Understanding biotic interactions in invaded pond communities in the Sundays River irrigation network, South Africa

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GENERAL ABSTRACT

The Sundays River valley irrigation ponds provide a unique opportunity to investigate biotic interactions within a biological invasions context, as they contain both native and non-native fish species. This study focusses on two native species (*Glossogobius callidus* and *Gilchristella aestuaria*) and two non-native species (*Oreochromis mossambicus* and *Gambusia affinis*). The ecology of the ponds was driven by physico-chemical variables, mainly temperature, but the interactions between fishes were a complex interplay between temperature, pond community ecology and food web structure. Seasonal changes in temperature and subsequent fluctuations in water levels resulted in changes in zooplankton community. Chlorophyll-*a*, temperature, *G. callidus* and *G. affinis* were the drivers of the seasonal changes in macroinvertebrate composition.

Stable isotope analysis identified substantial ontogenetic dietary shifts in all species, corresponding to changes in body size. Stable isotope analysis revealed that the niche space occupied by *G. affinis* was broad and overlapped with that of the other three focal species. Stable isotope metrics showed that *G. affinis* and *O. mossambicus* utilised a wide range of resources compared to *G. callidus* and *G. aestuaria*. Stomach content analysis showed that *G. callidus*, *O. mossambicus* and *G. affinis* fed predominantly on benthic resources, while *G. aestuaria* fed mainly plankton resources.

Functional response experiments revealed that *G. callidus* and *G. affinis* both displayed Type II functional responses. In single fish trials, *G. affinis* had significantly higher functional responses than *G. callidus.* In heterospecific *G. callidus*-*G. affinis* combinations the functional response of *G. callidus* was reduced by the presence of *G. affinis*, whereas, this combination greatly enhanced *G. affinis* functional response magnitudes. The functional response of *G. callidus*, *O. mossambicus* and *G. affinis* under two temperature treatments along with fish abundance data was used to determine temporal differences in the ecological impacts of each fish species between seasons. The relative impact potential of *O. mossambicus* was consistently higher than that of *G. callidus* and *G. affinis*. This study demonstrates how seasonal temperature fluctuations affect the relative impact capacities of introduced species. Overall, this thesis showed that high temperature along with life-history traits contributes to the biotic interactions between native and non-native species in novel environments.

Keywords: Freshwater; South Africa; Eastern Cape; pond; ecology; fish; zooplankton; macroinvertebrates; stable isotope; functional response; Relative Impact Potential

PREFACE

This thesis is comprised of a general introduction (Chapter 1), study site and study species (Chapter 2), data chapters (Chapters 3, 4, 5 and 6) and a general synthesis and conclusion (Chapter 7). All chapters are organized according to the Rhodes University thesis guidelines and some have been published (see below). All references have been combined in a single reference list at the end of the thesis to ensure limited repetition.

- Mofu, L., Dalu, T., Wasserman, R. J., Woodford, D. J., Khosa, D. & Weyl, O. L. F. (*under-review*). Seasonal variation and drivers of zooplankton, macroinvertebrates and littoral fish communities from irrigation ponds in a semi-arid region. *Inland Waters.*
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- Mofu, L., Cuthbert, R. N., Dalu, T., Woodford, D. J., Wasserman, R. J., Dick, J. T. A. & Weyl, O. L. F. (2019). Impacts of non-native fishes under a seasonal temperature gradient are forecasted using functional responses and abundances. *NeoBiota* **49**, 57-75.

DECLARATION

This thesis forms the compilation of original research that I conducted through the South African Institute for Aquatic Biodiversity (SAIAB) and the Department of Ichthyology and Fisheries Science, Rhodes University, between 2016 and 2020. I am aware of the University's policy on plagiarism. All of the work is my own. I have not included ideas, phrases, passages or illustrations from another person's work without acknowledging their authorship.

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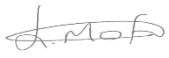
Signed: 

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CHAPTER 1

General introduction

The intentional or unintentional introduction of species into new geographical areas through human actions has facilitated biological invasions (Vitousek *et al*., 1996; Mack *et al*., 2000; Abellán *et al*., 2006). The introduction, establishment, and spread of non-native species is regarded as a major threat to global biodiversity (Olden *et al*., 2010; Reid *et al*., 2019). In particular, freshwater ecosystems are the most susceptible to invading species and fishes are the most commonly introduced aquatic animals (Gozlan *et al*., 2010). Non-native species can have negative effects on native species by disrupting food webs, modifying habitats and competing with native species, through predation, parasitism and disease, which can result in a decline in biodiversity (Cucherousset & Olden, 2011).

There are, however, major gaps in our knowledge as to why introduced species succeed or fail, with implications for our capacity to predict the outcome of future invasions (Dick e*t al*., 2017b). The nature of receiving environments is, however, thought to be important in regarding species-specific invasions, with native communities being key in this regard (Latombe *et al*., 2017). In novel environments, understanding how introduced species and native communities interact within disturbed systems could inform us to which species are most likely to establish and what environmental factors facilitate colonisation and invasion (Wellborn *et al*., 1996). Therefore, understanding the factors leading to the success of introduced species is of great practical and conceptual importance (Sato *et al*., 2010). The irrigation ponds in the Sundays River valley provide

a unique opportunity to investigate colonisation processes because they receive water, and propagules, from a single source from the Sundays River (Woodford *et al*., 2013).

Once a non-native species establish in a new region, the consequences are almost irreversible (Frederico *et al*., 2019). Non-native species are considered invasive once they have passed through every stage in the naturalisation-invasion continuum (Su *et al*., 2014). An understanding of the impact of non-native species will improve our ability to manage existing and emerging invaders (Mooney & Hobbs, 2000; McGeoch *et al*., 2015). However, impacts of invasive species are often speculative and the resources needed to manage invasive species, especially in freshwater ecosystems, are limited (Simberloff *et al*., 2013; Latombe *et al*., 2017; Jackson *et al*., 2017; Woodford *et al*., 2017).

During the invasion process, several factors must be considered at different invasion stages, such as introduction, establishment and spread (Blackburn *et al*., 2011). Therefore, to understand how these factors play in invasions success, it is important to incorporate these factors into the framework of biological invasions. The unified framework proposed by Blackburn *et al*. (2011) is an important tool in invasion biology as it combines stage-based and barrier models and provides categorisation for populations at different points in the invasion process. As a result, while the framework proposed by Blackburn *et al*. (2011) is well developed, understanding the impact of alien species is important for prioritizing efforts to prevent future introductions and to manage the spread of invasive species (Blackburn *et al*., 2014). The invasion process is complex and can be divided into a series of sequential stages (arrival, establishment and spread), and at each stage, there are barriers that need to be overcome for a species or population to pass on to the next

invasion stage (Blackburn *et al*., 2011) (Figure 1.1). In addition, Blackburn *et al*. (2011) suggested management actions for each stage, namely, preventing introductions, containment once introduced, mitigating spread once species established and eradication when feasible.

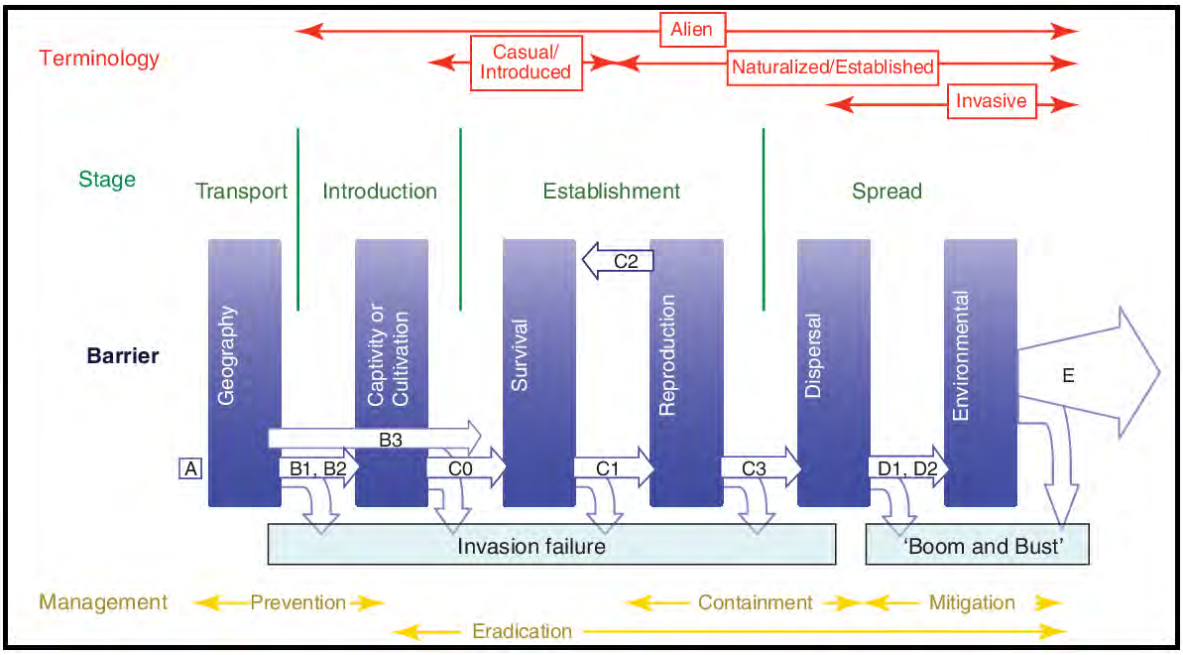

Figure 1.1: The invasion process and the stages needed to be overcome by an introduced species on the naturalisation-invasion continuum. Adapted from Blackburn *et al*. (2011). The unified framework for biological invasions incorporates the factors associated with invasions and the evolutionary effects occurring in the species' native ranges. Letters A = not transported beyond limits of native range; B1 = individual transported beyond limits of native range, and in captivity; B2 = individuals transported beyond limits of native, and in cultivation; B3 = individuals transported beyond limits of native range, and directly released into novel environment; C0 = individuals released into wild; C1 = individuals surviving in the wild location; C2 = individuals surviving in the wild in location where introduced, reproducing occurring but populations not self-sustaining; C3 = individuals surviving in the wild location where introduced, reproducing occurring, and population self-sustaining; D1 = self-sustaining population in the wild; D2 = self-sustaining populations in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction; E = fully invasive species, individuals dispersing, surviving and reproducing.

In many instances and systems, however, researchers and managers are restricted from using this framework from the *Establishment* and *Spread* stages, given that so many South African systems are already invaded (Ellender & Weyl, 2014). In addition, newly invaded systems are often only identified as such once a non-native population has become established, as encounter rates increase with increasing population size. As such, there are relatively few opportunities to explore invasion processes from the very beginning at the *Introduction* stage.

Propagule pressure (arrival stage)

There are numerous contributors to successful biological invasions. Propagule pressure or 'introduction effort' has been widely recognized as the primary determinant of invasion success and has been widely accepted as the 'null model' for biological invasions (Colautti *et al*., 2006; Lockwood *et al*., 2009; Simberloff, 2009; Britton & Gozlan, 2013). Propagule pressure describes the total number of individuals introduced into a new environment and acts as the first filter to establishment success (Briski *et al*., 2012; Blackburn *et al*., 2013). Several studies have found that higher propagule pressure often results in establishment success, while low propagule pressure is most likely to result in establishment failure (Drake *et al*., 2005, Britton & Gozlan, 2013; Brockerhoff *et al*., 2014). In agreement with these studies, Lockwood *et al.* (2005) found that with an increase in the number of released individuals, propagule pressure increased and thus the introduced species would establish (Colautti, 2003). Thus confirming, the success of the founding population (measured by population size and fitness) has been shown to be influenced by propagule pressure (Hufbauer *et al*., 2013). Previous studies have revealed that propagule pressure in conjunction with environmental disturbances is likely to increase the probability of invasion

success (Zenni *et al*., 2017). Previous studies have shown that greater probability of finding suitable habitat for introduced species through environmental disturbances and greater propagule pressure increases invasion success (Lozon & MacIsaac, 1997; Melborne *et al*., 2007).

Sinclair and Arnott (2016) conducted a mesocosm experiment to investigate the role of propagule pressure in the establishment of a sexually reproducing bloody-red mysid, *Hemimysis anomala* (Sars, 1907), across a range of propagule sizes and numbers. Their experiment showed that more introductions resulted in higher probabilities of survival than smaller, more frequent introductions. An experimental study to identify the long-term effect of propagule pressure on the probability of population establishment by Britton and Gozlan (2013) on the topmouth gudgeon, *Pseudorasbora parva* (Bleeker, 1859), using mesocosm ponds over three reproductive seasons, agreed with the previous literature (Lockwood *et al*., 2009) in that increased propagule pressure increased population establishment. A study by Woodford *et al.* (2013) examined the fish composition upstream, within and downstream of the Sundays River Valley irrigation network. They showed that the invaded ponds received more propagules than other ponds. In addition, they showed that both propagule pressure and life-history traits are likely to contribute to the successful establishment of species into novel environments. Overall, these studies offer insights into the dynamics of the colonisation process, yet more extensive field studies are needed as data on failed invasions is still relatively scarce.

Biology

The life-history traits of non-native fauna is a second key factor known to influence invasion success (Kolar & Lodge, 2001). The biology can influence a species' ability to establish a founding

population (Kolar & Lodge, 2001; Moyle & Marchetti, 2006; Masson *et al*., 2018; Milardi *et al*., 2018). Knowledge of the biology of invasive species can be used to explore their plasticity across geographically distant populations under different environmental conditions (Bøhn *et al*., 2004; Wei *et al*., 2017). For fish, several studies have revealed that size, high fecundity, high growth rates, and early maturity are the main characteristics that are likely to increase invasion success (Ruesink, 2005; Tayeh *et al*., 2015; Rooke & Fox, 2018). In addition, the biology of fishes are most likely to change in response to physico-chemical variables such as temperature, which will have an effect on the physiological mechanisms (i.e., metabolism and reproduction) (Brown *et al*., 2004; Jonsson *et al*., 2013).

The Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852) and the Western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853) are both species introduced globally for aquaculture and biocontrol, respectively (García-Berthou *et al*., 2005). The biology of these species has enabled them to establish, and thus they are both listed as 'World Worst Invasive Species' (Lowe *et al*., 2000). Overall, their wide range of environmental tolerance, diverse diet, flexibility in reproductive characteristics and competition for resources have resulted in the success of both species (García-Berthou *et al*., 2005; Rehage *et al*., 2019). A study by Jia *et al.* (2019), explored the role of life-history traits in the invasion success of *P. parva* on the Qinghai-Tibet Plateau. They observed that the life-history characteristics differed between native and introduced populations, and they showed that the introduced *P. parva* population adopted different life-history strategies to the native populations, such as smaller body size, higher fecundity, smaller oocytes and earlier maturation. A study by Gertzen *et al.* (2016) strongly supports the theory that the biology of fishes

plays an important role in biological invasions. Their study assessed the reproductive biology of the bighead goby, *Ponticola kessleri* (Günther, 1861), the round goby, *Neogobius melanostomus* (Pallas, 1814), and the monkey goby, *Neogobius fluviatilis* (Pallas, 1814). Their main findings showed that the invasive Gobiid species in the Lower Rhine had different reproductive modes to the native species, spawning lengths and phenotypic plasticity in reproductive traits, contributing to the success of the Ponto-Caspian invasive gobies.

Overall, a noteworthy observation from previous studies is that fish biology plays a very important roles in invasion success of introduced species mainly through plasticity in reproductive traits under changing environmental conditions allows them to optimise reproductive success (Alcaraz & García-Berthou, 2007; Ribeiro & Collares-Pereira, 2010). However, fish biology may change depending on environmental context, successful invasive species are likely to have wider tolerance ranges towards environmental variability, therefore it is likely that non-native species will have the required biological traits to out compete native species in order to colonise and establish in novel environments.

Abiotic interactions

The Environmental Matching Hypothesis predicts that individuals, which experience the same conditions in both their developmental and adult life-stages, which should have increased fitness relative to those that experience, mismatched conditions (Monaghan, 2008). Individuals that have adapted to environmental conditions such as oxygen concentrations, salinity, conductivity and temperature have a higher probability of establishment in novel environments (MacNeil *et al*.,

2004; Alcaraz *et al*., 2008; Kestrup & Ricciardi, 2009). Research has indeed shown that the successful establishment of non-native species has been driven by climate that closely matches that of their original range (Blackburn & Duncan, 2001; Duncan *et al*., 2003; Bomford *et al*., 2010; Britton *et al*., 2010). The successful establishment of species in novel environments depends on the species' ability to adapt to environmental conditions such as temperature (Garton *et al*., 1990; Moyle & Light, 1996; Diez *et al*., 2012). Temperature can limit the abundance and distribution of non-native species depending on the species' temperature tolerances (Lennon *et al*., 2001). Species differ in their physiological and behavioural responses in changing environmental conditions often can lead to interspecific competition and/or exclusion of native species if there is high overlap in their niches (Dick *et al*., 1993; Dick & Platvoet, 1996; Sorte *et al*., 2013).

If high temperature is more physiologically optimal for invaders, ecological impacts may be intensified (Ricciardi *et al*., 2013; Iacarella *et al*., 2015), however, temperatures beyond a species' optimum can result in performance declines (Schulte *et al*., 2011). Indeed, interaction strengths are known to vary even with slight changes to seasonal temperatures (Sanford, 1999). Therefore, failure to incorporate these factors into predictive approaches limits our ability to forecast invasive species impacts under changing environmental conditions across different spatiotemporal scales (Dick *et al*., 2013, 2014, 2017b). Carmona-Catot *et al*. (2013) examined the role of water temperature in interspecific competition between invasive mosquitofish, *Gambusia holbrooki* (Girard, 1859) and native Iberian toothcarp, *Aphanius iberus* (Valenciennes, 1846). They further examined food capture rates and aggressive interactions between the two species at three different temperatures (19, 24 and 29°C). They found that the mosquitofish exhibited greater aggression

together with greater food capture efficiency than the toothcarp, and at a higher temperature, the mosquitofish, therefore, had the potential to competitively displace the toothcarp through interference competition.

Biotic interactions

The establishment success of introduced species is also potentially dependent on their interactions with the resident species and, therefore, biotic interactions play a major role in invasion success (Tilman, 1980; Jones & Gomulkiewicz, 2011; Cucherousset *et al*., 2012). In addition, biotic interactions, along with environmental constraints can determine food web structure and community assembly through competition, predation, or facilitation (Gauzens *et al*., 2016). Interspecific competition is most often studied under controlled conditions and competition can occur through two mechanisms, namely exploitative competition and interference competition (Dunham *et al*., 2002). Exploitation competition occurs when one species exploits resources better than the other does, while interference competition occurs where one species excludes the other species through behaviour (Begon *et al*., 1996). Competition between invasive species and native species can be inferred through both indirect (habitat use and stable isotope analysis) and direct interactions (competition and predation) (Cucherousset *et al*., 2012). Biotic interactions such as competition can also influence community structure and thus affect the successful establishment of invaders and their impact (Hairston *et al*., 1960; Dick *et al*., 2017b; Britton *et al*., 2019). Several studies have shown that predation and competition differ across environmental gradients and are interrelated mechanisms that influence community structure. (Menge & Sutherland, 1976; Mofu *et al*., 2019a).

Successful invaders can be facilitated such as positive effects or inhibited by biotic interactions in part because natural enemies are absent (Ricciardi & Atkinson, 2004). On the other hand, resident species can reduce the success of invasive species through biotic resistance (Levine *et al*., 2004). Direct biotic interactions can drive trophic cascades through alterations of prey abundance and native predator fitness (Gallardo *et al*., 2016; Penk *et al*., 2017). Therefore, there is an urgent need for predictive methods to include environmental contexts as factors for their ability to forecast impact.

Several studies have focussed on exploitative competition as the key factor that determines the success of introduced species (McKnight *et al*., 2016; Dufour *et al*., 2018). There is also a growing interest in behavioural response as part of biotic interactions (Raw *et al*., 2013; Meuthen *et al*., 2016). According to Tilman (1987), interspecific competition occurs throughout natural communities and may occur through chemically mediated interactions. A study by Raw *et al.* (2013) assessed the interactions between three native gastropod species, *Assiminea* cf. *capensis* (Bartsch, 1915), *Melanoides tuberculate* (Müller, 1774), and *Coriandria durbanensis* (Tomlin, 1916) and an invasive gastropod, *Tarebia granifera* (Lamarck, 1816) in South Africa. Their main findings were that the invasion success of the invasive *T. granifera* was driven by chemical cues that drove the native gastropods *A. capensis*, *M. tuberculata* and *C. durbanensis* away and allowed the invasive gastropod *T. granifera* additional food and resources.

Caiola and de Sostoa (2005), conducted mesocosm and laboratory experiments between the Eastern mosquitofish, *Gambusia holbrooki* and two endemic Mediterranean cyprinodontids, the Iberian toothcarp, *Aphanius iberus* and Valencia toothcarp, *Valencia hispanica*. The mesocosm

was used to determine the individual survival of each adult fish species of approximately the same size (40-50 mm total length) through interactions and prey availability, where Gammarids and Chironomid larvae were quantified as food. The second experiment was conducted in an indoor laboratory aquarium at room temperature under natural light conditions. The experiments were conducted in four 250 L containers, where each container contained 5 cm layer of sand, *Ceratophyllum demersum* and stonefish shelters.

The laboratory experiment was conducted to assess interspecific agonistic behaviours. Their results revealed that the adult Eastern mosquitofish had a negative impact on the survival of the two native toothcarp species. Their experiment demonstrated that the endemic cyprinodonts displayed decreased feeding rates compared to the Eastern mosquitofish and the Eastern mosquitofish displayed more aggression toward the cyprinodonts. In addition, the mesocosm and the aquarium experiment demonstrated that the satiety and voracity of the Eastern mosquitofish were higher than that of the native cyprinodont forms and Iberian toothcarp. A study by Rehage *et al.* (2019) compared the population-level response of two mosquitofish species, the invasive *Gambusia affinis* and non-invasive *G. geiseri* in competition with each other. Their study revealed that the native populations of *Gambusia* were relatively smaller in the presence of the novel competitor and were rather higher in its absence. In addition, both *G. affinis* and *G. geiseri* had similar trophic roles, yet differed in their top-down predatory impacts in the absence of intrageneric and intraspecific competition.

One of the tools used to assess aspects of biotic interactions are Functional Response (FR) experiments. This approach was chosen for this study and is explained in two data chapters

(Chapters 4 and 5), and a brief overview of the FR method is outlined in this chapter (Chapter 1). The functional response of a consumer describes the relationship between *per capita* prey (or other resources) consumption and prey (or other resources) density (Solomon, 1949; Holling, 1959, 1965). Functional responses are thus a critical determinant in the outcome of interacting consumer-resource populations (Holling, 1959) and are a classic method used to understand predator-prey interactions and competition with regard to resource density (Solomon, 1949; Holling, 1959; 1965; Alexander *et al*., 2012; Toscano *et al*., 2014; Dick *et al*., 2017a). The types and magnitude of functional responses quantify whether consumers will regulate, stabilize or destabilize the resources in a population (Juliano, 2001).

The relationships between consumer resource uptake and resource densities result in three functional response types an increasing linear relationship with no handling time constraint (Type I, common in filter feeders) (Jeschke *et al*., 2004), an inversely density-dependent response characterised by high prey consumption at low prey density (Type II, resulting in rapid prey depletion at low densities), and a sigmoidal positive density-dependent relationship (Type III, where prey have a low-density refuge) (Holling, 1959) (depicted in Figure 1.2). Functional responses have been applied to various aspects of ecology (Abrams, 1982; Englund & Harms, 2001; Faria *et al*., 2004; Feldman, 2006). However, only more recently have comparative functional response analyses been used to predict the impacts invasive species can have on resources such as prey, whereby the impact of an invader is contrasted with functionally similar native species (Alexander *et al*., 2013; Dick *et al*., 2013, 2014, 2017b). In this regard, Dick *et al.* (2014) emphasized that functional responses provide a rapid, reliable, inexpensive and applicable

tool to assess invader ecological impact across taxonomic and trophic groups. In southern Africa, functional response experiments have been used across a wide range of taxa. In freshwater fish, functional response approach has been applied to offer insights into how prey populations may be regulated by predators (Alexander *et al*., 2013). In addition, functional response in varying environmental contexts has been used as a tool in predicting the ecological impact of an invasive species (Alexander *et al*., 2014a; Dick e*t al*., 2014; Wasserman *et al*., 2016; Cuthbert *et al*., 2018a; Khosa *et al*., 2020).

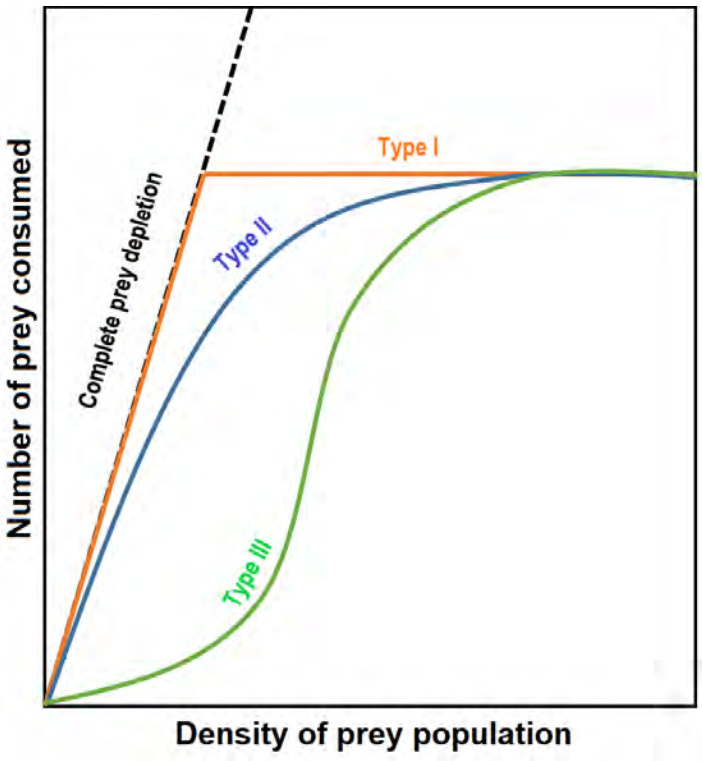

Figure 1.2: Graph showing categorical functional response types (Holling, 1959) describing the relationship between prey density and prey consumption (Type I, Type II and Type III). (Redrawn from Pritchard *et al*., 2017)

Irrigation ponds

Networks of artificial water bodies are increasingly being created globally in response to growing water demands (Davies *et al*., 1992). In arid regions, artificial water bodies are often established to facilitate agricultural practices in previously non-agrarian environments (James *et al*., 1999; Kingsford, 2000). Irrigation infrastructure in these regions often takes the form of canals, tunnels and water transfer schemes (Davies *et al*., 1992). Changes in landscape because of artificial water bodies are particularly pronounced in arid environments given that aquatic environments are novel habitats in dry regions (Abellán *et al.*, 2006). While the effects of introduced moisture on the landscape with regard to agricultural practices are well known (James *et al*., 1999; Kingsford, 2000), there are aspects of artificial water body introductions into landscapes that are less explored, such as nutrient flows, primary and secondary productivity dynamics, the flow of resources between the artificial aquatic and the receiving terrestrial ecosystems and the potential ecological services that they provide (Knight *et al*., 2005; Leroux & Loreau, 2008). Plankton (phytoplankton and zooplankton), macroinvertebrates, and fish often comprise significant components of the biological communities that establish within artificial water bodies (Abellán *et al*., 2006; Woodford *et al*., 2013). However, in many developing regions, basic ecological information on the aquatic faunal groups associated with artificial waters is understudied (Abellán *et al*., 2006).

Many agricultural water body networks are comprised of numerous small impoundments, with differing degrees of inter-impoundment connectivity and connectivity to water sources such as rivers (Downing *et al*., 2006; Powers *et al*., 2013; Woodford *et al*., 2013). Similarly, impoundment hydro periods, depth, age and other characteristics can be highly variable (Baxter, 1977; Pintar &

Resetarits, 2018). As such, these environments offer neat systems to explore biological and ecological questions, such as bottom-up and top-down drivers of population and community dynamics, community succession patterns and even food web structures. These environments can, under certain circumstances, also be used as experimental systems to assess ecological aspects within the context of biological invasions (Woodford *et al*., 2013). Since they can serve as 'novel' ecosystems in a landscape, faunal colonisation dynamics can potentially be explored in a replicated and somewhat controlled fashion. New impoundments are filled with water from a known source and propagules imported into the system can be readily quantified, as can colonisation dynamics, at the population- or community-level (Woodford *et al*., 2013). This study investigates aspects of the ecology of small agricultural impoundments in the Sundays River valley, with particular emphasis on the fish community, and within the context of interaction between colonising native and non-native species.

Research approach and thesis outline

This study builds upon previous work by Woodford *et al*. (2013) to improve our understanding of the ecology of the Sundays River valley agricultural ponds, focussing on the interactions between native and non-native species within the fish community. Previously Woodford *et al*. (2013) investigated the effects of propagule pressure and biological adaptations within the Sundays River valley irrigation network. They demonstrated that four species, *Glossogobius callidus* (Smith, 1937), *G. affinis*, *Gilchristella aestuaria* (Gilchrist, 1914) and *O. mossambicus* were the earliest species entering the irrigation network with the highest number of propagules and were, therefore, the first to establish. The Woodford *et al*. (2013) study was based on fish abundance data and

although they hypothesised that colonisation success was likely a combination of propagule pressure and fish biology, data on the biological aspects was lacking. Hence, the present study engaged in developing a deeper understanding of the biotic interactions within then pond ecosystems by describing the ecology by assessing the interactions between phytoplankton, zooplankton, macroinvertebrates and fishes from both, an abundance and a food web perspective in Chapters 3 and 4.

The main objective of Chapter 3 was to describe the pond community structure, where zooplankton, macroinvertebrates and the littoral fish are described in relation to physico-chemical variables and biological parameters. In this chapter, I was interested in the drivers of zooplankton, macroinvertebrates and the littoral fish community structure, and to better understand whether these were driven by environmental of biological variables.

Pond food web structure is investigated in Chapter 4. Here I investigate the potential for competition between fishes colonising irrigation ponds in the Sundays River system by comparing the diet composition of *G. callidus*, *G. aestuaria*, *O. mossambicus* and *G. affinis* through gut content analysis and by using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. I test whether stable isotope and gut content analyses will demonstrate overlap in feeding niches due to limited resources. Using the insights gained on potential interactions between the species, I subsequently test these interactions in Chapters 5 and 6, which assess the competitive interactions between members of the littoral fish community.

Chapter 5 investigates antagonistic interactions between *G. callidus* and *G. affinis* and uses the functional response experiments to compare the resource utilisation in individual experiments and in heterospecific experiments. This experiment builds on a study by Howell *et al*. (2013) who demonstrated negative spatial associations between *G. callidus* and *G. affinis* suggesting antagonistic interactions between them. A series of experiments was therefore developed to better understand the interactions between the two species by investigating multiple predator effects in an experimental setting.

Finally, to better understand the competitive interactions between the littoral fish community, the impacts of *O. mossambicus* and *G. affinis* relative to *G. callidu*s towards benthic prey are assessed across a seasonal temperature gradient using the Relative Impact Potential metric proposed by Dick *et al*. (2017b). The data chapters are then integrally discussed in the discussion chapter (Chapter 7), where a synthesis and recommendations for future research are provided.

CHAPTER 2

Study site and study species

Study Site

The Sundays River is about 310 km long and drains an extensive catchment of 20 792 km^2 from Graaff-Reinet to the Indian Ocean (Mackay & Schumann, 1990; Baird, 2001). The river flows through an arid region (Lombard *et al*., 2001). The vegetation in the Sundays River valley is dominated by thick dense succulent thicket and low Karoo shrub vegetation namely, *Pentzia incana* and *Rhigozum obovatum* (Hoffman & Cowling, 1990). The soil is characterised by red loamy to clayey soils about > 1 m deep around the Sundays River valley and Kirkwood (Hoffman & Cowling, 1990). The Fish-Sundays River Inter Basin Water Transfer Scheme (IBWTS), comprises a canal and tunnel system that supplies Orange River water from the Orange River first to the Great Fish River valley and then to the Sundays River valley in order to supplement the water supply in the Eastern Cape (Pech *et al*., 1995).

The Inter-Basin Water Transfer (IBWT) scheme enters the Sundays System through a Darlington Reservoir tributary (Woodford *et al*., 2013). Water released from this 4000 ha impoundment flows 50 km downstream to Korhaansdrift Weir, from where it is diverted into the irrigation network. From the network, water is transported downstream to Korhaansdrift and the irrigation off-take canal, which directs water away from the river and into the irrigation network of the Sunland district (Howell *et al*., 2013). Sunland is an area of intense citrus farming (DAFF, 2011). Within the network, water flows via gravity down several major and minor concrete canals, eventually feeding about 390 small off-channel irrigation ponds on private farms (Woodford *et al*., 2013).

The Sundays River irrigation ponds are located within a semi-arid region in Eastern Cape, South Africa (Kadye & Booth, 2013). The agricultural activities in the lower Sundays River have introduced high concentrations of ionic salts sodium, sulphate, bicarbonate and chloride (Russell, 1999). The natural flow of the river before the IBWT was seasonal and mainly characterised by periodic summer floods. After the IBWT was implemented, the flow was altered to perennial (Russell, 1999). Water is purchased on a weekly basis by each landowner from the Lower Sundays River Water Users Association (LSRWUA) that controls the opening and closing of sluices that connect each pond. Each pond receives water up to an annual quota that is approximately 9000 m^3 per hectare irrigated. Fish are distributed with the water to the irrigation canals and finally flushed into the farm ponds (Woodford *et al*., 2013). Each pond has a gravity-fed inflow, where the amount of water purchased by the landowner is introduced at irregular intervals from the canal system. Inflow canals or pipes may run dry most of the time and only be flushed for several hours per week.

The climate in the Sundays River valley is warm-temperate with average temperatures between 5-18°C in winter and 15-45°C in summer (Kadye & Booth, 2013) and an annual rainfall of 350-600 mm (Lombard et al., 2001). A Temperature logger (Sensor HOBO water temp PRO v2) which was placed in MLS during the period of 2013-2014 was used to measure water temperature (Onset Computer Corporation, Bourne MA, USA) (Figure 2.1). Inflow data were obtained from the Sundays River Irrigation Board, which was characterised by a peak flow into the dams in September and January and the lowest flow was between May and June (Figure 2.1).

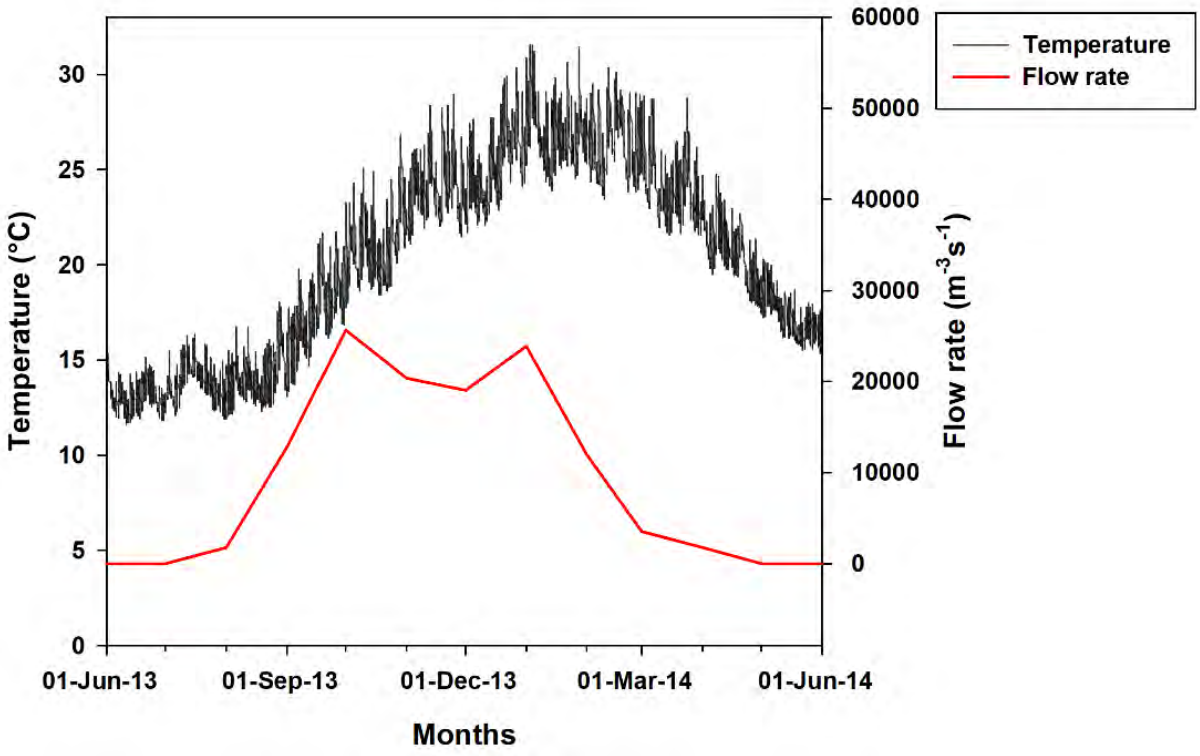

Figure 2.1: Daily temperature (black line) from ML Swart (MLS) irrigation pond for the period May 2013-July 2014, and monthly flow variability (red line) into ML Swart (MLS) irrigation pond in the Sundays River valley, Eastern Cape, South Africa. Flow rate ($m^{-3}s^{-1}$) pond inflow data were obtained from the Sundays River Irrigation Board and water temperature data were obtained from the HOBO logger that was placed in ML Swart (MLS) irrigation pond.

Fish species

The Sundays River flows through many distinct bioregions and provides varying habitats for fish and following Woodford *et al.* (2013), upon its completion in 1978, the Orange-Fish-Sundays IBWT scheme resulted in the passive introduction of 21 fish species in Darlington dam, the irrigation network and four distinct zones in the Sundays River main stem (Table 2.1). The outline of the biology and ecology of the four main colonisers of these irrigation ponds follows.

Table 2.1: Distribution of species in the Sundays River system from Darlington dam. Species are categorized as: Alien to the system (A); alien Orange River fauna (O); native freshwater spawning fish (N); catadromous eel (E); and marine spawning fishes (M). Faunal zones along the Sundays River are divided as follows: within Darlington reservoir (DR); river upstream of the off-take canal at Korhaansdrift (UR); inside the off-take canal (IO); inside the irrigation ponds (IP); river upstream of Cleveland weir in the middle reaches (CW); lower river between Cleveland weir and the Biggs causeway (LR); and below Biggs causeway in the estuarine-influenced zone (EI). Presence records are indicated with a 1 and shaded in grey. Extracted from Woodford *et al*. (2013).

Species	**Group**	**DR**	**UR**	**IO**	**IP**	**CW**	**LR**	**EI**
Common carp, *Cyprinus carpio* (Linnaeus, 1758)	A	1	1	1	1	1	1	1
Banded tilapia, *Tilapia sparrmanii* (Smith, 1840)	A	0	0	0	0	1	1	1
Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852)	A	1	1	1	1	1	1	1
Western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853)	A	1	1	1	1	1	1	1
Sharptooth catfish, *Clarias gariepinus* (Burchell, 1822)	O	1	1	1	1	1	1	1
Smallmouth yellowfish, *Labeobarbus aeneus* (Burchell, 1822)	O	1	1	1	1	0	0	0
Orange river mudfish, *Labeo capensis* (Smith, 1841)	O	1	1	0	1	0	0	0
Goldie barb, *Barbus pallidus* (Smith, 1841)	N	0	0	0	0	1	1	1
Moggel, *Labeo umbratus* (Smith, 1841)	N	1	1	1	1	0	1	0
River goby, *Glossogobius callidus* (Smith, 1937)	N	1	1	1	1	1	1	1
Round-herring, *Gilchristella aestuaria* (Gilchrist, 1914)	N	1	1	1	1	1	0	0
Longfin eel, *Anguilla mossambica* (Peters, 1852)	E	1	1	1	1	1	1	1

Table 2.1: Continued

Species	Group	DR	UR	IO	IP	CW	LR	EI
Mottled eel, *Anguilla marmorata* (Quoy and Gaimard, 1824)	E	0	0	0	0	0	1	1
Cape moony, *Monodactylus falciformis* (Lacépède, 1801)	M	0	0	0	0	0	1	1
Cape stumpnose, *Rhabdosargus holubi* (Steindachner, 1881)	M	0	0	0	0	0	0	1
Dusky kob, *Argyrosomus japonicus* (Temminck and Schlegel, 1844)	M	0	0	0	0	0	0	1
Flathead mullet, *Mugil cephalus* (Linnaeus, 1758)	M	1	0	0	0	0	1	1
Freshwater mullet, *Myxus capensis* (Valenciennes, 1836)	M	0	0	0	0	0	1	1
Smallspotted grutter, *Pomadasys commersonii* (Lacépède, 1801)	M	0	0	0	0	0	0	1
Threespot wrasse, *Liza trimaculatus* (Valenciennes, 1836)	M	0	0	0	0	0	0	1
White Steenbras, *Lithognathus lithognathus* (Cuvier, 1829)	M	0	0	0	0	0	0	1

Study ponds

A National Research Foundation-South African Institute for Aquatic Biodiversity (NRF-SAIAB) long-term monitoring programme has been assessing fish species composition and relative abundance since 2011. Four irrigation ponds (Dungbeetle 1; Dungbeetle 2, ML Swart and River Bend) were selected for this study as replicates, they represented a range of ages from first filling until recently, and had no history of stocking by the landowners. The sampling periodicity for the four study ponds during the NRF-SAIAB monitoring programme and during this PhD is summarised in Table 2.2. Details of the ponds are heighted below.

Table 2.2: Sampling events of Dungbeetle 1 (DB1), Dungbeetle 2 (DB2), ML Swart (MLS) and River Bend (RBD) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Sampling events are shaded in grey.

Year	Month	Season	Sampling	DB1	DB2	MLS	RBD
2011	August	Winter	SAIAB				
2011	November	Spring	SAIAB				
2012	January	Summer	SAIAB				
2012	March	Autumn	SAIAB				
2012	May	Autumn	SAIAB				
2012	August	Winter	SAIAB				
2012	December	Summer	SAIAB				
2013	May	Autumn	SAIAB				
2013	August	Winter	SAIAB				
2013	November	Spring	SAIAB				
2014	January	Summer	SAIAB				
2014	May	Autumn	SAIAB				
2014	October	Spring	SAIAB				
2014	December	Spring	SAIAB				
2015	February	Summer	SAIAB				
2015	May	Autumn	SAIAB				
2015	July	Winter	SAIAB				
2016	November	Spring	SAIAB & PhD				
2017	March	Autumn	PhD				

Table 2.2: Continued							
Year	**Month**	**Season**	**Sampling**	**DB1**	**DB2**	**MLS**	**RBD**
2017	June	Winter	PhD				
2017	September	Spring	SAIAB & PhD				
2017	December	Summer	PhD				

At each sampling event for all the ponds, water temperature (°C), pH and water depth (m) were routinely measured (see Table 2.3 for trends) and fish densities were determined from seine netting. Depending on the surface area of each pond, 3 or 4 hauls of a 30 x 2 m seine net with 12 mm mesh wings and an 8 mm mesh cod-end were conducted and these sampled areas of each pond were chosen as the sampling sites for all data chapters (Figure 2.2). Prior to each haul, the rope of the seine net was allowed to extend a minimum of 5 meters to a maximum of 25 meters from shore from one side of the pond and then the net was deployed in a semi-circle near the centre of the ponds from an inflatable boat and retrieved from the other side (Figure 2.2).

Table 2.3: Mean values (mean ± SD) of pond characteristics for each of the four ponds studied in the Sundays River valley irrigation ponds, Eastern Cape, South Africa.

	Autumn	**Winter**	**Spring**	**Summer**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Dungbeetle 1 (DB1)				
Temperature (°C)	24.5 ± 1.1	13.9 ± 1.3	20.3 ± 0.4	26.0 ± 1.7
pH	9.0 ± 0.4	8.8 ± 0.3	8.7 ± 0.4	9.2 ± 0.1
Water depth (m)	1.70 ± 0.34	1.54 ± 0.25	1.59 ± 0.41	1.68 ± 0.44
Dungbeetle 2 (DB2)				
Temperature (°C)	21.9 ± 6.1	11.4 ± 0.1	23.9 ± 4.9	24.9 ± 1.2
pH	9.3 ± 0.1	8.3 ± 0.2	8.8 ± 0.3	8.9 ± 0.4
Water depth (m)	2.92 ± 0.86	4.62 ± 2.80	3.47 ± 1.27	2.78 ± 1.28
ML Swart (MLS)				
Temperature (°C)	19.3 ± 1.3	13.1 ± 0.5	23.0 ± 5.0	21.8 ± 7.3
pH	9.2 ± 0.1	9.3 ± 0.2	9.1 ± 0.7	9.6 ± 0.3
Water depth (m)	2.01 ± 0.96	2.16 ± 0.88	2.45 ± 0.85	1.89 ± 0.71
River Bend (RBD)				
Temperature (°C)	20.7 ± 3.9	12.1 ± 0.1	24.9 ± 3.3	26.6 ± 2.8
pH	9.3 ± 0.1	8.4 ± 0.0	9.0 ± 0.0	9.5 ± 0.1
Water depth (m)	3.10 ± 0.91	3.61 ± 1.20	3.40 ± 1.21	3.21 ± 1.21

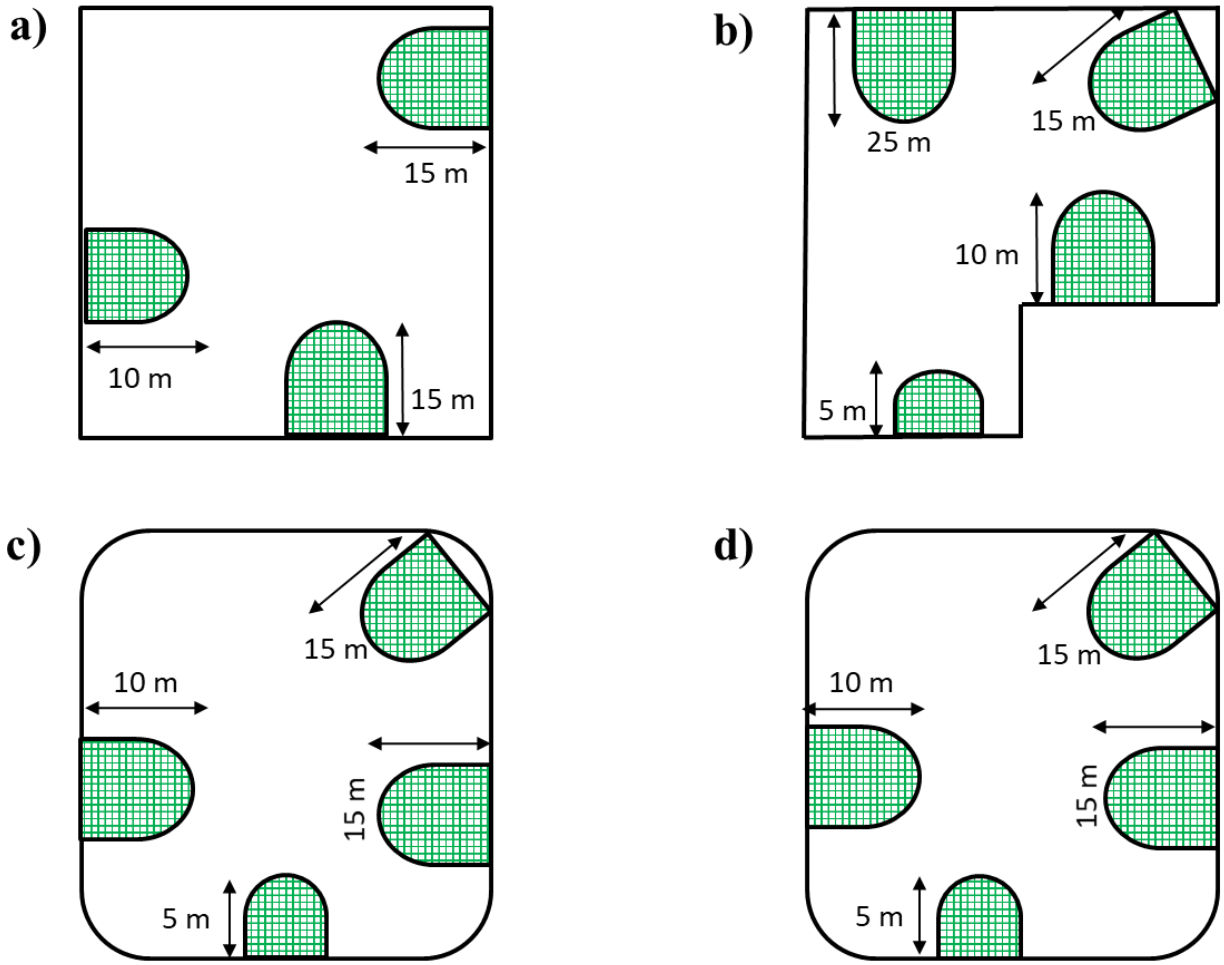

Figure 2.2: Schematic representation of the different seine hauls conducted per pond, a) Dungbeetle 1 (DB1); b) Dungbeetle 2 (DB2); c) ML Swart (MLS) and d) River Bend (RBD) irrigation ponds, Eastern Cape South Africa.

Dungbeetle 1

Dungbeetle 1 (33°26'33"S; 25°40'57"E), hereafter referred to as DB1 (Figure 2.3), was drained, refurbished and filled in May 2013. The pond is 150 m long and 95 m wide and covers an area of 14 250 m^2. Over the monitoring period water temperatures was 24.5 ± 1.1 °C in autumn, decreased

to 13.9 ± 1.3 °C in winter, 20.3 ± 0.4°C in spring and the highest temperature recorded from DB1 was 26.0 ± 1.7 °C in summer (Table 2.3). The pH in autumn was 9.0 ± 0.4, in winter pH was 8.8 ± 0.3, in spring pH was 8.7 ± 0.4 and in summer, pH was 9.2 ± 0.1 (Table 2.3). Water depth from DB1 was 1.70 ± 0.34 m in autumn, 1.54 ± 0.25 m in winter, 1.59 ± 0.41 m in spring and 1.68 ± 0.44 m in summer (Table 2.3).

Figure 2.3: Photograph illustrating Dungbeetle 1 (DB1) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. (Photo: Lubabalo Mofu).

The abundance data from DB1 for the four fish species under consideration in this study are shown in Table 2.4. *Glossogobius callidus* densities ranged from 0.6 ± 0.3 fish/100m^2 in September 2017 to 7.7 ± 0.5 fish/100m^2 in July 2015 (Table 2.4). *Gilchristella aestuaria* densities ranged from a

low of 0.2 ± 0.1 fish/100m^2 in May 2015 to 48.7 ± 23.1 fish/100m^2 in November 2016 (Table 2.4). The lowest post-establishment density of *O. mossambicus* was 0.4 ± 0.2 fish/100m^2 in November 2016 and the highest was 7.0 ± 2.7 fish/100m^2 in September 2017 (Table 2.4). *Gambusia affinis* density ranged from 0.2 ± 0.1 fish/100m^2 in December 2014 to 66.9 ± 54.7 fish/100m^2 in December 2014 (Table 2.4).

Table 2.4: Density (fish/100m^2) for *Glossogobius callidus* (GLC), *Gilchristella aestuaria* (GIA), *Oreochromis mossambicus* (ORM) and *Gambusia affinis* (GAA) from Dungbeetle 1 (DB1) irrigation ponds, Eastern Cape, South Africa.

Year	Pond age	Season	GLC (fish/100m^2)	GIA (fish/100m^2)	ORM (fish/100m^2)	GAA (fish/100m^2)
2014	0.75	Summer	2.6 ± 0.7	2.3 ± 0.6	0.0	2.7 ± 1.9
2014	1.08	Autumn	2.3 ± 0.9	0.2 ± 0.1	0.0	62.3 ± 19.2
2014	1.50	Spring	6.6 ± 2.2	8.8 ± 8.7	0.0	0.2 ± 0.2
2014	1.66	Summer	1.6 ± 0.3	0.9 ± 0.4	0.0	0.2 ± 0.1
2015	1.83	Summer	3.2 ± 1.1	2.7 ± 1.2	0.0	23.0 ± 22.4
2015	2.08	Autumn	4.6 ± 4.6	0.3 ± 0.3	0.0	1.4 ± 1.4
2015	2.25	Winter	7.7 ± 0.5	0.3 ± 0.2	0.0	3.7 ± 1.2
2016	3.58	Spring	0.0	48.7 ± 3.6	0.4 ± 0.2	66.9 ± 54.7
2017	4.41	Spring	0.6 ± 0.3	1.2 ± 0.9	7.0 ± 2.8	18.4 ± 9.8

Dungbeetle 2

Dungbeetle 2 (33°26’37”S; 25°40’45”E), hereafter referred to as DB2 (Figure 2.4), was first filled in December 2013. The pond is 270 m long and 105 m wide and covers an area of 28 350 m^2. Over

the monitoring period water temperatures was 21.9 ± 6.1 °C in autumn, 11.4 ± 0.1 °C in winter, 23.9 ± 4.9 °C in spring and 24.9 ± 1.2 °C in summer (Table 2.3). The pH in autumn was 9.3 ± 0.1, in winter pH was 8.3 ± 0.2, in spring pH was 8.8 ± 0.3 and in summer, pH was 8.9 ± 0.4 (Table 2.3). The recorded water depth from DB2 in autumn was 2.92 ± 0.86 m, increased in winter to 4.62 ± 2.80 m, decreased in spring to 3.47 ± 1.27 m and in summer water depth was 2.78 ± 1.28 m (Table 2.3).

Figure 2.4: Photograph illustrating Dungbeetle 2 (DB2) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. (Photo: Lubabalo Mofu).

The abundance data from DB2 for the four species under consideration in this study are shown in Table 2.5. From DB2, *G. callidus* had the lowest CPUE of 0.5 ± 0.1 fish/100m^2 in January 2014 and the highest CPUE of 21.2 ± 8.6 fish/100m^2 in May 2015 (Table 2.5). The lowest recorded

CPUE of *G. aestuaria* from DB2 was 0.3 ± 0.3 fish/100m^2 in July 2015, while the highest recorded CPUE was 107.4 ± 38.1 fish/100m^2 in September 2017 (Table 2.5). From DB2 the lowest recorded CPUE for *O. mossambicus* was 0.1 ± 0.1 fish/100m^2 in September 2017 and the highest CPUE of 13.5 ± 0.1 fish/100m^2 occurred in May 2015 (Table 2.5). From DB2, *G. affinis* had the lowest CPUE of 0.8 ± 0.7 fish/100m^2 in July 2015 and the highest CPUE of 26.4 ± 4.3 fish/100m^2 in February 2015 (Table 2.5).

Table 2.5: Catch per Unit Effort (fish/100m^2) for *Glossogobius callidus* (GLC), *Gilchristella aestuaria* (GIA), *Oreochromis mossambicus* (ORM) and *Gambusia affinis* (GAA) from Dungbeetle 2 (DB2) irrigation ponds, Eastern Cape, South Africa.

Year	Pond age	Season	GLC (fish/100m^2)	GIA (fish/100m^2)	ORM (fish/100m^2)	GAA (fish/100m^2)
2014	0.16	Summer	0.5 ± 0.1	0.0	0.0	0.0
2014	0.50	Autumn	0.0	0.0	0.0	1.3 ± 0.1
2014	0.91	Spring	1.3 ± 0.5	0.0	0.0	0.0
2014	1.08	Summer	1.4 ± 0.5	0.4 ± 0.3	0.0	1.0 ± 0.8
2015	1.25	Summer	17.2 ± 10.4	0.9 ± 0.6	0.0	26.4 ± 4.3
2015	1.50	Autumn	21.2 ± 8.6	0.2 ± 0.2	13.5 ± 0.1	11.3 ± 2.5
2015	1.66	Winter	10.1 ± 3.8	0.3 ± 0.3	0.0	0.8 ± 0.7
2016	3.00	Spring	7.1 ± 0.9	14.9 ± 11.1	0.0	0.9 ± 0.4
2017	3.83	Spring	2.3 ± 1.1	107.4 ± 38.1	0.1 ± 0.0	1.6 ± 0.6

ML Swart

ML Swart (33°24'33"S; 25°29'04"E), hereafter referred to as MLS (Figure 2.5), was first filled in February 2011. The pond is 86 m long and 57 m wide and covers an area of 4 902 m^2. Over the monitoring period water temperatures varied across seasons, in autumn the temperature was 19.3 ± 1.3 °C, the temperature decreased in winter to 13.1 ± 0.5 °C, 23.9 ± 4.9 °C was the highest recorded temperature in spring and 21.8 ± 7.3 °C was recorded in summer (Table 2.3). The pH in autumn was 9.2 ± 0.1, in winter pH was 9.3 ± 0.2, in spring pH was 9.1 ± 0.8 and in summer, pH was 9.6 ± 0.3 (Table 2.3). The recorded water depth from MLS in autumn was 3.05 ± 0.97 m, 2.16 ± 0.88 m in winter, increased in spring to 2.45 ± 0.85 m and in summer water depth was 1.89 ± 0.71 m (Table 2.3).

Figure 2.5: Photograph illustrating ML Swart (MLS) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. (Photo: Lubabalo Mofu).

The abundance data from MLS for the four species under consideration in this study are shown in Table 2.6. From MLS the lowest recorded CPUE for *G. callidus* was 0.4 ± 0.3 fish/100m^2 in August 2013, and the highest recorded CPUE was 0.6 ± 36.9 fish/100m^2 in January 2012 (Table 2.6). From MLS the lowest recorded CPUE for *G. callidus* was 0.4 ± 0.3 fish/100m^2 in August 2013, and the highest recorded CPUE was 0.6 ± 36.9 fish/100m^2 in January 2012 (Table 2.6). The lowest recorded CPUE for *G. aestuaria* from MLS was 12.8 ± 6.1 fish/100m^2 in September 2017 and the highest CPUE of 1120.2 ± 674.9 fish/100m^2 was recorded from MLS in August 2012 (Table 2.6). *Oreochromis mossambicus* had the lowest CPUE of 0.1 ± 0.1 fish/100m^2 in August 2013 and the highest CPUE of 15.5 ± 2.4 fish/100m^2 was recorded from MLS in March 2012 (Table 2.6). From MLS, *G. affinis* had the lowest CPUE of 0.2 ± 0.1 fish/100m^2 in August 2013 and the highest CPUE of 20.7 ± 10.6 fish/100m^2 in March 2012 (Table 2.6).

Table 2.6: Catch per Unit Effort (fish/100m^2) for *Glossogobius callidus* (GLC), *Gilchristella aestuaria* (GIA), *Oreochromis mossambicus* (ORM) and *Gambusia affinis* (GAA) from ML Swart (MLS) irrigation ponds, Eastern Cape, South Africa.

Year	Pond age	Season	GLC (fish/100m^2)	GIA (fish/100m^2)	ORM (fish/100m^2)	GAA (fish/100m^2)
2012	1.00	Summer	62.9 ± 36.9	192.3 ± 120.3	10.8± 6.1	2.1 ± 1.5
2012	1.16	Autumn	13.7 ± 5.4	550.5 ± 251.9	15.5 ± 2.4	20.7 ± 10.6
2012	1.33	Autumn	19.8 ± 8.3	290.1 ± 60.0	5.3 ± 1.5	3.6 ± 0.9
2012	1.58	Winter	4.9 ± 2.0	1120.2 ± 674.9	2.2 ± 0.6	0.3 ± 0.2
2012	1.91	Summer	12.9 ± 9.1	947.5 ± 272.0	4.4 ± 3.9	1.5 ± 1.0
2013	2.33	Autumn	2.1 ± 1.2	432.9 ± 177.5	2.7 ± 1.6	4.5 ± 2.4
2013	2.58	Winter	0.4 ± 0.3	145.4 ± 61.6	0.0 ± 0.0	0.2 ± 0.1
2014	3.00	Summer	0.0	0.0	1.9 ± 0.4	9.1 ± 8.8
2014	3.33	Autumn	0.0	0.0	0.0 ± 0.0	0.1 ± 0.1
2016	5.83	Spring	8.5 ± 2.7	16.0 ± 8.6	0.0	1.2 ± 0.7
2017	6.66	Spring	2.3 ± 3.1	12.9 ± 12.2	2.4 ± 2.8	0.2 ± 0.2

River Bend

River Bend (33°26’23”S; 25°42’25”E), hereafter referred to as RBD (Figure 2.6), was first filled in December 2013. The pond is 135 m long and 128 m wide and covers an area of 17 280 m^2. Over the monitoring period water temperatures was 20.7 ± 3.9 °C in autumn, 12.1 ± 0.1 °C in winter, 24.9 ± 3.3 °C in spring and 26.6 ± 2.8 °C in summer (Table 2.3). The pH in autumn was 9.3 ± 0.1, in winter pH was 8.4 ± 0.0, in spring pH was 9.0 ± 0.0 and in summer, pH was 9.5 ± 0.1 (Table

2.3). The recorded water depth from RBD in autumn was 3.10 ± 0.91 m, 3.61 ± 1.20 m in winter, decreased in spring to 3.40 ± 1.21 m and in summer water depth was 3.21 ± 1.21 m (Table 2.3).

Figure 2.6: Photograph illustrating River Bend (RBD) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. (Photo: Lubabalo Mofu).

From RBD, the lowest recorded CPUE for *G. callidus* was 4.3 ± 0.7 fish/100m^2, and the highest CPUE of 11.2 ± 3.3 fish/100m^2 was recorded in December 2014 (Table 2.7). The lowest recorded CPUE for *G. aestuaria* from RBD was 0.1 ± 0.1 fish/100m^2 in October 2014 and the highest CPUE was 69.9 ± 30.6 fish/100m^2 in November 2011 (Table 2.7). From RBD, the lowest CPUE for the *O. mossambicus* was 0.1 ± 0.1 fish/100m^2 in October 2014 and the highest CPUE of 20.1 ± 4.0 fish/100m^2 occurred in December 2016 (Table 2.7). The lowest recorded CPUE for the *G. affinis*

from RBD was 0.1 ± 0.1 fish/100m^2 in November 2016 and the highest abundance of 31.6 ± 21.1 fish/100m^2 occurred in February 2015 (Table 2.7).

Table 2.7: Catch per Unit Effort (fish/100m^2) for *Glossogobius callidus* (GLC), *Gilchristella aestuaria* (GIA), *Oreochromis mossambicus* (ORM) and *Gambusia affinis* (GAA) from River Bend (RBD) irrigation ponds, Eastern Cape, South Africa.

Year	Pond age	Season	GLC (fish/100m^2)	GIA (fish/100m^2)	ORM (fish/100m^2)	GAA (fish/100m^2)
2014	0.50	Autumn	4.9 ± 2.4	0.0	0.0	10.3 ± 7.0
2014	0.91	Spring	5.9 ± 1.7	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.4
2014	1.08	Summer	11.2 ± 3.3	1.6 ± 1.6	1.7 ± 1.5	6.9 ± 3.9
2015	1.25	Summer	6.5 ± 3.0	7.5 ± 4.4	14.1 ± 7.4	31.6 ± 21.1
2015	1.50	Autumn	4.3 ± 0.7	0.3 ± 0.3	17.1 ± 6.6	18.6 ± 10.2
2015	1.66	Winter	12.2 ± 0.1	14.2 ± 9.4	16.8 ± 0.1	12.7 ± 0.1
2016	3.00	Spring	5.4 ± 1.4	69.9 ± 30.7	10.1 ± 4.0	0.1 ± 0.1
2017	3.83	Spring	5.2 ± 2.4	17.3 ± 4.6	2.0 ± 0.3	0.5 ± 0.2

Study species

River goby, Glossogobius callidus (Smith, 1937)

Glossogobius callidus is a member of the family Gobiidae (Thacker, 2003) (Figure 2.7). Within southern Africa, *G. callidus* is native and is widely distributed in freshwater, estuarine and marine habitats (Strydom & Neira, 2006). This species are small-sized fish (larvae: 2.2-22.9 mm total length, adults: 60-140 mm total length) (Strydom & Neira, 2006; Mofu, 2016). Larvae have a moderate to large head and a long gut (Strydom & Neira, 2006), while adults have a depressed head and an elongated snout (Skelton, 2001). Recent studies have shown that *G. callidus* is a speleophil or hole-nester (Wasserman *et al*., 2015), and has an extended spawning season from spring to early summer (Table 2.8). In addition, *Glossogobius callidus* feed predominately on aquatic invertebrates such as Diptera, Hemiptera, Trichoptera, Odonata, Cladocera, Copepoda, Hydrachnidia, Amphipoda, Mollusca and Teleostei (Mofu *et al*., 2019c).

Figure 2.7: River goby, *Glossogobius callidus* (Photo: Darragh J Woodford taken from the Sundays River valley irrigation ponds, Eastern Cape, South Africa).

Table 2.8: Comparison of life-history parameters of *Glossogobius callidus*, *Gilchristella aestuaria*, *Oreochromis mossambicus* and *Gambusia affinis* from the Sundays River valley irrigation ponds, in Eastern Cape, southern Africa.

Species		L_∞ (mm)	Amax (years)	Spawning season	Reproductive guild	Diet	Reference
Glossogobius callidus	M	70	6	Spring & Summer	Speleophil	Diptera, Hemiptera, Tricoptera, Odonata, Cladocera, Copepoda, Hydracarina, Amphipoda, Mollusca & Teleostei	Wasserman *et al*., 2015; Mofu *et al*. 2019c
Glossogobius callidus	F	64	5	Spring & summer	Speleophil	Diptera, Hemiptera, Tricoptera, Odonata, Cladocera, Copepoda, Hydracarina, Amphipoda, Mollusca & Teleostei	Wasserman *et al*., 2015; Mofu *et al*., 2019c
Gilchristella aestuaria	M	29.77	2	Summer	Pelagic spawner	Phytoplankton, Zooplankton	Kunz (unpublished data); Kimberg *et al*., 2014; This study
Gilchristella aestuaria	F	32.51	2	Summer	Pelagic spawner	Phytoplankton, Zooplankton	Kunz (unpublished data); Kimberg *et al*., 2014; This study
Oreochromis mossambicus	M	168	2	Winter	Mouth brooder	Phytoplankton, Chironomidae, detritus & Algae	James & Bruton, 1992; This study

Table 2.8: Continued

Species		L_∞ (mm)	Amax (years)	Spawning season	Reproductive guild	Diet	Reference
Oreochromis mossambicus	F	186	2	Winter	Mouth brooder	Phytoplankton, Chironomidae, detritus & Algae	James & Bruton, 1992; This study
Gambusia affinis	M	12.2		Summer	Mouth brooder	Algae, crustaceans, insects, amphibian larvae and small fishes	Docherty, 2012; Pyke, 2005; Howell *et al*., 2013; Sloterdijk *et al*., 2015; This study
Gambusia affinis	F	21.45		Summer	Livebearer	Algae, crustaceans, insects, amphibian larvae and small fishes	Docherty, 2012; Pyke, 2005; Howell *et al*., 2013; Sloterdijk *et al*., 2015; This study

Roundherring*, *Gilchristella aestuaria (Gilchrist, 1914)

Gilchristella aestuaria, is a member of the family Clupeidae (Figure 2.8). *Gilchristella aestuaria* is native to the Sundays River valley and occurs in a wide range of salinities and is common in estuaries, rivers and coastal lakes of the east coast of South Africa (Cyrus *et al*., 1993; Whitfield, 1998). This species is a successful coloniser of the Sundays River valley irrigation ponds (Woodford *et al*., 2013; Kimberg *et al*., 2014). *Gilchristella aestuaria* is a pelagic spawner (Kimberg *et al*., 2014) and spawns throughout the year (Blaber *et al*., 1981) (Table 2.8). Length-at-50% maturity was 32.51 mm total length for females and 29.77 mm T_L for males, respectively (Table 2.8). According to Strydom *et al*. (2014), *G. aestuaria* spawns in the middle reaches of estuaries and a high abundance of larvae occurs in the mesohaline zones of estuaries (Strydom *et al*., 2014). *Gilchristella aestuaria* is planktivorous, feeding predominantly on phytoplankton and zooplankton and plays a key ecological role as a mid-trophic species (Blaber *et al*., 1981; White & Bruton, 1983; Whitfield & Harrison, 1996; Harrison, 2005).

Figure 2.8: Estuarine roundherring, *Gilchristella aestuaria* (Photo: Munetsi Zvavahera taken from the Sundays River valley irrigation ponds, Eastern Cape South Africa).

Mozambique tilapia*, *Oreochromis mossambicus (Peters, 1852)

Oreochromis mossambicus is a member of the family Cichlidae (Figure 2.9). Skelton (2001) showed that *Oreochromis mossambicus* attains sizes of up to 400 mm standard length with a moderately deep body. Juveniles and females have a straight head profile, while adult males have a concave head profile and enlarged jaws with forward projecting teeth (Skelton, 2001). Body colour is the main differentiating characteristic between juveniles and adults, whereby juveniles are silvery, with well-defined vertical bars (6-7) and three spots along the flanks (Skelton, 2001). Adults have a silvery olive to deep blue-grey colour, and the dorsal and caudal fins have red margins (Skelton, 2001).

Oreochromis mossambicus is extra-limital to the Sundays River valley irrigation ponds; its natural distribution of *O. mossambicus* ranges from southern Kenya along the inland and coastal regions of south-eastern Africa southwards to the Eastern Cape, South Africa (Holden & Bruton, 1992). Holden and Bruton (1992), reported that *O. mossambicus* occurs in a wide range of habitats as they are found in riverine, lacustrine and estuarine habitats. This species inhabits mud bottoms and well-vegetated areas and is widely distributed around the world. According to Russell *et al*. (2012), *O. mossambicus* exhibits wide environmental tolerances such as high concentrations of ammonia and nitrates, wide temperature regimes and low dissolved oxygen levels. Maddern et al. (2007) reported that *O. mossambicus* is a mouth brooding species and the spawning frequency differs across systems (Table 2.8). In addition, *O. mossambicus* can produce up to 5 brood per female (James & Bruton, 1992).

During the spawning seasons, male *O. mossambicus* form dense nests (arenas or leks); they defend the nests (pits) and attract females for mating (Oliveira & Almada, 1998; Amorim *et al*., 2003). *Oreochromis mossambicus* is considered an omnivorous species feeding on terrestrial vegetation, macrophyte, filamentous algae, phytoplankton, zooplankton, detritus and benthic organisms (Arthington & Bluhdorn, 1994) (Table 2.8).

Figure 2.9: Mozambique tilapia, *Oreochromis mossambicus* (Photo: Professor Olaf L. F Weyl taken from the Sundays River valley irrigation ponds, Eastern Cape South Africa).

Western mosquitofish*, *Gambusia affinis (Baird and Girard, 1853)

Gambusia affinis is a member of the family Poeciliidae (Figure 2.10). Skelton (2001) reported that *G. affinis* are small-sized fish that attain a size of 60 mm total length and are light brown in colour (Skelton, 2001). In addition, females have a deep abdomen and the anal fin is rounded and short. Males have a flattened head, and are more slender, with the anal fin greatly modified. *Gambusia affinis* is non-native to the Sundays River valley and is native to the lowland ponds, lakes and drainages of North America from Mexico to Alabama (Skelton, 2001; Pyke, 2008). Studies have shown that *G. affinis* have been introduced worldwide in efforts to control mosquitoes and the diseases they carry (Lloyd, 1986; Pyke, 2005).

According to Howell *et al*. (2013), *G. affinis* are live-bearing small-sized fish that spawn during the summer months. They are opportunistic omnivores with a diet consisting mainly of algae, crustaceans, insects, amphibian larvae and small fishes (Pyke, 2005). *Gambusia affinis* is counted amongst the *100 World's Worst Alien Invasive Species* (Lowe *et al*., 2000; Kulhanek *et al*., 2011). The ability of *G. affinis* to adapt to changing environmental conditions allows this species to minimize risks of predation (Polverino *et al*., 2018).

Negative impacts of *G. affinis* on native fish species include nipping fins, aggressive exclusion from microhabitat, competition for food and/or resources and the consumption of eggs and juveniles of native species (Keller & Brown, 2008). Studies have shown that *G. affinis* have negative effects on macroinvertebrates, zooplankton and tadpoles (Walton & Mulla, 1991; Baber & Babbitt, 2004). Pyke and White (2000) showed that *Gambusia affinis* threaten native fish and amphibian species through competition and predation dynamics. In addition, the aggression of *G. affinis* on other fish species can force them to move into habitats where they are far more exposed to other predators (Ayala *et al*., 2007).

Figure 2.10: Western mosquitofish, *Gambusia affinis* (Photo: Josie South taken from the Sundays River valley irrigation ponds, Eastern Cape, South Africa).

CHAPTER 3

Seasonal variation and drivers of zooplankton, macroinvertebrate and littoral fish communities.

Introduction

There has been a global increase in networks of artificial water bodies as societal water demands grow (Hill *et al*., 2018; Ouyang *et al*., 2018). Artificial water bodies are often established to facilitate agricultural practices in otherwise non-agrarian environments, particularly in arid regions (Apinda-Legnouo *et al*., 2014). Many agricultural irrigation networks are comprised of numerous small impoundments, with differing degrees of inter-impoundment connectivity and connectivity to water sources such as rivers (Downing *et al*., 2006; Powers *et al*., 2013; Woodford *et al*., 2013). The Sundays irrigation network forms part of a larger inter-basin water transfer (IBWT) scheme whereby water is moved from the Orange River basin to the Great Fish River and Sundays River basins. The Sundays irrigation network provides water to irrigation ponds in the Sundays River valley, which are used as reservoirs to irrigate dairy pastures and citrus orchards in the region (Baird, 2001; Woodford et al., 2013). These ponds are characterised by raised earth walls and so receive no external run-off. Water within these systems is, therefore, derived entirely from the Sundays River through the irrigation canals network or through direct rainfall.

Previous studies conducted on these ponds include a study by Woodford *et al.* (2013), where they examined the fish composition upstream, within and downstream of the Sundays River valley irrigation network. Their study showed that propagule pressure from the irrigation network contributed to the successful establishment of *G. callidus*, *G. aestuaria*, *O. mossambicus* and *G. affinis*. Howell *et al*. (2013) studied the population dynamics of *G. affinis* in the same irrigation ponds where *G. callidus* occurred and found that the abundance of *G. affinis* are not only driven

by water temperature but by also the presence of *G. callidus* and competition for resources. A recent study by Mofu *et al*. (2019) in these ponds examined the feeding ecology of *G. callidus* that has successfully colonised the ponds. They showed that *G. callidus* is a generalist invertebrate predator, feeding on any abundant prey including juvenile conspecifics and other fishes. However, there is no further information on the community structure of these ponds.

Plankton, macroinvertebrates, and fish comprise significant components of the biological communities that establish within artificial water bodies (Abellán *et al*., 2006; Woodford *et al*., 2013; Hill *et al*., 2017). However, in many developing regions, basic ecological information on the aquatic faunal groups associated with artificial waters is inadequately researched (Mimouni *et al*., 2015). Such ecological knowledge is important for interpreting information regarding fish feeding biology and even for determining productivity, which in turn might inform their utility for developing fisheries. The present study forms part of a larger investigation into the trophic ecology of these ponds and I specifically describe the community structure found in this extensive network of ponds in the Eastern Cape of South Africa. As such, these ponds represent relatively simple systems in which to explore biological and ecological questions, such as bottom-up and top-down drivers of population and community dynamics, community succession patterns and even food web structures. Bottom-up processes regulate nutrient availability to higher trophic levels, whereby a given trophic level is controlled by its resource, while top-down factors refer to the effects of predators on their prey (Hairston *et al*., 1960; McQueen *et al*., 1986; Lemmens *et al*., 2018). Both bottom-up and top-down factors determine primary production, although these factors differ across systems and under different environmental conditions (Pace *et al*., 1999; Carpenter *et al*., 2001; Matsuzaki *et al*., 2018).

This study describes the seasonal patterns in zooplankton, macroinvertebrates and littoral fish communities, and their relative abundances in response to seasonal variations in physico-chemical conditions and chlorophyll-*a* concentrations (as a proxy for phytoplankton) in the Sundays River valley in South Africa. I hypothesised that (i) high temperature and nutrient availability will cause changes in chlorophyll-*a* concentration and subsequently drive the seasonal changes in zooplankton, macroinvertebrates and the littoral fish community structure through bottom-up effects, (ii) seasonal changes in physico-chemical variables underlies differences in zooplankton, macroinvertebrates and littoral fish species richness and diversity.

Materials and methods

Sampling sites

Within the four study ponds (see Chapter 2), four sampling sites were used (see Figure 2.2) and are situated within the Sundays River valley (see Chapter 2 for site description)

Physico-chemical variables

From each pond, four sites were chosen as sampling sites for physico-chemical variables in March 2017, June 2017, September 2017 and December 2017. During each sampling event, physico-chemical parameters (i.e., water depth, temperature, pH, Secchi depth) were recorded at each sampling site. Water temperature (°C) and pH were measured *in-situ* using a multi-meter (HACH, LDO, Germany). Water transparency was measured using a Secchi disk (cm), while a graduated measuring tape with a weight on the tape end was used to measure the water depth (m) of each pond. Water samples (250 mL, n = 4 per pond) were collected and placed on ice for the determination of ammonium (mg/L) and phosphate (mg/L) concentrations within 24 hours in the laboratory. In the laboratory, a HI 83203 multiparameter bench photometer (Hanna Instruments Inc., Rhode Island) was used to analyse ammonium concentrations using the persulphate digestion

method (HACH method 10071) at precision of 0-1 mg/L ± 0.1 mg/L accuracy, while total phosphorus was determined by the PhosVer 3 with acid persulphate digestion method (HACH method 8190 and Standard Method 4500 P-E) at precision of 0-3 mg/L, ± 1 mg/L accuracy).

Chlorophyll-a analysis

Chlorophyll-*a* (chl-*a*) measurements in the water column were conducted to give a proxy of the phytoplankton biomass present and four samples were collected per site. During each sampling event, an additional 4 × 250 mL water samples per pond were collected from the surface for chl-*a* determination. Thereafter, the collected samples were kept on ice for analysis later in the laboratory. Chlorophyll-*a* concentrations were determined fluorometrically (Turner designs 10AU fluorometer) following recommendations by Holm-Hanses and Riemann (1978). In the laboratory, the 100 mL of sample were filtered through a Whatman GF/F 0.7 µm filter. Each filter was extracted in 90% acetone at -20°C for 24 hours in the dark. After 24 hrs, the samples were centrifuged at 5000 rpm for 5 minutes. A 7 mL subsample from the supernatant from the solution was decanted into a glass vial. The vial was inserted into a Turner 10AU fluorometer for readings (Welschmeyer, 1994). After the first reading, the vial was removed and two drops of hydrochloric acid were added and mixed. A second reading was taken and chl-*a* was calculated using the formula:

$$\text{Chl} - a = [7/100 \text{ X } [\text{Before Acid} - \text{After Acid}] \times 0.325] \text{ x } 40 \qquad \text{Equation. 3.1}$$

Zooplankton sampling

Zooplankton samples were collected by pulling a zooplankton net (40 cm diameter, 63 µm mesh size) through the water column from each of the four sampling sites (n = 16 per sampling season: 4 ponds × 4 sites), following the recommendation of Edmonson and Winberg (1971). When sampling was completed, the net was rinsed thoroughly with water to wash all remaining

zooplankton into a 250 mL container. The collected samples were preserved in 70 % ethanol and properly labeled. In the laboratory, the samples were decanted by removing the supernatant after allowing the samples to sediment for 48 hrs to concentrate the sample (Verlencar & Desai, 2004). The remaining ~25 mL concentrated sample was mixed, and a 4 mL subsample was transferred into a petri dish with grid marking for identification. Zooplankton samples were identified under an inverted Nikon TMS light microscope at ×100 and ×200 magnification from each site to the lowest taxonomic level with the aid of keys from Fernando (2002). Zooplankton abundances were presented as relative percentage abundances for each sampling site and pond.

Littoral macroinvertebrates sampling

Littoral macroinvertebrates were sampled by sweeping a length of at least 1.5 m using a 50 cm equilateral triangle-shaped net (0.9 m^2) with a 1.5 m long handle on the littoral zones of each pond. This involved walking along the littoral zones of the ponds and sweeping through the submerged macrophyte vegetation. Upon collection, the littoral macroinvertebrates transferred into a 250 mL plastic honey jar and preserved in 70% ethanol. In the laboratory, the littoral macroinvertebrates were identified to family level according to Gerber and Gabriel (2002). Littoral macroinvertebrates were presented as relative abundance for each sampling event and pond.

Littoral fish sampling

In the littoral zone, small-bodied fish were sampled using the same equipment and technique as for macroinvertebrates. Sampling involved walking along the littoral zones of the ponds and sweeping through the submerged macrophyte vegetation. Littoral macroinvertebrates were presented as relative abundance for each sampling event and pond. The sampling was consistent for each season. Upon collection, the littoral fish were euthanised using an overdose of 40 mg/L of clove oil that was added to the collection buckets containing sampled fish and pond water. Once

euthanised, the littoral fish were preserved in 70% ethanol for later analysis. All fish species were identified to species level and measured to the nearest 0.1 cm total length (T_L).

Data analysis

No raw data collected met assumptions required for parametric tests, such as normality as confirmed by the Shapiro-Wilk normality test. All physico-chemical variables were thus log-transformed and chl-*a* concentrations, zooplankton, littoral macroinvertebrates, and littoral fish abundances were square-root transformed prior analysis. The effects of pond identity and season on physico-chemical variables, chl-*a* concentrations were analysed using factorial ANOVA followed by a Tukey's *post-hoc* test in Statistica version 13.2 (StatSoft Incorporated, 2013). The Kruskal-Wallis test statistic was used to test for differences in zooplankton, macroinvertebrates and littoral fish diversity index (Shannon-Wiener index (H) and Evenness index (E)) across the four seasons. Zooplankton and littoral macroinvertebrate and littoral fish community composition across seasons were assessed using a permutation multivariate analysis of variance (PERMANOVA) with the PRIMER v7 statistical software package (Clarke & Warwick, 2001). Taxon richness, Shannon-Wiener index (H) and Evenness index (E) were calculated for each pond for zooplankton and littoral macroinvertebrates' relative abundance.

Detrended correspondence analysis (DCA) was performed on the transformed abundance data to determine the gradient length for the two axes. The analysis indicated that the maximum gradient length did not exceed 3 standard deviation units. Consequently, redundancy analysis (RDA) was used for the analysis. The direct gradient technique of the RDA was performed using the forward selection method with the Monte Carlo permutation test (9999 permutations), using physico-chemical variables as explanatory variables and the biological parameters as response variables. The individual effects of physico-chemical variables on chl-*a* concentration, zooplankton, littoral

macroinvertebrates, and littoral fish abundance were studied using CANOCO version 5 (ter Braak & Šmilauer, 2002).

Results

Physico-chemical variables

The seasonal variation in physico-chemical variables are summarised in Table 3.1 and Figure 3.1. Temperature (mean ± SD) (25.8 ± 2.3°C), pH (9.3 ± 0.4), water depth (3.1 ± 0.4 m) and the trophic state index (18.2 ± 4.3) were high in autumn, while water clarity (17.1 ± 5.0 cm), ammonium concentrations (0.3 ± 0.4 mg/L) and phosphorus concentrations (0.3 ± 0.2 mg/L) were highest in spring. Temperature significantly differed across pond identity ($P < 0.05$) and across seasons ($P < 0.05$) (Figure 3.1a), and there were significant pond identity × seasons interactions ($P < 0.05$) (Table 3.2). Water depth was observed to differ significantly across pond identity ($P < 0.05$) and across seasons ($P < 0.05$) (Figure 3.1b); however, there were no pond identity × seasons interactions ($P > 0.05$) (Table 3.2). Secchi depth significantly differed across pond identity ($P < 0.05$) and across seasons ($P < 0.05$) (Figure 3.1c), in addition, there were pond identity × seasons interactions ($P < 0.05$). Ammonium concentration significantly differed across pond identity ($P < 0.05$) and across seasons ($P < 0.05$) (Figure 3.1d), in addition, there were pond identity × seasons interactions ($P < 0.05$). The pH and phosphorus concentrations, however, did not differ across pond identity and across seasons ($P > 0.05$) (Table 3.2). A Tukey's *post-hoc* comparison revealed that water temperature significantly differed across ponds ($P < 0.05$) and across seasons ($P < 0.05$) (Table 3.2). Water depth significantly differed between ponds ($P < 0.05$) and across seasons ($P < 0.05$) (Table 3.2). Water clarity significantly differed across ponds ($P < 0.05$) and across seasons ($P < 0.05$) (Table 3.2). A Tukey's *post-hoc* comparison revealed that ammonium concentration significantly differed between ponds ($P < 0.05$) and across seasons ($P < 0.05$) (Table 3.2).

Table 3.1: Physico-chemical variables range and mean with standard deviation (mean ± SD) measured from Dungbeetle 1, Dungbeetle 2, ML Swart and River Bend irrigation ponds seasonally. Abbreviation: SD – standard deviation; Chl-a – Chlorophyll-a; NH+ – Ammonium; P – Phosphorus.

Environmental variable	**Autumn**		**Winter**		**Spring**		**Summer**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Temperature (°C)	23.5 – 29.8	25.8 ± 2.3	13.7 – 18.8	16.6 ± 1.6	17.2 – 20.6	18.3 ± 1.3	21.5 – 28.5	25.4 ± 2.1
pH	8.7 – 10.10	9.3 ± 0.4	8.8 – 9.4	8.7 ± 0.3	8.5 – 9.5	9.1 ± 0.4	8.6 – 9.5	9.3 ± 0.2
Water depth (m)	2.50 – 3.59	3.11 ± 0.40	1.32 – 3.62	2.50 ± 0.71	1.22 – 4.55	3.00 ± 1.11	1.42 – 3.00	1.93 ± 0.54
Secchi depth (cm)	5.00 – 15.00	14.44 ± 2.53	12.01 – 17.12	14.30 ± 1.21	10.00 – 26.01	17.21 ± 5.00	9.00 – 25.01	12.81 ± 3.94
NH^+ (mg/L)	0.1 – 0.5	0.2 ± 0.2	0.1 – 0.5	0.3 ± 0.1	0.0 – 1.2	0.3 ± 0.4	0.0 – 0.1	<0.10
P (mg/L)	0.0 – 0.6	0.2 ± 0.2	0.0 – 0.5	0.1 ± 0.2	0.1 – 0.6	0.2 ± 0.1	0.0 – 1.3	0.2 ± 0.3
Chl-*a* (µg/L)	3.0 – 250.3	89.2 ± 78.5	8.8 – 233.0	88.3 ± 63.3	2.8 – 48.1	11.1 ± 14.4	1.8 – 224.8	36.2 ± 60.3

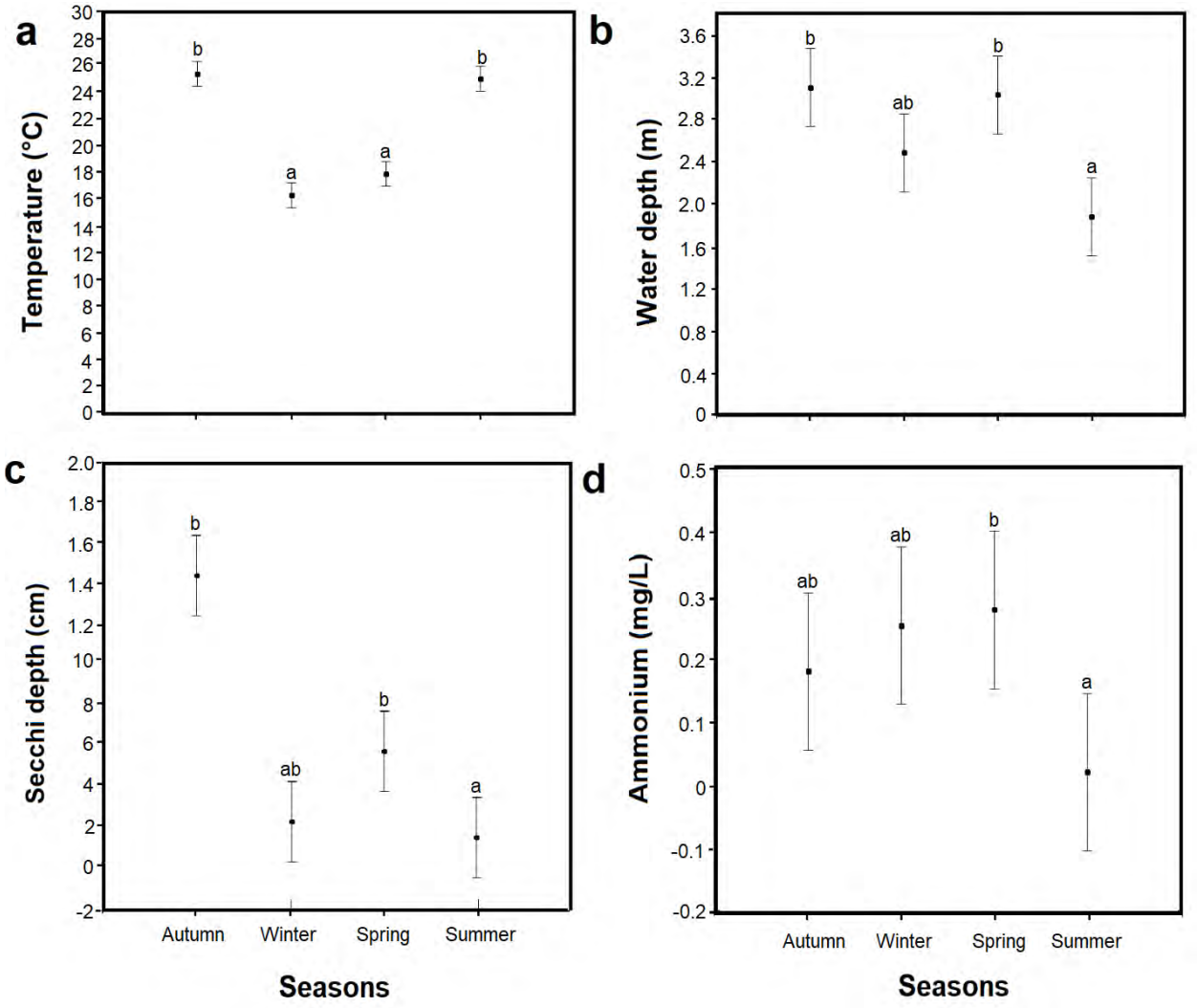

Figure 3.1: Seasonal fluctuation (mean ± SE) of (a) water temperature (°C), (b) water depth (m), (c) secchi depth (cm) and (d) ammonium (mg/L) from the Sundays River valley irrigation ponds. Different symbols (a, b) above bars indicate significant differences at the $P = 0.05$ level (Tukey's *post-hoc* comparison). Mean ± standard deviation of temperature, water depth, secchi depth and ammonium calculated across the four ponds. Note different y-axes scales.

Table 3.2: Factorial ANOVA results of physico-chemical variables and total chlorophyll-*a* (chl-*a*) concentration responses to pond identity and seasons. Significant values ($P < 0.05$) are indicated in bold.

Effect	SS	D*f*	F	*P*	Tukey p*ost-hoc*
Temperature (°C)					
Pond	0.01	3	9.5	**<0.001**	DB1 > DB2 (***P* < 0.001**); DB1 > MLS (***P* = 0.03**); RBD > DB2 (***P* < 0.001**)
Season	0.41	3	270.5	**<0.001**	Autumn > Spring (***P* < 0.001**); Autumn > Winter (***P* < 0.001**); Summer > Spring (***P* < 0.001**); Spring > Winter (***P* < 0.001**); Summer > Winter (***P* < 0.001**)
Pond × Season	0.03	9	7.6	0.396	
pH					
Pond	40	3	0.9	0.42	
Season	51	3	1.2	0.32	
Pond × Season	12	9	1.0	0.49	
Water depth (m)					
Pond	0.13	3	7.6	**<0.001**	DB2 > DB1 (***P* = 0.007**); MLS > DB1 (***P* = 0.003**); RBD > DB1 (***P* < 0.001**)
Season	0.23	3	13.7	**<0.001**	Autumn > Summer (***P* < 0.001**); Autumn > Winter (***P* = 0.02**); Spring > Summer (***P* < 0.001**); Winter > Summer (***P* < 0.001**)
Pond × Season	0.10	9	1.9	0.07	
Secchi depth (cm)					
Pond	0.99	3	26.4	**<0.001**	MLS > DB1 (***P* < 0.001**); MLS > DB2 (***P* < 0.001**); MLS > RBD (***P* < 0.001**)
Season	6.61	3	175.1	**<0.001**	Autumn > Spring (***P* < 0.001**); Autumn > Summer (***P* < 0.001**); Autumn > Winter (***P* < 0.001**); Spring > Summer (***P* < 0.001**); Spring > Winter (***P* < 0.001**)
Pond × Season	1.31	9	11.6	**<0.001**	

Table 3.2: Continued

Effect	SS	D*f*	F	*P*	Tukey p*ost-hoc*
NH^+ (mg/L)					
Pond	0.03	3	5.0	**0.004**	DB1 > MLS (***P* = 0.01**); DB1 > RBD (***P* = 0.007**)
Season	0.07	3	10.6	**<0.001**	Autumn > Summer (***P* = 0.008**); Spring > Summer (***P* < 0.001**); Winter > Summer (***P* < 0.001**)
Pond × Season	0.22	9	10.8	**<0.001**	
P (mg/L)					
Pond	0.03	3	2.3	0.08	
Season	0.02	3	1.5	0.21	
Pond × Season	0.07	9	1.7	0.10	
Chl-*a* (µg/L)					
Pond	39.93	3	1.2	0.33	
Season	430.90	3	12.8	**<0.001**	Autumn > Spring (***P* < 0.001**); Autumn > Summer (***P* = 0.01**); Winter > Spring (***P* < 0.001**); Winter > Summer (***P* = 0.008**)
Pond × Season	119.06	9	1.2	0.33	

Chlorophyll-a concentration

High chl-*a* concentrations were recorded in autumn (mean ± SD) (89.2 ± 78.5 µg/L) (Table 3.1; Figure 3.2). Chlorophyll-*a* concentration did not differ across ponds ($P > 0.05$), however, chl-*a* significantly differed across seasons ($P < 0.05$) (Figure 3.2), in addition, there were no pond identity × seasons interactions ($P > 0.05$) (Table 3.2). Chlorophyll-*a* was significantly higher in autumn *vs* spring ($P < 0.001$) and spring *vs* winter ($P < 0.05$) (Figure 3.2). The RDA separated the sites according to seasons (Figure 3.3). Water clarity was significant ($P < 0.005$) in explaining the variation in the chl-*a* concentration (Table 3.3; Figure 3.3). This variable accounted for 32.1 % (1st axes) and 67.9 % (2nd axes) of the species and physico-chemical variation. The species and physico-chemical correlations were 0.6 (eigenvalue = 0.3) for axis 1 and <0.1 (eigenvalue = 0.1) for axis 2. Spring was indicative of a close relationship with increased water clarity (Figure 3.3). High chl-*a* concentrations were associated with autumn and winter (Figure 3.3).

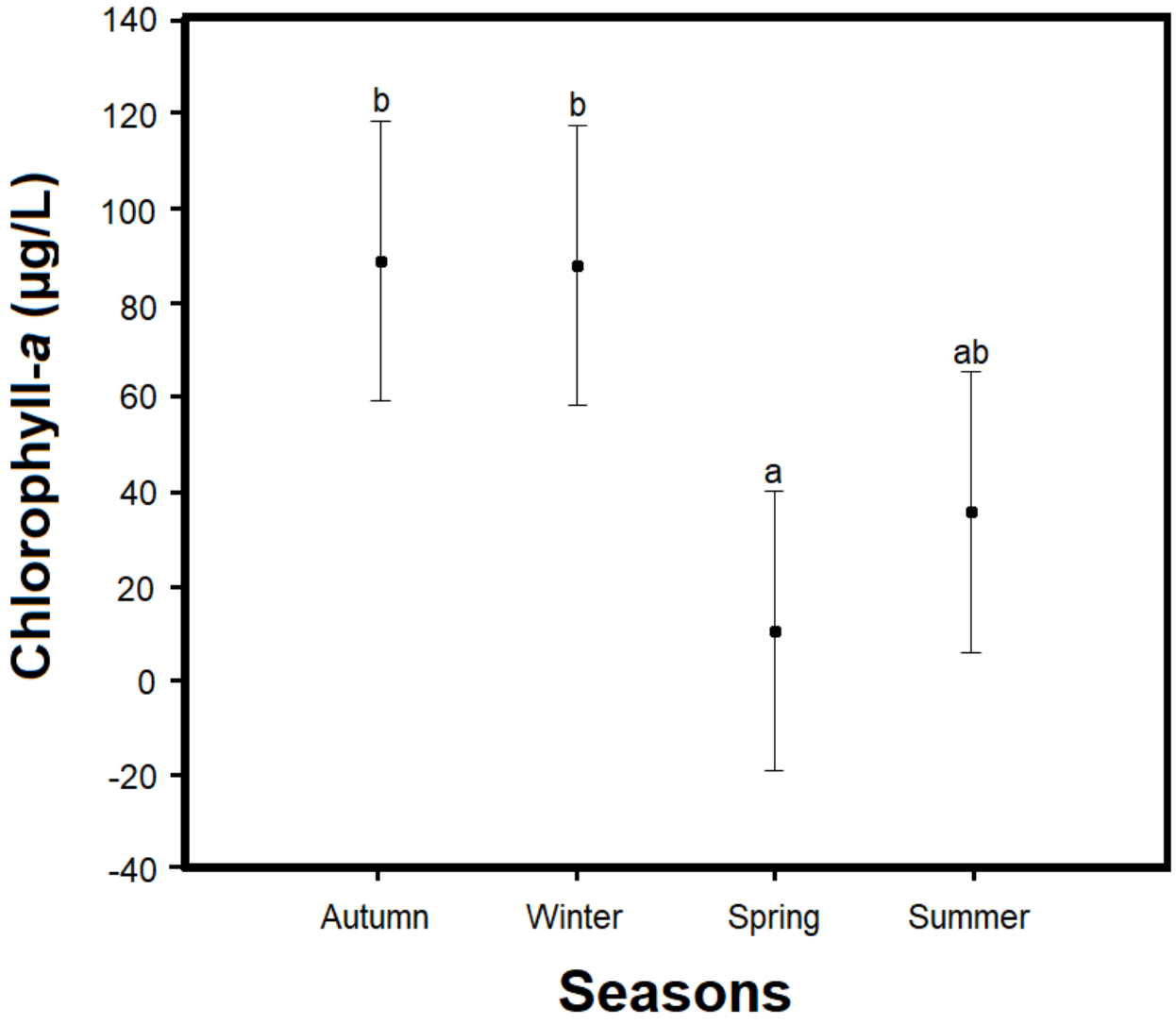

Figure 3.2: Seasonal fluctuation (mean ± SE) of chlorophyll-*a* (µg/L) from the Sundays River valley irrigation ponds. Different symbols (a, b) above bars indicate significant differences at the $P = 0.05$ level (Tukey's *post-hoc* comparison). Mean ± standard deviation of chlorophyll-*a* calculated across the four ponds.

Table 3.3: Results of RDA analysis of physico-chemical variables and biological parameters explaining the chlorophyll-*a* concentration. Significant physico-chemical variables and biological parameters are in bold. Abbreviation: % expl. var - % explained variance.

Physico-chemical variables and biological parameters	% expl. var.	F-ratio	*P*
Secchi depth (cm)	**56.6**	**6.6**	**0.017**
Cladocera	9.0	1.1	0.348
Water depth (m)	6.0	0.7	0.415
pH	4.0	0.5	0.508
Phosphorus (mg/L)	3.3	0.4	0.547
Copepods	2.9	0.3	0.592
Rotifers	1.2	0.1	0.720
Water temperature (°C)	<0.1	<0.1	0.941
Ammonium (mg/L)	<0.1	<0.1	0.970

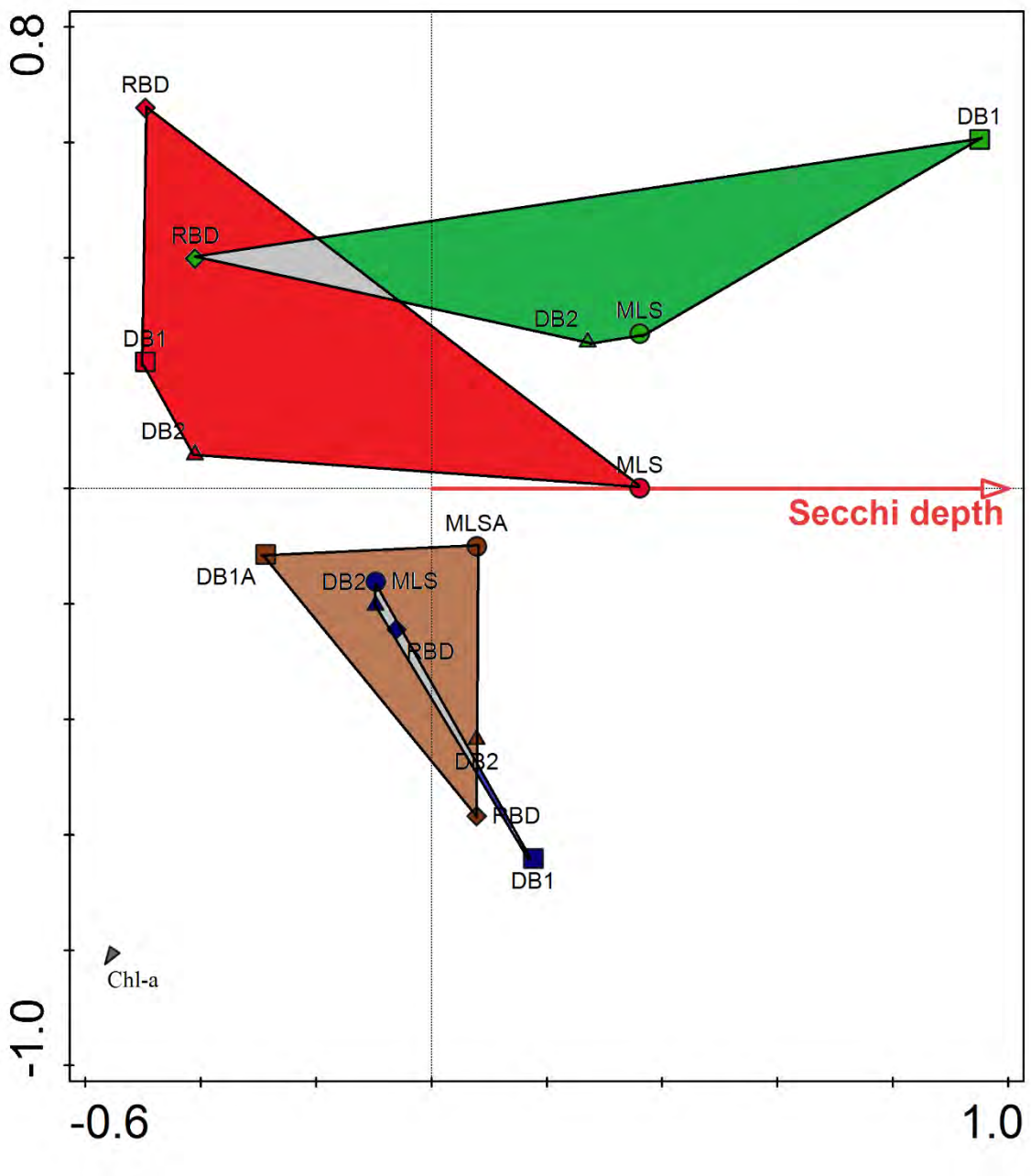

Figure 3.3: RDA ordination tri-plots of chlorophyll-*a* concentration (grey triangles) and associated significant physico-chemical variables (red arrows) collected from the Sundays River valley irrigation ponds (DB1: square; DB2: triangle; MLS: circle; RBD: diamond, Eastern Cape, South Africa seasonally (autumn: brown; winter: blue; spring: green; summer: red). Abbreviations: DB1 – Dungbeetle 1; DB2 – Dungbeetle 2; MLS – ML Swart; RBD – River Bend.

Zooplankton communities

Twenty-five zooplankton taxa (20 Rotifers, 4 Copepoda and 1 Cladocera) were recorded during the study (Table 3.4). Four Rotifera, (i.e. *Polyarthra vulgaris* (Carlin, 1943) (mean: 14.4 %), *Keratella tropica* (Apstein, 1907) (mean: 10.1 %), *Keratella tecta* (Gosse, 1851) (mean: 9.1 %) and *Trichocerca ruttneri* (Donner, 1953) (mean: 6.8 %)), 1 Cladocera (i.e. *Diaphanosoma* sp. (mean: 5.4 %) and two cyclopoid copepods (i.e. *Microcyclops* sp. (mean: 14.9 %) and *Mesocyclops* sp. (mean: 5.4 %)) were the most abundant taxa across all four seasons (Table 3.4). Zooplankton community (taxa) composition differed significantly among seasons (PERMANOVA, $F_{3,15} = 2.99$, $P = 0.002$).

The high taxa richness was observed in winter (11 taxa) (Table 3.4). The diversity index was high in autumn (mean ± SD) (H: 1.8 ± 0.1) and in summer (H: 1.8 ± 0.1), respectively (Table 3.4). Evenness was high in autumn (E: 0.7 ± 0.1) and in summer (E: 0.7 ± 0.1), respectively (Table 3.4). Taxon richness and Shannon-Wiener diversity index differed across seasons ($P < 0.05$), pair-wise comparison found differences (Kruskal-Wallis, $P < 0.05$) between autumn *vs* summer, and winter *vs* summer for the two indices. Evenness differed across seasons (Kruskal-Wallis, $P < 0.05$). Pair-wise comparison found differences (Kruskal-Wallis, $P < 0.05$) in evenness between spring *vs* summer and winter *vs* summer.

Table 3.4: Zooplankton taxa percentage of abundance in the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Dominant values (>10 %) are indicated in bold. Abbreviated names are used in Figure 4b.

Taxa	Abbreviation	Autumn	Winter	Spring	Summer
Rotifera					
Asplanchna sp.	ASP	0.8	0.1		
Brachionus diversicornis (Daday, 1883)	BRD		0.2		4.2
Brachionus falcatus (Zacharias, 1898)	BRFA			0.2	0.4
Brachionus cf. *havanaensis* (Rousselet, 1911)	BRH	7.8	0.8	2.1	**19.0**
Brachionus quadridentatus (Hermann, 1783)	BRQU				**12.6**
Branchionus calyciflorus f. amphiceros (Ehrenberg, 1838)	BRCA			0.6	
Branchionus dimidiatus (Bryce, 1931)	BRDI			1.9	
Cephalodella gibba (Ehrenberg, 1830)	DEG	9.5	9.1		
Euchlanis dilatata (Ehrenberg, 1832)	EUD		0.1	0.2	
Filinia sp.	FIL	0.1	2.8	**21.5**	
Keratella cochlearis (Gosse, 1851)	KECO			1.0	**12.1**
Keratella lenzi (Hauer, 1953)	KEL			6.8	**16.3**
Keratella tecta (Gosse, 1851)	KET	**25.0**	5.7	5.8	
Keratella tropica (Apstein, 1907)	KETR		**27.8**	0.4	**12.3**
Keratella valga (Ehrenberg, 1834)	KEV		0.8	3.7	
Macrochaetus collinsi (Gosse, 1867)	MAC		0.1		
Polyarthra vulgaris (Carlin, 1943)	POV	**12.9**	**10.1**	**34.0**	0.8
Pompholyx sulcata (Gosse, 1851)	POS	0.4	0.1		
Trichocera dongata (Meigen, 1803)	TRD	0.1	0.1	0.2	
Trichocerca ruttneri (Donner, 1953)	TRR		0.2	4.6	**22.4**
Cladocera					
Diaphonosoma sp.	DIA	9.9	**11.8**		
Copepoda/cyclopoida					
Mesocyclops sp.	MES	**12.5**	2.8	6.2	
Microcyclops sp.	MIC	**21.2**	**27.6**	**10.8**	
Taxa richness		11	17	16	9
Shannon-Wiener index		1.8 ± 0.1	1.5 ± 0.3	1.5 ± 0.5	1.8 ± 0.1
Evenness index		0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.1

The RDA analysis identified two significant factors ($P < 0.05$) affecting zooplankton variation; water temperature and water depth (Table 3.5; Figure 3.4). The water temperature and water depth accounted for 18.9 % (1st axes) and 10 % (2nd axes) of the physico-chemical variation. The species

correlations with physico-chemical variables were 0.7 (eigenvalue = 0.2) for axis 1 and 0.9 (eigenvalue = 0.1) for axis 2. The RDA separated ponds seasonally; ponds sampled in autumn were characterised by high temperature and high water depth (Figure 3.4). Zooplankton species associated with autumn were *Asplanca* sp., *Brachionus diversicornis* (Daday, 1883) and *Brachionus falcatus* (Zacharias, 1898). Ponds sampled in winter were characterised by low water depth and temperature, and the zooplankton species associated with winter were *Diaphonosoma* sp., *Macrochaetus collinsi* (Gosse, 1867) and *Filinia* sp. (Figure 3.4). The ponds sampled in spring were characterised by high water depth and associated with *Polyarthra vulgaris*, *Keratella valga* (Ehrenberg, 1834) and *Branchionus calyciflorus f. amphiceros* (Ehrenberg, 1838) (Figure 3.4). Finally, ponds sampled in summer were characterised by high water temperatures and associated with *Keratella lenzi* (Hauer, 1953), *K. cochlearis* (Gosse, 1851) and *Brachionus quadridentatus* Hermann, 1783) (Figure 3.4).

Table 3.5: Results of RDA analysis of physico-chemical variables explaining the zooplankton community. Significant physico-chemical variables and biological parameters are in bold. Abbreviation: % expl. var - % explained variance.

Physico-chemical variables and biological parameters	**% expl. var.**	**F-ratio**	***P***
Water temperature (°C)	**16.1**	**2.7**	**0.034**
Water depth (m)	**12.3**	**2.2**	**0.047**
Ammonium (mg/L)	14.3	2.3	0.061
Chlorophyll-*a* (µg/L)	13.6	2.2	0.069
Gambusia affinis	11.9	1.9	0.109
Glossogobius callidus	9.2	1.4	0.202
Phosphorus (mg/L)	8.3	1.3	0.288
Secchi depth (cm)	5.4	0.8	0.534
Oreochromis mossambicus	5.2	0.8	0.573
pH	5.1	0.7	0.592
Littoral macroinvertebrates	4.2	0.6	0.706

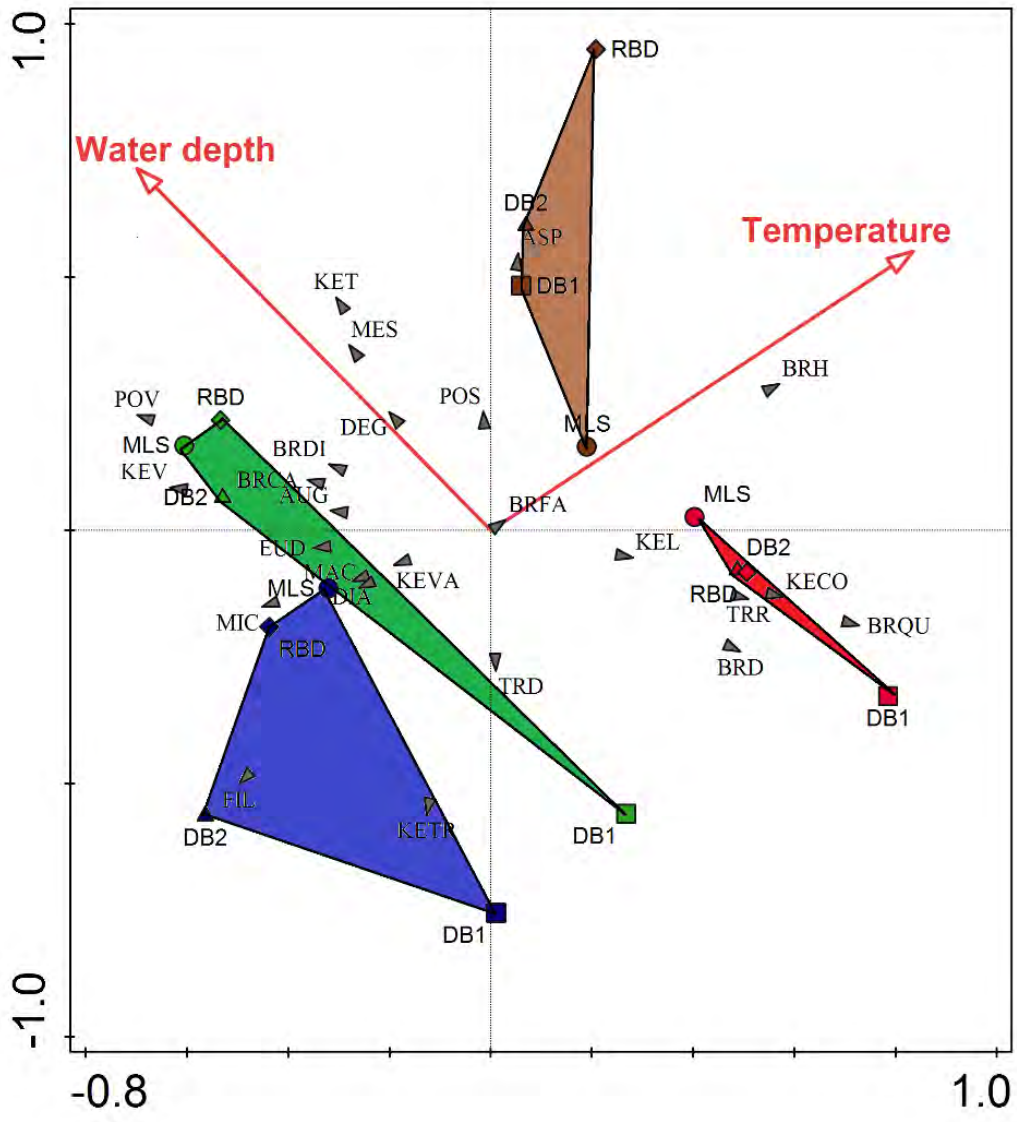

Figure 3.4: RDA ordination tri-plots of the zooplankton species (grey triangles) and associated significant physico-chemical variables and biological parameters (red arrows) collected from the Sundays River valley irrigation ponds (DB1: square; DB2: triangle; MLS: circle; RBD: diamond, Eastern Cape, South Africa seasonally (autumn: brown; winter: blue; spring: green; summer red). Abbreviations: DB1 – Dungbeetle 1; DB2 – Dungbeetle 2; MLS – ML Swart's; RBD – River Bend. Zooplankton species names are presented in Table 3.4.

Littoral macroinvertebrates communities

Twenty-one macroinvertebrates families were recorded during the study period. Insects were the most abundant taxa followed by Annelids and Mollusca across the four seasons. Two Insects, (Corixidae (78.8 %)) and 1 (Naucoridae (6.6 %)) and 1 Annelid (Hirudinea (3.3 %)) were the most abundant taxa across the four seasons (Table 3.6). Taxon richness (7 taxa), H diversity index (1.1 ± 0.7) and Evenness index (0.5 ± 0.1) were high in spring, and in summer, E (0.5 ± 0.1) (Table 3.6). Taxon richness and H differed significantly across seasons (Kruskal-Wallis, $P < 0.05$). Pair-wise comparisons in taxon richness found differences ($P < 0.05$) between autumn *vs* spring, winter *vs* summer, winter *vs* spring and spring *vs* summer. The Evenness index (E) was, however, similar across seasons (Kruskal-Wallis, $P > 0.05$).

Table 3.6: Littoral macroinvertebrates taxa percentage of abundance in the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Dominant values (>10 %) are indicated in bold. Abbreviated names are used in Figure 3.5.

Taxa	Abbreviation	Autumn	Winter	Spring	Summer
Insects					
Unid. Diptera larva	DIP	1.6		0.8	
Chironomidae larvae	CHI		1.8	9.9	0.2
Corixidae	COR	**52.6**	**92.1**	**73.7**	**96.8**
Gyrinidae	GYR			0.2	
Hydrophilidae	HYD		0.1		
Baetidae	BAE		0.1	8.6	
Philopotamidae	PHI	0.4			
Anisoptera	ANI	1.6			
Calopterygidae	CAL	2.0	0.1		0.2
Dytiscidae	DYT			1.6	
Gomphidae	GOM		0.2		
Lestidae	LES	1.2			
Libellulidae	LIB		0.5	0.3	
Naucoridae	NAU	**21.3**	3.5	0.3	1.3
Nepidae	NEP	0.4	0.1	0.2	
Notonectidae	NOT	0.8	0.2	0.6	0.3
Notonemouridae	NOTO			0.1	
Protoneuridae	PRO			0.5	
Pyralidae	PYR				0.1
Annelids					
Hirudinea	HIR	**12.9**			0.3
Mollusca					
Mollusca	PHY	5.2	1.6	3.2	0.8
Taxa richness		11	11	13	8
Shannon-Wiener index		0.8 ± 0.5	0.6 ± 0.6	1.1 ± 0.7	0.3 ± 0.3
Evenness index		0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1

The RDA analysis identified four variables (i.e., water temperature, chl-*a*, *G. callidus* and *G. affinis* as the most important in structuring littoral macroinvertebrates (Table 3.7; Figure 3.5). Physico-chemical variables were 1.0 (eigenvalue = 0.3) for axis 1 and 0.9 (eigenvalue = 0.2) for axis 2. The RDA separated the ponds according to seasons, where the ponds sampled in autumn

and summer were characterised by increased water temperature, high chl-*a* concentration and high abundances of *G. callidus* and *G. affinis* and were associated with Hirudinea and Anisoptera (Figure 3.5). The ponds sampled in winter and spring were characterised by low water temperature, chl-*a,* and *G. callidus* and *G. affinis* abundances (Figure 3.5). The littoral macroinvertebrate families associated with the ponds at these times included Hemiptera, Gyrinidae, and Dytiscidae (Figure 3.5).

Table 3.7: Results of RDA analysis of physico-chemical variables and biological parameters explaining the littoral macroinvertebrate community. Significant physico-chemical variables and biological parameters are in bold. Abbreviation: % expl. var. - % explained variance.

Physico-chemical variables and biological parameters	**% expl. var.**	**F-ratio**	***p***
Water temperature (°C)	**10.2**	**2.5**	**0.006**
Chlorophyll-*a* (µg/L)	**10.4**	**2.3**	**0.031**
Glossogobius callidus	**11.1**	**2.2**	**0.022**
Gambusia affinis	**24.1**	**4.4**	**0.001**
Water depth (m)	7.2	1.7	0.075
Oreochromis mossambicus	6.4	1.5	0.149
Cladocera	5.0	1.1	0.358
Secchi depth (cm)	4.5	1.0	0.435
Phosphorus (mg/L)	3.3	0.7	0.737
Ammonium (mg/L)	2.8	0.6	0.839
pH	2.6	0.5	0.884

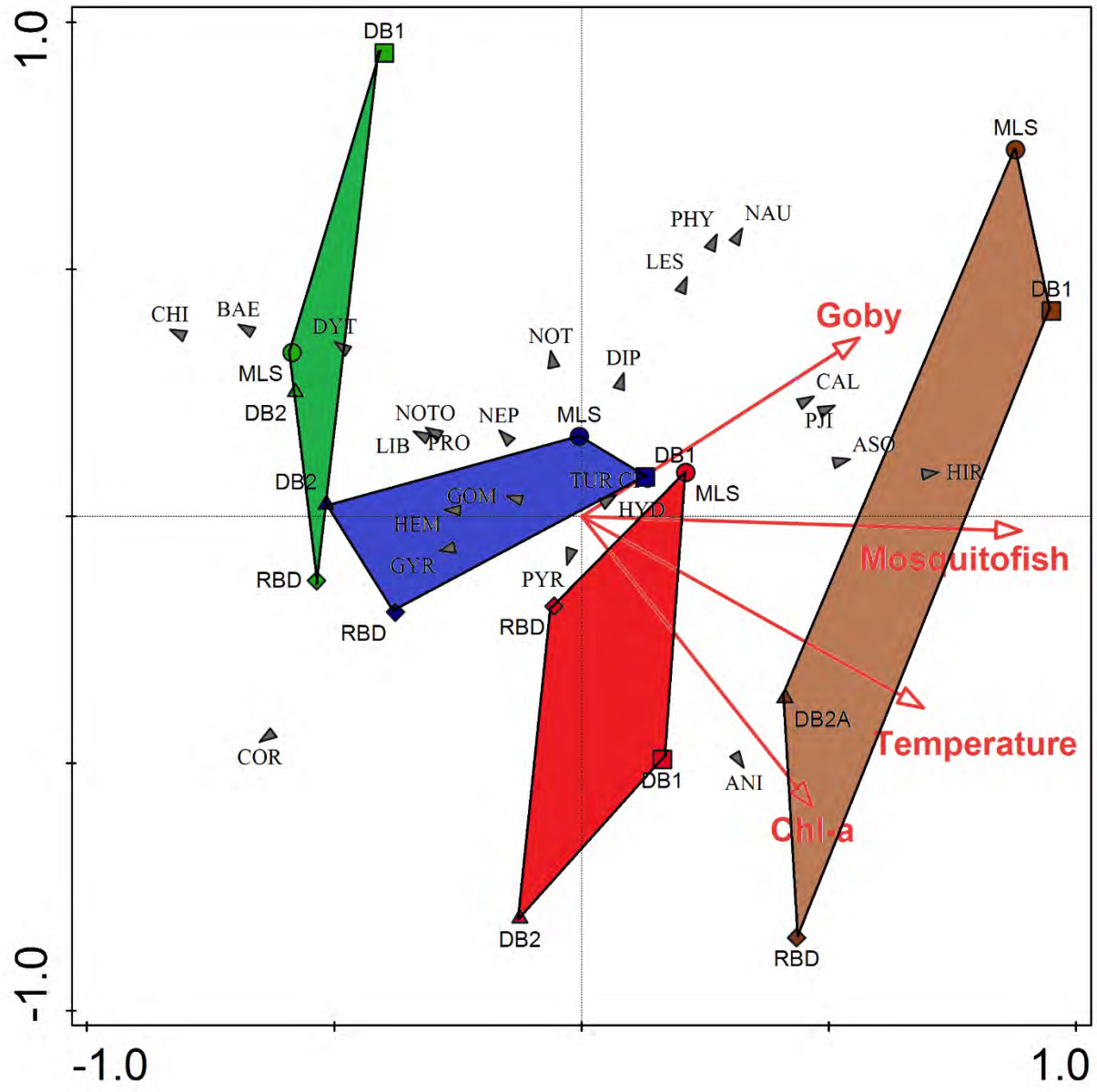

Figure 3.5: RDA ordination tri-plots of littoral macroinvertebrates families (grey triangles-abbreviated names) and associated significant physico-chemical variables and biological parameters (red arrows) collected from the Sundays River valley irrigation ponds (DB1: square; DB2: triangle; MLS: circle; RBD: diamond, Eastern Cape, South Africa seasonally (autumn: brown; winter: blue; spring: green; summer: red). Abbreviations: DB1 – Dungbeetle 1; DB2 – Dungbeetle 2; MLS – ML Swart's; RBD – River Bend; Chl-*a* – Chlorophyll-*a*. Abbreviated littoral macroinvertebrate taxa names are presented in full in Table 3.6.

Littoral fish communities

The littoral fish community was comprised of only three juvenile fish species namely, *G. callidus*, *O. mossambicus* and *G. affinis*. *Gambusia affinis* (72.9 ± 67.7 ind/m^2) with a total length of 3.0 ± 0.1 cm was the most abundant fish throughout the study period (Table 3.8). Taxon richness was high in autumn (3 taxa) and summer (3 taxa), respectively (Table 3.8). The Shannon-Wiener diversity index was high in summer (mean ± SD) (H: 0.4 ± 0.3) and Evenness index was high in winter (E: 1.0 ± 0.1) (Table 3.8). Taxon richness differed significantly across ponds and seasons (Kruskal-Wallis, $P < 0.05$), pair-wise comparison in taxon richness found differences ($P < 0.05$) between MLS *vs* DB2 and between MLS *vs* RBD. In addition, pair-wise comparison found differences ($P < 0.05$) between autumn *vs* winter and between winter *vs* summer. Shannon-Wiener index significantly differed across ponds and seasons (Kruskal-Wallis, $P < 0.05$), pair-wise comparison found differences ($P < 0.05$) between autumn *vs* winter, and winter *vs* summer. Evenness differed significantly across the season (Kruskal-Wallis, $P > 0.05$), pair-wise comparison found differences between autumn *vs* winter and winter *vs* summer.

Table 3.8: littoral fish percentage of abundance and total length (cm) ± standard deviation (SD), taxa richness, Shannon-Wiener index (H) and Evenness index (E) seasonally from the Sundays River valley irrigation ponds, Eastern Cape, South Africa.

Taxa	Autumn		Winter		Spring		Summer	
	Abundance	Length (Mean ± SD)	Abundance	Length (Mean± SD)	Abundance	Length (Mean ± SD)	Abundance	Length (Mean ± SD)
Glossogobius callidus	10.7	5.0 ± 0.3	6.9	3.0 ± 0.5			0.5	2.0 ± 0.4
Oreochromis mossambicus	1.3	2.1 ± 0.4			16.7	2.0 ± 0.5	31.8	4.0 ± 0.2
Gambusia affinis	87.9	3.0 ± 0.1	93.0	4.0 ± 0.3	83.3	2.0 ± 0.3	67.7	3.0 ± 0.3
Taxa richness	3		2		2		3	
Shannon-Wiener index	0.3 ± 0.3		0.1 ± 0.2		0.3 ± 0.3		0.4 ± 0.3	
Evenness index	0.5 ± 0.2		1.0 ± 0.1		0.9 ± 0.1		0.9 ± 0.1	

The RDA identified water temperature as significant ($P < 0.05$) in explaining the variation in fish abundances (Table 3.9; Figure 3.6). This variable accounted for 35.7 % (1st axes) and 33.8 % (2nd axes) of the species and physico-chemical variation. The species correlations with physico-chemical were 0.8 (eigenvalue = 0.4) for axis 1 and 0.8 (eigenvalue = 0.3) for axis 2. The RDA separated the sites according to seasons, with ponds sampled in winter and spring separated from ponds sampled in autumn and summer (Figure 3.6). *G. affinis* and *O. mossambicus* were associated with the autumn and summer season, which were characterised by high temperature, while *G. callidus* was found in high abundances in autumn (Figure 3.6).

Table 3.9: Results of RDA analysis of physico-chemical variables explaining the littoral fish community. Significant physico-chemical variables and biological parameters are in bold. Abbreviation: % expl. var - % explained variance.

Physico-chemical variables and biological parameters	% expl. var.	F-ratio	*p*
Water temperature (°C)	**45.4**	**7.8**	**0.003**
pH	6.1	1.7	0.179
Water depth (m)	5.6	1.6	0.210
Phosphorus (mg/L)	5.6	1.6	0.215
Rotifers	5.4	1.5	0.223
Chlorophyll-*a* (µg/L)	4.7	1.3	0.296
Macroinvertebrates	2.5	0.7	0.582
Cladocera	2.4	0.6	0.599
Ammonium (mg/L)	1.2	0.3	0.812
Copepoda	0.4	0.1	0.951
Secchi depth (cm)	0.3	<0.1	0.966

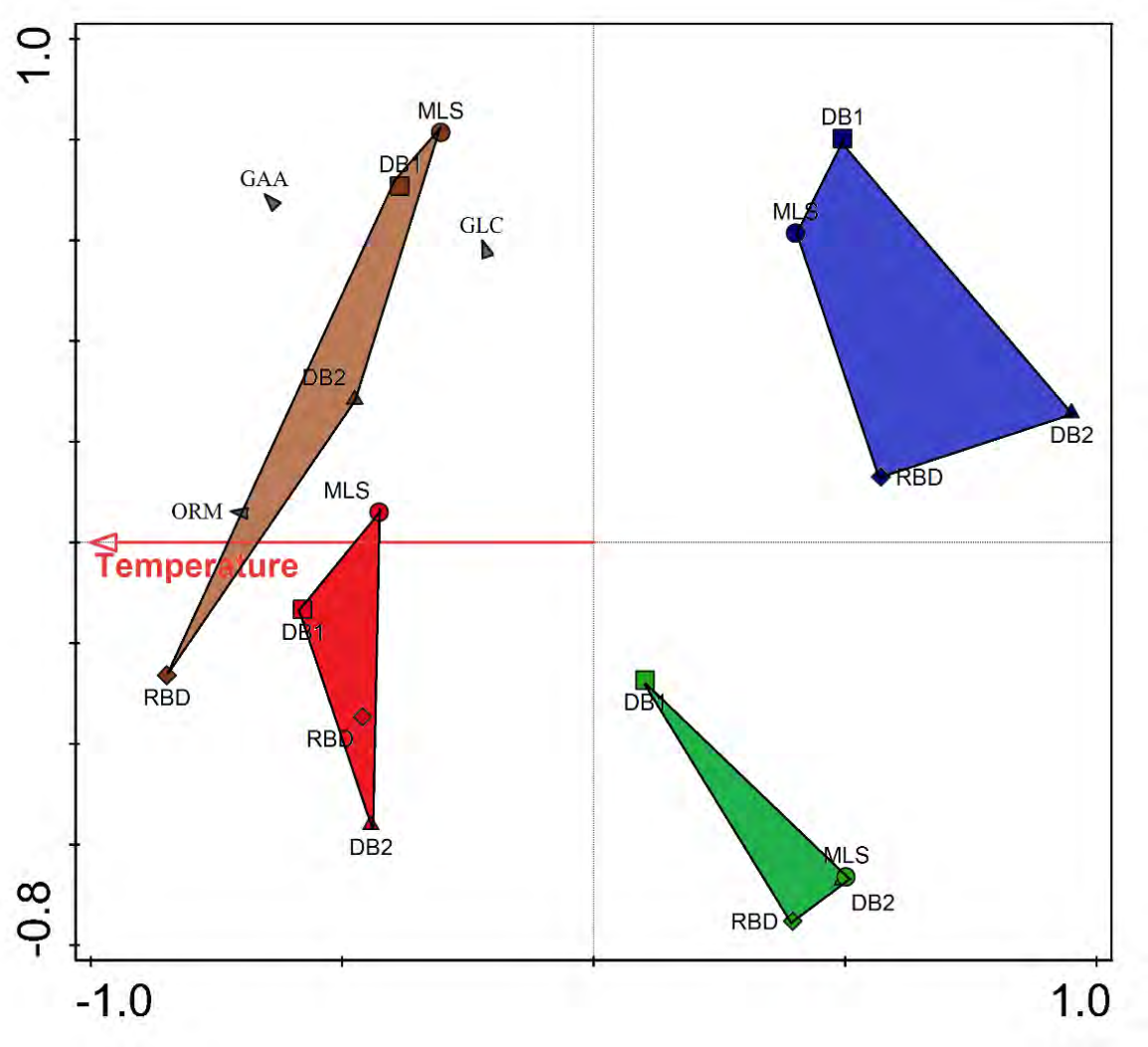

Figure 3.6: RDA ordination tri-plots of *Glossogobius callidus* (GLC), *Oreochromis mossambicus* (ORM) and *Gambusia affinis* (GAA) species (black triangles) and associated significant physico-chemical variables (red arrows) collected from the Sundays River valley irrigation ponds (DB1: square; DB2: triangle; MLS: circle; RBD: diamond, Eastern Cape, South Africa seasonally (autumn: brown; winter: blue; spring: green; summer: red). Abbreviations: DB1 – Dungbeetle 1; DB2 – Dungbeetle 2; MLS – ML Swart's; RBD – River Bend.

Discussion

The results of this chapter highlight that the Sundays River valley irrigation ponds are driven by physico-chemical variables mainly temperature. Overall, the analysis, which took into consideration seasonal effect, showed that water clarity was associated with bottom-up control of primary production in the Sundays River valley irrigation ponds. This is in agreement with studies that showed that primary production was limited by reductions in light availability, which was mainly caused by increases in suspended solids (Welch & Jacoby, 2004; Karlsson *et al*., 2009; Fukushima & Arai, 2015). It is evident from the present results that chl-*a* responded to seasonal changes in water transparency and that water temperature was the main driver of the seasonal changes in chl-*a* concentrations across seasons. This is in agreement with other studies, which found that chl-*a* concentrations are affected by physico-chemical variables such as nutrients (i.e. total phosphorus and total nitrogen), water transparency and temperature (Welch & Jacoby, 2004; Zhang *et al*., 2012; Nhiwatiwa *et al*., 2019). This chapter showed that the pond become turbid, with low chl-a concentration during the winter season and this was likely due to increased water depth.

With regard to the second hypothesis, it was shown that temperature had the greatest effect on the seasonal changes in diversity and abundance of zooplankton, macroinvertebrates and littoral fish. The RDA demonstrated that seasonal changes in water temperature and water depth influenced the relative abundance of the zooplankton community structure. Rotifers were the most dominant zooplankton taxa across all seasons, which was in agreement with the findings of other studies indicating that their small size, fast growth rate and parthenogenetic reproduction generally resulted in high abundances (Inaotombi *et al*., 2016). In this chapter, RDA demonstrated that the zooplankton community was significantly affected by water temperature and water depth. This

study is congruent with other studies that have shown that the seasonal dynamics of zooplankton can be influenced by temperature (Hussain *et al*., 2016). As such, different zooplankton species have a different adaptation to temperature (Florencio *et al*., 2020). In this chapter, during the colder seasons of winter and spring, zooplankton species such as *P. vulgaris* dominated, while in the warmer seasons (autumn and summer) thermophilic species such as *Brachionus* spp. were more abundant.

This chapter highlighted that water temperature and chl-*a* concentrations influenced the abundances, richness, and diversity of both the macroinvertebrate families and littoral fish. Overall, insects were the most abundant macroinvertebrate taxa, with Corixidae found to be the most abundant family across all seasons. Macroinvertebrates abundances were lower in spring and summer, which could suggest impacts of fish (i.e. *G. callidus* and *G. affinis*) predation. Miller and Crowl (2006) also found that the abundance of macroinvertebrates communities was reduced by fish predation. *Glossogobius callidus* and *G. affinis* feed on a variety of food sources such as crustaceans and insects (Pyke, 2005; Mofu *et al*., 2019c). This study was able to show the overarching patterns in terms of the change and response of macroinvertebrates across the sampled seasons. Throughout the study period there were strong environmental changes in physico-chemical variables, and, irrespective of pond characteristics, it was found that changes in physico-chemical variables and abundances of biological parameters corresponded to changes in littoral macroinvertebrates communities. Seasonal changes in physico-chemical variables ultimately resulted in structural changes in chl-*a* concentrations, zooplankton and macroinvertebrates communities. This study further suggests that macroinvertebrate composition may reflect food resource distribution, which ultimately structures the fish communities and drives competition for resources (Macadam & Stockan, 2015; Nhiwatiwa *et al*., 2017).

As the influence of physico-chemical and biotic variables differed between chl-*a* concentrations, zooplankton, littoral macroinvertebrate, and the littoral fish communities, the data from this study indicated that water temperature may strongly influence competition for resources and interactions among species. Smaller ponds are likely to be more greatly affected by seasonal changes than larger ponds (Olmo *et al*., 2016); this study provides insights into the ecology of these irrigation ponds and further illustrates the variation in bottom-up factors regulating the pond community ecology. With increasing water temperature, internal dynamics increased primary production and secondary production. Similar to other studies that investigated the importance of physico-chemical variables in influencing community structure of zooplankton, macroinvertebrates and littoral fish (De Meester *et al*., 2005; Christopher *et al*., 2016); this study showed that freshwater ponds undergo seasonal changes in community structure in response to seasonal changes in physico-chemical variables.

In conclusion, zooplankton, macroinvertebrates and littoral fish community structure may mirror food resource distribution and the findings of this study have contributed towards our understanding of the pond community structure. However, when linking food source distribution and resource use, it is important to have basic knowledge of the drivers of the pond community structure, as physico-chemical variables play an important role in driving what food sources are available. The data obtained from the seasonal sampling of these ponds suggest that food source distribution may be sufficient to structure the pond food web during the warm season (i.e. summer) and that temperature plays and important role in structuring the functioning of ecological communities and is well known to regulate of predator-prey dynamics. The next step is to perform stable isotope analysis to determine the assimilation patterns of the four species (see Chapter 4).

CHAPTER 4

Feeding ecology of fishes in the Sundays River valley irrigation ponds using gut content and stable isotope analyses

Introduction

Quantifying interspecific interactions between native and non-native species within a particular ecosystem is integral to understanding community biology, by providing data on food web dynamics such as competition, resource partitioning and predation (Braga *et al*., 2012; Weidner *et al*., 2017). Assessing the interactions between native and non-native species will provide knowledge on the trophic structure and assemblage composition change instigated by sympatric and successive invaders (Jackson *et al*., 2012; Lombard *et al*., 2017). Therefore, understanding the interactions between native and non-native species is pivotal in understanding changes in trophic structure and species composition (Jackson *et al*., 2012; Tran *et al*., 2015; Copp et al., 2017). The optimal foraging theory predicts that in situations of increased competition, individual species will widen their trophic niche (Stephens & Krebs, 1986). Conversely, sympatric non-native species having the same feeding strategy may exhibit a degree of dietary overlap with native species, which might potentially lead to strong interspecific competition when resources are limited (Jackson *et al*., 2012).

Many studies focusing on interspecific competition for food resources have used gut content analysis to try to understand trophic relationships between predators and prey (Hyslop, 1980; Happel *et al*., 2015; Foley *et al*., 2017). However, gut content analysis, despite being able to provide detailed diet information, does not reflect the predator's long-term assimilation of prey items (Cortés, 1997; Vander Zanden *et al*., 1997; Park *et al*., 2018) because gut content analysis provides only a snapshot of a consumer's diet (usually only hours prior to sampling) (Hyslop,

1980; Evans *et al*., 2018). Additionally, gut content analysis is a laborious technique that often requires considerable taxonomic expertise to identify prey items (Rybczynski *et al*., 2008), and it is necessary to decide whether the observed gut contents are representative of the nutrient intake of a species in general (Blüthgen *et al*., 2003). An alternative tool, therefore, is required, which can provide more information on longer-term nutrient pathways to better describe food web linkages over varying time periods.

The determination of food web structures using stable isotope analysis has increased our understanding of the dynamics of organisms belonging to different trophic levels, giving us a holistic view of ecosystem functioning (Abrantes *et al*., 2014; Vilizzi *et al*., 2019). However, distinguishing and estimating aquatic food web dynamics is exceptionally unpredictable, given the complex network of trophic linkages (Soto *et al*., 2013; Hussey *et al*., 2014). Stable isotope analysis has been used in the last few decades to take into account the complex community structure around a species as well as the presence of extra trophic levels and as a technique to measure variations in consumer trophic position (Anderson & Cabana, 2007; Vander Zanden *et al*., 2015). The stable isotope analysis method is comparatively cost-effective, temporally integrative and can provide information on the long-term assimilation of dietary resources, as well as inform the trophic ecology of predator-prey interactions (Grey, 2006; Rybczynski *et al*., 2008; Thomas & Crowther, 2015). Stable isotope analysis is an increasingly important tool in the study of ecological food webs and has been used to complement stomach content analysis (Peterson & Fry, 1987). As gut content analysis reveals recently ingested food, while stable isotope reflects the diet integrated over a longer period of time, the use of the two methods in combination can provide both long and short-term aspects of feeding ecology (Vander Zanden & Rasmussen, 1999).

In dietary studies of fish, the composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is the most commonly investigated (Peterson & Fry, 1987; Post, 2002). The $\delta^{13}C$ signature provides information on the habitat use of predators, the sources of carbon from the bottom of the food web and the overall diet preference of the predators (Davias *et al*., 2014), whilst $\delta^{15}N$ is used to calculate trophic position and can be the measure of energy transfer (Peterson & Fry, 1987, Vander Zanden *et al*., 1997). Both $\delta^{13}C$ and $\delta^{15}N$ signatures differ between sources, thus providing information on the diet of species (Vander Zanden *et al*., 1997). Stable isotopes provide detailed information on food sources and trophic relationships, allowing for the identification of main food chains, trophic niches, niche overlaps and interspecific diet variability (Vander Zanden *et al*., 1997; Bolnick *et al*., 2003; Post, 2003; Abrantes & Sheaves, 2010). In addition, stable isotope analysis has been extensively used to understand the trophic relationship between native and non-native species (Pilger *et al*., 2010).

The presence/abundance of fish in the littoral zone of the ponds was demonstrated to be a significant explanatory variable with regard to macroinvertebrate abundance in Chapter 3. Literature on the feeding ecology of these fishes suggests that *G. callidus* which primarily feeds on benthic invertebrates (Wasserman, 2012; Mofu *et al*., 2019c), the mostly planktivorous *G. aestuaria* (Blaber *et al*., 1981; Haigh & Whitfield, 1993) and *O. mossambicus*, which forages on phytoplankton (Pérez *et al*., 2006), will occupy well segregated trophic niches and that *G. affinis*, an opportunistic omnivore (Pyke, 2008), will occupy a trophic niche that overlaps that of the three specialist species.

The aim of this chapter is to describe and compare the food web structure and trophic fish community characteristics of the Sundays River valley irrigation ponds and assess the trophic interactions among the four fish species and discuss their potential influences in shaping the fish

community structure in these ponds (see Chapter 2 and 3). To further investigate for the potential for competition between fishes colonising irrigation ponds in the Sundays River system, the objective of this section of the study was to quantify and compare the diet composition of the four main colonising fishes, namely *G. callidus*, *G. aestuaria*, *O. mossambicus,* and *G. affinis* using a combination of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and gut content analyses. This was done during the summer months, when the littoral fish community structure had developed (see Chapter 3).

Stable isotope and gut content analyses were used to test the following hypothesis: 1) Stable isotope analysis and gut content analysis will both reflected resource partitioning among the species

Materials and methods

Study area

Samples for stable isotope and gut content analyses were collected in December 2017 from Dungbeetle 1, Dungbeetle 2, ML Swart and River Bend irrigation ponds in the Sundays River valley (see Chapter 2 for site description). The physico-chemical and biological characteristics of the study ponds have been described in detail in Chapters 2 and 3, and are therefore not repeated here.

Food webs

Stable isotope sample collection and analysis

Given the high diversity and abundance of zooplankton, littoral macroinvertebrates and littoral fish in summer in the study ponds (see Chapter 3); samples for isotope analysis were collected in summer. Samples collected were aquatic plants in the littoral zone, terrestrial plants, detritus, algae, phytoplankton, zooplankton, whole bodies of macroinvertebrates, muscle tissue of molluscs and fish. Sampling techniques for various components followed the procedures described in Chapters 2 and 3. Briefly, phytoplankton and zooplankton were vertically collected using a 20 µm and 63 µm plankton net, respectively. Macroinvertebrates were collected using a 50 cm equilateral triangle-shaped net a net with a 1.5 m long handle (to allow a sampling distance of 1.5 m) and fish were collected using a 30 long × 2 deep m seine net with 12 mm mesh wings and an 8 mm mesh size cod-end and plants and detritus were collected by hand. Upon collection of fish, samples were measured for total length (T_L) to the nearest 1.0 mm and a muscle tissue sample was taken from each individual fish from the caudal peduncle, with all the scales and skin removed. For small (< 10 cm) fish, the whole body was used after removing the head, intestines and eyes. Molluscs were separated from the shell material, while detritus, aquatic plants in the littoral zone and terrestrial plants were handpicked. All collected samples were placed in 1.5 ml Eppendorf tubes for later analysis.

On collection, all samples were stored separately according to site and pond in labelled bags and were kept on ice in a cooler box for processing in the laboratory, which was done within 24 h of collection. Phytoplankton and zooplankton samples were identified to family level using keys by John *et al*. (2002) and Fernando (2002), respectively. Macroinvertebrates were identified to family level according to Gerber and Gabriel (2002). Carbonate-containing shells of molluscs were

removed prior to drying. Once sorted and identified, samples were oven-dried at 60°C for at least 48 h, then ground into a homogenous powder with a pestle and mortar and sent for isotopic analysis at the Stable Isotope Laboratory, Mammal Research Institute (MRI) at the University of Pretoria, South Africa. The stable isotope analysis was carried out using a Flash EA 112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany). Standard delta notation (δ) was used to express stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The isotope ratios in parts per thousand (‰) differences from a standard reference material were expressed as follows:

$$\delta^{13}\text{C or } \delta^{15}\text{N} = \left[\left(\frac{Rsample}{Rstandard} - 1\right)\right] x\ 1000 \qquad \text{Equation. 4.1}$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively (Fry, 1991, Hobson & Clark, 1992). The C:N was in mass ratios. The standards used were referenced to atmospheric nitrogen for $\delta^{15}N$ (Ehleringer & Rundel, 1988) and $\delta^{13}C$ was referenced to Vienna Pee-Dee Belenmite (Craig, 1957). The average analytical precision was < 0.15‰ for $\delta^{13}C$ and < 0.1‰ for $\delta^{15}N$.

Analysis of stable isotope data

Preliminary exploratory analysis revealed that all four ponds had very similar trophic signatures (Figure 4.1). The four studied ponds received water from the same source (see Chapter 1), and contained similar phytoplankton, zooplankton, macroinvertebrate and fish communities (see Chapter 3). Given these similarities, samples from the different ponds were grouped for analysis. For baseline $\delta^{15}N$ values, long-lived primary consumers are typically used (Van Zanden *et al*., 1999; Post *et al*., 2000; Post, 2002). In this study, molluscs collected from the ponds were used to calculate an average $\delta^{15}N_{baseline}$ of 3.16‰ ± 0.87, $\delta^{13}C_{baseline}$ of -27.69‰ ± 2.57, δ^{13}Cmax of -22.31‰, δ^{13}Cmin of -30.69‰, and a carbon range $CR_{baseline}$ of 8.38‰.

All organisms in all ecosystems were then adjusted based on the following equations for $\delta^{15}N$ and $\delta^{13}C$:

$$Trophic\ position = \frac{\delta^{15}N_{organism} - \delta^{15}N_{baseline}}{\Delta^{15}N} + 2, \quad \text{Equation 4.2}$$

where 2 represents the trophic position of the baseline organism, $\Delta^{15}N$ represents the fractionation factor calculated as 3.4‰, $\delta^{15}N_{organism}$ is the isotope ratio of the organism, and $\delta^{15}N_{baseline}$ is the isotope ratio (3.16‰) of the primary consumers used for the baseline (Post, 2002).

$$\delta^{13}C_{corrected} = \frac{\delta^{13}C_{organism} - \delta^{13}C_{baseline}}{CR_{baseline}}, \quad \text{Equation 4.3}$$

where $\delta^{13}C_{corrected}$ is the corrected carbon isotope ratio of the consumer, $\delta^{13}C_{organism}$ is the uncorrected isotope ratio of the organism, $\delta^{13}C_{baseline}$ is the mean primary consumer isotope ratio -27.69‰, and $CR_{baseline}$ is the primary consumer carbon range (δ^{13}Cmax - δ^{13}Cmin = 8.38‰) (Olsson *et al*., 2009; Jackson & Britton, 2014).

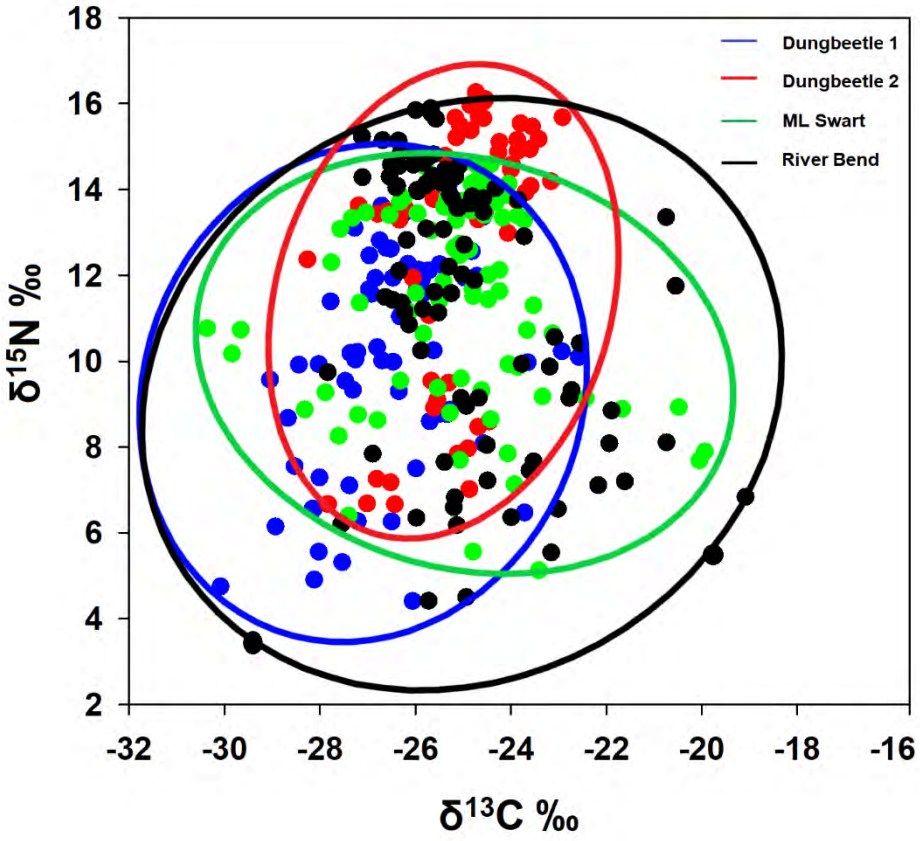

Figure 4.1: Isotopic signatures of the aquatic communities sampled from Dungbeetle 1, Dungbeetle 2, ML Swart and River Bend irrigation ponds, Eastern Cape, South Africa.

For analysis, resources were grouped into broad functional groups (Table 4.1), and fishes were grouped into two size classes as follows *G. callidus*: 40–80 mm T_L hereafter referred to as GLC1; *G. callidus*: 81–140 mm T_L hereafter referred to as GLC2; *G. aestuaria*: 20–40 mm T_L hereafter referred to as GIA1; *G. aestuaria*: 41–80 mm T_L hereafter referred to as GIA2; *O. mossambicus*: 10–40 mm T_L hereafter referred to as ORM1; *O. mossambicus*: 80–400 mm T_L hereafter referred to as ORM2; *G. affinis*: 10–40 mm T_L hereafter referred to as GAA1; *G. affinis*: 40–80 mm L_T T_L hereafter referred to as GAA2, as their diets were perceived to change with size. Fish $\delta^{13}C$ and $\delta^{15}N$ values per size class were compared by means of a one-way analysis of variance (ANOVA). Linear regression was used to assess the ontogenetic shift of $\delta^{13}C$ and $\delta^{15}N$ according to total length

(T_L) for individuals of each species. To analyse the isotopic niche width and overlap among the fish species using their $\delta^{13}C$ and $\delta^{15}N$ values, the Stable Isotope Bayesian Ellipses in R (SIBER) model was used (Layman *et al*., 2007; Jackson *et al*., 2011). Thereafter, to determine the isotopic niche breadths and width for all fish species, the convex hull areas and ellipses were described as per recommendation by Jackson *et al*. (2011).

The standard ellipse areas (SEA_c) were calculated using the corrected isotope data (TP and $\delta^{13}C_{corr}$) to measure the trophic niche width at the species and population level. To describe variation in trophic structure, six community-wide metrics based on stable isotope data (Layman *et al*., 2007) were used. The community-wide metrics illustrate the assemblage trophic niche space as the area occupied within a $\delta^{13}C$-$\delta^{15}N$ biplot: range in $\delta^{13}C$ (dCR_b), where CR_b is a measure of basal $\delta^{13}C$ variation; range in $\delta^{15}N$ (NR_b), where NR_b describes the food chain length or nitrogen range. The extent of trophic redundancy within the assemblage was measured by mean nearest neighbour distance ($MNND_b$) and standard deviation of the nearest neighbour distance ($SDNND_b$). According to Abrantes *et al*. (2014), greater trophic redundancy is indicated through smaller $MNND_b$ and $SDNND_b$. All metrics were bootstrapped ($n = 1000$, as indicated by the subscript 'b'). Total trophic niche space occupied by the assemblage is represented by total area SEA_c (where the subscript 'c' indicates that a small sample size correction was used); this was used to measure population niche size.

To measure the trophic niche size and to test whether trophic niche overlap was not equivalently weighted among species, a probabilistic method was used (Swanson *et al*., 2015). This method measures a given 95% probability niche size and provides directional estimates of pairwise niche overlap in multivariate space (Swanson *et al*., 2015). The proposed method defines the niche overlap of a particular species A onto species B as the fraction of the intersection area between

niche of species A and niche of species B over the total niche area of species B and vice versa. To assess the relative proportion of each food source in the diet of the four fish species across all size classes per species, a Bayesian stable isotope mixing model was used that runs on the R platform (SIAR; Parnel *et al*., 2010; R Development Core Team, 2017). Bayesian mixing models have the advantage of allowing the variation and uncertainties associated with isotopic estimates and trophic enrichment to be propagated through the model, with outputs being more reflective of the natural variability within a system. Prior to SIAR analyses, macroinvertebrates were grouped according to functional feeding groups to minimise the number of sources and to simplify the range of possible solutions (Phillips *et al*., 2005). The SIAR model was fitted via Markov Chain Monte Carlo methods, producing simulations of values of dietary proportions from sources consistent with the data using a Dirichlet prior distribution (Parnell *et al*., 2010).

Gut content analysis

Fish sampling for gut content analysis

Gut content analysis was undertaken to help interpret the results from the stable isotope analysis and to inform the inclusion of prey taxa in the mixing models. To ensure that all four species would be represented in the sample (see Chapter 2) during a time when all potential food resources were available (see Chapter 3); fishes were sampled during austral summer in December 2017 (which coincided with the period of isotope sampling). Sampling was conducted using a 30 m long x 2 m deep seine net with 12 mm mesh wings and an 8 mm mesh size cod-end from Dungbeetle 1, Dungbeetle 2, ML Swart and River Bend ponds (see Chapter 2 for site description). All collected fish were fixed in 10% formalin and later preserved in 70% ethanol for later analysis.

Gut content analysis

To assess the diet composition of the four study species, the gut contents of *G. callidus* (*n* = 98), *G. aestuaria* (*n* = 99), *O. mossambicus* (*n* = 98) and *G. affinis* (*n* = 45) were examined, and the total length (T_L) of each fish was measured to the nearest 1.0 mm and dissected. All items present in the gut were emptied into a 1 mm petri dish, with graduated increments of 1 x 1 mm at the bottom, as described by Wasserman (2012). Items were sorted according to the indirect volumetric method of gut content analysis (Hyslop, 1980). All gut content items were identified to the lowest taxonomic level possible using Day *et al*. (2003), De Moor *et al*. (2003), Gerber and Gabriel (2002). To obtain the volume of each prey item an indirect volumetric assessment was performed, by flattening the gut content items under a microscope slide to a depth of approximately 1 mm in thickness. The number (1 mm x 1mm) of blocks occupied by non-faunal prey groups was estimated as volume occupied. The volume was then calculated as the area an item covered (Wasserman, 2012). Gut contents were expressed as percentage volume (%V) of each prey category (Hyslop, 1980).

Analysis of gut content data

For analysis, fishes were grouped into the size classes as mentioned above. To identify the differences in diet between the fish, prey abundance (%N), frequency of occurrence (%F) and percentage volume (%V) were determined within each size class category per prey item as a percentage of all prey. As is common practice in gut content analyses (Hyslop, 1980), %N was calculated as the number of individuals of any prey item as a percentage of all prey items; %V was the volume occupied by any prey as a percentage of the total volume of all prey items and %F was the number of stomachs containing a given taxon as a proportion of all sampled stomachs. This allowed for the calculation of the Index of Relative Importance (IRI) for each prey category (Pinkas *et al*., 1971) as:

$$IRI = (\%N + \%V) \times \%F$$ Equation 4.4

as recommended by Hyslop (1980). For each prey taxon, the IRI was then expressed as a percentage of the IRI values of all prey categories (%IRI).

To assess dietary similarities between the species, the %IRI was used to construct a classification tree (Bray-Curtis, single link) and Analysis of similarities (ANOSIM) was used to test for significant differences in prey species composition using the PRIMER v7 statistical software package. Cluster analysis in the dendrogram format was identified and assessed using group average hierarchical sorting (Clarke, 1993). Additionally, the contributing taxa to the differences between the group's diet were identified using the SIMPER test.

The (%) of IRI values for each prey item were then later used for all comparative analyses including the determination of niche breadth. To do this the dietary diversity (Levin's niche breadth) equation was used:

$$B = 1/\Sigma\, Pi^2$$ Equation 4.5

where P*i* is the relative frequency of prey item *i*, in the diet of the predator P (Levins, 1968). As P*i* is the denominator, Larger *B* values represent greater dietary diversity. The modification to calculate standardize niche breadth (scale from 0–1) was estimated to Krebs (1989) as follows:

$$B_A = (B-1)/(n-1)$$ Equation 4.6

where B_A is the Levins standardized niche breadth and n is the number of food items.

Results

Food web

The mean $\delta^{13}C$ and $\delta^{15}N$ of the potential resources and fishes from the Sundays River valley irrigation ponds are shown in Table 4.1. A total of 349 samples were collected for stable isotope analysis, comprised of 138 basal resources (terrestrial plants - riparian zone (n = 17), (aquatic littoral zone plants (n = 23)), detritus (n = 15), phytoplankton (n = 12)), zooplankton (n = 16), macroinvertebrates (n = 55), and 210 individual fish samples (GLC1 (n = 26), GLC2 (n = 37), GIA1 (n = 17), GIA2 (n = 38), ORM1 (n = 24), ORM2 (n = 29), GAA1 (n = 29) and GAA2 (n = 11) (Table 4.1).

The mean $\delta^{13}C$ values for phytoplankton and zooplankton were -25.7‰ ± 1.9 and -25.4 ± 0.9‰, respectively (Table 4.1). The littoral zone aquatic and terrestrial plants had a mean $\delta^{13}C$ value of -16.1 ± 5.7‰ and -25.6 ± 6.6‰, respectively (Table 4.1). Detritus had a mean $\delta^{13}C$ values of -24.11 ± 5.2‰. The mean $\delta^{15}N$ for phytoplankton was 6.5 ± 1.5‰ (Table 4.1). Aquatic plants in the littoral zone had a mean $\delta^{15}N$ value of 10.3 ± 3.8‰, while terrestrial plants had a mean $\delta^{15}N$ value of 9.8 ± 2.6‰ (Table 4.1). Detritus had a mean $\delta^{15}N$ value of 6.8 ± 2.8,

For macroinvertebrate FFGs, scrapers, collectors and predators had mean $\delta^{13}C$ values of -24.8 ± 3.8‰, -28.4 ± 1.7‰ and -25.1 ± 1.9‰, respectively (Table 4.1). Scrapers, collectors and predators had a mean $\delta^{15}N$ value of 9.3 ± 2.1, 8.4 ± 2.2 and 9.1 ± 1.8‰, respectively (Table 4.1). The mean $\delta^{15}N$ for zooplankton was 7.9 ± 1.3‰ (Table 4.1). For fish species, the most depleted $\delta^{13}C$ (-22.6 ± 0.9‰) and $\delta^{15}N$ values (9.9 ± 2.1‰) were for ORM2 (Table 4.1). The most enriched $\delta^{13}C$ value was for GAA2 (-19.4 ± 0.6‰), while GIA2 was the most $\delta^{15}N$ enriched (14.4 ± 0.7‰) (Table 4.1). Using one-way ANOVA analysis, significant differences were observed among fish species size classes for $\delta^{13}C$ ($F_{7, 203}$= 19.1, $P < 0.05$).

Table 4.1: Carbon and nitrogen isotope values (mean ± standard deviation) of basal resources, zooplankton, macroinvertebrates and fish sampled from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Abbreviations: GLC1 = *Glossogobius callidus* (40–80 mm T_L); GLC2 = *Glossogobius callidus* (81–140 mm T_L); GIA1 =*Gilchristella aestuaria* (20–40 mm T_L); GIA2 = *Gilchristella aestuaria* (41–80 mm T_L); ORM1 = *Oreochromis mossambicus* (10–40 mm T_L); ORM2 = *Oreochromis mossambicus* (80–400 mm T_L); GAA1 = *Gambusia affinis* (10–40 mm T_L); GAA2 = *Gambusia affinis* (41–80 mm T_L). Abbreviation: *n* = number of samples, TP = trophic position, $\delta^{13}C$ = carbon stable isotope and $\delta^{15}N$ = nitrogen stable isotope.

Groups		*n*	TP	$\delta^{13}C$	$\delta^{15}N$	Functional group
Basal resources						
	Asteraceae	8		-15.13 ± 4.29	12.44 ± 3.89	Aquatic plants littoral zone
	Atriplex semibaccata	3		-27.44 ± 0.93	9.49 ± 0.20	Aquatic plants littoral zone
	Chloris sp.	4		-12.36 ± 0.28	12.27 ± 1.55	Terrestrial plants
	Cynodon dactylon	8		-12.78 ± 1.00	8.37 ± 3.54	Aquatic plants littoral zone
	Ficus burkei	13		-28.93 ± 0.78	10.04 ± 2.27	Terrestrial plants
	Salix mucronata	4		-27.87 ± 0.96	6.64 ± 1.26	Terrestrial plants
	Detritus	15		-24.12 ± 5.21	6.81 ± 2.81	
	Phytoplankton	12		-25.74 ± 1.89	6.50 ± 1.54	
Zooplankton						
	Zooplankton	16	1.5	-25.39 ± 0.91	7.94 ± 1.31	
Macroinvertebrates						
	Baetidae	5	1.3	-26.54 ± 1.61	7.44 ± 1.93	Scraper
	Belostomatidae	10	2.1	-24.54 ± 1.14	9.72 ± 1.00	Predator

Table 4.1: Continued

		n	TP	$\delta^{13}C$	$\delta^{15}N$	Functional group
	Chironomidae	4	1.7	-28.35 ± 1.66	8.44 ± 2.21	Collector
	Coenogronidae	2	2.9	-25.08 ± 0.01	12.34 ± 0.01	Predator
	Corduliidae	2	2.3	-24.88 ± 0.57	10.31 ± 2.22	Predator
	Corixidae	17	1.4	-24.86 ± 2.44	7.74 ± 1.40	Predator
	Hirudinea	3	2.4	-25.55 ± 0.81	10.97 ± 0.86	Predator
	Notonectidae	6	2.1	-26.27 ± 2.25	9.78 ± 0.53	Predator
	Physidae	4	1.9	-22.73 ± 5.09	9.40 ± 2.04	Scraper
Teleost						
	GLC1	26	4.92	-20.05 ± 0.84	12.61 ± 1.34	Generalist invertivore
	GLC2	37	5.42	-24.96 ± 0.84	14.23 ± 1.27	Generalist invertivore
	GIA1	17	5.17	-26.79 ± 0.60	13.40 ± 0.63	Planktivorous
	GIA2	38	5.47	-25.47 ± 0.76	14.37 ± 0.78	Planktivorous
	ORM1	24	4.11	-25.67 ± 1.55	9.99 ± 2.10	Omnivorous
	ORM2	29	4.45	-26.15 ± 1.11	11.09 ± 1.51	Omnivorous
	GAA1	29	4.75	-24.62 ± 1.61	12.06 ± 1.97	Omnivorous
	GAA2	11	5.37	-24.05 ± 0.65	14.07 ± 0.73	Omnivorous

Three trophic positions were identified with GLC2, GIA2 and GAA2 occupying trophic position 3. Trophic position 2 was occupied by GLC1, GIA1, ORM1, ORM2 and GAA 1 and majority of the macroinvertebrates, while zooplankton occupied trophic position 1 (Table 4.1).

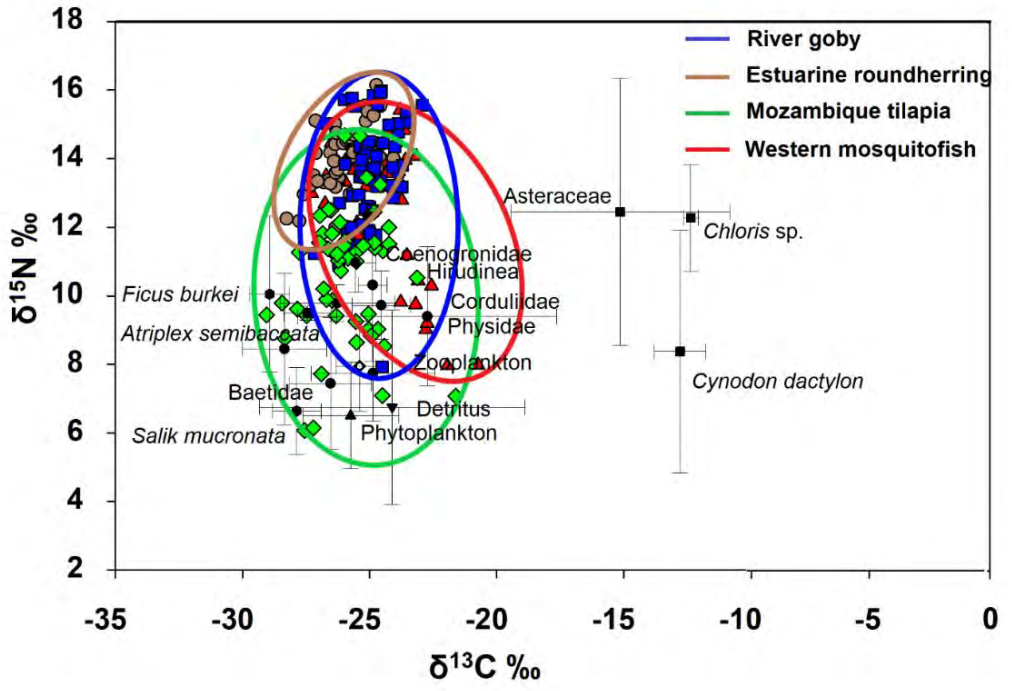

Figure 4.2: Corrected standard ellipse areas (SEA_c), representing core isotopic niche space of *Glossogobius callidus* (blue line), *Gilchristella aestuaria* (brown line), *Oreochromis mossambicus* (green line) and *Gambusia affinis* (red line), with potential food source riparian plants (hexagon), terrestrial plants (square), detritus (down triangle shape), zooplankton (diamond), macroinvertebrates (circle) determined through SIBER models (Jackson *et al*., 2011). Error bars show ± SE.

Ontogenetic shifts

Regressions of $\delta^{13}C$ and $\delta^{15}N$ against fish length revealed that there were no significant relationships between $\delta^{13}C$ signatures and T_L of *G. callidus* (Figure 4.3a) but that there was a positive relationship between $\delta^{15}N$ signatures and T_L for *G. callidus* (Figure 4.3b). This indicates that while the carbon source remained the same, larger fish fed at higher trophic levels.

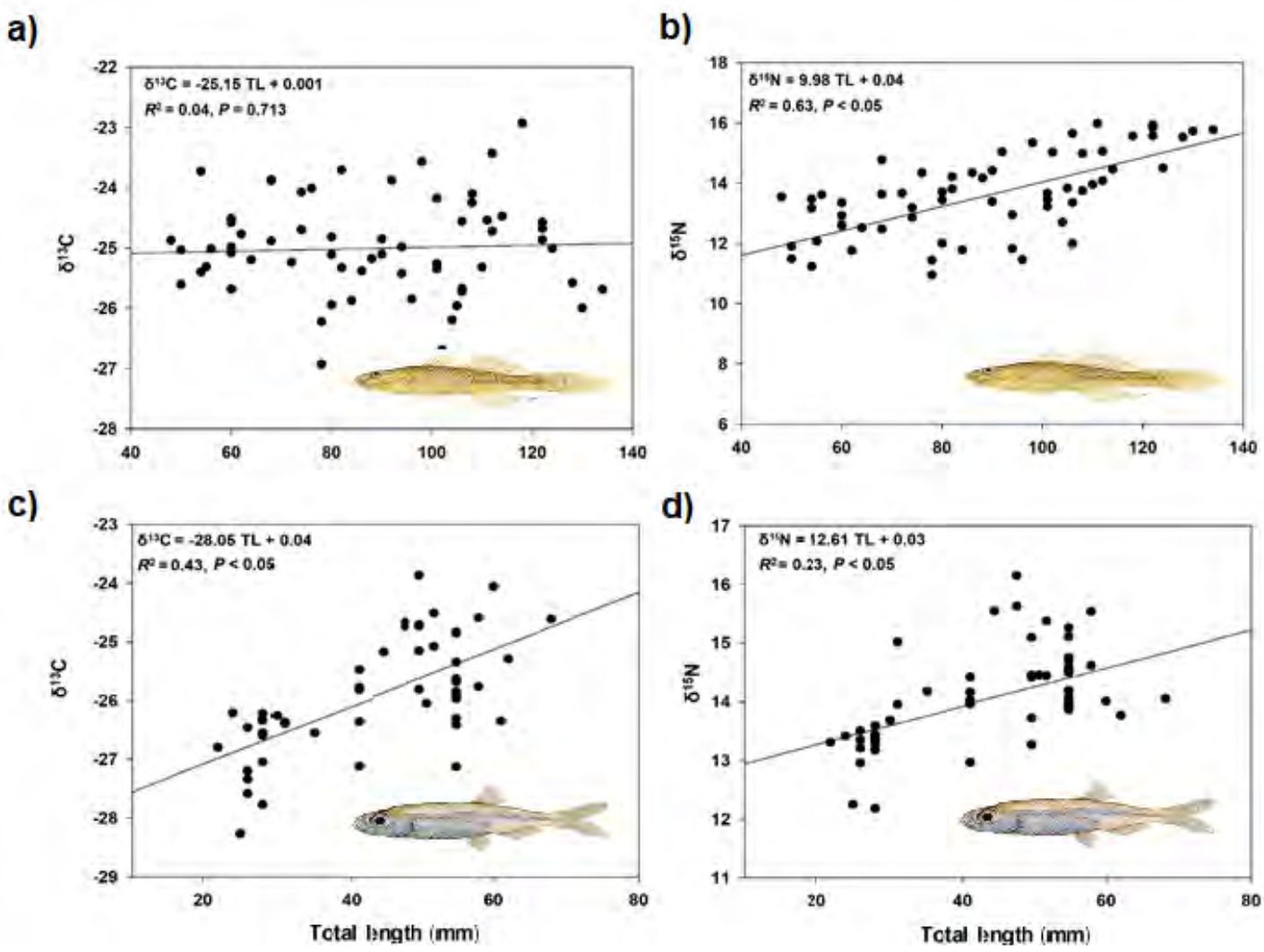

Figure 4.3: Linear regression plots illustrating relationships of *Glossogobius callidus* total length (T_L) to (a) $\delta^{13}C$, (b) $\delta^{15}N$ and linear regression plots illustrating the relationship of *Gilchristella aestuaria* total length (T_L) to (c) $\delta^{13}C$, (d) $\delta^{15}N$ sampled from the Sundays River valley irrigation ponds, Eastern Cape, South Africa.

For *G. aestuaria*, regressions of $\delta^{13}C$ and $\delta^{15}N$ against fish length revealed that there was a negative significant relationship between $\delta^{13}C$ signatures and T_L (Figure 4.3c, 4.3d). This indicates an ontogenetic shift in both their carbon source and trophic level.

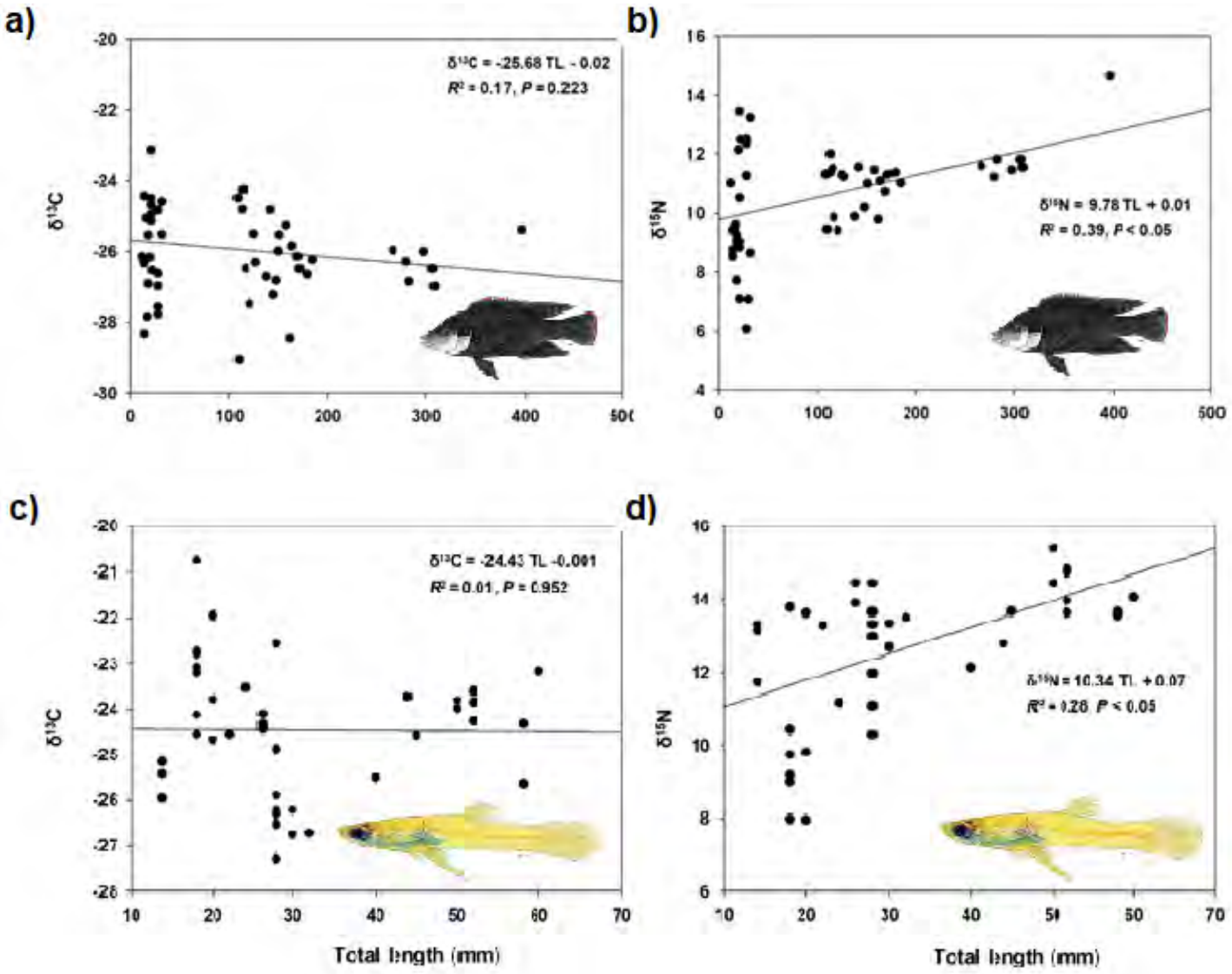

Figure 4.4: Linear regression plots illustrating relationships of *Oreochromis mossambicus* total length (T_L) to (a) $\delta^{13}C$, (b) $\delta^{15}N$ and linear regression plots illustrating the relationship of *Gambusia affinis* total length (T_L) to (c) $\delta^{13}C$, (d) $\delta^{15}N$ sampled from the Sundays River valley irrigation ponds, Eastern Cape, South Africa.

Regressions of $\delta^{13}C$ and $\delta^{15}N$ against fish length revealed that there were no significant relationships between $\delta^{13}C$ signatures and T_L for *O. mossambicus* (Figure 4.4a) but that there was a positive relationship between $\delta^{15}N$ signatures and T_L for *O. mossambicus* (Figure 4.4b). The relationship between $\delta^{15}N$ signatures and T_L indicates that while the carbon source remained the

same, larger fish fed at higher trophic levels. Regressions of $\delta^{13}C$ and $\delta^{15}N$ against fish length revealed that there was no significant relationships between $\delta^{13}C$ signatures and T_L for *G. affinis* (Figure 4.4a) but that there was a positive relationship between $\delta^{15}N$ signatures and T_L for *G. affinis* (Figure 4.4b). This indicates that while the carbon source remained the same, larger fish fed at higher trophic levels.

Layman's Bayesian community metrics

The dNR (description of the food chain length or nitrogen range) and dCR (measure of basal $\delta^{13}C$ variation) was higher for GAA1 (Table 4.2), suggesting that the trophic level and range of basal resources used by GAA1 was greater than that of the other three species (GLC1, GIA1, and ORM1). In addition, CD (a measure of trophic diversity) was higher for GAA1. Higher MNND (mean nearest neighbour distance) and SDNND (standard deviation of the nearest neighbour distance) values recorded for GAA1 indicated higher trophic diversity (Table 4.2). The core isotopic niche area (SEA_c) is illustrated in Figure 4.4. High values of SEA_c for ORM1 and GAA1 indicated that both species occupied a larger isotopic niche than GLC1, GLC2, GIA1, and GIA2 (Table 4.2; Figure 4.5a, b).

Table 4.2: Bayesian trophic niche metrics of GLC1 = *Glossogobius callidus* (40–80 mm T_L), GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA1 =*Gilchristella aestuaria* (20–40 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), GAA1 = *Gambusia affinis* (10–40 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L). Species collected and their isotope metrics: dNR_b = $\delta^{15}N‰$ range, dCR_b = $\delta^{13}C$ range, CD_b = mean distance to centroid, $MNND_b$ = mean nearest neighbour distance, $SDNND_b$ = SD mean nearest neighbour distance, SEA_c = standard ellipse area.

Species	**dNR_b**	**dCR_b**	**CD_b**	**$MNND_b$**	**$SDNND_b$**	**SEA_c**
GLC1	0.6	0.2	0.3	0.6	0.1	3.4
GLC2	1.3	0.4	0.6	1.3	0.1	3.4
GIA1	0.4	0.3	0.3	0.5	0.2	0.9
GIA2	0.2	0.2	0.1	0.3	0.1	1.9
ORM1	0.4	0.6	0.4	0.7	0.1	10.7
ORM2	0.7	0.3	0.4	0.8	0.1	4.8
GAA1	1.7	1.6	1.2	2.4	0.1	7.5
GAA2	0.5	0.1	0.2	0.5	0.2	2

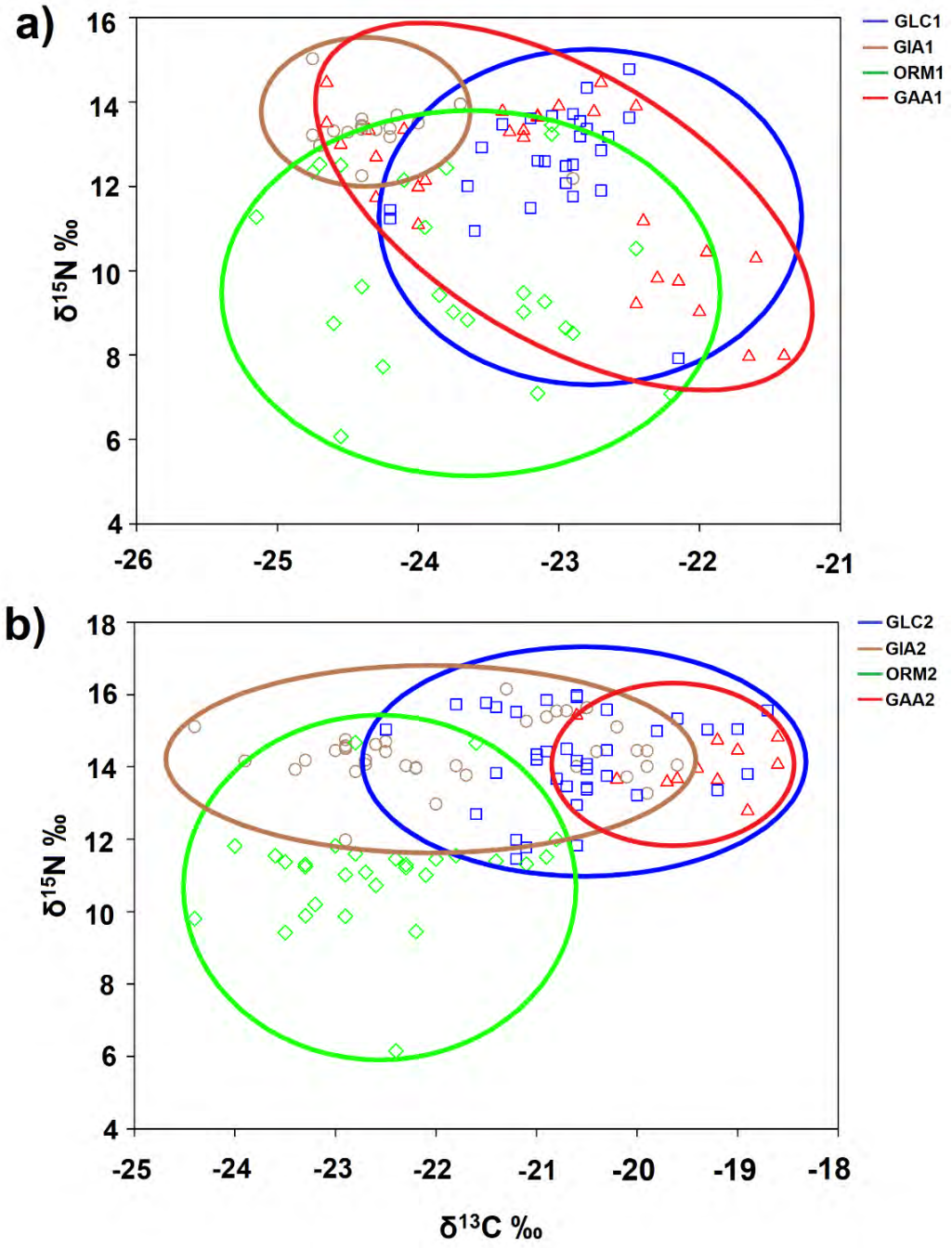

Figure 4.5: The core isotopic niche areas (SEA_c) of a) small size-matched fish (GLC1 = *Glossogobius callidus* (40–80 mm T_L), GIA1 =*Gilchristella aestuaria* (20–40 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), and GAA1 = *Gambusia affinis* (10–40 mm T_L)) and b) large size-matched fish (GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L)) from the Sundays River valley irrigation ponds, Eastern Cape, South Africa.

Mixing models

Glossogobius callidus

Overall, collectors (Chironomidae) constituted the largest proportion of the diet of both size-classes of *G. callidus* (Figure 4.6a, b). The SIAR mixing models indicated that collectors (Chironomidae) contributed the largest proportion (mean: 0.40) to the diet of GLC1 followed by GAA1 (mean: 0.18) and predator macroinvertebrates (mean: 0.10), respectively (Figure 4.6a). Results of the SIAR mixing model suggest that GAA1 (0.43) contributed the largest proportion of the diet of GLC2, followed by collectors (Chironomidae) (mean: 0.37) and predator macroinvertebrates (mean: 0.05) (Figure. 4.6b).

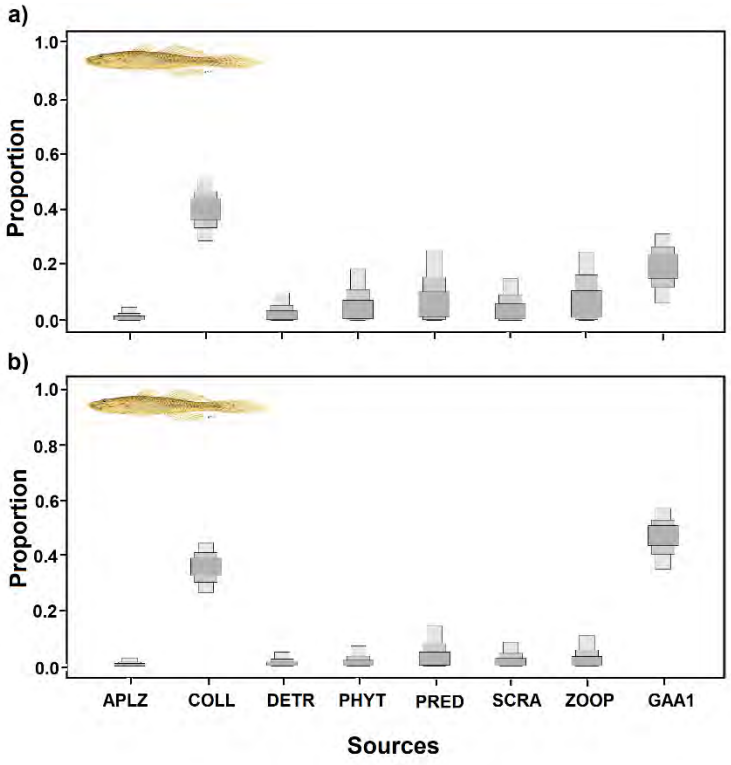

Figure 4.6: SIAR box plots showing estimated proportions of source contributions to the diet of (a) GLC1 = *Glossogobius callidus* (40–80 mm T_L) and (b) GLC2 = *Glossogobius callidus* (81–140 mm T_L) collected from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Widths of bars show 95%, 75% and 50% credibility intervals. APLZ = aquatic plants littoral zone; COLL = collectors; DETR = detritus; PHYT = phytoplankton; PRED = predators; SCRA = scrapers; ZOOP = zooplankton and GAA1 = *Gambusia affinis* (10–40 mm T_L).

Gilchristella aestuaria

The SIAR mixing models demonstrated that collectors (Chironomidae) constitute the largest proportion to the diet of both size-classes of *G. aestuaria* (Figure 4.7a, b). The SIAR mixing models indicated that collectors (Chironomidae) (mean: 0.69) contributed the most to the diet of GIA1, followed by predator macroinvertebrates (mean: 0.10) and scrapers (mean: 0.05) (Figure 4.7a). Results of the SIAR mixing model suggest that collectors (Chironomidae) (mean: 0.46) contributed the largest proportion of the diet of GIA2, followed by predator macroinvertebrates (mean: 0.23) and zooplankton (mean: 0.16) (Figure. 4.7b).

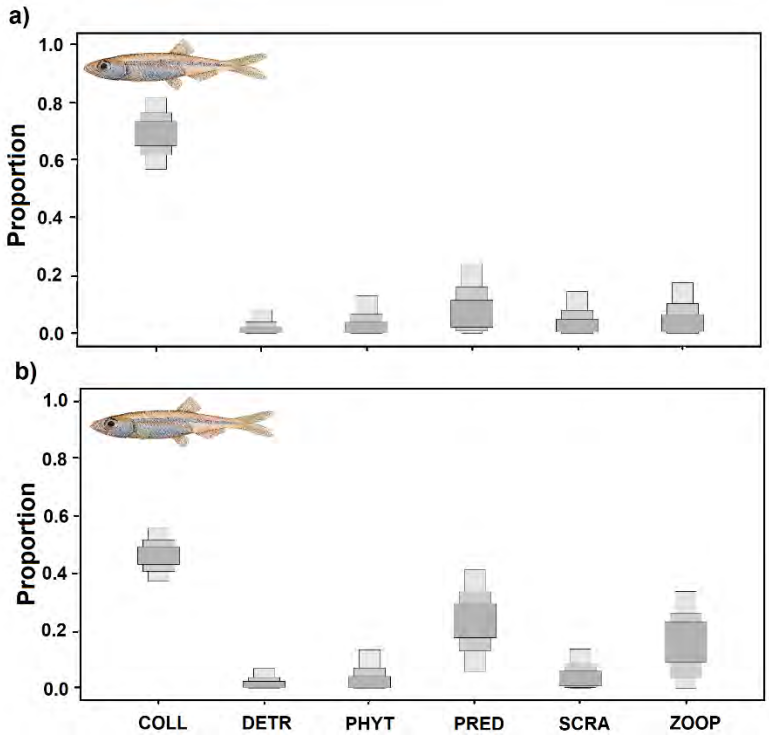

Figure 4.7: SIAR box plots showing estimated proportions of source contributions to the diet of (a) GIA1 = *Gilchristella aestuaria* (20–40 mm T_L) and (b) GIA2 = *Gilchristella aestuaria* (41–80 mm T_L) collected from the Sundays River Valley irrigation ponds, Eastern Cape, South Africa. Widths of bars show 95%, 75% and 50% credibility intervals. COLL = collectors; DETR = detritus; PHY = phytoplankton; PRED = predators; SCRA = scrapers; ZOO = zooplankton.

Oreochromis mossambicus

Overall, collectors (Chironomidae) contributed the largest proportion to the diet of both size-classes of *O. mossambicus* (Figure 4.8a, b). According to SIAR mixing models, collectors (Chironomidae) (mean: 0.35) contributed the largest proportion to the diet of ORM1, followed by phytoplankton (mean: 0.26) and detritus (mean: 0.11) (Figure 4.8a). According to the SIAR results the diet of ORM2 was mainly comprised of collectors (Chironomidae) (mean: 0.61), followed by phytoplankton (mean: 0.18) and zooplankton (mean: 0.04) (Figure 4.8b).

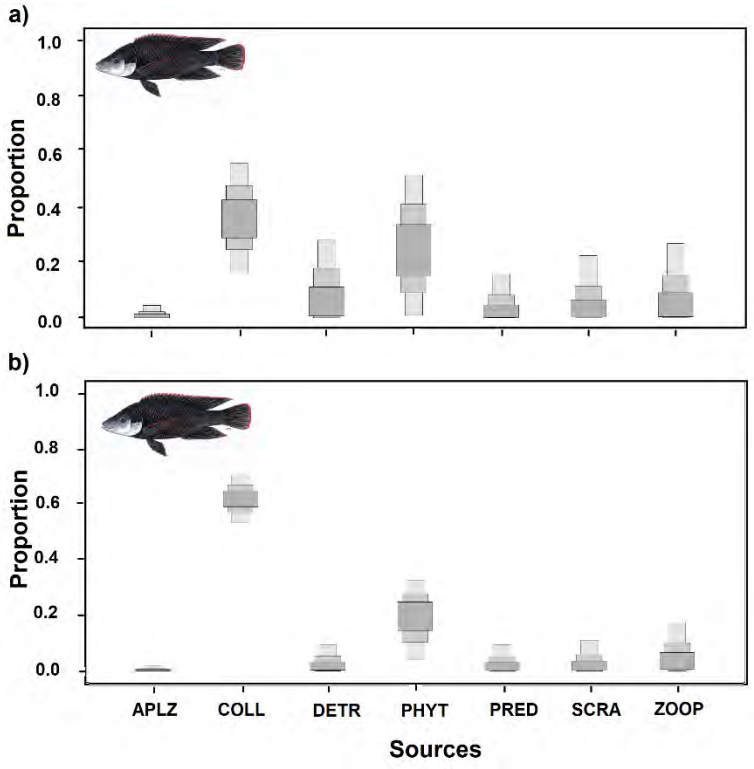

Figure 4.8: SIAR box plots showing estimated proportions of source contributions to the diet of (a) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L) and (b) ORM2 = *Oreochromis mossambicus* (80–400 mm T_L) collected from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Widths of bars show 95%, 75% and 50% credibility intervals. APLZ = aquatic plants littoral zone; COLL = collectors; DET = detritus; PHYT = phytoplankton; PRED = predators; SCRA = scrapers; ZOOP = zooplankton.

Gambusia affinis

There were differences in the diet composition between the size-classes of *G. affinis* (Figure 4.9a). According to SIAR mixing models collectors (Chironomidae) (mean: 0.23) contributed the largest proportion to GAA1, followed by predators (mean: 0.22) and scrapers (mean: 0.17), respectively (Figure 4.9a). The SIAR mixing models suggested that a wide range of resources contributed to the diet of GAA2, which included zooplankton (mean: 0.37) contributing the largest proportion, followed by predators (mean: 0.34) and collectors (Chironomidae) (mean: 0.10) (Figure 4.9b).

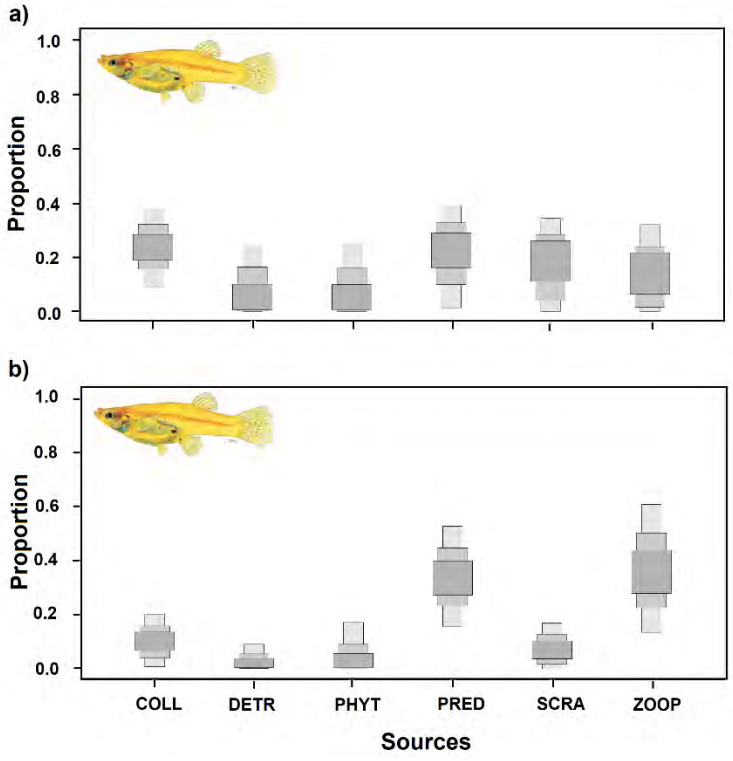

Figure 4.9: SIAR box plots showing estimated proportions of source contributions to the diet of (a) GAA1 = *Gambusia affinis* (10–40 mm T_L) and (b) GAA2 = *Gambusia affinis* (41–80 mm T_L) collected from the Sundays River valley irrigation ponds, Eastern Cape, South Africa. Widths of bars show 95%, 75% and 50% credibility intervals. COLL = collectors, DETR = detritus, PHYT = phytoplankton, PRED = predators, SCRA = scrapers, ZOOP = zooplankton.

Probabilistic niche overlap

The application of the Bayesian posterior distribution of the probabilistic niche overlap metric illustrated niche overlaps among the different size classes (Figure 4.10). Overlap values showed a high niche overlap between GAA1 and ORM1 (92.4%) and that the GAA2 niche overlapped with all the species except for that of GIA1 (Figure 4.10). In addition, GIA1 had a high niche overlap (>80%) with all the other species except for GAA2 and GLC1, while GIA2 had a high niche overlap with all the species except that of GIA1 (50%). A high niche overlap for GLC1 was observed for all fishes except for GIA1 and GIA2 (Figure 4.10). Likewise, GLC2 also had a high niche overlap with all the fishes expect for GIA1 and GIA2 (Figure 4.10). ORM1 had moderate niche overlaps with the other fish species, except for GAA2 and GLC2, where the species had a low niche overlap (Figure 4.10). However, ORM2 had a high niche overlap with GLC1 (80%) and ORM2 had a high niche overlap with ORM1 (Figure 4.10).

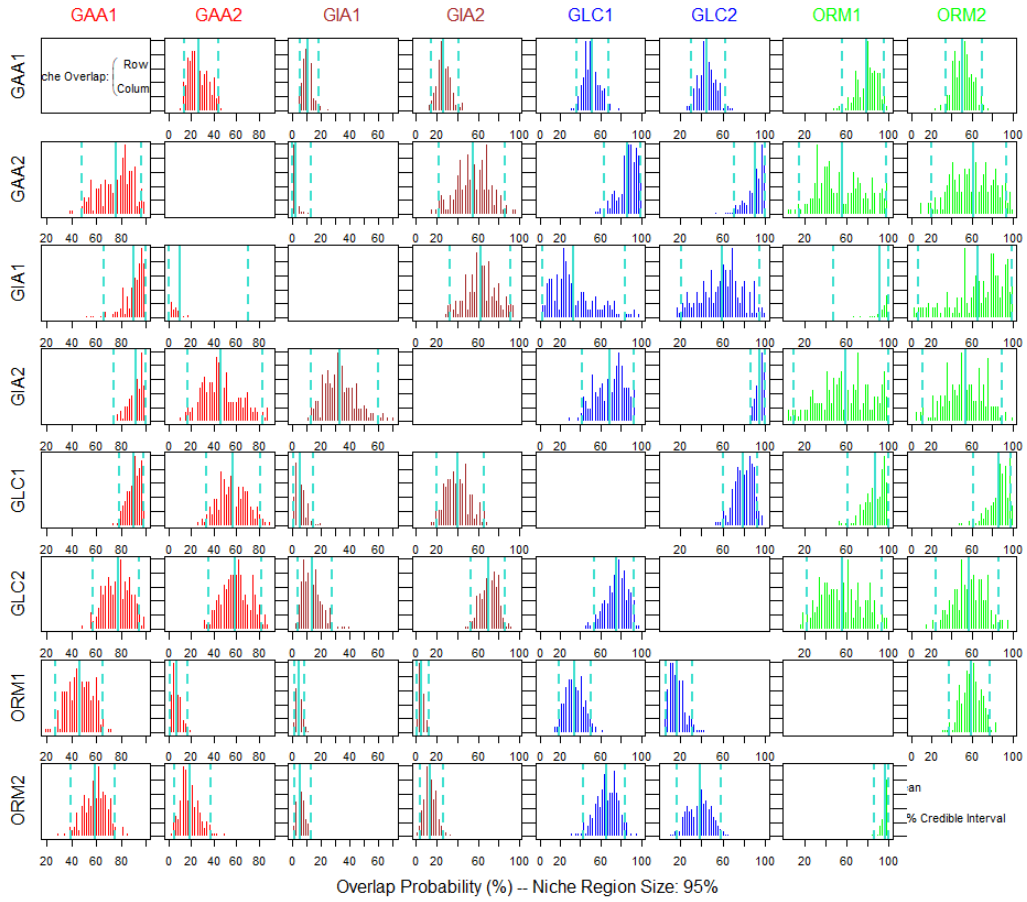

Figure 4.10: Bayesian posterior distribution of probabilistic trophic niche overlap for GLC1 = *Glossogobius callidus* (40–80 mm T_L), GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA1 =*Gilchristella aestuaria* (20–40 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), GAA1 = *Gambusia affinis* (10–40 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L). The probabilities of niche overlap (mean and 95% credibility intervals) are specified as the overlap of *Glossogobius callidus* onto the isotopic niche of *Gilchristella aestuaria*, *Oreochromis mossambicus* and *Gambusia affinis* and vice versa. The dark solid vertical line represent the mean, dotted line represent 95% credible interval".

Gut contents

Glossogobius callidus

Of the 98 *G. callidus* gut contents examined, only 0.03% were empty, which consisted of 35 GLC1 and 63 GLC2. A summary of the variation in %IRI across size classes is presented in Table 4.3. As predicted from the literature and the SIAR analysis, the diet composition of *G. callidus* was mainly dominated by Chironomidae. The gut contents of GLC1 contained mostly Chironomidae (Insecta) (%IRI = 66.22) and detritus (%IRI = 32.32) (Table 4.3; Figure 4.11a). The diet composition of GLC2 was Chironomidae (Insecta) (%IRI = 53.46) and detritus (%IRI = 42.10) (Table 4.3; Figure 4.11b). *Gambusia affinis* was found in the guts of both GLC1 and GLC2 (Table 4.3).

Table 4.3: Gut content analyses of 98 (35 samples of GLC1 = *Glossogobius callidus* (40–80 mm T_L); 63 samples of GLC2 = *Glossogobius callidus* (81–140 mm T_L)) from the Sundays River valley irrigation ponds. Values presented as %N = number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of guts containing a particular food item expressed as a percentage of all guts sampled; %V = the volume of each prey category expressed as a percentage of the total volume of all prey items; %IRI = Index of Relative Importance as a proportion of the total IRI of all prey items.

Taxa		**GLC1**					**GLC2**				
		n	**%N**	**%F**	**%V**	**%IRI**	***n***	**%N**	**%F**	**%V**	**%IRI**
Insecta											
	Caloperygidae	4	1.99	11.43	5.35	0.48	9	1.99	14.29	5.57	0.77
	Chironomidae	34	83.08	97.14	34.83	66.22	50	72.74	79.37	20.82	53.46
	Corixidae	4	4.23	11.43	5.50	0.64	12	11.55	19.05	11.31	3.13
Teleost											
	Gambusia affinis	1	0.25	2.86	1.96	0.03	2	0.36	3.17	2.73	0.07
Unidentified											
	Detritus	1	8.21	94.29	51.08	32.32	1	9.93	87	57.06	42.10
	Unidentified insects	5	2.24	14.29	1.26	0.28	9	1.62	14.29	2.43	0.41

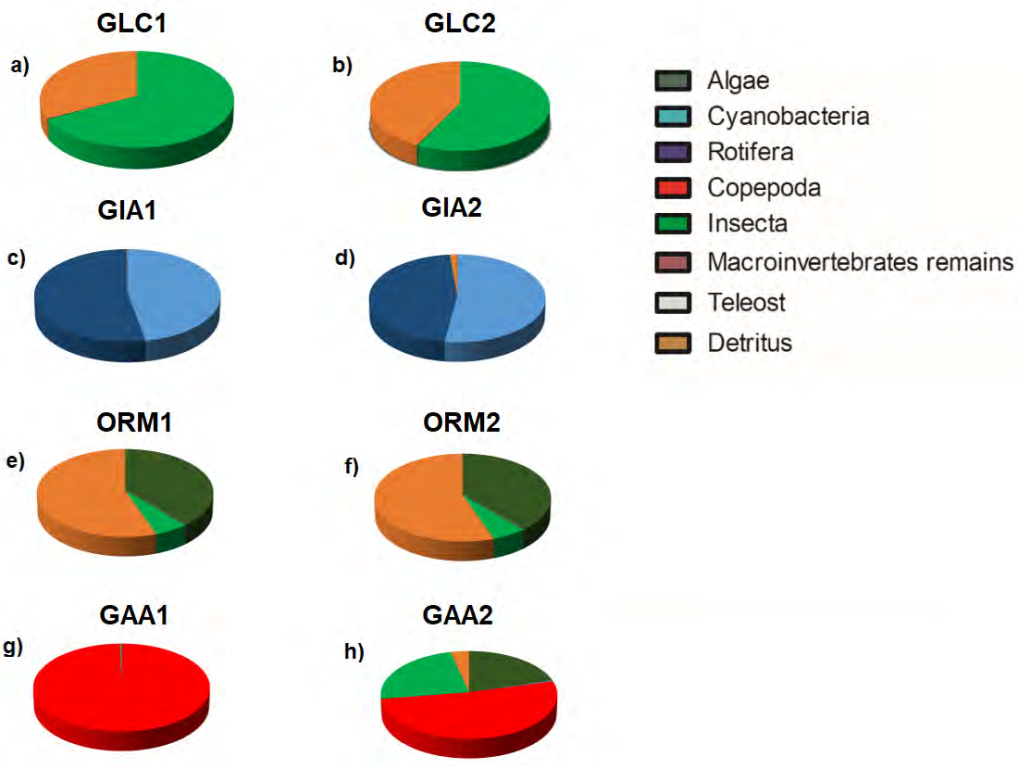

Figure 4.11: The percentage of relative importance (%IRI) of prey items from the gut contents of a) GLC1 = *Glossogobius callidus* (40–80 mm T_L) and b) GLC2 = *Glossogobius callidus* (81–140 mm T_L); c) GIA1 =*Gilchristella aestuaria* (20–40 mm T_L) and d) GIA2 = *Gilchristella aestuaria* (41–80 mm T_L) e) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L) and f) ORM2 = *Oreochromis mossambicus* (80–400 mm T_L) and g) GAA1 = *Gambusia affinis* (10–40 mm T_L) and h) GAA2 = *Gambusia affinis* (41–80 mm T_L). Sampled from the Sundays River valley irrigation ponds in the Eastern Cape, South Africa.

Gilchristella aestuaria

A total of 99 *G. aestuaria* gut contents were examined, which consisted of 10 GIA1 and 89 GIA2, and none of the examined guts were empty. A summary of the variation in %IRI across size classes is presented in Table 4.4. The GIA1 gut contents contained Cyanobacteria (*Xenococcus kerneri* (%IRI = 46.97)), Rotifera (*Trichocera similis* (%IRI = 32.43) and *Keratella tecta*. (%IRI = 20.25)) (Table 4.4; Figure 4.11c). GIA2 guts contained Cyanobacteria (*Xenococcus kerneri* (%IRI = 51.83) and Rotifers (*Trichocera similis* (%IRI = 36.60)) (Table 4.4; Figure 4.11d).

Table 4.4: Gut content analyses of 99 (10 samples of GIA1 =*Gilchristella aestuaria* (20–40 mm T_L); 89 samples of GIA2 = *Gilchristella aestuaria* (41–80 mm T_L)) from the Sundays River valley irrigation ponds. Values presented as %N = number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of guts containing a particular food item expressed as a percentage of all guts sampled; %V = the volume of each prey category expressed as a percentage of the total volume of all prey items; %IRI = Index of Relative Importance as a proportion of the total IRI of all prey items.

Taxa	GIA1					GIA2				
	n	**%N**	**%F**	**%V**	**%IRI**	***n***	**%N**	**%F**	**%V**	**%IRI**
Algae										
Euglena sp.						3	0.20	3.37	0.39	0.02
Unidentified algae						4	0.06	4.49	0.41	0.02
Cyanobacteria										
Pannus sp.						15	0.65	16.85	0.91	0.24
Xenococcus kerneri	9	46.47	90.00	46.74	46.97	53	56.66	59.55	36.94	51.83
Rotifers										
Brachionus sp.	1	2.58	10.00	2.09	0.26	23	1.59	25.84	4.84	1.55
Keratella tecta	10	19.79	100.00	16.37	20.25	40	9.89	44.94	9.64	8.16
Trichocera similis	9	30.98	90.00	33.38	32.43	57	30.55	64.04	30.91	36.60
Polyarthra vulgaris						1	0.09	1.12	0.84	0.01
Insecta										
Caloperygidae						3	0.05	3.37	3.37	0.11
Chironomidae						4	0.06	4.49	0.45	0.02
Unidentified										
Detritus	1	0.17	10.00	1.39	0.09	1	0.19	13.48	11.30	1.44

Oreochromis mossambicus

A total of 98 gut contents were examined and 0.01% were empty. The ORM1 guts contained mostly detritus (%IRI = 55.43) and algae (%IRI = 38.40), while ORM2 guts contained detritus (%IRI = 54.84) and Rotifera (*Keratella tecta* (%IRI = 12.43)) (Table 4.5; Figure 4.11e, f).

Table 4.5: Gut content analyses of 98 (51 samples of ORM1 = *Oreochromis mossambicus* (10–40 mm T_L); 47 samples of ORM2 = *Oreochromis mossambicus* (80–400 mm T_L)) from the Sundays River valley irrigation ponds. Values presented as %N = number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of guts containing a particular food item expressed as a percentage of all guts sampled; %V = the volume of each prey category expressed as a percentage of the total volume of all prey items; %IRI = Index of Relative Importance as a proportion of the total IRI of all prey items.

Taxa	**ORM1**					**ORM2**				
	n	**%N**	**%F**	**%V**	**%IRI**	***n***	**%N**	**%F**	**%V**	**%IRI**
Algae										
Euglena sp.						3	1.54	6.38	0.06	0.07
Melosira varians						3	7.72	6.38	0.22	0.36
Unidentified algae	1	66.87	86.27	7.18	38.40	1	13.62	40.42	3.85	5.03
Cyanobacteria										
Pannus sp.						24	11.07	51.06	1.63	4.62
Xenococcus kerneri	2	5.35	3.92	0.42	0.13	20	1.62	42.55	0.34	0.59
Rotifers										
Brachionus sp.						29	15.27	61.70	4.11	8.52
Cephalodella gibba						5	0.56	10.63	0.01	0.04
Keratella tecta						33	21.66	70.21	3.19	12.43
Trichocera similis	2	0.17	3.92	0.11	0.01	21	11.33	44.68	8.98	6.47
Polyarthra vulgaris						29	14.24	61.70	1.57	6.95

Table 4.5: Continued

Taxa		ORM1					ORM2			
		n	**%N**	**%F**	**%V**	**%IRI**	***n***	**%N**	**%F**	**%V**	**%IRI**
Copepoda											
	Nauplii	2	0.89	3.92	0.80	0.03					
Insecta											
	Chironomidae	21	22.23	41.18	1.89	5.97					
Unidentified											
	Detritus	1	4.46	98.04	89.60	55.43	1	1.21	100.00	75.73	54.84
	Unidentified insects						4	0.10	8.51	0.24	0.02

Gambusia affinis

A total of 45 *G. affinis* gut contents were examined and 0.05% were empty (Table 4.6; Figure 4.11g, h). The examined guts consisted of 30 GAA1 and 15 GAA2. A summary of the variation in %IRI across size classes is presented in Table 4.6. The GAA1 guts contained Copepoda (*Microcyclops* sp. (%IRI = 99.56)) and detritus (%IRI = 0.18), while the GAA2 guts contained Copepoda (*Microcyclops* sp. (%IRI = 51.62) and Insecta (Chironomidae (%IRI = 23.88)) (Table 4.6; Figure 4.11g, h).

Community structure

Prey assemblages differed significantly among species (ANOSIM: global R = 0.89, $p < 0.05$). Cluster analysis highlighted overlap in the diet composition of the studied species with two distinct groups at the 40% aggregation level (Figure 4.12). Group 1 comprised GLC1, GLC2, ORM1, GAA1 and GAA2 (Figure 4.12). Group 2, however, was comprised of GIA1, GIA2 and ORM2 (Figure 4.12). Subsequent SIMPER analysis revealed that the predators of group 1 (see Cluster results) fed mainly on detritus (47.10%), Chironomidae (30.42%) and Algae (18.91%.) (Table 4.7), while predators of group 2 (see Table 4.7) fed mainly on Rotifers (*Trichocera similis* (36.43%)), detritus 26.49% and *Branchionus* sp. (Table 4.11). Additionally, SIMPER analysis revealed that four taxa independently contributed more than 50% to the dissimilarity between group 1 and group 2. These were Rotifer *Trichocera similis* (20.61%), Insecta, Chironomidae (16.83%) and Algae (13.24%), which cumulatively contributed 50.61% of the dissimilarity between the two groups (Table 4.7).

Table 4.6: Gut content analyses of 45 (30 samples of GAA1 = *Gambusia affinis* (10–40 mm T_L); 15 samples of GAA2 = *Gambusia affinis* (41–80 mm T_L)) from the Sundays River valley irrigation ponds. Values presented as %N = number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of guts containing a particular food item expressed as a percentage of all guts sampled; %V = the volume of each prey category expressed as a percentage of the total volume of all prey items; %IRI = Index of Relative Importance as a proportion of the total IRI of all prey items.

Taxa		**GAA1**					**GAA2**				
		n	**%N**	**%F**	**%V**	**%IRI**	***n***	**%N**	**%F**	**%V**	**%IRI**
Algae											
	Melosira varians						4	5.19	26.67	24.79	20.62
Cyanobacteria											
	Pannus sp.						1	1.30	6.67	0.39	0.29
Rotifers											
	Brachionus sp.	1	1.13	3.33	2.04	0.15					
Copepoda											
	Microcyclops sp.	12	96.87	40.00	75.34	99.56	5	31.17	33.33	28.88	51.62
Insecta											
	Chironomidae	1	0.85	3.33	1.19	0.09	5	11.69	33.33	16.09	23.88
Unidentified											
	Detritus	1	0.28	3.33	3.57	0.18					
	Unidentified insects						2	5.19	13.33	5.20	3.57

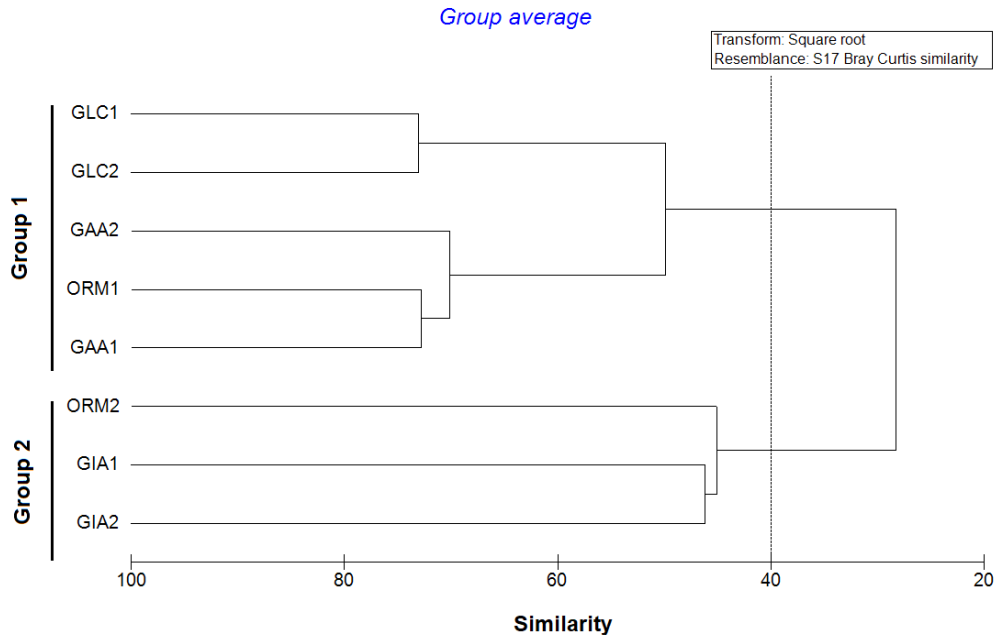

Figure 4.12: Dendrogram presentation of dietary similarities (%IRI) of GLC1 = *Glossogobius callidus* (40–80 mm T_L), GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA1 =*Gilchristella aestuaria* (20–40 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), GAA1 = *Gambusia affinis* (10–40 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L).

Table 4.7: SIMPER test results for GLC1 = *Glossogobius callidus* (40–80 mm T_L), GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA1 =*Gilchristella aestuaria* (20–40 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), GAA1 = *Gambusia affinis* (10–40 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L).from the Sundays River valley irrigation ponds. Group refers to size class per species as outlined in the similarity dendrogram (Figure 4.11).

Factor	Simper	Contribution (%)
	Dominant taxa	
Group 1		
	Detritus	47.10%
	Chironomidae	30.42%
	Algae	18.91%
Group 2		
	Trichocera similis	36.43%
	Detritus	26.49%
	Brachionus sp.	19.21%

Niche breadth

Levin's niche breadth varied among species (Figure 4.13). The ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), followed by GLC1 = *Glossogobius callidus* (40–80 mm T_L), had a greater niche breadth at a smaller size-class (<80 mm T_L), while, GAA1 = *Gambusia affinis* (10–40 mm T_L) had the lowest niche breadth (Figure 4.13).

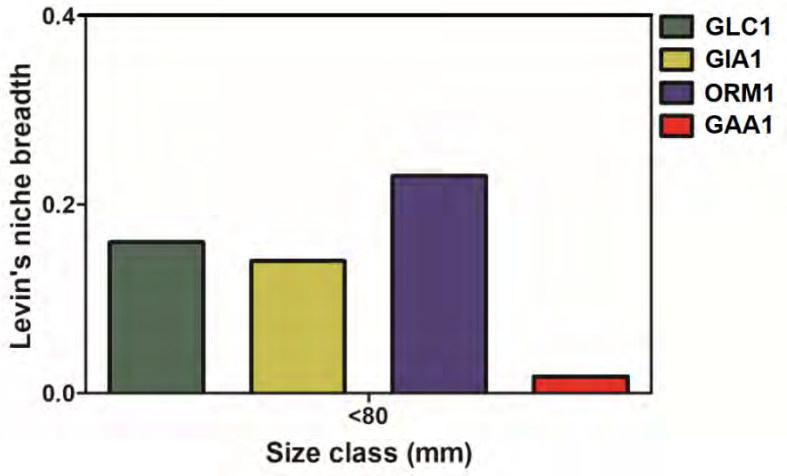

Figure 4.13: Illustration of Levin's niche breadth for GLC1 = *Glossogobius callidus* (40–80 mm T_L) (Olive), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L) (dark green), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L) (dark blue) and GAA1 = *Gambusia affinis* (10–40 mm T_L) (red).

Discussion

This study's aim was to quantify and compare the diet composition of the four primary colonising fish species in Sundays River valley irrigation ponds using $\delta^{13}C$ and $\delta^{15}N$ stable isotope and gut content analyses. With regard to the hypothesis of this Chapter, both stable isotope and gut content analysis showed that all four species shared the feeding niches and this nullified the hypothesis. The results of this study, based on stable isotope analyses and gut content analyses, showed that in the Sundays River valley irrigation ponds *G. callidus* feeds predominantly on benthic resources (Table 4.3; Figure 4.11a,b). These findings highlight the benthic nature of this species and this study corroborates that of with Mofu *et al*. (2019c), which found *G. callidus* to be a generalist invertivore in their previous assessment of the diet of this fish from the Sundays River valley irrigation ponds. This is also in concordance with research in estuaries, where Boulle (1990) showed that non-epi-benthic and terrestrial invertebrates dominated the diet of *G. callidus*. Regression analysis showed a significant increase in $\delta^{15}N$ with increasing fish size, suggesting an ontogenetic shift in the diet of *G. callidus*.

Ontogenetic niche shift is important particularly in fishes, as it helps maximize their fitness by reducing competition with conspecifics (Werner & Gilliam, 1984), reduces predation risk through changes in habitat (Werner *et al*., 1983) and increases growth rates through changes in diet (Aggus & Elliot, 1975). The ontogenetic shift of *G. callidus* in this study was from collectors (Chironomidae) to Teleost (i.e. *G. affinis*) and unidentified insects. The SIAR results supported the observation of a wide shift in food source contribution between the size classes of *G. callidus*. According to the results of the SIAR model, collectors (Chironomidae) made the largest contribution to the diet of the small-sized *G. callidus* (GLC1). This suggest insectivory, as

supported by the proportion of benthic macroinvertebrates in gut content analysis in this chapter (Table 4.3) and by Mofu *et al*. (2019c).

The SIAR results showed that *G. callidus* became more oriented towards piscivorous feeding behaviour with increasing size Figure 4.6b). These findings corroborate gut content analyses in this study and that by Mofu *et al*. (2019c). Both stable isotope analysis and gut content analysis showed an ontogenetic shift towards a preference in larger sized prey, including juvenile *G. affinis* (Table 4.3, Figure 4.6b). In contrast, the SIAR results did not find the inclusion of detritus in the gut content results (Table 4.4a) as reported by Mofu *et al*. (2019c).

The estimates from SIAR found a low contribution of detritus to the overall diet of both size classes of *G. callidus*, while gut content analysis found that detritus formed a bulk component (Table 4.3; Figure 4.6). This suggests that high contribution of detritus may be accidentally ingested, most likely during benthic foraging for invertebrates. Items in the diet of *G. callidus* did match items that were available in the benthic samples (see Chapter 3); for example Chironomidae are the most abundant bottom-dwelling macroinvertebrates in most freshwater habitats, inhabiting the uppermost sediments of both littoral and profundal environments (Gerber & Gabriel, 2002).

The methods used (stable isotope and gut content analyses) did not permit assessment as to whether the fish isotope signature and gut content data are attributed more so to scavenging on dead individuals than direct predation on live *G. affinis*. Both of these interactions, however, may be important and support the interactions between *G. callidus* and *G. affinis*. Howell *et al*. (2013) found negative spatial associations between *G. callidus* and *G. affinis* suggesting antagonistic interactions between them (Howell *et al*., 2013). These interactions are further investigated in Chapter 5 where a functional response experiment was used to understand predator-prey

interactions and competition, and it describes the relationship between *per capita* prey consumption and prey density.

Overall, stable isotope and gut content analyses' results of this study demonstrated that *G. aestuaria* is a generalist feeder and is capable of exploiting resources ranging from phytoplankton to collectors and scrapers (Figure 4.7). This further suggests patterns of resource partitioning, which could be achieved through selective feeding by *G. aestuaria*. To date, only limited literature exists on resource use by the *G. aestuaria*, and current literature is based on estuaries where the *G. aestuaria* feeds predominantly on zooplankton, including Copepod eggs, and Ostracoda (Blaber, 1979; Coetzee, 1981; Froneman & Vorwerk, 2003; Strydom *et al*., 2014). The stable isotope component of this study corroborates findings by Coetzee (1981), who showed that when phytoplankton and zooplankton densities are low, *G. aestuaria* is often seen feeding on larger prey items such as Chironomid larvae. The gut content found in this study agrees with the findings of Blaber *et al*. (1981), that the diet of *G. aestuaria* is mainly comprised of zooplankton.

Ontogenetic shifts in the diet of *G. aestuaria* have been described in the St. Lucia estuary, where size-based shifts in foraging resulted in increased predation of larger sized prey by larger *G. aestuaria* (Blaber, 1979). According to Blaber (1979), in estuaries, *G. aestuaria* fed predominately-on calanoid (*Pseudodiaptomus stuhlmanni*) and mysid (*Mesopodopsis africana*)*;* while in periods of low prey abundance *G. aestuaria* selectively feeds on larger benthic invertebrates (Coetzee, 1981). Changes in the diet of *G. aestuaria* with increasing length are caused by a decrease in the filtering efficiency of copepods, and an increased ability to feed on macroinvertebrates (Blaber, 1979; Coetzee, 1981). This is supported by the findings of Costalago *et al*. (2015), who demonstrated that in turbid water *G. aestuaria* adapted their feeding strategy to a non-filter-feeding strategy.

Results from stable isotope and gut content analyses suggest that *O. mossambicus* is an omnivorous feeder, which undergoes an ontogenetic shift in diet (Figure 4.4), whereby juveniles feed predominantly on collectors, and phytoplankton (Figure 4.8a) and adults feed mostly on collectors, phytoplankton and zooplankton (Figure 4.8b). Gut content analysis revealed that small-sized *O. mossambicus* fed on detritus and algae (Table 4.5), while large-sized *O. mossambicus* fed on detritus, rotifers, and cyanobacteria (Table 4.5). The trophic position occupied by the different size classes is in accordance with previous findings, that *O. mossambicus* have the ability to feed on a different kind of prey such as algae, phytoplankton, zooplankton, insects and fish (Zengeya *et al*., 2011; Dyer *et al*., 2013).

Ontogenetic shifts are well described in this species, whereby juvenile tilapia congregate in the shoals in shallow water and feed on different resources than adults (Whitfield & Blaber, 1978). According to Le Roux (1956), while these species are prone to be opportunistic feeders when resources are abundant, they are more likely to shift to a specialized diet when resources are less abundant. This is a similar finding to that of De Moor *et al*. (1986), who reported that the intestinal length of *O. mossambicus* varied with size, suggesting an ontogenetic adaptation from a carnivorous diet to an omnivorous diet. In the current study, the $\delta^{15}N$ value of *O. mossambicus* increased with total length and this indicates a broader food resource and an increased niche width. In addition, previous studies have highlighted that there are generally no dominant food sources in the diet of *O. mossambicus* (Carrasco *et al*., 2012), and that *O. mossambicus* will target any food resource available (Dyer *et al*., 2013).

The results obtained from stable isotope analyses and gut content analyses suggest that *G. affinis* has a broad dietary niche. This is similar to other research on this species, which has shown that *Gambusia affinis* feeds on a wide range of resources, including algae, detritus, zooplankton,

crustaceans and insects (Daniels & Felley, 1992; Garcia, 1999; Ruehl & DeWitt, 2005). Stable isotope analysis showed an ontogenetic shift in the diet of *G. affinis* with changes in $\delta^{15}N$ with increasing fish length, which corroborates the results from the gut content analysis. Stable isotope analysis showed that *G. affinis* feeds on a wide range of resources from zooplankton and collector/gatherer to predator macroinvertebrates (Figure 4.9). Small size *G. affinis* fed predominately on collectors, predators, scrapers and zooplankton, while large-sized mosquitofish fed mainly on predators and zooplankton. This suggests that large-size *G. affinis* had greater efficiency in predation and the ability to move up and down the water column. In addition, gut content revealed that small-sized *G. affinis* fed on Copepods and detritus and large-sized *G. affinis* fed on Copepods (*Microcyclops* sp.), Insects (Chironomidae) and Algae (*Melosira varians*).

The findings of this study are congruent with a study by García-Berthou (1999), who observed an ontogenetic shift in *G. affinis*. They showed that with increasing size, *G. affinis* fed on larger prey, such that small-sized fish fed on Cladocerans, nematoceran larvae, diatoms, and collembolan, while large-sized *G. affinis* fed on collembolan and nematoceran adults. The SIAR mixing models suggest that *G. affinis* feed across multiple trophic levels, and the SIAR estimates showed that a wide variety of food resources contributed to the diet of small-sized *G. affinis*, with no single dominant food item. The diet of large-sized *G. affinis* was mainly comprised of larger prey resources such as Coenogronidae and Hirudinea. Gut content analysis provided conflicting results, as small-sized *G. affinis* fed mainly on copepods, and large-sized *G. affinis* fed on copepods, algae, and insects. The observation that small-sized *G. affinis* are more specialized than large-sized *G. affinis* is likely rooted in their foraging behaviour. These results confirm that stable isotope analysis provides the long-term assimilation of resources, while gut contents provide a snapshot of a consumer's diet (Hyslop, 1980; Vander Zanden *et al*., 1997).

The differences in diet between the small and large size *G. affinis* are correlated with anatomical attributes such as gape size (Lazzaro, 1987). Small-sized *G. affinis* are found commonly in shallow water, whereas large size *G. affinis* are found mostly in deeper water (Pyke, 2005). Within the studied irrigation ponds, it was necessary pivotal to consider the interspecific competition. The gut content study by Mofu *et al*. (2019c) and the results from this chapter show that *G. affinis* are preyed on by *G. callidus*. In addition, Howell *et al*. (2013) suggested that *G. callidus* has the potential to alter the population dynamics of *G. affinis*. Even though *G. affinis* fed on benthic resources such as Chironomidae (Figure 4.9), changes in diet from Copepoda to a wide range of resources such as Copepoda, Insects, and algae were found, which could possibly infer competition for resources between it and *G. callidus*. The results from the stable isotope analysis and gut content analysis show that there is a niche overlap between *Glossogobius callidus* and *G. affinis* over a longer time period; this was reflected by the stable isotope analysis (Table 4.3, 4.6; Figure 4.11), however, the overlap may not be observed when prey diversity is high as reflected by the gut content analysis.

Despite differences in life-history traits (Woodford *et al*., 2013) and variation in abundances (see Chapter 2) among these four fish species within the Sundays River valley irrigation ponds, all these species appear to occupy similar trophic positions based on their diets (Figure 4.2). In fact, the isotopic niche of the small size-class of these species showed a variable niche overlap between species. However, despite the overall high degree of overlap observed from the SIBER results (Table 4.2), subtle differences were observed for large size-classes. The isotope metrics showed that both *G. affinis* and *O. mossambicus* utilized a wide range of resources and trophic levels and in addition, both species had the largest isotopic niche widths (SEA_c) compared to *G. callidus* and *G. aestuaria* (Table 4.2).

Cluster analyses showed two groups, where group 1 consisted of the littoral small-sized River gobies, small-sized *O. mossambicus*, and *G. affinis* (Figure 4.12). Group 2 was comprised of the large-sized *O. mossambicus* and *G. affinis* (Figure 4.12). This makeup of these groups suggests interaction between the species and possible competition for resources. These interactions will further be investigated in Chapters 5 and 6, respectively. The SIMPER results further revealed that Chironomidae and detritus were major contributors to the diet of these three species. In addition, cluster analysis showed that large-sized *O. mossambicus* grouped with *G. aestuaria* and this confirmed through stomach content analysis and SIMPER results that both species mainly fed on zooplankton. These differences between the groups further illustrate the need for the use of both stable isotope analysis and gut content analysis to understand resources partitioning among these co-occurring species (French & Jude, 2001; Britton *et al*., 2010; Jackson *et al*., 2012).

The study highlights the simplicity of the food web within the Sundays River valley, where all the small-bodied fishes (Figure 4.14) share food items. Stable isotope analysis suggested interactions between *G. aestuaria* and large-sized *O. mossambicus* as well as interactions between the littoral groups such as *G. callidus*, *O. mossambicus* and *G. affinis*. Stable isotope analysis revealed that there is overlap in the diet of the four species across size classes. Gut content analysis provided a snapshot of the diet of the four species. In addition, gut content analysis revealed that during summer, when fish, plankton (phytoplankton and zooplankton) (Figure 4.11) and macroinvertebrates abundances are high, there is increased overlap between the diet of *G. callidus* and *G. affinis* (Figure 4.11). Given that during the summer period there is high abundances of food sources, this could also suggest increased interactions between *G. callidus* and *G. affinis*, which both fed on Chironomid larvae. Thus, the results of this chapter suggest that there is potential interspecific competition between *G. callidus* and *G. affinis*. In light of this, the following chapter

on functional response, Chapter 5, will provide greater ecological insights into the interactions between native and non-native species.

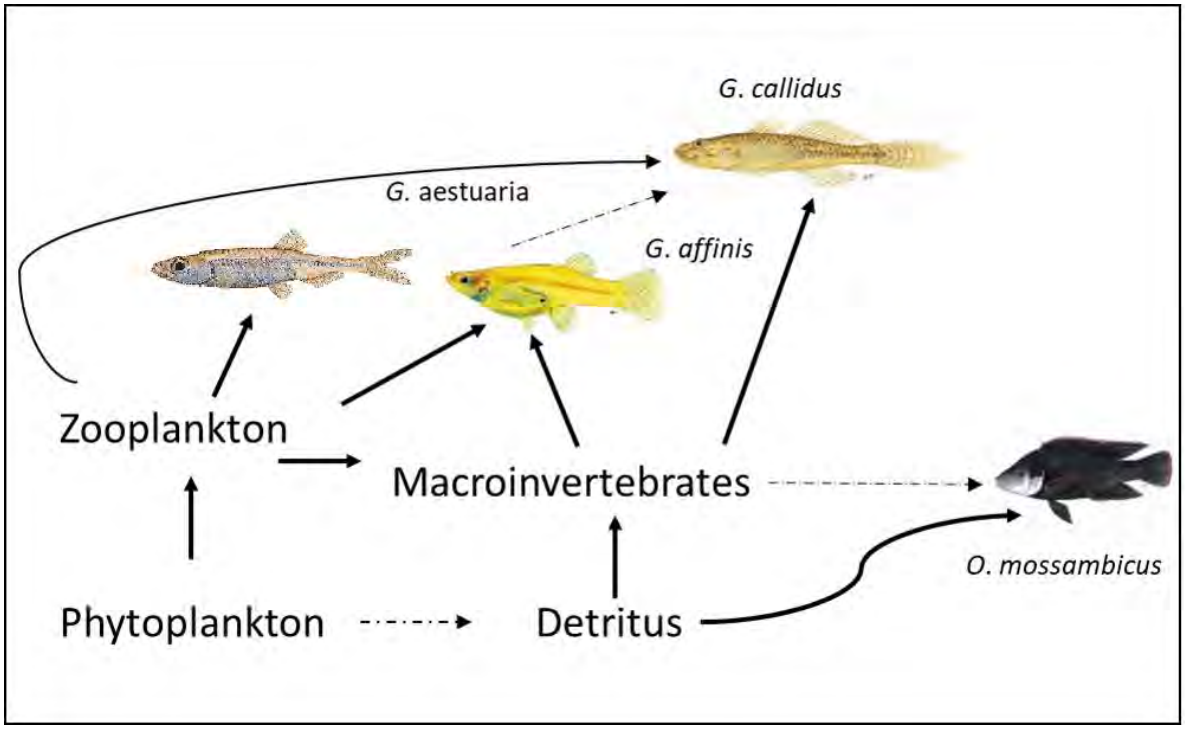

Figure 4.14: Conceptual diagram of the food web in the Sundays River valley irrigation ponds, illustrating the flow of energy and their integration by *Glossogobius callidus*, *Gilchristella aestuaria*, *Oreochromis mossambicus* and *Gambusia affinis*. Arrow thickness denotes the relative importance of each trophic transfer.

CHAPTER 5

Interspecific differences in invader and native fish functional responses illustrate neutral effects on prey but superior invader competitive ability

Introduction

Human-mediated vectors and pathways have increased the introduction of non-native species into new environments and, in particular, freshwater fishes are one of the most commonly introduced taxa (Gozlan, 2008). While introduction pathways for fishes are fairly well understood (e.g., Copp *et al.*, 2005), establishment success appears to be context-dependent and mediated by a variety of influences, including abiotic factors such as temperature (Kolar & Lodge, 2001; Jeschke & Strayer, 2006), biotic factors such as predatory and competitive interactions (Marchetti *et al*., 2004; Latombe *et al.*, 2017), and propagule pressure (Woodford *et al.*, 2013). Thus, understanding the drivers of establishment success or failure is of conceptual and practical importance (Sato *et al.*, 2010). Particularly important in this regard are biotic interactions between native and non-native species, which can have strong implications for community dynamics, as both consumptive and non-consumptive interactions will have direct effects on a predator's fitness and prey biomass (Britton *et al*., 2010; Barrios-O'Neill *et al.*, 2014a; Médoc & Spataro, 2015; Lopez *et al*., 2018).

A classic method used to understand predator-prey interactions and competition is the quantification of the 'functional response' (FR) (Holling, 1959; Murdoch & Oaten, 1975). The FR of a consumer describes the relationship between *per capita* prey (or other resources) consumption and prey (or other resources) density (Solomon, 1949; Holling, 1959, 1965). Functional responses are thus a critical determinant in the outcome of interacting consumer-resource populations (Holling, 1959). The types and magnitude of FRs quantify whether consumers will regulate,

stabilize or destabilize the resources in a population (Murdoch & Oaten, 1975; Juliano, 2001). In addition, differential FRs may reveal inter-specific competitive asymmetries (Dick *et al*., 2017a).

The relationships between consumer resource uptake and resource densities result in three functional response Types: linear Type I, hyperbolic Type II and sigmoidal Type III (Holling, 1959; Jeschke *et al*., 2004). Functional responses have been applied to various aspects of ecology (Abrams, 1982; Englund & Harms, 2001; Faria *et al*., 2004; Feldman, 2006), such as assessing adaptive behaviours (Abrams, 1982) and how resource abundance influences the *per-capita* effects of individual predators under a number of environmental variables such as temperature (Stephens & Krebs, 1986). However, only recently have comparative functional response analyses been used to assess invasive species' impacts on resources such as prey, whereby the impact of an invader is contrasted with functionally similar native and/or non-native species (Dick *et al.*, 2014; Xu *et al.*, 2016; Gebauer *et al.*, 2018).

Dick *et al.* (2014) highlighted that FRs provide a rapid, reliable, inexpensive and readily applicable tool to assess invader ecological impacts across taxonomic and trophic groups. A similar approach has also been applied to quantify multiple predator effects, by comparing predicted and observed functional responses based on single predator and multiple predator combinations and their consumption of prey (Soluk, 1993; Barrios-O'Neill *et al.*, 2014b; Wasserman *et al.*, 2016). As such, there is much potential for the use of comparative FRs for the assessment of dynamics between invasive and native species.

Introduced species enter ecological interaction networks and interact with resident communities, resulting in novel heterospecific interactions, which can include interactions among predators that have no co-evolutionary relationships (Jackson *et al.*, 2017). Consequently, there is a need to better

understand the effects of multiple species interactions in invasion and biotic-resistance contexts (Barrios-O'Neill *et al.*, 2014b; Wasserman *et al.*, 2016). These interactions are fundamental to the structure and functioning of ecological communities and are well-known regulators of predator-prey dynamics (Barrios-O'Neill *et al.*, 2014a).

In a food web context, predator-predator interactions are mediated by intermediate species and these are termed indirect effects. When one species modifies how the other species interact this is called trait-mediated indirect effects (Abrams *et al.*, 1996; Schmitz, 1998; Werner & Peacor, 2003). These interactions can lead to emergent multiple predator effects (MPEs). Quantifying and qualifying MPEs are necessary to understand how interactions between predator and prey may be altered by the addition of another predator, and how one predator may interfere with the predatory success of another (Soluk, 1993; Sokol-Hessner & Schmitz, 2002).

This study thus aims to determine the individual-level functional responses of *G. affinis* and the River goby, *Glossogobius callidus* towards a readily consumed prey, under multi-predator scenarios using the 'multiple predator functional response' (MPFR) approach (Barrios-O'Neill *et al.*, 2014a; Wasserman *et al.*, 2016). To achieve this, I used the novel approach of quantifying individual prey consumption from gut contents to assess the predator functional response of the introduced and native predators under multi-predator scenarios. This approach bridges a major gap where previous MPE research has not been able to assign predator consumption rates to individuals but has rather focussed on the overall effects by determining prey consumption from counts of prey surviving (Barrios-O'Neill *et al.*, 2014a; Wasserman *et al.*, 2016). This latter approach has the potential to mask the presence of MPEs if respective predatory facilitation and disruption occur simultaneously between two predator species, with no observed non-additive effects on prey. In this study chironomid larvae, a prey source that is easily quantified in gut content analysis, due to

their heads being relatively resistant to digestion, were used to test the hypotheses that (i) functional response will differ between native and the invasive species, (ii) multiple predator effects will be observed for both predator species through differences in individual and multi-predator scenario FRs, and (iii) MPEs may not be detectable based on surviving prey counts but may be revealed by gut contents analysis.

Material and methods

Species used

In this study, the MPEs between two trophically similar fish species that often co-occur in warm-temperate, South African river systems, namely, *G. affinis* and *G. callidus*, were assessed (see Chapter 2 for species description) (Woodford *et al.*, 2013).

Animal maintenance

Both species were collected in November 2016 from two ponds (33°27'53"S, 25°33'02"E; 33°27'26"S, 25°39'57"E) in the Sundays River valley, Eastern Cape, South Africa, where they co-occur, using a 30 x 2 m seine net with 12 mm mesh wings and an 8 mm mesh cod-end. Upon capture, the fish were transported to the South African Institute for Aquatic Biodiversity (SAIAB) in aerated containers and the two species were housed separately in 40 L holding tanks with 10 fishes per holding tank. All fish were acclimatized for seven days prior to experiments and maintained on a diet of live chironomid larvae *ad libitum* in a controlled temperature laboratory, maintained at 25°C. Experimental temperature selection was based on daytime summer temperatures recorded from the irrigation pond sites (Howell *et al.*, 2013).

Following Alexander *et al.* (2014), all fish were size-matched with regard to total length (T_L), to reduce the influence of size-related differences in prey consumption, and to more easily reveal any

species-specific differences in feeding (T_L, mean ± SD: *G. affinis* = 40.6 ± 0.56 mm; *G. callidus* = 40.5 ± 0.23 mm). Twenty-four hours prior to the individual (i.e. single predator), and mixed (one of each species) predator experiments, fish were randomly selected from the holding tanks and individually transferred to separate experimental arenas (grey 20 L spherical buckets: diameter = 290 mm; height = 400 mm) containing 5 litre of continuously aerated water in a controlled-temperature laboratory at 25°C. All fish were acclimated to experimental arenas for one day prior to the experiment without food, to allow for standardization of hunger levels (Alexander *et al*., 2013). In all experiments, fish were only used once.

Functional Response experiments

Both the single predator and mixed predator experiments were initiated at 09h00 through the addition to the above tanks of live chironomid larvae. After 2 hours of feeding, predators were removed and the total number of live prey items remaining was enumerated in each replicate. In the individual predator treatments, single fish were presented with larval chironomids (length = 4.35 ± 0.12 mm) at eight prey densities (2, 4, 8, 16, 32, 64, 96 and 120; n = 7 replicates per prey density), with separate experimental arenas used for each density and replicate. Here, FRs were based on surviving prey counts alone. In the mixed predator combinations, interspecific mosquitofish and *G. callidus* pairs were presented with larval chironomids (4.9 ± 0.11 mm) at nine prey densities (2, 4, 8, 16, 32, 64, 96, 120 and 392; n =7 replicates per prey density), with separate experimental arenas used for each density and replicate. In addition to talking surviving prey counts, both predators were euthanised by immersion in 40 mg/l of clove oil and fixed in formalin for stomach contents analysis to determine the proportion of predation for which each species was responsible. As the heads of chironomid larvae are relatively resistant to digestion (Wasserman *et al.*, 2012), individual consumption within fish pairs was inferred by counting numbers of larval

chironomid heads in the stomach contents. In both single and multiple predator trials, controls consisted of larval chironomids in experimental arenas at each prey density (n = 2), but in the absence of predators.

Data analysis

The 'frair' package in R was used to perform functional response analysis (Pritchard *et al.*, 2017). Logistic regression, testing first and second order linear coefficient, were fitted using maximum likelihood to determine the Type of FR (Bolker, 2010). Here, a significantly negative first-order term identifies the FR as Type II. Functional responses were modelled using the 'Random Predator Equation' (Rogers, 1972; Equation 5.1) due to prey not being replaced as they were consumed. Maximum Likelihood Estimation (MLE; Bolker, 2010) was used to estimate values for 'a' (attack rate), 'h' (handling time) and the maximum feeding rate (1/hT), where T was fixed at 1.

$$Ne=N_0\ (1\text{-}exp\ (a\ (Neh\text{-}T))) \qquad \text{Equation 5.1}$$

where Ne is the number of prey eaten and N_0 is the initial density of prey. The Lambert W function was used to fit the model to the data (Bolker, 2008). Non-parametric bootstrapping (n = 2000) was used to generate 95% bias-corrected and accelerated confidence intervals around the response curves. Bootstrapping allows direct population-level inference and visual comparisons of the confidence intervals around the FR, where the overlapping of the bootstrapped clouds indicates no significant differences between the curves. We used the Juliano (2001) difference (delta) method, which allows for the differentiation of coefficients of attack rates and handling time between the treatments.

To assess whether the functional response of the mixed predators could be predicted by summing individual FRs, the predicted combined consumption was calculated using the Soluk (1993) multiplicative model:

$$C_{ab} = N_p (P_a + P_b - P_a P_b) \quad \text{Equation 5.2}$$

where C_{ab} is the predicted consumption for certain initial prey density (N_p) and P_a and P_b are the probabilities of being consumed by each predator present (fish a and fish b), respectively, over two hours of exposure. The predicted combined consumption calculated by the multiplicative model cannot exceed the total number of prey densities used in the experiments. The predicted null FR model for both predators (fish a and fish b) was generated from underlying individual FR data, with which actual multiple predator consumption could be compared. The observed consumption was calculated from the actual number of prey consumed as determined from stomach content analysis from mixed predator experiments in order to test whether the multiplicative model was valid.

The functional response parameters were compared with respect to (1) the species individually, that is, *G. affinis* versus *G. callidus* with no multiple predator treatment; (2) those derived from multiple predator treatment both between species and within species, that is, *G. affinis* compared to *G. callidus* in a heterospecific pair, *G. affinis* alone compared to *G. affinis* in the presence of *G. callidus*, and *G. callidus* alone compared to *G. callidus* in the presence of *G. affinis*; and (3) the observed (stomach content) parameters compared to the parameters from the expected (multiplicative model). All statistical analyses were carried out in R version 3.5.1 (R Development Core Team 2017).

Results

In the controls for both individual and mixed predator experiments, no chironomid mortalities were observed, therefore, in the presence of predators, 100% of prey mortality was attributed to predation and further evidenced through the direct observations and stomach content analysis.

Individual Predator

Both *G. affinis* and *G. callidus* exhibited Type II FRs (Table 5.1; Figure 5.1a). Attack rate did not differ between species ($z = 0.2$, $P = 0.81$), however, *G. affinis* had a significantly shorter handling time, and hence higher maximum feeding rate, compared to *G. callidus* ($z = 7.2$, $P < 0.001$; Table 5.1; Figure 5.1a).

Table 5.1: First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with functional parameters (a, h and $1/hT$) and significance levels from Rogers' random predator equation. Expected functional response was calculated from the combined performance of individual predators of each species and observed functional responses were determined by gut content analysis of each species from mixed predator trials of mixed predator pairs of *Gambusia affinis*, and *Glossogobius callidus* toward larval chironomid prey. a = attack rate; h = handling time; $1/(hT)$ = maximum feeding.

Treatment	Predator	First-order term	*P*	*a*	*P*	*h*	*P*	1/*h*T
Individual	*Gambusia affinis*	-0.016	<0.001	3.796	<0.001	0.016	<0.001	62.5
Individual	*Glossogobius callidus*	-0.025	<0.001	3.64	<0.001	0.029	<0.001	34.482
Mixed	*Gambusia affinis* in the presence of *Glossogobius callidus*	-0.011	<0.001	2.235	<0.001	0.007	<0.001	142.857
Mixed	*Glossogobius callidus* in the presence of *Gambusia affinis*	-0.011	<0.001	0.289	<0.001	0.021	<0.001	47.62
Mixed	Observed	-0.011	<0.001	4.953	<0.001	0.006	<0.001	166.666
Mixed	Expected	-0.011	<0.001	2.573	<0.001	0.006	<0.001	166.666

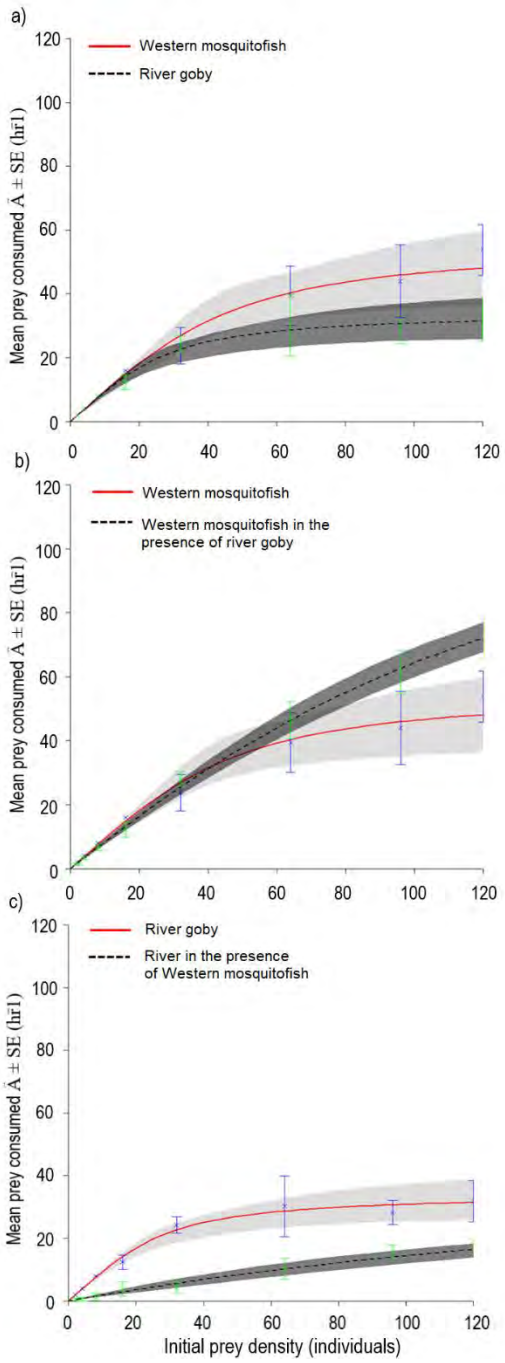

Figure 5.1: Functional responses towards larval chironomid prey of a: (a) *Gambusia affinis* alone and *Glossogobius callidus* alone; (b) *Gambusia affinis* alone and *Gambusia affinis* in the presence of *Gambusia affinis*; and (c) *Glossogobius callidus* alone and *Glossogobius callidus* in the presence of *Gambusia affinis*. Shaded areas are bootstrapped 95% confidence intervals.

Multiple predators

Based on individual consumption data, both species in the multiple predator treatment had Type II FRs (Table 5.1; Figure 5.1b, c). When considering the multiple predator heterospecific combination, *G. affinis* had a significantly higher attack rate ($z = 19.5$, $P < 0.001$) and lower handling time, and hence a higher maximum feeding rate, than *G. callidus* ($z = 7.4$, $P < 0.001$; Figure 5.1b, c).

In comparison with its performance in individual trials, when in the presence of *G. callidus*, *G. affinis* attack rate was significantly higher ($z = 3.4$, $P < 0.001$) and handling time was significantly lower, with consequent higher maximum feeding rate ($z = 10.2$, $P < 0.001$, Figure 5.1b). Conversely, in the presence of *G. affinis*, *G. callidus* attack rate was significantly reduced ($z = 3.1$, $P < 0.001$) and the handling time was significantly longer, with a consequently lower maximum feeding rate ($z = 2.3$, $P < 0.001$), Figure 5.1c).

When the overall MPE observed (stomach content) and the predicted functional response (multiplicative model) for *G. affinis* and *G. callidus* (*G. affinis* + *G. callidus*) were assessed, the FR was also Type II (Table 5.1; Figure 5.2). In the MPE, the overall observed attack rate was significantly higher than expected ($z = 6.6$, $P < 0.001$), but handling time and therefore maximum feeding rate did not differ significantly between observed and expected FRs ($z = 0.6$, $P = 0.53$).

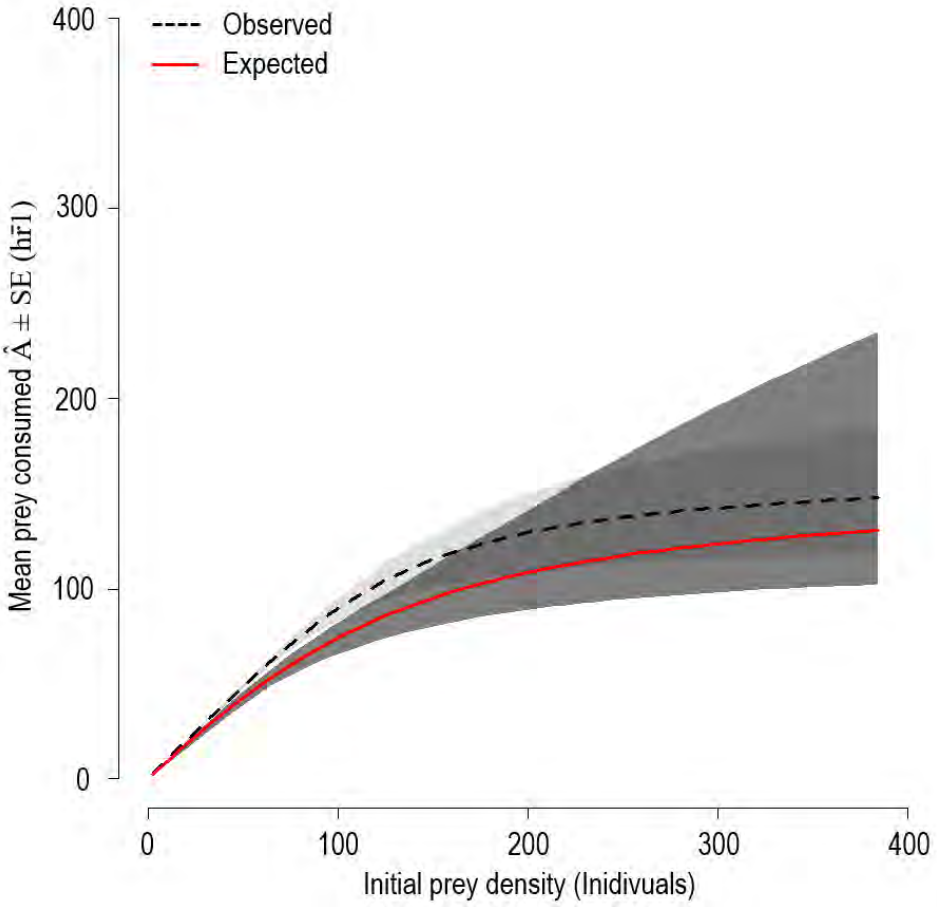

Figure 5.2: Expected and observed functional responses of mixed predator pairs of *Gambusia affinis*, and *Glossogobius callidus* toward larval chironomid prey. Expected functional responses are calculated from the combined performance of individual predators of each species (Figure 5.1). Observed functional responses were determined by gut content analyses of each species from mixed predator trials. Shaded areas are bootstrapped 85% confidence intervals.

Discussion

Both *G. affinis* and *G. callidus* displayed potentially population destabilizing Type II functional responses (Alexander *et al.*, 2014a), in both individual and combined trials. However, this must be caveated as single prey species experiments do not allow for prey switching, which could result in Type III FRs (Dick *et al.*, 2014). With regard to the first hypothesis, This study showed that the individual-level functional response of the invasive *G. affinis* was higher towards chironomid larvae than its native counterpart, *G. callidus*, as predicted by FR theory (Dick *et al.*, 2014). For the second hypothesis, MPEs in the heterospecific treatments occurred and were driven by *G. affinis*, which increased its FRs in the presence of *G. callidus*, while *G. callidus* reduced its FRs in the presence of *G. affinis*. The heterospecific treatments resulted in neutral impacts on prey and there was clear inter-species interference in favour of *G. affinis*. For the final hypothesis, this study showed that only the attack rates of observed FR in the mixed predator pairs were significantly higher than the predicted FR, and there were no significant differences in handling times and maximum feeding rates between the observed FR and the predicted FR from the multiplicative model. This study revealed how multiple predators can alter the shape of individual FRs while masking obvious MPE effects when assessing impacts on surviving prey numbers alone.

The impacts of *G. affinis* were found to be more profound when differences in magnitudes of the FR between the two species were considered. The classic comparative approach, therefore, highlights that *G. affinis* have the ability and feeding efficiency to cause greater field ecological damage, which further exacerbates its known global ecological impacts on native macroinvertebrate communities (Linden & Cech Jr, 1990; Lee *et al.*, 2018). However, growing literature on interactions between predators has shown that overall predation can result in both positive and negative MPE (Davenport & Chalcraft, 2013; Barrios-O'Neill *et al.*, 2014a; Palacios

et al., 2018) and there are a number of ways in which MPE can be effectively determined (Sih *et al*., 1998; McCoy *et al*., 2012; Wasserman *et al.*, 2016; Sentis & Boukal, 2018). In addition, the interaction between predator and prey in ecological communities, and the shape of the functional response affects the stability of the populations of interacting species (Kalinkat *et al.*, 2013; Barrios-O'Neill *et al.*, 2016).

When considering *G. affinis* predation in the presence of the native competitor, this study showed that the interactions between *G. affinis* and *G. callidus* resulted in a negative MPE for *G. callidus*. The reduction in the consumption rate of *G. callidus* in the presence of *G. affinis* and the enhanced consumption rate by *G. affinis* in the presence of *G. callidus* implies net-neutral effects on prey populations. However, this simultaneously showed evidence of competition between the species for resources, with the invader being superior in this regard. At the individual level, both species illustrated high FRs, which could suggest that intraspecific interactions could maximise each species' performance. However, heterospecific pairings showed trait-mediated effects, whereby the FRs of *G. callidus* was greatly reduced while the FRs of *G. affinis* was enhanced.

These findings suggest that the presence of *G. affinis* may have profound impacts on *G. callidus* when competing for food and this may explain the negative association that has been shown between these species in the field (Howell *et al.*, 2013). This study also revealed that the magnitude of the FR differed significantly among predator species when presented as individuals, as was indicated by the attack rates and handling time coefficients. These observed differences in attack rates and handling times offer insights into how species interactions are dependent on prey densities (Lampropoulos *et al*., 2013; Sun *et al.*, 2018).

Although the non-native species achieved a higher FR asymptote than the native species, the combination of *G. affinis* and *G. callidus* resulted in neutral effects on overall prey consumption. These results, therefore, suggest that both predator facilitation and disruptive MPEs occur between interacting *G. affinis* and *G. callidus*. The presence of *G. callidus* enhanced *G. affinis* FR (facilitation), while *G. callidus* FR was reduced (disruption) in the presence of *G. affinis*, possibly due to agonistic behavioral interactions during feeding. According to Bollache *et al.* (2008), the individual-level functional response may not fully express the non-native species' impact, as they do not account for interactions with native species, and therefore it is imperative to include multiple-predator scenarios (Barrios-O'Neill *et al.*, 2014a). Findings with respect to the hypothesis reiterate the importance of such an approach. The findings associated with the second hypothesis emphasize the importance of assessing predator-predator interactions, as this will offer insights on resource-use differentiation between individual-level FR and in multiple-predator scenarios.

This study supports the direct FR approach by adding a novel method of integrating stomach content analysis into the FR procedure when assessing multiple species interactions. This approach will reduce biases in the detection and interpretation of predator functional responses, as it uses direct enumeration of prey items in the predator's stomach. When predator individuals are combined, non-additivity may occur regardless of species identity, such that the combination of two individuals may cause predators to decrease their rates of prey consumption, thereby reducing predation risk for prey (Griffen & Byers, 2006; Sun *et al.*, 2018). In this study, non-additive effects on prey were not observed, as the combined effects of *G. affinis* facilitation and *G. callidus* disruption resulted in a risk-neutral scenario for prey, compared to the effects of the single predator experiment results. These results suggest that the presence of *G. affinis* may hinder *G. callidus* in foraging dynamics in the wild. A previous field study suggested that the population dynamics of

the mosquitofish were regulated by seasonal temperatures and potentially influenced by the presence of *G. callidus* (Howell *et al.*, 2013). They hypothesized that the negative relationship between *G. callidus* and mosquitofish abundance was due to competitive or predator-prey interactions, with *G. callidus* abundance suppressing *G. affinis* densities (Howell *et al.*, 2013). The experimental data, however, suggest that the opposite may be occurring.

The observed changes in prey consumption across treatments suggest that predator-predator interactions have the ability to alter the food intake of native predators, which in turn may result in changes in native population dynamics, with implications for the broader community (Palacios *et al.*, 2018). We suggest that future studies assessing MPEs using FR approaches consider the assignment of prey consumption to individual predators to strengthen MPE identification. This approach revealed the elevated impact potential of mosquitofish compared to the native species, under multi-predator scenarios. This study shows that interactions between non-native and native species may facilitate the success of introduced species, at the cost of the native species. This mechanistic understanding of interaction outcomes between the focal fish species is useful in the broader invasion biology context. However, in the Sundays River irrigation ponds it is highly likely that such interference is restricted to the warmer periods where fish abundance are high and competition for food resources likely heightened. As such, considering temperature effects on FRs, and incorporating this information with abundance data from the ponds across seasons would be useful to obtain a clearer picture on top-down pressures exerted by the fish species on the invertebrate communities.

CHAPTER 6

Impacts of non-native fishes under a seasonal temperature gradient are forecast using functional responses and abundances

Introduction

Biological invasions can occur through numerous pathways, such as human-mediated introduction, climate change and the connectivity of systems, thereby allowing extra–limital movement of species (Latombe *et al*., 2017). On arrival in a new environment, non–native species can cause ecological impact on native species assemblages through a range of biotic interactions such as predation, competition, and parasitism (Vitousek *et al*., 1996; Thomsen *et al*., 2011; Havel *et al*., 2015; Seebens *et al*., 2018). Competition and predation play particularly important roles in the structuring of ecological communities (Paine, 1980). Although the impact of invaders through predation and competition is well documented, the context-dependency of these processes is often overlooked. In particular, direct biotic interactions (predation) can drive trophic cascades through alterations of prey abundance and native predator fitness (Gallardo *et al*., 2016; Penk *et al*., 2017).

Despite the considerable work conducted on invasive species, being able to predict ecological impacts of biological invasions has remained elusive (Simberloff *et al*., 2013; Dick *et al*., 2014). Ricciardi *et al*. (2013) highlighted context–dependency as the key factor for impact predictions in invasion biology. Therefore, robust predictive methods should include environmental contexts in invasion studies to improve impact forecasting. In particular, the temperature regime is a key abiotic context that is pervasive across all ecosystem types, and particularly in aquatic ecosystems (Lang *et al*., 2017). Specifically, temperature is a central determinant of the strength of predator-prey interactions and mediates food web stability (Rall *et al*., 2010, 2012; Englund *et al*., 2011). In freshwater, fish have physiological mechanisms (that is metabolism and reproductive success)

which are directly and/or indirectly dependent on temperature (Roessig *et al*., 2004). These mechanisms may differ between native and non–native species given differences in geographical origins and their physiological tolerances (Sorte *et al*., 2013). If high temperature is more physiologically optimal for invaders, ecological impacts may be intensified (Iacarella *et al*., 2015), and seasonal changes, coupled with ongoing climatic warming, are key drivers of such temperature change in aquatic ecosystems. Indeed, interaction strengths are known to vary even with slight changes to seasonal temperatures (Sanford, 1999). Therefore, failure to incorporate these factors in predictive approaches limits our ability to forecast invasive species impacts under changing environmental conditions across different spatiotemporal scales (Dick *et al*., 2013, 2014, 2017b).

Methodological developments, which incorporate native/non–native species' resource utilization 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) quantifies resource consumption as a function of resource density, and, in a predator-prey context, can quantify *per capita* ecological impacts of predators towards lower trophic groups (Holling, 1959; Adams, 1980; Dick *et al*., 2013, 2014, 2017a; Alexander *et al*., 2014; Cuthbert *et al*., 2018b). The types and magnitude of FRs quantify whether consumers will likely stabilize or destabilize resource populations (Murdoch & Oaten, 1975; Rip & McCann, 2011; Uszko *et al*., 2017). The relationships between consumer resource uptake and resource densities results in three broad functional response Types, and each type has a different effect on resource population stability. An increasing linear relationship with no handling time constraint indicates a Type I, mechanistically exclusive to filter feeders (Jeschke *et al*., 2004). An inversely density-dependent response characterised by high resource consumption at low resource density indicates a Type II, resulting in rapid resource depletion at low densities, and a sigmoidal positively

density-dependent relationship indicates a Type III, where resources have a low–density refuge (Holling, 1959). Despite the fact that the two functional response components, of attack rate and handling time, are strongly associated with variations in temperature (Englund *et al*., 2011; Rall *et al*., 2012; Sentis *et al*., 2015; South *et al*., 2017; Cuthbert *et al*., 2018a), there is very limited information on how temperature mediates species interactions at the population-level (Viherluoto & Viitasalo, 2001; Fussmann *et al*., 2014; O'Gorman *et al*., 2017). Temperature and/or season effects may differ depending on how species' functional traits directly influence responses, and these traits may also change along environmental gradients (Chapin *et al*., 2000).

Classically, the functional response has been combined with the 'numerical response' to determine the 'total response' of consumers (Solomon, 1949; Holling, 1959). The numerical response describes the consumer population-level response to changes in resource densities, while 'total response' can be defined as the multiplication of species' numerical responses with functional response (Solomon, 1949; Holling, 1959). Given that the numerical response, in comparison to the functional response, is difficult to ascertain, consumer abundance has recently been proposed as a proxy for numerical response in the development of the 'impact potential' and 'relative impact potential' metrics (Dick *et al*., 2017b; Dickey *et al*., 2018). The 'impact potential' is the product of functional responses and abundance of consumers, while the relative impact potential compares the impact of the invader to that of a native (Dick *et al*., 2017b). The strength of the relative impact potential metric lies in its ability to incorporate both species abundance and functional response under different environmental conditions (such as. temperature change) and thus predict the influence of context–dependencies on invader impact (Laverty *et al*., 2017). This metric provides a novel approach for assessing existing and potential ecologically damaging species with actual field abundance data under different environmental conditions.

This study sought to comparatively assess the potential relative ecological impacts of *O. mossambicus* and *G. affinis* relative to the River goby, *Glossogobius callidus* towards native benthic prey across a seasonal temperature gradient.

Materials and methods

Functional response experimental design

Glossogobius callidus, *O. mossambicus*, and *G. affinis* individuals were sourced using a 30 m × 2 m seine net with 12 mm mesh wings and an 8 mm mesh cod-end from Dunbrody (33°27'53"S; 25°33'02"E) and Disco Chicks (33°27'26"S; 25°39'57"E) irrigation ponds, Eastern Cape, South Africa (see Chapter 2 for site description and SAIAB long-term monitoring method details). Upon capture, fish were transported to NRF–SAIAB, Grahamstown in continuously aerated containers with source water. Each fish species was housed separately in a controlled temperature and light laboratory and kept under a 12:12 light: dark cycle. The temperature was maintained at either 18 ± 2 °C or 25 ± 2 °C (experimental temperature groups) for seven days prior to experimentation, with each species acclimated separately in 40 L fish tanks in a closed recirculating system. All fish were maintained on a standardized diet of larval chironomids *ad libitum*. The chironomid larvae were collected by kick sampling from the Bloukrans River (33°19'06"S; 26°34'22"E) using a kick net (1000 μm). The chironomids were then strained twice through first 2.0 mm and then 1.0 mm sieves to obtain the experimental size class (total length (T_L) ± standard deviation ((SD) 1.5 ± 0.11 mm), and then rinsed thoroughly with deionized water to remove any other food sources.

Functional response experiments were performed at 18°C and 25°C, reflecting respective spring and summer temperatures at the sampling locations. Following Alexander *et al*. (2014a), all fish were size-matched (T_L (mean ± SD): *G. callidus* = 41.50 ± 4.10 mm; *O. mossambicus* = 41.70 ± 4.10 mm; *G. affinis* = 41.60 ± 4.10 mm), in order to eliminate the influence of size-related

differences in prey consumption and to focus on species-specific differences (Rall *et al*., 2012). Individuals of *G. callidus*, *O. mossambicus*, and *G. affinis* were randomly selected from the holding tanks 24 hours prior to the trial and transferred into experimental arenas (opaque 20 L spherical arenas: diameter: 290 mm; depth: 400 mm) containing 5 L of continuously aerated rainwater. In individual experimental arenas, each assigned fish was held for 24 hours prior to the experiment without food to allow for acclimatization and standardization of hunger levels. Individual fish were then presented with chironomid larvae at one of eight prey densities (n = 2, 4, 8, 16, 32, 64, 96 and 120; n = 7 replicates per prey density). At the end of each experimental period, predators were removed and the total number of live prey items remaining, and hence numbers consumed, enumerated. One set of experiments (i.e. one randomized fully factorial replicate per experimental temperature group) was conducted in a day, and the experiments were initiated at 09h00 am, during photoperiod, with prey consumption examined after 2 h. Controls consisted of larval chironomids in experimental tanks at each prey density in the absence of predators (n = 2 replicates per experimental group). Predators were only used once and there was no reuse within or across experimental groups.

Fish abundances

The fish predator abundance data were obtained from the NRF–SAIAB`s monitoring programme of irrigation ponds in the Sundays River valley, Eastern Cape, South Africa (see Chapter 2). Fish abundance from two irrigation ponds were used, ML Swart, and River Bend (see Chapter 2 for site description). These ponds were selected on the basis that they were surveyed in both spring and summer and that all three species were captured to give abundance estimates. During each survey, the irrigation pond water temperatures were measured using a HANNA HI98129 combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, USA). Spring (18°C) and

summer (25°C) abundance estimates were used in this study as they were in line with the experimental temperatures, and reflect seasonal temperature means.

The ponds were surveyed using a 30 m × 2 m seine net with 12 mm mesh wings and an 8 mm mesh cod–end. At least three hauls were conducted per pond and, upon completion of a single haul; all fish were kept alive in a continuously aerated container (20 L) until every seine haul was completed within a pond. Fish were then identified to species–level, enumerated and released back into the water. The abundance data were based on maximum catch field abundances using mean catch per 100 m^2.

Data analysis

Generalised linear models (GLMs) assuming a Poisson error distribution and log link were used to analyse overall prey consumption with respect to species, temperature, and prey supply. Likewise, GLMs were used to compare fish abundances with respect to species, season and pond. Non–significant terms and interactions were removed stepwise to obtain minimum adequate models (Crawley, 2007). Tukey's comparisons were used to undertake *post-hoc* tests of significant effects in each resulting model (Hothorn *et al*., 2008).

To distinguish between Type II and III functional responses, logistic regression of the proportion of prey consumed as a function of initial prey density was performed (but see also Rosenbaum & Rall, 2018; Chapter 5). The selection between Type II and Type III models was further confirmed via comparison of Akaike's information criterion. A significantly negative first-order term indicates a Type II functional response, whereas a significantly positive first-order term followed by a significantly negative second-order term indicates a Type III response (Juliano, 2001).

Rogers' random predator equation was used to model functional responses, as prey were not replaced, as they were consumed (Rogers, 1972; Chapter 5).

The relative impact potential (RIP) of *G. callidus*, *O. mossambicus* and *G. affinis* species was measured using the mean bootstrapped functional response maximum feeding rate and abundance (AB) for the three species at each season and pond (Dick *et al*., 2017b), where:

$$\text{RIP} = \left(\frac{\text{FR non–native}}{\text{FR native}}\right) \times \left(\frac{\text{AB non–native}}{\text{AB native}}\right) \qquad \text{Equation. 6.1}$$

when RIP < 1, the predicted impact of the non–native fish is predicted to be less than the native; when RIP = 1, there is no difference in impact between the fish species; whereas when RIP > 1, the non–native has a greater impact than the native. To integrate uncertainty into the RIP score, a probability density function (pdf) was applied using the standard deviation (SD) of the FR and AB estimates and this generated 80% confidence intervals (CIs) (Dick *et al*., 2017). Biplots were then generated to illustrate the RIP for both the non–native *O. mossambicus* and *G. affinis* relative to the native *G. callidus* at each season between ponds (Laverty *et al*., 2017). All analyses were carried out in R v. 3.4.2 (R Development Core Team 2017).

Results

Functional response

Prey survival of larval chironomids was 99% in control groups with predators absent, and thus prey mortality in the experimental groups was attributed to predation. Overall consumption was significantly different among fish species (χ^2 = 221.67, *df* = 2, $P < 0.001$). *Glossogobius callidus* consumed significantly fewer prey than both *O. mossambicus* ($z = 14.61$, $P < 0.001$) and *G. affinis* ($z = 8.43$, $P < 0.001$). *O. mossambicus*, in turn, consumed significantly more prey than *G. affinis* overall ($z = 6.41$, $P < 0.001$). Consumption was also significantly greater at the higher temperature,

analogous with the summer season (χ^2 = 179.61, *df* = 1, *P* < 0.001), and consumption increased with temperature for all species as there was no significant 'predator × temperature' interaction (χ^2 = 3.54, *df* = 2, *P* = 0.171; Figure 6.1). In addition, consumption increased significantly with increasing prey supply (χ^2 = 2019.88, *df* = 1, *P* < 0.001).

Table 6.1: Parameter estimates from first-order logistic regression of the proportion of consumed prey as a function of prey density, with functional response estimates, *a* = attack rate; *h* = handling time 1/*h* = maximum feeding rate.

Predator	Season	First-order term, *p*	*a*	*P*	*h*	*P*	1/*h*
Glossogobius callidus	Spring	-0.035, <0.001	4.340	<0.001	0.050	<0.001	20.000
Oreochromis mossambicus	Spring	-0.025, <0.001	5.230	<0.001	0.023	<0.001	43.478
Gambusia affinis	Spring	-0.026, <0.001	4.741	<0.001	0.036	<0.001	27.778
Glossogobius callidus	Summer	-0.025, <0.001	3.647	<0.001	0.029	<0.001	34.482
Oreochromis mossambicus	Summer	-0.014, <0.001	2.237	<0.001	0.009	<0.001	111.111
Gambusia affinis	Summer	-0.016, <0.001	3.796	<0.001	0.017	<0.001	58.823

At 18°C (spring temperature), all three fish species displayed a Type II functional response (Table 6.1; Figure 6.1a). Attack rates did not differ significantly between fishes (*G. callidus* and *O. mossambicus*: *z* = 1.03, *P* = 0.301; *G. callidus* and *G. affinis*: *z* = 0.42, *P* = 0.675; *O. mossambicus* and *G. affinis*: *z* = 0.51, *P* = 0.611). However, *G. callidus* exhibited significantly longer handling times than both *O. mossambicus* (*z* = 9.67, *P* < 0.001) and *G. affinis* (*z* = 4.36, *P* < 0.001). Accordingly, maximum feeding rates were considerably higher in the non–native as compared to native fishes (Table 6.1). In turn, *O. mossambicus* had significantly shorter handling times, and thus higher maximum feeding rates, than *G. affinis* (*z* = 6.27, *P* < 0.001) (Figure 6.1a).

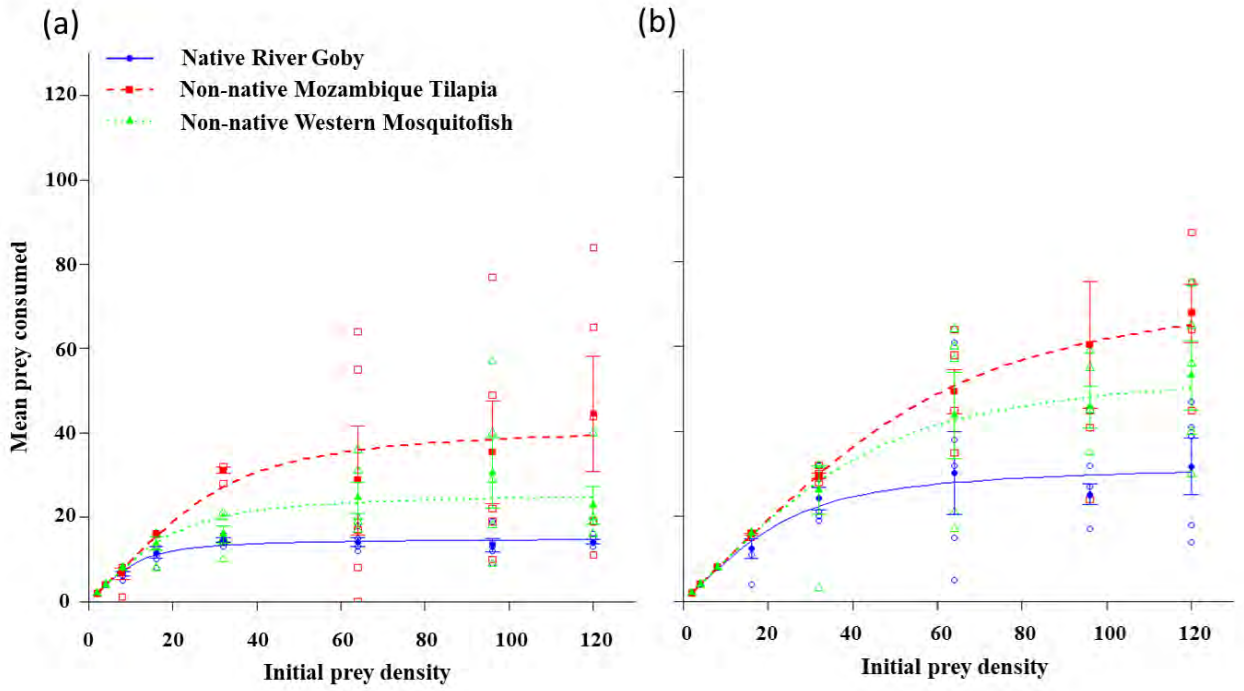

Figure 6.1: Functional response curves for *Glossogobius callidus* (blue circles, solid lines), *Oreochromis mossambicus* (red squares, dashed lines) and *Gambusia affinis* (green triangles, dotted lines) at 18°C (a) and 25°C (b). Means are ± SE. Filled points are means and unfilled points are raw data.

At 25°C (summer temperature), all three fish species also exhibited a Type II functional response (Table 6.1; Figure 6.1b). There were significant differences in attack rates between the River goby and *O. mossambicus* ($z = 2.62$, $P = 0.008$). Attack rates between the River goby and *G. affinis* did not differ significantly ($z = 0.24$, $P = 0.811$). However, *O. mossambicus* had significantly lower attack rates than *G. affinis* ($z = 2.88$, $P = 0.004$). The River goby displayed significantly longer handling times than *O. mossambicus* ($z = 12.55$, $P < 0.001$) and *G. affinis* ($z = 7.18$, $P < 0.001$), again driving substantially higher maximum feeding rates by *O. mossambicus* and *G. affinis* (Table 6.1). In turn, *O. mossambicus* had significantly shorter handling times than *G. affinis* ($z = 6.92$, $P < 0.001$), and exhibited the highest maximum feeding rate (Figure 6.1b).

Fish abundances

There was a significant 'species × season × pond' interaction ($\chi^2 = 92.54$, $df = 2$, $P < 0.001$; Figure 6.2), with seasonal responses of fish species abundance differing between the two ponds. From ML Swart in spring, *G. callidus* abundances were not significantly different to that of *O. mossambicus* ($z = 0.63$, $P = 0.988$), but were more abundant than *G. affinis* ($z = 4.44$, $P < 0.001$). In turn, *O. mossambicus* were also more abundant than *G. affinis* ($z = 4.73$, $P < 0.001$). In summer, abundances of *G. callidus* in ML Swart did not differ significantly either to *O. mossambicus* ($z = 0.48$, $P = 0.990$) or *G. affinis* ($z = 2.71$, $P = 0.070$). In addition, there were no significant differences between *O. mossambicus* and *G. affinis* abundances ($z = 2.23$, $P = 0.223$). In contrast, in spring at RBD, *G. callidus* were significantly more abundant than both *O. mossambicus* ($z = 4.52$, $P = 0.001$) and *G. affinis* ($z = 6.28$, $P < 0.001$). *Oreochromis mossambicus* abundances was significantly greater than that of *G. affinis* ($z = 3.51$, $P = 0.006$). In summer, however, *G. callidus* was significantly less abundant than *O. mossambicus* ($z = 10.74$, $P < 0.001$) yet more abundant than *G. affinis* ($z = 4.12$, $P < 0.001$). Similarly, *O. mossambicus* was more abundant than *G. affinis* at this site ($z = 5.74$, $P < 0.001$).

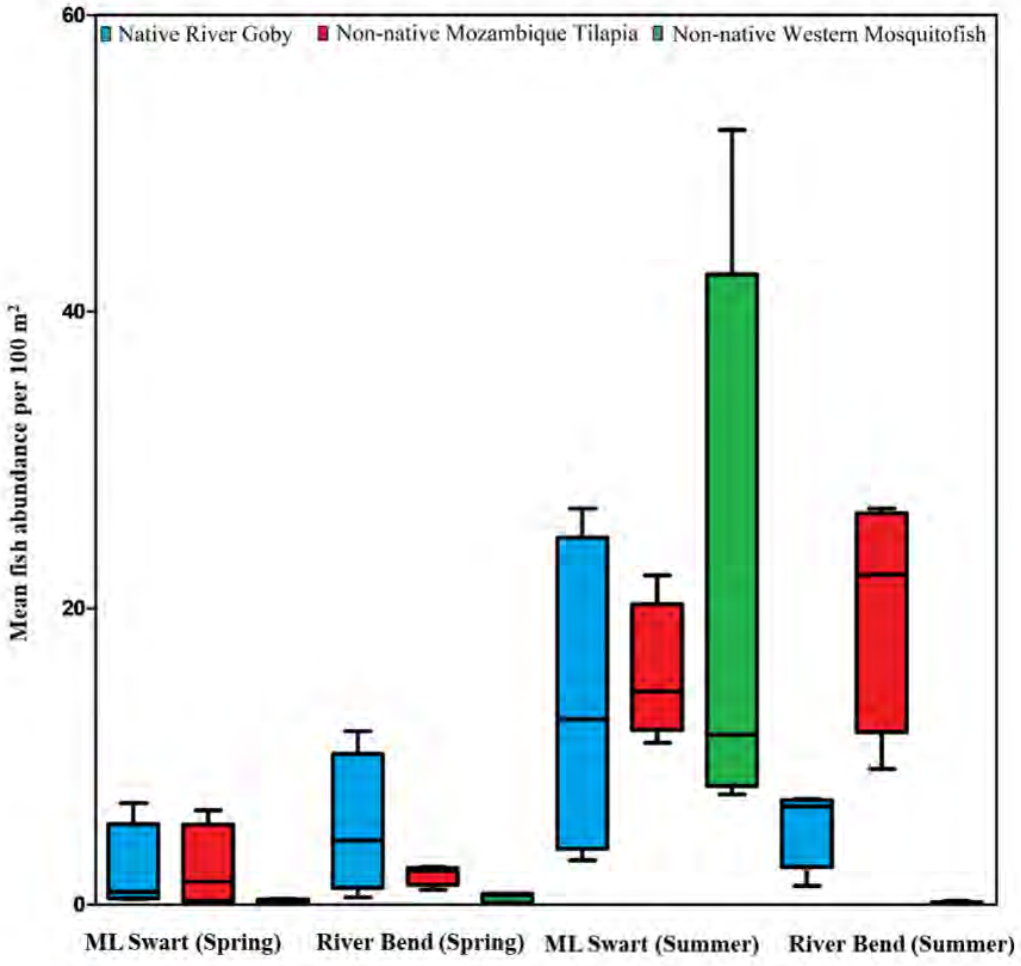

Figure 6.2: Abundance box plots for *Glossogobius callidus* (blue), *Oreochromis mossambicus* (red) and *Gambusia affinis* (green) from ML Swart and River Bend irrigation ponds, Eastern Cape, South Africa. Sampling occurred in spring (18°C) and summer (25°C).

Relative impact potential

Under both spring and summer treatments, *O. mossambicus* consistently displayed relative impact potential scores > 1 relative to *G. callidus* irrespective of focal ponds, suggesting a greater impact than the native species (Table 6.2). In contrast, *G. affinis* had relative impact potential scores of < 1 from ML Swart and approximately 1 from River Bend in spring, respectively suggesting lower or similar impacts to the native *G. callidus* (Table 6.2). In summer, *G. affinis* had a relative impact

potential score > 1 from ML Swart, but a relative impact potential score < 1 from River Bend. This suggests a lower impact in River Bend and a higher impact in ML Swart, relative to *G. callidus*.

The relative impact potential biplots concur with the relative impact potential scores (Figure 6.3). In spring, *O. mossambicus* had the highest impact potential followed by *G. callidus* and trailed by *G. affinis* in both ML Swart and River Bend (Figure 6.3a, b). In summer, there was an inconsistency between the ponds, as *G. callidus* has the lowest relative impact potential in ML Swart compared to *O. mossambicus* and *G. affinis* (Figure 6.3c). The relative impact potential biplots from River Bend in summer are more reflective of the trends observed in both ponds in spring, where *O. mossambicus* had the highest impact potential followed by *G. callidus* and lastly *G. affinis*, which had no impact owing to its absence at this site (Figure 6.3d).

Table 6.2: Relative Impact Potential (RIP) using mean bootstrapped maximum feeding rates for *Oreochromis mossambicus* and *Gambusia affinis* against *Glossogobius callidus*. Field abundance data are integrated from ML Swart (MLS) and River Bend (RBD) ponds in spring and summer. Uncertainties are reflected through 80% confidence intervals (CIs).

Species	Season	Pond	Mean FR maximum feeding ± SD	Mean field abundance ± SD	RIP	CIs	$P_{rip}>1$
Oreochromis mossambicus, *Glossogobius callidus*	Spring	MLS	45.40 ± 11.31, 19.96 ± 3.53	2.41 ± 2.84, 2.25 ± 3.10	7.25	0.42 – 16.32	75.17
Oreochromis mossambicus, *Glossogobius callidus*	Spring	RBD	45.40 ± 11.31, 19.96 ± 3.53	2.04 ± 0.69, 5.22± 4.80	1.69	0.35 – 3.57	55.21
Gambusia affinis, *Glossogobius callidus*	Spring	MLS	26.68 ± 2.87, 19.96 ± 3.53	0.19 ± 0.17, 2.25 ± 3.10	0.35	0.29 – 0.78	70.78
Gambusia affinis, *Glossogobius callidus*	Spring	RBD	26.68 ± 2.87, 19.96 ± 3.53	2.04 ± 0.69, 5.22 ± 4.80	1.01	0.22 – 2.11	33.40
Oreochromis mossambicus, *Glossogobius callidus*	Summer	MLS	125.02± 54.57, 32.60 ± 4.10	15.50 ± 4.80, 13.70 ± 11.10	7.30	1.58 – 15.35	96.40
Oreochromis mossambicus, *Glossogobius callidus*	Summer	RBD	125.02 ± 54.57, 32.60 ± 4.10	20.10 ± 8.02, 5.41 ± 2.77	18.26	5.21-36.15	99.98
Gambusia affinis, *Glossogobius callidus*	Summer	MLS	97.17 ± 148.60, 32.60 ± 4.10	20.70 ± 21.20, 13.70 ± 11.10	7.58	0.30 – 16.56	69.64
Gambusia affinis, *Glossogobius callidus*	Summer	RBD	97.17 ± 148.60, 32.60 ± 4.10	0.06 ± 0.13, 5.41 ± 2.77	0.05	0.00 – 0.09	40.20

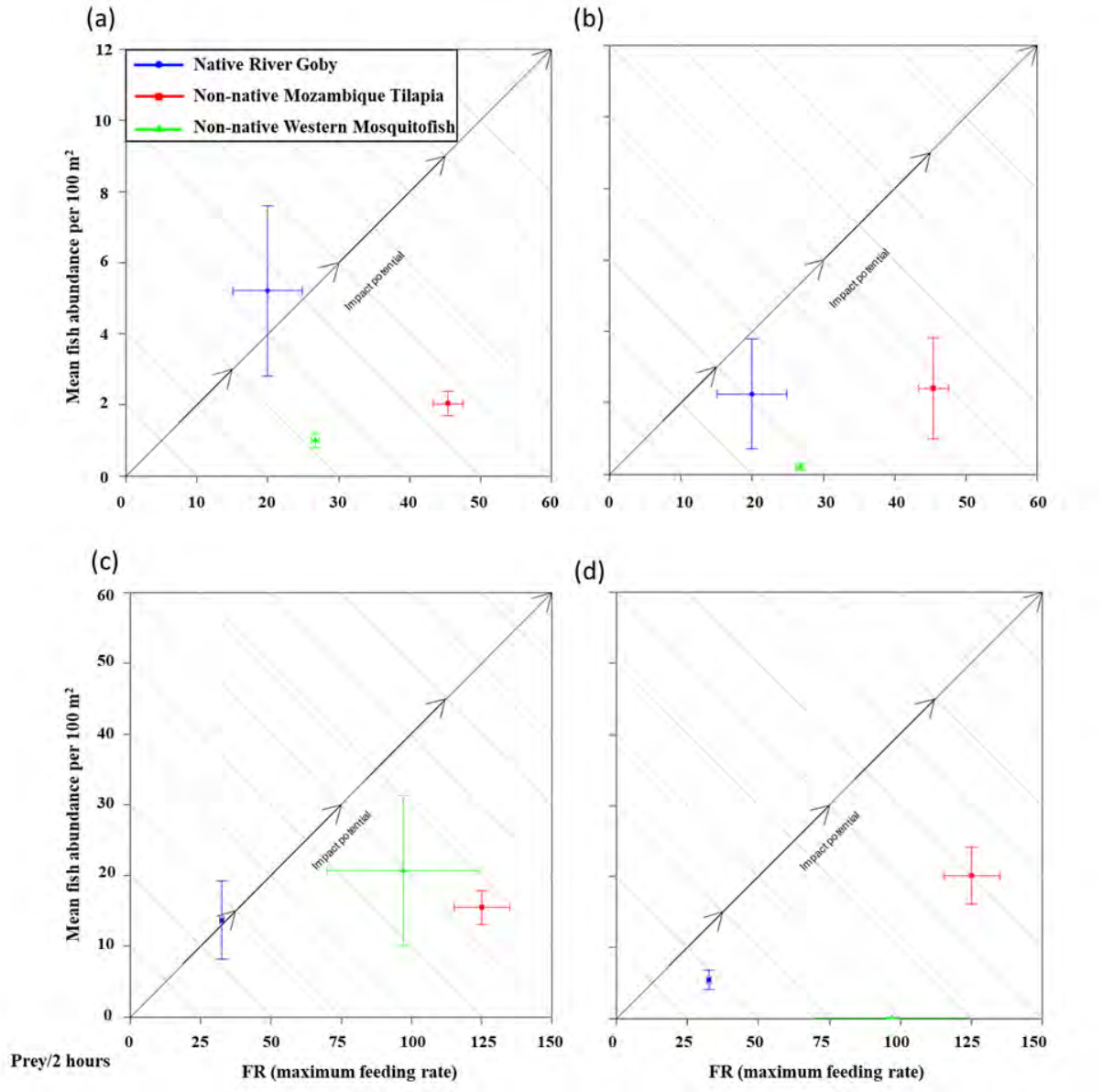

Figure 6.3: Relative impact potential (RIP) biplots (see also Table 6.2) of *Glossogobius callidus* (blue circles), *Oreochromis mossambicus* (red squares) and *Gambusia affinis* (green triangles) in spring (18°C): (a) ML Swart (b) River Bend; and in summer (25°C): (c) ML Swart and (d) River Bend. Ecological impact increases from bottom left to top right. Note differences in axes scaling. Values are mean ± SD.

Discussion

Using the relative impact potential metric proposed by Dick *et al*. (2017b), this study provides insights into how the ecological impacts of non–native species are mediated by temporal variabilities associated with seasons, through the multiplying of functional responses and population abundances. Irrespective of seasonal variations, the results of this chapter corroborate studies which have identified *O. mossambicus* as an impactful species (Canonico *et al*., 2005; Maddern *et al*., 2007), whilst *G. affinis* impacts have been observed to be less pronounced. The results show that all three fish species displayed a Type II functional response across the seasonal gradient, indicative of high resource utilisation at low densities (see Table 6.1).

Whilst Type II functional responses are common in comparative laboratory-based studies (e.g., Dick *et al*., 2013) if included experimentally, additional context–dependencies such as habitat structure may have driven a significant impact on functional response form (Vucic-Pestic *et al*., 2010a; Vucic-Pestic *et al*., 2010b; Kalinkat *et al*., 2013; Barrios–O'Neill *et al*., 2016). Moreover, greater incremental low–density prey resolution, different feeding durations and larger experimental aquaria volumes may further alter functional response forms (for example, to Type III) (Sarnelle & Wilson, 2008; Uiterwaal & DeLong, 2018). In this study, interspecific variation in functional responses between the species showed that both *O. mossambicus* and *G. affinis* exerted higher *per capita* impacts than *G. callidus* on native prey, and that predatory impacts were more profound during the summer season (Figure 6.1). These findings concur with a considerable number of studies comparing the impact between invasive and native species (Alexander *et al*., 2014b; Dick *et al*., 2014; Cuthbert *et al*., 2019).

Temperature differences had a significant effect on the functional response parameters, wherein attack rates were high in spring (18°C) and were reduced in summer (25°C). This result concurs

with Grigaltchik *et al*. (2012), who observed that an increase in temperature resulted in reduced attack rates, but contrasts with other studies (e.g., Wasserman *et al*., 2016) who found that attack rates exhibit a non–monotonic temperature response. In addition, this chapter shows that during the summer season, handling times were reduced, and therefore these species exhibited higher maximum feeding rates. The findings of Englund *et al*. (2011) corroborate the results of this chapter. This effect is mostly related to predators' metabolic rate changes. For example, for a predator's metabolic activity to reach its maximum efficiency (that is high *per capita* effects), temperatures need to be optimal, however, if temperatures are too high this will result in reduced metabolic rates through catabolism (Clarke & Johnston, 1999).

Secondly, the abundance results showed that there was significant variation in fish abundances among species according to the season, and between ponds (Figure 6.2). Such variation in fish abundances seems to be a common theme, especially in fish communities that co-occur in environments, which is driven by spatial and temporal variation in life-history traits (Amezcua & Amezcua-Linares, 2014). All three fish species were generally less abundant in spring and more abundant in summer. *Oreochromis mossambicus* was the most abundant species overall, followed by *G. callidus* and lastly *G. affinis*. By combining the fish maximum feeding rates and abundances (as per Dick *et al*., 2017b) to give the relative impact potential score, this study showed that *O. mossambicus* had the highest impact consistently across seasons, whereas, in the majority of cases, the impacts of *G. affinis* were less apparent than that of *G. callidus* given currently low abundances (Figure 6.3).

Changes in relative impact potential scores with seasonal temperature fluctuations and fish abundances from different localities demonstrate how such context-dependencies can have a critical effect on the relative field impact capacities of introduced species through time (Dick *et*

al., 2017b). The effects of temperature regime shifts on interaction strengths are profound across habitat types and trophic groups (Englund *et al*., 2011; Rall *et al*., 2012), and increasing temperatures may exacerbate invader ecological impacts as species approach thermal optima (Iacarella *et al*., 2015). This is supported by the heightened functional responses observed in this study for *O. mossambicus* and *G. affinis* as the experimental temperature was increased to near their thermal preferendum (~28˚C; Jobling, 1981). Therefore, the explicit inclusion of temperature change should be critical in future studies that seek to predict invader impacts across regime shifts associated with climatic warming and seasonal variability. As the relative impact potential metric was 100% predictive of ecological impact across taxonomic and trophic groups (Dick *et al*., 2017b), these results, which showed high relative impact potential for *O. mossambicus*, indicates that this species could be used to forecast to cause major ecological impacts.

This study further demonstrates the usefulness of numerical response proxies such as abundances in rapid assessments of the potential impacts of introduced species. Indeed, in many cases, impact predictions are inherently limited if based on *per capita* impacts alone, given the importance of abundances in discernments of overall offtake rates by consumer populations (Dick *et al*., 2017b). Importantly, the results of this chapter suggest that ecological impacts of non–native species are likely to change across seasonal gradients associated with both changing functional responses and abundances, with summer impacts generally more profound than those in spring. This study thus proposes that further research should incorporate such seasonal variability. This study demonstrates that species-specific shifts in abundances may alter interaction strengths within ecosystems towards native populations. Therefore, quantitative assessments of species abundances can ultimately bridge the gap in decision-making and can be used to forecast future invader impacts under different climatic conditions when combined with *per capita* effects. In addition, this study

demonstrates that individual systems (such as ponds) can differ substantially in predator community composition over time, and this system-specific population variability should be considered in future studies.

Overall, this study provides further evidence of the strength of the relative impact potential metric in predicting ecological impacts of species and provides an extension to the framework by integrating an environmental gradient, which reflects seasonal temperature fluctuations. The identification of temporal shifts in impact across seasons and habitats in this study presents novel insights into invader impact and top-down pressure potential on invertebrate populations. In many ecosystems, data on species abundances are still lacking, but as the relative impact potential metric enables impact predictions for species without invasion histories, we recommend more surveys to estimate the abundance of potential invaders and/or for practitioners to incorporate other proxies (such as fecundity) into the metric (Dickey *et al*., 2018). Crucially, the ability of both *O. mossambicus* and *G. affinis* to thrive in novel habitats highlights their ecological plasticity, and with an increase in environmental temperatures, their impacts may be intensified through changes to functional responses and fish abundances. The relative impact potential metric thus allows for rapid assessment of current and future invasive species under shifting environmental contexts and can identify priority species for management. This study further highlights that seasonal effects on the fish communities are considerable, even for the invasive species. The incorporation of both field abundance and laboratory FR information proves that temperature is a major mediator of trophic dynamics in these systems with likely implications for fish population and invasion success.

CHAPTER 7

Synthesis and conclusions

This study demonstrates that the biotic interaction between the River goby, *Glossogobius callidus*, Estuarine roundherring, *Gilchristella aestuaria*, Mozambique tilapia, *Oreochromis mossambicus* and Western mosquitofish, *Gambusia affinis* was driven by a complex interplay between temperature, pond community ecology and food web structure. The pond community ecology (consisting of seasonal dynamics of the zooplankton, macroinvertebrates and the littoral fish community structure) was described in detail. The pond food web structure was noted emphasising the trophic positions of *G. callidus*, *G. aestuaria*, *O. mossambicus* and *G. affinis*, inferring inter-specific food resource utilisation and overlap. Gut content analysis was also employed to assess dietary overlap dynamics between the four fish species; however, gut content analysis revealed an increased overlap between *G. callidus* and *G. affinis*. The interactions among key species were further assessed using laboratory-based functional response experiments. Finally, an integration of functional response and abundance data across different temperatures was performed to assess the Relative Impact Potential of each species across different seasons.

An overview of all four data chapters provides insight into the ecology of these irrigation impoundments and further offers the discernment on factors regulating native and non-native fish populations (Figure 7.1). The pond community ecology, food web and the interactions between the four fish species were mainly driven by temperature. As water temperatures increased, internal dynamics increased in primary and secondary productivity, along with fish abundance. This pattern was evident for all fish species across all ponds. The warmer sampling periods were associated with higher fish catches in the littoral habitats (see Chapter 3) and in general; food webs that are more complex (see Chapter 4). The assessment of the trophic position of the fish showed

that many of the fish are consuming similar resources, implying that competition for food may be a biotic factor structuring population dynamics when food resources become limiting.

Given that of the four study species, *G. callidus* and *G. affinis* are the most similar in size and feeding ecology, it is not surprising that gut content dynamics revealed that in summer these two species had particularly pronounced similarities in their diet. This prompted the competition experiment (Chapter 5), which showed that during certain periods, under certain conditions, *G. affinis* might competitively exclude *G. callidus* from acquiring food resources. However, ultimately *G. affinis*, along with the other fish species, seem to be limited by temperature. Abundance and predatory performance (FR) data, using the RIP approach, highlighted the role of temperature in limiting predation pressure potential during cooler periods when littoral fish abundance was low (see Chapter 6). Overall, fish abundance, environmental conditions and life-history traits influenced the biotic interactions among the four studied species. Competition among the species changes seasonally mainly through differences in abundances.

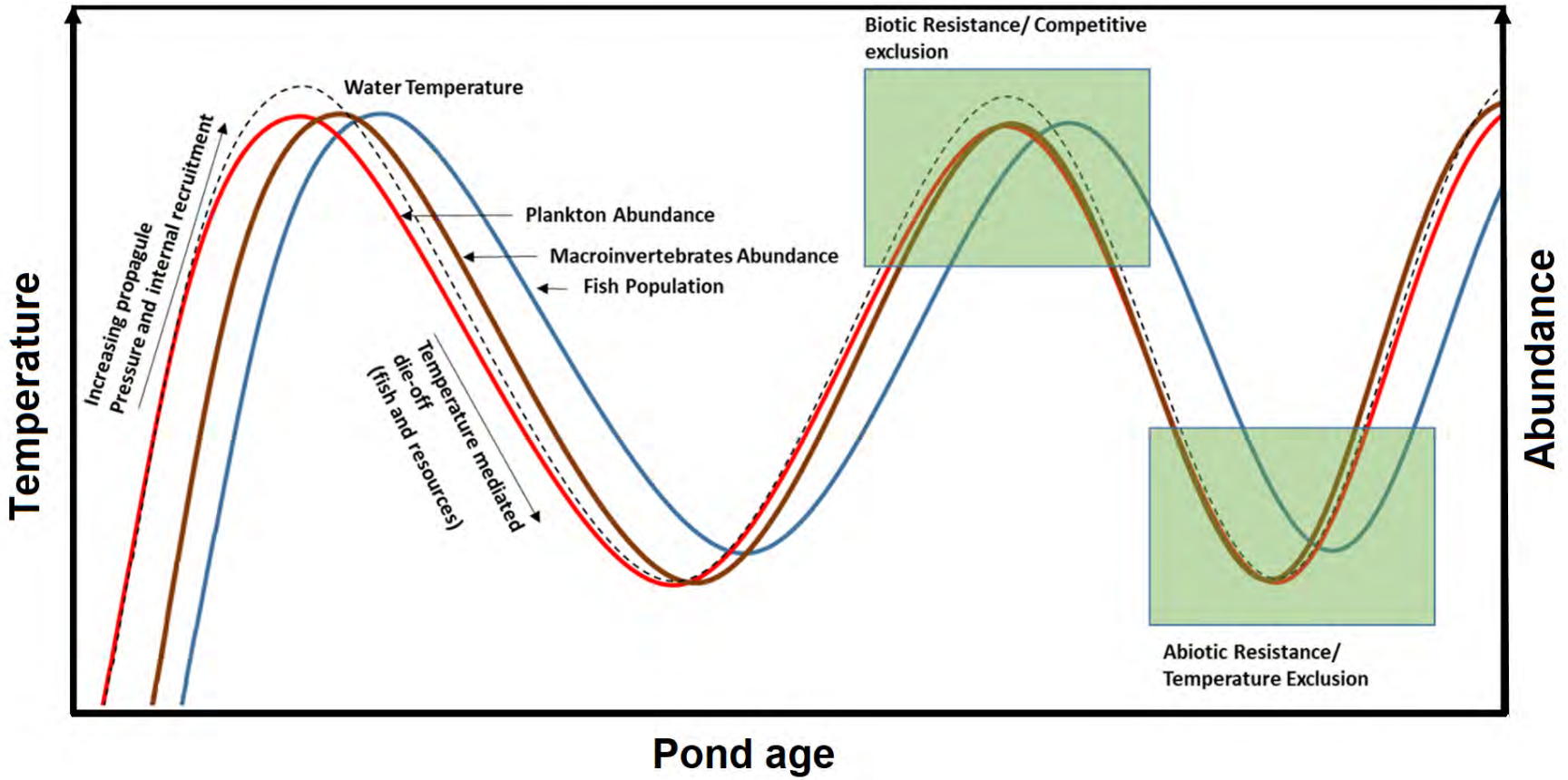

Figure 7.1: Conceptual model outlining ecological dynamics across seasons in the Sundays River irrigation ponds. Seasonal temperature fluctuations are considerable, resulting in temporal shifts in drivers of fish populations. Increases in temperature are associated with increased fish propagule pressure and primary and secondary productivity. Increased fish abundance increases competition interaction potential, with implications for native species (*Glossogobius callidus*) prey acquisition. Decreases in temperature are associated with system-level abundance reductions, including fish population size. As such, in winter months invasive species numbers are reset by low temperatures, with implications for impact potential during these times.

In this thesis, it is shown that during peak inflow period there was high plankton productivity (see Chapter 3). Interactions among the species influenced the communities within these ponds, but only during the warmer periods when fish abundances were high. This study also showed that the three species, namely *G. callidus*, *O. mossambicus* and *G. affinis* occupied similar trophic positions and overlapping isotopic niches suggesting high potential for competition for dietary resources (see Chapter 4).

Chlorophyll-*a*, temperature, *G. callidus* and *G. affinis* were the drivers of the seasonal changes in macroinvertebrate composition. Zooplankton, macroinvertebrates and littoral fish abundances, species richness, diversity and assemblage composition differed significantly across seasons with temperature identified as the main driver of these differences. This study, therefore, demonstrates the bottom-up driven nature of the irrigation pond communities in the Sundays River valley and highlights the relevance of seasonal abiotic drivers in structuring these communities. As shown in the data chapters, temperature was important in determining the biotic interactions among the four fish species. Given the temperature effects on fish abundance in winter, propagule pressure seems to be a major driver of population persistence, as fish populations tend to be reintroduced as temperatures increase.

The flow of materials and nutrients in ecosystems can be mapped by food webs, which are macrodescriptors of the feeding interactions within a community (Jepsen & Winemiller, 2002; Schiesari *et al*., 2009). Food webs in aquatic ecosystems may differ in productivity; resource abundance and community structure (Ottonello & Romano, 2011). Therefore, trophic ecology plays a pivotal role in trophic connections among taxa in ecological communities (Yule & Gomez, 2009). The findings of Chapter 3 highlight the potential role of temperature and water fluctuations in structuring the ponds' zooplankton, macroinvertebrates, and the littoral fish community. In

addition, the high abundance of zooplanktivorous fish is a result of high chlorophyll-*a* concentrations, as is common in reservoirs (Prchalová *et al*., 2009). These changes in zooplankton abundance mainly due to temperature may cascade down the food web. The littoral fish community structure differed across seasons and this is likely a result of reproductive success of each individual species, diet, competition for resources, escape from predators, water level fluctuations and water temperature (Fischer & Echmann, 1997; Vašek *et al*., 2006; Brosse *et al*., 2007). Within these irrigation ponds, temperature affects all levels of the food web mainly due to its impact on the physiological performances. These findings are congruent with those of several studies, which reported that temperature affected phytoplankton growth, zooplankton and littoral community structure and fish through changes in consumption and metabolic rates (Hairston & Hairston, 1993; Dippner *et al*., 2000).

During the time the ponds were filling up (that is, the time of increased propagule pressure), species abundances in terms of fish, zooplankton, and littoral macroinvertebrates increased mainly to utilise the available space and food resources in an attempt to complete their life cycles (Wilbur & Alford, 1985; Wilbur, 1987). As competition for resources increases and space and/or habitat increases, species with rapid growth rates, early maturation and the ability to compete for resources are favoured such as *G. callidus* and *G. affinis*.

An additional tool for predicting the success of non-native species is an understanding of life-history traits (Mooney & Hobbs, 2000; Bøhn *et al*., 2004). Non-native species need several traits to be successful in a new environment and this includes rapid growth, early maturity and short life span (Morton, 1996). Life-history traits might be affected by a variety of factors such as ecosystem productivity and physico-chemical variables (Schlosser, 1990). Previous studies on *G. callidus*, *G. aestuaria* and *G. affinis* found that these three fish species differed in their life-history traits from

high fecundity, early maturity, parental care, short life span and longevity and these seem to have affected their success in these ponds (Docherty, 2012; Kunz, 2013; Mofu, 2016).

Conclusions

The biotic interactions between the four species exemplifies the difficulties in predicting the colonisation success of non-native species into novel environments. Overall, the fast colonisation patterns exhibited by *G. callidus* and *G. affinis* seemed to be a response to high food availability, during filling up of the ponds. Early maturity and, generalist feeding behaviour are traits that enabled *G. callidus* and *G. affinis* to thrive in these ponds. The different kinds of substrates (rocks, and aquatic littoral vegetation) allowed the fish protection from predation for recruiting fishes, provided food for the littoral small-bodied fish and reduced the strength of predator-prey interactions (Diehl, 1992). The simple structure of the ponds may also be a driving factor in the interactions between these four fish species, as it renders prey easily available. In a broader context, this study has demonstrated conclusively that *G. callidus*, *G. aestuaria*, *O. mossambicus*, and *G. affinis* can colonize novel environments if they receive adequate propagules. However, without the constant input of propagules from another source location, it is unclear whether populations would persist. Stable isotope analysis suggested an overlap in the diet of the four species and the study observed potential interactions between *G. aestuaria* and large-sized *O. mossambicus*. Gut content suggested a greater overlap in the diet of *G. callidus* and *G. affinis*, thus inferring competition for food resources.

A combination of stable isotope derived-population metrics and functional response experiments revealed a complex interaction between *G. callidus* and *G. affinis* mainly driven by temperature. Understanding of the interaction (*per capita* effect) between *G. callidus*, *O. mossambicus* and *G. affinis* could illuminate the driving forces behind the pond community structure and the species

population abundances. The abundance of resources (Chapter 3) during the warm season suggests that both *G. affinis* and *O. mossambicus* may have an advantage due to increased attack rates and reduced handling times. For the most part, biotic interactions among the four species was influenced by a combination of water temperature and food web structure. Overall, the results presented in this thesis produce a complex picture of abiotic and biotic drivers of biotic interactions of species in novel environments.

Recommendations for future research

The ecology of these ponds needs to be explored further to obtain a comprehensive understanding of the complex process controlling the interaction of species in new environments. Although Chapters 2 to 4 provide the foundation of the biology and abundances of the four fish species, there are still considerable gaps in our knowledge pertaining to the biology and population dynamics of their juveniles. Future research should focus on age and growth, physiology and behavioural adaptation of these four fish species as well as the stochastic events within these ponds by teasing out the dynamics of top-down and bottom-up effects for each individual fish species. In this study, there was evidence of seasonal changes in zooplankton, littoral macroinvertebrates and the littoral fish community structure across ponds. These changes need to be measured, as well as the importance of nutrients concentration in driving the success of these fish species.

In Chapter 3, the seasonal community structure of zooplankton and littoral macroinvertebrates was described in detail, and further studies should incorporate multiple sampling methods for macroinvertebrates in the ponds. In addition, there is a need to understand the ecology of the Sundays River as well as to understand the ecological mechanisms and the strength of the connection between the Sundays River and the irrigation ponds. The stability of food webs is altered by the flow of energy across ecosystems between recipient and donor ecosystems. In this

study, knowledge was provided on the food web structure of these ponds, however, this needs to be investigated further, seasonally by collecting stable isotope samples from all basal resources including, plankton, macroinvertebrates and fish, and perhaps also including autotrophic inputs as a source of food. The food web structure of the Sundays River also needs to be understood to provide an overall picture of the food web and compare the lotic with the lentic environment. The results in Chapter 5 and 6, clearly show competition for food resources, under different water temperatures. This was an important factor in the identification of competition for resources. Further investigation of habitat use, chemical cues and multiple food sources, which were not investigated in this thesis, is warranted. In addition, functional morphological studies should also be included with functional response studies as well as a detailed description of the metabolism of each species.

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