ellender et al. (2014), so that policies for established non-native fisheries are supported by all stakeholders before they become a "wicked problem" (Woodford et al. 2016).

The lack of data on establishment, spread, and ecological impacts of crayfishes are important knowledge gaps as there is insufficient evidence to compel policymakers to implement legislation or management actions to minimise further introductions or spread. Future research is required on generating cross border, contextually appropriate, and robust abundance and distribution data for invasive crayfish and functionally similar native species to form baseline assessments as invasions progress. Such data can inform ecological niche models to predict presence/absence and prioritise monitoring sites (Nunes et al. 2017c). As current research efforts are geographically, temporally, and methodologically disjunct, this will require the development of a regular monitoring scheme employing standardised sampling protocols to detect new invasions and robustly compare density estimates and population structure across regions. Once a cohesive method has been established the data should be incorporated into a database of locality records of formal and informal presence/absence data for crayfish and freshwater crab species at a national scale. In more contained invasions, such as within dam environments, a focus should be on evaluating the efficacy and potential biases of intensive

manual depletion on crayfish populations. With regards to invasion impacts the specifics of trophic interactions with native species should be assessed, especially along an invasion gradient as this can give insights on the effect of crayfish abundance on nutrient pathways as well as acting as a forecasting system as the invasion progresses. Prediction of impact in situ is troublesome, especially considering the large scales of the invaded systems, therefore contextually relevant experimental approaches should be combined with comparable field data to ground truth predictions. This should involve competitive and consumptive resistance as well as species specific impact assessments via contest experiments, functional response analysis, and mesocosm experiments. Researchers should maintain a focus on native freshwater crab co-occurrence and population dynamics over a long term and whole systems scale to assess changes in community composition as basic ecology of the continents closest functional analogue is lacking.

Understanding crayfish impacts on specific ecosystem services is necessary not only for their regulation and management, but also to guard against detriment to human wellbeing in a continent where food security and water resources are already precarious (Egoh et al. 2020). This is particularly relevant as some of the crayfish species were introduced for aquaculture and there are ongoing requests for the use of crayfishes, and indeed other species, in both inland fisheries and for aquaculture (Nunes et al. 2017c; Moshobane et al. 2020). As economic information on fisheries and aquaculture development based on invasive crayfish is lacking, research into the factors driving the success, or failure, of such enterprises could provide important information for decision makers needing to consider economic and environmental factors in decision making. As crayfish invasions contribute to economic losses through consumption of catches and destruction of fishing gear, nonetheless the actual realised economic impact of such scavenging behaviour has not yet been quantified and therefore may

be potentially over or under-estimated. Quantitative surveys should address contextually different socio-economic impacts of different crayfish species and address this with regards to whether the communities affected can also derive positive benefits from the invasion.

CHAPTER 3

STANDARDISATION OF *CHERAX QUADRICARINATUS* SAMPLING GEAR IN SOUTHERN AFRICA

Abstract

Freshwater crayfish are damaging invaders across southern Africa, however, monitoring techniques and efforts are disparate as different sampling methods have been used across the region. To develop a standard method for assessing redclaw crayfish *Cherax quadricarinatus* abundance, a survey was conducted to assess for differences in detection and catch per unit effort (CPUE) in Lake Kariba. Two sampling approaches were compared, opera traps baited with cooked maize meal, historically used in crayfish surveys in Zimbabwe, and promar traps baited with dry dog food that have been used for assessments in South Africa and Swaziland. Baits were compared in the Barotse floodplain in Zambia using the promar® trap. Detection Probability ($P_{capture}$) and CPUE were significantly lower for opera traps baited with cooked maize meal than the promar traps baited with dry dog food. Sex ratio and carapace length of crayfish sampled did not differ between sampling methods. Due to higher CPUE, and the prevalence of the method in the area, we consider the promar trap baited with dog food approach to be the better method for determining crayfish population abundance. Future comparisons of abundance should take this into consideration by applying the conversion factors presented here, if different methods are applied.

Introduction

Multiple freshwater crayfishes have escaped from captivity and invaded African freshwater ecosystems (Madzivanzira et al. 2020; Chapter 2). The Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) has invaded the Zambezi River Basin and several other rivers in southern Africa, where it is rapidly spreading (Madzivanzira et al. 2020; Chapter 2). In order to understand the invasion process, implement management measures or undertake impact assessments, comparable data on presence and abundance are required (Larson and Olden 2016; Madzivanzira et al. 2020; Chapter 2).

Common sampling methods for crayfish include active methods such as hand collecting, electrofishing and direct underwater or bankside observation and passive methods such as the use of baited traps (Williams et al. 2014; Larson and Olden 2016). Baited traps are the most commonly applied method (Larson and Olden 2016). While a variety of traps have been used in crayfish surveys in Africa (see Madzivanzira et al. 2020; Chapter 2), two designs: opera traps (Marufu et al. 2014, 2018a) and collapsible promar traps (Nunes et al. 2017a); are most commonly applied methods for sampling *C. quadricarinatus* in southern Africa. Bait also differs between surveys, with choices including cooked maize meal (e.g., Marufu et al. 2014, 2018a, 2020) and dry dog food pellets (e.g., Nunes et al. 2017a). Recently, Mhlanga et al. (2020) investigated the effectiveness of three trap designs and three bait types to identify the best means for harvesting *C. quadricarinatus* in Lake Kariba. Cylindrical, rectangular and opera traps were used which were baited with liver, cooked maize meal and fish heads were compared. As both trap design and bait type have been shown to influence freshwater crayfish catch rates (Huner 1988; Huner and Paret 1995), different sampling methods can result in disparate results in surveys seeking to estimate abundance or even the distribution of crayfish. Research into developing standardised field sampling approaches is therefore required for

cohesive assessment of invasion status and population dynamics (Madzivanzira et al. 2020; Chapter 2).

In this study two field experiments are presented, one to compare the sampling approaches employed by Marufu et al. (2014, 2018a, 2020) and Nunes et al. (2017a) in Lake Kariba, Zimbabwe, and one to assess the influence of bait choice in the Barotse floodplain, Zambia. The central hypothesis was that there would be no difference between methods with regard to detection probability (the proportion of traps containing at least one crayfish), catch rate (number of individuals per trap per night), crayfish size and sex ratio. This study further re-calculates catch data across the sampled regions in order to have cohesive population abundance estimates.

Materials and methods

Study sites

The gear comparison study was carried out in the Sanyati Basin of Lake Kariba, which is to the north-eastern end of the lake (Fig. 3.1) where *C. quadricarinatus* populations have established (Madzivanzira et al. 2020). Lake Kariba is the largest man-made lake (by volume) and is located along the Zambezi River and shared between Zambia and Zimbabwe, forming part of the Middle Zambezi Biosphere Reserve (Magadza et al. 2020). Sampling was carried out between 27 November to 5 December 2018 at 10 locations in the basin (Fig. 3.1a).

The bait study was carried out in the Barotse floodplain in Zambia where *C. quadricarinatus* populations have established (Madzivanzira et al. 2020). The Barotse floodplain is the largest

wetland in the Zambezi Basin endowed with rich biodiversity and is considered to be a centre for biological diversification (Tweddle 2010). The annual inundation in the Barotse floodplain is influenced by the Zambezi River and its tributaries such as Lungwebungu and Luanginga emanating from the Lungwebungu and Luanginga Basins, respectively in Angola as well as the Kabompo River in Zambia (Zimba et al. 2018). Sampling was done between the 16th and 18th of October 2019 at 3 locations in the floodplain (Fig. 3.1b).

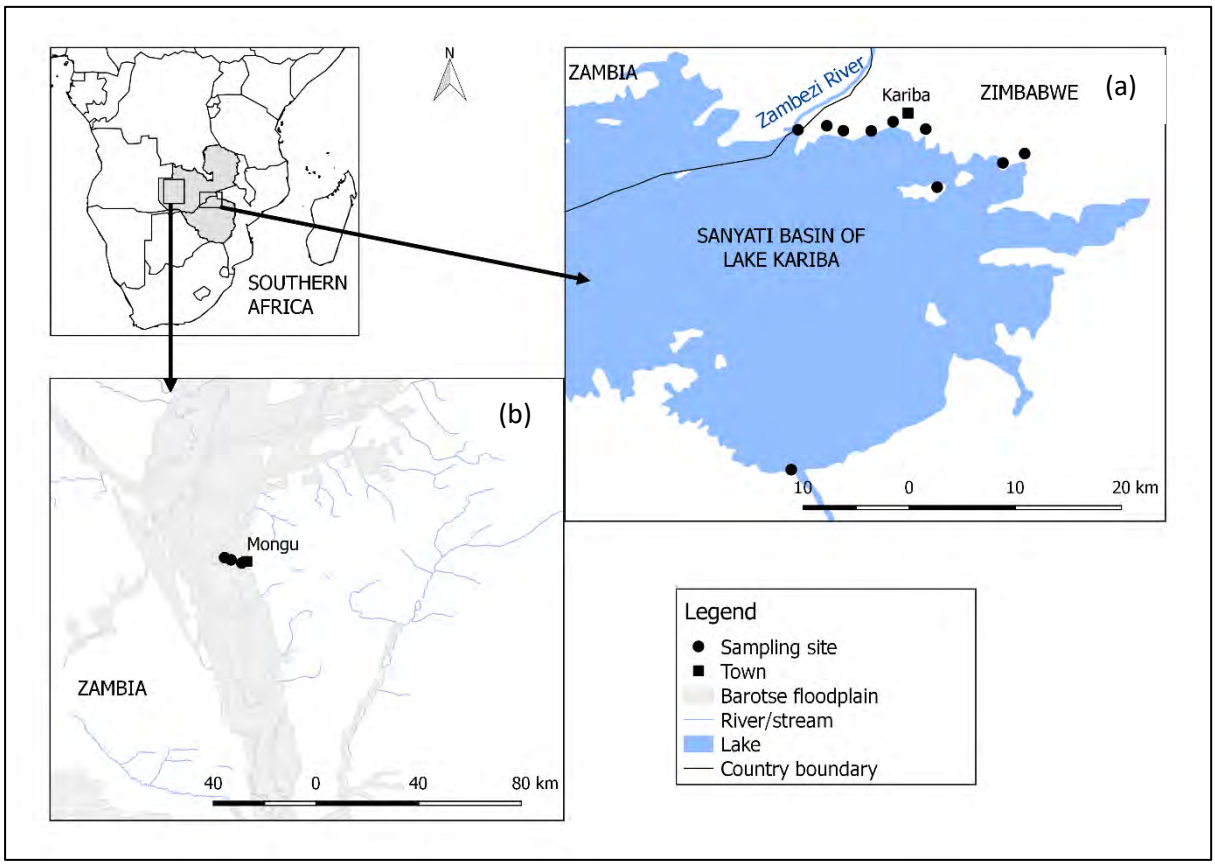

Figure 3.1. Sampling sites in: (a) the Sanyati Basin of Lake Kariba where opera traps baited with cooked maize meal and promar traps baited with dog food were compared; and (b) sampling sites on the Barotse floodplain where baits were compared.

Gear comparison

The opera trap (dimensions: 100 × 50 × 30 cm; mesh size: 10 mm) and the collapsible promar® crayfish trap (dimensions: 61 × 46 × 20 cm; mesh size: 10 mm) were used in this survey (Plate 3a). The opera trap entrance has a fixed circular area of 78.5 cm^2 whilst that of the promar collapsible trap is flexible and can widen to a rectangular area of 460 cm^2. The opera traps were baited with approximately 100 g of cooked mealie meal and the promar traps were baited with 100 g of Bobtail® dry dog food pellets.

Plate 3a. (a) The opera trap used for crayfish surveys in Zimbabwe and (b) the promar trap used for crayfish surveys in South Africa and Swaziland. (Photo: Takudzwa C Madzivanzira, 2018).

At each sampling locality the physico-chemical variables (temperature, pH, dissolved oxygen saturation, turbidity, electrical conductivity and salinity) were measured and traps were deployed in pairs, comprising of an opera trap and one collapsible trap deployed at least 10 m apart to minimise potential interaction between baits. Traps were deployed overnight at depths of ≤ 5 m. On retrieval, the number of crayfish caught in each trap was recorded and each crayfish was measured for carapace length (CL), weighed (to the nearest g) and sexed.

Bait comparison

The bait study was carried out in the Barotse floodplain to compare bait efficiency in the promar traps. Fifty-six promar traps baited with either cooked mealie meal or dog food were deployed overnight at depths of ≤ 5 m. These were also deployed 10 m apart. On retrieval, the number of crayfish caught in each trap was recorded and each crayfish was measured for CL, weighed (to the nearest g) and sexed.

Data analysis

The STATISTICA software package (version 7.1; Statsoft, Inc.) was used to conduct the analysis. Differences in the habitat as well as physico-chemical variables in this survey were not considered factors as the gears with different baits were deployed simultaneously in the same environment. Detection probability ($P_{capture}$) was expressed as the number of traps containing at least one crayfish as a proportion of all traps set. Catch rate was expressed as catch per unit effort (CPUE) as the mean number of individuals caught per trap per night set. The Shapiro–Wilk test of normality failed to reject the null hypothesis that CPUE data were normally distributed for the $promar_{df}$ sampling method ($w = 0.86$, $p > 0.05$), and rejected the null hypothesis that CPUE data were normally distributed for the $opera_{mm}$ sampling method ($w = 0.82$, $p < 0.03$), and therefore the data was log-transformed. A t-test was used to test for CPUE differences between the sampling methods. The $P_{capture}$ were analysed with contingency tables, and the differences in $P_{capture}$ between the two gears were tested with a Chi-square statistical test. The relationship between CPUE from the sampling methods was explored using linear regression to estimate a conversion factor for data standardisation. A Kolmogorov-Smirnov two-sample test was used to test whether CL distributions from the sampling methods differed significantly. Finally, sex ratios between the sampling methods were compared using contingency tables and tested using Chi-square analysis.

Results

Gear comparison

In Lake Kariba 572 crayfish were caught during this survey. Of these, 116 were caught from the $opera_{mm}$ method and 456 were caught in the $promar_{df}$ method. Overall, $P_{capture}$ for the $opera_{mm}$ method (0.41) was significantly lower ($\chi^2 = 13.90$, df = 1, $p < 0.001$) than that for the $promar_{df}$ (0.67) method. Mean (± SE) CPUE for the $opera_{mm}$ method (1.19 ± 0.24 ind.·trap^{-1}·night^{-1}) was significantly lower ($t_{(18)} = 2.84$, $p < 0.05$) than that for the $promar_{df}$ method (4.53 ± 0.82 ind.·trap^{-1}·night^{-1}). The CL distribution of *C. quadricarinatus* (see Fig. 3.2 for CL distributions) did not differ significantly (K-S, D = 0.05; $p = 0.98$). The overall female to male ratio for $opera_{mm}$ (1:1.06) was not significantly different ($\chi^2 = 0.54$, df = 1, $p = 0.49$) from that of the $promar_{df}$ method (1:1.22). Regression analysis indicated a weak correlation between CPUE of the two sampling approaches ($R^2 = 0.40$) which was best explained by the relationship: CPUE$promar_{df}$ = 2.68·CPUE$opera_{mm}$ + 1.28 (Fig. 3.3). Data collected using $opera_{mm}$ can therefore be up-calculated using the equation in Fig. 3.3 (see Table 3.1 for final CPUEs).

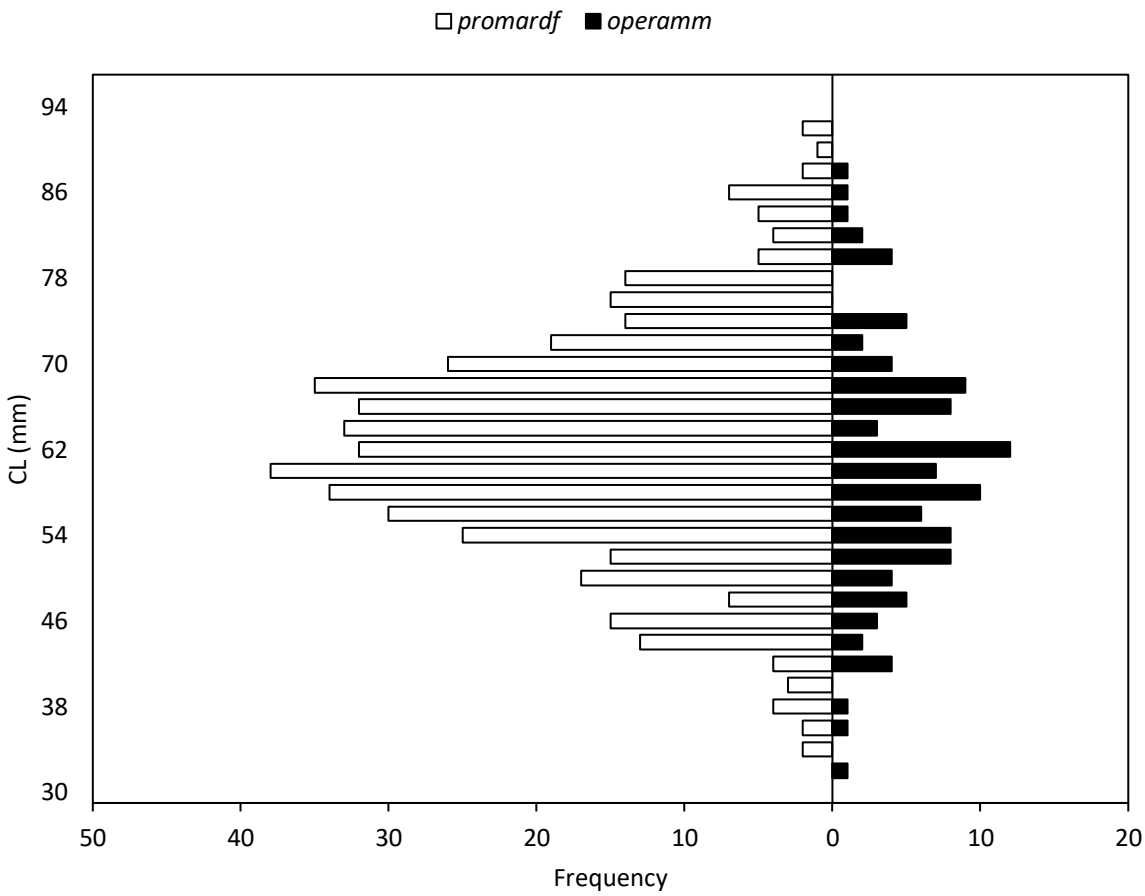

Figure 3.2. Carapace length (CL) frequency distributions of *Cherax quadricarinatus* in the $opera_{mm}$ and $promar_{df}$ methods in the Sanyati Basin, Lake Kariba, November-December 2018.

Table 3.1. Standardised CPUEs from Marufu et al. (2014, 2020) using regression parameters from Fig. 3.3.

Site	CPUE (ind.$^{-1}$. trap^{-1}. night^{-1}.)	Slope	Constant	Predicted standardised CPUES (ind.$^{-1}$. trap^{-1}. night^{-1})
1	2.00	2.68	1.28	6.64
2	0.00	2.68	1.28	1.28
3	4.00	2.68	1.28	12.00
4	0.20	2.68	1.28	1.82
5	2.17	2.68	1.28	7.09
6	1.33	2.68	1.28	4.85
7	1.67	2.68	1.28	5.75
8	0.50	2.68	1.28	2.62
9	0.20	2.68	1.28	1.82
10	0.00	2.68	1.28	1.28
11	0.67	2.68	1.28	3.07
12	0.50	2.68	1.28	2.62

Bait study

In the bait experiment on the Barotse floodplain, 121 of the 128 *C. quadricarinatus* were caught in traps baited with dog food and 7 in traps baited with cooked maize meal. Overall, $P_{capture}$ for traps with dogfood (0.89) was significantly higher ($\chi^2 = 23.63$, df = 1, $p < 0.001$) than that for cooked maize meal (0.29). Mean (± SE) CPUE for dog food bait (4.29 ± 0.83 ind.·trap^{-1}·night^{-1}) was significantly higher ($t_{(2)} = 3.43$, $p < 0.05$) than cooked maize meal bait (0.25 ± 0.17

ind.·trap^{-1}·night^{-1}). Insufficient crayfish specimens were captured with maize meal bait for meaningful comparison on CL and sex ratios.

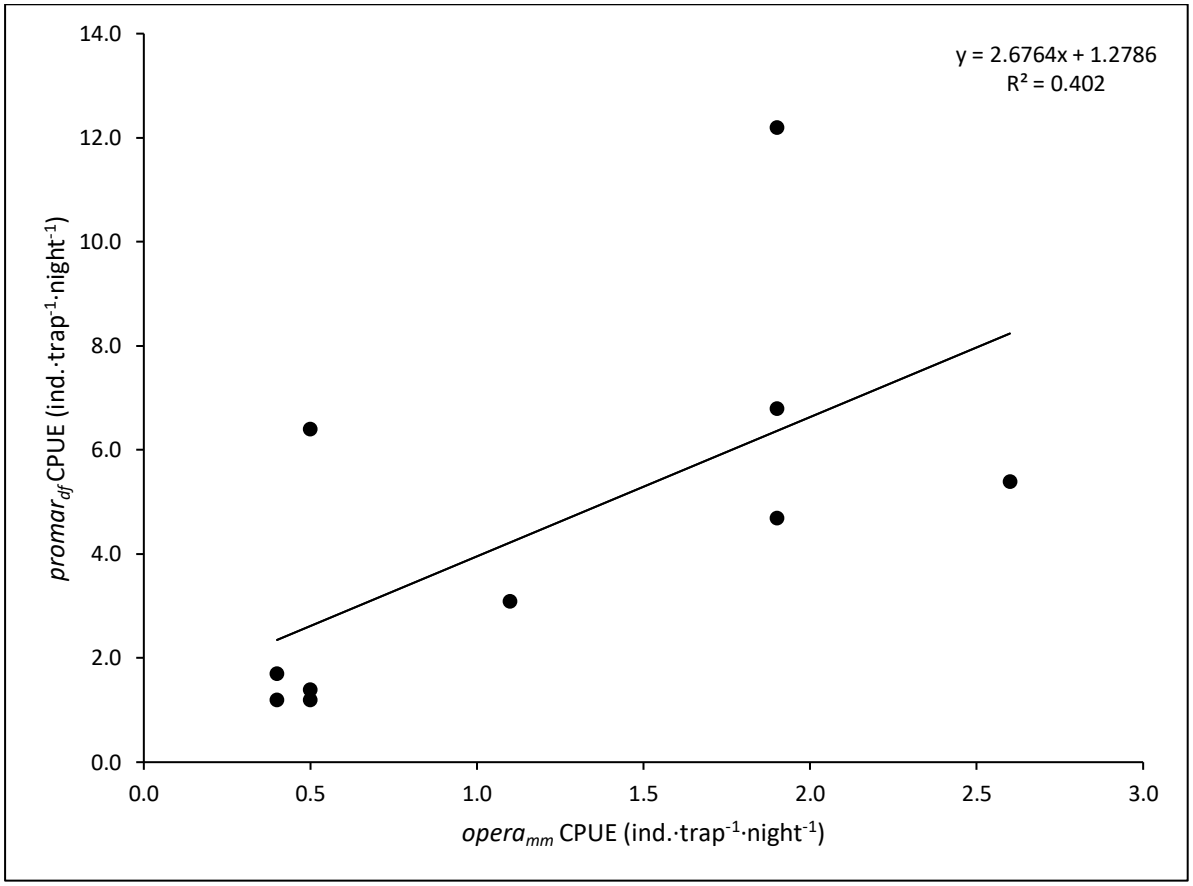

Figure 3.3. CPUE of the *opera*$_{mm}$ sampling method versus the *promar*$_{df}$ sampling method from 10 sampling localities in the Sanyati Basin, Lake Kariba, November-December 2018.

Discussion

The first objective of this study was to determine the more effective of the two sampling approaches used for *C. quadricarinatus* in southern Africa, an important step towards standardising crayfish sampling methods in Africa (Madzivanzira et al. 2020; Chapter 2). In Lake Kariba, detection probability and CPUE were significantly higher for *promar*$_{df}$ than for *opera*$_{mm}$. Sex ratio and size structure did not however, differ between sampling approaches.

The second objective was to determine whether there was a difference between bait type effectiveness. The bait type experiment demonstrated that the use of dried dog pellets as bait resulted in higher CPUE and superior detection probability over cooked maize meal. The results demonstrate that, CPUE data from the $opera_{mm}$ method require up-calculation by a factor of 2.68 to enable comparisons with data obtained using the $promar_{df}$ method for *C. quadricarinatus.*

Neither the proportion of females to males as well as the CLs differed between the two sampling approaches. However, it should be noted that although the two sampling approaches captured a wide range of crayfish sizes with total and carapace length ranges of 65 to 197 mm and 32 to 91 mm respectively, juveniles with CL less than 32 mm were poorly represented overall. As crayfish trapping is a passive sampling method, it is not only dependent on the overall crayfish abundance, but also their behaviour, and this will unavoidably interact with trapping results in potentially complex ways (Dorn et al. 2005). Large and aggressive crayfish which normally favour baited traps (Brown and Brewis 1978; Capelli and Magnuson 1983) often tend to defend traps as habitat and exclude smaller individuals (Ogle and Kret 2008) which may account for the observed poor percentage of juveniles.

Effectiveness of the dog food bait in attracting crayfish may be related to its high crude protein content (180 g/kg) compared to that of cooked maize meal (50-84 g/kg). Baits with high-protein content are considered effective for crayfish (Huner and Barr 1980). Other high protein baits have been found to produce similar catch results to that of the dog food (Larson and Olden 2016). Somers and Stechey (1986) did not find any significant CPUE differences (for *Cambarus bartonii* (Fabricius 1798) and *Faxonius virilis* (Hagen 1870)) between liver, fish,

chicken, canned cat food and dog food baits. In Lake Kariba, the CPUE of traps baited with liver, cooked maize meal and fish heads were not significantly different (Mhlanga et al. 2020). The traps used by Mhlanga et al. (2020) differed in size, with large traps catching more crayfish than the smaller traps. Dog food is suitable as a standard bait as it is easy to handle and store and requires less time to prepare for fieldwork and can be standardised. Other baits, for example, fish and liver, would need to be standardised according to species, size and condition, which would make the standardisation process across the region cumbersome. An example of such bait use is the sampling of *Procambarus clarkii* (Girard 1858) in Mimosa Dam, Free State Province in South Africa using fish head bait (common carp *Cyprinus carpio,* Orange River mudfish *Labeo capensis* and moggel*, Labeo umbratus*) (Barkhuizen et al. Unpublished data). The other types of bait can be suitable for crayfish exploitation purposes and population suppression as suggested by Mhlanga et al. (2020) which should be an integrated approach for it to be effective (Gherardi et al. 2011). The intensive crayfish removal programme to suppress crayfish populations, while practical in small contained water bodies and reservoirs, is virtually not possible in vast systems like Lake Kariba.

Standardisation and affirmation of crayfish trapping gear capacities in the southern African region is essential information for further work on the subject (Madzivanzira et al. 2020; Chapter 2). Due to higher $P_{capture}$ and CPUE across multiple sampling sites, the collapsible promar crayfish trap baited with dog food could be considered to be the most appropriate gear for crayfish abundance studies in Africa. The findings that the two types of gear used in this study differed in their efficiency shows that correction is necessary when comparing population abundance estimates in the region.

CHAPTER 4

DISTRIBUTION AND ESTABLISHMENT OF THE ALIEN AUSTRALIAN REDCLAW CRAYFISH, *CHERAX QUADRICARINATUS*, IN THE ZAMBEZI BASIN

Abstract

The Australian redclaw crayfish *Cherax quadricarinatus* has established and is spreading in several southern African freshwater systems, including the Zambezi River Basin. To determine the invasion status and extent of invasion of *C. quadricarinatus*, surveys were conducted across the Zambezi Basin from 2017 to 2019. This study confirmed the establishment of the recently reported *C. quadricarinatus* in the Barotse Floodplain, Upper Zambezi Floodplains freshwater ecoregion. The probability of capture ($P_{capture}$), catch per unit effort (CPUE) and population characteristics of *C. quadricarinatus* from the Barotse floodplain were compared with older invasions from Lake Kariba and Kafue River (invaded in the early 2000s). The $P_{capture}$ and CPUE of *C. quadricarinatus* in the invaded region of the Barotse floodplain were similar to that of the older invasions. Both mass and carapace length of *C. quadricarinatus* from the Barotse floodplain were significantly lower than that of *C. quadricarinatus* from the older invasions. The sex ratio (female:male) of *C. quadricarinatus* from the invaded regions was significantly different, with more females present in the more recent Barotse floodplain invasion. Although no crayfish were detected in the Zambezian Headwaters and the Okavango Floodplains ecoregions, *C. quadricarinatus* have the potential to spread at a downstream and upstream rate of 49 and 12 km·year^{-1}, respectively. We recommend a continued long-term monitoring programme of the regions at risk of new invasions to limit the need for costly and resource-intensive control programmes.

Introduction

Major biodiversity declines are occurring worldwide and across all ecosystems (Ceballos et al. 2015; Pimm et al. 2014; Tickner et al. 2020). Freshwater species are particularly affected; however, a strategy has been outlined to bend the curve of biodiversity declines, which includes tackling invasive alien species (IAS) (Tickner et al. 2020; Jarić et al. 2020). In addition to their negative impacts on biodiversity and ecosystems, IAS can also confer a considerable cost to economy not only as a direct impact upon ecosystem services, but also in terms of management costs (Marbuah et al. 2014; Bradshaw et al. 2016; Barbet-Massin et al. 2020; van Wilgen et al. 2020). Additionally, Aichi Target 9, which set out to identify IAS and their pathways, to control or eradicate them and put in place measures to manage pathways to prevent their introduction and establishment, has not been achieved. Major set-backs impeding objective achievement are scarcity of information regarding distribution, abundance and impacts, as well as control legislation (Pergl et al. 2019; Faulkner et al. 2020). Therefore, identifying, quantifying and monitoring the distribution and abundance of IAS, in a standardised manner, is key to developing effective and long-term management solutions (Pergl et al. 2019).

Multiple freshwater crayfish species are now widespread globally as a result of aquaculture and aquarium trade-related invasion pathways (Lodge et al. 2012; Madzivanzira et al. 2020; Chapter 2). Impacts of introduced crayfish include direct and indirect effects of predation upon native species via polytrophic, generalist feeding behaviours; spreading of parasites and diseases; habitat modifications; and loss of macrophytes (Gherardi 2007; Lodge et al. 2012; Twardochleb et al. 2013; Souty-Grosset et al. 2016; Madzivanzira et al. 2020; Chapter 2). Generally, crayfish introductions have also been shown to cause strong disruptions to native food webs (Ficetola et al. 2012). Crayfish can also cause significant economic costs through

burrowing activities and damage to agricultural infrastructure which affect agriculture production, also by scavenging on fish catches and destruction of fishing gear (Lodge et al, 2012; Madzivanzira et al. 2020; Chapter 2). Despite the documented impacts, crayfish still remain one of the most widely introduced freshwater taxa (Hobbs et al. 1989; Gherardi 2010; Battisti and Scalici 2020).

Nine crayfish species have been introduced to mainland Africa (Madzivanzira et al. 2020; Chapter 2), which has no native freshwater crayfish species (Crandall and Buhay 2008). In southern Africa, the Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) and Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852) have established populations in the wild (Madzivanzira et al. 2020; Chapter 2). In southern Africa *Procambarus clarkii* is currently only reported in three localities, but *C. quadricarinatus* is spreading rapidly, with populations reported from South Africa, Swaziland, Mozambique, Zambia and Zimbabwe (Madzivanzira et al. 2020; Chapter 2).

The first documented introduction of *C. quadricarinatus* into the Zambezi system was in 2001 when this species was introduced from Swaziland to two fish farms in the Zambezi system, one at the eastern end of the Kafue Flats (Kafue freshwater ecoregion *sensu* Abell et al. 2008), and the other at Siavonga on the shore of Lake Kariba (Middle Zambezi-Luangwa freshwater ecoregion) in Zambia (Madzivanzira et al. 2020; Chapter 2). Wild populations of *C. quadricarinatus* were first reported in the Kafue River in 2001 and in 2002 in Lake Kariba (Douthwaite et al. 2018). Victoria Falls is a natural barrier to the unaided spread of *C. quadricarinatus* from Lake Kariba into the Upper Zambezi River system. However, in November 2014 *C. quadricarinatus* was reported on the Barotse Floodplain (in the Upper

Zambezi Floodplains ecoregion) (Nunes et al. 2016), the largest wetland in the Upper Zambezi, after they escaped from an open tank kept by road construction workers who were working on a road traversing the floodplain (Madzivanzira et al. 2020; Chapter 2).

Although there is some literature available on the presence of crayfishes in the Zambezi Basin, the lack of standardisation of survey methods limits the comparative utility of these surveys (see Chapter 3). Data on distribution, abundance and population structure of IAS are an essential component of their management because they provide environmental managers and policy makers with the information necessary to target and undertake appropriate management actions (Sibley et al. 2002). Baseline distribution surveys of IAS determines current presence and absence across an invasion gradient, potential areas at risk of invasion, and a starting point for future long-term monitoring campaigns. Early detection of *C. quadricarinatus* can facilitate rapid response actions, which can reduce the long-term costs of managing this IAS and associated ecological impacts. This study, based on surveys using a standardised trapping approach, aimed to provide data on the extent of occurrence and relative abundance of *C. quadricarinatus* across the middle and upper Zambezi Basin. Crayfish population characteristics have been shown to vary along invasion gradients (Hudina et al. 2017; Messager and Olden 2019). Therefore, this study compared *C. quadricarinatus* population characteristics, including abundance, mass, carapace length and sex ratio from more established invasions (Kafue and Lake Kariba) with a recent invasion (Barotse Floodplain), to accurately reflect the population dynamics across the area. Comparing more established invasions with a recent one means that the data will act as a forecasting tool to better understand how the Barotse floodplain invasion may progress in the future.

Methods

Study areas

The study was conducted in the Zambezian Headwaters (ecoregion 555), Upper Zambezi Floodplains (ecoregion 556), Okavango Floodplains (ecoregion 569), Middle Zambezi-Luangwa (ecoregion 558) and Kafue (ecoregion 557) freshwater ecoregions (*sensu* freshwater ecoregions of Africa, Abell et al. 2008). The water in these ecoregions is critical for sustainable economic growth and poverty alleviation and supports the basic needs of more than 30 million people (Thieme et al. 2005). By sustaining a rich and diverse fish faunal diversity and natural environment, it plays a central role in the economies of eight countries (Thieme et al. 2005). Threats to these freshwater ecoregions include overexploitation, land clearance, fire, pollution, dams and modification of hydrology, climate change and introduction of exotic species (Darwall et al. 2008; Darwall et al. 2009). Dominant invaders include the water hyacinth *Eichhornia crassipes*, red water fern *Azolla filiculoides*, water lettuce *Pistia stratiotes*, Kariba weed *Salvinia molesta*, Nile tilapia *Oreochromis niloticus* (Tweddle 2010; Zengeya et al. 2015) and *C. quadricarinatus* (see Madzivanzira et al. 2020).

Zambezian Headwaters Ecoregion

The Zambezian Headwaters Ecoregion extends from the headwaters of the Okavango catchment in the west to the Kafue River headwaters in the east and includes the headwaters of the Upper Zambezi River (Thieme et al. 2005). The majority of the rivers in this ecoregion drain the interior of Angola, but parts of the upper Kafue and upper Zambezi flow through northern Zambia. Sampling in this ecoregion was conducted in the Kabompo River and its West Lunga tributary in Zambia (Fig. 4.1). The Kabompo River is characterised by diverse freshwater habitats that include forested headwaters in the main channel, backwaters, floodplains, rapids and pans, with unconsolidated sandy bottoms dominating in the main

channel. Along the Kabompo River ten sites were sampled comprising eight sites upstream of the Kabompo River before its confluence with the West Lunga River, one at the confluence and one upstream of West Lunga River before the confluence. Overall, 200 traps were set in this ecoregion.

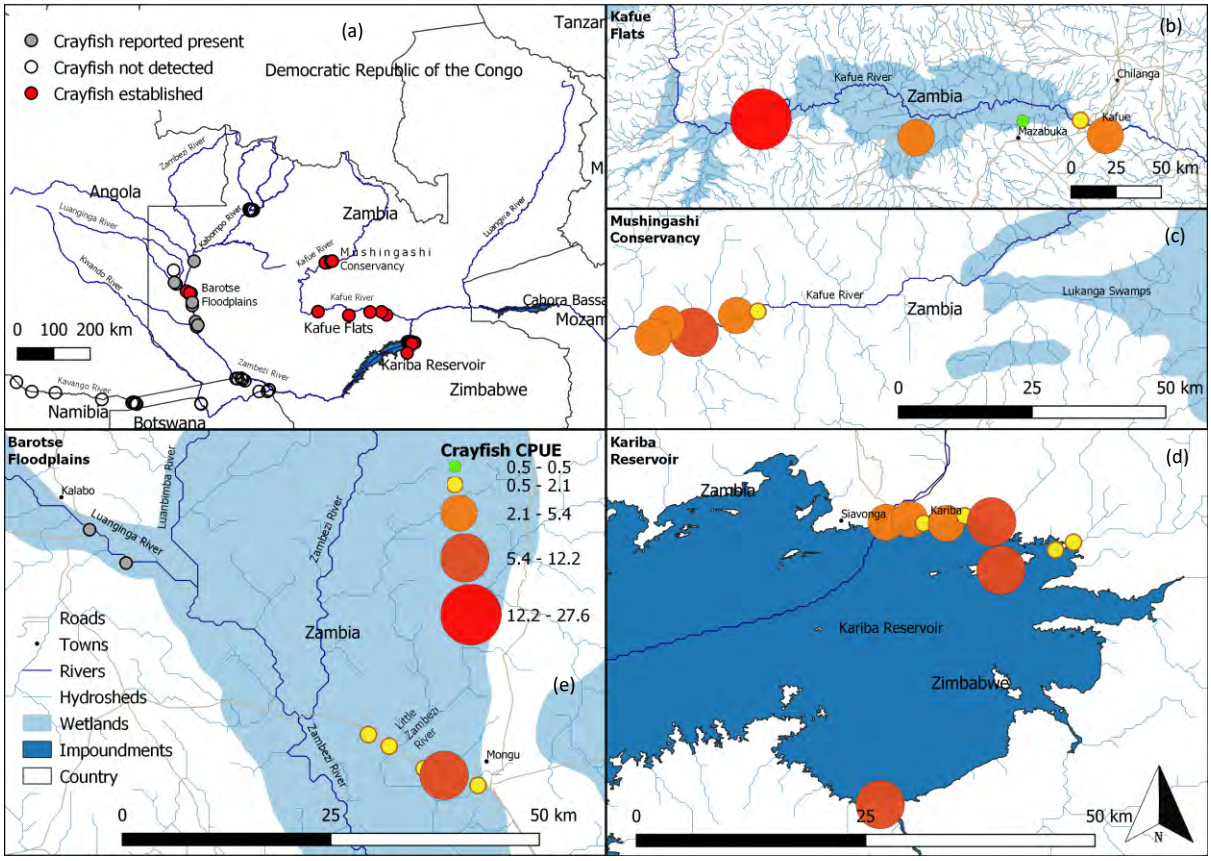

Figure 4.1. Study area in Zambia, Namibia, Botswana and Zimbabwe. (a) General overview of the sampling regions showing the 68 sites sampled in this study. The status of *Cherax quadricarinatus* in the sampled regions is represented by: grey circle = reported present, white circle = not detected, and red circle = established. Area specific CPUE is shown as inserts (b) Kafue Flats; (c) Mushingashi Conservancy on the upper Kafue River; (d) Lake Kariba; and (e) Barotse Floodplain.

Upper Zambezi Floodplains

This ecoregion is located across south-eastern Angola, south-western Zambia, the Zambezi Region of Namibia, and the northern edge of Botswana (Thieme et al. 2005). It begins at the top of the Barotse Floodplain demarcated by the confluence of the Kabompo River and the Zambezi River in the East. The Zambezi River then flows in a southerly direction through the Barotse Floodplain and turns east before descending the Victoria Falls gorge (Thieme et al. 2005) which demarcates the middle and upper sections of the Zambezi River (Davies 1986). The Zambezi River in the Barotse Floodplain is characterised by shallow loose sandy bottoms with a very slight river gradient, which results in calm slow-moving waters (Moore et al. 2007). The floodplain is characterised by grassy swamps with few to no trees, which provide breeding and feeding grounds for a moderately rich fish fauna (Winemiller and Kelso-Winemiller 2003). The river channel branches extensively with natural and man-made canals. The Barotse floodplain experiences a peak in inundation approximately 3 months after the peak of the rainy season in January–February (Zimba et al. 2018). By April the flood has usually reaches its peak and recedes from May to December (Zimba et al. 2018). Fifteen sites were sampled in the Barotse Floodplain, extending from the inflow of the Kabompo River to the end of the floodplain at Senanga (n = 257 traps), three sites were sampled on the Liuwa plains (n = 55 traps), and five sites were sampled downstream of the Barotse Floodplain along the Zambezi River main stem, in Namibia (n = 79 traps) (Fig. 4.1).

Okavango Floodplains

This ecoregion covers the Okavango Basin which flows south and east to diverge into the many arms of the Okavango Delta in Botswana (Beadle 1981). Sampling in this ecoregion was conducted in the Kavango, Kwando (Namibia) and Chobe (Botswana) rivers (Fig. 4.1). The Kavango River is characterised by numerous backwaters and floodplain lagoons that become

contiguous during the flood (Taylor et al. 2017). Floods in the Kwando River are not usually sufficient to inundate the floodplain, and backwaters may be cut off from main channel habitats by dense mats of plants (Taylor et al. 2017). The area around the Chobe River consists of riverine woodlands, open woodlands, as well as lagoons, with the rest of the region mainly consisting of annually inundated floodplains (Pricope 2013). The Chobe River joins the Upper Zambezi River at the eastern end of the 120 km long Zambezi Region floodplain (Tweddle 2010). In years of exceptionally high flood levels in the Okavango River, it overflows from the Okavango Delta to the northeast via the Selinda (Makwegana) Spillway to the Chobe/Linyanti and from there to the Upper Zambezi River (Tweddle 2010). Thirteen sites were sampled in the Kavango River and floodplain (n = 219 traps), four in the Kwando River and backwaters (n = 20 traps), and four in the Chobe River (n = 40 traps).

Middle Zambezi-Luangwa

The Middle Zambezi-Luangwa Ecoregion extends from Victoria Falls (Zambia and Zimbabwe) to the Cahora Bassa dam wall (Mozambique) along the Zambezi River and from the headwaters of the Luangwa River (Zambia) to its confluence with the Zambezi River (Thieme et al. 2005). The Middle Zambezi River flows through narrow gorges and fault-defined valleys and has been extensively modified by two hydroelectric reservoirs (lakes Kariba and Cahora Bassa) (Thieme et al. 2005). In this ecoregion, sampling was conducted in Lake Kariba, Zimbabwe which is a monomictic lake characterised by mostly, a pelagic (open water) lentic habitat (Magadza et al. 2020). The major rivers that drain directly into the lake are Lufua, Luizilukulu and Sengwe on the Zambian side and Sanyati, Gache-gache, Charara, Nyaodza, Ume and Sengwa on the Zimbabwean side. In Lake Kariba, ten sites in the littoral zone of the Sanyati Basin (n = 102 traps) characterised by greater size diversity of benthic substrates (from clay to bedrock substrates) were sampled (Fig. 4.1a).

Kafue Ecoregion

The Kafue Ecoregion encompasses the Kafue River drainage basin from central Zambia to the Kafue Gorge where the river enters the Middle Zambezi River. Two reservoirs, Lake Itezhi-Tezhi and Kafue Gorge Dam were constructed on the Kafue River mainly for power generation (Tweddle 2010). This ecoregion contains the extensive and seasonally inundated Kafue Flats floodplains and Lukanga swamps (Thieme et al. 2005). The Kafue Flats region is characterised by a shallow gradient, and diverse habitats, with delayed flood pulses. The floodplains generally become inundated between December and May thereby driving the productivity of the flats. In this ecoregion, six sites were sampled on the Kafue Flats (n = 129) and five sites on the Kafue River above Lake Itezhi-Tezhi (n = 95 traps) (Fig. 4.1).

Sampling

Sampling in these ecoregions was conducted over a period of 2 years, Kafue in 2017, Middle Zambezi-Luangwa in 2018, and Upper Zambezi Headwaters, Upper Zambezi Floodplains and Okavango Floodplains in 2019. At each sampling locality the physico-chemical variables (temperature, pH, dissolved oxygen saturation, turbidity, electrical conductivity, total dissolved solids (TDS) and salinity) were measured (Table 4.1). Collapsible Promar® crayfish traps (dimensions: 61 × 46 × 20 cm; mesh size: 10 mm) baited with approximately 100 g of Bobtail® dry dog food pellets steak flavour (the standardised approach recommended for sampling *C. quadricarinatus*, Madzivanzira et al. Accepted; Chapter 3) were then deployed at least 20 m apart at depths of ≤ 5 m and left overnight. A total of 1196 traps were deployed in all the five ecoregions. On retrieval, the number of crayfish caught in each trap was recorded and each crayfish was measured for carapace length (CL), weighed (to the nearest g) and sexed. Individuals that could not be sex differentiated, due to possession of both male and female genitals, were recorded as intersex individuals.

Table 4.1. Mean (±SE) of physico-chemical variables from the sampled regions.

Sampling region	Temperature (°C)	pH	DO ($mg \cdot l^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)	TDS ($mg \cdot l^{-1}$)	Salinity	ORP
Kavango	23.56±0.18	7.76±0.06	9.03±0.12	37.58±0.50	24.11±0.29	0.01±0.00	-
Kwando	25.92±0.17	7.65±0.08	6.62±0.60	94.67±1.60	62.00±1.15	0.03±0.00	-
Lwanginga	20.07±0.10	8.08±0.05	10.07±0.38	196.53±57.16	127.53±37.20	0.08±0.02	-
West Lunga	22.81±0.10	8.63±0.24	6.77±0.27	293.70±4.68	190.70±3.96	0.09±0.00	-30.12±9.34
Kabompo	24.44±0.05	8.46±0.02	7.71±0.16	295.94±7.12	251.46±1.02	0.12±0.00	-17.34±1.22
Zambezi	25.36±0.05	6.94±0.14	7.30±0.06	137.47±1.55	89.87±0.98	0.04±0.00	8.39±10.84
Barotse floodplain	26.15±0.07	7.78±0.19	9.23±0.76	177.22±11.79	116.44±7.72	0.06±0.00	-26.34±13.17
Kafue	22.40±0.35	7.52±0.07	5.32±0.19	201.96±8.32	-	-	-
Kariba	28.39±0.40	7.66 ±0.10	7.43±0.83	229.50 ±4.90	147.65±2.76	0.07±0.01	55.6±0.00

Data analysis

Detection probability ($P_{capture}$) was expressed as the proportion of traps containing at least one crayfish, and abundance of crayfish was expressed as catch per unit effort (CPUE, as the mean number of individuals or biomass) caught per trap per night. The CPUE data set were zero inflated, therefore a zero corrected delta-X transformation model was used for CPUE standardisation to prevent invalid standard errors (Lo et al. 1992; Ellender et al. 2010). Differences in CPUE were determined using a One-Way ANOVA. A quantile-quantile (Q-Q) plot was used to inspect the CL and mass distribution data of *C. quadricarinatus* from the Kafue River, Lake Kariba and Barotse floodplain, for normality (Thode 2011). The Q-Q plot residuals for CL were normally distributed, however the mass data was not (See Appendix 4.1). Differences in CL and mass of *C. quadricarinatus* between the three regions were therefore determined using One-Way ANOVA and Kruskal-Wallis tests, respectively (with a Dunn test post-hoc, with P-values adjusted for multiple comparisons using Holm-Bonferroni corrections). The $P_{capture}$ and sex ratios between invaded regions were analysed with 2 × 3 contingency tables, and differences between the invaded regions were tested with a Chi-square test.

The rate of spread was calculated based on *C. quadricarinatus* spread from the introduction point to extreme sites reported in the Upper Zambezi Floodplains ecoregion. This was achieved by dividing the distance from the introduction point, to the extreme sites where *C. quadricarinatus* were reported (measured in km using Google Earth) by the number of years *C. quadricarinatus* took to reach those sites (Nunes et al. 2017a).

Results

Crayfish presence/absence and abundance

In the Upper Zambezi Floodplains ecoregion, *C. quadricarinatus* were only detected on the Barotse floodplain around Mongu, Zambia (Fig. 4.1). The point of introduction in this ecoregion was ascertained to be a pond in Lealui village, Mongu in the Barotse floodplain, Zambia (Madzivanzira et al. 2020). On the Barotse floodplain, crayfish were present in 16% of all traps set and were represented at 6 of the 15 sites sampled. Using only the sites where *C. quadricarinatus* were detected on the Barotse floodplain, the $P_{capture}$ (0.59) was not significantly different (χ^2 = 1.37, df = 2, p = 0.50) from that of Lake Kariba and Kafue River ($P_{capture}$ = 0.53 and 0.62, respectively) where *C. quadricarinatus* were represented at all sampling sites. A total of 1 202, 456 and 190 *C. quadricarinatus* were caught in the Kafue River, Lake Kariba and Barotse floodplain respectively. The CPUE between the Kafue River (5.34 ± 2.35 ind.·trap^{-1}·night^{-1}), Lake Kariba (4.47 ± 1.11 ind.·trap^{-1}·night^{-1}) and Barotse floodplain (0.59 ± 0.44 ind.·trap^{-1}·night^{-1}) did not differ significantly ($F_{(2, 26)}$ = 2.52, p > 0.05) (Fig. 4.2). The mean CPUE (with respect to both number and mass) and relative abundance (of males, females and intersex) of respective sites sampled are shown in Table 4.2. The highest CPUE between the sampled sites was 27.65 ± 4.11 ind.·trap^{-1}·night^{-1} (from Namwala site, Kafue River) and the lowest was 0.2 ± 0.47 ind.·trap^{-1}·night^{-1} (Matongo site, Barotse floodplain) (Table 4.2). Crayfish were not detected in the Zambezian Headwaters ecoregion (Zambia) and the Okavango Floodplains ecoregion (Namibia and Botswana) (Fig. 4.1).

Table 4.2. Sampling site, coordinates, sampling region, number of traps set, CPUE (mean ± SE), CL and mass (mean ± SE) and number of males and females where *Cherax quadricarinatus* was detected.

Site	Coordinates	Sampling region	Traps N	CPUE (mean±SE) ind./trap/night	CPUE (mean±SE) g	CL (mean±SE) mm	Mass (mean±SE) g	N Males	N Females	N Intersex
Pond 2	S-15.2611830°, E23.0813490°	Barotse floodplain	10	8.60±1.49	367.30±3.32	53.12±1.55	41.62±3.26	28	55	6
Lealui	S-15.2267240°, E23.0217350°	Barotse floodplain	11	0.64±0.90	28.60±12.58	57.50±1.96	44.94±3.53	2	5	0
Nalusa	S-15.2746740°, E23.0665080°	Barotse floodplain	21	1.81±0.90	56.54±74.48	37.22±1.14	12.61±1.20	4	32	2
Matongo	S-15.2746740°, E23.0665080°	Barotse floodplain	10	0.2±0.47	7.23±13.55	55.97±0.27	36.17±0.35	0	2	0
Mongu Harbour	S-15.2721010°, E23.1189090°	Barotse floodplain	18	0.89±0.04	14.44±21.98	46.32±0.54	24.48±1.85	1	11	4
Little Zambezi	S-15.2155490°, E22.9967910°	Barotse floodplain	18	2.11±0.39	103.11±5.30	57.98±1.28	48.84±3.46	15	19	4
DDF	S-16.5316640°, E28.7589350°	Kariba	10	3.1±0.72	214.33±5.05	66.58±1.62	69.14±5.05	12	18	1
I&J	S-16.5301650°, E28.7797570°	Kariba	10	5.4±1.17	272.50±3.19	60.76±1.31	50.46±3.19	21	33	0
Carribea Bay	S-16.5333340°, E28.7917140°	Kariba	10	1.4±0.40	68.35±8. 76	57.81±3.78	48.82±8.76	6	8	0
Cutty Sark	S-16.5323140°, E28.8172410°	Kariba	10	4.7±0.72	290.35±4.20	63.30±1.24	60.93±4.22	25	22	0
Kasese	S-16.5263810°, E28.8374830°	Kariba	10	1.2±0.47	56.80±10.02	57.34±3.49	47.33±10.02	6	6	0
Chawara	S-16.5340440°, E28.8634580°	Kariba	10	12.2±1.29	602.60±2.74	57.96±1.00	49.39±2.74	48	73	1
Charara	S-16.5524890°, E28.9549080°	Kariba	10	1.2±0.47	67.25±9.30	61.72±2.73	56.04±9.30	4	8	0
Nzou	S-16.5613470°, E28.9342600°	Kariba	10	1.7±0.46	147.30±6.85	71.93±1.83	86.65±6.86	12	5	0
Antelope	S-16.5765940°, E28.8498940°	Kariba	15	6.8±1.15	462.39±2.96	62.86±1.01	58.36±3.00	52	49	1

Sanyati	S-16.5742080°, E28.8756640°	Kariba	7	6.40±0.38	372.07±4.88	58.81±1.75	50.24±4.82	17	28	0
Kapalaushi	S-14.4664000°, E26.6894400°	Kafue	30	8.33±1.56	504.00±2.90	66.04±13.44	63.27±45.82	157	91	4
Kanunga	S-14.4524000°, E26.6413400°	Kafue	14	3.14±1.00	222.50±5.87	68.41±13.45	84.19±59.30	20	24	1
Deali	S-14.4749600°, E26.6186600°	Kafue	30	3.37±1.47	159.03±4.38	62.19±13.26	61.17±40.65	65	36	1
Kaulundeya	S-14.4374500°, E26.7635300°	Kafue	26	3.31±0.98	176.31±6.67	63.14±11.29	58.03±30.54	43	40	3
Lenda Burton	S-14.4302200°, E26.8004800°	Kafue	29	0.72±0.48	38.24±6.67	59.05±12.40	52.81±30.58	14	7	0
Chunga Lagoon	S-15.8465830°, E27.2303833°	Kafue	15	1.73±0.36	-	-	-	14	8	0
Chunga harbour	S-15.8422667°, E27.2334500°	Kafue	15	3.07±0.30	-	-	-	10	6	0
Kafue	S-15.8303167°, E28.2149833°	Kafue	15	3.20±1.39	-	-	-	17	29	2
Kafue edges	S-15.7523500°, E28.0856667°	Kafue	15	0.93±0.28	-	-	-	6	7	1
Mazabuka	S-15.7556833°, E27.7864667°	Kafue	15	0.53±0.38	-	-	-	2	6	0
Namwala	S-15.7463333°, E26.4282333°	Kafue	20	27.65±4.11	-	-	-	179	119	37

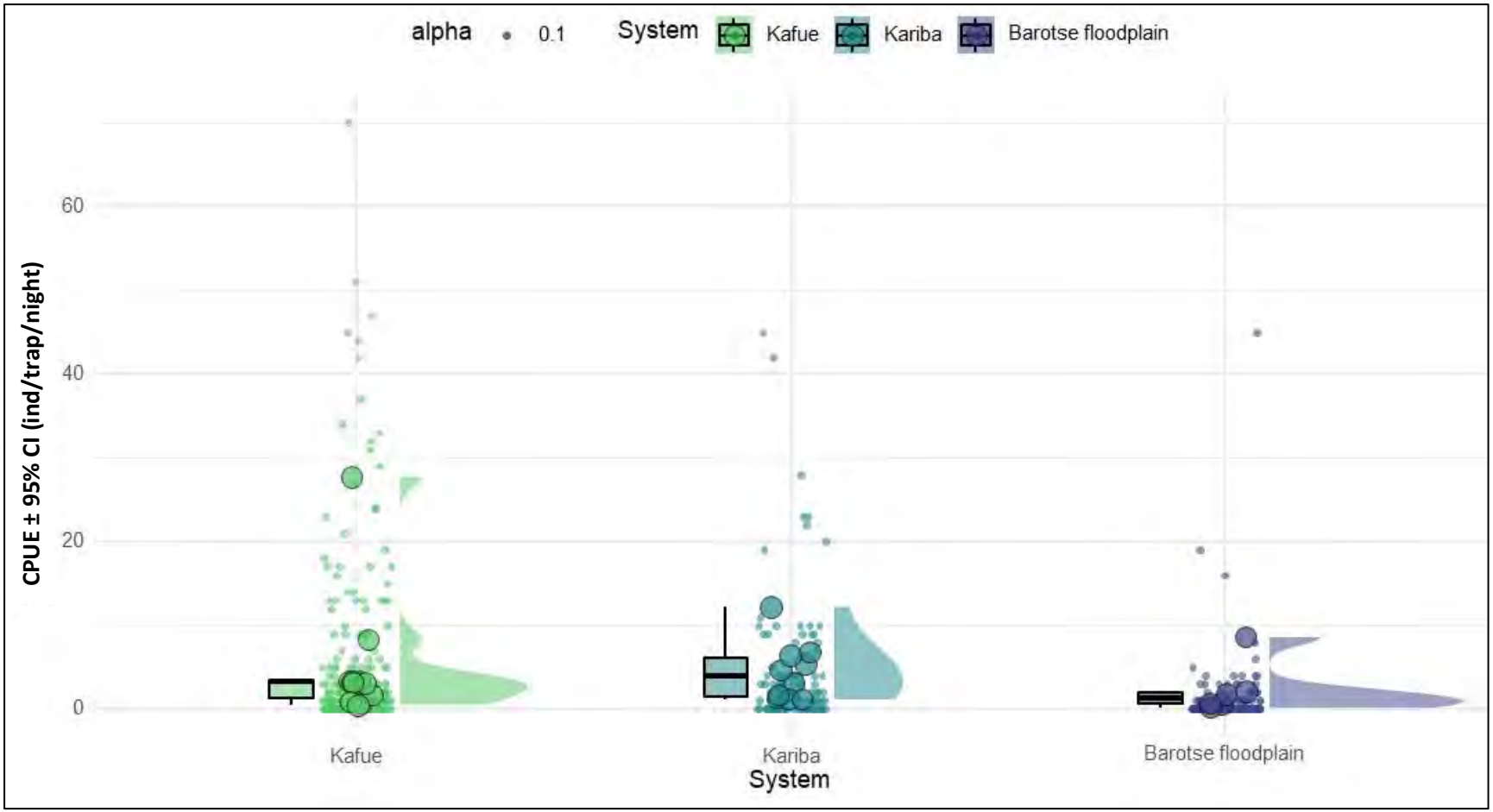

Figure 4.2. Mean CPUE of sampling region, sites, and number of individuals per trap of *Cherax quadricarinatus* in the Kafue River, Lake Kariba and Barotse floodplain. Box plot shows the overall median and interquartile range per system, small round circles indicate the number of individuals per trap, large circles represent the CPUE per site and the CPUE kernel density distribution is shown by the violin plot.

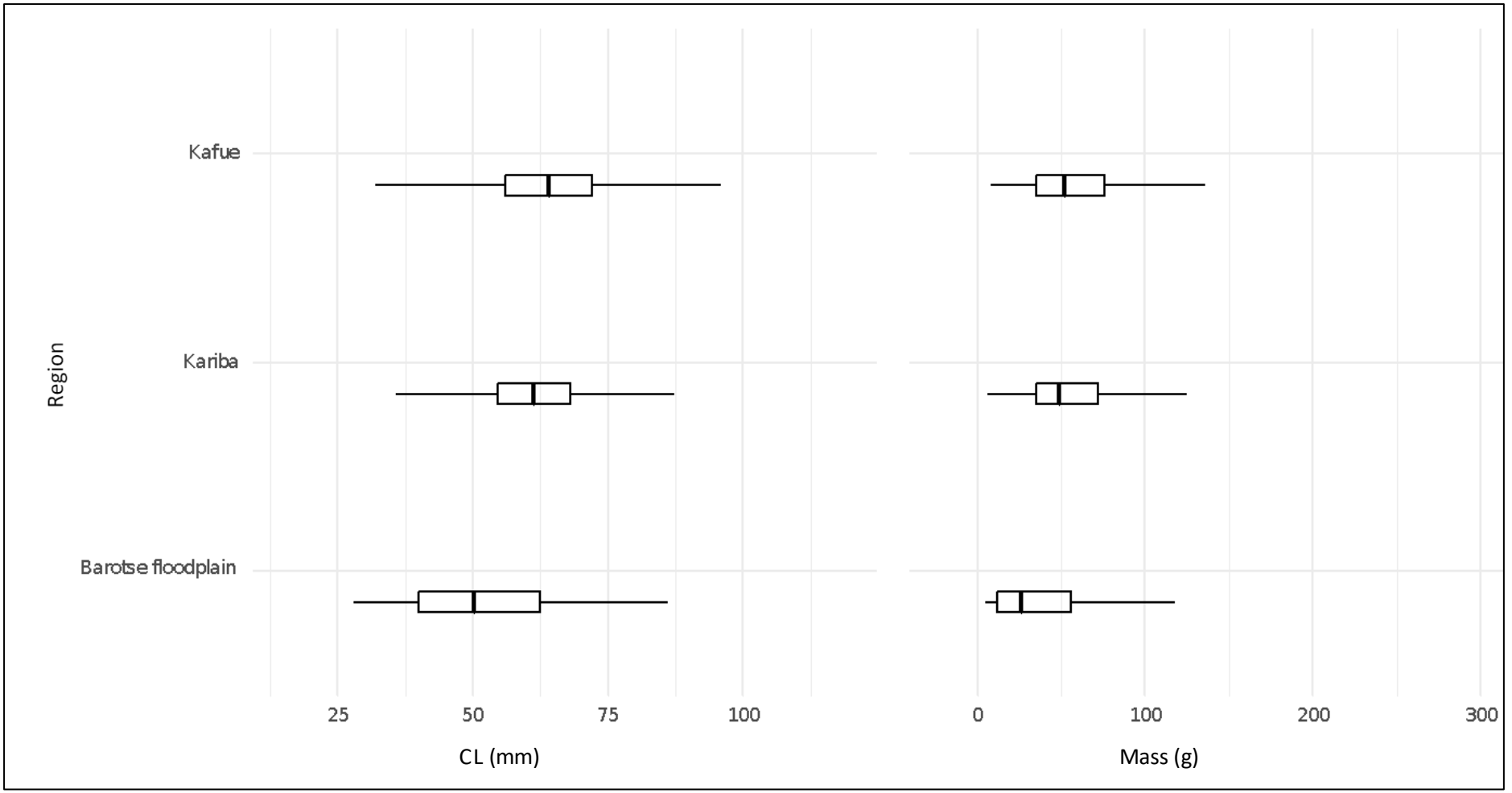

Figure 4.3. Carapace length (CL) and mass of *Cherax quadricarinatus* from Kafue River, Lake Kariba and the Barotse floodplain. Boxplots indicate median and interquartile range.

Size structure

The mean CL of *C. quadricarinatus* differed significantly ($F_{(2, 1097)} = 78.08$, $p < 0.05$) between the three invaded regions, where mean CL for the Barotse floodplain (51.32 ± 1.04 mm) was significantly less than that of Lake Kariba (61.21 ± 0.51 mm) ($p < 0.001$) and Kafue River (64.69 ± 0.61 mm) ($p < 0.001$) (Fig. 4.3). The mean CL of *C. quadricarinatus* from Lake Kariba was significantly less compared to the Kafue River ($p < 0.001$). Individuals with a CL range of 30 to 70 mm constituted 91% of *C. quadricarinatus* sampled in the Barotse floodplain; 44 to 78 mm represented 91% of *C. quadricarinatus* sampled in Lake Kariba; and 42 to 92 mm constituted 94.6% of *C. quadricarinatus* sampled in the Kafue River.

The mass of *C. quadricarinatus* individuals differed significantly ($H_{(2)} = 80.81$, $p < 0.001$) between the three invaded regions, where mean mass from Barotse floodplain (37.18 ± 2.17 g) was significantly lower than that of Lake Kariba (55.85 ± 1.43 g) ($p < 0.001$) and Kafue River (63.22 ± 2.05 g) ($p < 0.001$) but mass was comparable between Kafue River and Lake Kariba (Fig. 4.3).

Sex ratio

The overall female to male sex ratio between the sampled regions was significantly different ($\chi^2 = 85.86$, df = 2, $p < 0.001$).

Crayfish caught in the Kafue River comprised 56.0% males and 38.8% females and 5.2% intersex (Table 4.2). In the Kafue River, 11 ovigerous female *C. quadricarinatus* (mean CL and mass of 70.82 ± 4.12 mm and 74.63 ± 18.54 g, respectively) were caught, with four of

those carrying hatchlings and juvenile crayfish. The CL and mass ranges for the ovigerous *C. quadricarinatus* in the Kafue River were 53 – 101 mm and 64 – 255 g, respectively.

Crayfish caught in Lake Kariba comprised 44.3% males, 54.8% females and 0.9% intersex (Table 4.2). Five ovigerous female *C. quadricarinatus* (mean CL and mass of 76.30 ± 1.39 mm and 98.00 ± 5.04 g, respectively) were caught, with 3 of those carrying hatchlings and juvenile crayfish. The CL and mass ranges for the ovigerous *C. quadricarinatus* in Lake Kariba were 71 – 80 mm and 84 – 112 g, respectively.

Crayfish caught in the Barotse floodplain comprised 26.3% males, 65.3% females and 8.4% intersex (Table 4.2). Fifteen ovigerous female *C. quadricarinatus* (mean CL and mass of 65.24 ± 1.70 mm and 61.81 ± 4.58 g, respectively) with eggs were caught in the Barotse floodplain. The CL and mass ranges for the ovigerous *C. quadricarinatus* in the Barotse floodplain were 51 – 78 mm and 30 – 94 g, respectively.

Rate of spread

In the Upper Zambezi Floodplains ecoregion, *C. quadricarinatus* were found at maximum distances of 315 km downstream from the introduction point (along the Zambezi River at Katima Mulilo, Namibia in 2017), 100 km downstream from the introduction point (along the Zambezi River at Senanga, Zambia in 2017), 95 km upstream from the introduction point (along the Zambezi River at Lukulu, Zambia in 2017) and 40 km upstream from the introduction point (along the Luanginga River, Zambia in 2017) (Madzivanzira et al. 2020; Chapter 2). This therefore means the estimated average rate of spread of *C. quadricarinatus* is

49 ± 29 km year^{-1} downstream and 12 ± 7 km year^{-1} upstream (using 2012 as the year of first introduction into the Barotse floodplain).

The river distance from the introduction point to the Zambezi-Kabompo confluence is 125 km. At a rate of upstream dispersal of 12 km year^{-1}, *C. quadricarinatus* might reach Kabompo River where it meets with the Zambezi main channel in 10 years from the time at which they were introduced in the Barotse floodplain. The river distance from the invasion front to the Zambezi-Chobe confluence and to the main stem of the Okavango Delta is 460 and 825 km, respectively. The invasion of the Okavango Delta could take approximately 17 years from the year *C. quadricarinatus* was introduced in the Barotse floodplain.

Discussion

It is impossible to manage any species if basic distribution and abundance are unknown, and this is compounded when considering IAS (Magurran and Henderson 2003; Harvey et al. 2009). The lack of data on establishment and spread of crayfishes in Africa constrains the development of management strategies (Madzivanzira et al. 2020; Chapter 2). This study confirmed the presence and establishment of *C. quadricarinatus* in the Barotse floodplain, Upper Zambezi Floodplains ecoregion, which invaded the floodplain in 2012. The abundances and population characteristics of *C. quadricarinatus* in the Barotse floodplain were compared with established populations from Lake Kariba and Kafue River which were invaded in the early 2000s. While the $P_{capture}$ and CPUE of *C. quadricarinatus* in the Barotse floodplain were similar to that of Lake Kariba and Kafue River, the study detected morphometric differences among *C. quadricarinatus* populations sampled from these invaded regions. The high CPUE of *C. quadricarinatus* in some of the sites sampled in the Barotse floodplain indicates

considerable invasion and establishment success (Nunes et al. 2017a). This is likely due to environmental matching between the tropical floodplains of the invaded regions and the billabongs of the Australian systems where *C. quadricarinatus* is native, (see McLoughlan 2014). Crayfish were, however, below detection levels in the Kavango, Kabompo, Chobe, West Lunga and Kwando rivers. Most sites where *C. quadricarinatus* were currently absent or below detection, had similar environmental variables as those from the invaded sites, which indicates that several of these sites have the capacity to become invaded as the range of *C. quadricarinatus* expands throughout the ecoregions.

Crayfish from the Barotse floodplain were significantly smaller with respect to CL and mass than those from Lake Kariba and Kafue River. This could be due to population trait changes associated with the invasion core and invasion front populations (this is the leading-edge hypothesis) (Hudina et al. 2017). In this scenario, as the population of crayfish expands along an invasion gradient, individual growth is compromised due to intra-specific competition in the high-density core populations, which may reduce resource availability, foraging time and consumption rates (Guan and Wiles 1999; Corkum and Cronin 2004). Such processes may in turn reduce the per-moult increment (and growth rates), consequently resulting in lower abundance of large crayfish in a population (see Hudina et al. 2017) as observed for *C. quadricarinatus* sizes from the Barotse floodplain. These population characteristics will vary over time as the species becomes established, in response to changes in population size and, consequently, resource availability (e.g., Bøhn et al. 2004).

The overall number of female *C. quadricarinatus* was greater than that of males in the Barotse floodplain, whilst the sex ratios between the three invaded systems were significantly different.

Recruitment is occurring within the *C. quadricarinatus* population as evidenced by the capture of juveniles and berried females in all the systems as well as multiple size classes being detected across the invasion gradient. *Cherax quadricarinatus* like most parastids has relatively short breeding periods (3 – 20 weeks) and undergoes multiple spawning during an extended spawning breeding period (Reynolds 2002). In addition, crayfish are also *r*-selected cyclic breeders and *C. quadricarinatus* is able to spawn at least three times a year (Barki et al. 1997).

The proportion of berried females caught in this survey was 12.1%, 2.0% and 5.4% for the Barotse floodplain, Lake Kariba and Kafue River, respectively. The higher proportion of berried females from the Barotse floodplain could also be due to population expansion associated changes where reproduction investment is high at the front of the invasion gradient (see Hudina et al. 2017). Capture of berried females is considered rare in trap catches (Leland et al. 2012), probably because berried females are less active and, therefore, less susceptible to capture (Masser and Rouse 1997). Therefore, the capture of a high proportion of berried females using traps in the Barotse floodplain highlights the presence of an established breeding population with higher propagule pressure in the Barotse floodplain invasion, which has implications for dispersal potential both up- and downstream.

In this survey, intersex *C. quadricarinatus* were sampled from all populations. Intersex crayfish are morphologically and functionally males but possess both male and female genital openings, a testis and a sperm duct with attached androgenic gland (AG) in the lateral half displaying the male opening, and a previtellogenic ovary in the contralateral half (Sagi et al. 1996). Intersex *C. quadricarinatus* behave like males with respect to fighting and contests and they mate with receptive females (Barki et al. 2006). Crosses between *C. quadricarinatus* intersex and normal

females have been shown to yield a 1:3 (male:female) sex ratio in most crosses (Parnes et al. 2003). Crossing normal males with F1 females (progeny of intersex fathers) yielded almost 100% females (Parnes et al. 2003). This greatly increases the reproductive potential of the population as males are able to mate with many females as the cost of reproduction is generally lower for males than for females (Trivers 1972). In one of the ponds in the Barotse floodplain, more than 90% of *C. quadricarinatus* specimens, collected by drag netting, were females (pers. obs. 2019). The relatively high level of intersex individuals (8.4%) on the Barotse floodplain may be contributing to the skewed sex ratio and increasing the reproductive potential of the population. These population dynamics are likely to drive high propagule pressure and high densities of *C. quadricarinatus* across the invasion gradient, which may be driving the high abundances seen in the more established invasions. Long term monitoring and research is required to understand this population trait, as it was prominent in an invasion in its infancy (Barotse floodplain) and as well as in the more mature invasion in the Kafue River. This will provide comparative evidence of both temporal and spatial changes in *C. quadricarinatus* population dynamics as the invasion progresses. Efforts to reduce the number of intersex individuals or females may then become a possible management strategy at the start of invasions; this is particularly important to understand as *C. quadricarinatus* is an emerging invader worldwide.

Cherax quadricarinatus exhibited the potential to disperse both down- and upstream of the introduction point, similar to dynamics registered from the Inkomati Basin (Nunes et al. 2017a). In the Upper Zambezi Floodplains ecoregion, *C. quadricarinatus* specimens were observed in the Zambezi main channel 315 km downstream from the introduction point and 90 km upstream in Lukulu. The dispersal rates are a cause for concern as *C. quadricarinatus* might

spread to other pristine regions such as the Kabompo and West Lunga rivers upstream, and the Okavango Delta (seasonally connected to the Upper Zambezi River) downstream. The rates of upstream movement are generally lower than for downstream because water flow impedes the upstream movement (Bubb et al. 2004; Kerby et al. 2005; Bernardo et al. 2011). The mean rate of spread of *C. quadricarinatus* calculated in this study is however, higher than that for the Inkomati Basin in South Africa (8 and 4.7 km.year^{-1} downstream and for upstream spread, respectively) (Nunes et al. 2017a). This could be due to the mean discharge rate of the Zambezi system, 1 181 m^3·s^{-1} (Beilfuss 2012) which is higher than that of the Komati River, 2.55 m^3·s^{-1} (Roux et al. 2018). In addition, numerous obstructions exist in the Komati system in the form of weirs with crest height ranging from 0.9 m to 5.6 m, which affects the rate of spread of *C. quadricarinatus* in the Komati River (Roux et al. 2018). These estimated rates of spread to other systems can either be decelerated or accelerated depending on propagule pressure (Kolar and Lodge 2001), hydrological cycles (Gido and Brown 1999), species richness, composition and environmental variability (Gido and Brown 1999; Ricciardi and MacIsaac 2011) and biotic resistance (deRivera et al. 2005; Alofs and Jackson 2014; South et al. 2020).

Impoundments or lentic artificial aquatic systems have been shown to function as invasion 'hubs' for freshwater invaders, facilitating their subsequent spread and establishment into natural aquatic systems (Johnson et al. 2008). Lake Kariba might act as a secondary source of crayfish invasions in a similar manner to the irrigation ponds around the Komati River (Nunes et al. 2017a). *Cherax quadricarinatus* could also naturally migrate up rivers that feed into Lake Kariba as well as downstream, through the hydroelectric turbines to the Zambezi River and invade Lake Cahora Bassa. Lake Cahora Bassa could also be a recipient of *C. quadricarinatus* moving downstream from the Kafue River. Regions connected to the Zambezi River (e.g. Lake

Malawi through the Shire tributary) could eventually be recipients of combined *C. quadricarinatus* populations from the Kafue River, Lake Kariba and the Barotse floodplain. Spread into further reaches of the Zambezi basin represents a serious biodiversity concern, as the ecosystems are integral for the successful attainment of sustainable development goals including zero hunger and no poverty, through fisheries which provide food and income to the riparian communities of the Zambezi Basin (Tweddle et al. 2015).

Along with natural dispersal throughout the system, when considering invasions by species initially introduced for aquaculture purposes, there is always the possibility of further human-assisted translocations. Assisted translocations of crayfish have been noted in the Barotse floodplain around Mongu, predominantly by the local communities associated with specific ephemeral pans (pers. obs.). In addition, most crayfish have been known to disperse over land (Riek 1969; Lodge et al. 2000; Furse and Wild 2002). In Kenya, *P. clarkii* used the footprints of hippopotamuses as refugia and by doing so, effectively extended their range beyond Lake Naivasha up to 40 m into the riparian zone (Grey and Jackson 2012). Isotopic analysis confirmed that *P. clarkii* was using terrestrial subsidies, facilitated by the presence of aquatic mega-fauna (Grey and Jackson 2012). In the Kafue River, *C. quadricarinatus* was observed walking on land approximately 1 km from the river (BR Ellender, WWF Zambia pers. comm. 2020). It is likely that this is the same in the Barotse floodplain, which means that *C. quadricarinatus* can easily move from one pool that is drying out, into permanently flooded pools.

In this study, the extent of the *C. quadricarinatus* invasion in the Upper Zambezi Floodplains freshwater ecoregion was successfully described thereby providing essential baseline

information for its presence and absence throughout a data scarce system. The recorded differences in population structures between the invaded Upper Zambezi Floodplains, Middle Zambezi-Luangwa and Kafue freshwater ecoregions is important evidence regarding the invasion dynamics of *C. quadricarinatus*. This has global relevance as *C. quadricarinatus* is an emerging invader in numerous countries (e.g. Mexico, Jamaica, Singapore, China, as well as many European countries (Lodge et al. 2012; Pienkowski et al. 2015). Established differences can also provide insights into how to target and develop management responses aimed at *C. quadricarinatus* control, on both a local and transboundary level. The potential of *C. quadricarinatus* to invade and establish into other pristine catchments in Southern Africa is a matter of concern, especially given the rapid dispersal rates calculated in this study. The environmental variables in the region match their native habitat, which aids their successful establishment (Nunes et al. 2017a). The high abundance, fecundity, large sizes and high propagule pressure of *C. quadricarinatus* are also stereotypical traits of highly successful invasive species. Human mediated translocation and the flood regime dynamics makes control and containment of *C. quadricarinatus* exceedingly difficult. Community education and engagement is needed to prevent further spread of *C. quadricarinatus* through human mediated activities. More research needs to be done to address the ecological impacts of *C. quadricarinatus,* as information globally still remains scant (Madzivanzira et al. 2020; Chapter 2). The study recommends continued long term monitoring of the systems at risk of new invasions, with a specific integration of wider monitoring of the whole ecological system to detect species affected by these invasions, and to determine appropriate management approaches. In this way management can be tailored to be as cost effective as possible. For example, targeted removal of intersex and female crayfish in early invasions may be used to reduce propagule pressure and further spread. The eight governments that share these freshwater ecoregions should work together to develop cohesive legislation to restrict further

movement of *C. quadricarinatus* and other invasive species. Legislation should not only restrict movement of crayfish (both within country and across borders), but also improve screening techniques and early warning systems to prevent new introductions.

CHAPTER 5

INVASIVE CRAYFISH OUTPERFORM POTAMONAUTID CRABS AT HIGHER TEMPERATURES

Abstract

Data on ecological impacts of freshwater crayfish invasions in Africa are scarce but invasion history suggests the likelihood of negative implications for biodiversity. To evaluate the potential for ecological impacts we describe the consumer-resource dynamics of two established crayfish species (*Cherax quadricarinatus* and *Procambarus clarkii*) in comparison with the native trophic analogue, the freshwater crab *Potamonautes perlatus* preying upon *Clarias gariepinus* fry using comparative functional responses (FRs) and FR ratio (FRR). Experiments were conducted under dark and light conditions as well as low (19°C) and high (28°C) temperature treatments. All three species exhibited potentially population destabilising Type II (hyperbolic) FRs towards prey, which was significantly higher in the dark than in the light. At low temperatures, *P. perlatus* exhibited the highest maximum feeding estimate although the FR curve was not significantly different from *C. quadricarinatus* at the highest prey densities. Both crayfish species had higher attack rates at both temperatures and consumed significantly more prey at the high temperature than *P. perlatus*. The FRR of both crayfish species at the high temperature treatment was higher than that of *P. perlatus* due to high attack rate and low handling parameters. At 19°C *P. perlatus* had a higher or negligible relative FR magnitude compared to *P. clarkii* and *C. quadricarinatus* respectively, which suggests some degree of biotic resistance at this temperature, however this resistance is decreased at 28°C. *Cherax quadricarinatus* consistently had disruptive FR parameters across both temperatures. Findings from this study represent an important step towards understanding the impacts of crayfish in Africa for public authorities and environmental managers. The two invasive crayfish

species have the potential to exert greater *per capita* impacts on benthic prey communities in invaded systems as shown by their high FRs and FRRs. Furthermore, *C. quadricarinatus* is an emerging invader globally and our results provide evidence of potential for negative ecological impact regardless of thermal conditions. African countries and their respective environmental managers should therefore maximise efforts to prevent the introduction and spread of these invaders to conserve native biodiversity.

Introduction

Freshwater biodiversity is declining rapidly on a global scale, and at an accelerated rate compared to terrestrial ecosystems (Albert et al. 2020). Biological invasions represent a major anthropogenic stressor as many confer negative impacts on biodiversity ranging from behavioural shifts of native species to complete restructuring of food webs and extirpation of entire faunas (Gallardo et al. 2015). High economic costs are associated with the prevention, management and mitigation of biological invasions and as such, these programs are notoriously difficult to maintain (Day 2018; Barbet-Massin et al. 2020; Diagne et al. 2020b). Due to insufficient information, public awareness, and policy frameworks, the effects of invasive alien species (IAS) are consistently underestimated (Early et al. 2016).

Freshwater crayfish are among the most successful freshwater invaders and are considered keystone species as they are polytrophic omnivores and ecosystem engineers (Gherardi 2007). Global crayfish introduction pathways are aquaculture and fisheries, the aquarium trade, biological control of disease vectors and for research purposes (Lodge et al. 2012), with wild populations establishing due to accidental and/or deliberate release (Geiger et al. 2005; Lodge et al. 2012; Kouba et al. 2014; Oficialdegui et al. 2019). Negative impacts of invasive crayfish can be direct (consumptive) or indirect (non-consumptive) and include: the loss of ecosystem services such as food provisioning through reduction in native species used in subsistence fisheries or of economic value; disruption of community food webs; disease vectoring, and increased costs to agriculture and water management (Lodge et al. 2012).

Africa has been a recipient of nine crayfish species introductions, the two most invasive species, Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) and

Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852), are spreading rapidly across the continent (Madzivanzira et al. 2020). The main driver of crayfish introductions was to provide socio-economic benefits through fisheries development and aquaculture but there is limited evidence of success (Madzivanzira et al. 2020). *Cherax quadricarinatus* is native to Northern Australia and south-eastern Papua New Guinea (Riek 1969). In Africa *C. quadricarinatus* has established feral populations in parts of South Africa, Swaziland, Zambia and Zimbabwe (see Madzivanzira et al. 2020). *Procambarus clarkii* is native to southern and south-eastern USA and northern Mexico (Hobbs 1972). In Africa, populations are reported from Kenya, Uganda, Sudan, Egypt, South Africa, Zambia, Morocco and Tunisia. Both crayfish species have traits typical of successful invaders: high fecundity, behavioural and phenotypical plasticity, broad tolerance of abiotic variables and are polytrophic omnivores (Jones et al. 2000; Veselý et al. 2015).

Most crayfish research has been concentrated in Europe and North America, thus cohesive information on crayfish in Africa is scant, and the consequences and impacts of *C. quadricarinatus* and *P. clarkii* on southern African ecosystems are not well documented (Madzivanzira et al. 2020). In Africa, prevailing negative socio-economic impacts of *P. clarkii* are linked to damage to fishing gear, declining fisheries performance and damage to agricultural water infrastructure. Although the ecological impacts of *C. quadricarinatus* and *P. clarkii* are not well studied in Africa, the two species are considered to cause detrimental impacts once established in the natural environment (Tricarico et al. 2010; Zeng et al. 2019). Crayfish consume resources across all trophic levels including aquatic macrophytes, as well as aquatic invertebrates, amphibians and fish (Twardochleb et al. 2013). In light of their invasion history and taxonomic traits (Ricciardi 2003; Veselý et al. 2015), research on their impacts in

African freshwater systems is an urgent requirement (Madzivanzira et al. 2020). Knowledge gaps on crayfish impacts in Africa constrain conservation efforts of native biodiversity, as there is insufficient evidence to compel policymakers to implement legislation or management interventions.

Trophically and functionally analogous native species have high potential to confer biotic resistance or to be susceptible to competitive exclusion by the invading species (Elton 1958; Tilman 1999; Alofs and Jackson 2014). Despite the negative invasion history and traits typical of a damaging invader, it is an oversight to infer that there will be persistent trends in low biotic resistance, especially in highly dynamic and biodiverse African systems (Elton 1958; Tilman 1999). Given the absence of native crayfish on mainland Africa, it is predicted that negative impacts on native trophic analogous decapods, such as freshwater crabs from the genus *Potamonautes* are likely to be strong (de Moor 2002; Jackson et al. 2016; Nunes et al. 2016) but see South et al. (2020) regarding trait inferred measures of biotic resistance. Potential impacts by freshwater crayfish on African freshwater crabs are a cause for concern because freshwater crabs are the major shredder species present in African freshwaters, forming the vital intermediate trophic link between detritus and predatory species such as fish, otters and birds (Cumberlidge 2011). In Africa 28% of the 94 non-data deficient potamonautid crabs are currently assessed as being at risk of extinction due to their narrow range and exposure to a variety of stressors including habitat destruction, invasive species and climate change (Cumberlidge 2011). While impacts of invasive crayfishes on potamonautid crabs are poorly documented, the potential spill-over of diseases such as crayfish plague, a disease caused by the fungus *Aphanomyces astaci* that has been shown to cause mortalities in Asian crabs (see

Svoboda et al. 2014), and competition for resources such as food and habitat are major concerns (Madzivanzira et al. 2020).

Species interactions shape community dynamics through both consumptive and non-consumptive manners such as intra- and interspecific competition for resources such as food, shelter, and reproduction (Sih et al. 2010; Lopez et al. 2019; Mofu et al. 2019b; Zeng et al. 2019). Evidence of effects of crayfish introductions upon freshwater crabs are unequivocal across regions due to context dependencies largely relating to co-evolutionary history (Gherardi and Cioni 2004; Savvides et al. 2015; Mazza et al. 2017; Zeng et al. 2019).

Functional Response (FR) analysis, and its derived metrics such as the FR Ratio (FRR), have been useful in relative impact assessments as the FR describes resource density dependent predation (Holling 1959; Holling 1966) and provides the associated parameters of attack (or search efficiency) [a], handling (or processing time) [h] and maximum feeding estimate [$1/h$] (Dick et al. 2013, Dick et al. 2014; Cuthbert et al. 2019). Functional responses can be linear (Type I), hyperbolic (Type II), or sigmoidal (Type III) (Holling 1966) and Type II responses in particular may potentially destabilise prey populations and lead to localised extirpations (Dick et al. 2013; Dickey et al. 2020). Invasion impacts are highly context dependent with regards to abiotic and biotic characteristics of the recipient environment, thus FR provides a standardised method to compare resource consumption across contexts (Mofu et al. 2019a; Uiterwaal and DeLong 2020; Strayer 2020). The parameter FRR [a/h] combines variables to elucidate potential intermediate impact (where previously distinguishing differences between FR curves was problematic) and may give a further mechanistic insight into the ultimate driver of impact (Cuthbert et al. 2019; South et al. 2019; Dickey et al. 2020).

Given the vast geographical spread of the two crayfish species invasions in Africa there are differences in thermal regimes across the invaded range even without taking into account climate warming scenarios (Nunes et al. 2017c). Furthermore, crayfish and crabs are generally nocturnal and locate their prey using a variety of chemical and sensory cues (Barki et al. 2011). Therefore, both light and thermal regimes have the potential to mediate feeding behaviour, and thus have implications for vulnerable prey species and resources across the invaded area (Fitzpatrick et al. 2013; South et al. 2017; Uiterwaal and DeLong 2020). Consequently, we present the first investigation of consumer-resource dynamics of two invasive crayfish species *C. quadricarinatus* and *P. clarkii* compared to the native African freshwater crab *P. perlatus*, which has a broad geographic distribution (Daniels 2003; Cumberlidge 2011) that includes areas invaded by both crayfish species, under light and dark conditions and two temperatures.

Given that ecologically damaging invasive species are presumed to consume higher proportions of resources than their native counterparts (Dick et al. 2013; Ricciardi et al. 2013; Dickey et al. 2020), our hypothesis was that 1) all species will exhibit a Type II response (Holling 1959; Lee and Kang 2004), and 2) both crayfish species will possess a higher magnitude FR and FRR than the crab under all treatments.

Methods

Animal collection and maintenance

Sixty *P. perlatus* samples were collected from Eastern Cape Dams (S -33.324570°, E 26.527735°; S -33.321060°, E 26.520957°; S -33.294321°, E 26.515702°; S -33.414137°, E 26.506825°). One hundred and fifty live *C. quadricarinatus* samples were collected from sugarcane irrigation ponds in Nkomazi, Komatipoort (Mpumalanga Province), Komati River

catchment (S -25.552418°, E 31.904307°). *Cherax quadricarinatus* established in the Komati River System as a result of escapees from an aquaculture farm next to the Sand River Dam in Swaziland (in the late 1990s) from where specimens later spread into South Africa and were first detected in the Komati River in 2002 (Nunes et al. 2017a). Eighty seven live *P. clarkii* samples were collected from Mimosa Dam (S -27.881976°, E 26.694330°) in Free State Province South Africa. *Procambarus clarkii* specimens were deliberately released by aquarists in 2016 and were first detected in 2018 (Madzivanzira et al. 2020). Both Mimosa Dam and Komatipoort represent invasion core populations of *P. clarkii* and *C. quadricarinatus.* A standard gear developed for *C. quadricarinatus* using collapsible crayfish trap (promar®, California USA) baited with dry dog food (Bobtail®, South Africa) was used for trapping both *C. quadricarinatus* and *P. perlatus.* The same gear plus rectangular minnow traps baited with fish heads was used to capture *P. clarkii*. All traps were deployed at 1600 hrs and retrieved at 0800 hrs. All animals caught were placed in 60 L cooler boxes with regularly replaced fresh water from the respective source with battery powered air pumps and transported to the bio-secure facility at the South African Institute for Aquatic Biodiversity (SAIAB).

Animals were maintained in species specific and sex specific holding tanks (60 L) with constantly filtered and dechlorinated tap water, which was periodically replaced to maintain good water quality. Specimens were kept in the holding tanks for at least 1 month prior to experimentation to acclimate them to lab conditions. *Cherax quadricarinatus* has an optimal thermal range of 23 – 31°C (Jones 2000), whereas the optimal range of *P. clarkii* is between 21 – 27°C (Huner and Barr 1991; Ackefors 1999). Thermal ranges for *P. perlatus* are not known but as they are widely distributed throughout southern Africa it is assumed that they are broad and as such will encompass the same ranges (Daniels et al. 2006). Water temperature was maintained at 22 ± 1°C using a computer controlled recirculating air conditioning unit and

the laboratory was held under a 12:12 light:dark regime with white light and total darkness. All animals were maintained on cabbage leaves and cultured *Eisenia* sp. worms.

Prey used was sharptooth catfish *Clarias gariepinus* fry as they are ubiquitous across the African continent including the invaded ranges (Bruton 1979; Skelton 2001), are a commercially important aquaculture and fisheries species, and their morphology (a broad flattened head and a tail) and swimming modality are similar to a tadpole (Woodward and Quinn 2011; pers. obs. 2020) thus also representing an amphibian proxy in terms of movement and size. Further, considering the nocturnal behaviour of all predator species, they are likely to exert predatory pressure on native prey that have evolved to avoid interactions with native predators during the day. In this case *C. gariepinus* is a relevant prey species as it both chemosensory and nocturnal (Bruton 1979). Live *C. gariepinus* fry (total length mean ± SE: 25.82 ± 0.18 mm, mass mean ± SE: 1.01 ± 0.01 g) was supplied by Aquaculture Innovations in Makhanda, and therefore naïve to all predator species. These were maintained in a 200 L holding tank in the lab under identical conditions to the crayfish and crabs.

Experimental setup

Functional response experiments were carried out in 12 L of dechlorinated tap water in 15 opaque rectangular tanks (dimensions 550 mm × 365 mm × 205 mm) in a randomised order. Tanks had a layer of river sand substrate (≈ 20 mm). No shelter or burrow was included in the experimental tank as only *P. clarkii* is a burrowing species, but also to avoid extra complexity introduced into the results, as this experiment was not intended to quantify the effect of structural complexity on FR. Individual animals were randomly selected from the holding tank and patted dry before measurements were taken. Neither moulting nor individuals with regenerating/deformed/missing chelae or walking legs were used in the experiments. The predators were placed individually into experimental tanks and were not fed for 24 h prior to the experiment to standardise satiation level. Animals were allowed to acclimate to experimental arenas for 1 h before prey was supplied. Prey were supplied to each of the predators at densities of 2, 5, 10, 20, 40, 60, 150, 200 (n = 5 at each density per species per treatment). Experiments were run for 3 h, after which the predators were removed and number of prey items consumed was enumerated.

Two functional response experiments were completed: 1) under light (daylight proxy) and dark (night proxy) conditions at 19°C and 2) under 19°C and 28°C in dark conditions. The two light conditions represent the diurnal and nocturnal periods and the two temperature treatments are based on realistic field conditions in the crayfish-invaded range in southern Africa (Madzivanzira et al. In Review; Chapter 4) and the thermal requirements of the prey species, *C. gariepinus*, which spawns at temperatures exceeding 18°C (Bruton 1979; Hecht et al. 1988). While these temperatures are high compared to other invaded ranges (i.e. *P. clarkii* and *C. quadricarinatus* in Europe) this is the first southern African assessment of both species with a

native comparison and prey, it was considered important that temperature treatments were representative of field conditions.

Prior to functional response experiments all animals (predator and prey) were acclimated to the desired temperature at a rate of 1°C per day and allowed to acclimate to the two temperatures for a week before experiments were conducted. No predators were re-used more than once per temperature and light treatment. As the experimental set up was not fully randomised (See Appendix 5.1), due to the temperature acclimation procedure, there is a potential that some animals were used in the subsequent experiment at the higher temperature. Although in this case, no animal would be used more than twice, therein the capacity for predator learning is limited, especially considering the length of time between experiments (≈ 2 weeks). Controls were four replicates of each prey density in the absence of the predators to assess natural mortality and cannibalism among the prey. This was not a fully factorial experiment as the light and dark treatment experiment was completed first at 19°C. In addition we were constrained by prey availability and ethics considerations regarding reducing live prey use, so an experiment under daylight conditions at 28°C was not conducted as all three predators are mainly nocturnal.

Statistical analysis

All analysis was carried out in R v3.6.1 (R Development Core Team 2019). Morphometric data for predators were square root transformed for the data to meet the assumptions of normality, and differences in weight and carapace length between species were determined using one-way ANOVA tests and Dunn test post-hoc. Two separate generalised linear models (GLM) were used to assess prey consumption with regards to: 1) light and dark conditions, using fixed

factors *predator species*, *light*, and *density* (i.e., prey supplied), and 2) temperature differences under dark conditions, using fixed factors *predator species*, *temperature*, and *density*. For the light and dark experiment, a GLM with quasipoisson error distribution was used as data were over-dispersed, however, for the temperature experiment a poisson error distribution was assumed as data were over-dispersed. Differences between factor levels were assessed post-hoc using linear contrasts via the R package, emmeans (Lenth 2018).

The R package 'frair' (Pritchard 2014) was used to model the FR type. Functional responses were modelled using maximum likelihood estimation (MLE; Bolke 2010) and Rogers' (1972) Random Predator Equation (Equation 1; see Chapter 1), due to the prey not being replaced as they were consumed. If the proportion of prey consumed decreases with an increase in prey density then the logistic regression will produce a significantly negative result and thus the FR type can be classed as Type II, if the logistic regression produces a significantly positive result the response will be classed as Type III (Juliano 2001).

The data were non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals around the mean functional response curve for each treatment, this allows the curves to be considered for population level inferences (Pritchard et al. 2017). The FR data were assessed in a phenomenological manner, therefore, overlapping confidence intervals suggest a lack of significant difference in the magnitude FR.

The FRR (per Cuthbert et al. 2019) (Equation 2) was calculated for each predator using the parameter estimates of a and h derived from the maximum likelihood estimate results of Equation (1). In this case the FRR is used as a diagnostic tool to determine whether there were

differences that were not clearly observed from the usual FR outputs. Therefore, the higher the FRR value the higher the inferred impact (Cuthbert et al. 2019).

Results

The CL of the decapods used for the experiments differed significantly ($F_{(2, 357)} = 91.95$, $p < 0.001$) where *P. perlatus* and *P. clarkii* had a mean CL which was significantly lower than *C. quadricarinatus* ($p < 0.001$). With respect to mass, there were significant differences between the three decapods ($F_{(2, 357)} = 158.90$, $p < 0.001$) where *P. perlatus* weighed more than the two crayfish species ($p < 0.001$).

Catfish fry prey survival was > 99% under all control treatments and there was no mortality of predators during the experimental period.

Interaction of factors

For all the experimental conditions, there was a significant three-way interaction affecting the number of catfish fry prey consumed between 'species × light × density' (Table 5.1 – 5.2, Fig. 5.1). Due to the three-way interaction we describe our results only with respect to the significant interaction effect.

At 19°C under dark conditions at prey density 20, both crayfish species consumed significantly more than *P. perlatus* (both $p < 0.05$) (Fig. 5.1). At the three highest prey densities (60, 150, 200) both *C. quadricarinatus* and *P. perlatus* consumed significantly more prey than *P. clarkii*

(all $p < 0.05$) but there was no difference in prey consumption between *C. quadricarinatus* and *P. perlatus* at these prey densities.

Table 5.1. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in prey consumption with regards to factors *species*, *light* and *density*, using a Type 3 Anova and χ^2 to report the effects at the low temperature.

Effect of light and dark conditions at 19°C			
Model term	Chisq	df	*p*-value
Species	28.49	2	< 0.001
Light	294.22	1	< 0.001
Density	1547.87	7	< 0.001
Species * Light	37.43	2	< 0.001
Species * Density	41.14	14	< 0.001
Light * Density	12.65	7	0.13
Species * Light * Density	26.90	14	< 0.05

Table 5.2. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in prey consumption with regards to factors *species*, *light* and *density*, using a Type 3 Anova and χ^2 to report the effects at the high temperature.

Effect of temperature change under dark conditions			
Model term	Chisq	df	*p*-value
Species	59.56	2	< 0.001
Temperature	8.36	1	< 0.01
Density	2048.48	7	< 0.001
Species * Temperature	60.81	2	< 0.001
Species * Density	32.62	14	< 0.01
Temperature * Density	25.56	7	< 0.001
Species * Temperature * Density	34.97	14	< 0.01

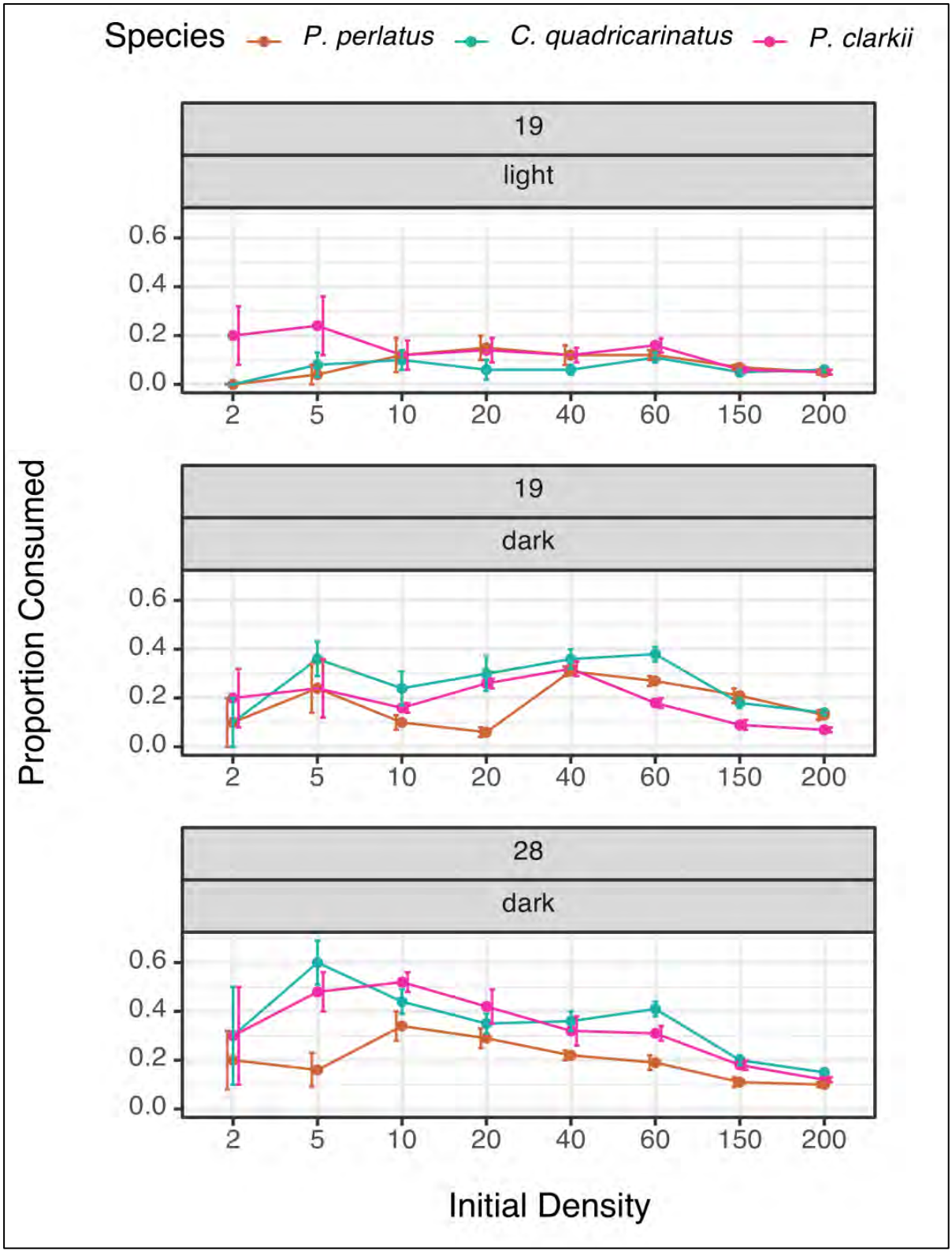

Figure 5.1. Mean ± SE proportion of *Clarias gariepinus* prey consumed by *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* in dark and light treatments under the temperatures 19°C and 28°C.

Under dark conditions at prey density 20 both crayfish species consumed significantly more prey than *P. perlatus* at 28°C compared to 19°C (all $p < 0.05$). At the three highest prey densities (60, 150, 200) both crayfish species consumed significantly more than *P. perlatus* at 28°C compared to 19°C (all $p < 0.05$) and *C. quadricarinatus* consumed significantly more than *P. clarkii* at 28°C compared to 19°C.

Functional response results

All predator species and light and temperature treatment combinations resulted in significant Type II FRs (Table 5.3, Fig. 5.2 and 5.3).

Table 5.3. First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with FR Type, functional parameters (a, h, and 1/h), associated significance levels from Rogers' random predator equation, bias accelerated and corrected 95% confidence intervals for a and h, and the functional response ratio (a/h) with regards to *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* predating upon *Clarias gariepinus* fry under different temperatures and light conditions.

a = attack parameter; *h* = handling parameter; 1/*h* = maximum feeding estimate.

Species	Light	Temperature (°C)	First order term (*p*-value)	FR Type	*a* (*p*-value)	*a* 95% BCa CI	*h* (*p*-value)	*h* 95% BCa CI	Maximum Feeding Estimate (1/*h*)	Functional Response Ratio (*a*/*h*)
P. perlatus	Light	19	-0.005, <0.001	II	0.15, < .001	0.10-0.24	0.053, < .001	0.032-0.077	18.7	2.95
C. quadricarinatus	Light	19	-0.007, <0.05	II	0.09, < .001	0.06-0.14	0.037, < .05	0.003-0.067	26.5	2.51
P. clarkii	Light	19	-0.007, <0.001	II	0.23, < .001	0.15-0.36	0.071, < .001	0.049-0.103	14.0	3.29
P. perlatus	Dark	19	-0.003, <0.001	II	0.29, < .001	0.22-0.39	0.013, < .001	0.004-0.024	76.3	22.52
C. quadricarinatus	Dark	19	-0.007, <0.001	II	0.63, < .001	0.53-0.75	0.023, < .001	0.020-0.027	42.9	27.16
P. clarkii	Dark	19	-0.008, <0.001	II	0.42, < .001	0.33-0.58	0.052, < .001	0.039-0.065	18.9	8.15
P. perlatus	Dark	28	-0.006, <0.001	II	0.36, < .001	0.27-0.47	0.036, < .001	0.024-0.048	27.5	10.01
C. quadricarinatus	Dark	28	-0.008, <0.001	II	0.72, < .001	0.58-0.90	0.023, < .001	0.019-0.029	42.5	30.74
P. clarkii	Dark	28	-0.008, <0.001	II	0.71, < .001	0.54-0.92	0.029, < .001	0.024-0.035	33.7	24.23

Functional response under light and dark at 19°C

For the light and dark experiments at 19°C, all species exhibited a higher maximum feeding estimate under dark conditions than under light conditions (Fig. 5.2). This was driven by consistently higher attack parameter values and lower handling parameter values in the dark treatment (Table 5.3, Fig. 5.2). However, in the dark treatment, both crayfish species had a higher attack parameter (initial FR slope steepness) than *P. perlatus* (Table 5.3, Fig. 5.2). Attack and handling parameters in the light resulted in similar values and thus maximum feeding estimates and FRR values for all three predator species (see overlapping confidence intervals for all species FR under light conditions) (Table 5.3, Fig. 5.2). The low handling parameter estimates for *P. perlatus* drove high maximum feeding estimates for *P. perlatus* in the dark treatment, however the attack parameter had a very slight increase between treatments (Table 5.3, Fig. 5.2). In contrast, both crayfish species showed a considerable increase in attack parameter in the dark compared to the light treatment (Table 5.3). The high attack parameter and lower handling parameter exhibited by *C. quadricarinatus* in the dark treatment resulted in the highest FRR (*a/h* ratio). The magnitude of *P. clarkii* FR curves did not differ significantly at the lowest and highest catfish fry prey densities. In the dark treatment, *C. quadricarinatus* and *P. perlatus* did not differ in per capita impact at the higher prey densities as the confidence intervals of the FR curves overlapped. However, at lower densities *C. quadricarinatus* had significantly higher per capita impact than *P. perlatus* and *P. clarkii*. The confidence intervals of the FR curves for *P. perlatus* and *P. clarkii* overlapped until around prey density 60 at which point they diverged (Fig. 5.2).

Functional response under dark at 19°C and 28°C

For the temperature experiments under dark conditions, *P. perlatus* had the highest maximum feeding estimate driven by the low handling parameter at 19°C, however, the FR curve was not

significantly different from *C. quadricarinatus* at 19°C at the highest prey densities (Fig. 5.3). This low handling time estimate drove predatory impact here as the attack parameter was lower for the crab than for both crayfish species but nonetheless resulted in a high FRR, similar to that of *C. quadricarinatus*. *Procambarus clarkii* had a long handling time at 19°C which resulted in a low maximum feeding estimate and FRR overall at the lower temperature. In contrast to the two invasive crayfish species, when the temperature was raised to 28°C, *P. perlatus* FR magnitude significantly decreased overall resulting in the lowest maximum feeding estimate for this treatment (Fig. 5.3). This was driven by a longer handling parameter than at the 19°C treatment. Both crayfish species had higher attack parameters at 28°C than at 19°C and low handling parameters, which resulted in high maximum feeding estimates and FRR values. *Cherax quadricarinatus* maximum feeding estimates and thus FR magnitude were the same at both 19°C and 28°C as the handling parameter was the same at both temperatures (Table 5.3, Fig 5.3). *Procambarus clarkii* FR magnitude increased significantly with increased temperature as a result of an increased attack parameter and decreased handling parameter but the confidence intervals overlapped with those of *C. quadricarinatus* at 28°C, showing non-significant differences in high temperature FR (Fig. 5.3).

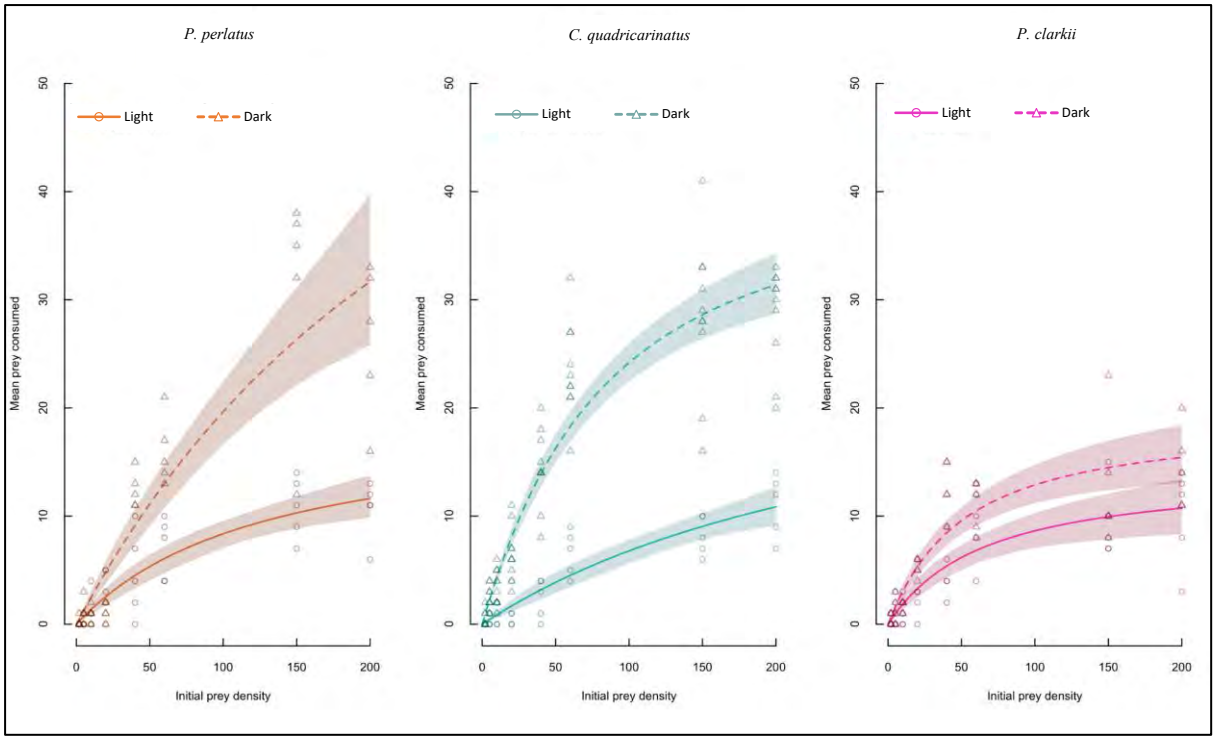

Figure 5.2. Functional response curves of *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* preying on *Clarias gariepinus* fry in dark and light treatments at 19°C (modelled using the Rogers' random predator equation for a Type II functional response). Points are raw data of number of prey consumed at each prey density. Shaded areas are bootstrapped 95% confidence intervals.

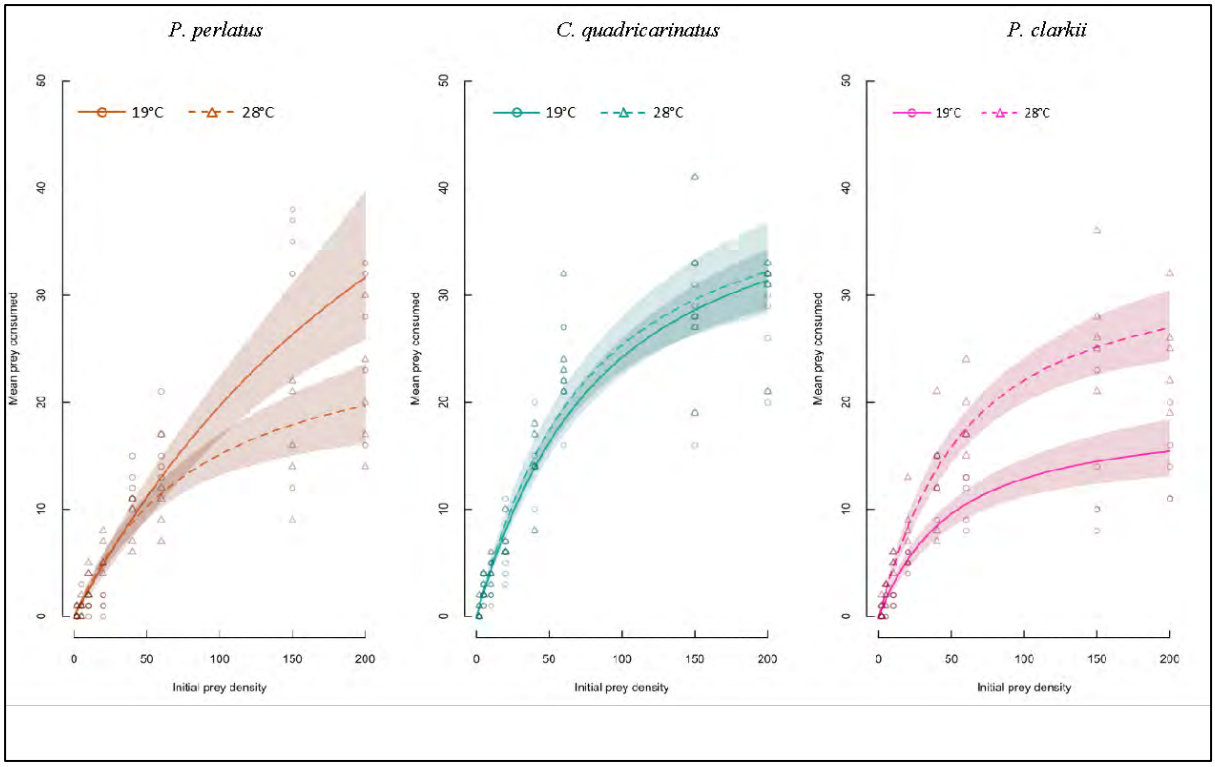

Figure 5.3. Functional responses curves of *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* preying on *Clarias gariepinus* fry across temperature treatments 19°C and 28°C in dark conditions (modelled using the Rogers' random predator equation for a Type II functional response). Points are raw data of number of prey consumed at each prey density. Shaded areas are bootstrapped 95% confidence intervals.

Discussion

Negative impacts conferred by IAS are usually trophic and characterised by the invader consuming and depleting resources at a higher magnitude relative to native species (Dick et al. 2013; Ricciardi et al. 2013; Dickey et al. 2020). We compared predatory impacts of three decapods: the invasive *C. quadricarinatus* and *P. clarkii*, and the native crab *P. perlatus* on catfish fry under light and dark conditions as well as under two temperature treatments. Both the invasive crayfish species and the native crab displayed potentially population destabilising

Type II FRs (Dick et al. 2013) under all conditions. Overall, their FR magnitudes were enhanced under dark conditions compared to light and at a higher magnitude at 28°C than at 19°C except for *P. perlatus*, which experienced a thermally induced dampening of FR at the high temperature. This data also represents the first quantitative estimates of *P. perlatus* predatory efficiency.

While we hypothesised higher consumption rates and asymptotes of the FR curves of both crayfish species compared to *P. perlatus*, our results showed that the *per capita* predation by *P. perlatus* and *C. quadricarinatus* consistently exceeded that of *P. clarkii* under almost all of the experimental conditions (apart from the raised temperature treatment). Crabs and crayfish are both benthic omnivores with similar feeding mechanisms (Jackson et al. 2016) and readily consumed *C. gariepinus* fry in the lab. As such, any differences detected in the consumption rates and FR parameters between these decapods are unlikely to be due to major differences in their trophic ecology nor functional traits. Using trophically analogous native species as comparison species can provide ecologically meaningful inferences which allows the results to be interpreted in a contextually relevant manner (Dick et al. 2013; Dickey et al. 2020; South et al. 2020). In all laboratory experiments there is some degree of confinement effect for both predator and prey behaviour. Predation can be mediated by structural complexity (amongst other factors) for example by changing predator free space (Barrios-O'Neill et al. 2015), increasing shelter use leading to less active foraging (Wood and Moore 2020), or acting as a prey aggregator (Persson and Eklov 1995). Therefore, these results are relative within the context of this experiment, although FR results have been shown to be predictive and meaningful in terms of ecological impact across many taxa (Dick et al. 2017a; Cuthbert et al. 2019).

As expected, all three species fed more in the dark, but consumption does occur under daylight conditions in natural scenarios as well (Cumberlidge 2011; Jones et al. 2016). Light wavelength has been documented to affect predation in a number of species (Becker et al. 2013; Jones et al. 2016; South et al. 2017). Crayfish predominantly detect prey through a chemosensory pathway and therefore do not rely on visual foraging cues (Corotto and O'Brien 2002; Meakin et al. 2009) so foraging occurs in crepuscular or nocturnal periods as an anti-predator strategy (Styrishave et al. 2007). Since freshwater crabs are also nocturnal (Cumberlidge 2011) they are likely to interact and share common resources with crayfish during the night when both species emerge to forage. There have been some reports of freshwater crab declines as an inferred impact of crayfish invasions in some African river systems, either through competitive exclusion or through direct consumption (Foster and Harper 2007; Jackson et al. 2016). As a result, it is recommended that further research focuses on interactions under nocturnal conditions to avoid temporal biases in effects.

Our results support the ecological matching hypothesis which predicts that the performance of IAS are higher at temperatures that most closely match their thermal optima (Iacarella et al. 2015). As per the metabolic theory of ecology (Huey and Stevenson 1979; Jobling 1981; Englund et al. 2011), temperature increase will also have increased prey metabolism, increasing the frequency of prey movement, speed and boldness (although these effects will also be apparent in the predator) (van Baalen et al. 2001; Nowicki et al. 2012; South and Dick 2017). As a result, more stimuli are provided to the predator and the encounter rate (i.e. attack parameter) is increased (South and Dick 2017) as observed when temperatures were increased to 28°C in this study. Mismatches in foraging and digestion capacity will occur when the effects

of temperature change are of different magnitude and direction between species, however this might only be detected at different time scales (i.e. acclimation duration) (South and Dick 2017).

Temperature differences, however, had dissimilar effects on the handling parameter of the three species. The temperature tolerance and preference ranges for *P. perlatus* are as yet unknown due to data deficiency. Nonetheless, *P. perlatus* had a low and high FRs under the high and low temperatures respectively, which suggests a cooler thermal range than the other species that may be a result of adaptation to the cooler Eastern Cape water bodies (Dallas 2008). While the native range of *P. perlatus* is large and overlaps with regions that have been invaded by the two crayfish species in South Africa, as well as spreading into Namibia (Barnard 1950; Gouws et al. 2002; Cumberlidge and Daniels 2007), it should be emphasised that FRs can be population specific (Boets et al. 2019; Grimm et al. 2020). Similar results (i.e. FR magnitude decrease with temperature increase) have been found for cold-water adapted fish (e.g. South et al. 2018). However, FRs generally follow a hump shaped relationship with temperature regardless of taxon (Uiterwaal and DeLong 2020). This may indicate that the optimal foraging temperature for *P. perlatus* is somewhere between 19°C and 28°C. *Procambarus clarkii* experienced a reduced handling parameter with increased temperature, reflecting the assumptions in the metabolic theory of ecology and resulting in increased per capita effect (Brown et al. 2004; Englund et al. 2011; Dell et al. 2011). As such, *P. clarkii* is likely to exert higher relative impact than the other two species when compared between systems with different thermal regimes, which may result in faster growth rates and higher maximum body sizes of *P. clarkii* in warmer areas. Contrastingly, handling times were similar for *C. quadricarinatus* at each temperature, suggesting that attack parameter is the driver of increased impact under high temperatures. The consistency of handling parameter values for *C.*

quadricarinatus across temperature treatments implies that its broad thermal tolerance will enable high ecological impact across the invaded range, whereas *P. clarkii* is somewhat constrained by thermal regime. In any case, both invasive crayfish have a competitive advantage over *P. perlatus* at higher temperatures. These results emphasise the importance of contextualising impact assessments to specific localities or ecoregions to avoid over or under-estimating possible impacts of IAS. Although some populations may be more or less heat tolerant than others, but this does not detract from the overall message. Furthermore, due to climate warming, invasive crayfish may potentially establish in previously unavailable or unsuitable habitats thereby increasing their invasion range (Hellmann et al. 2008; Rahel and Olden 2008). Our data suggest that such warmer conditions may also reduce the potential for freshwater crabs to resist crayfish invasions.

The FRR method was used to determine subtle differences between impact predictions between standard FR curves *C. quadricarinatus* and *P. perlatus* which were inconclusive at the low temperature treatment under light conditions. *Cherax quadricarinatus* has potential to destabilise prey populations as the FFR was higher than for *P. perlatus*. The FRR of both crayfish species at the high temperature treatment was higher than that of *P. perlatus* due to the dual impact of high attack and low handling parameters. The high FRR values for the two crayfish species are of concern as they show their potential impacts on the fry of benthic fish species (here represented by *C. gariepinus*). Crayfish negatively impact benthic fish populations in other systems, for example in the Ouse River, England, where the presence of signal crayfish *Pacifastacus leniusculus* (Dana 1852) resulted in increased benthic fish mortalities due to direct predation (Guan and Wiles 1997). More recently, long-term monitoring results showed the capacity for signal crayfish to cause complete extirpations of some benthic fish species in the River Tees catchment, UK (Galib et al. 2020). Similar results

have been detected for the Rusty crayfish *Faxonius rusticus* (Girard 1852) in Illinois, USA (Thomas and Taylor 2013). Further research should assess propensity for density dependent predation (i.e. prey switching capacities) specifically in African systems to determine whether other resources are consumed over fish (Geiger et al. 2004).

Populations of predators and prey usually follow predictable adaptive evolution cycles that can lead to niche divergence and thus facilitate coexistence in predator–prey communities (Chesson 2000; Cortez and Weitz 2014). Due to the ubiquity of potamonautid crabs and clariid catfishes throughout African waterbodies they have co-evolutionary history, subsequently predatory impacts of crabs upon native fishes have never been investigated. Relative to crab consumption, the crayfish species did not exert pressure upon *C. gariepinus* at lower temperatures, however the mismatch occurred between *P. perlatus* and *C. quadricarinatus* at the higher temperature. It is however possible that at lower temperatures, resources that were not previously threatened by the crabs will be subjected to multiple predators after the invasion which will place increased pressure on resources. Co-existence of native and invasive species may lead to evolutionary changes in both species as they will be competing for the same limiting resource (Pekkonen et al. 2013). Alternatively, if the competitively dominant species does not change and is able to expand its niche, it may reduce niche space available for the other species. *Procambarus clarkii* showed niche breadth reduction when found in sympatry with *P. loveni* in Lake Naivasha, Kenya, where they affected leaf litter breakdown due to direct consumption (Jackson et al. 2016). Furthermore, *P. clarkii* is capable of exerting predatory pressure on planktonic as well as benthic prey in the laboratory, although the pressure exerted differs between substrate types (South et al. 2019).

Procambarus clarkii has been named as one of the world's worst invasive species (Lowe et al. 2000), however, it is also one of the most well studied species (Twardochleb et al. 2013), unlike *C. quadricarinatus* and *P. perlatus*, which are essentially data deficient in terms of ecology (Cumberlidge and Daniels 2007; Souty-Grosset 2016; Weyl et al. 2020). This study hypothesised higher FRs from the two crayfish under all conditions. This was however not the case as *C. quadricarinatus* and *P. perlatus* had a significantly high FR under low temperature conditions and *C. quadricarinatus* had a higher FR under the high temperature treatment. Therein, the unexpectedly comparatively poor performance of *P. clarkii* was only evident at the lower temperature. Consumptive impacts conferred by *P. clarkii* are variable and resource specific: it may switch to predominantly herbivory during heatwave conditions and alters its resource assimilation efficiency (Carreira et al. 2017). Furthermore, reported impacts are mainly on primary producers (e.g. macrophytes and algae) (Matsuzaki et al. 2009) altering trophic cascades and changing ecosystem structure and function through indirect effects (Twardochleb et al. 2013). The results presented here confirm the necessity to use relevant comparison species, indeed in terms of trait performance (closing force) *P. clarkii* was outperformed by the same two focal species (South et al. 2020), as well as consider a broader range of resources when assessing impact. The documented impacts in field and laboratory experiments may be related to resource availability in the field (i.e. density dependent consumption) (Bondar et al. 2005; Veselý et al. 2020), feeding specialisations via functional traits and chelae dentition (West et al. 1991; South et al. 2020), or even some degree of survivorship bias with regards to ethical barriers to using live vertebrate prey in laboratory experiments. Our results highlight that in some cases invasion history, especially Palearctic and Nearctic results, might not be robust enough to transplant into Afrotropical settings. Accordingly, full food web effects of crayfishes on African freshwater biota should be pursued through further research, such as more complex resource consumption experiments, stable

isotope analysis, and invader abundances, so as to fully capture possibilities of trophic cascades.

Accumulating quantitative evidence of IAS impacts is an integral factor in making informed management decisions as well as IAS classification and assessment (Kumschick et al. 2012; Blackburn et al. 2014; Hawkins et al. 2015). While comparative data from more natural settings are desirable, the data comprising this study supports the use of FRs and FRR as part of a toolbox to predict and understand the potential impacts of *C. quadricarinatus* and *P. clarkii* and represents the first step in assessing ecological impact in challenging African freshwater systems. This data provides novel evidence of the impacts of two established invasive crayfish in Africa and is thus the first attempt to determine relative impact of both invasive crayfishes compared to native African crabs, especially relevant considering the multiple crayfish introductions in the continent (Madzivanzira et al. 2020; Chapter 2). The per capita differences between the crayfish and native crabs seen at higher temperatures may contribute to heightened success of the invaders in specific regions, as well as causing biodiversity and biomass declines of native species in the invaded systems through predation. Furthermore, the crayfish could confer stronger impacts in the field than anticipated, owing to patterns in population abundance, fecundity and growth rates. This is an important caveat in lab-based predictions in invasion ecology as resource consumption is exacerbated by invader abundance and the function of both denotes overall *per capita* impact (Parker et al. 1999; Dick et al. 2014; Dickey et al. 2018, Dickey et al. 2020). Future studies should prioritise the integration of field and laboratory data, especially concerning abundance and distribution.

CHAPTER 6

PREDATORY IMPACTS OF INVASIVE CRAYFISH IN AFRICA ARE PREDICTED BY FUNCTIONAL RESPONSES

Abstract

There are few estimates of ecological impacts of invasive crayfish in Africa, yet invasion history elsewhere suggests the likelihood of negative impacts on various ecosystem components. Two global invaders, the Australian redclaw crayfish *Cherax quadricarinatus* and the Louisiana red swamp crayfish *Procambarus clarkii* have been reported to be spreading rapidly across the African continent. To evaluate the potential for ecological impacts of these two crayfish species, comparative functional responses (FRs) and FR ratio (FRR) were employed to describe their consumer-resource dynamics in comparison to the native trophic analogue, the freshwater crab *Potamonautes perlatus*. Three different resources were used. One that represents an aquatic terrestrial link (earthworms), one benthic (chironomid larvae) and one pelagic (mosquito larvae). Experiments were conducted under dark conditions at two temperature treatments, 19°C and 28°C. Our prediction that invasive crayfish would have greater maximum feeding rates on all prey items than the native crab was not supported as the FR was prey and temperature dependant. All three predators exhibited potentially population destabilising Type II (hyperbolic) FRs towards all the prey species at all the temperatures. At least one of the crayfish species had a higher attack parameter than *P. perlatus* in all the prey experiments except for the earthworms under low temperatures. At low temperatures, *P. perlatus* exhibited the highest maximum feeding estimates on most prey items, and although the FR curves at the high temperature treatment were not significantly different between the predators, the two crayfish species had high FRRs due to high attack and low handling parameters. Results from this study indicate that FR parameters such as high attack parameter,

and high FRRs for crayfish species may contribute to their invasion success and negative impacts on native species, as well as decreased biodiversity in invaded systems. In cases where the FR for crabs was similar or higher than at least one of the crayfish species, resources will be subjected to multiple predators which will threaten the resources. Management options in Africa should thus aim at preventing introductions and further spread to reduce associated negative impacts of these crayfish species.

Introduction

Biological invasions are a key contributor to dwindling biodiversity (Vilà et al. 2011; Galiana et al. 2014; Doherty et al. 2016) and are deemed to be the second most important global driver of biodiversity loss (MEA 2005). One taxonomic group at the forefront of this are freshwater crayfish species which have been spread globally for aquaculture, fisheries and ornamental purposes that have facilitated their establishment beyond their native ranges (Lodge et al. 2012; Twardochleb et al. 2013; Madzivanzira et al. 2020; Chapter 2). Introduced crayfishes have been shown to negatively impact ecosystems, through several mechanisms including predation, competition, spreading of diseases and environmental modification, which are well described in Chapter 1.

The two focal crayfish species in this thesis: Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) and Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852) lack contextual assessments within an Afrotropical setting, and *C. quadricarinatus* is essentially data deficient worldwide. Since freshwater crabs and the invasive crayfish species are functionally similar (Cumberlidge 2011; Jackson et al. 2016), they are likely to interact, and share common resources. Ecological impacts have been predicted to be strong on native trophic analogous decapods, such as freshwater crabs from the genus *Potamonautes* (de Moor 2002; Jackson et al. 2016; Nunes et al. 2016; Madzivanzira et al. 2021; Chapter 5) although biotic resistance by the freshwater crab has been shown (South et al. 2020).

A standardised method for inferring the relative impact of IAS is to compare functional response (FR) of a native species and IAS. The FR describes the causal relationship between resource consumption and resource (e.g. prey) density (Solomon 1949; Holling 1959). Studies

using FR have consistently demonstrated that IAS consume available resources at a higher rate than analogous native species (Dick et al. 2014, 2017; Dickey et al. 2020). Further, higher FR magnitudes for more invasive and ecologically damaging species may be due to differences in physical characteristics, such as resource acquisition and metabolism, or the lack of co-evolutionary history between IAS and native resource (Ricciardi et al. 2013). Functional responses can be linear (Type I), hyperbolic (Type II), or sigmoidal (Type III) (Dick et al. 2014, 2017; Dickey et al. 2020) and are parsed into parameters: attack (search parameter [a]), handling (a conglomerate of behaviours [h]) and maximum feeding estimate [1/h]. Type II responses in particular may potentially destabilise prey populations and lead to localised extirpations. Unlike predation studies, which only seek to directly measure the impact of a non-native species in a particular location or on a particular species, this method uses simplified experimental conditions to generate relative parameter values that can be compared across numerous biotic and abiotic contexts, something that is particularly important in invasion studies, as impacts are highly context-dependent (Dick et al. 2014, 2017). More recently, progress into developing the FR approach towards predictive capacity has resulted in the combination of '*a*' and '*h*' to derive a "Functional Response Ratio" (FRR), in order to elucidate potential intermediate impact where previously distinguishing differences between FR curves were problematic (Cuthbert et al. 2019; South et al. 2019; Dickey et al. 2020). This is centred in the assertion that high attack parameter and low handling parameter both imply high impact, and thus the parameters are combined to create a ratio of a/h, higher values of which are found in high-impact invaders (Cuthbert et al. 2019).

In Africa, both FR and FRR have been successfully employed to infer impact of invasive crayfish species in comparison to *Potamonautes perlatus* using a benthic resource, African

sharptooth catfish *Clarias gariepinus* fry as prey (Madzivanzira et al. 2021; Chapter 5). The overall FR magnitudes of the crayfish species were enhanced under dark conditions compared to light and at a higher magnitude under the high temperature than low temperature treatment except for *P. perlatus* which experienced a thermally induced dampening of FR at the high temperature treatment (Madzivanzira et al. 2021; Chapter 5). There is, however, little empirical data quantifying and comparing the impacts of crayfish across different native prey resources in Africa (but see Madzivanzira et al. 2021; Chapter 5; Grey and Jackson 2012). Crayfish are able to use both aquatic and terrestrial resources (Twardochleb et al. 2013; Jackson et al. 2016; South et al. 2019) and therefore it is integral to assess impacts across different types of resources. These different native resources play distinct roles in ecosystems, for example how macroinvertebrates drive the productivity of the fisheries on the floodplain (Wallace and Webster 1996). In Africa, their ability to utilise both terrestrial and aquatic resources depends on the dynamics of floodplains, for example in the Zambezi Basin (Madzivanzira et al. 2020; Chapter 2). When ponds on the floodplains dry up, the crayfish are seen walking on the ground (Grey and Jackson 2012; Madzivanzira et al. 2020; Chapter 2) and stable isotope analysis showed the presence of terrestrial resources in the diet of crayfish (Grey and Jackson 2012).

Understanding the impacts of *C. quadricarinatus* and *P. clarkii* on different native resources is an urgent requirement in Africa. Hence, this study investigates the variability in consumer-resource dynamics of two alien crayfish species *C. quadricarinatus* and *P. clarkii* in comparison to a native freshwater crab *P. perlatus* on three different three macroinvertebrates resources: one that represents an aquatic terrestrial link (earthworms), one benthic (chironomid larvae) and one pelagic (mosquito larvae). The prediction is that, although all species will exhibit a Type II FR, the two crayfish species will possess a higher magnitude FR and FRR

than the crab under all treatments based on crayfish invasion history on native resources. This study among a few others in Africa attempts to predict the potential impacts of crayfish species that have established invasive populations in the region.

Methods

Animal collection and maintenance

The animals used in Chapter 5 were rested for at least a month before being reused for experiments in this chapter. Animals were maintained in species specific and sex specific holding tanks (60 L) with constantly filtered and dechlorinated tap water, which was periodically replaced to maintain good water quality. Specimens were acclimated to lab conditions in the holding tanks for at least 1 month prior to experimentation. Water temperature was maintained at 22 ± 1 °C and the laboratory was held under a 12:12 light:dark regime with white light and total darkness. Crayfish and crabs are omnivores (Geiger et al. 2005; Gherardi 2007; Reynolds and Souty-Grosset 2012) and hence all animals were maintained on cabbage leaves and pieces of dead fish (*O. mossambicus*) purchased from Aquaculture Innovations in Makhanda.

Prey chosen were live: earthworms *Eisenia* sp. (total length mean ± SE: 58.51 ± 0.35 mm), common midge Chironomidae (total length mean ± SE: 17.98 ± 0.37 mm) and mosquito larvae *Culex* sp. (total length mean ± SE: 9.06 ± 0.15 mm). *Eisenia* sp. were collected from a compost culture at SAIAB and represent a terrestrial resource which can be utilised by the crayfish, as they have been observed to walk on land around Lake Naivasha, Kenya (Grey and Jackson 2012) and in the Zambezi Basin (Madzivanzira et al. 2020; Chapter 2). Chironomid larvae were collected from ponds around Makhanda and represent a benthic aquatic resource. Chironomid

larvae are most commonly found amongst benthic debris (Griffiths et al. 2015). Mosquito larvae were collected from buckets and bins filled with water at SAIAB. Mosquito larvae typically live near the surface of the water, but periodically travel downwards and upwards through the water column (Griffiths et al. 2015) and therefore represent a pelagic resource. All the macroinvertebrates used in this study are ubiquitous across the invaded range in Africa (Griffiths et al. 2015).

Experimental setup

Functional response experiments were carried out in 12 L of dechlorinated tap water in 15 opaque rectangular tanks (dimensions 550 mm × 365 mm × 205 mm) in a randomised order. Tanks had a layer of river sand substrate (~ 20 mm). Individual animals were randomly selected from the holding tank and patted dry before measurements were taken. Neither moulting nor individuals with deformed/regenerating/missing chelae or walking legs were considered for the experiments. *Procambarus clarkii* (carapace length (mean ± SE): 56.7 ± 0.7 mm; mass: 60.1 ± 1.1 g), *C. quadricarinatus* (carapace length: 61.7 ± 0.7 mm; mass: 69.9 ± 1.7 g) and *P. perlatus* (carapace length: 61.7 ± 0.7 mm; mass: 52.78 ± 0.6 g) were used for the experiments. All experimental animals were starved for 24 h prior to the experiment to standardise satiation level. Animals were allowed to acclimate to experimental arenas for 1 h before prey were supplied. Prey were supplied to each of the predator at densities of: 2, 5, 10, 15, 20, 30, 40 (n = 5 at each density per species per treatment) for earthworms, and 2, 5, 10, 20, 40, 60, 150, 200 (n = 5 at each density per species per treatment) for chironomid and mosquito larvae. These prey densities were determined by the preliminary predation trials that were run, for example, the predators can consume approximately 40 *Eisenia* sp. compared to approximately 200 *Culex* sp. in the given time period because the earthworms have a considerably larger body size. In

order for the FR curves to plateau, the consumer must be provided with an overabundance of resource with which it can satiate. Unfortunately, in the case of chironomid and mosquito larvae, prey supply was limited by the amount needed for each experiment (i.e. 14610 for each full experiment, three predators and two temperatures), therefore due to prey availability and logistical issues, the highest prey density was capped at 200. Experiments were run for 3 h, after which the predators were removed, and number of prey items consumed were enumerated. No predators were re-used more than once per prey treatment. Controls were four replicates of each prey density in the absence of the predators to assess whether there was natural mortality and cannibalism among the prey.

Two functional response experiments were completed under 19°C and 28°C (which are realistic field winter and summer temperatures conditions of the invaded ranges in Southern Africa, see Madzivanzira et al. 2021; Chapter 5). All trials were run in dark conditions as the three decapods were shown to consume more under dark than light conditions (Madzivanzira et al. 2021; Chapter 5). Prior to functional response experiments all animals (predator and prey) were acclimated to the desired temperature at a rate of change of 1°C per day and allowed to acclimate to the two temperatures for a week before experiments were conducted.

Statistical analysis

All analysis was carried out in R v3.6.1 (R Development Core Team 2019). Three separate Generalised Linear Models (GLM) were used to assess prey consumption with regards to temperature differences using fixed factors *predator species*, *temperature*, and *density*. Three separate GLMs were used since prey body size were not comparable and prey with different densities were used. The R package 'frair' (Pritchard 2014) was used to model the FR type.

Functional responses were modelled using maximum likelihood estimation (MLE; Bolker 2010) and Rogers' (1972) Random Predator Equation (Equation 1), due to the prey not being replaced as they were consumed. If the proportion of prey consumed decreases with an increase in prey density, then the logistic regression will produce a significantly negative result and thus the FR type can be classed as Type II (Juliano 2001).

The data were non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals around the mean functional response curve for each treatment; this allows the curves to be considered for population level inferences. The FR data were assessed in a phenomenological manner, therefore, overlapping confidence intervals suggest a lack of significant difference in the magnitude FR. Differences in a and h were assessed within species across the two temperatures using the the z-method via *frair::frair_compare* function (Juliano 2001) in Pritchard et al. (2017).

The FRR (per Cuthbert et al. 2019) (Equation 2) was calculated for each predator using the parameter estimates of a and h derived from the maximum likelihood estimate results of Equation (1). The FRR was used as a diagnostic tool to determine whether there were differences that were not clearly observed from the usual FR outputs. Therefore, the higher the FRR value the higher the inferred impact (Cuthbert et al. 2019).

Results

Prey survival was > 99% under all control treatments and there was no mortality of predators during the experimental period.

Earthworm prey

There was a two-way interaction between 'species × temperature' and 'temperature × density' (Table 6.1). For the species × temperature interactions, *P. perlatus* consumed significantly more prey than the two crayfish species at both temperature treatments and *C. quadricarinatus* consumed significantly more prey than *P. clarkii* at both temperature treatments (Appendix 6.1). For the temperature × density interaction, *P. perlatus* consumed significantly more prey ($p < 0.05$) than the two crayfish species at prey densities above 5 at 19°C. *Cherax quadricarinatus* consumed significantly more prey ($p < 0.05$) than *P. clarkii* at prey densities above 5. At 28°C, *P. perlatus* and *C. quadricarinatus* consumed significantly more prey ($p < 0.05$) than *P. clarkii* at prey densities above 5 and 15, respectively (Appendix 6.1).

Table 6.1. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in earthworm *Eisenia* sp. prey consumption by *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* with regards to factors *species*, *temperature* and *density*, using a Type 3 Anova and χ^2 to report the effect size.

Model term	Chisq	df	*p*-value
Species	179.92	2	< 0.001
Temperature	45.72	1	< 0.01
Density	303.68	7	< 0.001
Species × Temperature	33.71	2	< 0.001
Species × Density	12.16	14	0.43
Temperature × Density	16.32	7	< 0.05
Species × Temperature × Density	12.28	14	0.42

Chironomid larvae prey

There was a significant two-way interaction between 'species × temperature' (Table 6.2). At 19°C, *P. perlatus* and *C. quadricarinatus* consumed significantly more prey ($p < 0.05$) than *P. clarkii* and at 28°C there was no difference in consumption between the three species ($p > 0.05$) (Appendix 6.1).

Table 6.2. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in chironomid larvae prey consumption by *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* with regards to factors *species*, *temperature* and *density*, using a Type 3 Anova and χ^2 to report the effect size.

Model term	Chisq	df	*p*-value
Species	6.40	2	< 0.05
Temperature	122.20	1	< 0.01
Density	7035.50	7	< 0.001
Species × Temperature	89.80	2	< 0.001
Species × Density	46.50	14	0.43
Temperature × Density	13.40	7	0.06
Species × Temperature × Density	17.50	14	0.23

Mosquito larvae prey

There was a two-way interaction between 'species × temperature' (Table 6.3). There were no significant differences ($p > 0.05$) in consumption between the three species at 19°C, and at 28°C *C. quadricarinatus* consumed significantly more prey ($p < 0.05$) than *P. perlatus* and *P. clarkii* (Appendix 6.1).

Table 6.3. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in mosquito larvae *Culex* sp. prey consumption by *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* with regards to factors *species*, *temperature* and *density*, using a Type 3 Anova and χ^2 to report the effect size.

Model term	Chisq	df	*p*-value
Species	24.40	2	< 0.001
Temperature	1.70	1	0.20
Density	7616.90	7	< 0.001
Species × Temperature	79.40	2	< 0.001
Species × Density	9.40	14	0.80
Temperature × Density	1.60	7	0.98
Species × Temperature × Density	5.70	14	0.97

Functional response results

All predator species and temperature treatment combinations resulted in a significant Type II FRs (Table 6.4; Figs. 6.1 – 6.3).

FR earthworms

All species exhibited a higher maximum feeding estimate under the high temperature treatment in comparison to the low temperature treatment (Table 6.4; Fig. 6.1). This was driven by handling parameter values which were significantly lower ($p < 0.001$) in the high temperature treatment than in the low temperature treatment (Table 6.5).

At 19°C, the FR curves for all the predators do not differ significantly at the lowest prey densities but all diverged at prey densities < 2 resulting in significant differences with *P. perlatus* having the highest FR and *P. clarkii* having the lowest (Fig. 6.1). At 28°C, the magnitude of *P. clarkii* FR curve does not differ significantly at the lowest prey density with *P. perlatus* and *C. quadricarinatus* but diverges at prey densities < 5 resulting in significant differences. At 28°C, *C. quadricarinatus* FR curve does not diverge from *P. perlatus* FR curve at the highest and lowest prey densities (Fig. 6.1). The maximum feeding estimate of *P. perlatus* consistently exceeded that of the two crayfish species at both temperature treatments (Table 6.4). Due to the high attack parameter and lower handling parameter values exhibited by *P. perlatus* in both temperature treatments it results in the highest FRR ratio (Table 6.4).

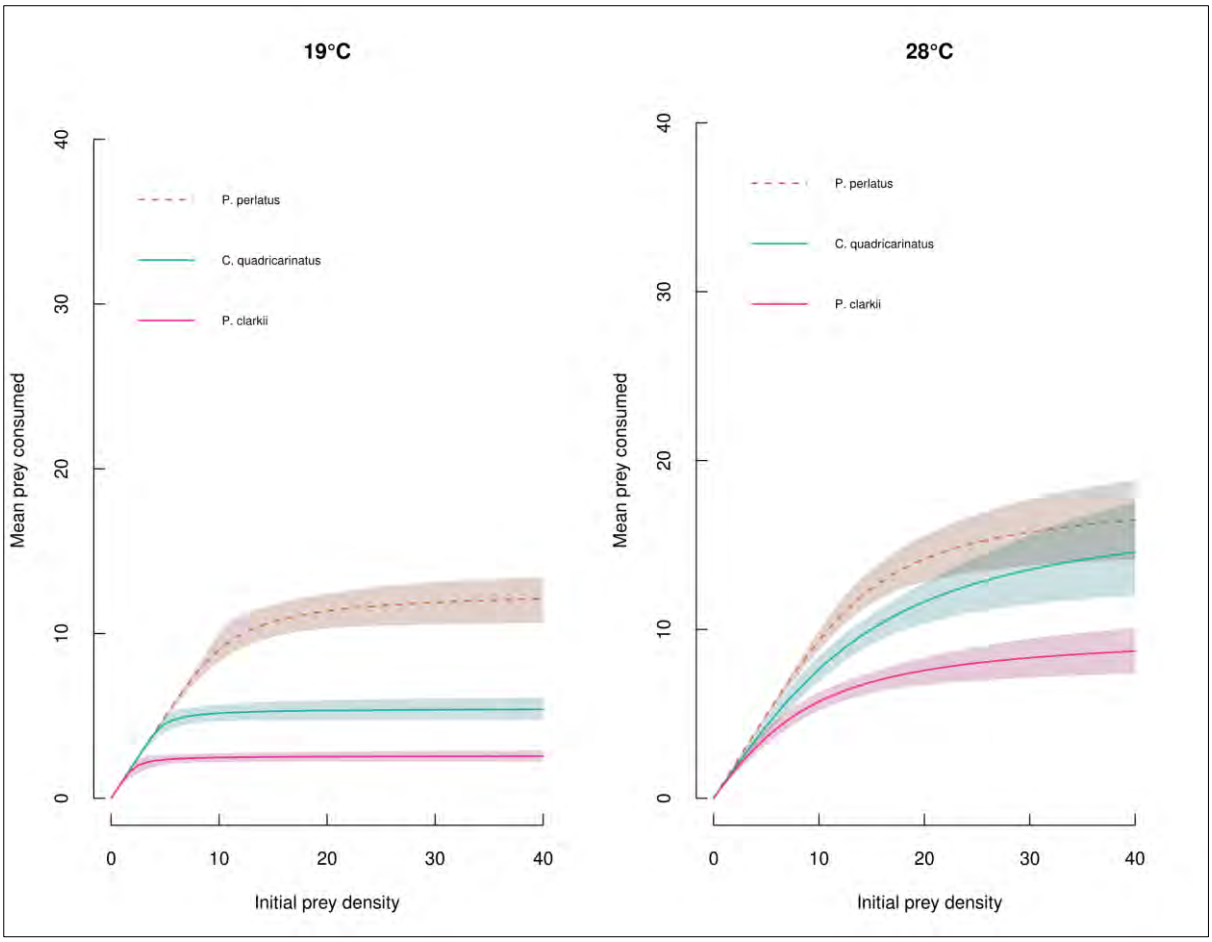

Figure 6.1. Functional response curves of *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* preying on earthworms *Eisenia* sp. under 19°C and 28°C temperature treatments (modelled using the Rogers' random predator equation for a Type II functional response). Shaded areas are bootstrapped 95% confidence intervals.

Table 6.4. First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with FR Type, functional parameters (*a*, *h*, and 1/*h*), associated significance levels from Rogers' random predator equation (for Type II) with regards to *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* predating upon earthworms *Eisenia* sp., Chironomid larvae and mosquito larvae *Culex* sp. under different temperature treatments.

a = attack parameter; *h* = handling parameter; 1/*h* = maximum feeding estimate.

Species	Prey	Temperature (°C)	First order term (*p*-value)	FR Type	*a* (*p*-value)	*a* 95% BCa CI	h (*p*-value)	*h* 95% BCa CI	Maximum Feeding Estimate (1/h)	Functional Response Ratio (a/h)
P. perlatus	Earthworm	19	-0.096, <0.001	II	8.10, <0.001	4.56-27.51	0.079, <0.001	0.067-0.090	12.68	102.62
C. quadricarinatus	Earthworm	19	-0.074, <0.001	II	13.73, >0.05	4.93-105.73	0.184, <0.001	0.158-0.208	5.45	74.85
P. clarkii	Earthworm	19	-0.066, <0.001	II	7.17, >0.05	0.95-44.03	0.388, <0.001	0.331-0.440	2.58	10.46
P. perlatus	Earthworm	28	-0.087, <0.001	II	5.55, <0.001	3.47-10.71	0.055, <0.001	0.045-0.068	18.22	101.16
C. quadricarinatus	Earthworm	28	-0.056, <0.001	II	2.53, <0.001	1.63-4.25	0.056, <0.001	0.039-0.074	17.75	44.92
P. clarkii	Earthworm	28	-0.057, <0.001	II	2.00, <0.001	1.31-3.20	0.101, <0.001	0.080-0.125	9.92	19.89
P. perlatus	Chironomid larvae	19	-0.004, <0.001	II	0.93, <0.001	0.64-1.28	0.007, <0.001	0.003-0.012	154.30	142.96
C. quadricarinatus	Chironomid larvae	19	-0.002, <0.001	II	0.71, <0.001	0.59-0.88	0.006, <0.001	0.003-0.008	166.70	118.33

P. clarkii	Chironomid larvae	19	-0.008, <0.001	II	1.70, <0.001	1.32-2.15	0.009, <0.001	0.007-0.012	108.00	184.03
P. perlatus	Chironomid larvae	28	-0.007, <0.001	II	1.67, <0.001	1.19-2.24	0.005, <0.001	0.001-0.008	76.30	185.44
C. quadricarinatus	Chironomid larvae	28	-0.008, <0.001	II	1.94, <0.001	1.48-2.52	0.005, <0.001	0.003-0.007	190.19	369.50
P. clarkii	Chironomid larvae	28	-0.008, <0.001	II	1.62, <0.001	1.22-2.03	0.010, <0.001	0.007-0.013	100.83	163.20
P. perlatus	Mosquito larvae	19	-0.004, <0.001	II	0.93, <0.001	0.95-1.33	0.007, <0.001	0.002-0.006	142.86	132.86
C. quadricarinatus	Mosquito larvae	19	-0.002, <0.05	II	0.69, <0.001	0.56-0.88	0.003, <0.05	0.000-0.005	333.33	230.00
P. clarkii	Mosquito larvae	19	-0.003, <0.001	II	1.70, <0.001	0.65-1.01	0.009, <0.001	0.003-0.007	111.11	188.89
P. perlatus	Mosquito larvae	28	-0.002, <0.001	II	0.71, <0.001	0.57-0.96	0.004, <0.001	0.001-0.006	250.00	177.50
C. quadricarinatus	Mosquito larvae	28	-0.003, <0.001	II	1.20, <0.001	0.95-1.48	0.005, <0.001	0.000-0.006	200.00	240.00
P. clarkii	Mosquito larvae	28	-0.003, <0.001	II	0.79, <0.001	0.64-1.05	0.004, <0.001	0.002-0.007	250.00	197.50

Table 6.5. Frair compare test for the attack '*a*' and handling '*h*' parameters for each predator species (*Cherax quadricarinatus*, *Procambarus clarkii* and *Potanounates perlatus*) between two temperature treatments (19°C and 28°C) using earthworms *Eisenia* sp., Chironomid larvae and mosquito larvae *Culex* sp. as prey.

Species combination	Prey	Estimate		SE		*z*-value		*p*-value	
		a	*h*	*a*	*h*	*a*	*h*	*a*	*h*
C. quadricarinatus	Earthworms	7.83	0.12	6.64	0.02	1.18	7.58	0.23	< 0.001
P. clarkii	Earthworms	5.20	0.29	5.67	0.05	0.92	5.99	0.36	< 0.001
P. perlatus	Earthworms	2.55	0.02	2.41	0.01	1.06	3.74	0.29	< 0.001
C. quadricarinatus	Chironomid larvae	-1.23	< 0.01	0.13	< 0.01	-9.52	0.19	< 0.001	0.84
P. clarkii	Chironomid larvae	0.09	< 0.01	0.18	< 0.01	0.49	-0.73	0.63	0.46
P. perlatus	Chironomid larvae	-0.74	< 0.01	0.13	< 0.01	-5.96	1.16	< 0.001	0.25
C. quadricarinatus	Mosquito larvae	-0.51	< -0.01	0.09	< 0.01	-5.44	-5.57	< 0.001	0.57
P. clarkii	Mosquito larvae	0.01	< 0.01	0.08	< 0.01	0.16	0.70	0.87	0.48
P. perlatus	Mosquito larvae	0.42	< 0.01	0.09	< 0.01	4.58	0.49	< 0.001	0.63

FR chironomid larvae

The confidence intervals of all three species FR curves overlapped at 19°C, indicating no difference in pressure on prey between the three predators (Fig. 6.2). At 28°C, the FR confidence intervals of *P. clarkii* do not overlap with the other two species at densities of ~60 and above, indicating significantly lower consumption under these conditions (Fig. 6.2). Both *C. quadricarinatus* and *P. perlatus* had attack parameters that were significantly higher in the high temperature treatment than the low temperature treatment and the FR curves for both species do not differ at 28°C. The handling parameter values for each predator were, however, not significantly different ($p > 0.05$) between the two temperature treatments (Table 6.5). The FR curves for all the three predators do not plateau at 28°C which means the parameter estimates will be under-estimated as they have not reached satiation. The higher attack parameter exhibited by *P. clarkii* at the low temperature treatment results in the highest FRR ratio whilst the higher attack parameter and lower handling parameter values exhibited by *C. quadricarinatus* at the high temperature treatment results in the highest FRR ratio (Table 6.4 – 6.5).

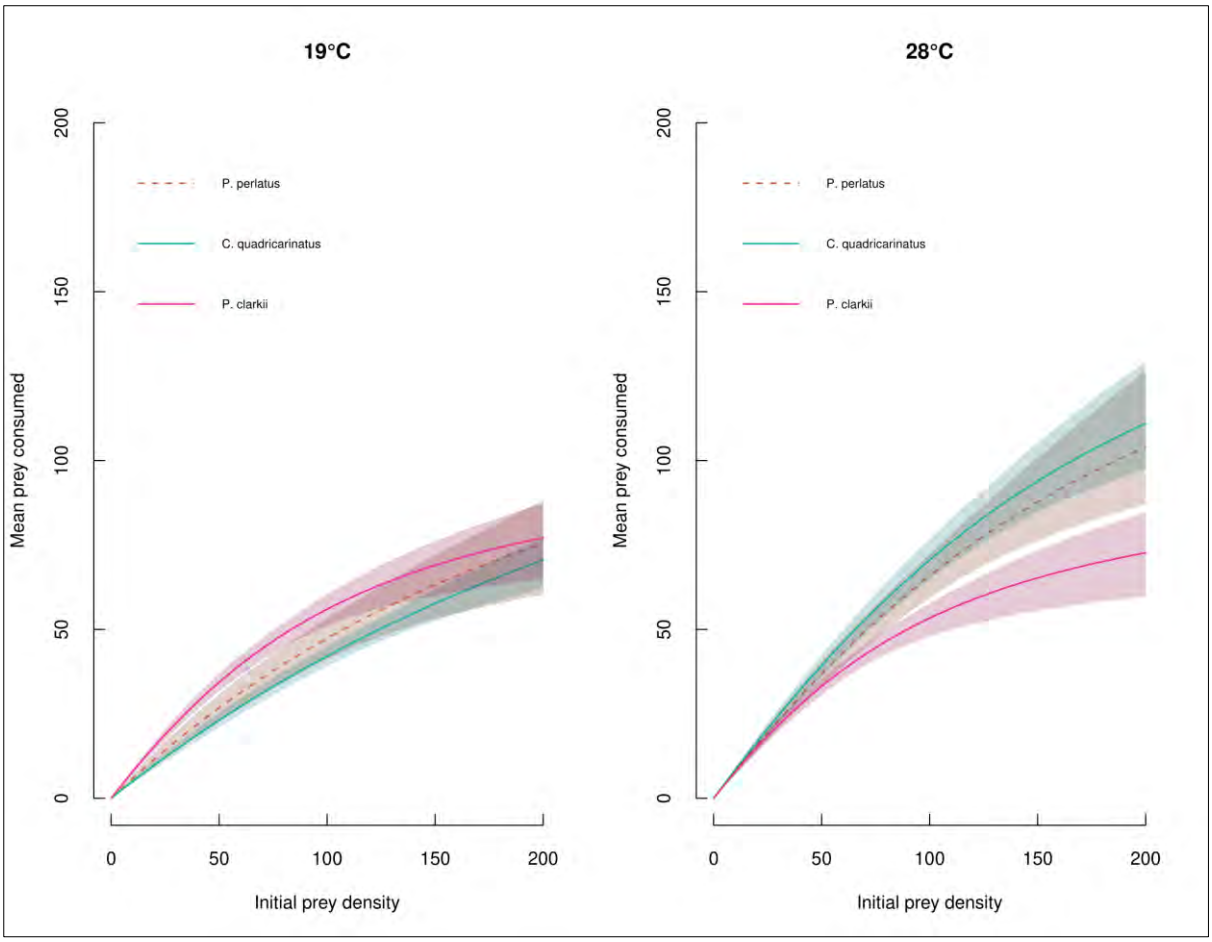

Figure 6.2. Functional response curves of *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* preying on Chironomid larvae prey under 19°C and 28°C temperature treatments (modelled using the Rogers' random predator equation for a Type II functional response). Shaded areas are bootstrapped 95% confidence intervals.

FR mosquito larvae

All the species except *C. quadricarinatus* had higher maximum feeding estimate under the low temperature treatment than the high temperature treatment (Table 6.4; Fig. 6.3). At 19°C, the FR curves for all the predators do not differ significantly at the lowest prey densities but the FR curves for *C. quadricarinatus* and *P. clarkii* diverged from the *P. perlatus* FR curve at~40

and above prey densities (Fig. 6.3). The FR curves for the two crayfish species were not significantly different at 19°C. At 28°C, the magnitude of *C. quadricarinatus* FR curve does not differ significantly at the lowest prey density with *P. perlatus* and *P. clarkii* which diverge at prey densities of ~40 resulting in significant differences. The FR curves for *C. quadricarinatus* and *P. clarkii* however do not differ significantly at the highest prey density at 28°C as their confidence intervals overlap (Fig. 6.3).

The handling parameter values for each predator were, however, not significantly different ($p > 0.05$) between the two temperature treatments (Table 6.5). The attack parameter values for *P. perlatus* and *C. quadricarinatus* were significantly higher ($p < 0.001$) for the high temperature treatment than the low temperature treatment and did not differ significantly for *P. clarkii* (Table 6.5). The low handling parameter exhibited by the three species at the high temperature treatment results in the highest FRR ratio whilst the higher attack parameter and lower handling parameter values exhibited by *C. quadricarinatus* at the high temperature treatment results in the highest FRR ratio (Table 6.4). The FR curves do not plateau at both temperatures which means the maximum feeding estimates will be under-estimated as they have not reached the satiation point (Fig. 6.3).

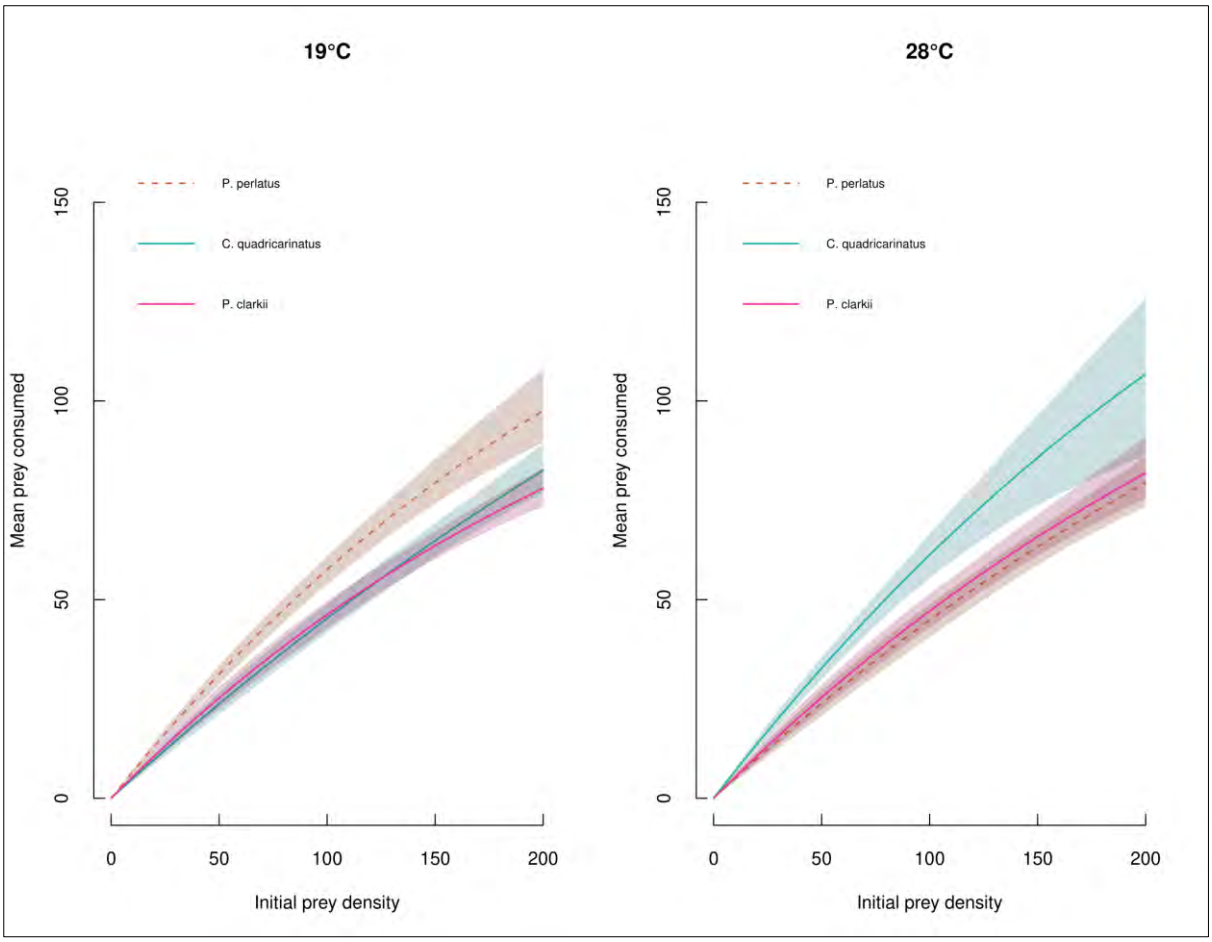

Figure 6.3. Functional response curves of *Potamonautes perlatus*, *Cherax quadricarinatus* and *Procambarus clarkii* preying on mosquito larvae *Culex* sp. under 19°C and 28°C temperature treatments (modelled using the Rogers' random predator equation for a Type II functional response). Shaded areas are bootstrapped 95% confidence intervals.

Discussion

Invasive crayfish species are proliferating in Africa and their invasion history on other continents necessitates risk assessment and management prioritisation (Lodge et al. 2012; Madzivanzira et al. 2020; Chapter 2). Understanding the impacts of invasive crayfish on native biota is an urgent requirement and hence, this study among a few others in the continent was conducted to fill in this knowledge gap using FRs, which have been advocated as predictors of field impacts (Grimm et al. 2020; Linzmaier and Jeschke 2020). Using the FR approach, this study demonstrated that Type II FRs were found to be the best descriptor of all the three decapods feeding on all the prey items under all temperature treatments. This high proportional depletion of resources at low resource density regardless of temperature is typical of highly damaging invasive predators (Dick et al. 2013; Dick et al. 2014; Alexander et al. 2014; South et al. 2017, 2018, 2019; Mofu et al. 2019a; Khosa et al. 2020; Madzivanzira et al. 2021; Chapter 5). These results are relative within the context of this experiment, but FR results can be highly predictive and meaningful in terms of predicting ecological impact across many taxa (Dick et al. 2017a; Cuthbert et al. 2019).

Our prediction that invasive crayfish would have greater maximum feeding rates than the native crab, irrespective of prey type, was not supported, as the FR was prey and temperature dependant. The *per capita* predation on earthworms by *P. perlatus* consistently exceeded that of the two crayfish species under all the temperature treatments, although the FR curve was not significantly different from that of *C. quadricarinatus* at the highest prey density (28°C). When using chironomid larvae as prey, the maximum feeding rate increased with temperature only for *C. quadricarinatus*, and the attack parameter and handling parameter parameters increased and decreased, respectively, with temperature for *P. perlatus* and *C. quadricarinatus*. For the

mosquito larvae experiment, maximum feeding rate increased with temperature for *P. perlatus* and *P. clarkii* due to the decreased handling parameter. At least one of the crayfish species had a higher attack parameter than the native crab in all the prey experiments except for the FR earthworm prey experiment under low temperatures. Given that the attack parameter represents the initial slope (a measure of relative consumption rate at low resource density) of the FR curves, this can impart instability on resource populations, as high attack parameters intuitively predict invasiveness and ecological impact (Dick et al. 2013, 2014; Xu et al. 2016). These results are inconsistent with Madzivanzira et al. (2021; Chapter 5), where the attack parameter of the two crayfish was higher than that of *P. perlatus* using a benthic fish resource, *Clarias gariepinus* fry, as prey. High attack parameters have also been shown for other IAS, such as alien herbivorous snails (Xu et al. 2016); the invasive European green crab (*Carcinus maenas*) (Howard et al. 2018); and the Asian multicoloured ladybird *Harmonia axyridis* (Crookes et al. 2018).

The temperature treatments resulted in divergent handling parameters of the three decapods. For the earthworm prey experiment, the handling parameter for all the predators decreased with temperature increase. For chironomid larvae and mosquito larvae prey, the handling parameters decreased with temperature except for *P. clarkii* and *C. quadricarinatus*, respectively. This is due to effects of the prey body size and response to temperature in both predator and prey (South and Dick 2017). Context-specific experiments need to be considered to further explain the observations. The three predators also had different handling parameters using *C. gariepinus* in Madzivanzira et al. (2021; Chapter 5). Southern Africa freshwater systems where these two crayfish species have established are vulnerable systems, at risk due to climate change factors and other anthropogenic activities. Climate change effects include temperature

rises, which may facilitate range expansions of IAS through thermal acclimation (South et al. 2017). In this study, the performance of crabs on most prey resources decreased at the high temperature treatment. These results corroborate the findings of Madzivanzira et al. (2021; Chapter 5) who noted that the FR of *P. perlatus* preying on *C. gariepinus* reduced with temperature. The predicted warming in the region may favour the range expansion of the two crayfish species with associated ecological impacts in invaded regions (Haubrock et al. 2021). The biotic resistance conferred by crabs, may be affected by the predicted climate change events, as the performance of crabs will be reduced under the warming scenarios.

Relative to crab consumption, the crayfish species did not exert pressure upon most resources. By comparing the FR curves for each prey species, this study was able to distinguish whether one prey species may have higher pressures exerted upon it by crayfish species. Both the benthic (chironomid larvae) and pelagic (mosquito larvae) resources were equally utilised by both invasive crayfish species. The predators showed that they have the capacity to exert substantial predatory pressure on these aquatic resources, as they were able to consume up to 100 live prey during the experimental period. Had more prey been provided (i.e. > 200) there may have been higher levels of consumption, as the FR curves did not plateau, making our results a conservative estimation. For the terrestrial resource, earthworms, the predators consumed less despite the ease of capture, which was due to the size of the resource, and as a result, the FR curves plateaued under 20 prey items. These prey resources are generally abundant in the field in Africa (Griffiths et al. 2015) and there has been no documented adverse impact by native crab species, due to co-evolutionary history and predator-prey dynamics being in equilibrium. It is, however, possible that these resources that were not previously threatened

by the crabs only will be subjected to multiple predators after invasion and a change in overall predator biomass, which will lead to biodiversity loss (Madzivanzira et al. 2021; Chapter 5).

The FRR method was utilised to determine subtle differences between impact predictions between standard FR curves, which were inconclusive due to large overlaps of confidence intervals, such as with the FR experiment at high temperature, chironomid larvae as prey, and the FR experiment at low temperature with mosquito larvae as prey. Under these conditions, *C. quadricarinatus* would potentially destabilise native prey populations, as the FFR was higher than that of *P. perlatus*. The assertion is that high attack parameter values indicate high impact, while low handling parameter values also imply high impact, and thus the two parameters are combined to give the FRR, higher values of which are found in high-impact IAS (Cuthbert et al. 2019). *Cherax quadricarinatus* was also shown to exhibit high FRR on *C. gariepinus* fry (Madzivanzira et al. 2021; Chapter 5). These high FRR values for the crayfish species are of concern as they show their potential impacts on various native prey resources.

Studies to infer crayfish impacts on various prey items have only been carried out using *P. clarkii* (see South et al. 2019). *Procambarus clarkii* was shown to utilise both benthic and pelagic resources although the extent can be altered by substrate type and prey morphology or behaviour which increases the handling parameter (South et al. 2019). Results from this study show that all three of the decapods will readily consume all aquatic and terrestrial resources. Crayfish impacts will be more pronounced due to the high attack parameter and all the prey items are likely to be impacted by the invasive crayfish species. The fact that *P. perlatus* had a higher or negligible relative FR magnitude compared to the two crayfish species suggests some

degree of biotic resistance, as also shown by Madzivanzira et al. (2021; Chapter 5). The crabs are likely to interact, and share common resources, with crayfish when both species emerge to forage at night (Madzivanzira et al. 2021; Chapter 5), which can result in competition and predation of the inferior species. When these predators are in abundance, the probability of interaction and overlaps is likely to increase. The fate of the co-existence of native crabs and either of the invasive crayfish species is well described in Madzivanzira et al. (2021; Chapter 5). As crabs and crayfish will be competing for the same limiting resource, the competitively dominant species does not change and might expand its niche, and thus reduces niche space available for the other species (Madzivanzira et al. 2021; Chapter 5) e.g. in Lake Naivasha, Kenya whereby *P. clarkii* exhibited a pattern of niche breadth reduction when found in sympatry with *P. loveni*, where they affected leaf litter breakdown due to direct consumption (Jackson et al. 2016).

Using comparative FR experiments, invasiveness and ecological impacts of alien crayfish species were predicted, providing a convenient way to predict the interactions between alien consumers and native resources. Our simulations enhance our confidence in the use of FR and FRR as an effective tool in predicting the impact of invasive crayfish species. The crayfish species have the potential to negatively impact benthic, pelagic and terrestrial prey resources due to high FR and high FRR values, especially at higher temperatures. This will disrupt food webs at certain trophic levels, which will destabilise ecosystem structures and, potentially, floodplain food web dynamics. Further to the per capita predation impacts, crayfish are likely to bring additive predatory pressure on resources that previously were not threatened by the native crabs. The results of these experiments contribute to a growing literature base on the application of comparative FRs to investigate crayfish invasion ecology (e.g. Rosewarne et al.

2016; South et al. 2019; Grimm et al. 2020; Linzmaier and Jeschke 2020; Madzivanzira et al. 2021; Chapter 5). The inclusion of population parameters and habitat associations can help increase the realism and complexity of the comparative FR models. These changes would then enable population parameter responses to be predicted, that could also account for changes in the body size of the invader. This is important given the capacity of invasive species to attain relatively large sizes compared with many native fishes (Britton et al. 2019), for example, the large sizes of crayfish to native crabs in Southern Africa (Madzivanzira et al. In Review; Chapter 4; South et al. Unpublished data). With broadened use, this predictive impact methodology would be able to move beyond its current constraints of individual FRs, providing impact predictions at a higher level of ecological complexity and realism. As the experimental setups in this study were rather shallow and devoid of natural hydrological characteristics, the results should be interpreted relative to each other rather than as a direct estimation of consumption in the invaded rivers, dams and lakes. The management application of these invasion studies is their ability to inform invasion risk assessment.

CHAPTER 7

POTENTIAL IMPACTS OF INVASIVE CRAYFISH ON AQUATIC MACROPHYTES AND AN ARTISANAL FISHERY IN AFRICA

Abstract

Quantifying the impacts of invasive species, relative to native analogues, is crucial for management and policy development. Two freshwater crayfish species, *Cherax quadricarinatus* and *Procambarus clarkii*, have established naturalised populations across southern-central Africa. Concerns are being raised on their impacts on native biodiversity as documented in other continents, however, there is a paucity of information on impacts from Africa. To fill this literature gap, this study used laboratory experiments to determine potential ecological and socioeconomic impacts conferred by the crayfish species relative to a functionally similar native analogue, the river crab *Potamonautes perlatus*, on two static but different resources. Consumption rates were derived for *Oreochromis mossambicus* and *Potamogeton nodosus* under different temperatures with maximum feeding rate used to infer impact. Scavenging on dead *O. mossambicus* was used as proxy for fish catches in artisanal gillnet fisheries, while *P. nodosus* represents ecologically important nutrient cycling macrophytes, as well as habitat for juvenile fish. Consumption of both resources by all the decapods increased with temperature. The damage by *C. quadricarinatus* on fish was significantly higher than that caused by the other two decapods, whilst *P. clarkii* had a significantly higher consumption of macrophytes than the other two decapods at all temperatures. This study demonstrated that the introductions of crayfish species can cause highly destructive ecological and socio-economic impacts to invaded systems.

Introduction

Anthropogenic activities have facilitated the occurrence and spread of invasive alien species (IAS) globally, at unprecedented rates (IPBES 2019; Meyerson et al. 2019; Seebens et al. 2020). Invasive alien species are widely recognised as drivers of negative ecological change (Ricciardi et al. 2013; Blackburn et al. 2014; Tickner et al. 2020). Invasions result in new species interactions which confer to a variety of negative effects upon indigenous populations such as: direct predation (Fritts and Rodda 1998); hybridisation (Mooney and Cleland 2001; Luquet et al. 2011); disease transfer (Prenter et al. 2004) and competition for resources (Raymond et al. 2015). Invasions also cause socio-economic impacts by affecting the different constituents of human livelihoods and well-being (security; health; material and non-material assets) (Bacher et al. 2017).

Informing policy makers and the general public on the economic losses and expenditures due to impacts of IAS appears to be a fundamental step to raise public awareness and compel policymakers to prioritise IAS management (Diagne et al. 2020a,b). Although significant progress has been made recently in collating and analysing information on economic data associated with IAS (e.g. InvaCost (Diagne et al. 2020a)), most of the studies attempting to broadly quantify IAS impacts have so far individually focused on either a few model taxonomic groups (often at species level) (Bradshaw et al. 2016), or specific economic sectors (Paini et al. 2016) and/or restricted spatial scales (local or regional) (Hoffmann et al. 2016). Many regions (such as Africa), however, still lag behind in basic assessment and quantification of economic impacts associated with many IAS. Invasion costs in Africa have only been estimated in South Africa ($1 billion per year for all IAS) (van Wilgen et al. 2020); this is also likely to be a conservative estimate due to lack of cohesive reporting protocols. Economic losses and reductions in ecosystem services have serious implications for human wellbeing, especially in

contexts where socioeconomic status is disparate and many of the population directly depend on ecosystem services for their livelihoods (Shackleton et al. 2019a).

Freshwater crayfish are among the most notorious and destructive IAS (Gherardi 2007; Lodge et al. 2012). Crayfish invasions in Africa, their distribution and spread have been described in Madzivanzira et al. (2020) (Chapter 2). Two of the five crayfish species established in Africa: Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) and Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852) are spreading rapidly on the continent (Madzivanzira et al. 2020; Chapter 2). The ecology of these two invasive species is described in detail in Chapter 1 and the current spread of *C. quadricarinatus* is described in Chapter 4. Considering the damaging invasion history of crayfish species, there is alarmingly little current research on the possible impacts of crayfish invasions in Africa (Madzivanzira et al. 2020; Chapter 2). In Africa, documented impacts of crayfish include decline in macrophytes, predation on other invertebrates and damage to fishermen gear and fish, however most are anecdotal and have not been quantified in a meaningful manner (Madzivanzira et al. 2020; Chapter 2, but see Chapter 5 both Functional Response results), hence the requirement for empirical studies.

Aquatic macrophytes are ecosystem engineers as they can alter the water and sediment physico-chemistry as well as influence the cycling of nutrients (Yu et al. 2019). Macrophytes are also crucial in food webs as they are consumed by vertebrates and invertebrates in the form of leaves as well as detritus (Gomes et al. 2012; Yu et al. 2019). Crayfish diets can comprise high amounts of macrophytic content (de Moor 2002; Gherardi and Barbaresi 2007). In Lake Kariba, Zimbabwe, macrophytes comprised 57% and 64 – 97% of all *C. quadricarinatus* size classes'

diet with respect to stable isotope analysis (SIA) and frequency of occurrence, respectively (Marufu et al. 2018b). In Lake Naivasha, Kenya, food items in *P. clarkii* guts were predominantly plant material and approximately 60% of adult crayfish had empty stomachs where macrophytes were absent (Smart et al. 2002). According to SIA results, macrophytes comprised 17% of the diet of adult *P. clarkii* in Lake Naivasha (Grey and Jackson 2012). The introduction of *P. clarkii* into Lake Naivasha also coincided with notable declines in the water lily *Nymphaea nouchalii var. caerulea* suggesting consumptive impacts on this macrophyte (Lowery and Mendes 1977). Submerged, floating and emergent macrophytes are also a spawning habitat, a place where fish nests are constructed, and serve as nurseries for initial life stages and juveniles, and valuable refugia for the survival and population growth of prey species such as plankton, invertebrates, and juvenile fish as macrophyte stands increase habitat complexity (Gomes et al. 2012; Choi and Kim 2020). As such, macrophyte biomass can indirectly promote fisheries recruitment (Pouilly and Rodríguez 2004).

Macrophyte and leaf litter breakdown is a critical step in transferring energy and nutrients from basal resources to higher trophic levels (Gomes et al. 2012; Choi and Kim 2020). The invasion by crayfish in Africa is likely to accelerate macrophytes and leaf litter breakdown, a role played by freshwater crabs of the Potamonautes genus (Jackson et al. 2016), which are predicted to be negatively impacted as a result of crayfish invasion (de Moor 2002; Jackson et al. 2016; Nunes et al. 2016). Potamonautid crabs, like crayfish, are omnivores, scavengers and are nocturnal (Dobson 2004), but are not vastly abundant in southern Africa (South et al. Unpublished data). Despite the similarities between crayfish and crabs, experimental studies suggest that replacement of the native crabs by invasive crayfish will considerably alter key ecosystem services such as fishery production and water quality (Jackson et al. 2016). This was supported by the findings in Madzivanzira et al. (2021; Chapter 5) where the consumer resource dynamics

between invasive crayfish and the widely distributed freshwater crab in South Africa, *Potamonautes perlatus*, were compared using a benthic resource as prey. The per capita magnitudes of two invasive crayfish were enhanced at high temperatures compared to low temperatures and *P. perlatus* experienced a thermally-induced dampening of per capita consumption at the high temperatures. This confirms that the crayfish species have the potential to exert greater per capita impacts on benthic prey communities and are in competition with crabs in invaded systems (Madzivanzira et al. 2021; Chapter 5). Further, 28% of the 94 non-data deficient potamonautid crabs are currently assessed as being at risk of extinction in African freshwater systems due to their narrow range and exposure to a variety of anthropogenic stressors (Cumberlidge 2011).

Invasive crayfish have also been shown to affect the livelihoods of communities across many invaded regions (Lodge et al. 2012; Madzivanzira et al. 2020; Chapter 2). Artisanal fishermen have reported anecdotally that crayfish affect their catches through partial consumption of fish caught on static gillnets (Weyl et al. 2017; Madzivanzira et al. 2020; Chapter 2). Fish makes a vital contribution to the food and nutritional security of over 200 million Africans and provides income for over 10 million (Isaacs and Witbooi 2019). In the Kafue area in Zambia, people earn between 40 – 77% of their income from fishing (Ngoma 2010; Aquatic Ecosystem Services and WWF 2020) and about 63% of their animal protein consumption from fishing (Ngoma 2010). Artisanal fisheries typically use beach seines, open water seines, gill nets, traps and longlines (Weyl et al. 2010; Deines et al. 2013; Aquatic Ecosystem Services and WWF 2020). Impact on artisanal fisheries by crayfish is incurred as the crayfish are attracted to any dead fish caught in the net and will partially consume the catch, while simultaneously spoiling the value of the catch (Weyl et al. 2017) (Plate 7a). In doing so, the crayfish often become entangled in fishing gear which decreases their fishing efficiency (Weyl et al. 2017) (see plate

7a). The nets entangled with crayfish are at times either discarded or simply abandoned at the site (Weyl et al. 2017; TC Madzivanzira and J South pers. obs. 2019 (Plate 7b)). Fish partially consumed are not marketable as potential buyers consider the fish to be spoilt (pers. obs). Although fishers use the spoiled fish for home consumption, the value is not the same (BR Ellender WWF Zambia, pers. comm. 2021). Owing to the significant contribution which fishery contributes to livelihoods as a source of protein, income, or supplementary income (Aquatic Ecosystem Services and WWF 2020), the losses attributed to crayfish are of concern.

Plate 7a. Catch spoilage by *C. quadricarinatus* in Kafue River, Zambia (Photo: Bruce R Ellender, 2018).

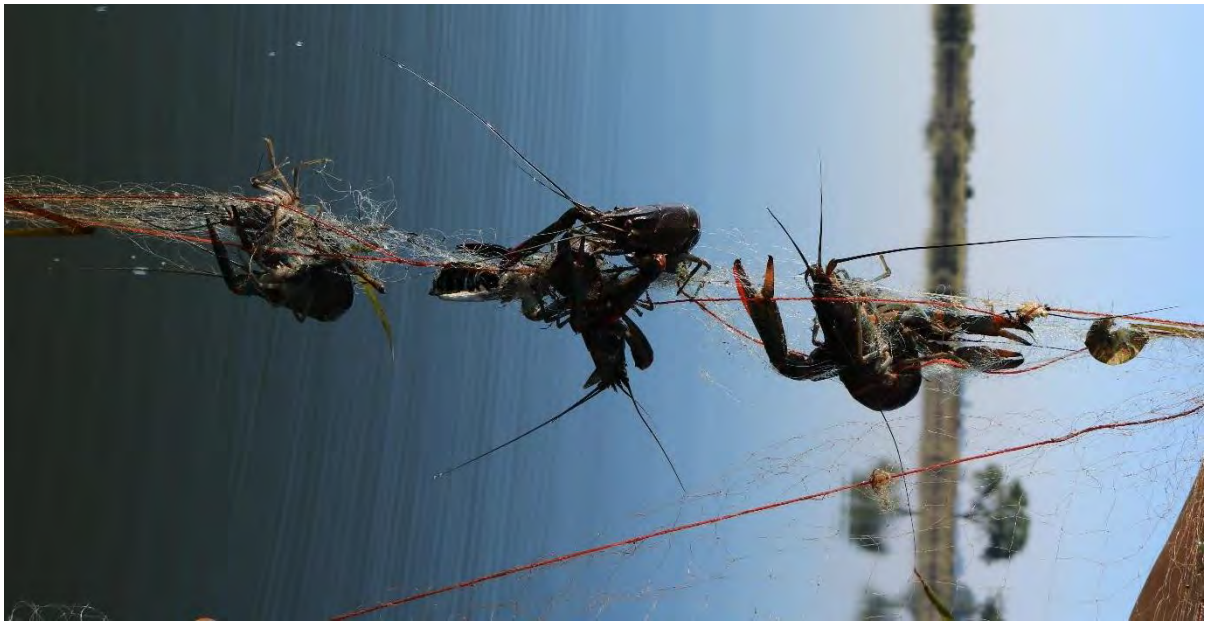

Plate 7b. Damage to nets by *C. quadricarinatus* in Kafue River, Zambia (Photo: Bruce R Ellender, 2018).

The IUCN adopted protocol for assessing ecological impact [Environmental Impact Classification for Invasive Species (EICAT)] relies upon previously documented ecological impacts (Hawkins et al. 2015). Management actions are thus based on their invasion history and impacts documented elsewhere (Ricciardi et al. 2013), however this precludes the speculative assessment of novel or potential invaders (Laverty et al. 2017). Documenting field impact can often take a long time and many resources, therefore, to rapidly infer impact of IAS, various consumption rate experiments may be carried out in the laboratory (but see Griffen et al. 2020 on the limitation of these methods). These methods have also allowed for the incorporation of context-dependencies, which were once viewed as an impediment to robust impact prediction, for example, how differential impacts of IAS and native species on native resources are likely to change under altered conditions (e.g. temperature) (see Dickey et al. 2020 for review).

To quantify the ecological and socioeconomic impacts caused by the invasive crayfish species relative to a native analogue, this study attempts to estimate the consumption of two static but different resources; dead fish Mozambiaque tilapia *Oreochromis mossambicus* (Peters 1852) and long-leaved pondweed *Potamogeton nodosus* (Poir). *Oreochromis mossambicus* is native to eastward flowing rivers of central and southern Africa (Skelton 2001). The fish species, together with other *Oreochromis* species, is commonly referred to as 'breams' in the Zambezi Basin, and comprises more than 50% of fishers' catch (Tran et al. 2019). *Potamogeton nodosus* is a heterophyllous monocotyledonous aquatic plant with both floating and submerged leaves (Ryan 1985), present in most freshwater systems in Africa (Kaplan and Symoens 2005). *Cherax quadricarinatus* and *P. clarkii* will be used in comparison with a functionally native analogue, the Cape River crab *Potamonautes perlatus* (Edwards 1837) (see Chapter 5 and 6 regarding comparative benefit of using African freshwater crabs). It is difficult to quantify socioeconomic and ecological impacts of crayfish in the field and hence the use of controlled experiments which could predict patterns that should be seen in the field if the hypotheses are correct. As temperature affects resource utilisation (Niimi and Beamish 1974; Zweifel et al. 1999; South et al. 2017, 2018; Khosa et al. 2020; Madzivanzira et al. 2021; Chapter 5), the consumption rates of macrophytes as well as scavenging and damage on fish at two selected temperatures, representative of field conditions will be tested. The hypothesis was that: invasive crayfish species will have higher maximum feeding rates at both temperatures and thus have higher negative ecological and socio-economic impacts than the native crab.

Materials and Methods

Animal collection and maintenance

The animals used in Chapter 5 and 6 were rested for at least a month before being reused for experiments in this chapter. Animals were maintained in species specific and sex specific

holding tanks (60 L) with constantly filtered and dechlorinated tap water, which was periodically replaced to maintain good water quality. Specimens were acclimated to lab conditions in the holding tanks for at least 1 month prior to experimentation. Water temperature was maintained at 22 ± 1 °C and the laboratory was held under a 12:12 light:dark regime with white light and total darkness. Crayfish and crabs are omnivores (Geiger et al. 2005; Gherardi 2007; Reynolds and Souty-Grosset 2012) and hence all animals were maintained on cabbage leaves and cultured *Eisenia* sp. worms.

Experimental setup

All experiments were carried out in 15 L of dechlorinated tap water in 15 rectangular tanks (dimensions 550 mm × 365 mm × 205 mm) in a randomised order. Tanks had an approximate river sand substrate of 20 mm. *Cherax quadricarinatus* and *P. clarkii* perform well at temperature ranges between 23 – 31°C (Jones 2000) and 21 – 27°C (Huner and Barr 1991; Ackefors 1999), respectively. *Potamonautes perlatus* has a broad geographic distribution (Daniels 2003; Cumberlidge 2011) and is found in areas invaded by the two crayfish species in South Africa, at temperatures ranging between 18°C and 28°C. Experiments were completed at both 19°C and 28°C, which are average winter and summer temperatures in the invaded ranges in Southern Africa (see Madzivanzira et al. 2021; Chapter 5). To prevent thermal shock associated with temperature changes to attain the experimental temperatures, temperatures were decreased/increased by 1°C a day and animals were acclimated for at least 1 week at the selected temperature, before the experiment.

Macrophyte consumption experiment

Potamogeton nodosus was collected from a pond in Makhanda, South Africa (S-33.2°, E26.5°). In the lab, plant matter was rinsed thoroughly under tap water to remove any attached macroinvertebrates. Dry mass is generally regarded as the most reliable method of acquiring precise biomass measurements of aquatic macrophytes (Madsen 1993; Bickel and Perrett 2015) as it accounts for the ecological variability in the water content of the macrophytes (Bickel and Perrett 2015). Therefore, a wet–dry conversion equation was determined by drying a known mass of fresh pondweed (5, 10, 15, 20, 25. 30, 35, 40, 45 and 50 g; n = 3) in an oven at 60°C for 24 hrs. The subsequent equation was derived, where dry mass = -0.0043 + 0.1134·wet weight (Fig. 7.1) (Bickel and Perrett 2015).

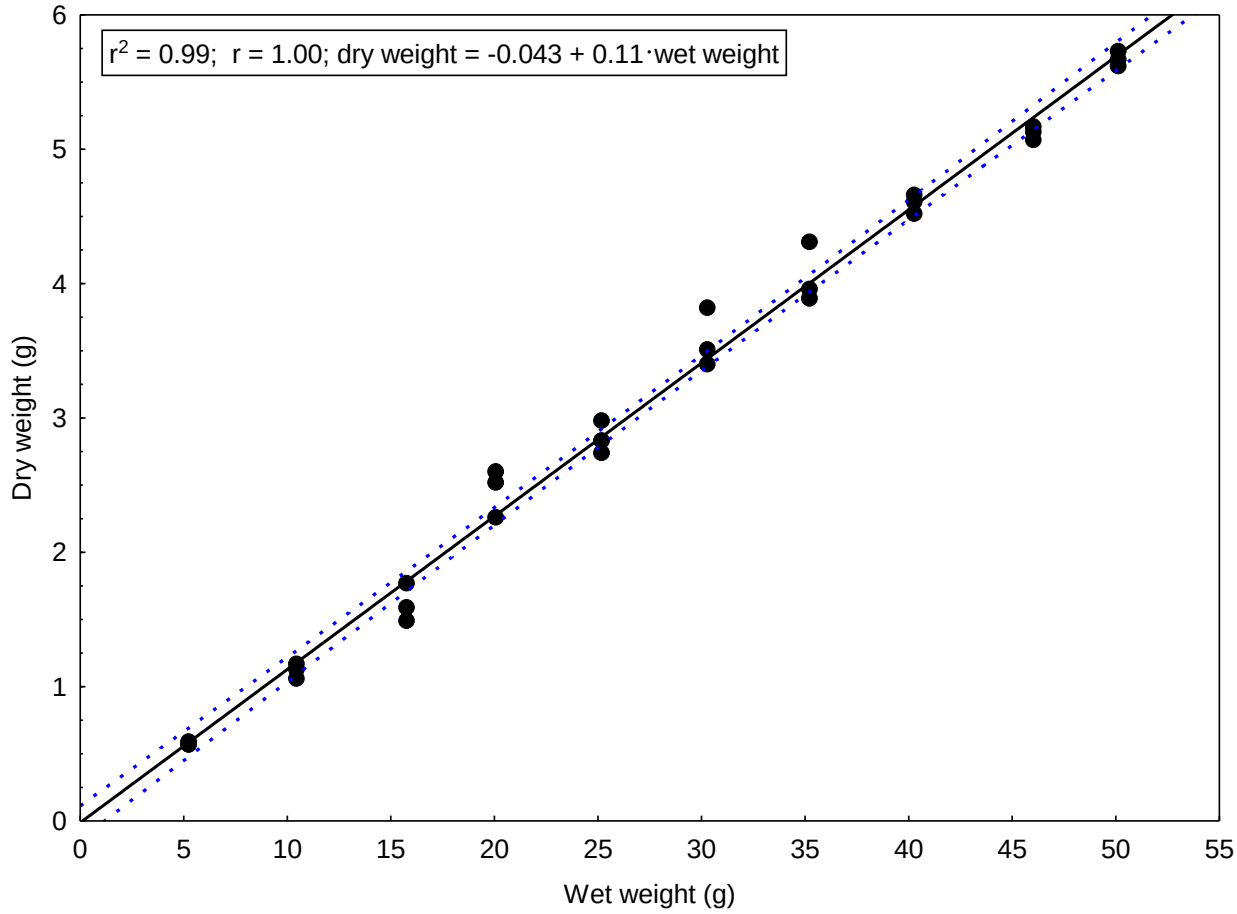

Figure 7.1. Determination of dry weight from wet weight of the pondweed *Potamogeton nodosus* using linear regression with 95% confidence intervals shown by dashed lines. Individual points indicate raw data points for each wet weight.

Prior to experimentation, the pondweed was patted dry with a paper towel and weighed, then an average of 45.65 ± 0.27 g (equivalent to 5.13 ± 0.03 g dry mass) was put into each experimental tank with an animal. These animals were randomly selected from the holding tanks and patted dry before morphometric measurements were taken. *Cherax quadricarinatus* (60.01 ± 1.31 mm mean ± SE CL; 68.83 ± 2.82 g mean ± SE mass, n = 20), *P. clarkii* (56.24 ± 1.14 mm mean ± SE CL; 59.63 ± 1.22 g mean ± SE mass, n = 20) and *P. perlatus* (53.28 ± 1.16 mm mean ± SE CL; 87.72 ± 4.92 g mean ± SE mass, n = 20) were acclimated to the

experimental tanks and deprived of food for 24 hrs before the pondweed was added. Control experiments were run at each temperature with the pondweed and no predators. The experiments were run under a 12:12 light:dark regime for 24 hrs. As this was a static resource, the max consumption per g of predator was chosen as the most informative measure, as the respective parameters from FR analysis are somewhat less meaningful, and this allows for quantification of the maximum feeding rate (i.e. the constituent of the RIP equation) per g of predator. Therefore, for the aims of this chapter, only maximum feeding rate (i.e. veracity) is determined in order to extrapolate results to field data. After the experiment, the remaining macrophytes were patted dry, weighed and dried in an oven to determine the dry weight.

Fish consumption experiment

In this experiment, dead *Oreochromis mossambicus* (160.65 ± 1.26 mm, mean total length ± SE, 74.54 ± 1.59 g mean mass ± SE) were purchased from Aquaculture Innovations in Makhanda. Experimental fish were kept frozen and defrosted prior to experimentation. Individual animals were randomly selected from the holding tank and patted dry before morphometric measurements were taken. *Cherax quadricarinatus* (63.20 ± 1.10 mm mean ± SE carapace length; 67.34 ± 2.52 g mean ± SE mass, n = 20), *P. clarkii* (58.62 ± 1.53 mm mean ± SE CL; 59.54 ± 1.58 g mean ± SE mass, n = 20) and *P. perlatus* (53.27 ± 1.02 mm mean ± SE CL; 96.29 ± 4.95 g mean ± SE mass, n = 20) were deprived of food for 24 hrs prior to experimentation, and were allowed to acclimate to experimental arenas for 1 h before the fish were supplied.

Fish were patted dry and the total length and mass for each fish was recorded. A 50 g sinker was then inserted in their guts through the mouth (Plate 7c) so that the fish sank to the bottom.

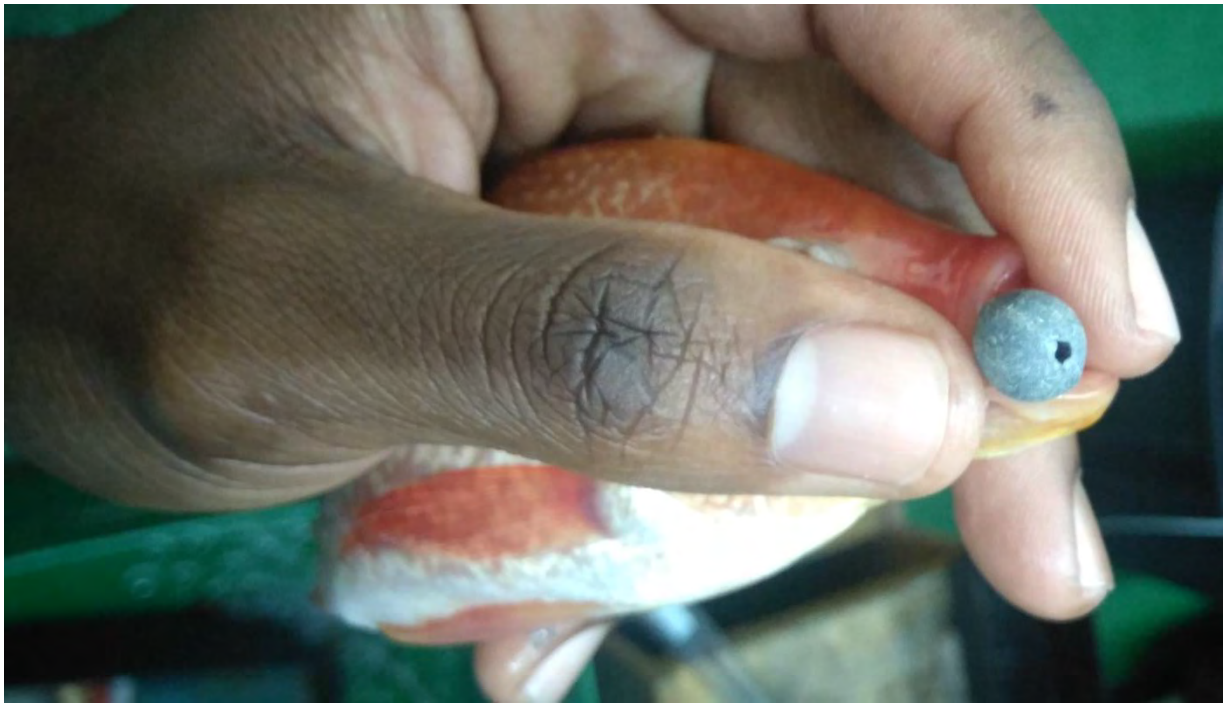

Plate 7c. A 50 g sinker being inserted in the gut of dead *Oreochromis mossambicus* for the scavenging experiments. (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

The fish were then introduced to the tanks with a predator in each tank. Controls were also run where fish were placed in tanks without predators. Fishermen in the Zambezi system deploy their gillnets around 1600 hrs and retrieve them around 0600 hrs (pers. obs.). To simulate these conditions, experiments were run over the same day/night period in the dark from 1600 hrs and terminated at 0600 hrs. At the end of the experiment, crayfish were removed and placed in respective holding tanks. The remains of the fish were removed from the water and placed in a tray with blotting paper to remove excess water. The sinkers were removed from the fish. The fish were patted dry and the mass was recorded as well as the parts that were eaten. The parts of fish damaged by the decapods were expressed as the proportion (%) of fish with damage '*i*' where '*i*' is the area (mouth, eyes, abdomen, fin, gut) damaged by the predator. As it was

possible for one fish to have several parts damaged, a single fish could have multiple damage categories.

Data analysis

Morphometric data for predators were square root transformed, and differences in weight and carapace length between species were determined using one-way ANOVA and Dunn test post-hoc. We used dry mass as a benchmark to gauge the accuracy of macrophyte wet mass measurements (Bickel and Perrett 2015) and the dry mass was used for all macrophyte-associated analyses. In order to compare consumption rates between species we calculated the mass of resource consumed per gram of decapod per hour from equation 9 to have all values in $mass^{-1}$ g^{-1} h^{-1} (reported as consumption in this chapter):

$$Mass^{-1} \cdot g^{-1} \cdot h^{-1} = (N_e/Mass)/T \quad (6)$$

Where N_e is the dry/wet weight of resource; Mass is the mass of individual; and T = experimental duration.

A t-test was used to test for loss in mass of resource before and after the experiment in the absence of a decapod for the control treatment. As resources were presented separately, and dry mass of plant matter used was compared to wet mass of fish, two separate generalised linear models (GLM) were used to assess, 1) macrophyte consumption using temperature and species as factors and determining their interaction, and 2) scavenging of fish using temperature and scavenger as factors and determining their interaction. Differences between factor levels were assessed using a Dunn-test post-hoc.

The parts of fish damaged by the decapods were analysed with 3 × 7 contingency tables, and differences were tested with a Chi-square test.

Results

The CL of the decapods used for the experiments differed significantly ($F_{(2, 117)} = 16.77$, $p < 0.001$), where *P. perlatus* and *P. clarkii* had a mean CL which was significantly lower than *C. quadricarinatus* ($p < 0.001$). With respect to mass, there were significant differences between the three decapods ($F_{(2, 117)} = 43.47$, $p < 0.001$), where *P. perlatus* weighed more than the two crayfish species ($p < 0.001$).

There was no significant change in resource mass ($p > 0.05$) before and after each control experiment, at either of the temperatures, and therefore all decreases in resource mass were deemed to be due to consumption by the three decapods.

Macrophytes consumption experiment

Species interacted significantly ($p < 0.001$) with temperature on the consumption rate of macrophyte *P. nodosus* (Table 7.1) and consumption of all the three species was significantly higher at 28°C than at 19°C (Table 7.2). The mean consumption (per hour per gram) of *P. clarkii* was significantly higher ($p < 0.05$) than that for *C. quadricarinatus* and *P. perlatus* at all temperatures (Fig. 7.2).

Table 7.1. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in macrophytes consumption with regards to factors '*temperature*' and '*species*', using a Type 3 ANOVA and χ^2 to report the effects.

Model term	Chi- Square	df	*p*-value
Temperature	64.64	1	< 0.001
Species	37.57	2	< 0.001
Temperature × Species	79.37	1	< 0.001

Table 7.2. Mean (±SE) consumption of macrophyte *Potamogeton nodosus* (in 24 hrs) by *Cherax quadricarinatus*, *Procambarus clarkii* and *Potamonautes perlatus* at 19°C and 28°C.

Species	Temperature (°C)	Wet mass	Dry mass
C. quadricarinatus	19	4.88 ± 0.62	0.55 ± 0.07
C. quadricarinatus	25	9.08 ± 0.62	1.02 ± 0.07
P. clarkii	19	7.29 ± 0.41	0.82 ± 0.05
P. clarkii	25	11.48 ± 0.41	1.29 ± 0.05
P. perlatus	19	3.59 ± 0.59	0.40 ± 0.07
P. perlatus	25	7.79 ± 0.59	0.87 ± 0.07

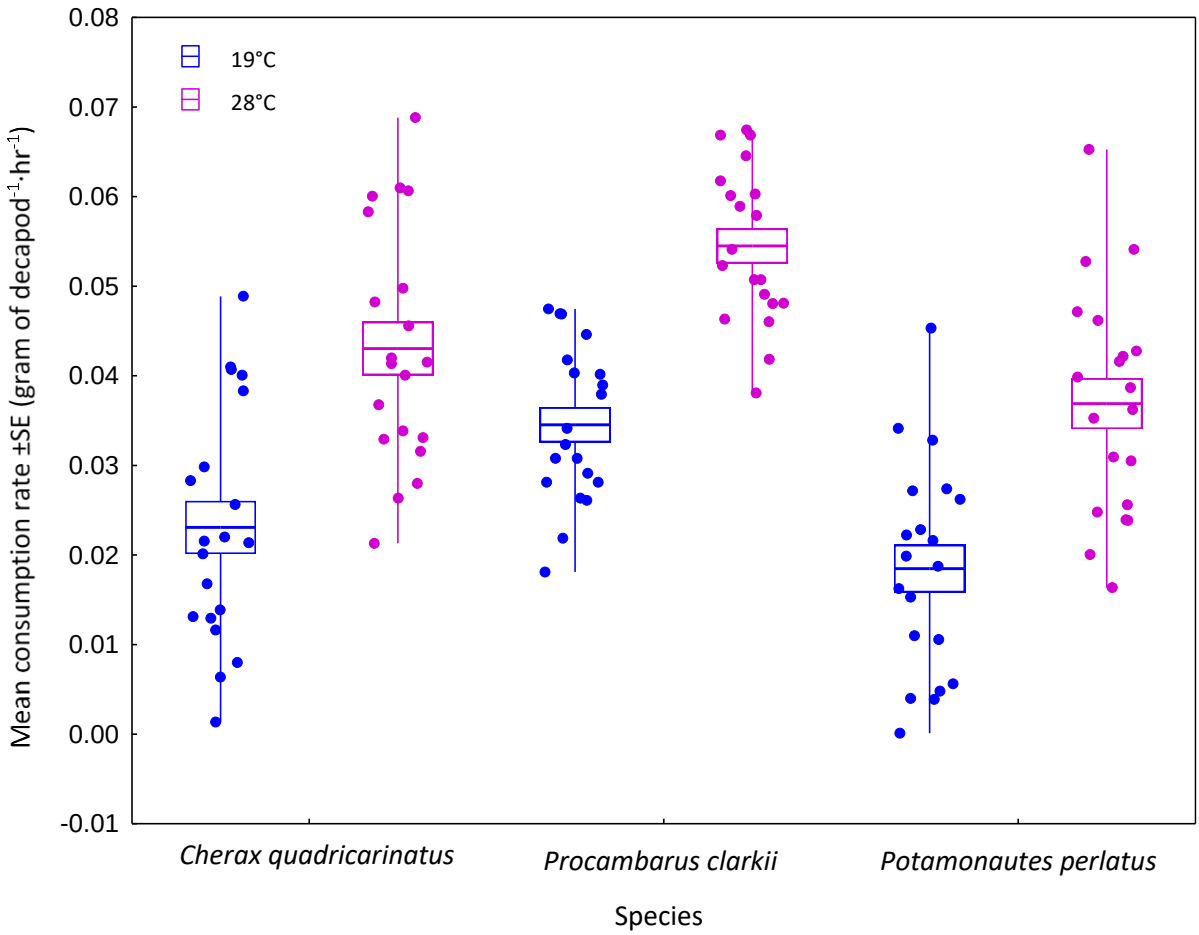

Figure 7.2. Mean consumption of macrophyte *(Potamogeton nodosus)* per hour per gram of *Cherax quadricarinatus*, *Procambarus clarkii* and *Potamonautes perlatus* at 19°C and 28°C. Points indicate raw data values, boxplots indicate ± Standard Error and solid lines on the box represents the inter-quartile range.

Fish scavenging experiment

Species, temperature and their interaction had a significant effect ($p < 0.001$) on the scavenging of *O. mossambicus* (Table 7.3) and consumption of all three species was significantly higher ($p < 0.001$) at 28°C than at 19°C. The mean consumption rate of *C. quadricarinatus* was

significantly higher (all $p < 0.05$) than that for *P. clarkii* and *P. perlatus* at all temperatures (Fig. 7.3) with no difference between ($p > 0.05$) *P. clarkii* and *P. perlatus*.

Table 7.3. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in scavenging with regards to factors '*temperature*' and '*species*', using a Type 3 Anova and χ^2 to report the effects.

Model term	Chi- Square	df	*p*-value
Temperature	85.11	1	< 0.001
Species	114.42	2	< 0.001
Temperature × Species	143.18	1	< 0.001

All the three decapods caused aesthetic damage to the fish through consumption (See Plate 7d – f). Each scavenger caused significantly different damage to different areas of *O. mossambicus* ($\chi^2 = 152.68$, df = 12, $p < 0.001$). The two crayfish species mostly damaged the tail, abdomen and the fins (Proportion > 80%) whilst the crab damaged mostly the head (Proportion = 100%) (Table 7.4; Plate 7g).

Table 7.4. Proportion of fish with different categories of damage *i*.

Species	Temperature (°C)	Tail	Abdomen	Fin	Guts	Mouth	Head	Eyes
C. quadricarinatus	19	20	19	19	0	0	0	1
P. clarkii	19	20	20	20	1	0	0	0
P. perlatus	19	1	4	0	0	20	20	20
C. quadricarinatus	28	20	17	17	3	4	0	1
P. clarkii	28	20	20	20	1	0	0	0
P. perlatus	28	0	4	0	1	20	20	20

Plate 7d. Experimental catch spoilage by *Cherax quadricarinatus*. (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

Plate 7e. Experimental catch spoilage by *Procambarus clarkii*. (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

Plate 7f. Experimental catch spoilage by *Potamonautes perlatus*. (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

Plate 7g. *Potamonautes perlatus* damaging mostly the head of *Oreochromis mossambicus*. (Photo: Takudzwa C Madzivanzira taken from SAIAB biosecure controlled environment laboratory, 2019).

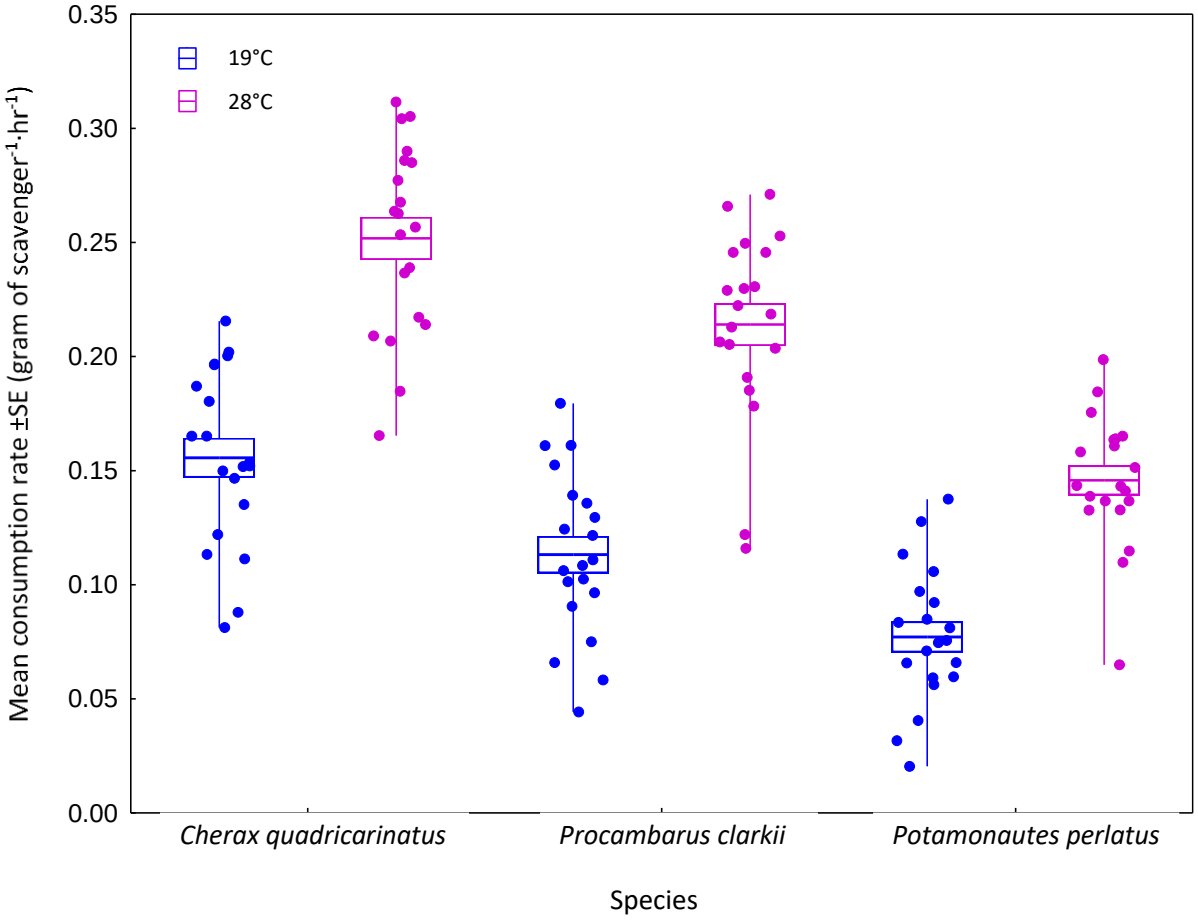

Figure 7.3. Mean consumption of fish (*Oreochromis mossambicus*) per hour per gram of *Cherax quadricarinatus*, *Procambarus clarkii* and *Potamonautes perlatus* at 19°C and 28°C. Points indicate raw data values, boxplots indicate 95% Confidence Interval and solid lines on the box represents the inter-quartile range.

Discussion

High direct consumption of native resources, relative to that of a native analogue, is indicative of high impact IAS according to the Resource Consumption Hypothesis (Bollache et al. 2008, Dick et al. 2013, Ricciardi et al. 2013, Paterson et al. 2015; Laverty et al. 2017; Dick et al. 2017a). The results from this study support the hypothesis that the two crayfish species had high consumption rates and caused greater damage on both a plant and an animal resource in

comparison to the native crabs. There were, however, differences in performance between the two invasive species according to resource type.

All the three decapods consumed *P. nodosus,* with *P. clarkii* having the highest consumption rates. The adverse impacts of *P. clarkii* on aquatic macrophytes globally are well documented (see Matsuzaki et al. 2009; Lodge et al. 2012; Twardochleb et al. 2013; Loureiro et al. 2015; Madzivanzira et al. 2020; Chapter 2). *Procambarus clarkii* strongly prefers macrophytes even in the presence of an alternative immobilised animal prey despite the assimilation efficiency and higher nutritional value of the latter (Gherardi and Barbaresi 2007). *Procambarus clarkii* has been implicated as being one of the world's worst invasive species, however, it is also one of the most well studied species (Twardochleb et al. 2013) unlike *C. quadricarinatus* and *P. perlatus* which are data deficient (Cumberlidge and Daniels 2007; Souty-Grosset 2016; Weyl et al. 2020). The high consumption rate of macrophytes by *P. clarkii* compared to the other two species as recorded in this study could be related to chelae morphology, as *P. clarkii* has thin chelae, with low closing force (South et al. 2020) and their gastric mill (Chisaka and Kozawa 2003; McGaw and Curtis 2013) may specialise them for processing plant matter over other resources. Additionally, *P. clarkii* uses chelipeds to destroy more macrophyte tissues than they consume (Nyström and Strand 1996) and to cut plant stems, and therefore the macrophytes are unlikely to remain connected to their root systems in the presence of crayfish (Smart et al. 2002). Harper et al. (1990) and Hofkin et al. (1991) strongly implicated this feeding strategy as the main cause of the decline in abundance of the blue water lily *Nymphaea nouchalii var. caerulea* in Lake Naivasha, Kenya. While this impact is already detrimental to native plant stands, it also could facilitate invasion by other IAS by spreading plant matter throughout a system, which increases propagule pressure. As *C. quadricarinatus* is an emerging invader in various regions, documented impacts on macrophytes and other biotic components are sparse.

The only impact of *C. quadricarinatus* on macrophytes was recorded in the Pilbara region of Australia whereby its introduction resulted in the complete loss of macrophyte cover (Pinder et al. 2019). This could be circumstantial, but the observation is not surprising as macrophytes were shown to dominate the diet of *C. quadricarinatus* in Lake Kariba, Zimbabwe (Marufu et al. 2018b). In most African countries there is no monitoring of ecological systems, which makes pre-invasion data unavailable and hence, impacts by IAS often go unnoticed.

Although all the species used in this study are omnivores and are shredder species that can cause impact on native resources, it is the relative difference in damage that is important and hence, the comparison with a native crab was essential. Scavenging by crabs occurs throughout their range, although this has not been reported as causing any appreciable impacts. The abundance of crabs is, however, relatively low in Southern Africa (South et al. Unpublished data), therefore while they will incur some impact, it is the relative difference that can be used as a baseline to compare native impacts with crayfish invasions. This, however, emphasises the need to take contextually relevant field abundance patterns into account when inferring magnitude impacts (Dick et al. 2017b; Zeng et al. 2019).

In the fish consumption experiment, the two crayfish species damaged mostly the posterior parts of the fish whilst the crabs damaged mostly the anterior parts (Plate 7d – 7g). The differences in the damaged parts of fish could be related to the differences in chelae strength between the scavengers. *Potamounates perlatus* was shown to exhibit higher chelae strength than both the focal crayfish species, albeit related to sex and mass, (South et al. 2020). This may facilitate the higher amount of damage to the anterior parts of the fish, which are tougher than the soft parts (abdomen and guts) which were more likely to be damaged by the crayfish

species. The fish head damaged by the crabs contains the bulk of the nutrients compared to other body parts of the fish (Petricorena 2014). The damage to fish was however, more pronounced from *C. quadricarinatus*, which significantly consumed more than the other scavengers. This result corroborates with reports from fishers in regions that have been invaded by *C. quadricarinatus* (e.g. Kafue River, Zambia and Lake Kariba, Zimbabwe). The crayfish partially consume fish caught on gillnets, making the fish unmarketable. Losses incurred have not been quantified, which is a cause for concern as fishers could be losing a significant amount of catch and income due to catch spoilage by *C. quadricarinatus*. The socioeconomic losses therefore need to be calculated, as the impacts will be clearer if framed in an economic perspective (Diagne et al. 2020a).

An increase in temperature should theoretically increase foraging demands in poikilotherms due to an amplified metabolic rate (Gilbert et al. 2014). In this study, consumption of both resources by all three decapods was significantly higher under the high temperature than the low temperature treatment. This is because temperature drives metabolism (Brown et al. 2004), locomotion (Dell et al. 2011, 2014) and rate of resource consumption (Uiterwaal and DeLong 2020). The increase in consumption by the two crayfish species corroborates with functional response (FR) results found by Madzivanzira et al. (2021; Chapter 5) whereby temperature increases resulted in increased maximum feeding rates of *C. quadricarinatus* and *P. clarkii* on *Clarias gariepinus* prey. Introduced crayfish were also shown to exhibit higher FRs and generalist feeding behaviours than natives in other studies (Haddaway et al. 2012). However, contrary to this study, *P. perlatus* showed decreased maximum feeding rates on *C. gariepinus* prey at higher temperatures (Madzivanzira et al. 2021; Chapter 5). The resource used in this study was static and therefore could not escape consumption and hence the high maximum feeding rate of *P. perlatus* (compared with Madzivanzira et al. 2021; Chapter 5) at the higher

temperatures was driven solely by the species' metabolic requirements. Global annual mean temperatures are projected to increase by 1.5°C between 2030 and 2052 if temperatures continue to increase at the current rate (IPCC 2018). The impact of crayfish species in invaded systems will likely increase with the projected climatic changes, as demonstrated in this study. The warming of regions such as the Barotse floodplain is likely to affect artisanal fishery, especially during the summer season when water recedes (see Madzivanzira et al. In Review: Chapter 4) which therefore increases both the likelihood of catch in gillnets as well as decreasing predator free space, which will in turn increase scavenging risk. These impacts are also enhanced by warming, which increases consumption rates (assumption based on results from Madzivanzira et al. (2021; Chapter 5) and this study).

Through lab experiments, this study demonstrated the socio-ecological impacts that the introductions of crayfish species confer to the invaded systems. The crayfish species consumed both resources at a higher rate than the native crabs. These differences in consumption rates could facilitate niche differentiation between the invasive decapods if they should occur in sympatry (see South et al. 2020), given the many translocations of both crayfishes around Africa and indeed Europe, with *P. clarkii* utilising mainly macrophytes. Nonetheless, results from this study, irrespective of temperature, corroborate with literature which identify introduced crayfish as highly damaging species (Gherardi 2007; Lodge et al. 2012; Twadorchleb et al. 2013; Loureiro et al. 2015; Stebbing 2016; South et al. 2019). These irreversible impacts are likely to persist and worsen, especially considering the low level of conservation management resources in many African countries. Results from this study should be combined with regional abundances of these decapods to determine the Relative Impact Potential (RIP) metric, which has been successfully used in predicting the likelihood and degree of impact caused by IAS (Dick et al. 2017b; Dickey et al. 2020). The value of the RIP

metric is in its consideration of species abundances (Dick et al. 2017b; Dickey et al. 2020), and in Southern Africa, the abundance of crayfish is significantly higher than that of native crabs (Madzivanzira et al. In Review; Chapter 4; South et al. Unpublished data; Barkhuizen et al. Unpublished data). Thermal dependency on abundance, physiology and environmental stochasticity is likely to pressurise ecosystem services in invaded regions. The pressing issue of unhindered crayfish invasions, especially in Africa, needs to be prioritised, as the food security of livelihoods in invaded regions will be affected. There is a need to investigate whether results from this study translate to actual declines in catches through fish catch assessments and value chain analysis, and also by taking abundance patterns into consideration. Respective countries should implement robust regulations, channel resources to conservation and work with communities as well as other countries, especially on shared aquatic systems, in order to implement transboundary mitigation measures to limit further spread.

CHAPTER 8

THE RELATIVE IMPACT POTENTIAL AND SOCIOECONOMIC IMPACTS OF INTRODUCED CRAYFISH SPECIES IN AFRICA

Abstract

The establishment of introduced crayfish species in Africa is a cause for concern owing to their documented impacts and invasion history outside the continent. Functional response (Chapters 5 and 6) and consumption (Chapter 7) experiments have been conducted to infer impact of two invasive crayfish species, namely, the Australian redclaw crayfish *Cherax quadricarinatus* and Louisiana red swamp crayfish *Procambarus clarkii* in Africa. Given the importance of abundances in discernments of the overall offtake rates by consumers, basing impact prediction on only FRs limits the estimation of field impact of abundances. Recently, the relative impact potential (RIP) metric was developed to predict the ecological impacts of invasive alien species, which compares the FRs of invader and native species and incorporates the abundances of both invader and existing similar native species. This study utilised this new metric to calculate the RIP of the two introduced crayfish species in Africa. By multiplying the maximum feeding rates with abundances of each species across the seasonal gradient, the RIP of the two crayfish species were consistently higher in all the invaded regions compared to that of the native crabs of the *Potamonautes* genus, which are functionally similar to crayfish. This is due to the high feeding rates of the crayfish species and their high field abundances compared to the native crabs. Temperature was shown to play a role as crayfish RIP values were higher in the summer than the winter season. This study further estimated impacts by calculating the potential socioeconomic losses that are due to catch spoilage by *C. quadricarinatus*, using data from the heavily invaded Kafue River Basin in Zambia and spoilage rates from the results documented in Chapter 7. The calculations from this study showed that *C. quadricarinatus* can

cause up to 1500 t catch losses per year, which translates to an overall potential loss of US$ 2 million per year in the Kafue Flats. The data from this study have huge conservation and management implications, as these crayfish species have the potential to threaten food security as well as incurring personal losses to fishers via damage-related cost. This warrants efforts to invest in early management and functional eradication in other invasions (such as the Barotse floodplain) to avoid larger costs in the future and to discourage their introductions, preventing further spread.

Introduction

Non-native crayfish species have impacted numerous ecosystem services well documented for Europe and North America (Holdich 1999, 2003; Gherardi 2007; Lodge et al. 2012). Information on crayfish impacts is however scant in Africa where five crayfish species have established naturalised populations (Madzivanzira et al. 2020; Chapter 2). The current status and distribution of these crayfish species are described in Chapters 2 and 4. Presently, invaders may be prioritised for management based on their invasion history and impacts documented elsewhere (Kulhanek et al. 2011; Ricciardi et al. 2013) which precludes the assessment of novel or potential invaders (Laverty et al. 2017).

Scientists and practitioners have been frustrated by a lack of predictive methodologies to reliably identify potentially damaging invaders and their likely magnitude of ecological impact (Laverty et al. 2017). Predicting which future IAS are likely to exert ecological impacts, and how such impacts are likely to change under different biotic and abiotic contexts, are vital objectives for global biodiversity conservation (Dick et al. 2017a; IPBES 2019). Methodological developments, which incorporate native/IAS resource utilisation across context-dependencies, have recently provided robust predictive tools for invasion science (Laverty et al. 2015; Dick et al. 2017a; Dickey et al. 2018; Cuthbert et al. 2019). In particular, the functional response (FR) metric quantifies resource consumption as a function of resource density, and, in a predator-prey context, can quantify per capita ecological impacts of predators towards prey of respective trophic groups (Holling 1959; Dick et al. 2013, 2014, 2017a; Alexander et al. 2014). To infer the impact of the Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) and Louisiana red swamp crayfish *Procambarus clarkii* (Girard 1852) in Africa, Chapters 5 – 7 described consumer resource dynamics using various

resources that are ubiquitous across the continent. The use of FR and consumption experiments showed the potential impacts of the two crayfish species on native resources in invaded ecosystems. However, in many cases, impact predictions of IAS are inherently limited if based on per capita (FR) impacts alone, given the importance of abundances in discernments of overall offtake rates by consumer populations (Dick et al. 2017b).

Recently, the relative impact potential (RIP) was developed by Dick et al. (2017b) to predict impacts of IAS. The metric (which is described in Chapter 1), compares invader and native species' 'maximum feeding estimates' and incorporates the abundances/biomasses of both the invader and the existing similar native species (Dick et al. 2017b; Dickey et al. 2020). The invader impact will thus be compared to the existing baseline impact of native species. The value of the RIP metric is in its consideration of species abundances/biomasses, which can be estimated in the field or may already be available from monitoring data (see Mofu et al. 2019a). It is necessary to consider the FRs (or the maximum feeding estimates), because the rate at which an IAS consumes native resources strongly influences their ecological impact. The metric was successful at associating IAS with significant impacts on native populations (Dick et al. 2017b; Dickey et al. 2020).

In recent years, much research effort has been invested in understanding the ecological impacts of IAS across ecosystems and taxonomic groups (Cuthbert et al. 2021). However, studies of detailed economic impacts of IAS are limited (Bradshaw et al. 2016) and the studies are biased towards certain taxonomic groups, communities and regions (Pimentel et al. 2000, 2005; Kettunen et al. 2009; Cuthbert et al. 2021; Haubrock et al. 2021). In Africa, information on the economic costs of IAS is scant and only documented for South Africa (US$ 1 billion per year

(van Wilgen et al. 2020). Of concern is the lack of information on the economic impacts of invasive crayfish in Africa while they are rapidly spreading in the region (Kouba et al. In Review), and also the lack of information on *C. quadricarinatus* economic impacts globally. Crayfish costs in Europe, North America and Asia have been largely attributed to damages or resource losses, with about 10% attributed to management expenditures on prevention, control or eradication. Determining cost trends in Africa would provide a useful resource for communications with policymakers and the general public (Cuthbert et al. 2021) as impacts expressed in economic terms are more tangible and comprehensible than complex ecological impacts (Diagne et al. 2020a). The existence of information on costs inferred from IAS is therefore of utmost importance; a lack thereof can incite false impressions and minimize the awareness and preoccupation regarding IAS (Kouba et al. In Review).

Considering the scarcity of literature on crayfish impacts in Africa (Madzivanzira et al. 2020), and the multi-disciplinary resources needed for economic impact assessments (Diagne et al. 2020a) the RIP metric can be used as means of rapidly assessing impacts. These results can then be translated into legislation, conservation management, and planning. This study determines the RIP of the two crayfish species in comparison to the existing baseline impact of the native crabs and hypothesises that the two crayfish species will have a greater impact on various native resources across different invaded regions in Southern Africa compared to the native crabs. Despite these advances in invasion science to confirm the ecological impacts of invasive freshwater crayfish, information on their negative economic impacts is scarce, resulting in minimal investments into research, early detection and prevention (Kouba et al. In Review). Crayfish have been implicated in the artisanal fishery catch losses in Africa

(Madzivanzira et al. 2020; Chapter 2), and this study further estimates and quantifies the economic losses in catch that the fishers experience.

Methods

Relative Impact Potential

The RIP of native (i.e. *P. perlatus*) and non-native (i.e. *C. quadricarinatus* and *P. clarkii*) species were calculated using the mean maximum feeding rate and biomass (CPUEg). Using the maximum feeding rates of the predators from Chapters 5 – 7, and taking their respective biomass data in the region from: Madzivanzira et al. (In Review; Chapter 4), South et al. (Unpublished data), Nunes et al. (2017a), Barkhuizen et al. (Unpublished data) the RIP was calculated using the formula by Dick et al. (2017b) (Equation 5 in Chapter 1).

When the RIP score is less than one, this indicates that the invader is expected to have less of an impact than similar native species; an RIP higher than one indicates that the IAS is expected to have a greater impact than similar native species. As the RIP metric increases above one, the invader impact is also predicted to increase (Dick et al. 2017b). The biomass (g/trap/night) was used because the FR experiments were run using adult individuals, and some individuals caught in the traps were juveniles, and therefore biomass was a more reasonable comparison. In cases where the biomass data were absent, (for example, in the Kafue Flats), the product of the CPUE and average mass of one crayfish was used. Using biomass instead of abundance gives a more realistic demonstration of impact for IAS (Dickey et al. 2020). The Kariba CPUE was combined with that of Kafue Flats in constructing the RIP biplots, as there is no crab data for the former, and the Kafue population is genetically similar, in part, to that of Kariba (Weyl et al. Unpublished data).

Since impacts are likely to change under different biotic and abiotic contexts (Dick et al. 2017a), the two temperature treatments used in Chapters 5 – 7 (19°C and 28°C) which represents the winter and summer seasons, respectively of the invaded ranges in Southern Africa were used to predict the impact of these crayfish species during the two seasons (see temperature data in: Nunes et al. 2017a,b,c; Madzivanzira et al. In Review; Chapter 4; Madzivanzira et al. 2021; Chapter 5).

Catch loss estimation

Scavenging data from Chapter 7 were used to calculate the potential economic loss in catch using high and low water flow catch data, (which refer more to temperature in the high and low flow seasons rather than water flow and velocity), from the predefined Kafue River strata (Aquatic Ecosystem Services and WWF Zambia Report 2020) (Table 8.1). Data used in estimating the loss in catch included: soak time for the gillnets (15 hrs); price of fish per kg; exchange rate of Zambian Kwacha (ZMW) to United States Dollar (US$); fishing effort; and number of fishing days. The consumption per gram scavenger (from Chapter 7) was used to calculate the potential loss per day, per week, per season and per year using the catch data from Aquatic Ecosystem Services and WWF Zambia Report (2020). The data of catch losses were used to calculate the income losses due to *C. quadricarinatus*. The monetary loss per day and annually was expressed in ZMW using ZMW 18.80/kg (equivalent to ≈ US$ 1.30; rate calculated from First National Bank, FNB Zambia rate of 2020) as the market price for fish (Aquatic Ecosystem Services and WWF Zambia Report 2020). The following equations were used to calculate the economic losses due to crayfish:

$$catch\ loss\ per\ day\ (15\ hrs) = \frac{crayfish\ consumption\ per\ day\ (15\ hrs) \times crayfish\ CPUE}{1000} \quad (7)$$

$$catch\ loss\ per\ week = \frac{Fish\ CPUE - catch\ loss\ per\ day}{number\ of\ days\ fished} \quad (8)$$

$$catch\ loss\ per\ season\ = catch\ loss\ per\ week\ \times season\ fishing\ days \quad (9)$$

$$monetary\ loss\ per\ season\ = catch\ loss\ per\ season\ \times price\ of\ fish\ per\ kg \quad (10)$$

The overall calculations took into account the 3-month fishing ban (December to February, ≈90 days) in the Kafue Flats.

Table 8.1. Fish and crayfish catch data from Kafue River Zambia during the low and high flow seasons (Source: Aquatic Ecosystem Services and WWF Zambia Report 2020).

Stratum	Flow season	CPUE fish (kg/net/night)	CPUE crayfish (kg/trap/night)
1	low	0.20	0.16
	high	0.10	0.07
2	low	0.90	0.04
	high	2.80	0.01
3	low	4.30	0.02
	high	2.80	0.04
4	low	2.30	0.55
	high	9.10	0.49

Results

RIP

Under both winter and summer seasons, the crayfish species consistently displayed RIP scores > 1 relative to the native crab irrespective of region (Table 8.2). The RIP scores for *C. quadricarinatus* in all the regions were consistently higher in the summer compared to the winter season (Table 8.2) and the RIP biplots concurred with the RIP scores (Figs. 8.1 – 8.6). The RIP scores for *C. quadricarinatus* from the Kafue Flats were consistently higher than for other regions in the for the summer season. In the winter season, the RIP scores for Barotse floodplain were consistently higher than that for the Inkomati Basin (Table 8.2). This high RIP scores were driven by both abundance and per capita impact and further enhanced by temperature effects.

Catch loss estimation

Using the consumption per g decapod of fish from Chapter 7, the loss per day per fisher in all the strata ranged between 0.07–2.05 kg (low water) and 0.02–1.17 kg (high-water season) (Table 8.3). This all translates to seasonal losses of 0.10–5.46 t (low flow season) and 0.34–0.76 t (high flow season) per fisher. When all the losses are combined, 11–709 t and 17–178 t of catch are likely to be lost due to crayfish in the low and high flow seasons in each stratum (Fig. 8.8). This translates to economic losses of between ZWK 1800 (US$ 130) and ZWK 103 000 (US$ 7100) per fisher per year. When all the losses are combined, a total income of between US$ 14 000 and US$ 953 000 is likely to be lost due to crayfish invasion in each stratum (Table 8.3; Fig. 8.8). This brings the total loss in the entire Kafue Flats Fishery to US$ 2 million per year. These losses are likely to be higher during the low water flow season than the high-water flow season, and higher in stratum 2 than in the other strata (Fig. 8.8).

Table 8.2. Relative Impact Potential (RIP) using mean maximum feeding rates for non-native *Cherax quadricarinatus* and *Procambarus clarkii* against native *Potamonautes perlatus* predicted using prey: sharptooth catfish fry *Clarias gariepinus*, earthworms *Eisenia* sp., common midge Chironomidae and mosquito larvae *Culex* sp.; and static resources: aquatic macrophyte *Potamogeton nodosus* and dead fish *Oreochromis mossambicus*. Field biomass data are integrated from the Barotse floodplain, Kafue River, Lake Kariba, Komati River and Mimosa Dam in the winter and summer seasons.

Species	Region	Resource	Mean FR maximum feeding invader		Mean FR maximum feeding native		Mean CPUE (g/trap/night) invader		Mean CPUE (g/trap/night) native		RIP	
			Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*C. gariepinus*	42.90[1]	42.50[1]	76.30[1]	27.50[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	2.15	4.59
C. quadricarinatus, *Potamonautes* sp.	Kafue	*C. gariepinus*	42.90[1]	42.50[1]	76.30[1]	27.50[1]	-	520.50[2]	-	9.25[2]	-	86.94
C. quadricarinatus, *Potamonautes* sp.	Kariba	*C. gariepinus*	42.90[1]	42.50[1]	76.30[1]	27.50[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Inkomati	*C. gariepinus*	42.90[1]	42.50[1]	76.30[1]	27.50[1]	6.09	115.51[4]	1.63	4.89[4]	2.10	36.48
P. clarkii, *Potamonautes* sp.	Mimosa	*C. gariepinus*	18.90 [1]	33.70[1]	76.30[1]	27.50[1]	23.24[5]	-	1.36[5]	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*Eisenia* sp.	5.45[1]	17.75[1]	12.68[1]	18.22[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	1.65	2.89
C. quadricarinatus, *Potamonautes* sp.	Kafue	*Eisenia* sp.	5.45[1]	17.75[1]	12.68[1]	18.22[1]	-	520.50[2]	-	9.25[2]	-	54.81
C. quadricarinatus, *Potamonautes* sp.	Kariba	*Eisenia* sp.	5.45[1]	17.75[1]	12.68[1]	18.22[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Inkomati	*Eisenia* sp.	5.45[1]	17.75[1]	12.68[1]	18.22[1]	6.09	115.51[4]	1.63	4.89[4]	1.61	22.99
P. clarkii, *Potamonautes* sp.	Mimosa	*Eisenia* sp.	2.58[1]	9.92[1]	12.68[1]	18.22[1]	23.24[5]	-	1.36[5]	-	3.47	-

C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*Chironomidae*	166.70[1]	190.19[1]	154.30[1]	76.30[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	4.14	7.40
C. quadricarinatus, *Potamonautes* sp.	Kafue	*Chironomidae*	166.70[1]	190.19[1]	154.30[1]	76.30[1]	-	520.50[2]	-	9.25[2]	-	140.23
C. quadricarinatus, *Potamonautes* sp.	Kariba	*Chironomidae*	166.70[1]	190.19[1]	154.30[1]	76.30[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Inkomati	*Chironomidae*	166.70[1]	190.19[1]	154.30[1]	76.30[1]	6.09	115.51[4]	1.63	4.89[4]	4.04	58.83
P. clarkii, *Potamonautes* sp.	Mimosa	*Chironomidae*	108.00[1]	100.83[1]	154.30[1]	76.30[1]	23.24[5]	-	1.36[5]	-	11.92	-
C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*Culex* sp.	200.00[1]	333.33[1]	142.86[1]	250.00[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	5.36	3.96
C. quadricarinatus, *Potamonautes* sp.	Kafue	*Culex* sp.	200.00[1]	333.33[1]	142.86[1]	250.00[1]	-	520.50[2]	-	9.25[2]	-	75.01
C. quadricarinatus, *Potamonautes* sp.	Kariba	*Culex* sp.	200.00[1]	333.33[1]	142.86[1]	250.00[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Inkomati	*Culex* sp.	200.00[1]	333.33[1]	142.86[1]	250.00[1]	6.09	115.51[4]	1.63	4.89[4]	5.23	31.47
P. clarkii, *Potamonautes* sp.	Mimosa	*Culex* sp.	111.11[1]	250.00[1]	142.86[1]	250.00[1]	23.24[5]	-	1.36[5]	-	13.24	-
C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*P. nodosus*	0.07[1]	0.13[1]	0.04[1]	0.09[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	6.63	4.27
C. quadricarinatus, *Potamonautes* sp.	Kafue	*P. nodosus*	0.07[1]	0.13[1]	0.04[1]	0.09[1]	-	520.50[2]	-	9.25[2]	-	80.99
C. quadricarinatus, *Potamonautes* sp.	Kariba	*P. nodosus*	0.07[1]	0.13[1]	0.04[1]	0.09[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *P. Potamonautes* sp.	Inkomati	*P. nodosus*	0.07[1]	0.13[1]	0.04[1]	0.09[1]	6.09	115.51[4]	1.63	4.89[4]	6.47	33.98
P. clarkii, *Potamonautes* sp.	Mimosa	*P. nodosus*	0.12 [1]	0.19[1]	0.04[1]	0.09[1]	23.24[5]	-	1.36[5]	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Barotse Floodplain	*O. mossambicus*	10.5 [1]	16.77[1]	7.59[1]	13.89[1]	48.44[2]	52.55[2]	12.65[3]	17.70[3]	-	3.58
C. quadricarinatus, *Potamonautes* sp.	Kafue	*O. mossambicus*	10.5 [1]	16.77[1]	7.59[1]	13.89[1]	-	520.50[2]	-	9.25[2]	-	67.92
C. quadricarinatus, *Potamonautes* sp.	Kariba	*O. mossambicus*	10.5 [1]	16.77[1]	7.59[1]	13.89[1]	-	261.95[2]	-	-	-	-
C. quadricarinatus, *Potamonautes* sp.	Inkomati	*O. mossambicus*	10.5 [1]	16.77[1]	7.59[1]	13.89[1]	6.09	115.51[4]	1.63	4.89[4]	-	28.50

P. clarkii, *Potamonautes* sp.	Mimosa	*O. mossambicus*	6.92 [1]	12.89[1]	7.59[1]	13.89[1]	23.24[5]	-	1.36[5]	-	-

Sources

[1]Madzivanzira et al. (In Preparation; Chapter 7)

[2]Madzivanzira et al. (In Review; Chapter 4)

[3]South et al. (Unpublished data)

[4]Nunes et al. (2017a)

[5]Barkhuizen et al. (Unpublished data)

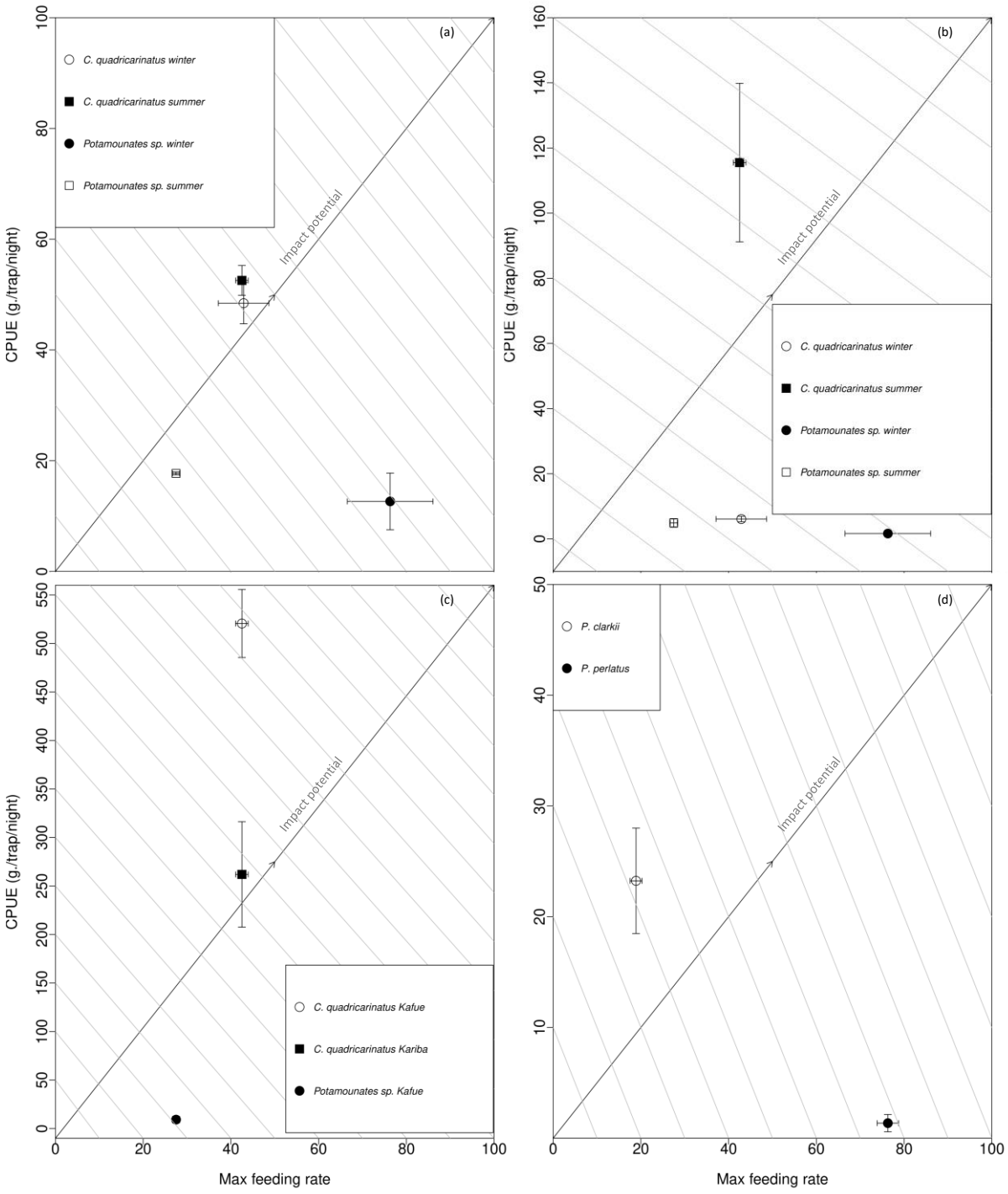

Figure 8.1. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using sharptooth catfish *Clarias gariepinus* in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

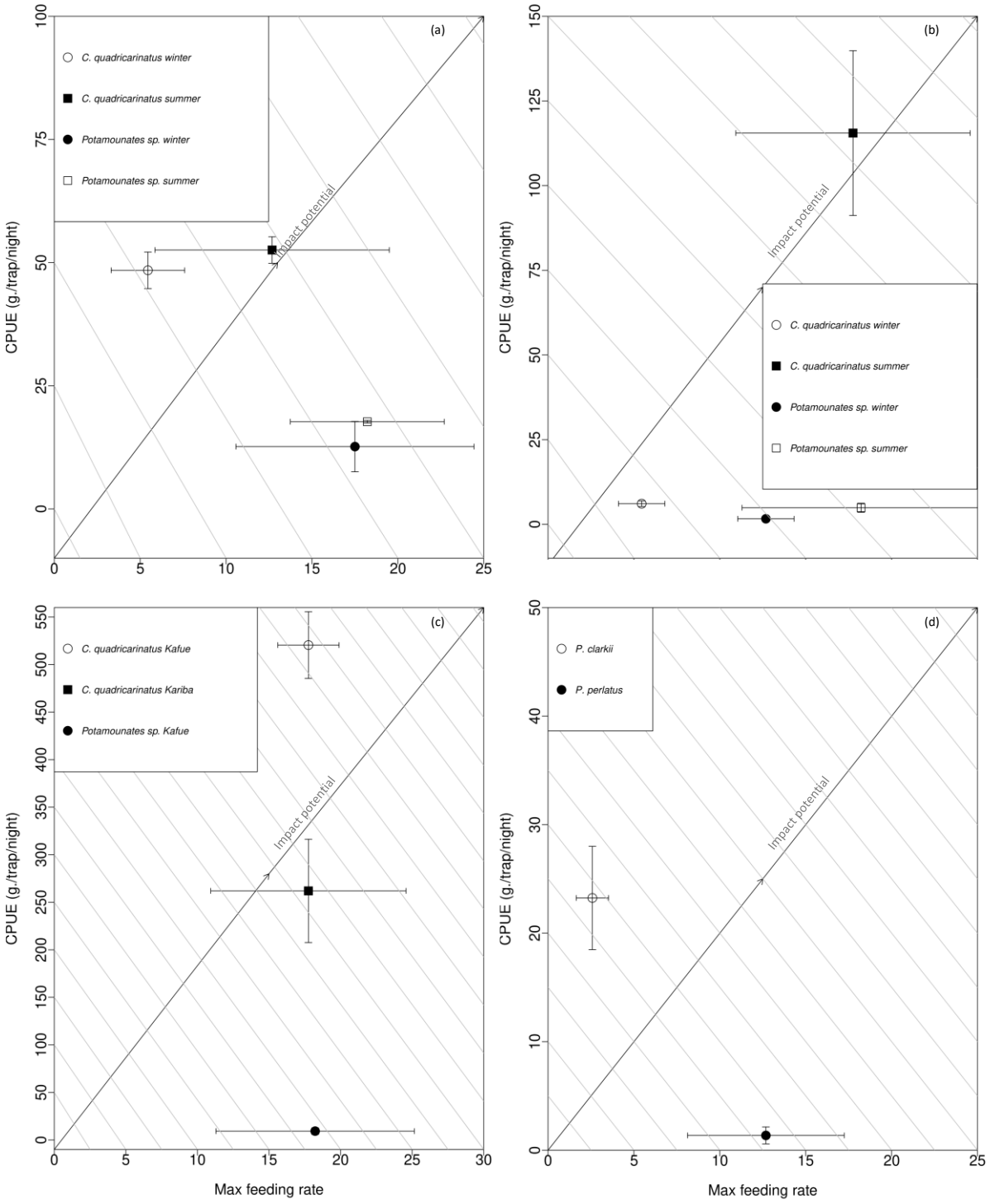

Figure 8.2. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using earthworms *Eisenia* sp. in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

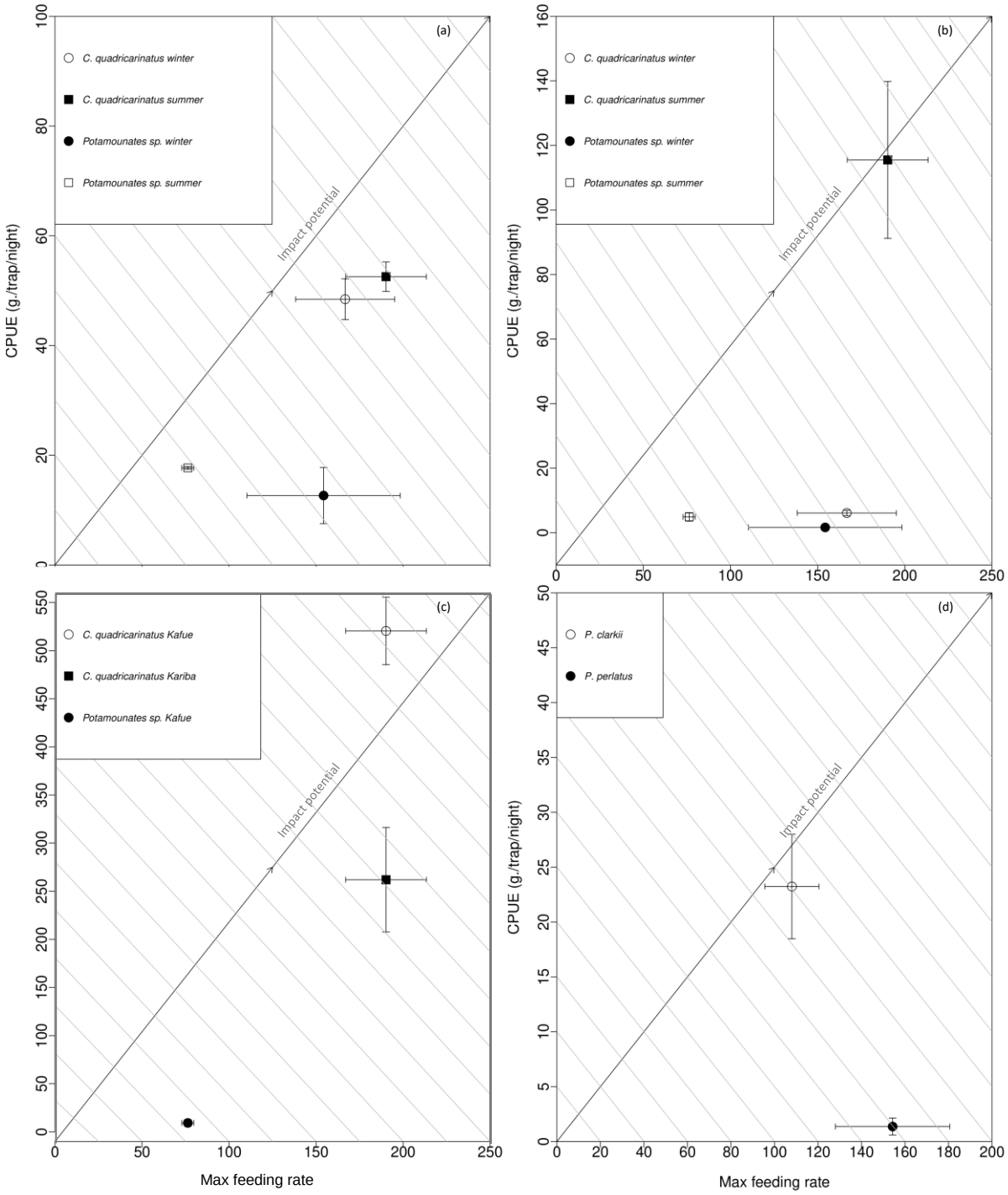

Figure 8.3. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using common midge Chironomid larvae in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

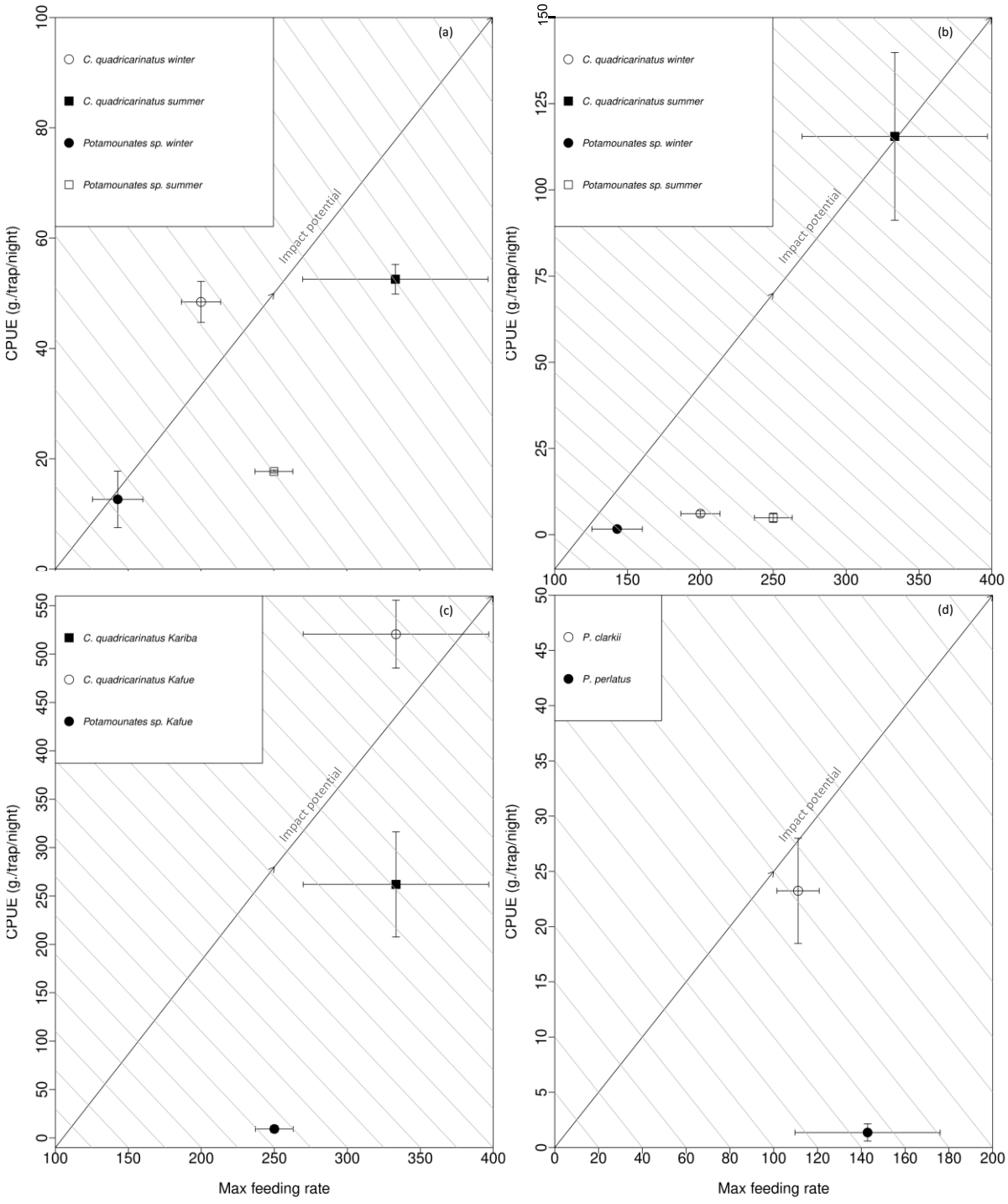

Figure 8.4. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using mosquito larvae *Culex* sp. in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

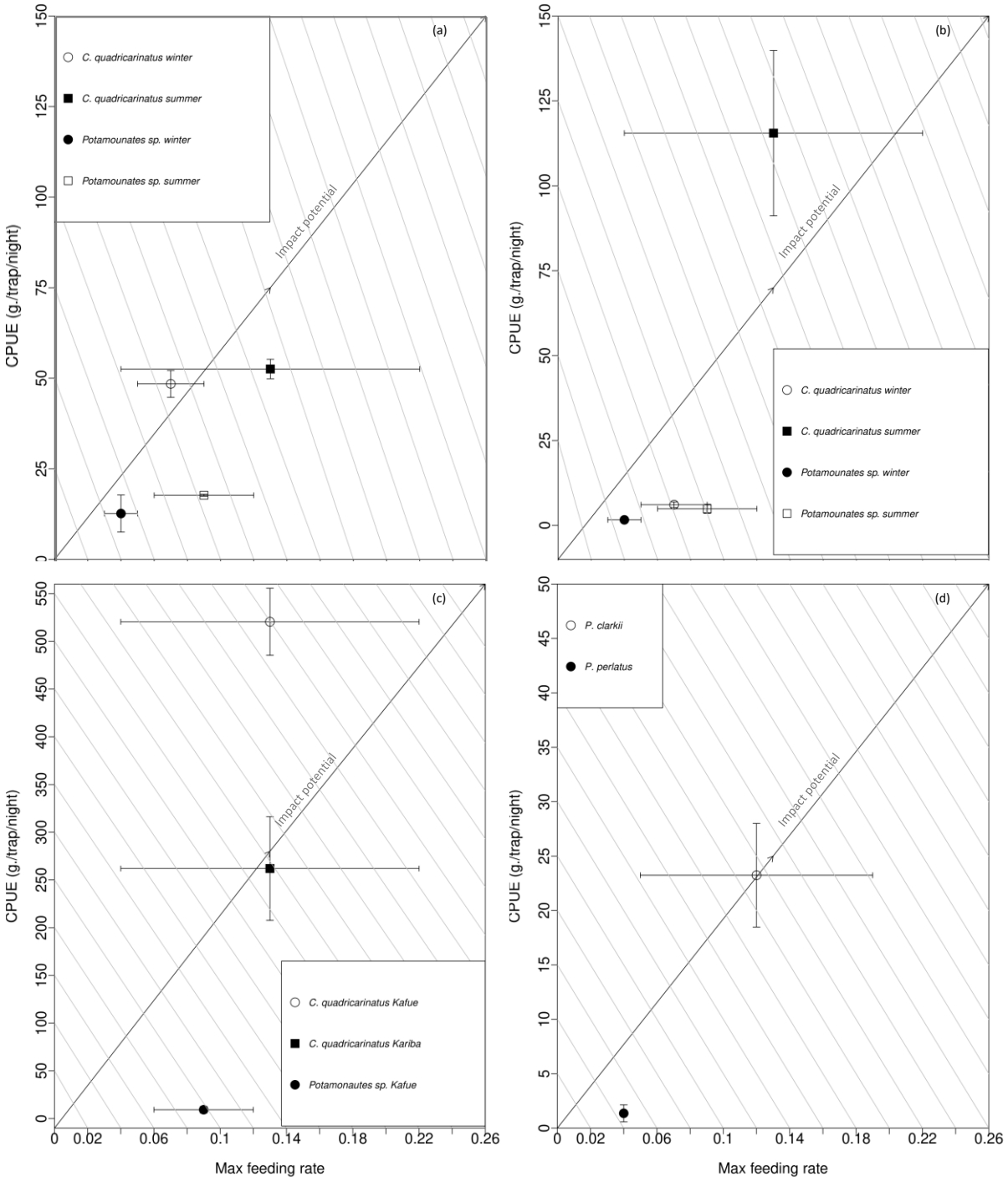

Figure 8.5. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using aquatic macrophyte *Potamogeton nodosus* in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

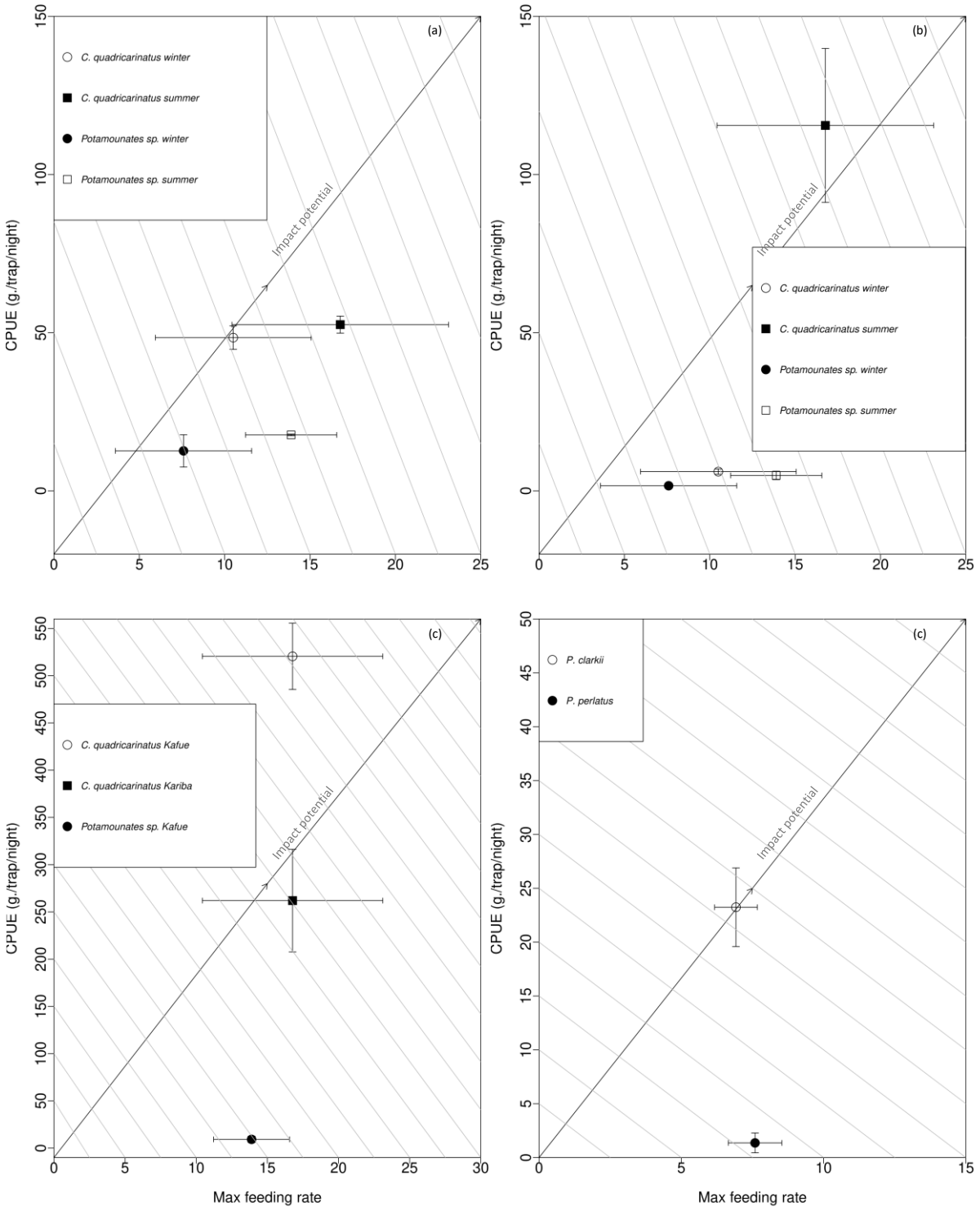

Figure 8.6. Relative impact potential (RIP) biplots (see also Table 8.1) of non-native *Cherax quadricarinatus*, *Procambarus clarkii* and native *Potamonautes perlatus* predicted using dead Mozambique tilapia *Oreochromis mossambicus* in summer (28°C) and winter (19°C) for: (a) Barotse floodplain; (b) Komati River; (c) in summer (28°C) for Kafue River and Lake Kariba; and (d) in winter (19°C) for Mimosa Dam. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

Table 8.3. Relative loss in fish catch and income due to *Cherax quadricarinatus* calculated for the Kafue River strata, Zambia, from fish scavenging experiments in Chapter 7. Monetary loss was recorded in Zambian Kwacha (ZWK) and converted to United States dollars (US$) using global rates of 2020 (i.e. 14.40:1 ZWK:USD).

Flow	Temperature (°C)	Stratum	Crayfish CPUE (g/trap/night)	Crayfish consumption/15h (kg/15h)	CPUE fish (kg/day)	Days fished	Catch loss/week (kg)	Overall catch loss/season (kg)	Fish price/kg (ZWK)	Catch loss/year/fisher (t)	Monetary loss/year/fisher (ZWK)	Monetary loss/year/fisher (USD)	Fisher effort	Overall loss/year (t)	Overall monetary loss/year (USD)
low	28	1	163.19	0.61	36.20	3.30	117.44	2818.57	18.80	2.82	52989.16	3679.80	79	222.67	299010.28
low	28	2	44.11	0.17	60.00	3.80	227.37	5456.91	18.80	5.46	102589.99	7124.30	130	709.40	952621.34
low	28	3	17.64	0.07	13.70	3.50	47.72	1145.24	18.80	1.15	21530.58	1495.18	178	203.85	273745.89
low	28	4	546.91	2.05	3.20	3.60	4.14	99.28	18.80	0.10	1866.49	129.62	106	10.52	14131.96
high	19	1	66.16	0.16	9.10	3.30	29.51	354.07	18.80	0.35	6656.56	462.26	47	16.64	22347.01
high	19	2	8.82	0.02	12.70	3.20	40.57	486.87	18.80	0.49	9153.10	635.63	180	87.64	117682.75
high	19	3	35.28	0.08	15.90	4.00	63.26	759.14	18.80	0.76	14271.75	991.09	235	178.40	239561.55
high	19	4	488.69	1.17	8.70	3.80	28.60	343.24	18.80	0.34	6452.87	448.12	119	40.85	54849.40

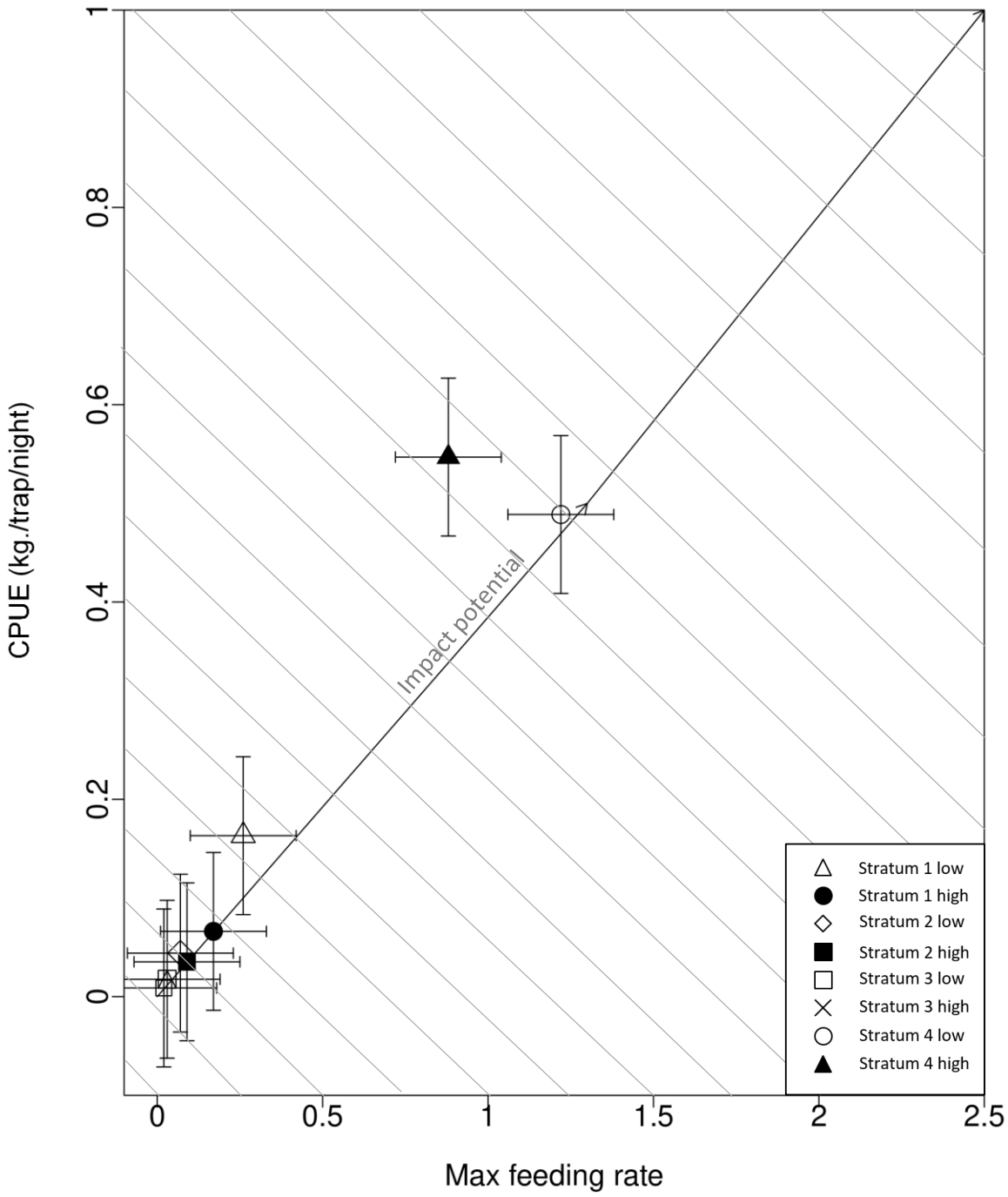

Figure 8.7. Relative impact potential (RIP) biplots of non-native *Cherax quadricarinatus*, predicted using catch from Kafue River strata, Zambia. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

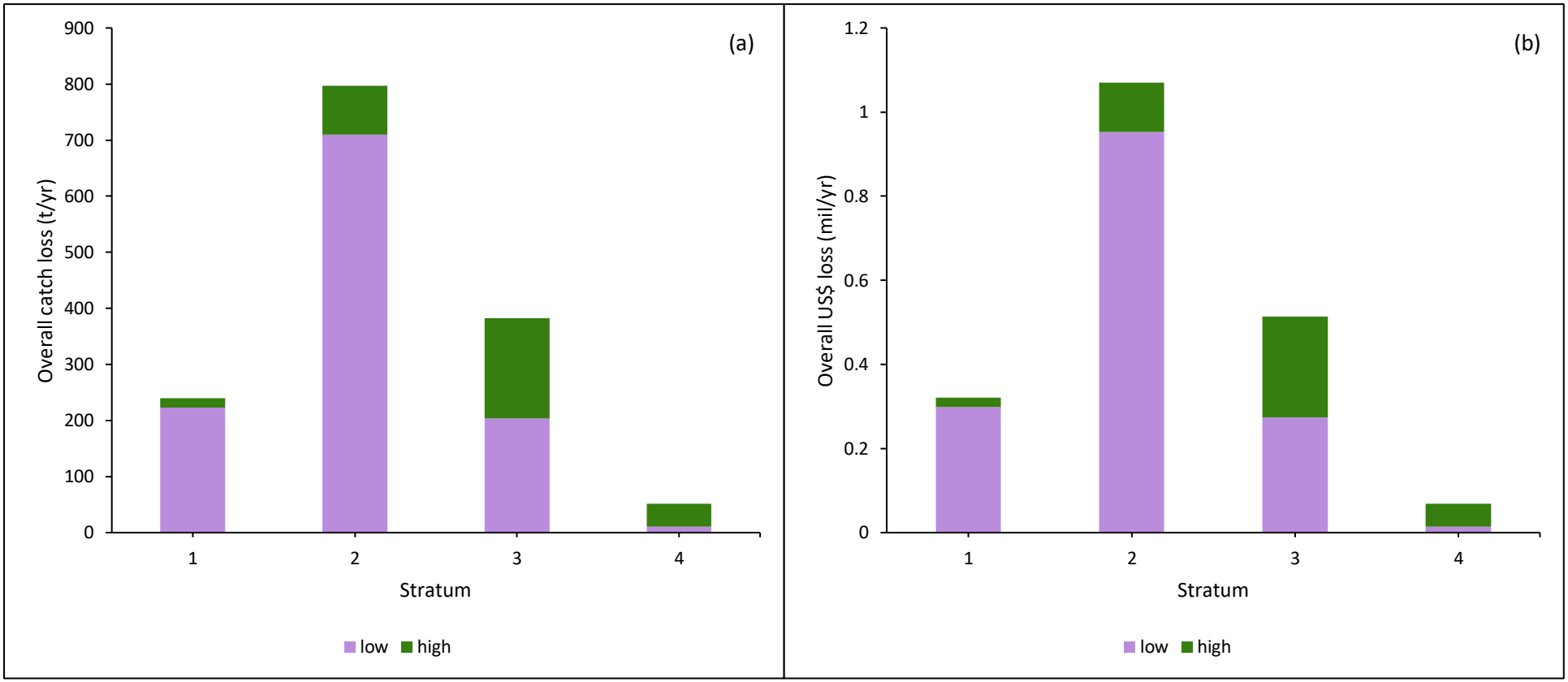

Figure 8.8. Predicted overall losses in (a) catch from Kafue River, Zambia strata, and (b) corresponding overall income losses during the low and high flow seasons.

Discussion

Developing predictive methods to forecast the impacts of IAS is critically important to biodiversity conservation (García-Díaz et al. 2020). Using the RIP metric proposed by Dick et al. (2017b), this study provided insights into the ecological impacts of IAS and how their impacts could be mediated by temporal variabilities associated with seasonal temperature changes through multiplying the maximum feeding rates and population biomasses. The RIP of the two crayfish species under both seasons and in all the invaded regions was consistently higher relative to the native crab suggesting greater impact than the native species regardless of context (Dick et al. 2017b; Dickey et al. 2020). The high abundance of crayfish in the invaded regions increase the per capita effects of these invaders relative to native crabs, and hence, potential high ecological impacts of the crayfish regardless of location.

This study predicted the impacts to be highly pronounced in the summer season, that is September – December, (characterised by high temperatures in southern Africa), as the relative abundance of *C. quadricarinatus* increases (see Nunes et al. 2017a; Marufu et al. 2014, 2017a, 2019; pers. obs). Crayfish predation and consumption impacts also increases with temperature (Madzivanzira et al. 2021; Chapters 5-7). Changes in RIP scores with seasonal temperature differences and decapod relative abundances from different regions demonstrate how such context-dependencies can have critical effects on the RIP capacities of IAS (Dick et al. 2017b; Mofu et al. 2019a). The temperature increase likely exacerbates the invader ecological impacts, especially for tropical species such as *C. quadricarinatus*, as it approaches thermal optima (Iacarella et al. 2015). Further to these, the summer season before significant rains (September – December) is characterised by low water levels especially in the Barotse floodplain ponds, which increases the rate of crayfish-prey/resource encounters, and this drives the additive

impact effect during this season. A combination of these factors could act in tandem and this will cause more deleterious ecological and potentially devastating socioeconomic impacts during the summer season. This study additionally demonstrated that individual regions can differ substantially in invasive species' consumptive impacts. A spatial resolution of the RIP between the invaded regions predicted high impacts in the older invasions (Kafue Flats and Lake Kariba). However, zooming in into the invasion core of the recently invaded Barotse floodplain, the abundance of *C. quadricarinatus* is similar to that of the older invasions (Madzivanzira et al. In Review; Chapter 4) and therefore, the impacts could be equivalent.

When an ecosystem is invaded, the structure or functioning of the system often changes, and may manifest at population or community level, while some, usually at later stages of invasion, may produce ecosystem-level impacts (Pyšek and Richardson 2010). Impacts of IAS are sometimes rapid and dramatic, especially where they result in noticeable ecosystem transformations. Some impacts for example, ecological impacts, often go unnoticed (and are often difficult to assess using traditional and monetary approaches), but with regard to *C. quadricarinatus*' invasion in Southern Africa, fishers reported that they began to affect their artisanal fishery soon after the establishment (G. Chisule, Ministry of Fisheries and Livestock Zambia pers. comm. 2019; AT Chakandinakira, Department of Fisheries Zimbabwe pers. comm. 2019). The impacts of *C. quadricarinatus* make it a food security concern in a region associated with high levels of poverty. When crayfish causes a percentage of the catch to be unmarketable (Chapter 7), targets are not met and the impacts cascade through the value chain. It is therefore vital to calculate the losses associated with *C. quadricarinatus* invasions, as socioeconomic impacts are more easily perceived and more likely to be addressed by stakeholders. Using the invaded Kafue Flats as an example, this study showed that annual

potential losses in catch and income could be up to US$ 2 million due to spoilage by *C. quadricarinatus* during the high and low water flows. This amount lost is significant, considering the contribution of fish to socioeconomic livelihoods. The potential overall loss in catch and income shown in this study could be even greater in the field, because the mass per gram consumed in the lab was used to up-calculate the overall mass lost due to crayfish spoilage. This overall mass underrepresents the spoiled catch as when crayfish consumes a small amount/part of the fish in the lab and field, the whole fish is regarded as spoiled (see Plate 7a, d – f in Chapter 7) and can only be used for home consumption. This therefore means that what is calculated as a 10 g loss due to crayfish consuming a small part of fish could translate to the loss of the entire fish as a tradable commodity (with a probable weight of 0.5 kg – 2.0 kg). While this study gives a snapshot of the potential losses due to crayfish invasions, field surveys are more appropriate to calculate the realistic losses in catch and income. Over- and underestimation of the losses can result in assumptions such as that crayfish feed on fish caught in the gillnets (over estimation in this case), fishing continues during the fishing ban (under estimation), and as discussed, not considering that small amounts consumed ruin the entire fish for sale (under estimation).

The effect of location within a region could be contributing to the high CPUE, as one stratum could be more productive than the other strata, as well as the effects of flow on CPUE for both crayfish and fish. The Kafue Basin has an altered flow rate due to the dams, which impact the biotic communities, and consequently, fishing activities, as seen in the fisher CPUE data from Aquatic Ecosystem Services and WWF Zambia Report (2020). The impact of crayfish increases as their numbers increase, resulting in significant losses as discussed in the paragraph above. As not all fishers will have crayfish impacts, and not all of the time, the data in this

chapter are the maximum likely impact, and therefore an over estimation of possible impact. This needs to be verified in the field through catch damage quantification from fisheries dependent and independent surveys. The low flow season is characterised by high temperatures and this corroborates with data from the previous chapters, which demonstrated that high temperatures result in increased per capita and consumption rates of the invasive crayfish species on various resources (Chapters 5 – 7). The effects of water level and water flow on crayfish feeding has been documented in other species and locations (e.g., Lake Naivasha, Grey and Jackson 2012), and this is likely having an effect in the floodplains as the ecology is driven by the flood pulses which change resource availability and predator prey dynamics, as well as human fishing behaviours. Additionally, the crayfish are not only feeding on scavenged fish, but also on other biotic components such as plant matter and invertebrates (Grey and Jackson 2012; Marufu et al. 2018b). This needs to be assessed with respect to crayfish abundance to document the intra-specific competitions on resources.

The RIP metric used in this study provided a standardised, user-friendly means of calculating the impacts of the established crayfish species, relative to a trophically analogous equivalent. The two invasive crayfish species have the potential to exert greater impacts on native resources in invaded systems, as shown by their high RIP, thereby destabilising ecosystem processes and functions. The RIP was consistently higher for the Barotse floodplain, which is a recent establishment. This study also quantified the socioeconomic impacts of crayfish species in the invaded systems, which makes it both a food security and an economic concern. These impacts are for a well-established invasion. Trends are likely similar across systems and will get worse as invasions establish in newly invaded habitats such as the Barotse Floodplain. Governments should strengthen efforts to prevent their introduction and spread, as economic

information on fisheries and aquaculture development based on invasive crayfish in Africa is lacking.

CHAPTER 9

GENERAL DISCUSSION

Main findings

This thesis focused on freshwater crayfish introductions in Africa, under three broad themes, namely: the current status (Chapters 2 – 4), ecological impacts (Chapters 5 – 7) and socioeconomic impacts (Chapters 7 – 8). A combination of extensive field surveys and laboratory experiments provided a better understanding of the current distribution of crayfish and the potential environmental and socioeconomic impacts they generate. A systematic review was conducted to compile current information to better understand the factors that have driven crayfish introductions in Africa and to identify knowledge gaps to better prioritise their management (Chapter 2). The review revealed that the continent has been a recipient of nine introduced crayfish species, with five of those: *Astacus astacus*, *Cherax quadricarinatus*, *Faxonius limosus*, *Procambarus clarkii*, and *Procambarus virginalis* establishing feral populations in fourteen African countries (Madzivanzira et al. 2020; Chapter 2). *Astacus astacus* and *F. limosus* are present in Morocco and *P. virginalis* is limited to Madagascar. *Cherax quadricarinatus* and *P. clarkii* are spreading at a fast rate in the continent and have established in five (Mozambique, South Africa, Swaziland, Zambia and Zimbabwe) and six (Egypt, Kenya, Morocco, South Africa, Tunisia, Uganda) countries, respectively. The main driver of these crayfish introductions was to provide socio-economic benefits through fisheries development and aquaculture, but there is limited evidence of success. The review identified the following research priorities: standardisation of crayfish sampling methods, determining abundance, distribution and spread of crayfishes and assessing ecological and socioeconomic impacts to inform management decisions (Madzivanzira et al. 2020; Chapter 2). Chapters 3 to

8 attempted to address the knowledge gaps around crayfish introductions in Africa as identified from Chapter 2.

The first step was to develop standardised gear and protocols for sampling *C. quadricarinatus* in Africa (Chapter 3). Among the two methods that have been used for *C. quadricarinatus* abundance studies in Southern Africa, Chapter 3 recommended the collapsible promar® crayfish trap baited with Bobtail® dry dog food pellets (steak flavour) as the most appropriate gear for *C. quadricarinatus* abundance studies in Africa, due to high detection probability, high CPUE (compared to the other gear) and the suitability of the dog food as a standard bait. This standard gear was then used for crayfish field surveys across a large portion of the Zambezi Basin in Botswana, Namibia, Zambia and Zimbabwe to determine relative abundance and the extent of invasion of *C. quadricarinatus* (Chapter 4). *Cherax quadricarinatus* were widespread and abundant in the middle and upper Zambezi Basin with evidence of multiple introductions, rapid spread and establishment where introduced. The study confirmed the establishment of *C. quadricarinatus* in the recently invaded Barotse Floodplain. Although the probability of capture and CPUE of *C. quadricarinatus* in the Barotse Floodplain were similar to that of Lake Kariba and Kafue River (the older invasions), morphometric differences among *C. quadricarinatus* populations sampled from these invaded regions were detected. The *C. quadricarinatus* samples from the Barotse Floodplain are mostly investing in reproduction and low investment in body size due to competition and time to establish, compared to the more established invasions. The potential for *C. quadricarinatus* to invade and establish in other pristine catchments in Southern Africa is a matter of extreme concern, especially given the rapid dispersal rates that were calculated in this study (Madzivanzira et al. In Review; Chapter 4).

Comprehensive laboratory assessments were conducted to infer the impacts of the two crayfish species that are spreading across the continent (*C. quadricarinatus* and *P. clarkii*) on various native resources (Chapters 5, 6 and 7). These included Functional Response (FR) and scavenging and consumption laboratory experiments on various native resources, to provide insights into the relative impacts of the two crayfish species in comparison to the native *Potamonautid* crabs. The use of FR experiments provided insights into how the two crayfish species are likely to utilise and impact the available native resources of invaded ecosystems. This in most cases was due to their high attack parameter which also contributed to higher FRRs compared to the native crabs. The crayfish FRs and FRRs were mostly enhanced under the high temperature treatment, and *C. quadricarinatus* in particular has the potential for negative impacts regardless of thermal conditions. To take this a step further, the FR, scavenging and consumption data were then combined with field abundances of crayfish and crabs (Chapter 8) to calculate the Relative Impact Potential (RIP), which provided a valuable quantitative means of assessing the potential impacts of *C. quadricarinatus* and *P. clarkii* in Africa. Under both winter and summer seasons, the crayfish species consistently displayed RIP scores > 1 relative to the native crab irrespective of region, suggesting greater impact of invaders compared to the native species (Dick et al. 2017b; Dickey et al. 2020). The study further estimated the economic losses due to catch spoilage caused by *C. quadricarinatus*, using Kafue Flats fishery data and scavenging data from Chapter 7. Fishers in Kafue Flats are likely to lose up to a maximum estimate of 1500 t per year (equivalent to up to US$ 2 million) of fish due to catch spoilage by *C. quadricarinatus* (Chapter 8). This is the first estimate for economic cost of *C. quadricarinatus* globally, and the first economic assessment for crayfish and indeed aquatic species economic cost in Africa (see Cuthbert et al. 2021; Kouba et al In Review for current costs). This provides the motivation for more in-depth assessments to

determine whether lab work and associated field work is efficient enough to match intensive fisheries surveys to determine the actual economic losses.

Conclusion and recommendations

This thesis contributes greatly to the understanding of the impacts of invasive crayfish species that have established naturalised populations in Africa. Of concern, specifically to Africa, are the species: *C. quadricarinatus*, *P. clarkii* and *P. virginalis* (Madzivanzira et al. 2020; Chapter 2). *Cherax quadricarinatus* and *P. virginalis* are emerging invaders globally, and *P. clarkii* is an established invader globally. By identifying the context dependencies of impact and the mechanisms which facilitate invasions by crayfish it is possible to predict and triage future spread and consequences. The establishment of a recently reported population of *C. quadricarinatus* in the Upper Zambezi Basin, as confirmed by this study using a standardised approach, raises concerns about their potential spread to important pristine ecosystems such as Kabompo River, Okavango Delta and Lake Malawi, which are all rich in biodiversity and endemic species. Detailed accounts of predation, scavenging and consumption parameters enabled us to mechanistically predict crayfish consumption rates, and the data suggest stronger impacts by the two crayfish species, which corroborates their invasion history as documented for other continents. Although crabs, which are the functional equivalent of crayfish in Africa, were shown to consume native resources in almost a similar pattern as that of the two crayfish species, their overall impact was not deemed to be significant, owing to their low abundances in Southern Africa as well as their co-evolutionary relationships with native prey. Therefore crayfish are likely to outcompete crabs due to their high FRs, FRRs, RIP, consumption, scavenging and field abundances. The underperformance of crabs at higher temperatures compared to crayfish is likely to decrease their degree of biotic resistance, which makes it easier for crayfish to expand their range in the invaded regions. Furthermore, due to the

predicted climate warming, invasive crayfish may potentially establish in habitats that were previously unsuitable, thereby increasing their invasion ranges in the region. Crayfish establishing in African freshwater systems pose a risk to native biota across all trophic levels, which will destabilise ecosystems. This will also be reflected in socioeconomic impacts as crayfish affect fisheries directly through scavenging of fish caught on gillnets, as reported in this thesis, and indirectly through their impact on fish recruitment. These irreversible impacts are likely to worsen and persist in the region, especially considering the low level of conservation management resources in Africa.

Clearly, prevention of IAS introduction is the most desirable outcome, but if a species is already introduced it may be possible to prevent their establishment and subsequent spread through early detection (Juette et al. 2014). Modern methods, for example environmental DNA (*e*DNA), increasingly being recognised as a useful tool for invasion biology, can be incorporated into crayfish monitoring, which can become an important approach for early detection of invasive crayfish. As described in Madzivanzira et al. (2020; Chapter 2), citizen science also plays an important role in management of IAS. For progress to be made in crayfish management, policy makers, environmental managers and conservationists should work together with communities directly benefiting from the invaded systems. The communities need to be educated about the impacts of these invasive crayfish species so that they desist from spreading them. Communities could also be encouraged to make use of these crayfish species as feed for their domestic animals, because most people around the invaded regions do not eat crayfish species due to their appearance (Madzivanzira et al. 2020, Chapter 2, TCM pers. obs.). This will help in supressing crayfish populations to minimise the associated socioeconomic and ecological impacts. Advocating for people to utilise crayfish should, however, be treated with caution because when the benefits become substantial, this could

promote the propagation and translocation of crayfish, as observed in the Barotse floodplain, Zambia, and might lead to considerable conflicts between fisheries agencies and the local communities.

Although various control methods have been considered to eradicate invasive crayfish species (such as proposed in South Africa), it is evident that no single method can yield the desired solution to the problem (see Madzivanzira et al. 2020; Chapter 2). The only practical solution is to supress crayfish populations which is only feasible in smaller contained dams and reservoirs (e.g. the invaded Mimosa Dam in Free State Province, South Africa), but there is not much evidence of its success because of crayfish ecological behaviours such as burrowing. It is rather difficult to control *P. clarkii* due to its burrowing behaviour. Further studies are needed in this regard, with a view to improving the performance of existing methods or inventing new methods that will be more effective, so that ecosystems do not continue to lose biodiversity. Introduction via the aquarium pet trade is of growing concern as many crayfish species are readily available in pet stores and over the internet (such as in South Africa). In most cases these crayfish species outgrow aquaria and are dumped into local watercourses, which act as an incubator. Once introduced, invasive crayfish species are often spread through anthropogenic activities, for example, being used as live bait by anglers. Environmental managers and conservationists should work with angling organisations to educate anglers and other stakeholders of the negative impacts associated with translocating invasive crayfish and other IAS in general.

Once crayfish establish it is difficult to eradicate them as shown by a few attempts to eradicate crayfish described in this thesis, all of which failed. The first management action by states and

governments should be to prevent crayfish introductions in order to avoid the potential negative impacts documented in this thesis. Policies for shared aquatic systems, for example, the SADC Protocol on Fisheries which prohibits the introduction of non-indigenous species into aquatic ecosystems without the approval of all riparian states, should be strengthened. In South Africa, four crayfish species (including the species studied in this thesis) are listed under the National Environmental Management: Biodiversity Act (NEM: BA)'s national list of invasive species, which have certain strict regulations with regards to their importation and translocation within the country. According to the NEMBA regulations, possession of crayfish without a permit from the Department of Environmental Affairs may lead to a jail sentence or a fine of up to R2 million (L Barkhuizen FSDESTEA, pers. comm. 2019). Prescripts in the Act also require the immediate eradication of this species. Other southern African countries such as Botswana, Namibia, Zambia and Zimbabwe have established several inland fisheries based on introduced fishes (Tanganyika sardine *Limnothrissa. miodon* fishery in Lakes Kariba and Itezhi-tezhi; Nile tilapia *Oreochromis niloticus* fishery in Lake Kariba, Kafue River; and Largemouth bass *Micropterus salmoides* in reservoirs Botswana and Zimbabwe reservoirs) (Ellender *et al*., 2014). Unfortunately, their conservation policies regarding introduction of non-native species are not stringent (Ellender et al. 2014). These contrasting policies on introduction of non-native species renders the southern African situation more problematic, and the lack of adequate regulatory frameworks constrain conservation of biodiversity. There is a need to develop and strengthen national policies that support regional frameworks and international treaties, such as the SADC Protocol on Fisheries and the Convention on Biological Diversity (CBD), to prevent the introduction and propagation of crayfish as well as other IAS. Some introduction routes can be controlled whilst others such as unaided translocations, are almost impossible to control. An example is the invasion of the Zambezi River which, at 2574 km, is the fourth longest river in Africa, encompassing eight countries with a catchment area of 1.39 million

km^2. The vastness of this system makes it impossible to prevent the unaided translocation of crayfish from one invaded region to another, which has resulted in the documented spread of *C. quadricarinatus* (Madzivanzira et al. 2020; Chapter 2; Madzivanzira et al. In Review; Chapter 4).

Crayfish distribution surveys should continue, especially in surveying and monitoring pristine regions at risk of invasion, such as the Kabompo River, Okavango Delta and Lake Malawi. The populations of the two crayfish species and the native crabs and their dynamics should continue to be monitored to identify differences and to determine whether they are following the same patterns. Species distribution models and ecological niche models, for example, MAXENT, can be used to identify suitable areas for crayfish establishment in Africa, to inform relevant authorities to be wary of these invaders in the near future. The diet of these crayfish species in different regions needs to be analysed using stable isotope analysis to determine the biotic components which are likely to be impacted, and to determine the spatiotemporal trophic changes as a result of their invasion. The data on establishment, spread, and ecological impacts of crayfishes provided in this thesis provide sufficient evidence to inform environmental managers and policy makers regarding minimisation of further introductions or spread and to prioritise their management.

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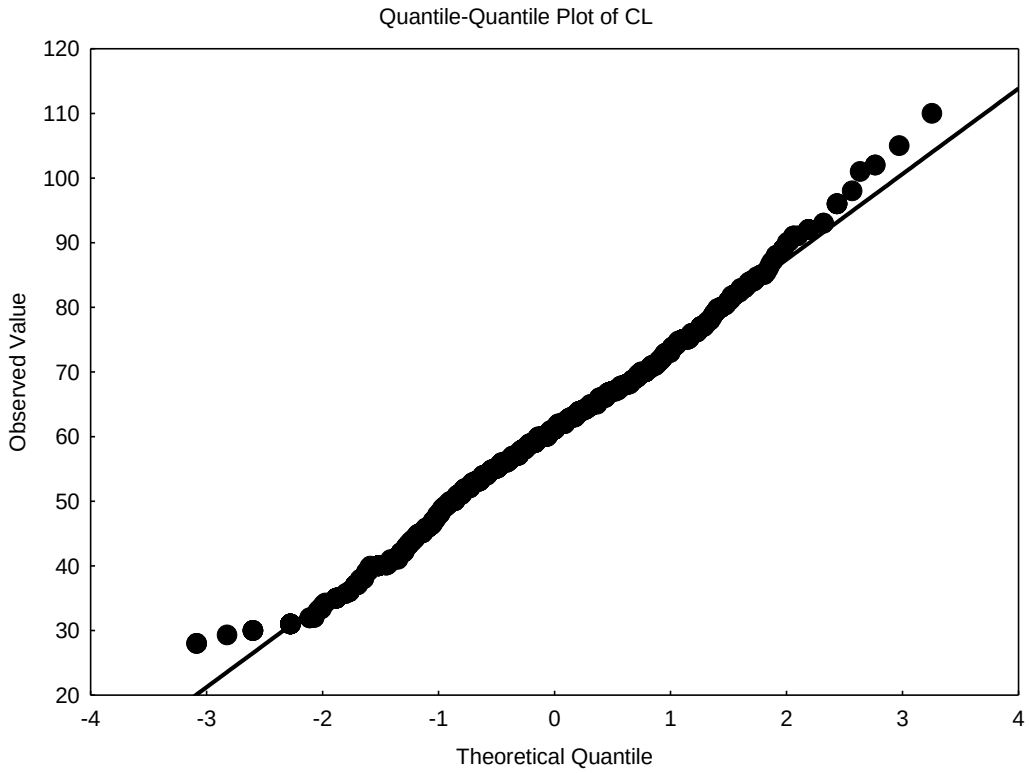
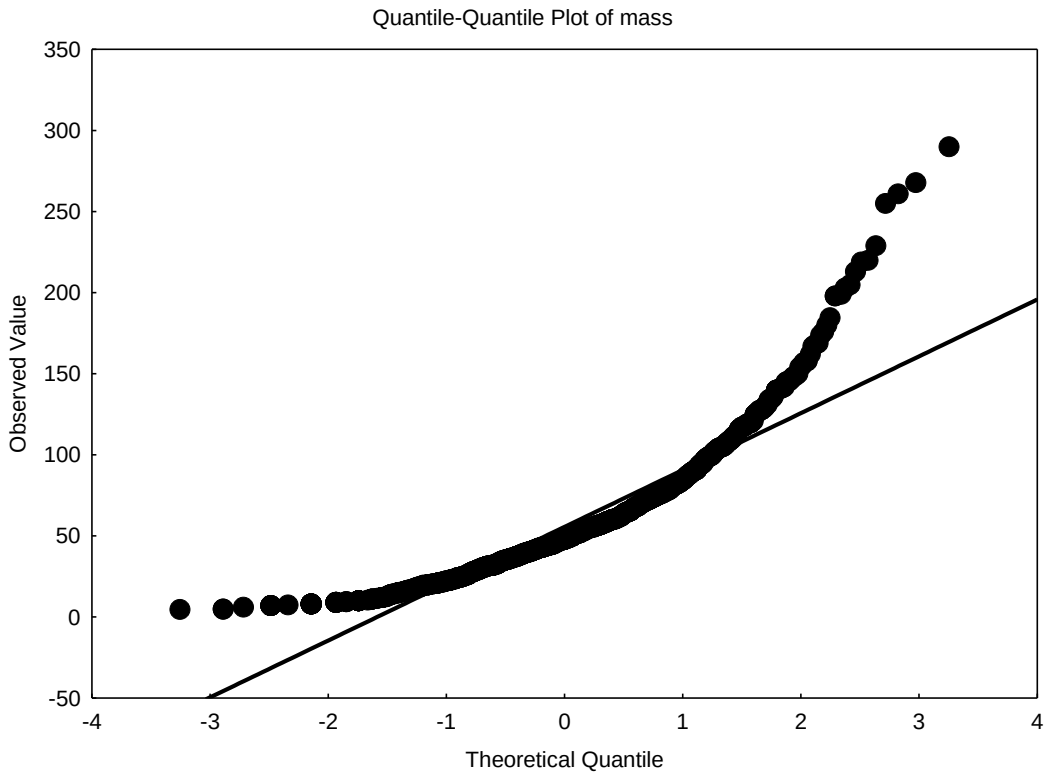
Appendices

Appendix 4.1. Quantile-quantile plot of *Cherax quadricarinatus* carapace length and mass from Kafue River, Lake Kariba and Barotse floodplain

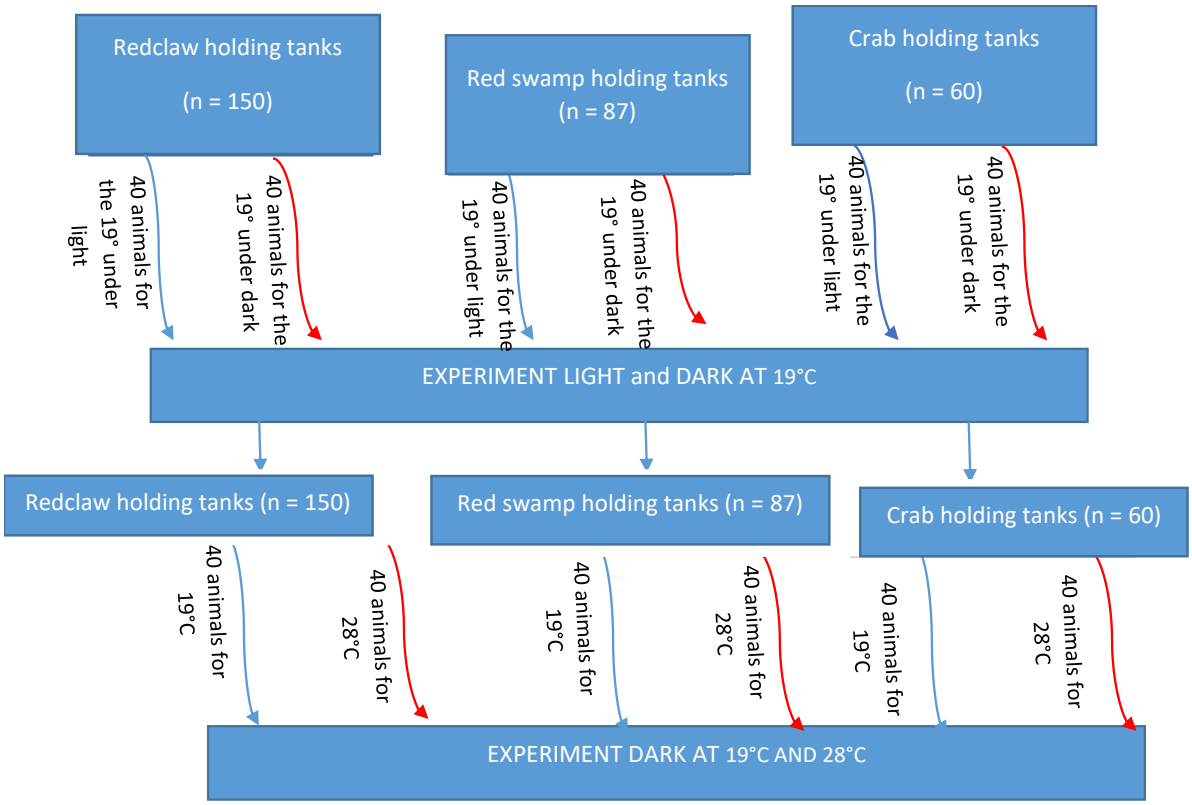


Quantile-quantile plot of *Cherax quadricarinatus* carapace length from Kafue River, Lake Kariba and Barotse floodplain



Quantile-quantile plot of *Cherax quadricarinatus* mass from Kafue River, Lake Kariba and Barotse floodplain

Appendix 5.1. Experimental set up for Functional Responses experiments from Chapter 5



Appendix 6.1. Species pairwise estimates

Eathworm at 19°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.741	0.0840	Inf	8.819	<.0001
cherax - pota	-0.466	0.0651	Inf	-7.157	<.0001
clarkii - pota	-1.207	0.0802	Inf	-15.040	<.0001

Eathworm at 28°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.342	0.0772	Inf	4.422	<.0001
cherax - pota	-0.150	0.0712	Inf	-2.100	0.0357
clarkii - pota	-0.491	0.0757	Inf	-6.490	<.0001

Chironomid larvae at 19°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	-0.415	0.134	Inf	-3.093	0.0020
cherax - pota	-0.110	0.149	Inf	-0.739	0.4602
clarkii - pota	0.305	0.122	Inf	2.499	0.0125

Chironomid larvae at 28°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.1342	0.107	Inf	1.257	0.2087
cherax - pota	0.0855	0.112	Inf	0.761	0.4466
clarkii - pota	-0.0487	0.112	Inf	-0.437	0.6624

Mosquito larvae at 19°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	-0.1094	0.0996	Inf	-1.098	0.2720
cherax - pota	-0.1716	0.1099	Inf	-1.562	0.1182
clarkii - pota	-0.0622	0.1024	Inf	-0.608	0.5431

Mosquito larvae at 28°C

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.306	0.132	Inf	2.319	0.0204
cherax - pota	0.418	0.153	Inf	2.725	0.0064
clarkii - pota	0.112	0.163	Inf	0.685	0.4933

Earthworm at 19°C

density = 2:

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.000	0.284	Inf	0.000	1.0000
cherax - pota	0.000	0.284	Inf	0.000	1.0000
clarkii - pota	0.000	0.284	Inf	0.000	1.0000

density = 5:

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	0.383	0.212	Inf	1.803	0.0714
cherax - pota	-0.128	0.185	Inf	-0.689	0.4907
clarkii - pota	-0.511	0.207	Inf	-2.465	0.0137

density = 10:

species_pairwise	estimate	SE	df	z.ratio	p.value
cherax - clarkii	1.163	0.230	Inf	5.061	<.0001
cherax - pota	-0.405	0.145	Inf	-2.800	0.0051
clarkii - pota	-1.569	0.221	Inf	-7.113	<.0001

density = 15:

```
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii    1.157 0.219 Inf  5.278  <.0001
 cherax - pota      -0.693 0.131 Inf -5.277  <.0001
 clarkii - pota     -1.851 0.206 Inf -8.993  <.0001

density = 20:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii    0.865 0.189 Inf  4.575  <.0001
 cherax - pota      -0.506 0.130 Inf -3.879  0.0001
 clarkii - pota     -1.371 0.178 Inf -7.717  <.0001

density = 30:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii    0.728 0.206 Inf  3.527  0.0004
 cherax - pota      -0.822 0.141 Inf -5.818  <.0001
 clarkii - pota     -1.551 0.187 Inf -8.306  <.0001

density = 40:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii    0.887 0.201 Inf  4.404  <.0001
 cherax - pota      -0.708 0.133 Inf -5.324  <.0001
 clarkii - pota     -1.595 0.186 Inf -8.577  <.0001
```

Earthworm at 28°C

```
density = 2:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.0000 0.330 Inf  0.000  1.0000
 cherax - pota      0.0000 0.330 Inf  0.000  1.0000
 clarkii - pota     0.0000 0.330 Inf  0.000  1.0000

density = 5:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.4274 0.245 Inf  1.745  0.0811
 cherax - pota     -0.0834 0.213 Inf -0.391  0.6959
 clarkii - pota    -0.5108 0.241 Inf -2.119  0.0341

density = 10:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.2429 0.182 Inf  1.336  0.1815
 cherax - pota     -0.3023 0.167 Inf -1.810  0.0702
 clarkii - pota    -0.5452 0.170 Inf -3.208  0.0013

density = 15:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.1398 0.160 Inf  0.876  0.3812
 cherax - pota     -0.2318 0.146 Inf -1.590  0.1118
 clarkii - pota    -0.3716 0.152 Inf -2.449  0.0143

density = 20:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.5162 0.153 Inf  3.366  0.0008
 cherax - pota     -0.2423 0.125 Inf -1.935  0.0530
 clarkii - pota    -0.7585 0.147 Inf -5.158  <.0001

density = 30:
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.5293 0.142 Inf  3.729  0.0002
 cherax - pota     -0.0790 0.120 Inf -0.659  0.5099
 clarkii - pota    -0.6082 0.140 Inf -4.348  <.0001

density = 40:
```

```
 species_pairwise estimate    SE  df z.ratio p.value
 cherax - clarkii   0.5349 0.145 Inf  3.684  0.0002
 cherax - pota     -0.1082 0.122 Inf -0.890  0.3733
 clarkii - pota    -0.6431 0.142 Inf -4.516  <.0001
```

Chironomid larvae at 19°C

```
density = 2:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -1.2040 0.8561 Inf -1.406  0.1596
 cherax - pota     -0.6931 0.9196 Inf -0.754  0.4510
 clarkii - pota     0.5108 0.6716 Inf  0.761  0.4469

density = 5:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.2877 0.4442 Inf -0.648  0.5172
 cherax - pota      0.6286 0.5694 Inf  1.104  0.2696
 clarkii - pota     0.9163 0.5440 Inf  1.684  0.0921

density = 10:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.3124 0.3125 Inf -1.000  0.3174
 cherax - pota      0.0000 0.3358 Inf  0.000  1.0000
 clarkii - pota     0.3124 0.3125 Inf  1.000  0.3174

density = 20:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.4177 0.2279 Inf -1.833  0.0668
 cherax - pota     -0.3285 0.2321 Inf -1.415  0.1570
 clarkii - pota     0.0892 0.2078 Inf  0.429  0.6676

density = 40:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.4714 0.1767 Inf -2.668  0.0076
 cherax - pota     -0.2412 0.1853 Inf -1.302  0.1930
 clarkii - pota     0.2303 0.1646 Inf  1.399  0.1619

density = 60:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.3827 0.1463 Inf -2.616  0.0089
 cherax - pota     -0.1001 0.1556 Inf -0.643  0.5202
 clarkii - pota     0.2826 0.1421 Inf  1.989  0.0467

density = 150:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.0712 0.1098 Inf -0.649  0.5166
 cherax - pota     -0.0539 0.1102 Inf -0.489  0.6251
 clarkii - pota     0.0173 0.1083 Inf  0.160  0.8728

density = 200:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.1737 0.0918 Inf -1.891  0.0586
 cherax - pota     -0.0906 0.0936 Inf -0.968  0.3332
 clarkii - pota     0.0831 0.0897 Inf  0.927  0.3541
```

Chironomid larvae at 28°C

```
density = 2:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.1054 0.6094 Inf -0.173  0.8627
 cherax - pota      0.2513 0.6684 Inf  0.376  0.7069
 clarkii - pota     0.3567 0.6537 Inf  0.546  0.5853

density = 5:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.0000 0.4093 Inf  0.000  1.0000
 cherax - pota      0.0488 0.4144 Inf  0.118  0.9063
 clarkii - pota     0.0488 0.4144 Inf  0.118  0.9063

density = 10:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.0488 0.2930 Inf  0.166  0.8678
 cherax - pota      0.1268 0.2991 Inf  0.424  0.6717
 clarkii - pota     0.0780 0.3025 Inf  0.258  0.7966

density = 20:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.1040 0.2141 Inf -0.486  0.6271
 cherax - pota     -0.1522 0.2117 Inf -0.719  0.4721
 clarkii - pota    -0.0482 0.2060 Inf -0.234  0.8150

density = 40:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.0344 0.1555 Inf  0.221  0.8251
 cherax - pota      0.2787 0.1661 Inf  1.678  0.0934
 clarkii - pota     0.2443 0.1674 Inf  1.460  0.1443

density = 60:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.4074 0.1309 Inf  3.112  0.0019
 cherax - pota     -0.0154 0.1166 Inf -0.133  0.8946
 clarkii - pota    -0.4229 0.1305 Inf -3.240  0.0012

density = 150:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.5393 0.0990 Inf  5.446  <.0001
 cherax - pota      0.1060 0.0873 Inf  1.214  0.2247
 clarkii - pota    -0.4332 0.1011 Inf -4.287  <.0001

density = 200:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.2532 0.0870 Inf  2.911  0.0036
 cherax - pota      0.0403 0.0822 Inf  0.490  0.6240
 clarkii - pota    -0.2129 0.0878 Inf -2.426  0.0153
```

Mosquito larvae 19°C

```
density = 2:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.5596 0.5710 Inf -0.980  0.3271
 cherax - pota      0.2877 0.6958 Inf  0.413  0.6793
 clarkii - pota     0.8473 0.6287 Inf  1.348  0.1778

density = 5:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.0953 0.3981 Inf -0.239  0.8108
 cherax - pota     -0.2624 0.3832 Inf -0.685  0.4936
 clarkii - pota    -0.1671 0.3732 Inf -0.448  0.6545

density = 10:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.0445 0.2717 Inf -0.164  0.8700
 cherax - pota     -0.1278 0.2663 Inf -0.480  0.6312
 clarkii - pota    -0.0834 0.2632 Inf -0.317  0.7514

density = 20:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.1542 0.1916 Inf -0.805  0.4210
 cherax - pota     -0.1942 0.1898 Inf -1.023  0.3064
 clarkii - pota    -0.0400 0.1823 Inf -0.220  0.8263

density = 40:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.0839 0.1411 Inf -0.594  0.5523
 cherax - pota     -0.4700 0.1298 Inf -3.620  0.0003
 clarkii - pota    -0.3861 0.1266 Inf -3.050  0.0023

density = 60:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii  -0.0121 0.1003 Inf -0.121  0.9038
 cherax - pota     -0.2567 0.0947 Inf -2.710  0.0067
 clarkii - pota    -0.2446 0.0944 Inf -2.590  0.0096

density = 150:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.0217 0.0718 Inf  0.303  0.7623
 cherax - pota     -0.1741 0.0685 Inf -2.544  0.0110
 clarkii - pota    -0.1958 0.0689 Inf -2.844  0.0045

density = 200:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.0530 0.0647 Inf  0.818  0.4131
 cherax - pota     -0.1753 0.0612 Inf -2.863  0.0042
 clarkii - pota    -0.2283 0.0621 Inf -3.674  0.0002
```

Mosquito larvae 28°C

```
density = 2:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.4055 0.8067 Inf  0.503  0.6152
 cherax - pota      1.0986 1.0204 Inf  1.077  0.2816
 clarkii - pota     0.6931 1.0823 Inf  0.640  0.5219

density = 5:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.2683 0.4604 Inf  0.583  0.5602
 cherax - pota      0.2683 0.4604 Inf  0.583  0.5602
 clarkii - pota     0.0000 0.4902 Inf  0.000  1.0000

density = 10:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.3567 0.3556 Inf  1.003  0.3158
 cherax - pota      0.2657 0.3464 Inf  0.767  0.4430
 clarkii - pota    -0.0910 0.3772 Inf -0.241  0.8094

density = 20:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.3977 0.2464 Inf  1.614  0.0406
 cherax - pota      0.5480 0.2581 Inf  2.123  0.0337
 clarkii - pota     0.1503 0.2802 Inf  0.536  0.5918

density = 40:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.3399 0.1784 Inf  1.905  0.0568
 cherax - pota      0.3280 0.1778 Inf  1.845  0.0650
 clarkii - pota    -0.0118 0.1923 Inf -0.062  0.9509

density = 60:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.1748 0.1270 Inf  1.376  0.1689
 cherax - pota      0.2386 0.1293 Inf  1.845  0.0650
 clarkii - pota     0.0638 0.1346 Inf  0.474  0.6356

density = 150:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.1697 0.0912 Inf  1.860  0.0428
 cherax - pota      0.2700 0.0938 Inf  2.878  0.0040
 clarkii - pota     0.1002 0.0975 Inf  1.028  0.3039

density = 200:
 species_pairwise estimate     SE  df z.ratio p.value
 cherax - clarkii   0.3346 0.0829 Inf  4.037  0.0001
 cherax - pota      0.3244 0.0826 Inf  3.926  0.0001
 clarkii - pota    -0.0102 0.0893 Inf -0.114  0.909
```