Influence of the invasive fish, *Gambusia affinis*, on amphibians in the Western Cape

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“The whole land is made desolate,
but no man lays it to heart.”

JEREMIAH 12:11

DECLARATION

I, Roxanne Conradie, declare that this dissertation is my own, unaided work, except where otherwise acknowledged. It is being submitted for the degree of M.Sc. to the North-West University, Potchefstroom. It has not been submitted for any degree or examination at any other university.

(Roxanne Conradie)

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ABSTRACT

The worldwide dispersal and establishment of invasive fish is partly responsible for the global decline in amphibian species. Unfortunately, this is a significant problem in the Western Cape in South Africa, as the region is both a hotspot for alien freshwater fish and an area of critical conservation concern for endemic and threatened amphibians. Research abroad has shown that one of the invasive fish that occur in the region, the Western mosquitofish (*Gambusia affinis*), is a threat to the persistence of amphibians. However, there are no South African studies that examine the impact of mosquitofish on local species, and consequently this study arose from this research need. Consequently, the influence of mosquitofish on native amphibian species was examined by determining the extent of mortalities and injuries inflicted on amphibians by way of mesocosm trials. These trials revealed that these alien fish have the potential to completely obliterate amphibians within a micro-habitat. However, mosquitofish predation effects were different between amphibian species, suggesting that certain species are more susceptible to mosquitofish predation than others. This phenomenon was further examined by way of predation experiments, which in combination with the mesocosm trials suggest that local non-bufonid species are at a higher risk of mosquitofish predation than bufonids. Indirect predation effects were also examined during the mesocosm trials, and it was revealed that surviving tadpoles were severely impacted through retarded development, stunted growth, limb and tail injuries, and the manifestation of stress-induced behaviour. The mechanisms of mosquitofish predation were also further explored, and it was found that attack behaviour on tadpoles is socially facilitated, which indicates that predation intensifies as group sizes of mosquitofish increase. Furthermore, the extent of their spread within the Cape Town area was determined by conducting a literature review and preliminary field survey. This information was further used to determine areas where mosquitofish occur in sympatry with threatened amphibians. It was found that mosquitofish are widely distributed throughout this area, and also that numerous endemic amphibians are vulnerable and likely to be negatively impacted by this invasive fish. There is, however, only one endemic species with an IUCN Threatened status that is potentially jeopardised by the mosquitofish.

Key terms: *Gambusia*, Anura, Cape Floristic Region, invasive species, predation, tadpole, conservation.

OPSOMMING

Die wêreld-wye verspreiding van indringer visspesies is een van die groot oorsake in die afname van amfibieë. Ongelukkig is hierdie probleem dubbelvoudig in die Wes-Kaap, aangesien daar in die streek 'n groter aantal indringer visspesies voorkom, en ook omdat dit 'n sensitiewe bewaringsarea is, wat krities bedreigde en endemiese amfibieë ondersteun. Huidige internasionale navorsing bewys dat een van hierdie indringer visspesies wat in die Wes-Kaap voorkom, die muskietvis (*Gambusia affinis*), 'n bedreiging vir amfibieë-spesies is, maar ongelukkig is daar tot dusvêr geen Suid-Afrikaanse studies wat die impak van muskietvisse op amfibieë ondersoek nie. Die doel van hierdie studie was dus om die invloed van die muskietvis op plaaslike amfibieë te bepaal. Deur middel van mesokosm studies, is daar ondersoek tot watter mate muskietvisse beserings en vrektes van paddavissies meebring. Die proewe het bewys dat die muskietvis paddavissies binne 'n mikro-habitat kan uitwis. Die mate van die impak van die muskietvis op paddavissies het verskil tussen amfibieë-spesies, wat 'n aanduiding is dat sekere spesies meer kwesbaar as ander is. Dié verskynsel is bevestig deur predasieproewe, waar dit verder openbaar is dat plaaslike nie-'bufonid' spesies heel waarskynlik meer kwesbaar is as 'bufonid' spesies. Indirekte predasie effekte is ook tydens mesokosm proewe ondersoek. Dit was duidelik sigbaar dat paddavissies wat aanvanklik predasie oorleef het, hewig beïnvloed was deur die vertraging van ontwikkeling en groei, beserings, asook die verandering van gedrag as gevolg van spanning. Daar is ook verder bevind dat die aanvalsgedrag op paddavissies toeneem soos die muskietvisgroep groter raak, met ander woorde dat die visse mekaar beïnvloed om meer intense aanvalle op hul prooi te loots. Ten slotte word daar in hierdie studie verwys na die verspreiding van die muskietvis in die wyer areas rondom Kaapstad, wat bepaal is deur middel van 'n literatuurstudie asook 'n praktiese opname. Hierdie ingligting is gebruik om te bepaal waar muskietvisse saam met bedreigde amfibieë-spesies voorkom. Bevindinge bewys dat die muskietvis reeds baie wyd verspreid voorkom, en dat verskeie endemiese spesies kwesbaar is. Daar is egter slegs een IUCN Bedreigde spesie wat moontlik negatief deur die muskietvis beïnvloed kan word.

Sleutel terme: *Gambusia*, Anura, Wes-Kaap, indringerspesie, predasie, paddavis, natuurbewaring

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ACRONYMS

ABM	Africa Biodiversity Management
ADU	Animal Demography Unit
BioNet	Biodiversity Network
CBA	Critical Biodiversity Area
CESA	Critical Ecological Support Area
CFR	Cape Floristic Region
CIB	Centre for Invasion Biology
CoCT	City of Cape Town
CSIR	Council for Scientific and Industrial Research
DAFF	Department of Agriculture, Forestry and Fisheries
DEA	Department of Environmental Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EOL	Encyclopaedia Of Life
FBNR	False Bay Nature Reserve
FISK	Fish Invasiveness Screening Kit
FRAI	Fish Response Assessment Index
FROC	Frequency of Occurrence
GBIF	Global Biodiversity Information Facility
GCTA	Greater Cape Town Area
GISD	Global Invasive Species Database
IPI	Impact of Predation Index
ISSG	Invasive Species Specialist Group
IUCN	International Union for the Conservation of Nature
LWR	Length: width ratio
OESA	Other Ecological Support Area
PII	Predation Intensity Index
PRI	Predator Response Index
RHP	River Health Programme
SAIAB	South African Institute for Aquatic Biology
SANBI	South African National Biodiversity Institute
TBNR	Table Bay Nature Reserve
WWTW	Waste Water Treatment Works
WRC	Water Research Commission

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The global persistence of amphibians is an escalating concern, with 48% of known species in rapid decline (Stuart *et al.* 2004). Natural habitat deterioration and loss through anthropogenic modification is one of the primary drivers of these declines (Stuart *et al.* 2004; Hayes *et al*. 2010). Atmospheric change, environmental pollutants, invasive species, exploitation and amphibian diseases are also a significant cause of dwindling amphibian populations (Beebee and Griffiths 2005; Hayes *et al*. 2010), and these causative factors interact to amplify their impact on amphibians (Hayes *et al*. 2010). Those amphibians particularly at risk are endemic species with small populations and localized geographical ranges, since endemism is a determinant of extinction risk (Purvis *et al.* 2000).

Amphibian biodiversity is particularly rich in South Africa, as more than 150 known species occur in the country (see Du Preez and Curruthers 2009; Channing *et al*. 2013a; Channing *et al.* 2013b; Conradie 2014; Channing *et al*. 2017; Minter *et al*. 2017; Turner and Channing 2017). The subtropical north-eastern area of the country is particularly high in amphibian species richness, but most of the endemic species in South Africa occur in the Cape Floristic Region (CFR) of the Western Cape, an area of global conservation concern (Cowling *et al*. 2003). Of the 40 local species that occur in the CFR, 32 are endemic, and the highest concentration of IUCN Red Data Listed amphibian species occur here (Stuart *et al*. 2008). These facts demonstrate the importance of this area for amphibian conservation.

Unfortunately and ironically, the CFR is not only rich in natural biodiversity, but is also an invasive species hotspot with the highest number of alien and extralimital invasive species in South Africa (Picker and Griffiths 2011), making it the most invaded area in the country (Wilson *et al*. 2014). Invasive fish species have been introduced into most of the river catchments of the CFR, with 18 alien fishes recorded for the region to date (see Marr *et al*. 2012). Despite the high number of invasive fishes in the CFR and their widespread distribution, there are less than 10 studies that document their impacts on the natural biota (Ellender *et al*. 2017). Most of these studies have focussed on the impacts of smallmouth bass, *Micropterus dolomieu* (Woodford *et al*. 2005; Ellender *et al*. 2011; Shelton *et al*. 2014; Weyl *et al.* 2013, 2014), and a few other studies on the largemouth bass, *Micropterus salmoides* (Shelton *et al*.

2008; Ellender *et al*. 2011), and rainbow trout, *Oncorhynchus mykiss* (Woodford and Impson 2004; Shelton *et al*. 2014). The majority of this research is limited to invader impacts on the native fish taxa, with only one impact study on invertebrate fauna (Lowe *et al*. 2008; reviewed by Ellender and Weyl 2014). There is no knowledge on how these invasive fish influence the amphibians of this region.

Studies have shown that alien fish have the potential to cause amphibian population declines and local extinctions (reviewed by Kats and Ferrer 2003). Invasive fish harm amphibian populations the most by decreasing the survivorship of tadpoles, through the predation of eggs and larvae (Kats and Ferrer 2003). However other indirect effects at the larval stage, such as predator-induced stress and injury, can reduce metamorphic size and vigour and are likely to result in the reduced fitness of adult amphibians (Segev *et al*. 2009). Some amphibians might also be more susceptible to mosquitofish than others, due to differences in palatability (Kats, Petranka and Sih 1988), and through the differential selection of prey that may alter whole community structures (Shulse *et al.* 2013).

The Western mosquitofish, *Gambusia affinis* (Baird and Girard 1853), is one of the invasive fish species that currently occupies the Western Cape. This alien is one of the most globally widespread invasive species, initially introduced into many countries as a bio-control agent for mosquitoes (Courtenay and Meffe 1989). It was introduced into South Africa in 1936 for this same purpose and also as a fodder fish for bass (De Moor and Burton 1988), and now occupies at least half of the country’s waterways (Van Rensburg *et al.* 2011). There is surmounting evidence that this invasive fish species poses a threat to amphibians (Goodsell and Kats 1999; Lawler *et al*. 1999; Segev *et al*. 2009, Shulse *et al*. 2013), but there are no South African studies that have examined this for native amphibian taxa. Segev *et al*. (2016) did investigate the predation efficiency of mosquitofish in comparison with local fishes, but there remains a dearth of information regarding this species. In fact, there is an overall paucity of knowledge regarding all invasive fish in the country, with little known about their mechanisms of invasion (Ellender and Weyl 2014), their drainage specific distributions (Ellender and Weyl 2014), and their impacts (Lowe *et al*. 2008). However, an understanding of these factors is required before the impacts and spread of invasive fish can be managed, prevented, or diminished (Thieme *et al*. 2005).

This study arose out of these current gaps in South African literature. The influence of mosquitofish on amphibian species was examined, with a focus on the amphibians of the Western Cape region. It was hypothesized that mosquitofish negatively affect amphibian larval development, growth, and survival. It was also hypothesized that certain species would be more vulnerable to predation than others, due to their increased palatability towards the mosquitofish, as well as due to overlapping distributions with the invasive species. In order to understand the impacts of the invasive mosquitofish to local amphibian tadpoles, the following aims and objectives were formulated for this study. Please see the relevant chapter for the theoretical background underlying each aim. The outline of this disseration is summarised at the end of this chapter.

1.2 Research aims

(1) Determine the direct and indirect effects of mosquitofish predation on tadpole species of the Western Cape (Chapter 2).

(2) Investigate whether certain species are more susceptible to mosquitofish predation than others (Chapters 2 and 3).

(3) Investigate whether tadpoles at different stages of development are more susceptible to mosquitofish predation than other stages (Chapter 3).

(4) Determine whether the presence of invertebrate prey alters the impacts of mosquitofish on amphibians (Chapter 3).

(5) Determine whether mosquitofish predation is socially facilitated, i.e. that attack behaviour intensifies as mosquitofish group size increases (Chapter 4).

(6) Determine what areas in the greater Cape Town area mosquitofish occur in sympatry with threatened amphibians, in order to determine the species most at risk (Chapter 5).

1.3 Research objectives

- Conduct a mesocosm study in order to investigate the impacts of mosquitofish on tadpoles through the direct effects of predation (mortality rate and infliction of injuries) and the indirect effects of predation, namely (stress behaviour, stunted growth and decreased developmental rates) (Aim 1).

- Repeat mesocosm trials using different amphibian species, in order to determine whether impacts of mosquitofish are varied among amphibian species (Aim 2).

- Perform laboratory feeding trials to examine differences in tadpole palatability towards mosquitofish when tadpoles are at different developmental stages (Aim 1 and 3).
- Incorporate invertebrate alternate prey in feeding trials in order to determine whether it reduces tadpole predation (Aim 5).
- Repeat the above laboratory feeding trials using different tadpole species, so that differences in mosquitofish predation among amphibian species can be detected (Aim 2).
- Conduct feeding trials with different sized groups of mosquitofish, in order to determine whether comparative tadpole predation per fish is amplified when group size increases (Aim 5).
- Undertake a comprehensive literature review and conduct a preliminary field survey, so that the distribution of mosquitofish in the greater Cape Town area can be determined. This information will also be used to ascertain which threatened amphibians occur in sympatry with this invasive fish (Aims 2 and 6).

1.4 Dissertation outline

Following the introduction, the primary content of the dissertation is divided into four main sections. The first three sections, which each focus on different aspects of mosquitofish predation on tadpoles, are examined in Chapters 2-4. The first section (Chapter 2) investigates the impact of mosquitofish predation on tadpoles by way of mesocosm trials. The second section (Chapter 3) consists of predation experiments that determine interspecific and ontogenic shifts in palatability of tadpoles towards mosquitofish. The influence of invertebrate prey on tadpole predation is also examined in this section. This is followed by the third section (Chapter 4), which examines the social behaviour of mosquitofish and how this characteristic influences tadpole predation. The last section (Chapter 5) largely consists of a description of the distribution of mosquitofish and explores the potential risk that this fish could impose on sympatric amphibians. These four main sections are followed by a summative discussion of all the work (Chapter 6), with recommendations for the management of mosquitofish. This is followed by a reference list using the referencing style of *African Zoology*, and the final section of the dissertation contains an appendix with addenda of additional tables, results, maps, and information.

Chapter 2 – Influence of the invasive mosquitofish (*Gambusia affinis*) on three amphibian species of the Western Cape

2.1 Introduction

The Western mosquitofish, *Gambusia affinis* (Baird and Girard 1853) is listed as one of the world's 100 worst invasive species (Lowe *et al*. 2000), and studies performed worldwide have shown that they are causing significant damage to natural ecosystems. *Gambusia affinis* and its close relative, *Gambusia holbrooki* (Hubs and Lagler 1947), originate from south-eastern and central North America (Lee *et al*. 1980), but have collectively become the most widespread invasive freshwater fish (Pyke 2008), and occur on all continents except Antarctica (Pyke 2005). The western mosquitofish has also become widespread in South Africa (Van Rensburg *et al*. 2011), and has inhabited the country for roughly eight decades since its introduction in 1936 (De Moor and Burton 1988). Their marked tolerance to a wide variety of environmental conditions has enabled their successful establishment in certain areas, and their spread to new regions. Not only do they inhabit freshwater systems in temperate regions, but have also been shown to exhibit euryhaline characteristics and are able to inhabit estuaries (Pyke 2005). Their success can also be attributed to their high fecundity, as this aquatic invader can produce a few generations of young in a single breeding season (Pyke, 2005). This species also modifies its life-history patterns and tolerates a wide variety of environmental conditions (Daniels and Felley 1992), which are characteristics that bolster the invasion success of mosquitofish populations.

Although brought into most countries for mosquito control (Courtenay and Meffe, 1989), these fish do not only eat mosquito larvae but consume a wide variety of biota due to their omnivorous feeding habits, (Kramer *et al.* 1987), and at higher rates than their local poecilid relatives (Rehage *et al*. 2005). Their broad diets and high feeding rates have caused serious declines in several localized populations of fish and amphibians in particular, as well as invertebrates (Meffe 1985; Gamradt and Kats, 1996; Howe *et al*. 1997; Goodsell and Kats 1999, Rehage *et al*. 2005). Studies have also shown that the mosquitofish does not only impact biota at the population level, but that this alien also adversely modifies biotic community structures through predation at multiple trophic levels (Hurlbert *et al*. 1972; Shulse *et al*. 2013).

The increasing awareness in academia abroad, concerning the dangers of mosquitofish, has not spilled over to South African research to the same degree, with local studies on this species being relatively sparse. Although these fish have been located in waterways country-wide, impact studies are restricted to the Sundays River valley system (Howell *et al*. 2013) and the Wilderness Lakes system (Olds *et al*. 2011; Sloterdijk *et al*. 2015). Mosquitofish occurrences are documented in a few other papers (Clarke *et al.* 2009; Viskitch *et al*. 2016), but there has been no specific research thus far regarding their impact on local biota (Howell *et al*. 2013), and in particular no research on their effect on amphibians. It is reported that 37% of all South African frogs are affected by invasive species (Measey 2011). However, it is unclear to what extent the mosquitofish contributes to this problem in comparison with the impacts of other exotic fish species. Invasive fish are not well-studied in South Africa overall, despite the country being listed as one of six invasive fish species hotspots in the world (Van Rensburg *et al*. 2011). Studies of introduced fish are largely biased towards the larger predatory fish, namely *Micropterus spp.* (Ellender and Weyl 2014); however serious aquatic ecosystem impacts do not only occur via large piscivorous species, but also small omnivorous fishes such as the mosquitofish (Moyle and Light 1996).

The most ecologically sensitive freshwater ecosystems in South Africa occur in the Western Cape, where the largest abundances of regionally threatened and endemic fish, plant, and invertebrate species occur in the Olifants and Berg river systems (IUCN 2017). The predatory habits of the mosquitofish, as well as its high success as an invasive species, make it a potential threat to South African freshwater biota, and particularly might jeopardise already threatened frog species. Although numerous studies on amphibians and invasive fish have been conducted abroad, research in the South African context is sparse and long overdue (Minter *et al*. 2004; Measey 2011).

Studies abroad show that mosquitofish are causing amphibian population declines due to the predation of eggs, embryos, and tadpoles (Smith and Smith 2015), and due to the infliction of injuries on tadpoles during predation attempts, resulting in sub-lethal effects (Shulse and Semlitsch 2014). Mosquitofish predation can even result in the extirpation of local amphibian populations (Goodsell and Kats 1999). It has also been shown that predatory fish may prefer certain amphibian species above others (Kats, Petranka and Sih 1988), due to differences in palatability and other anti-predator adaptations in tadpoles. Differences in prey preference can put certain species at greater risk than others, and therefore should be examined in order to determine the most susceptible species.

The aim of this research is to contribute to the knowledge base for this alien fish, by determining the extent of mosquitofish predation on amphibian species that occur within the Western Cape, South Africa. Direct effects of predation will be determined by monitoring injury and mortality in tadpoles exposed to mosquitofish, and comparing the effects of mosquitofish predation between amphibian species. Indirect effects such as reduced growth and development, and changes in tadpole behaviour, will also be examined. The latter will be determined because even sublethal effects have been shown to cause amphibian population declines (Beebee and Griffiths 2005).

2.2 Materials and methods

2.2.1 Experimental design and rationale

In order to investigate the direct and indirect effects of mosquitofish on anuran larvae, artificial ponds were set up within a greenhouse, at the Department of Biodiversity and Conservation Biology, University of the Western Cape, Cape Town, South Africa. Thirty tadpoles and ten mosquitofish were used for each experimental treatment, and ponds stocked only with tadpoles were used as controls. A minimum of three ponds was used for experimental and control groups each, for every amphibian species tested. The number of replicates per experiment was restricted due to the large space needed for each pond, and the area limitations of the greenhouse.

Three tadpole species from different ecomorphological guilds were used in this study so that predation effects could be determined between guilds. The three species that were chosen, according to availability and accessibility, were *Strongylopus grayii*, *Sclerophrys pantherina*, and *Tomopterna delalandii*. The ecomorphological guild occupied by each respective species is lentic-benthic, benthic-profundal and *excitus-parageios* (Botha 2014). Lentic-benthic tadpoles are bottom-dwellers, and prefer the shallower, well-vegetated areas of standing waters (Altig and Johnston 1989). Benthic-profundal tadpoles are also bottom-dwelling, but generally inhabit the deeper profundal zone of a water body in preference to the littoral zone (Altig and Johnston, 1989). Tadpoles of the guild *excites-parageios* are distinguished by their rapid (Latin = *citus*) development (Latin = *exitus*), and their association with shallow (Latin = *parageios*), temporary water bodies (Botha 2014). Except for these characteristics, South African *excites-parageios* tadpoles are similar in behaviour and morphology to tadpoles of the benthic-profundal and lentic-benthic guilds (Botha 2014). The particular habitat types occupied by species of these three guilds are also ideal for and utilised by mosquitofish (Pyke 2005).

Each artificial pond was stocked with ten fish, i.e. a stocking density of one fish per 15 L of water. This density was decided upon by using an average of densities employed by seven other similar studies (please see Table A1, Addendum A, for a list of stocking densities and their literature sources). The average density was 19.8 L per fish, equating to 9.8 fish per 150 L, which approximates to 10 fish per 150 L.

Use of a closed greenhouse system for the ponds was preferred over open-air property, and was advantageous for the following reasons: 1) the prevention of insect colonisation, and 2) the prevention of tadpole predation by external predators, 3) increased control of environmental factors, and 4) prevention of human vandalism or tampering. Insect colonisation by dragonfly nymphs would not only have influenced food control but also have introduced a secondary predator into the ponds, thereby confounding data.

2.2.2 Field collections and maintenance of experimental animals

Mosquitofish were collected on the 3rd of August and on the 17th of December 2016 from the Kuils River, Durbanville, Cape Town (33°50'46.6"S, 18°40'06.1"E). Specimens were sampled at random using sweep nets (2.5 mm and 1.0 mm mesh), but fish smaller than 15 mm were not collected. Fish were held and transported in buckets using water from the original habitat, and then placed into a holding tank until experiments could be performed. In order to prevent osmolality shock, fish were acclimatized to aged tap water by performing 50% water changes until TDS (total dissolved solids) had reached 150 ppm or lower, with a minimum of 2 h between water changes. Water changes were also performed to replace the polluted water originating from the fishes' previous habitat with fresh water, and to lower relatively high TDS levels (> 550 ppm). Water was aerated during the acclimatization process. Fish were maintained in holding containers filled with aged tap water, ranging from 15 L and 45 L in size, which were filled with macrophytes, namely *Ceratophyllum demersum* (water hornwort). Fish were fed a mixed diet of Marltons™ cold water fish flakes (Marltons™, Durban, South Africa) and Takara Sakana-II™ floating-type fish pellets (Takara Sakana-II™, Kian Weng Trading Co, Selangor, Malaysia), three times a week, and occasionally wild-caught aquatic macro-inverts.

Tadpoles of *Strongylopus grayii* (Clicking stream frog) were collected from a temporary urban stream in Burgundy Estate, Cape Town (33°50'14.5"S, 18°33'04.8"E) in the morning on the 10th of August and on the 23rd of August 2016. *Tomopterna delalandii* (Cape sand frog) tadpoles were collected from the same stream as for *S. grayii*, but at a different site downstream (33°50'14.7"S, 18°33'03.9"E), on the 12th and 16th of January 2017. *Sclerophrys pantherina* (Western leopard toad) tadpoles were reared from eggs that had been collected from a small dam on the 20th of August 2016 in Noordhoek, Cape Town (34°06'03.0"S, 18°22'55.1"E). After collection, the eggs were placed in a holding container with aerated water for four weeks until tadpoles had reached Gosner stage 25. Water changes were performed regularly, and any unfertilized eggs were removed to prevent rotting and to keep the water fresh. Procedures for the collection, transport, and acclimatisation of tadpoles and eggs were similar to those used for the mosquitofish. Tadpoles were maintained in 2–5 L plastic containers, and provided with aquatic vegetation (*Ceratophyllum demersum)* for foraging and shelter. No mosquitofish were observed in the original tadpole habitats, therefore the tadpoles were assumed to be naïve with no previous exposure to mosquitofish.

2.2.3 Construction and maintenance of artificial ponds

Artificial ponds were set up using 150 L oval black plastic containers, placed on concrete benches 1 metre from the ground. Ponds were placed in rows alongside each other, with alternating experimental and control treatments. Containers were filled with aged tap water, and water levels were maintained at approximately 5 cm below the container edge. Water was aged or de-chlorinated by allowing it to stand in open-top containers for at least 24 h. Water quality was maintained by performing 10% water changes at least once a week with previously prepared aged tap water.

Structural complexity within the ponds was created by adding freshwater plants and also artificial refugia, which provided media for tadpoles to hide in. The plants also provided food for the tadpoles and aided water quality by provision of dissolved oxygen, and metabolism of carbon dioxide and nitrates. Water hornwort (*Ceratophyllum demersum*), an indigenous submergent plant, was chosen for this purpose because it is rootless. This aided the monitoring process as plant material could easily be removed from the pond without the plants being harmed. This also eliminated the need for a substrate, which would otherwise complicate the tadpole sampling process. Plants were collected from a suburban recreational dam in Burgundy Estate, Cape Town (33°50'14.7"S, 18°33'09.4"E). Nymphal macro-invertebrate predators within plant material were killed by removing the plants from the water, allowing them to drain, and air-dried for 30 to 45 min. This allowed sufficient time for the macro-inverts to suffocate, while not excessively harming the aquatic plants. Several other methods were also tested, like raising water temperature and adding chlorine, but nymphs were quite resilient to these other methods. After removing nymphs, plant material (250 g wet mass) was added to each pond and allowed to settle for at least one week before experiments commenced. The establishment of plants was necessary in order to encourage algae, diatom and bacterial growth, as an additional food source for tadpoles. During each trial, aquatic plants were weighed once a week and the original mass maintained, either by trimming in the case of overgrowth, or by adding extra plants when die-back had occurred. The addition or removal of plants was minimal over the course of the study. Decomposing plant material, detritus and animal excrement were siphoned out of the water when necessary, to prevent ammonia build-up and fluctuations in pH levels.

An artificial refuge was constructed for each pond by creating open-top wire baskets (height: 11 cm, diameter: 23 cm) made of chicken wire, and filling them with roughly 2 L of small rocks, which were similar in appearance, mineral composition, and size. The collective volume of rocks was measured by the displacement of water. For pond experiments with *S. grayii*, beach rocks were used from Blaauwbergstrand, Cape Town (33°46'55.4"S, 18°27'00.9"E), that had been soaked in water for at least three days, and rinsed thoroughly under running tap water. Although these rocks did not cause remarkable shifts in TDS and pH, an acid test (5% acetic acid on cracked rock) at the end of the *S. grayii* experiments revealed that the rock composition was high in carbonates, which might have increased pH and TDS levels. As a precautionary measure, river rocks were used instead of beach rocks for *S. pantherina* and *T. delalandii* experiments. Because these were bought and not personally sourced, their location of origin was unknown. To remove any possible free carbonates and bicarbonates that would influence TDS and pH, rocks were treated with acetic acid (1.25% solution, pH 4.1) for 48 h, and rinsed thoroughly with water. Rocks were soaked again in clean water for 2 h and rinsed, which was repeated twice.

Water quality parameters were monitored by testing pH and total dissolved solids (TDS) at least three times a week. Water quality was monitored to detect fluctuations in water chemistry, which might be caused by nutrient cycling, decaying plant matter, or depleted oxygen levels. Large deviations from optimum conditions had to be detected and prevented to minimize confounding factors and to prevent environmental stress in the experimental animals. Although optimum pH ranges for mosquitofish are between 6.5 and 7.5 (Brannan 2016), average pH was maintained at pH 8.4 ± 0.7, due to the slightly more alkaline levels of both municipal and natural water. The measurement of water quality using a TDS meter was preferred over ammonia test strips, as TDS gives the concentration of all dissolved ions, not only the ammonium (NH_4^+) ion.

The photoperiod was determined by the natural light regime of the season. For mesocosm trials with *S. grayii* and *S. pantherina,* temperature was not controlled but recorded three times a week, due to the mild climate and moderate temperatures of the greenhouse, in August and September 2016. However, summer temperatures were high in January 2017, and therefore the greenhouse was air-conditioned at a constant temperature (25° C), for experiments with *T. delalandii.*

2.2.4 Experimental procedure and data collection

2.2.4.1 Mesocosm trials with *Strongylopus grayii*

On Thursday the 11th of August, ten mosquitofish and thirty Gosner stage 25 tadpoles of *S. grayii* were added to each experimental pond. Control ponds were set up in the same manner, but without predatory fish. Fish averaged 24.7 mm in total length (SE: 2.97, $n = 20$; 16.8–31.6 mm) and 4.8 mm in body depth (SE: 0.71, $n = 20$; 3.6–6.5 mm). Fish were selected at random from their holding container. Tadpoles had an average total length of 10.1 mm (SE: 1.30, $n = 120$; 7.6–16.1 mm) and an average body width of 2.5 mm (SE: 0.48, $n = 120$; 1.5–4.3 mm). Experimental animals were first acclimatized to the pond water before being released into each respective artificial pond. This was carried out by replacing 50% of the water in their respective holding containers with the new pond water, and leaving the animals to adjust for at least 30 min before release.

Initially, three experimental ponds were run, but during the study one pond trial was terminated because fish had jumped out of the pond. The other two experimental trials ended on the 22nd of August, after all the tadpoles had been completely consumed by mosquitofish. Two days later, three additional experimental trials were started. Fish for the second batch of trials averaged 23.6 mm in total length (SE: 4.42, $n = 30$; 16.6–31.2 mm) and 4.9 mm in body depth (SE: 0.89, $n = 30$; 3.4–6.8 mm), and tadpoles averaged 9.2 mm in total length (SE: 1.23, $n = 90$; 7.8–13.8 mm) and averaged 2.4 mm in body width (SE: 0.46, $n = 90$; 1.5–3.9 mm). Therefore three controls and five experimental treatments were performed for *S. grayii.*

Tadpole abundance was sampled every three days to track tadpole mortality over time. Sampling was performed by netting and siphoning, and searching carefully within vegetation and artificial refuges for tadpoles. It was assumed that all the tadpoles within the artificial pond were accounted for, as the water was clear and tadpoles were easily visible. Monitoring was conducted in the morning for the majority of the time.

Tadpole size measurements were taken once a week to track growth and development and thereby determine sublethal effects caused by mosquitofish. Growth was determined by measuring the total body length (from the snout to the tip of the tail) and the body width (broadest horizontal length of the abdomen, dorsal view) of the tadpole, using a digital calliper (see Figure 2.1). Tadpoles were classed into developmental stages using Gosner's identification table (Gosner 1960). The number of tadpoles with wounded tails was also noted while taking size measurements to determine injury prevalence. Size measurements for tadpoles of the control group were taken at the start, middle, and at the end of the trial (10th, 17th and 23rd of August 2016, respectively). For the experimental treatments with *S. grayii*, the entire tadpole population of a pond was consumed at a much quicker rate than predicted, therefore there are only two sets of tadpole size measurements for the first two experimental ponds (10th of August 2016), and only one set of tadpole measurements for the last three trials (23rd of August 2016). Tadpoles were returned to their respective ponds after measuring.

Fish size measurements were taken at the start of each trial, measuring the total length, i.e. from the snout to the tip of the tail fin, and the body depth, which is the vertical distance from the dorsal margin of the body, to the ventral margin of the body measured at the base of the pectoral fin (see Figure 2.1). Fish were captured by netting and counted regularly, to make sure numbers remained the same. Fish were fed 10 ml of Marltons™ cold water fish flakes (Marltons™, Durban, South Africa) every third day to provide an additional food source for the fish. At the termination of every mesocosm trial, mosquitofish and any remaining tadpoles were euthanised. For each new trial that was set up, artificial refugia and plants were rinsed with fresh water and re-used, but the water was replaced.

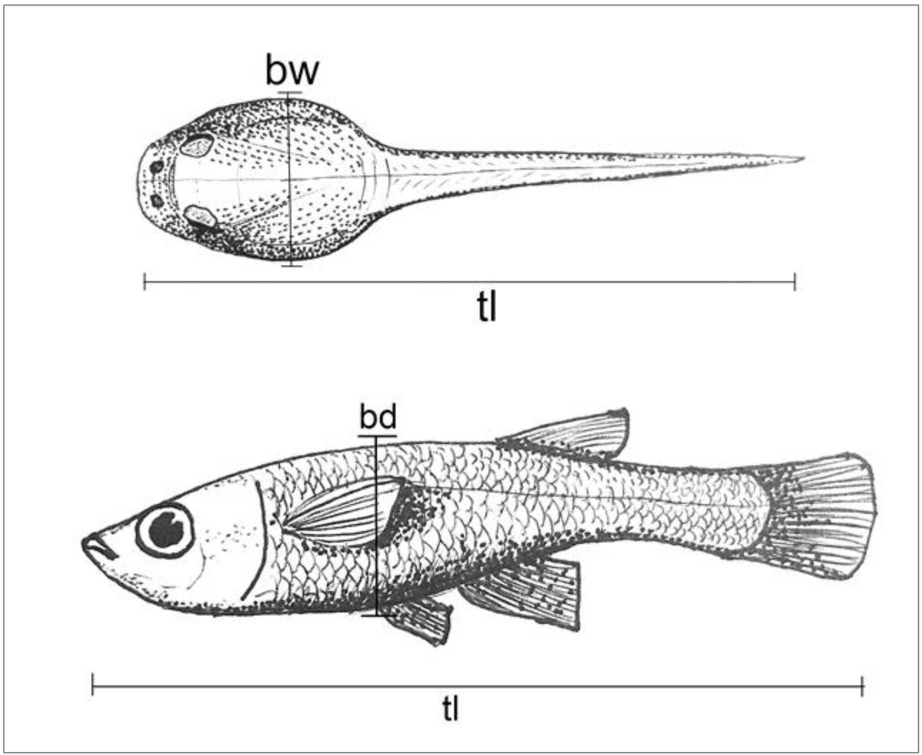

Figure 2.1. Measurements that were taken for tadpoles and mosquitofish. Tadpole measurements (above) were taken at the body width (bw) and total length (tl) of the animal. Fish measurements (below) were taken at the body depth (bd) and the total length (tl) of the fish. Illustration by Roxanne Conradie.

2.2.4.2 Mesocosm trials with *Sclerophrys pantherina*

Mesocosm trials with *S. pantherina* began on the 21st of September 2016. Tadpoles had an average total length of 11.8 mm (SE: 3.52, *n* = 180; 9.6–15.5 mm) and average body width of 1.2 mm, (SE: 0.41, *n* = 180; 2.6–4.6 mm), while fish had an average total length of 24.78 mm (SE: 2.97, *n* = 30; 20.4–32.2 mm), and body depth of 4.9 mm (SE: 0.71, *n* = 30; 3.2–6.6 mm). The experimental design and experimental methods for *S. pantherina* were the same as for *S. grayii*, with the exception that three control treatments and three experimental treatments were performed. Tadpole size measurements were taken at the start, middle and end of the study (20th and 28th of September, and 8th of October, respectively), for all trials, because fish had not consumed tadpoles to the same degree as for *S. grayii.*

While monitoring ponds with *S. pantherina*, the occupation of artificial refugia by tadpoles was documented in addition to tadpole mortality. This allowed anti-predator behaviour in *S. pantherina* tadpoles to be quantified and comparisons to be made between experimental and control groups, and possible associations to be made with tadpole mortality. The monitoring of tadpole abundance and use of artificial refugia was carried out by first counting the tadpoles within the pond, but outside the refuge. The refuge was then lifted to mid-water level and gently rocked back and forth for least 20 seconds. The total number of tadpoles was then counted, with the difference between the two counts being the number of tadpoles occupying the shelter.

2.2.4.3 Mesocosm trials with *Tomopterna delalandii*

On the 16th of January 2017, mesocosms with *T. delalandii* were initiated by introducing thirty tadpoles to each pond, having an average total length of 13.5 mm (SE: 3.50, *n* = 180; 10.1–18.9 mm) and average body width of 1.2 mm (SE: 0.41, *n* = 180; 2.4–4.7 mm). Ten fish were added to every experimental pond, with an average total length of 20.1 mm (SE: 1.70, *n* = 30; 16.8–25.8 mm), and an average body depth of 3.8 mm (SE: 0.46, *n* = 30; 2.9–4.9 mm). Procedures used in trials with *S. grayii* and *S. pantherina* were also applied to mesocosm trials with *T. delalandii.* Three control treatments and three experimental treatments were performed for *T. delalandii*, in the same manner as for *S. pantherina.* Three tadpole size measurements were taken at the start (16th of January), in the middle (23rd and 24th of January), and at the end (31st of January) of the study. Monitoring was performed five times a week, and not three times a week as for *S. grayii* and *S. pantherina*. The number of tadpoles injured by mosquitofish was not only noted while taking weekly size measurements, but also while monitoring for tadpole mortality. The number of tadpoles within aquatic vegetation was also recorded in addition to occupation of the artificial refugia. This was done to determine foraging behaviour in addition to anti-predator behaviour, in the absence or presence of mosquitofish. We assumed that foraging behaviour was an indication of the degree that tadpoles felt threatened by the fish, and that threatened tadpoles would spend more time in refugia.

2.2.5 Data analysis

In order to determine the degree of injury, the total length: width ratio (LWR; adapted from Segev *et al*. 2009) was determined for each tadpole measured, using total lengths and body widths obtained from the tadpole size data. Tadpole tails are most often the first body part to be injured by mosquitofish (Segev *et al*. 2009; Shulse and Semlitsch, 2014), therefore an increased number of injuries would reduce the tail length and the tadpole total length. However, the tadpole body width would remain the same, except in the case of growth. Assuming that the growth of tadpole length and body width is isometric, both growth and injuries in tadpoles could be compared using the total length: body width ratio. It was assumed that as injuries increased, the total length and therefore the numerator of the ratio would become smaller, thereby decreasing the ratio overall. It was expected that the LWR would decrease over time as injuries increased. However, the LWR could not be used to compare injuries between species, as different amphibian families have different body plans, and therefore the LWR would be different for each. For instance, *Amietia spp*. have relatively long tails in comparison with other species, while the bufonids are generally stout. However, the LWR would still be useful in order to compare injury and growth between experimental treatments and controls within the same species.

In order to assess the combined effects of injury and mortality in tadpoles for every species, an impact of predation index (IPI) was developed based on a similar index by Shulse and Semlitsch (2014). The mortality and injury of tadpoles recorded from both monitoring data and measurement data were used. Tadpoles were assigned ranks using three different classes: 1 – unaffected, 2 – injured, and 3 – dead, from the weekly measurement data. The coefficient of each rank (1–3) was the assigned IPI for each tadpole. The total IPI for each pond per monitoring effort was calculated by multiplying the coefficients of each class by the number of tadpoles in that class, and then the subtotals for each class were added to give the total index score.

Predation data was statistically analysed using univariate hierarchical linear mixed (HLM) models, with the different artificial ponds as primary unit of measurement. A model for each of the following response variables was run, for all three amphibian species: 1) tadpole mortality, 2) the impact of the predation score, 3) fish attack rate and 4) the body LWR of tadpoles. The proportion of tadpoles injured was also assessed for *T. delalandii.*

HLM models were also used to investigate sublethal effects in tadpoles, in the form of hampered growth and development, by using tadpole total length, body width and Gosner stage as response variables. In this case, all three amphibian species were tested. Sublethal effects by way of prey behaviour changes were also examined. The number of tadpoles making use of the refuge (for *S. pantherina* and *T. delalandii)* and the number of tadpoles found in aquatic vegetation (only for *T. delalandii*) were also used as dependent variables. Figure 2.2 summarises the response variables used for each tested predator-prey effect. Each model was constructed in SPSS v. 24 (SPSS, Inc.; Chicago, Illinois), with species, group, day, species interaction with day, species interaction with group, and species, group and day interactions as fixed effects. The subject (or each artificial pond) was chosen as random effect, and a random slope added at the subject level. A restricted maximum likelihood estimation method and an unstructured covariance structure were used. The practical significance of the results was determined by calculating the effect sizes from the differences between means, using the following formula adapted from Ellis and Steyn (2003), where MSE is the sum of the residual error estimate and the variance due to the different artificial ponds:

$$d = \frac{\left| \overline{x}_i - \overline{x}_j \right|}{\sqrt{MSE}}$$

These effect sizes take into account the spread of the data, and are also independent of units and sample size (Steyn 1999, 2000). The effect sizes were interpreted according to the guidelines given by Cohen (1988): (a) small effect: $d = 0.2$, (b) medium effect: $d = 0.5$ and (c) large effect: $d = 0.8$. Data with $d \geq 0.8$ were considered practically significant. Non-parametric correlations between response variables were determined by performing simple linear regression analysis in SAS v. 9.4 (SAS Institute Inc., Cary, NC). The coefficient of determination (R^2) was calculated, and the correlation coefficient (r) evaluated by taking the root. When the correlation coefficient was greater than 0.3, it was considered practically significant. The direction of the association was determined by the coefficient sign.

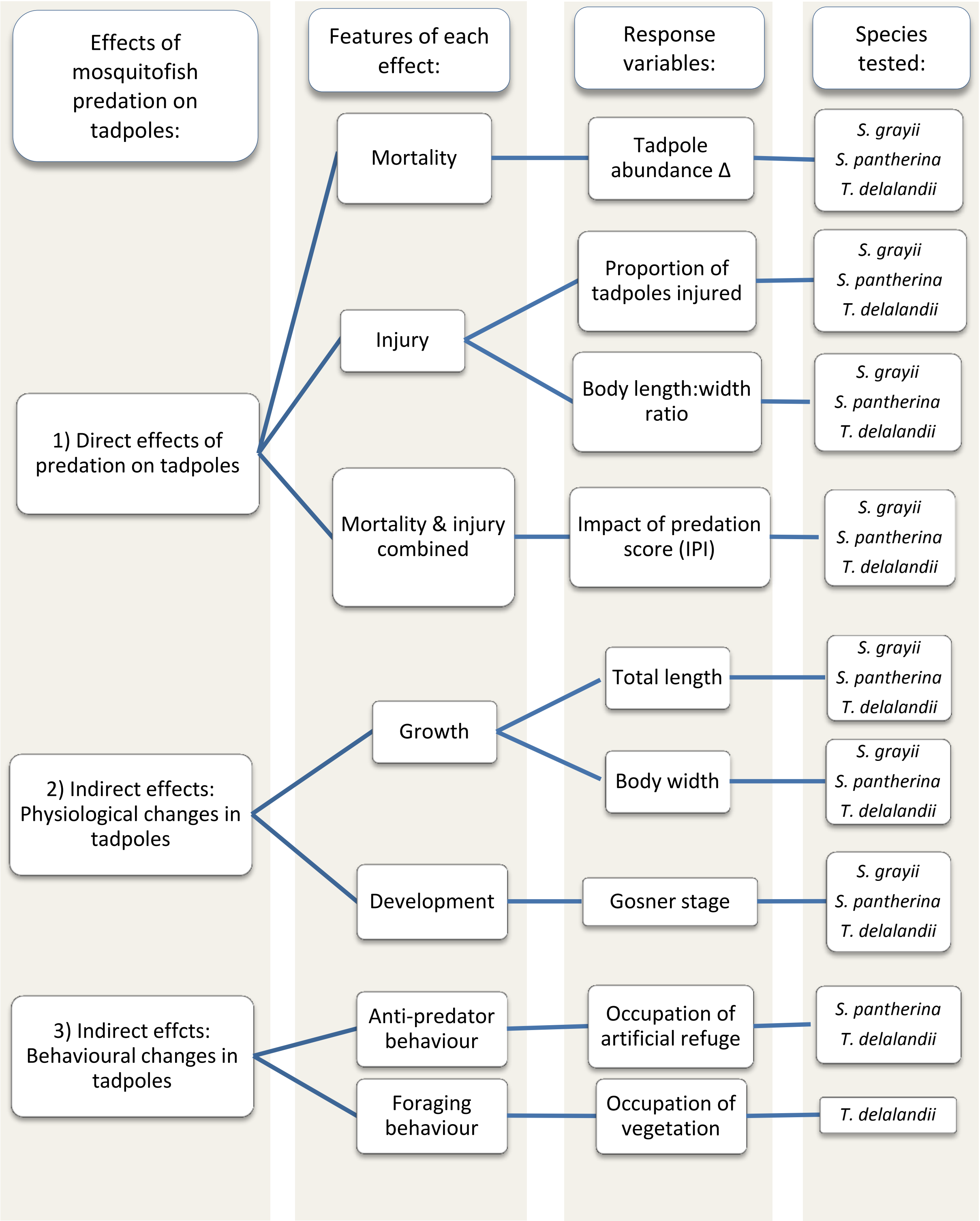

Figure 2.2. Schematic representation of the response variables tested for each predator-prey effect in the mesocosm trials. The amphibian species that were tested (*Strongylopus grayii*, *Sclerophrys pantherina*, *Tomopterna delalandii*) for each variable are indicated.

2.3 Results

2.3.1 Direct effects of predation

2.3.1.1 Tadpole mortality

The introduction of mosquitofish to amphibian sub-populations within mesocosms caused a significant decrease in the tadpole populations of *S. grayii* and *T. delalandii*, but population decrease for *S. pantherina* was not significant (see details for Type III tests of fixed effects in Addendum A). Consumption of *S. grayii* occurred very fast, with an average of 91.7% of the population being wiped out within seven days of exposure to mosquitofish (see Figure 2.3), despite the provision of shelter for tadpoles and alternate food for the fish. All *S. grayii* tadpoles were completely eaten by the twelfth day, with mortality between control and experimental treatments being significantly different (*d* = 14.03). For tadpoles of *T. delalandii,* population decrease occurred at a slower rate than for trials with *S. grayii* (see Figure 2.3). In the first week, an average of 21.1% of the *T. delalandii* tadpoles had been consumed, and by the twelfth day mosquitofish had consumed half of the population. On day 16, on termination of the study, 92.2% of the *T. delalandii* tadpoles had been consumed (*d* = 12.77). Although population decline was at a slower rate than for *S. grayii*, the effect of predation on tadpoles had already reached significant levels by the third day (*d* = 1.07). In contrast to the results for *S. grayii* and *T. delalandii*, overall mortality in *S. pantherina* was low (see Figure 2.3), and had no practical significance (*d* = 0.43), although the HLM models showed a significance between fixed effects in Type III tests (see Addendum A).

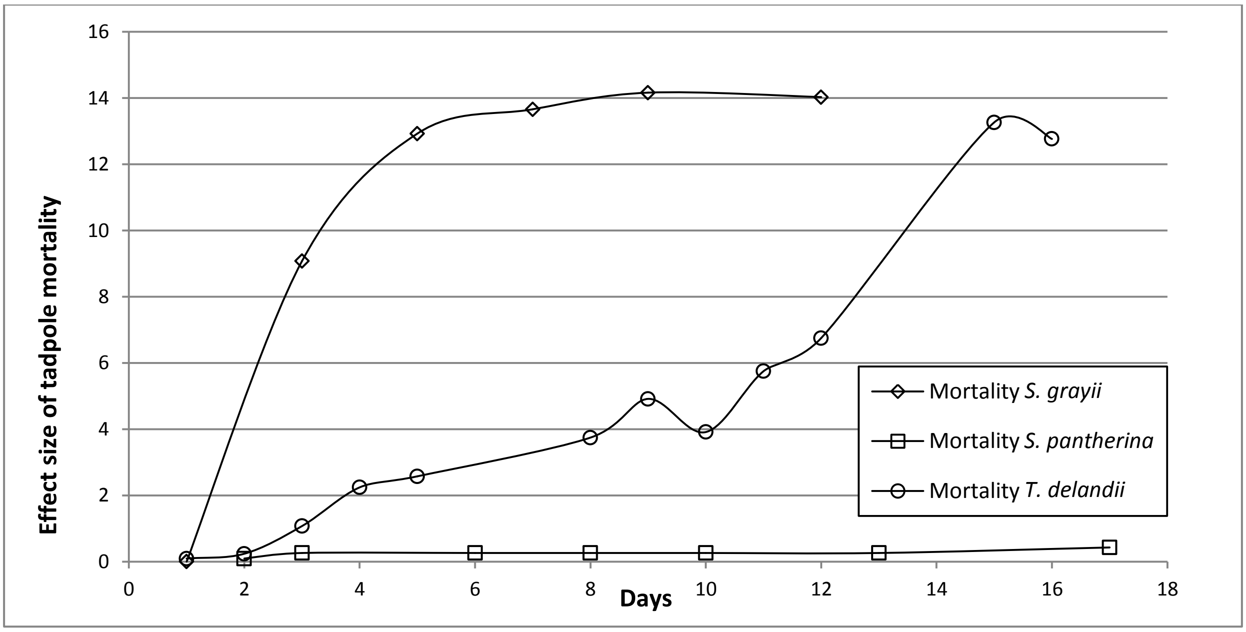

Figure 2.3. Effect sizes of tadpole mortality. These were calculated by comparing means of tadpole mortality in experimental and control treatments.

2.3.1.2 Tadpole injury

HLM analyses showed injury prevalence in *T. delalandii* tadpoles was significant ($p < 0.001$; $d = 3.38$) as well as for *S. pantherina* ($p < 0.001$; $d = 0.99$). Injuries inflicted on *S. grayii* were also statistically significant ($p < 0.001$), but the overall comparison of means showed insignificance in relation to the effect size ($d = 0.64$). Injury prevalence was generally the highest for tadpoles of *T. delalandii* (see Figure 2.4). In trials with this species, mosquitofish caused injuries in 86.6% ($d = 5.28$) of the tadpoles just after the first week, and thereafter injury prevalence rose to 100% ($d = 6.66$) after the second week. Injury prevalence in *S. pantherina* was not as high as for *T. delalandii*, and did not exhibit the same pattern of gradual increase throughout the study (see Figure 2.4). Instead, there were no injuries observed in the first week, but after the second week a large and significant number of tadpoles (45.8%, $d = 2.96$) had been injured by the fish. See details for Type III tests of fixed effects for the HLM analyses in Addendum A.

2.3.1.3 The Length: Width Ratio (LWR)

The overall LWR was lower for tadpoles exposed to mosquitofish in comparison with control treatments, and the differences were statistically significant for all three species ($p < 0.001$; see Type III tests of fixed effects in Addendum A). However, *T. delalandii* was the only species that displayed large differences in the LWR both at the end of the first week ($d = 1.23$) and at the end of the study ($d = 1.73$; see Figure 2.5), between experiemental and control treatments. For *S. pantherina* there was virtually no practical significance in the LWR in the first week ($d = 0.03$), but in the second week the LWR for experimental groups was significantly lower than for control groups, with a large effect size but smaller than for *T. delalandii* ($d = 0.97$). There was no practical significance for the LWR of *S. grayii* after the first week ($d = 0.53$). The LWR was not documented in the second week for *S. grayii* as all the tadpoles had been completely consumed.

Trends were similar for both LWR and injury prevalence, for all three species (compare Figure 2.4 and Figure 2.5). Simple linear regression analysis indicated a moderate negative correlation between LWR and injury prevalence in *T. delalandii*, with a correlation coefficient of –0.52 ($F_{(1, 427)} = 158.53$, $p < 0.0001$), as well as a weak correlation between the two variables in *S. pantherina* ($r = -0.39$ ($F_{(1, 520)} = 51.05$, $p < 0.0001$). There was no significant correlation between injury and body ratio in *S. grayii.*

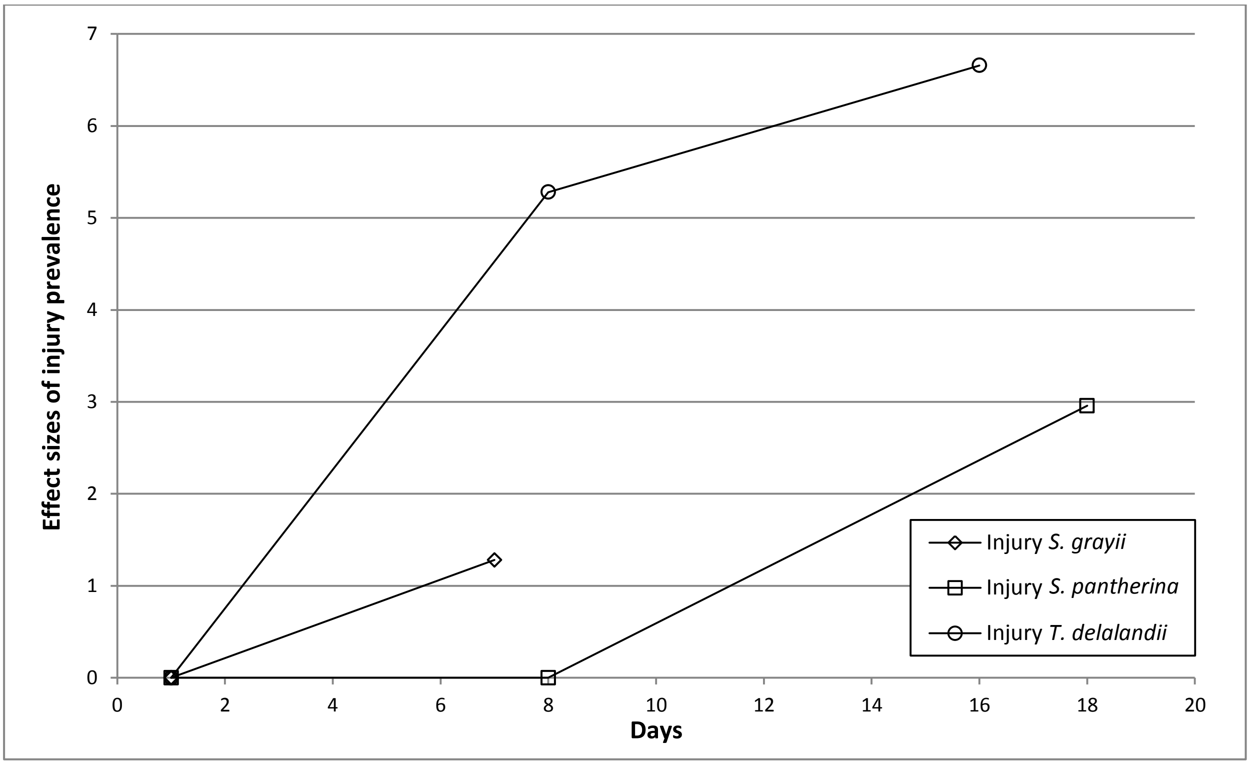

Figure 2.4. Effect sizes of injury prevalence. These were calculated by comparing means in experimental and control groups.

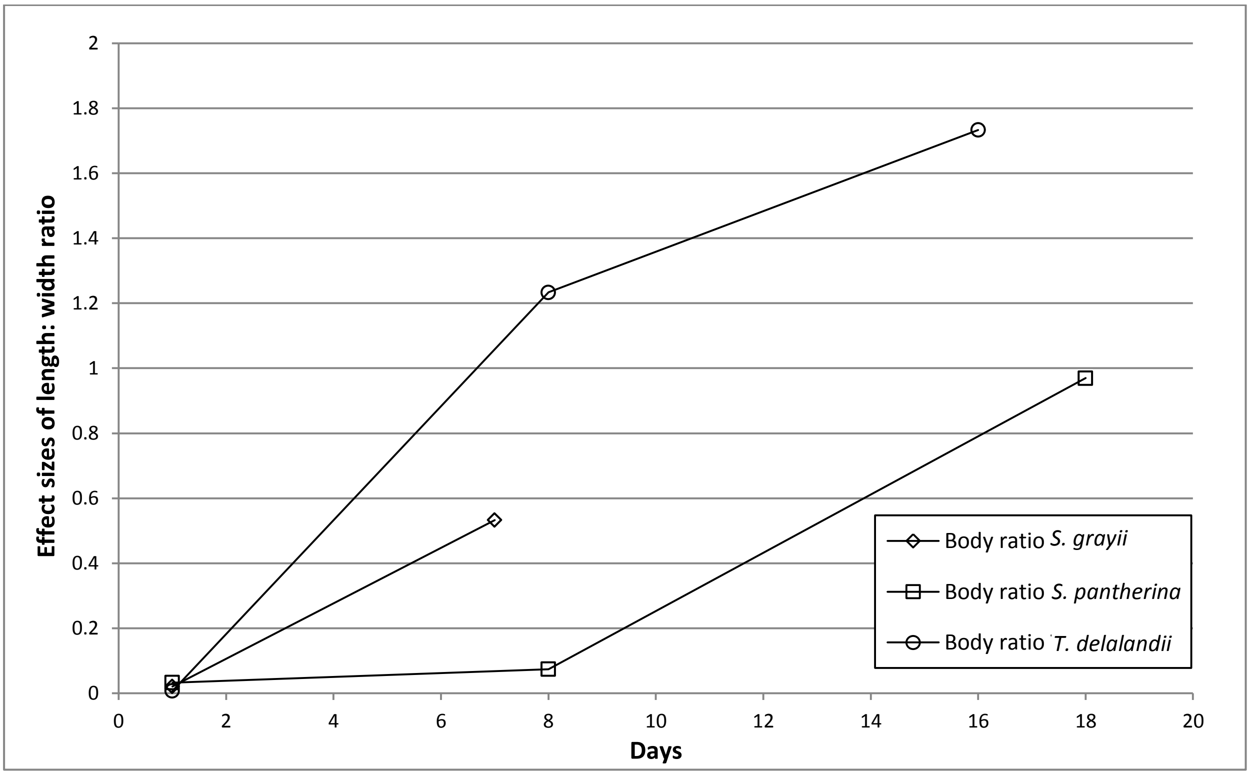

Figure 2.5. Effect sizes of the length: width ratio in tadpoles calculated by comparing means between experimental and control treatments.

2.3.1.4 Combined effects of mortality and injury: Impact of Predation Index (IPI)

HLM results of the total IPI scores for all three tadpole species showed that the collective effects of mortality and injury negatively impacted every species, albeit to varying degrees (see Figure 2.6). The IPI in experimental treatments was significantly higher in tadpoles exposed to mosquitofish, in comparison with controls (p <0.001, see Addendum A for Type III tests of fixed effects). Mosquitofish predation had the least effect on *S. pantherina* (d = 3.74), whilst effects of predation were very high in *T. delalandii* (d = 16.67), and in *S. grayii* (d = 17.65). The rate of increase in IPI scores was much more rapid in *S. grayii* than in *T. delalandii*, but both were much higher than *S. pantherina* (see Figure 2.6). Predation effects were evident in *T. delalandii* and *S. grayii* from the second day onwards, whilst predation effects only started showing in *S. pantherina* after the 13th day.

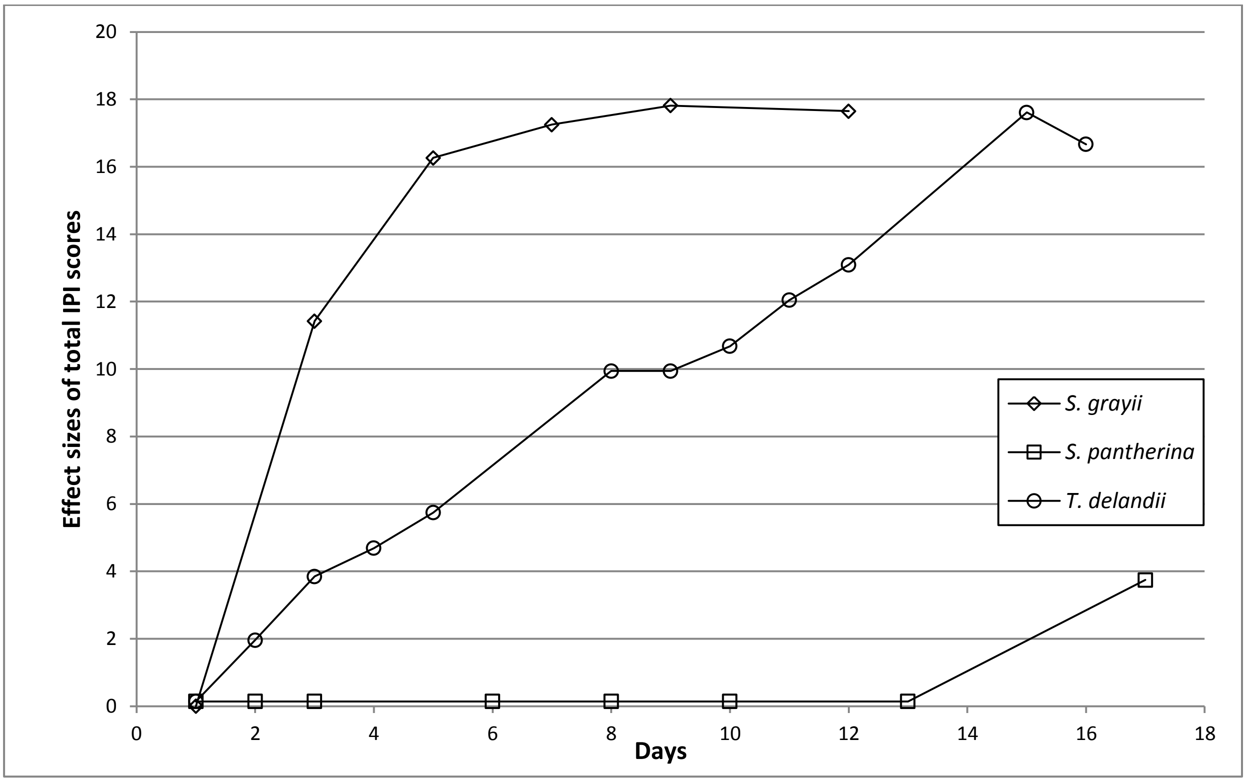

Figure 2.6. Effect sizes of total IPI scores, calculated by comparing the means of control and experimental treatments.

2.3.2 Indirect physiological changes due to predation

2.3.2.1 Growth and development in tadpoles

There was no significant difference for total length in *S. pantherina* at the end of the study ($d = 0.29$) between experimental and treatment groups. However, body width was significantly larger ($d = 1.13$) in experimental treatments, and these tadpoles had also reached a more advanced stage of development ($d = 0.89$) than control treatments. Changes in total length were different between the first monitoring period (day 1–8) and in the second monitoring period (day 9–18) of experimental groups. Mean total length increased from 11.5 to 13.8 mm (SE: 0.24) in the first period ($d = 1.28$), but in the second period, the mean total length decreased to 13.4 mm (SE: 0.24, $d = 0.23$). This is in contrast to the control groups, that increased in total length from 11.3 mm to 12.5 mm (SE: 0.23, $d = 0.62$) between day 1 and day 8, and then by the 18th day had increased to 12.9 mm (SE: 0.23, $d = 0.87$).

However, the changes in width and Gosner stage for *S. pantherina* contrast the above-mentioned trends for total length. Instead, a steady increase in both width and Gosner stage was observed from the start until the end of the study, and the overall increase is greater in experimental treatments than in controls. Body width in tadpoles of control treatments increased in from 3.4 to 4.0 mm (SE: 0.09, $d = 0.73$), whereas those for experimental treatments increased from 3.5 to 4.7 mm (SE: 0.10, $d = 1.81$). Development of tadpoles of control and experimental groups remained stable, at Gosner stage 25, within the first week. Development then increased in control groups, from Gosner stage 25 to a mean Gosner stage of 26 (SE: 0.14, $d = 1.08$) at the end of the study. Development was more pronounced in experimental groups, which had reached an average of Gosner stage 26.8 (SE: 0.14, $d = 1.20$) at the end of the study. Therefore growth was more generally more pronounced in experimental groups than in treatment groups, except for total length in the second monitoring period.

For tadpoles of *S. grayii*, total length increased in control groups from 10.3 mm to 12.3 mm (SE: 0.24, $d = 1.12$) in the first week. Growth was less in experimental treatments, where total length only increased from 10.3 mm (SE: 0.18) to 11.7 mm (SE: 0.80, $d = 0.80$). These growth trends between the two groups was significantly different in the first week ($p < 0.001$, $d = 0.97$). In the second week, total length of control groups grew from 12.3 mm to a further 12.9 mm (SE: 0.24, $d = 0.36$). This could not be compared with experimental groups, as total length measurements could not be taken as all the tadpoles had been consumed by mosquitofish. For body width, the increase was similar for both experimental and control treatments in the first week, where control groups increased from 2.5 mm to 3.2 mm (SE:

0.10, *d* =1.02), and experimental treatments increased from 2.5 mm (SE: 0.07) to 3.2 mm (SE: 0.31, *d* = 0.99). In the second measuring period, body width in control groups decreased slightly from 3.2 mm to 3.1 mm (SE: 0.10, *d* = 0.16). As mentioned previously, experimental tadpoles had all been consumed by the second week, and therefore body width could also not be measured. Differences in body width in the first week showed no practical significance (*d* = 0.03), nor did Gosner stage (*d* = 0.05).

For tadpoles of *T. delalandii,* total length increased in control treatments but decreased in experimental treatments. The mean total length of control tadpoles increased significantly from 13.7 mm at the start of the trials, to 20.1 mm (SE: 0.23, *d* = 3.61) by the last day. For experimental tadpoles, the mean total length decreased slightly over the trial period from 13.5 mm (SE: 0.24) to 13.1 mm (SE: 0.643, *d* = 0.22). At the end of the trials, total lengths of experimental tadpoles were significantly shorter than controls (*d* = 13.06).

Tadpoles of *T. delalandii* that had been exposed to mosquitofish displayed retarded growth, in comparison with control treatments. Body widths in experimental treatments were significantly smaller (*d* = 1.02), and development was significantly slower than in controls (*d* = 1.11). Mean tadpole width and Gosner stage increased in both experimental and control treatments, but growth was more pronounced in controls. The mean width of control tadpoles increased from 3.5 mm to 7.7 mm (SE: 0.09, *d* = 5.97) by the last day (day 16), while for experimental treatments the increase was slightly less, from 3.5 mm (SE: 0.10) to 6.7 mm (SE: 0.25, *d* = 5.05). Development was also more advanced in tadpoles of control treatments, which reached a mean Gosner stage of 29.0 (SE: 0.14, *d* = 4.33) by the last day, whilst those for experimental treatments reached a mean Gosner stage of 27.9 (SE: 0.34, *d* = 3.20).

The general trend for tadpoles of *T. delalandii* over the two-week period was that both control treatments and experimental treatments experienced growth over time, although the growth in experimental treatments was less than for controls. However, a different pattern can be seen when only the second monitoring period is observed (days 8–16), which displayed a reverse of the previously-mentioned phenomenon: growth in control groups was less than in experimental groups. For instance, tadpole body width in controls increased from 7.2 to 7.7 mm (SE: 0.09), with a moderate effect size (*d* = 0.64), but for experimental treatments the effect size was much larger (*d* = 1.03), when widths increased from 6.5 mm (SE: 0.10) to 7.0 mm (SE: 0.25). This pattern is also demonstrated in the Gosner stage measurements, where Gosner stage increased from 28.5 to 30 (SE: 0.13, *d* = 0.53) in controls, yet for experimental groups Gosner stage increased from 26 (SE: 0.15) to 28 (SE: 0.34), with a higher practical significance (*d* = 2.15).

2.3.3 Indirect behavioural changes in tadpoles due to predation

2.3.3.1 Occupation of the artificial refugia

There were notable differences in behaviour between tadpoles of *S. pantherina* and *T. delalandii* when exposed to mosquitofish. While monitoring in the first week, tadpoles of *S. pantherina* showed no sign of distress or hiding, but instead were observed swimming at leisure amongst mosquitofish in the ponds and were widely dispersed. However, this behaviour started to change at the end of the second week, when an increased number of tadpoles were occupying the refugia. This was in contrast to *T. delalandii*, where from the start of the experiments tadpoles could be seen in or near the shelter, and often at the bottom of the ponds. They were also inactive and seemingly under stress due to the presence of fish. The behaviour of control tadpoles was completely different, where tadpoles were widely dispersed and swimming to and fro throughout the pond.

HLM analyses showed that behaviour differences between experimental and control groups of both *S. pantherina* and *T. delalandii* were statistically significant ($p < 0.001$, see Addendum A for Test III of fixed effects). There was no overall practical significance in the number of tadpoles using the shelter for *S. pantherina* ($d = 0.34$), although on the last day the effect sizes between experiments and controls were large and significant ($d = 2.25$). There is a sharp contrast between the use of the shelter in the first and second week, increasing from an average of 11.11% in the first week, to 60.0% at the beginning of the third week and at the end of the trials. Use of the shelter was observed to a much greater degree in *T. delalandii* ($d = 8.36$), throughout the whole duration of the study. Occupation of the artificial refugia increased gradually from an average of 49.54% in the first week, to 90.41% in the second week (see Figure 2.7).

For both *T. delalandii* and *S. pantherina,* simple linear regression analysis indicated that occupation of the artificial refuge was strongly correlated with IPI scores ($r = 0.70$, $F_{(1, 42)} = 53.94$, $p < 0.0001$). There was also a very strong correlation between use of the shelter and injury prevalence in *T. delalandii* ($r = 0.88$, $F_{(1, 428)} = 3767.22$, $p < 0.0001$), and a moderate correlation between use of the shelter and injury prevalence in *S. pantherina* ($r = 0.58$, $F_{(1, 520)} = 219.58$, $p < 0.0001$).

2.3.3.2 Foraging behaviour in *Tomopterna delalandii*

There was a significantly higher degree of foraging behaviour in control groups of *T. delalandii* compared to experimental treatments ($F_{(1, 48)} = 8.67$, $p = 0.005$), with a high practical significance of 0.69. Although not recorded, control groups of *S. pantherina* and *S. grayii* were also observed foraging within vegetation to a larger degree than those tadpoles cohabiting with mosquitofish.

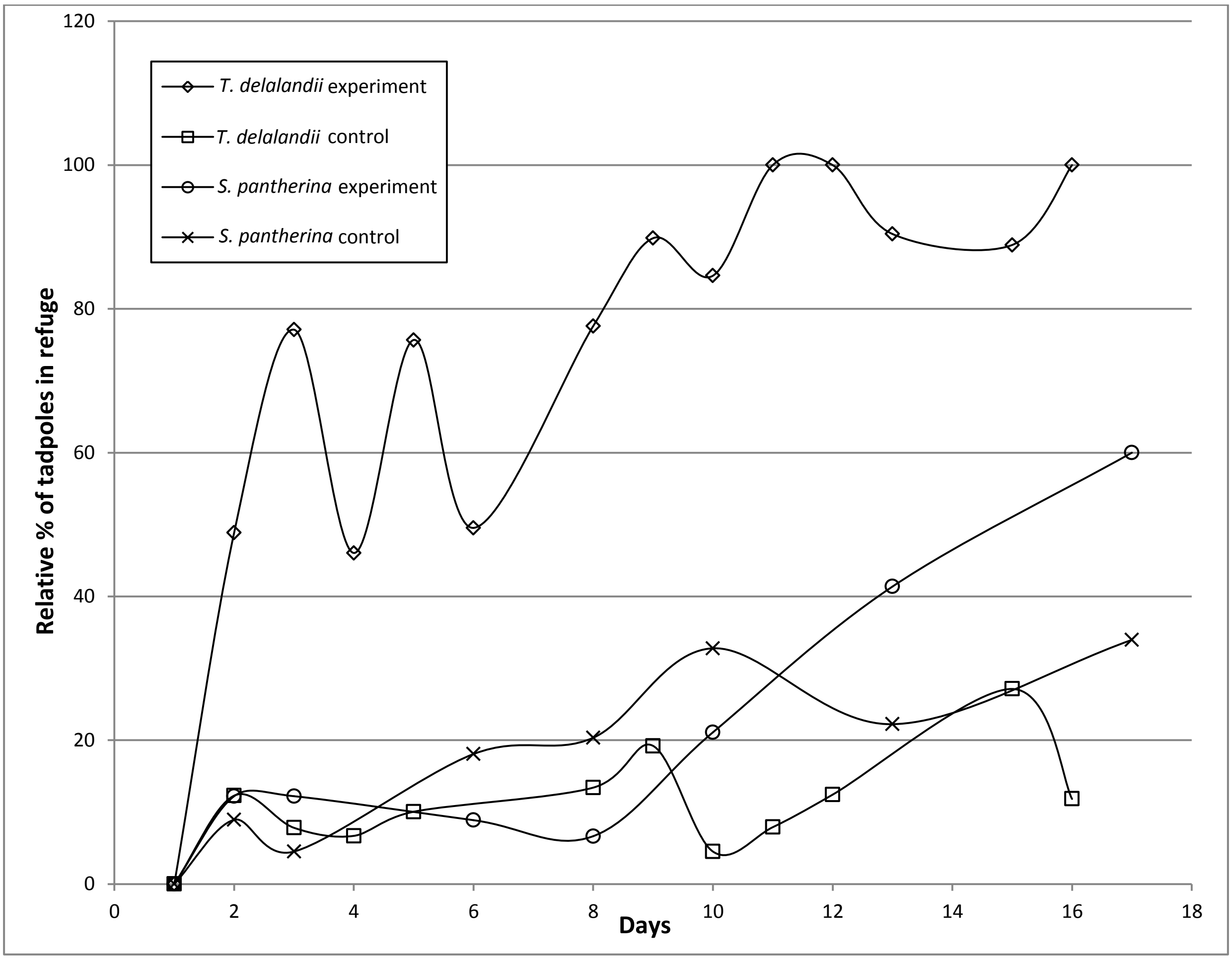

Figure 2.7. Relative percentages of tadpoles occupying artificial refugia over time.

2.4 Discussion

In this study, mosquitofish completely exterminated populations of *S. grayii* and *T. delalandii* within a relatively short space of time, despite the provision of an additional food source and the provision of refugia for tadpoles. The results of this study indicate that mosquitofish predation may have a detrimental effect on amphibian populations, causing a decrease in tadpole abundance and an increase in the infliction of injuries, albeit to different degrees between species. These results are in agreement with similar studies performed by other researchers, who have shown that mosquitofish have a strong negative effect on amphibian survival and development (Lawler *et al*. 1999; Segev *et al*. 2009; Shulse and Semlitsch 2014), and that mosquitofish prefer preying on some amphibian species more than others (Grubb 1972; Zeiber *et al*. 2008). This study also shows that stress rises with increased injury and mortality in tadpoles, which causes decreased foraging activity and hampers tadpole development and growth. Shulse and Semlitsch (2014) have also shown that even if tadpoles are not fatally attacked by mosquitofish, they are still affected by their presence.

Total IPI scores showed that mosquitofish predation had the greatest negative effect on *S. grayii* and *T. delalandii*, while *S. pantherina* was impacted the least. However, the two main predation effects, mortality and injury, had different contributing factors to the total IPI scores for each species. Mortality had the greatest contribution to total IPI scores for *S. grayii*, while injury prevalence was low. But in the case of *S. pantherina*, the reverse was true, with injury prevalence being the main factor that contributed to IPI scores and where mortality in *S. pantherina* was not practically significant. These results for *S. pantherina* were in contrast to those for *S. grayii* and *T. delalandii*. Tadpole populations of *T. delalandii* experienced both mortality and injuries at a gradually increasing rate until the entire population had died.

2.4.1 Direct effects of predation

Differences in palatability are the most likely explanation for the varying degrees of mosquitofish predation between species. Amphibians display various active defence strategies in avoiding or preventing attack by fish, and it is possible that the lower mortality of *S. pantherina* is due to this species displaying a chemical defence tactic which makes these tadpoles offensive to predatory fish. Adult South African bufonids are known to display varying degrees of noxiousness (Pantanowitz *et al*. 1998), although this has not been proven locally. Other studies abroad that are similar to the experiments of this chapter, have found American bullfrogs less palatable to mosquitofish in comparison with other ranid species (Preston *et al.* 2012; Shulse and Semlitsch 2014). Gregoire and Gunzburger (2008) also found that American leopard frogs were less palatable to *G. holbrooki* in comparison with gopher frogs.

These observations do not, however, explain the later onset of injuries in *S. pantherina*, where fish had started nipping the tails of tadpoles at a later stage but did not consume them entirely. This phenomenon was unexpected, although it is possible that an ontogenic shift in palatability was taking place. It has been found that the eggs of Australian bufonids possess a high amount and diversity of bufadienolides, chemical toxins which are poisonous and unpalatable to predators (Hayes *et al.* 2009). This toxin profile decreases with larval development, but then later increases after metamorphosis (Hayes *et al.* 2009). It is possible that the toxin profile in *S. pantherina* was decreasing in the second week, explaining the onset of mosquitofish attack at this time. However, it is likely that the tadpoles still possessed a degree of noxiousness, as mosquitofish wounds were not fatal and tadpoles were not entirely consumed.

Chemical defence is a common means of avoiding predator attack in tadpoles of the Bufonidae family (Hayes *et al.* 2009), but there are also several other tactics employed to repel predators. Wassersug (1973) has reasoned that bufonids are generally more conspicuous due to darker colouration and the formation of large aggregates of tadpoles, which advertises noxiousness. Most other anuran species are generally non-aggregating and cryptically coloured. However, the conspicuous nature of bufonid tadpoles can be to their detriment in developmental stages of decreased palatability (Heyer *et al.* 1975,) as their vulnerability is increased. This might have been the case in the experiments of *S. pantherina.*

Tadpole populations of *S. grayii* and *T. delalandii* were completely consumed by mosquitofish, suggesting that larvae of these species are highly palatable. It is likely that these species, which prefer breeding in temporary waters, are not as well adapted to fish predators as amphibian species that inhabit permanent waters, and lack the necessary anti-predator defence tactics which are needed to repel fish (Grubb 1972; Gregoire and Gunzburger 2008). Mosquitofish consumed *S. grayii* tadpoles in a very short space of time, indicating that certain characteristics of this species made them more palatable or easier to attack than others. Tadpoles of *S. grayii* were smaller than those of *T. delalandii* when at the same developmental stage, which could mean that *S. grayii* tadpoles were easier to capture and consume. However, other studies have shown that mosquitofish are not necessarily limited by the size of prey, and are able to consume a tadpole at any stage by constant nipping at the body (Pyke and White 2000; Baber and Babbitt, 2004). Although anti-intuitive, the low injury prevalence in *S. grayii* might also be an indication of high palatability. A decreased amount of injuries may not necessarily mean that a fish is less attracted to a certain prey, but because mortality for *S. grayii* was so high it can be concluded that the tadpoles where highly palatable and easy to attack and consume entirely, thus there were no surviving tadpoles that maintained injuries. Another possible explanation for the low injury prevalence in *S. grayii* is that because the population was declining so rapidly, there were not enough observations in the short amount of time to detect the injuries.

There was a strong negative correlation between tadpole LWR and injury prevalence, which suggests that increased fish-induced injuries were causing the decrease of tadpole total lengths. It is likely that tadpole injuries were the result of failed predation attempts or repeated nips at tadpole tails. The mosquitofish is a gape-limited predator, and if prey cannot be eaten whole then consumption occurs by nipping at muscular tissue (Baber and Babbitt 2004; Segev *et al.* 2009). Mosquitofish are known to attack the tails of tadpoles first, followed by the attack of the limbs and other body parts (Segev *et al.* 2009; Shulse and Semlitsch 2014), with the tail being the most vulnerable area. In some species, conspicuous tails are a trait which deliberately focuses predator attack away from more vital body parts, such as the head and abdomen. This phenomenon is known as the 'lure-effect' (Van Buskirk *et al.* 2003). However, tail injuries severely hamper swimming speed, due to the reduced tail length and area. This increases the probability of a future attack being fatal (Semlitsch 1989). Therefore, initially non-lethal injuries can accumulate to cause mortality, as shown by Harris (1989), and Shulse and Semlitsch (2014). It is likely that the high injury prevalence in *T. delalandii* spurred on the death of the population. It has been shown that tadpole populations sustaining high levels of injury may result in lower portions of the population reaching metamorphosis (Babbitt 2001), and even if tadpoles are able to survive to metamorphosis, injuries are likely to result in reduced fitness (Segev *et al.* 2009). In a study of mosquitofish predation on California red-legged frogs (Lawler *et al.* 1999), newly metamorphosed juveniles from experimental treatments weighed 34% less than controls, an effect which was attributed to the injuries in experimental treatments caused by fish.

2.4.2 Indirect effects of predation

Trends in growth differed among all three species. It was hypothesised that growth would be higher in treatments that were not subject to mosquitofish, but this was only observed to a significant degree in *T. delalandii*. Differences in growth trends between experiments and controls were not really detectable for *S. grayii,* although this could be due to the speed at which tadpoles of experimental treatments were being consumed. For *S. pantherina*, tadpoles exposed to mosquitofish were growing to a greater degree than in control treatments, which was unexpected. A possible explanation is that the tadpoles of experimental ponds were growing faster than controls due to the fish food that was provided, whereas control treatments were not receiving food. Also, because mosquitofish did not attack *S. pantherina* in the first week, these tadpoles were not under predation stress like tadpoles of *S. grayii* and *T. delalandii*. The absence of stress would have supported high growth rates (Kulkarni and Gramapurohit 2016). The overlap in diet between tadpoles and fish may have resulted in competition, and caused hunger levels in mosquitofish to rise. This may have been one reason why injuries in *S. pantherina* were initiated later in the study, combined with the effects of ontogenic changes in palatability.

The decrease in growth and development in tadpoles exposed to mosquitofish for *T. delalandii* was likely due to increased stress. The high correlation between IPI scores and occupation of the refugia may indicate that fish predation was generating an anti-predator response in the tadpoles. The increased tadpole inactivity by hiding in refugia, and reduction in foraging activity was significant in treatments exposed to mosquitofish, which also increased as tadpole injury and mortality increased. It has been shown that inactivity in tadpoles is an anti-predator response (Gregoire and Gunzburger 2008), and decreased activity of prey in the presence of mosquitofish has also been documented in other amphibians (Lawler *et al.* 1999). This predator-induced stress is linked to the corticosteroid levels in tadpoles, and when stress occurs for long periods of time and at high levels it results in retarded tadpole growth and development (Kulkarni and Gramapurohit 2016). Stress during larval development also reduces the fitness of metamorphs, due to poor jumping performance (Kulkarni and Gramapurohit 2016), decreased adult sizes (Maher *et al.* 2013) and delayed sexual maturity (Semlitsch *et al.* 1988). These above-mentioned changes in activity and foraging behaviour have been shown to cause population declines in amphibians (Beebee and Griffiths 2005).

It is another possibility, however, that the high correlation between IPI scores and occupation of the refugia was linked to individual behavioural tendencies between tadpoles. Individuals may consistently display either bold or cautious behaviour to a greater extent than others (Burns *et al.* 2012, Magnhagen and Bunnefeld 2009). Therefore it may have been that bold individuals were easier prey, and that the cautious tadpoles inhabiting refugia were the same individuals throughout the study. Therefore increased refuge use would not be a factor of time, but a factor of population size and behavioural diversity within a population. This is however difficult to elucidate, as it would require either the use of only one tadpole per treatment, or individual personality differences in tadpoles would have to be determined before experimental trials, and the identity of individuals would have to be recognizable throughout the study.

2.4.3 Implications of mosquitofish predation in the Western Cape

The findings of this study suggest that the presence of mosquitofish in dams and waterways in South Africa may have serious effects on local amphibian populations, by preying directly on tadpoles or by decreasing growth and development through predation stress. Therefore when mosquitofish and threatened amphibian species occur in the same aquatic system, it may have serious implications for the vulnerable species. These results are confirmed by studies that have been conducted abroad, which show that mosquitofish are a threat to amphibian conservation. For instance, the golden bell tree frog (*Ranoidea aurea*) and ornate burrowing frog (*Opisthodon ornates*) in Australia (Komak and Crossland 2000; Hamer *et al.* 2002), the frosted flatwoods salamander (*Ambystoma cingulatum*), the squirrel tree frog (*Dryophytes squirella*) and the red-legged frog (*Rana draytonii*) in the United States (Lawler *et al.* 1999; Baber and Babbitt 2004; Whiles *et al.* 2004) and the fire salamander (*Salamandra semenovi*) in Israel (Segev *et al.* 2009) are all under threat due to either western mosquitofish or its congeneric species, *G. holbrooki*. These impacts may serve as a warning and motivate further research on local interactions between mosquitofish and amphibian communities. Amphibians in the Western Cape are particularly at risk, due to their high endemicity and because of the sensitivity of the province's aquatic ecosystems (IUCN, 2017).

It is ironic that of the three species tested in this study, it was the endangered *S. pantherina* that was least affected by the mosquitofish. However, injury prevalence caused by predation attempts was nonetheless significant, even though *S. pantherina* possesses the noxious skin secretions that are present in bufonid tadpoles. *Sclerophrys pantherina* is one of five bufonid species in the Western Cape that is of conservation concern, the other four namely *Capensibufo deceptus*, *Capensibufo magistratus, Capensibufo rosei,* and *Capensibufo tradouwi*. It is presumed that these species will not be as threatened due to unpalabitiliy. Threatened species of other non-bufonid amphibian families in South Africa are likely to be palatable and non-offensive to mosquitofish, as they lack this chemical defence mechanism. Further studies should be performed in order to determine which species, that are sympatric with mosquitofish, are most vulnerable to attack. This can help in management decisions, where mosquitofish removal can be prioritised to areas where the most threatened amphibians co-occur with the invasive fish.

Mosquitofish may not only impact amphibians at the population level but also entire communities, through species-specific predation. This differential predation may cause shifts in amphibian community assemblages, as species diversity and assemblages are influenced by predation pressure (Shulse *et al.* 2013). A proliferation of some species and decline of others may create an imbalance and dysfunction in previously stable trophic interactions, and thereby increase the sensitivity of previously threatened aquatic systems. As mentioned previously, the most threatened aquatic systems in South Africa are those of the Western Cape (IUCN 2017), and the presence of mosquitofish in this province may deteriorate already threatened aquatic systems by the preference for certain prey species. However, the reverse is also true, where differential predation may be beneficial. The reduction of certain dominant tadpole species in an ecosystem may reduce interspecific competition, and thereby promote certain species that would otherwise be less prolific (Walls *et al.* 2002). In this way, differential predation may have a positive impact on amphibian communities in the Western Cape. Although indigenous to South Africa, there are three amphibian species that are locally invasive in Cape Town and the surrounding areas. This is due to extended ranges, in the case of *Hyperolius marmoratus* and *Xenopus laevis*, whereas a *Sclerophrys gutturalis* invasion occurred due accidental introductions (see De Villiers 2006). Local amphibians, which are threatened by these competitively superior invaders, may benefit from mosquitofish predation if it is concentrated to the invasive species. However, it is rather likely differential predation will be focused on the vulnerable amphibian species, amplify the negative effects of the invasive amphibians. This phenomenon has been observed in a study on the endangered California red-legged frog, which was threatened by American bullfrogs and mosquitofish (Lawler *et al.* 1999).

Anthropogenic habitat modification may also amplify the impact of invasive fish on amphibians. The draining and damming of wetlands for urban development and agricultural use change the natural hydrological regime of an ecosystem (Brock *et al.* 1999). Seasonal water regimes produce habitat diversity, as they change periodically, and the local biota is adapted to and depends on these fluctuations (Brock *et al.* 1990). Amphibians that are dependent on seasonal seeps, streams, and wetlands are restricted by a reduced number of temporary water bodies when wetlands are drained. In the case of damming, they are forced to breed in conditions that they are not adapted to. Such hydrological changes in urban areas are increasingly associated with the introduction of invasive fish, in areas that were previously fish-free (Hamer *et al.* 2002; Pilliod *et al.* 2012). Amphibian species dependent on and primarily adapted to ephemeral waters lack the necessary defensive mechanisms that prevent attack by fish, which increases their susceptibility to predation. Invasive fish in urban environments have been associated with the reduction of species richness and decline of amphibians in these areas (Hamer and Parris 2011, 2013). This study shows that both *S. grayii* and *T. delalandii* are highly palatable to mosquitofish and highly susceptible to predation. However, there is no clear evidence yet that their high palatability is linked with being ecologically more suited to temporal water bodies. They are known to utilise both permanent and temporary waters for breeding (Minter *et al.* 2004), however, it is likely that their use of permanent waters is an adjustment to land-use changes, and not due to preference. The majority of permanent water bodies in South Africa are man-made ponds, impoundments and dams, with more than 500 000 impoundments in the country (DWAF 1986). Naturally occurring permanent standing waters are virtually non-existent (Dallas and Day 2004), with the majority of natural lentic habitats being wetlands that are only seasonally inundated. Due to their widespread distribution and utilisation of a variety of habitats, the conservation of both species is of least concern (IUCN 2017), however anthropogenic land-use changes and the spread of invasive fish species is likely to influence local populations of *S. grayii* and *T. delalandii* in the future. Due to these country-wide land-use changes, it is also likely that mosquitofish will affect the many other non-bufonid species that occur in other parts of South Africa.

These potential implications of mosquitofish predation require further study in order to elucidate their magnitude, as well as to understand the mechanisms that determine their impact. In-field assessments are likely to be more useful than simplified laboratory trials in order to attain a true representation of the degree of mosquitofish impacts on natural biota, as such impacts are likely to be influenced by a complex web of factors. Where field studies are not possible, mesocosm and pond studies are the next best option because they incorporate a higher degree of complexity, in comparision with laboratory experiments. However, a few factors were a hindrance to the mesocosm trials performed in this study. Firstly, the mesocosms used in this study were limited by space constraints. Secondly, ethical considerations restricted the number of experimental animals available for use, and these two limitations minimized the number of possible replicates. Ideally, more replicates should have been performed with different predator-prey ratios, as dissimilar densities may result in different predation effects (see Chapter 4). A fixed predator-prey ratio of 1:3 was chosen in order to compare predation results between amphibian species, but this fixed ratio also does not accurately represent the natural setting where prey items are generally unlimited. Therefore it is recommended that field studies be performed in order to further examine the predation impacts of mosquitofish on amphibian tadpoles.

Chapter 3: The palatability of tadpoles to mosquitofish (*Gambusia affinis*): inter-specific variation, ontogenic variation and the influence of alternative prey

3.1 Introduction

The Western mosquitofish, *Gambusia affinis*, was introduced into South Africa in 1936 primarily for mosquito control (De Moor and Burton 1988). However, mosquitofish do not only eat mosquito larvae but consume a wide variety of biota due to their omnivorous feeding habits (Kramer *et al.* 1987), and at higher feeding rates than their local poecilid relatives (Rehage *et al.* 2005). Furthermore, studies have shown that the invasive fish is generally unreliable at controlling mosquito larvae (Hoy *et al.* 1972; Kramer *et al.* 1987; Bellini *et al.* 1994). However, these studies conflict in opinion with research that shows the fish to be an invaluable tool in mosquito control (Meisch 1995; Miura *et al.* 1984). As a result, biases in attitudes exist concerning the value of mosquitofish, which has been apparent since the 1960's (Pyke 2008; Rupp 1996). Despite its assumed usefulness as a mosquito control agent, the predatory habits of the mosquitofish, as well as its high success as an invasive species, makes it a potential threat to already jeopardised amphibian species. Therefore the worth and use of the mosquitofish has to be evaluated in the light of non-target impacts that it has on other organisms, and particularly in areas that are ecologically sensitive.

Although it is known that mosquitofish have the potential to prey heavily on amphibian eggs and larvae (Shulse and Semlitsch 2014; Smith and Smith 2015), it has also been found that certain amphibian species may be preferred by mosquitofish in comparison to others (Grubb 1972; Zeiber *et al.* 2008). Amphibians display various active defence strategies in avoiding or preventing attack by fish, which may reduce or enhance the susceptibility of a species. As mentioned in the previous chapter, bufonids have been found less palatable than ranid species (Preston *et al.* 2012; Shulse and Semlitsch 2014). Differences in palatability have been found between species of the same genus (Gregoire and Gunzburger 2008). Not only does the literature show inter-specific variations in palatability, but that differential predation on amphibians may be associated with ontogeny of tadpoles. Studies have shown that mosquitofish attack certain amphibian developmental stages with greater intensity in comparison with others (Smith and Smith 2015; Pyke and White 2000). Moreover, these trends are not similar amongst all amphibians. The eggs of one species may be preyed upon heavily by mosquitofish, or they may be completely unpalatable (Grubb, 1972). Smaller tadpoles at younger developmental stages may either be more palatable (Formanowicz and Brodie 1982) or less palatable (Adams *et al.* 2011; Brodie *et al.* 1978) than tadpoles of the same species at later developmental stages. Due to these varied responses of mosquitofish toward amphibian prey, it is necessary to use a variety of anuran species, as well as a variety of developmental stages of each species, in order to gain a realistic interpretation when assessing the impacts of mosquitofish. The resulting information can also be useful in determining future impacts, as differential predation causes the alteration of amphibian community structures (Shulse *et al.* 2013), which can affect entire ecosystems. Isolating the particular species and developmental stages that are most prone to mosquitofish predation is also useful when planning management strategies and mitigation measures, as the treatment of the problem can be pinpointed during management efforts.

In this chapter, the predation of different amphibian species by the mosquitofish was further assessed. This was based on the interspecific differences in predation found in the experiments of the previous chapter. Not only were species differences further explored, but also a variety of developmental stages were used in order to determine ontogenic variations in palatability. The presence of mosquito larvae as an additional prey item and their effect on tadpole palatability was also assessed. Alternative prey was provided, firstly because studies that have investigated the impacts of an introduced predator on native biota have been critiqued in the past, due to an absence of alternative prey provided during such tests (Goodsell and Kats 1999). It is argued that in such cases, the study is unrealistic as the predator is forced to feed on the provided prey item. Secondly, mosquito larvae were used in order to see if the presence of an alternative prey reduced tadpole predation. If mosquitofish feed on tadpoles regardless of alternative prey, the preference may indicate that local amphibian populations may be adversely affected by this species. This would also show the mosquitofish to be impotent as a biocontrol agent, in systems that harbour abundant amphibian diversity.

3.2 Materials and Methods

In order to examine the interactions between mosquitofish and larval anurans, the palatability of four different tadpole species were assessed: *Capensibufo magistratus*, *Sclerophrys capensis*, *Tomopterna delalandii* and *Xenopus laevis*. More than one anuran species was tested because prey palatability is only useful in a relative sense (Lawler and Hero 1997). In other words, a measure of palatability for one species can only be determined by comparing it with the palatability of another prey species. The amphibian species that were used for this study were chosen according to availability and accessibility. As many as possible different developmental stages were also tested, in order to determine if there were ontogenic shifts in palatability in the tadpoles, or if differences in size in relation to the gape width of the fish was affected. This would help determine which larval stages of an amphibian species are most prone to predation. The presence of alternative prey was also tested as a third effect, in order to see if predation intensity was significantly different in the presence and absence of alternative prey. Mosquito larvae would be used as an alternative prey item. In the light of these three tested effects, the palatability of tadpoles was evaluated using two methods which assess different aspects of palatability. The first was performed by measuring tadpole injury and mortality, or the predation intensity on tadpoles. The second measure was the behavioural response of fish towards the tadpoles. In the one method the tadpole was observed, in the other the fish was observed. These methods are discussed in detail in a later paragraph.

Tadpole development classes were grouped into nominal classes instead of using the ordinal normal tables of Gosner (1960) for *C. magistratus*, *S. capensis*, and *T. delalandii*; or Nieuwkoop and Faber (1956) for *X. laevis*. This was necessary because the palatability of certain developmental stages had to be compared between all four of the different amphibian species. Because normal tables are mostly species-specific, and dissimilarities between Gosner tables and Nieuwkoop and Faber tables occur, it was necessary to use a system of classification that was able to synchronise these two tables. Tadpoles were grouped into the following developmental classes: Embryonic stage, hatchling stage, pre-metamorphic stage, pro-metamorphic stage, and metamorphic climax (Etkin 1968). The pre-metamorphic stage has little hindlimb development, with growth mainly occurring in the trunk and tail, while the pro-metamorphic stage is characterised by rapid growth of the hindlimbs, mediated by thyroid function (McDiarmid and Altig 1999). The metamorphic climax is the last few stages of larval development that is characterised by marked changes in physiology, with visible changes being tail resorption and fore-limb development (McDiarmid and Altig 1999). Because tadpole size is an important determinant of tadpole palatability, the two classes described by Etkin (1968), namely pre- and pro-metamorphic classes, were further divided into early and late pre- and pro-metamorphic classes in order to prevent large disparities in tadpole size. It was decided to assign the Gosner stage 25, or the equivalent Nieuwkoop and Faber stage 45, to its own nominal class, instead of grouping it with the early pre-metamorphic class. This is because tadpoles can remain at this stage for a long period of time, where a large amount of growth in body size can occur before limb-buds appear. Therefore a separate class was necessary in order to keep size ranges of tadpoles as uniform as possible, called the free-feeding stage. See Table 3.1 for the different stages of Gosner and Nieuwkoop and Faber that were used to construct the developmental stages used in this study. Nominal classes based on development were used in preference to size classes, as it was important to distinguish the different ontogenic changes that were occurring through tadpole development. These are linked with hormonal changes in the tadpole (McDiarmid and Altig 1999) as mentioned previously, and could be related to any differences in palatabilty (Hayes *et al.* 2009).

Table 3.1. Nominal developmental classes assigned to tadpoles, constructed by combining the normal tables of Gosner (1960) and Nieuwkoop and Faber (1956).

Developmental class	Gosner (1960)	Nieuwkoop and Faber (1956)
Embryonic stage	1–19	1-32
Hatchling stage	20-24	33-44
Free feeding stage (pre-metamorphosis)	25	45
Early pre-metamorphosis	26–30	46–59
Late pre-metamorphosis	31–35	50–53
Early pro-metamorphosis	36–38	54–55
Late pro-metamorphosis	39–41	56–57
Metamorphic climax	42–46	58–66

3.2.1 Collection and maintenance of experimental animals

Mosquitofish were collected on the 3rd of August and on the 17th of December 2016 from an urban stream, the Kuils River, at Durbanville, Cape Town (33°50'46.6"S, 18°40'06.1"E). Sampling, collection, and handling methods were identical to the methods described in Chapter 2. Fish were maintained in holding containers filled with aged tap water, ranging from 10–70 L in size, which were filled with macrophytes (*Ceratophyllum demersum*) for structural elements. Fish were fed a mixed diet of Marltons™ cold water fish flakes (Marltons™, Durban, South Africa) and Takara Sakana-II™ floating-type fish pellets (Takara Sakana-II™, Kian Weng Trading Co, Selangor, Malaysia), three times a week, and occasionally wild-caught aquatic macro-inverts. Fish were held in captivity and trained to feed on artificial fish food at least three weeks before experiments commenced. Water temperature was not artificially regulated and fish were subject to the natural light regime of the season. Experiments were conducted at room temperature, which was measured but not regulated, and varied from 20–24°C.

The amphibian larval stages for this study were collected from a diverse range of water bodies and various districts within the Western Cape area. Tadpoles of *S. capensis* were collected at a farm dam near Porterville (32°59'04.0"S, 19°01'37.9"E), on the 30th of October, 2016. Tadpoles of *X. laevis* were collected from four different urban localities in Burgundy Estate, Cape Town (temporary streams: 33°50'23.6"S, 18°32'49.9"E; 33°50'27.2"S, 18°32'55.9"E; 33°50'24.1"S, 18°33'06.2"E and an ephemeral pool: 33°50'35.2"S, 18°33'14.2"E) on different days in mid-October and early November in 2016 and late January 2017. Tadpoles of *C. magistratus* were collected from Landdroskop, Hottentots Holland Mountains, on the 20th of September 2016 (32°02'55.1"S, 19°00'13.7"E). In order to obtain eggs and tadpoles of *T. delalandii,* adults in amplexus were caputured by hand at night at a recreational dam at Burgundy Estate, Cape Town, on the 12th of October 2016. These were held in captivity overnight, and the deposited eggs acquired the following morning. These eggs were stored in a refrigerator (4° C) to retard development until needed. After roughly five days, eggs were removed from the refrigerator and allowed to reach room temperature. These eggs were either used directly in predation trials, or they were allowed to develop at room temperature to a certain Gosner stage before being used in experiments. Water changes were performed regularly, and any unfertilized eggs were removed to prevent rotting and to keep the water fresh. Besides rearing from eggs, tadpoles of *T. delalandii* were also collected from an urban stream in Burgundy Estate (33°50'14.7"S, 8°33'03.9"E) on the 16th of January 2016.

Procedures for the collection, transport, and acclimatisation of tadpoles and eggs were similar to methods described in Chapter 2. Care was taken to minimize the trauma of handling and captivity. Tadpoles were maintained in plastic containers, ranging in size from 2–5 L, and housed in a room at an ambient light cycle. They were held in captivity for a minimum of 24 h from capture before being used in experiments. Tadpoles were provided with aquatic vegetation (*Ceratophyllum demersum*) for food, as well as Takara Sakana-II™ floating-type fish pellets (Takara Sakana-II™, Kian Weng Trading Co, Selangor, Malaysia) which supplemented the tadpole diet with additional proteins and nutrients.

Fish were likely to have had previous experience with tadpoles of different species, but the tadpoles, with the exception of *S. capensis*, all came from temporary water bodies, and therefore it is assumed that they were naïve to fish predators. No mosquitofish were observed in the farm dam from which *S. capensis* tadpoles were acquired.

3.2.2 Experimental layout

Palatability trials took place from October 2016 until January 2017. Experiments of tadpoles with mosquitofish had two treatment levels: with and without alternative prey. These two treatments were tested using four amphibian species (*C. magistratus*, *S. capensis*, *T. delalandii* and *X. laevis*) in the PII observations and three species in the PRI observations (all except *C. magistratus*). Each amphibian species was tested separately. Different developmental stages were also tested within species, according to availability. There were at least five replicates for every treatment level performed. See Table 3.2 for a complete outline of all the executed experiments.

Table 3.2. Outline of all experiments performed to test the palatability of amphibians toward mosquitofish.

Species	Developmental stage	Date	No mosquitoes	With mosquitoes
Capensibufo magistratus	Late pro-metamorphosis	6 Oct 2016	5 repetitions	5 repetitions
Sclerophrys capensis	Free feeding stage	2 Nov 2016	5 repetitions	5 repetitions
	Late pre-metamorphosis	2 Nov 2016	5 repetitions	5 repetitions
	Late pro-metamorphosis	9 and 18 Jan 2017	7 repetitions	5 repetitions
Tomopterna delalandii	Embryonic stage	10 Nov 2016	10 repetitions	10 repetitions
	Hatchling stage	13 Oct 2016	5 repetitions	–
	Free feeding stage	24 Oct 2016	5 repetitions	5 repetitions
	Late pre-metamorphosis	9 and 13 Jan 2017	5 repetitions	6 repetitions
	Late pro-metamorphosis	9,13,26 and 30 Jan 2017	11 repetitions	–
Xenopus laevis	Free feeding stage	10 and 16 Nov 2016, 18 Jan 2017	10 repetitions	9 repetitions
	Early pre-metamorphosis	13 Oct 2016 and 18 Jan 2017	10 repetitions	5 repetitions
	Late pre-metamorphosis	13 January 2017	5 repetitions	5 repetitions

No control treatments were performed (by making observations of tadpoles in the absence of fish), as tadpole mortality over such a short space of time is generally negligible (Pyke and White 2000). No individual fish or tadpole was used more than once in these experiments. All experimental fish and any surviving tadpoles were euthanised at the end of each trial. The fish were then sexed, and if the sex was indistinguishable the fish was classed as a juvenile. Body size measurements were taken by measuring the total length (from the snout to the tip of the tail fin) and the body depth (see Figure 2.1 in Chapter 2). The body depth was measured as the vertical distance from the dorsal margin to the ventral margin of the body, measured at the base of the pectoral fin for females and at the gonopodial base for males. The fish gape height was measured by opening the mouth and measuring the vertical distance from the top jaw to the bottom jaw, viewed laterally (Figure 3.1). The gape width was measured by taking the horizontal distance of the open mouth, viewed dorsally, measured at the point where the two jaws meet. A fish's mouth was opened by pressing the dorsal side of the fish backwards with the index finger, and pressing the ventral side of the fish forward with the thumb, which depressed the lower jaw until the mouth was opened at its maximum. The gape area was then calculated using the formula for the area of an ellipse:

$$Gape\ area = \pi \times \left(\frac{Gape\ height}{2}\right) \times \left(\frac{Gape\ width}{2}\right)$$

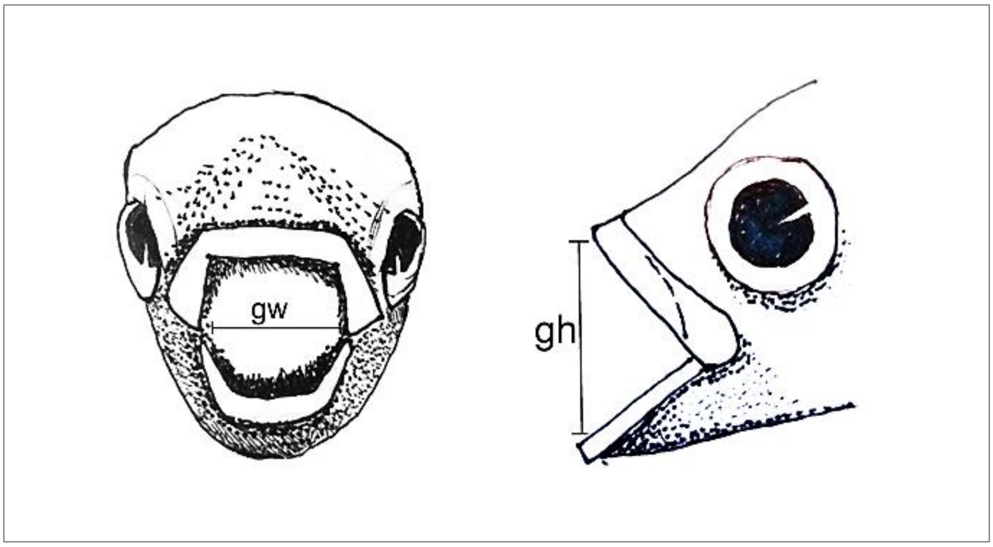

Figure 3.1. Gape width measurements for mosquitofish. Gape width (gw) is measured horizontally (left sketch) and gape height (gw) measured vertically (right sketch). Illustration by Roxanne Conradie.

For each experiment, mosquitofish were placed individually in a clean white plastic 2 L container, filled up with dechlorinated tap water until 2 cm from the brim. Water was dechlorinated by allowing it to stand in an open-top container for at least 12 h before use. The water was not aerated while experiments were running, and the water temperature was measured but not regulated. No structural elements or substrate was provided, as maximum visibility was required to observe the experimental animals.

After being released into containers, fish were presented with a single food pellet (Takara Sakana-II™). If the fish had not eaten the pellet after the 24 h period, the fish was considered behaviourally incapable of feeding and was not included in the experiment. Fish that had consumed the pellet were deprived of food for another 24 h. When the starvation period was complete, each fish was presented with three tadpoles by pouring them into the 2 L container, with approximately 200 ml of the water that tadpoles had previously been placed in prior to the experiment. This initiated a fright response in the fish, preventing them from attacking tadpoles immediately as they had been conditioned to take food artificially. Prior to each experiment, the total length and body width of each tadpole was measured, and grouped into its respective developmental class. Most experiments were started late morning to midday.

In order to determine different degrees of differential predation in fish, two different ways of measuring palatability were developed: the behavioural response of the fish towards the tadpoles, which gave an indication of the willingness to attack. The second measure of palatability was the predation intensity, or the degree of injury that fish exerted on the tadpole. These two methods are described in the following two sections.

3.2.2.1 Fish attack behaviour

The first measure of prey palatability was determined by examining the response of the fish toward tadpoles. From the moment the tadpoles were released into the container, the behaviour of the fish towards the tadpoles was monitored until 3 min had passed. In the case of eggs being tested, fish were monitored for 2 min. An index system was used to classify the behavioural responses of the fish, hereafter referred to as the Predator Response Index (PRI). The behaviour of the fish towards the tadpoles/eggs was scored according to six different levels:

(1) Fish showed no interest in the tadpoles.
(2) Showed interested and/or slowly approached a tadpole, but did not chase it.
(3) Chased or rammed into a tadpole, but did not bite it.
(4) Chased, followed or rammed into a tadpole, and bit it once.
(5) Repeatedly chased, followed or rammed into tadpoles and bit them.
(6) Consumed a tadpole (a tadpole was taken up into the fishes mouthparts).

Fish were assigned a coefficient (1–6) according to the class that they fell in, per minute. The PRI score for the entire observation period was calculated by adding the coefficients per minute, and then dividing by the number of minutes. Any other phenomena or trends in fish behaviour were also noted.

3.2.2.2 Predation intensity

The second measure of prey palatability was determined by measuring the predation intensity that fish exerted on the tadpoles. This was measured by visually assessing the amount of tadpole biomass consumed by the fish, which was based on the assumption that the amount of tadpole biomass consumed would increase with tadpole palatability. This would also be synonymous with the severity of injury inflicted on the tadpoles, which could be used to assess the extent of sublethal effects that mosquitofish can incur upon tadpoles. This was recorded using an injury index and assigning classes/scoring coefficients (coefficient 1 through to 10) to each tadpole, each time the experiment was monitored. The injury inflicted on tadpoles while they were still alive was classed according to scores from 1–6. When injuries were fatal to the tadpole, further tadpole consumption by the fish was assessed and classed according to scores 7–10. A total injury score for the monitoring period was given by adding the injury index coefficients of all three tadpoles, and then taking the average. The predation intensity index (PII) classes are as follows:

Class 1 – No observed injury
Class 2 – Tail nipped less than 2 mm, tadpole alive.
Class 3 – Between 2 mm and less than ¼ of tail length removed, tadpole alive.
Class 4 – Between ¼ and less than ½ of the tail length removed, tadpole alive.
Class 5 – Between ½ and less than ¾ of the tail length removed, tadpole alive.
Class 6 – Between ¾ and all of the tail removed, tadpole alive.
Class 7 – Between 0 to ½ of the tail removed, tadpole dead.
Class 8 – Between ½ to all of the tail removed, tadpole dead.
Class 9 – Part of the tadpole head consumed, tadpole dead.
Class 10 – Entire tadpole consumed.

In most cases, additional trials were performed where mosquito larvae (*Culex spp.*) were provided as alternative prey for the fish. These were collected from stagnant waters in Burgundy Estate (33°50'24.1"S, 18°33'06.2"; 33°50'14.5"S, 18°33'04.7"E) and Eversdal, Bellville (33°51'33.2"S, 18°38'37.1"E). Twenty mosquito larvae were added to each container, in addition to three tadpoles. Mosquito larvae were introduced to the fish at the same time as what the tadpoles were introduced. Five of the twenty mosquito larvae were measured prior to each trial, from the head to the end of the abdomen, or just before the siphon. From the first 2 min onwards, predation intensity in tadpoles was monitored at ever-increasing intervals. The number of mosquito larvae remaining in the container was also counted per observation. The time points were approximately 10 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, and thereafter increasing intervals ranging in duration. Trials ended when all the tadpoles had been eaten completely, or if after 48 h it was evident the fish showed no interest in the tadpoles. The maximum duration of the trials was 72 h.

3.2.3 Statistical analysis

3.2.3.1 Predation Intensity Index (PII)

In order to assess the effect of predation on tadpoles by mosquitofish, a univariate hierarchical linear mixed (HLM) model was used with each repetition as primary unit of measurement, and with the PII score as response variable. This model was constructed in SPSS v. 24 (SPSS, Inc.; Chicago, Illinois), with species, developmental stage and alternative prey (present or absent) as fixed effects. Because observations were measured at time points, which were not always precisely the same across all trials, the data was grouped into time intervals and this added as the fourth fixed effect in the HLM model. Interactions between fixed effects were tested for significance. The subject (experimental repetition) was chosen as random effect, and a random slope added at the subject level. A restricted maximum likelihood estimation method and an unstructured covariance structure were used. The practical significance of the results was determined by calculating the effect sizes from the differences between means. See Chapter 2 for the formula and the interpretation of the effect sizes. Non-parametric correlations were determined between PII scores, fish gape area, fish length, average tadpole length, and the number of mosquito larvae performing simple linear regression analysis in SAS v. 9.4 (SAS Institute Inc., Cary, NC). The strength of the correlation coefficient was determined using the same methods as in Chapter 2.

Previous studies have found that fish sex plays a role in the predation habits of fish (Smith and Smith 2015), and because fish were selected randomly and not according to sex it was necessary to determine if this factor was affecting the data. Therefore an additional HLM was performed, using PII score as response variable, and fish sex and time as fixed effects. The same parameters for model construction were used as the first model.

3.2.3.2 Predator Response Index (PRI)

A three-way ANOVA analysis was performed to determine the effects of different factors on fish behaviour, using SPSS. Amphibian species, developmental stages and the presence or absence of alternative prey were used as main effects, and interactions between these main effects were analysed. Factorial analysis was used instead of hierarchical mixed model analysis, because individual fish were being tested, unlike the predation intensity experiments where groups of tadpoles were assessed together per replicate (hierarchical data). An alpha level of 0.05 was chosen and an intercept included. The practical significance of the results was determined by calculating the effect sizes from the differences between means, in the same manner as discussed previously.

In order to determine if fish sex had an influence on the PRI data, a one-way ANOVA was performed using the mean PRI scores for female, male and juvenile fish. This was tested within each amphibian species, with alpha level of 0.05. Tukey's post hoc tests were performed on the ANOVA results, to determine significant differences in the mean PRI scores for the three different fish sex classes.

In order to determine if there were any significant correlations between the gape area of the fish, predatory response of the fish and the predation intensity on the tadpoles, two-tailed Spearman's rank correlation tests were performed. These tested for non-parametric relationships between the following dependent variables between the following variables: (1) PII scores at 24 h, (2) total PRI scores, (3) gape area of the fish, and (4) the average length of tadpoles. *Capensibufo magistratus* was not included in these tests as PRI observations were not performed when the experiments with this species were executed. A correlation coefficient (r) of 0.3 was a weak association, 0.5 was considered moderate, and a coefficient of 0.8 was a strong association. Correlations were considered significant at the $p < 0.05$ level, and the direction of the monotonic relationship was determined by the coefficient sign.

3.3 Results

3.3.1 Predation Intensity Index results

There was marked interspecific variation of predation intensity inflicted by mosquitofish. Injuries were so few in trials with *S. capensis* and *C. magistratus*, to the point of being negligible, while the effects of predation were clearly evident in trials and across all treatments with *X. laevis* and *T. delalandi* (see Figure 3.2 for photographs showig the intensity of tadpole injuries). The HLM for predation intensity observations showed significance for all four fixed effects (species, developmental stage, alternative prey, and time), and for all interactions except for the three-way interaction between species, developmental stage and alternative prey (see Tables B1 and B2 in Addendum B for detailed results and model parameters). Of all the four fixed effects, the *F* statistic revealed that time had the largest contribution to PII scores ($F_{(23,\ 1914)} = 108.11$, $p < 0.001$), in comparison with low *F* statistic scores for the other fixed effects . As time progressed, predation intensity increased significantly through all treatments, from a PII score of 1 to 5.90 at 40–50 h ($d = 3.17$), and to 7.12 ($d = 3.95$) after 60 h. The effect of interspecific variation, ontogeny, and alternative prey on predation intensity is further discussed in more detail below.

3.3.1.1 Predation intensity and interspecific variation

Predation intensity differed markedly amongst the four amphibian species. The lowest predation intensity was for *C. magistratus and S. capensis,* where average PII values were almost negligible and predation intensity was statistically insignificant. In fact, for *C. magistratus*, no injuries were observed whatsoever (PII = 1, $d = 0$). In some trials with *S. capensis*, fish nipped the tail slightly (max. PII = 2, see Figure 3.2) but tail nipping only occurred after 40 h after experiments had commenced. In contrast to results of these two species, predation intensity was significantly higher in treatments with *T. delalandii* and in *X. laevis*. At the end of the study with *T. delalandii*, (at time interval 40–50 h) the average PII across all treatments (developmental stages, and for treatments with and without alternative prey) the average PII score was 7.40 ($d = 4.13$) and for *X. laevis* it was 8.47 ($d = 4.81$). Therefore at the end of the trials, most tadpoles were dead as scores were higher than Class 7, yet mosquitofish consumed tadpoles of *X. laevis* to a greater degree than for *T. delalandii*. See Figure 3.2 to compare the degree of injury between *T. delalandii* and *S. capensis.*

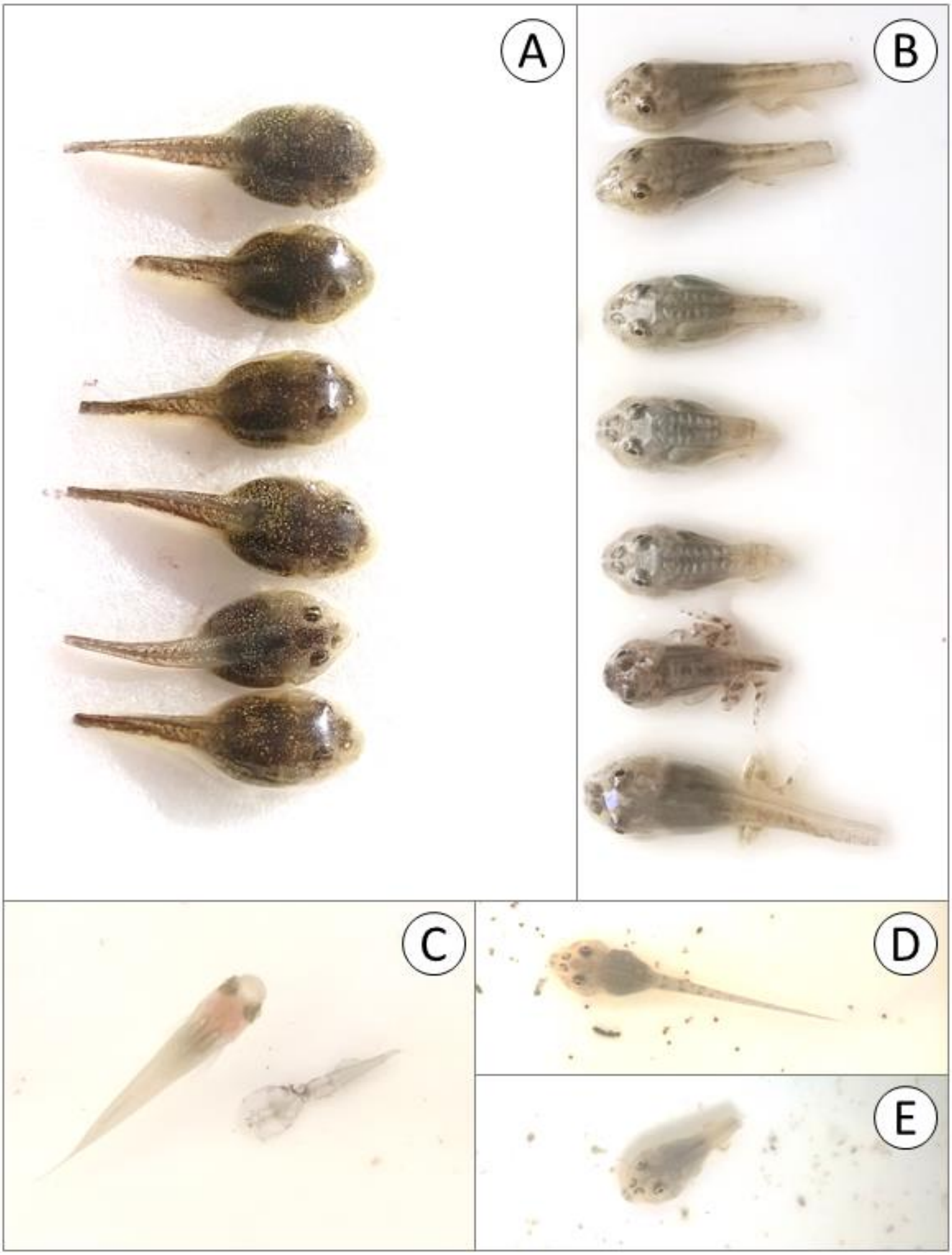

Figure 3.2. Injuries in tadpoles exposed to mosquitofish. (A) Injuries in tadpoles of *S. capensis* at the late pre-metamorphic stage. (B) Injuries in late pro-metamorphic tadpoles of *T. delalandii*. (C) Mosquitofish and an eaten carcass of an early pre-metamorphic *X. laevis* tadpole. (D) Photo of a healthy *T. delalandii* tadpole, in comparison with (E) an injured *T. delalandii* tadpole. Photos by Roxanne Conradie.

3.3.1.2 Predation intensity and ontogenic variation

The three–way interaction of species, developmental stage and time in the HLM was significant ($F_{(38,\ 1913)} = 3.342$, $p < 0.001$). When comparing PII values across developmental stages and species (see Figure 3.3), it is clear that palatability is much higher for *T. delalandii* and *X. laevis,* across all stages. The PII scores were practically significant for all developmental stages of these two species, ranging in effect size (*d*) from 2.51–5.75 (see Table 3.3). No PII scores of developmental stages of either *S. capensis* or *C. magistratus* were practically significant (*d* = 0.00–0.27, see Table 3.3), and PII scores of developmental stages of *S. capensis* were not significantly different from each other. Only one developmental stage of *C. magistratus* was tested, therefore comparisons could not be made across different stages of this species. There is a trend in developmental stages of *T. delalandii* where the PII increases significantly from the embryonic stage to the hatchling (*d* = 5.75) and free-feeding stages (*d* = 5.57). Thereafter the PII decreases significantly from the free-feeding stage to the late pre-metamorphic stage (*d* = 2.38). The PII is even further decreased in the early pro-metamorphic stage, but this is not significantly different from the late pro-metamorphic stage (*d* = 0.68). For *T. delalandii*, there are therefore three statistically distinct groups: low palatability in the embryonic stage, high palatability in the hatchling and free-feeding stages, and moderate palatability (yet still near lethal) in the late pre- and pro-metamorphic stages). For *X. laevis*, predation intensity was highest in the free-feeding and early pre-metamorphic stages, with no practical statistical difference between them (*d* = 0.52). However, predation intensity was significantly lower in the late pre-metamorphic stage, in comparison with the free-feeding stage (*d* = 0.99) and the early pre-metamorphic stages (*d* = 1.52).

Table 3.3. PII scores and their effect sizes when compared with the null hypothesis, at 40–50 h.

Species	***Tomopterna delalandii***		***Xenopus laevis***		***Sclerophrys capensis***		***Capensibufo magistratus***	
	PDI score	Effect size (*d*)	PDI score	Effect size (*d*)	PDI score	Effect size (*d*)	PDI score	Effect size (*d*)
Embryonic stage	6.60	3.61	–	–	–	–	–	–
Hatchling stage	9.91	5.75	–	–	–	–	–	–
Free feeding stage	9.63	5.57	8.71	4.97	1.03	0.02	–	–
Early pre-metamorphosis	–	–	9.52	5.50	–	–	–	–
Late pre-metamorphosis	5.94	3.18	7.17	3.98	1.06	0.04	–	–
Late pro-metamorphosis	4.89	2.51	–	–	1.43	0.27	1.00	0.00

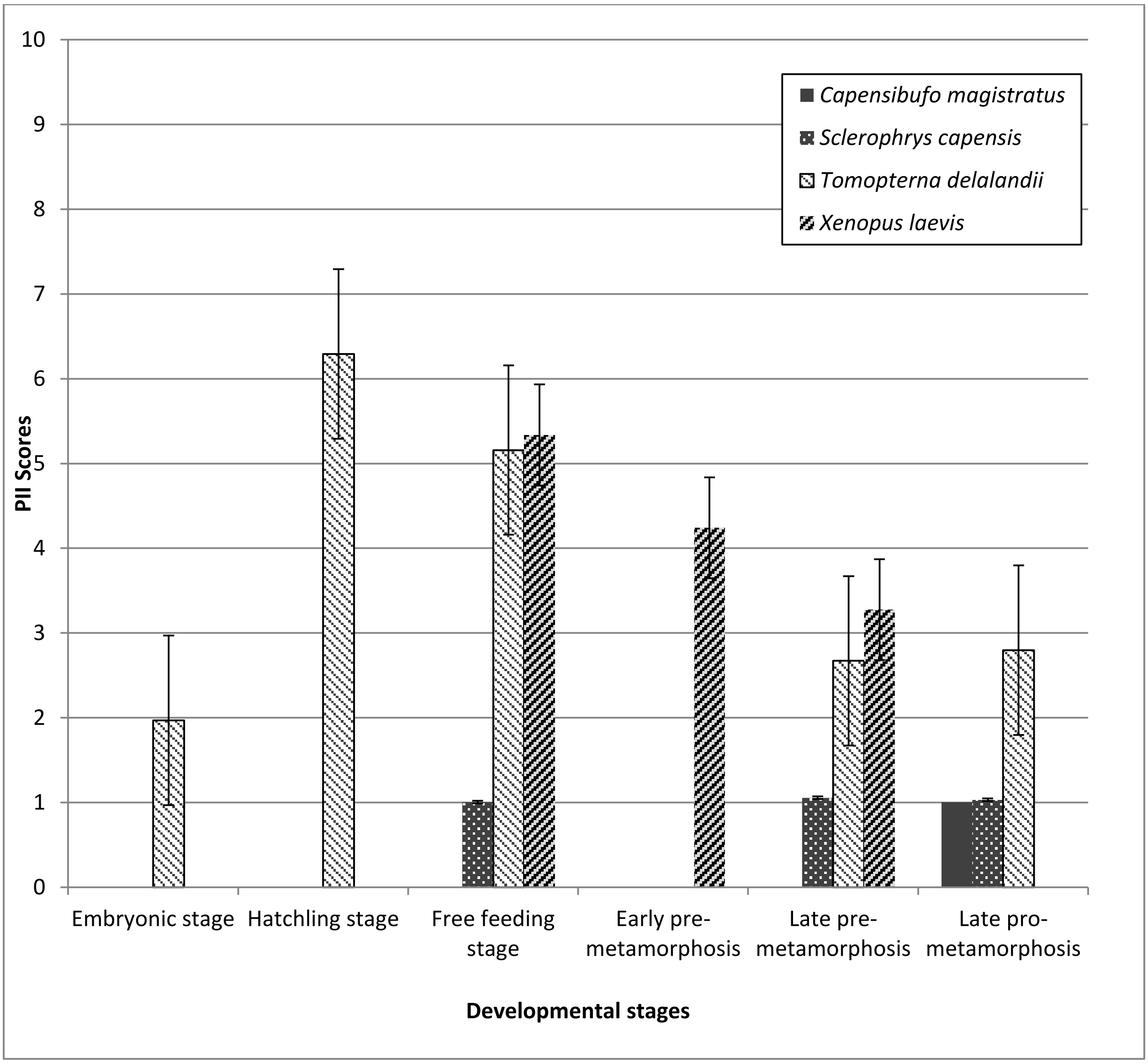

Figure 3.3. PII scores for all developmental stages tested per amphibian species, at time interval 40–50 h.

3.3.1.3 Predation intensity and the role of alternative prey

The four-way interaction of species, developmental stage, time and presence and absence of alternative prey was analysed in order to detect significant differences amongst groups with and without alternative prey. Interactions between all four fixed effects were significant ($F_{(14,\ 1913)} = 2.40$, $p = 0.003$). By comparing means between treatments with and without alternative prey, within developmental stages and within species and taking the effect sizes, it was found that alternative prey had no significant effect on all developmental stages of *T. delalandii* except for the late pre-metamorphic stage ($d = 2.14$), where the treatment with mosquito larvae had a lower mean PII of 4.28 (SE: 0.63), in comparison with the treatment that did not have mosquito larvae (PII = 7.60, SE: 0.69, see Figure 3.4). For *X. laevis*, mosquito larvae significantly reduced PII scores in comparison to non-mosquito larvae treatments, for tadpoles of the free-feeding stage (*d* = 1.23) as well as those of late pre-metamorphosis (*d* = 0.82). However, for the early pre-metamorphic stage, mosquito larvae had no significant effect on the already high PII scores (PII = 9.43, SE: 0.79; *d* = 0.12). For all developmental stages of *C. magistratus* and *S. capensis*, the role of alternative prey had no significant effect on the predation intensity of the tadpoles (see Table 3.4 and Figure 3.4). Overall predation intensity was low in both species, regardless of the presence or absence of mosquito larvae. Correlations performed by linear regression analysis showed that there was a weak negative correlation between the number of mosquitoes in the container, and the level of predation intensity on the tadpoles ($r = -0.28$, $p < 0.0001$, see Table 3.5).

Table 3.4. Effect sizes between mean PII scores of treatments with and without mosquito larvae, at time interval 40–50 h. Effect sizes are marked with (*) to indicate significance.

Species	***T. delalandii***			***X. laevis***			***S. capensis***			***C. magistratus***
Developmental stage	Embryonic stage	Free-feeding stage	Late pre-metamorphosis	Free-feeding stage	Early pre-metamorphosis	Late pre-metamorphosis	Free-feeding stage	Late pre-metamorphosis	Late pro-metamorphosis	Late pro-metamorphosis
Effect size (*d*) between treatments with and without alternative prey	0.00	0.50	2.14*	1.25*	0.11	0.82*	0.00	0.11	0.27	0.00

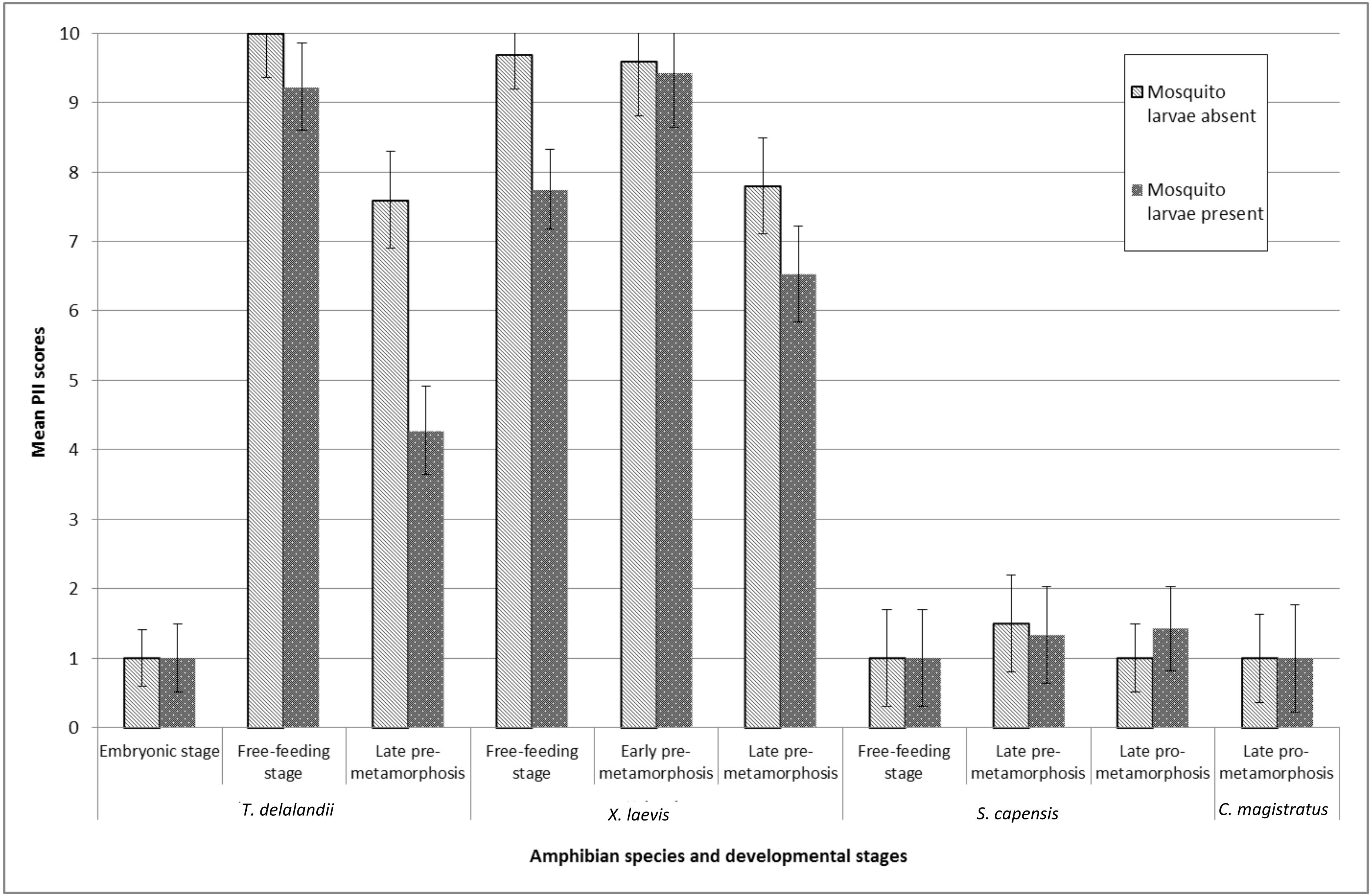

Figure 3.4. Mean PII scores of treatments with and without mosquito larvae, at time interval 40–50 h.

3.3.2 Predator Response Index results

The results from the three-way ANOVA showed that the three-way interactions between the three species, different developmental stages and the role of alternative prey were both not significant ($F_{(2,\,113)} = 0.208$, $p = 0.81$; see Table B3 of Addendum B for detailed statistical parameters). However, there two-way interactions were significant, between species and developmental stage ($F_{(3,\,113)} = 3.24$, $p = 0.025$), and species and alternative prey ($F_{(2,\,113)} = 3.71$, $p = 0.028$). Of the main effects, amphibian species ($F_{(2,\,113)} = 17.15$, $p < 0.001$) and the presence or absence of mosquitoes ($F_{(1,\,113)} = 12.65$, $p < 0.001$) had the largest significant effect on fish attack behaviour scores, while developmental stage had a much lower, yet still significant, effect ($F_{(5,\,113)} = 3.60$, $p = 0.005$). These results are further broken down according to the significant main effects and interactions, and discussed in terms of practical significance in the following paragraphs.

3.3.2.1 Two-way interaction between species and developmental stage

ANOVA results showed that fish behaviour towards all developmental stages of *S. capensis* was not practically significant (see Figure 3.5). For *T. delalandii*, PRI scores were also not significant for embryonic stages and the free feeding stage. For all other stages of *T. delalandii*, mean PRI scores did not exceed mere interest in the tadpoles, and did not go over to chasing or biting. However, the interest that fish showed towards tadpoles was still practically significant when compared with a null hypothesis of no reaction towards tadpoles. PRI scores were significant for the hatchling stage (PRI = 2.07, d = 1.19), the late pre-metamorphic stage (PRI = 1.74, d =0.84), and late pro-metamorphic stages (PRI = 1.818, d = 0.92). For trials with tadpoles of *X. laevis,* PRI scores were practically significant for all developmental stages tested. The highest significant effect was found in the free feeding stage of *X. laevis* (PRI = 3.48, d = 2.78), and fish had the highest level of reaction to this stage and species in comparison with all others (PRI = 3.48). PRI scores were also significant in late pre-metamorphic (PRI = 1.27, d = 1.27) and early pre-metamorphic (PRI = 1.82, d = 0.91) stages of *X. laevis.* At the beginning of the experiments, when PRI was measured, fish responded more antagonistically towards tadpoles in *X. laevis* than in *T. delalandii*. For the former species, not only was chasing and ramming behaviour observed, but also biting and nipping.

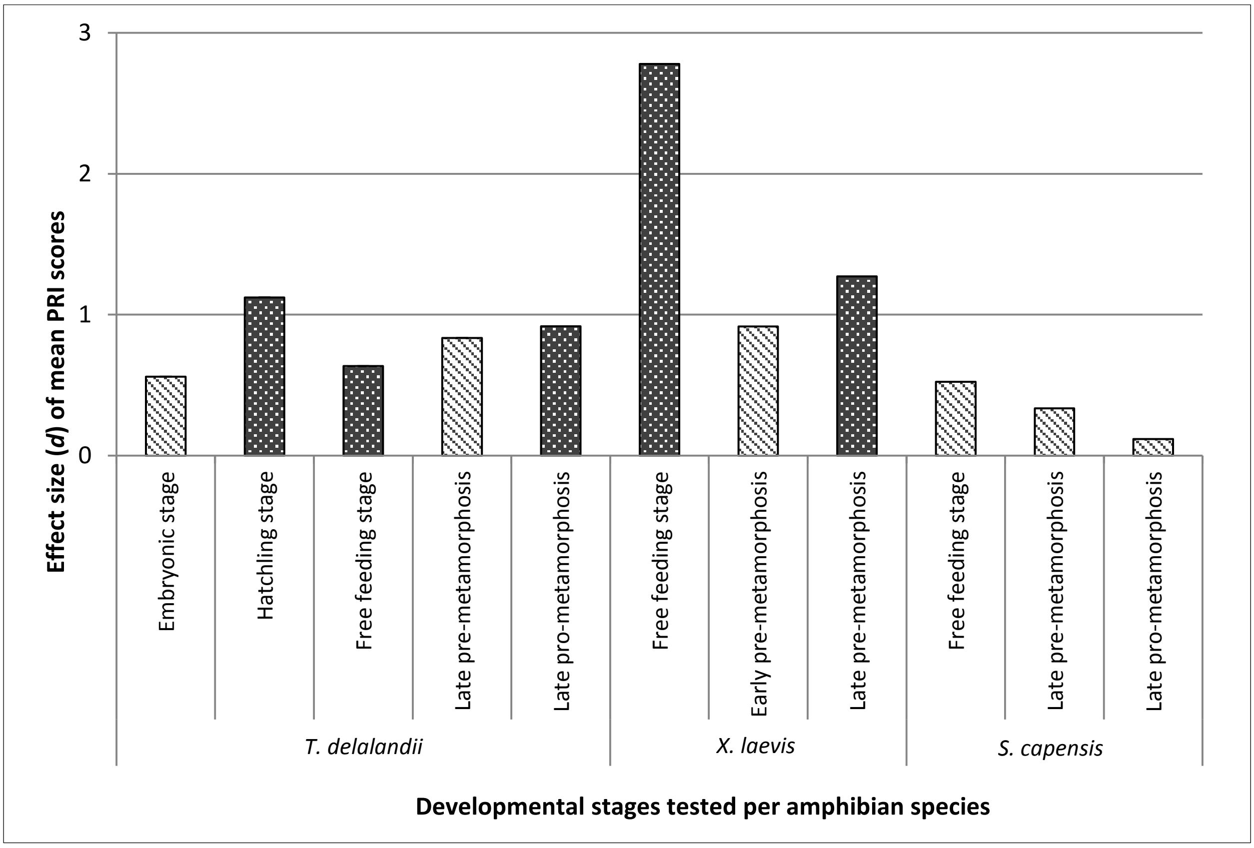

Figure 3.5. Effect sizes (d) of mean PRI scores according to developmental stages of different species. Darkly coloured bars indicate significance (d > 0.8).

3.3.2.2 Two-way interaction between species and the presence or absence of alternative prey

For *S. capensis,* the presence of mosquito larvae had no significant effect on the mean PRI score (*d* = 0.09). However, for *T. delalandii*, fish in treatments with mosquito larvae had a significantly lower attack response in comparison with those that did not have mosquito larvae (*d* = 0.92). This same phenomenon was observed in fish with *X. laevis*, but the difference in predator response was much larger (*d* = 1.47; see Figure 3.6). Overall, the highest predator response was observed in *X. laevis*, regardless of the presence or absence of mosquito larvae. There was no significant difference in fish response scores for trials with alternative prey between the three species, where the effect size between means of *S. capensis* and *T. delalandii* was *d* = 0.18, between *S. capensis* and *X. laevis* was *d* = 0.54, and between *X. laevis* and *T. delalandii* was *d* = 0.72. However, there were significant differences among fish response scores for trials without alternative prey, where the effect size between means of *S. capensis* and *T. delalandii* was *d* = 0.84, between *S. capensis* and *X. laevis* was *d* = 2.12, and between *X. laevis* and *T. delalandii* was *d* = 1.28. Correlations with Spearman's rho showed that there was a positive moderate correlation between the predation intensity (PII scores) and predator behaviour response (PRI scores) ($r = 0.45$, $p < 0.001$, see Table 3.6).

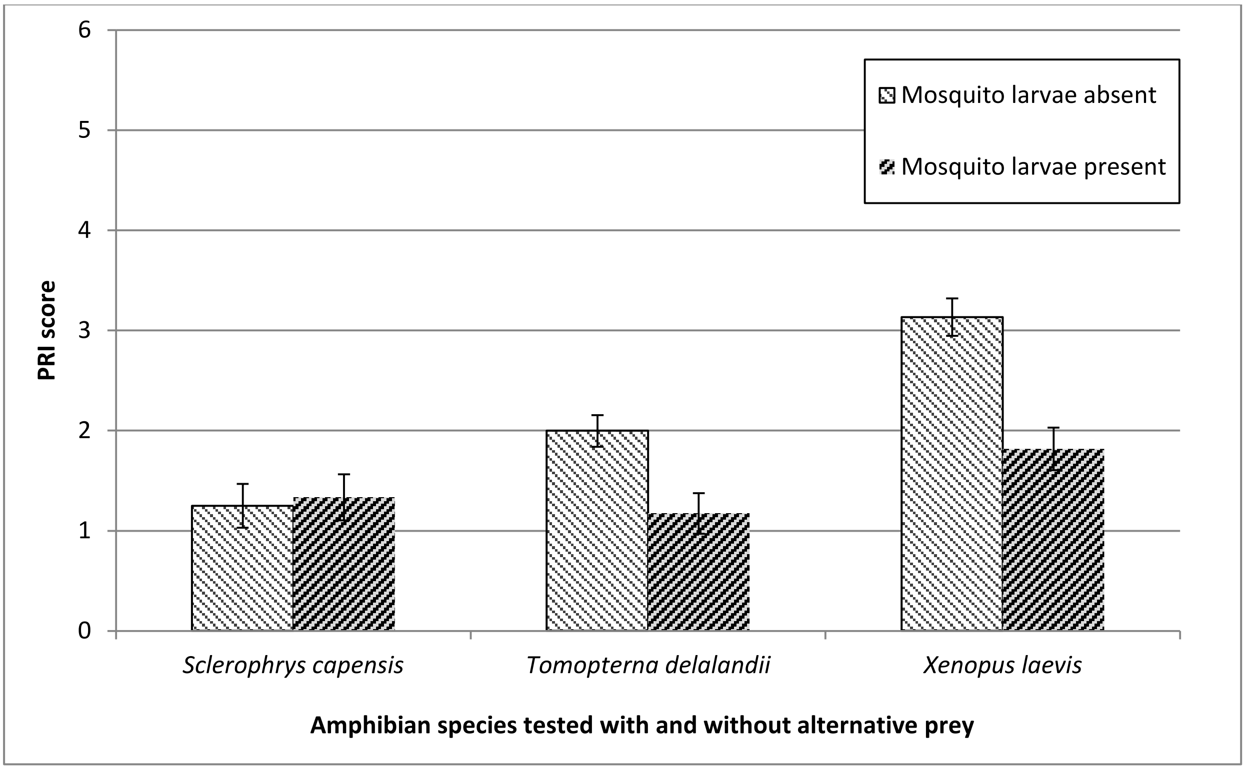

Figure 3.6. Mean PRI scores of *G. affinis* on three different amphibian species with and without alternative prey.

3.3.3 The influence of fish sex on PII and PRI

3.3.3.1 Fish sex and Predation Intensity Index results

Results of the HLM showed that fish sex and time had a significant effect on the PII ($F_{(23, 2150)} = 29.16$, $p < 0.001$; $F_{(3, 314)} = 19.71$, $p < 0.001$) as well as the two-way interaction between the two variables ($F_{(56, 2149)} = 3.172$, $p < 0.001$; see Table B4 and B5 in Addendum B for detailed statistical results). Time had a larger effect on the PII than fish sex, which was evident due to the larger F statistic. Females exerted the highest degree of response toward tadpoles (PII = 3.82, SE: 0.22), second highest was adult males (PII = 2.22 SE: 0.30) and the lowest was juveniles (PII = 1.83, SE: 0.43). However, effect sizes between PII means revealed that there was no practical significant difference between females and males ($d = 0.56$), between females and juveniles ($d = 0.69$) and between males and juveniles ($d = 0.13$).

3.3.3.2 Fish sex and Predator Response Index results

One-way ANOVA analysis showed that overall fish sex had no influence on the PRI scores for *S. capensis* and *T. delalandii*. However, fish sex had a significant effect on the PRI in *X. laevis* ($F_{(2,\,41)} = 3.40$, $p = 0.043$; see Table B6 in Addendum B for detailed results), although Tukey's post hoc tests showed no difference between mean PII scores in the different sexes. One-way ANOVA analysis and Tukey's post hoc tests also showed that fish sex had no influence on the PRI scores between treatments with and without mosquito larvae ($F_{(2,\,52)} = 2.278$, $p = 0.113$). Analyses on different developmental stages also showed that fish sex had no influence on PRI scores, except for the free-feeding stage ($F_{(2,\,36)} = 4.58$, $p = 0.017$). Tukey's post hoc tests identified PRI scores of males and females as statistically distinct from each other. There was no difference between PRI scores for male and juvenile fish, and female and juvenile fish.

3.3.3.3 The influence of tadpole size on PII and PRI

Correlations performed by linear regression analysis showed that there was a weak, but significant, negative correlation between tadpole length and the level of predation intensity on the tadpoles ($r = -0.21$, $p < 0.0001$, see Table 3.5). The Spearman's rank correlations showed a similar result for the relationship between PII and tadpole length ($r = -0.367$, $p < 0.001$, see Table 3.6). However, there was no significant association between the PRI scores and the average length of tadpoles ($r = -0.139$, $p = 0.143$).

3.3.3.4 The influence of fish gape size on PII and PRI

Correlations performed by linear regression analysis showed that there was a weak positive correlation between PII scores and fish gape area ($r = 0.23$, $p < 0.0001$, see Table 3.5), which was supported by Spearman's rank correlations ($r = 0.23$, $p = 0.007$, see Table 3.6). Regression analysis also showed a weak positive association between PII scores and average fish length ($r = 0.22$, $p < 0.0001$). However, there was no significant relationship between PRI scores and the gape area of fish ($r = -0.039$, $p = 0.653$, see Table 3.6), or between the gape area of fish and the average length of tadpoles ($r = 0.053$, $p = 0.576$).

Table 3.5. Correlations by way of linear regression analysis, between PII scores and other variables.

Dependent variable	**Independent variable**	**Coefficient of determination**	**Correlation coefficient (*r*)**	**Numerator DF**	**Denominator DF**	**F-Ratio**	**Sig. (*p*-value)**
		R-Square	sqrt R-Square				
PII score	Number of mosquitoes	0.08	-0.28	1	2229	955.0	<.0001
PII score	Average tadpole length	0.05	-0.21	1	1979	144.5	<.0001
PII score	Gape area of fish	0.05	0.23	1	2089	120.6	<.0001
PII score	Fish length	0.05	0.22	1	2209	128.7	<.0001

Table 3.6. Correlations by way of Spearman's rank correlation tests. Significant correlations are marked *.

Dependent variable	**Independent variable**	**Correlation coefficient (*r*)**	**Significance (2-tailed) (*p*-value)**	**N**
PII score (at 24h)	PRI total score	0.451*	0.000*	133
PII score (at 24h)	Gape area of fish	0.232*	0.007*	133
PII score (at 24h)	Average length of tadpoles	-0.367*	0.000*	113
PRI total score	Gape area of fish	-0.039	0.653	133
PRI total score	Average length of tadpoles	-0.139	0.143	113
Average length of tadpoles	Gape area of fish	0.053	0.576	113

3.4 Discussion

These experiments show that mosquitofish found the tadpoles of *C. magistratus* and *S. capensis* unpalatable, but those of *T. delalandii* and *X. laevis* were highly palatable. This indicates that certain amphibian species may be at a higher risk of mosquitofish predation than other species, which corresponds with the previous results of the mesocosm trials in Chapter 2. These previous experiments showed that tadpoles of *S. grayii* and *T. delalandii* were preyed upon to a greater degree than *S. pantherina*. Other researchers have also revealed that mosquitofish have a strong negative effect on amphibian survival (Lawler *et al.* 1999; Segev *et al.* 2009; Shulse and Semlitsch 2014), and that mosquitofish predation on amphibians may be higher in some species than others (Grubb 1972; Zeiber *et al.* 2008). It is likely that the unpalatability of *S. capensis* and *C. magistratus* is due to a chemical defence tactic, as both of these species are bufonids, which are known to possess noxious chemical substances in their skin that repel predators (Hayes *et al.* 2009). This was also discussed in Chapter 2, concerning the unpalatability of the bufonid *S. pantherina*. It is likely that the tadpoles of *T. delalandii* and *X. laevis* are more palatable due to an absence of such an anti-predator defence mechanism.

Furthermore, there are differences in ontogenic palatability between species. Tadpoles of *S. capensis* were unpalatable regardless of developmental stage. The eggs of *T. delalandii* were also completely unpalatable, while the smaller larval stages of *T. delalandii* and *X. laevis* were highly palatable to mosquitofish. Smaller larval stages of palatable species were also preferred to the larger developmental stages. This suggests that the hatchling, free feeding, and early premetamorphic developmental stages are the most vulnerable to mosquitofish predation, for *X. laevis* and *T. delalandii*. However, mosquitofish predation on tadpoles of larger developmental stages was still significant, with a high degree of tadpole injuries and mortalities. This shows that larger tadpoles can still be impacted by mosquitofish predation to a significant degree, and also shows that tadpole predation is not limited by the gape size of mosquitofish. Gape size and fish length were correlated to PII scores, and PII scores also decreased as tadpoles size increased, but gape size and tadpole size was not correlated.

The PII experiments showed that overall, the presence of mosquito larvae did not reduce the predation impacts of mosquitofish in palatable species over long periods of time, yet there were a few exceptions for specific developmental stages within certain species. When observed over shorter time periods, via the PRI experiments, the study showed the presence of alternative prey did reduce the predation impacts on tadpoles, with the exception of *S. capensis.* In this case, tadpoles of this species were unpalatable to the fish, regardless of the presence or absence of mosquito larvae. These two main findings, namely the role of ontogenic shifts in palatability, and the role of alternative prey on palatability, are discussed further in the paragraphs that follow.

3.4.1 Ontogenic shifts in palatability

The two different methods used to determine tadpole palatability did not yield the same results. The short-term PRI tests showed no apparent ontogenic trends in palatability, but the long-term PII experiments suggested that there were certain patterns between developmental stages, although not the same between amphibian species. PII scores showed that palatability was low for *S. capensis* and that there were no significant differences between different developmental stages. In the case of *C. magistratus,* different developmental stages could not be compared as only one developmental stage was available for use in experiments. In contrast to *S. capensis*, there was variation in palatability between different developmental stages of both *T. delalandii* and also *X. laevis.* Eggs of *T. delalandii* had the lowest palatability and PII scores were not statistically significant, but the hatchling and free-feeding stages were highly palatable as indicated by the high level of injury and mortality in the PII scores. Injury and mortality were also significant for the later developmental stages where *T. delalandii* tadpoles were larger (late pre- and late pro-metamorphic stages). However, PII scores were significantly lower than for the hatchling and free-feeding stages of *T. delalandii*. A similar trend was also seen in *X. laevis*, where the free-feeding stage and early pre-metamorphic stages where highly palatable, yet PII scores decreased significantly for larger tadpoles at the late pre-metamorphic stage. Although injury and mortality in the late pre-metamorphic stage was statistically distinct from the earlier developmental stages, PII scores in injury were still high and statistically significant (see Figure 3.3 and Table 3.3), similar to *T. delalandii*. However, when assessing the PRI data, there were no apparent ontogenic trends in palatability. The only visible trend is that high effect sizes of mean PRI scores in a certain amphibian species correspond with high PRI scores in that same species (compare Figure 3.3 and Figure 3.6). One unusual result of the PRI results was that the the free-feeding stage of *X. laevis* had the highest PRI score, and this was higher than for all other developmental stages. Possible reasons for these patterns in palatability amongst developmental stages and species are discussed below.

3.4.2 Larval stages of *Sclerophrys capensis*, *Tomopterna delalandii* and *Xenopus laevis*

Other studies have also documented ontogenic shifts in palatability, but there are differences in literature regarding the trends between larval stages. The results for *T. delalandii* and *X. laevis* were similar results found by Pyke and White (2000), where mosquitofish ate more hatchlings and small tadpoles of *Ranoidea aurea* than either eggs or larger tadpoles. Komak and Crossland (2000) and Lawler and Hero (1997) also reported the same trend for larval stages of *Bufo marinus,* where only the hatchlings and some free-feeding stage tadpoles were consumed. A similar trend was also found in *Duttaphrynus melanostictus* (Fan *et al.* 2016). Formanowicz and Brodie (1982) also demonstrated a decreasing trend in palatability in five amphibian species, where metamorphic stages of these species were least palatable. This unpalatability was attributed to the development of granular glands in their skin (Formanowicz and Brodie 1982).

In contrast, other studies have reported an opposite pattern in ontogenic variation, where palatability is lowest in the early stages and increases throughout development. For example, Adams *et al.* (2011) showed that five of twelve amphibian species tested were noxious to a variety of predators, but palatability increased as tadpole development progressed. This phenomenon was also found in a literature review by Gunzburger and Travis (2005), as they concluded that eggs and hatchlings were more noxious than tadpoles across most amphibian families. This was also found in *Bufo americanus charlesmithi* by Brodie *et al.* (1978) and *Pelophylax nigromaculatus* by Fan *et al.* (2016). These results conflict with those studies mentioned in the previous paragraph and also the results of this study for *T. delalandii* and *X. laevis*. However, Hayes *et al.* (2009) found that the toxin profiles of *B. marinus* were initially high at the embryonic stage, particularly the toxin bufadienolide, which then decreased throughout development, but then rose again at metamorphosis with the initiation of granular gland development in the skin. This may explain some of the differing trends in palatability in the literature, where some assess only egg and hatchling stages, and where others only observe premetamorphic stages and metamorphic stages. A complete analysis of palatability from the embryonic to metamorphic stages are likely to represent a more accurate picture, where palatability at the beginning is increased due to the remnant toxins of the egg diminishing through development, but which then is decreased at later stages due to the development of toxins in the adult frog skin. However, this explanation can only be attributed to the bufonids, or in species that are known to possess chemical defence tactics against predators. Therefore this reason for differences in palatability cannot be attributed to *T. delalandii* and *X. laevis,* as they are not known to possess chemical defensive mechanisms.

The literature also shows that in some species there are no ontogenic trends in palatability whatsoever. No ontogenic shifts in palatability were found *Bufo boreas halophilus* by Peterson and Blaustein (1992), although the predators were invertebrates and not fish. No ontogenic variation was also found in a study by Komak and Crossland (2000). In this case, all stages of *Opisthodon ornatus* were highly palatable to *G. holbrooki,* regardless of the developmental stage. These results are similar to the results of this study for the bufonid *S. capensis*, where tadpoles showed a consistent pattern of unpalatability throughout development. Mosquitofish were generally disinterested in the *S. capensis* tadpoles that were presented to them, regardless of developmental stage. By the end of the study, when fish had already been without food for four days, some tails had been slightly nipped, but these injuries were not statistically significant. If the study was prolonged the mosquitofish attacks might have increased in intensity due to hunger, but this cannot be said for certain. It is most likely that the overall unpalatability of *S. capensis* was due to noxious chemicals that serve as an anti-predator defence. It is however unclear why there were no shifts in palatability during development, as demonstrated in the literature for many other bufonid species.

The tadpoles of *T. delalandii* and *X. laevis* were initially highly palatable at very young stages, and then palatability decreased as development progressed. Because it is unlikely that the ontogenic shifts in palatability are due to changes in their chemical make-up, as they are not known to possess such anti-predator defences, it is more likely that there are other factors playing a role. The immobility and smaller size of hatchlings may be two large determining factors in increasing predation susceptibility (Webb and Joss 1997). Satiation of the fish may also be indirectly influenced by the physical size of the tadpoles consumed (Pyke and White 2000). Tadpole size may be a legitimate determining factor of palatability in this study, as results showed that PII scores and tadpole size were negatively correlated. These three factors, namely tadpole immobility, tadpole size, and satiation in fish, and their role and influence on palatability are discussed in the following paragraphs.

It has been suggested in previous literature that hatchlings and tadpoles at younger developmental stages may be more susceptible to predation as they are not able to actively avoid predators, due to their immobility (Webb and Joss 1997; Reynolds 2009). This has been used to explain ontogenic shifts in palatability in other studies (Webb and Joss 1997; Reynolds 2009). However, immobility may be more advantageous than movement, as this may attract the attention of mosquitofish (Smith and Smith 2015). It was found in this study that as soon as a tadpole darted around within the container due to a fright reaction, it promoted aggression and attack behaviour in the mosquitofish. This may explain the high palatability of *X. laevis* tadpoles, as the black tip at their tail pulsates and moves to keep it in the same position within the water column, even though the tadpole itself is stationary. This is likely to have attracted the mosquitofish, as this was often the first part of the tadpole to be bitten. It was also observed, that while tadpoles remained stationary they generally did not invite much interest from the mosquitofish, but occasional wiggling and intense swimming movements attracted the fish, regardless of the amphibian species used. Zeiber *et al.* (2008) and Pyke and White (2000) also found that mosquitofish aggressively responded to tadpole movement. The magnitude of movement also determined the degree of mosquitofish attraction: when tadpoles darted around due to a fright reaction, it initiated a greater response in the mosquitofish, which resulted in the fish aggressively chasing and nipping at the tadpole. If the tadpole just gently adjusted its position at the container edge, the mosquitofish would slowly align its line of sight with the tadpole, and would sometimes swim slowly toward it and nip at the tadpole's tail. Because of these observations, it is probable that the mobility of tadpoles played a role in their susceptibility to attack, rather than their immobility. However, it was observed that movement in the largest tadpoles (roughly 30–35 mm in length) did not attract mosquitofish to the same degree as movement by smaller tadpoles. Although this was not quantified, it is possible that increased tadpole size reduced the boldness of mosquitofish attack. This may provide a better explanation why there was a general decreasing trend in predation intensity as tadpole size increased. However, if tadpole movement alone was the determinant of palatability, we would expect that tadpoles of larger sizes (with greater swimming movements) would be more susceptible to predation than the smaller tadpoles, and this was not what was observed. It is likely that factors other than tadpole mobility play a stronger role in determining palatability.

Correlations between prey size and palatability have been found in other studies. For example, Heyer *et al.* (1975) found that an increase in tadpole size reduced predation by Odonate naiads and cannibalistic tadpoles, and predicted that larger body size was a characteristic utilized by tadpoles to avoid fish predators. Similarly, Adams *et al.* (2011) found that Odonate naiads, crayfish, and two fish species (*Lepomis spp.*) were unable to consume tadpoles that had reached large sizes at late developmental stages (*Rana spp.*), and also suggested that tadpole size may be an important anti-predator defence mechanism in late-stage tadpoles, where chemical noxiousness may play a larger role when tadpoles are too small to escape predators (Adams *et al.* 2011). However, these two anti-predator mechanisms, i.e. chemical noxiousness and larger size, are not normally found in the same amphibian clades. Heyer *et al.* (1975) suggested that larger body sizes in late-stage tadpoles is an anti-predator mechanism against fish, a characteristic employed by the Ranidae, whereas the predominant mechanism in bufonid species is chemical noxiousness. However, Hero *et al.* (2001) argued against the suggestion that large late-stage tadpole size was an anti-predator mechanism, as they found that there was no correlation between tadpole size and survival in the presence of a fish predator, even though relatively large tadpole sizes were used (up to 57 mm). These authors argued that it is more likely that the larger body size of these tadpoles can be attributed to the permanent water bodies that they occupy, where larger sizes can be attained as they are not threatened by desiccation, as in ephemeral situations. Hero *et al.* (2001) also pointed out that tadpole size at the hatchling stage is usually small (< 25 mm), regardless of species. Therefore if larger body size was an anti-predator defence mechanism of the Ranidae, one could expect larger hatchling sizes. There are also other studies that show no significant variation in predation between small and large body sizes. Webb and Joss (1997) initially found a correlation between tadpole size and survival from predators, according to their observations at 10 min into the trial. At this time, it was found that mosquitofish initially attacked small tadpoles (10 mm) of *Pengilleyia peronii* at a faster rate than larger tadpoles. However, when the trials were analysed after 24 h had passed, they found that the predation effects were not significantly different when comparing all size classes (10 mm, 15 mm and 20 mm). Zeiber *et al.* (2008) also found no differences between the extent of predation by mosquitofish on four amphibian species with varying body sizes, as species from both large (≈15 mm) and small (≈9.3 mm) size classes were preyed upon heavily.

These differing trends in the relationships between prey size and palatability are not clear, but it is likely that increased tadpole size is advantageous against gape-limited predators. In this case, tadpoles are more likely to resist and survive attacks by exceeding the gape threshold of the fish (Semlitsch and Gibbons 1998). However, studies have shown that despite the small size of mosquitofish, they are not gape-limited predators, which may explain the contrasting literature discussed previously. They are able to consume tadpoles regardless of body size, by first nipping at the tail until the tadpole is immobilized, and then consuming the rest of the tadpole until the fish is satiated (Baber and Babbitt 2004). Komak and Crossland (2000) found that eggs and hatchlings of *Opisthodon ornatus* were consumed immediately by mosquitofish, but from the free-feeding stage onward mosquitofish attacked tadpoles by tail-nipping until the tadpole was immobilised. In this study it was observed that mosquitofish were able to capture and consume tadpoles of smaller sizes (roughly 6–12 mm in length) by a few strikes, sometimes only one. However, larger tadpoles were bitten on the tail repeatedly, until a reduction in tail size made the tadpole unable to swim. Death of the tadpole soon followed immobilisation, as the fish nipped at the abdomen and head. These observations also show that mosquitofish are not gape-limited, and that large tadpoles are not at a lesser risk of predation or injury than smaller tadpoles.

Other results from this study showed that PII scores were weakly correlated with the gape size of fish, which contrasts with the above observations. However, it may be that the association between gape size and PII scores was indirectly related. Larger gape size would also be an indication of larger fish size, and fish with a higher biomass would require more food to reach a level of satiation. This idea is also supported by the significant correlation between fish length and PII scores. It is likely that predation intensity was higher in the smaller tadpole stages, because of the increased amount of tadpole biomass required to satiate the starved mosquitofish. It is likely that similar quantities of biomass were consumed in the trials with larger tadpoles, but the magnitude of injury was decreased due to greater tadpole body sizes. Bence and Murdoch (1986) as well as Webb and Joss (1997) also showed that mosquitofish predation on tadpoles was influenced by their own level of hunger, and not so much by tadpole size or density. Pyke and White (2000) also showed that the level of mosquitofish predation on *Ranoidea aurea* eggs, hatchlings and tadpoles was heavily dependent on the satiation level of the fish. They found that an increased number of eggs or tadpoles were eaten per fish the longer the fish were starved, and the larger the fish biomass was relative to tadpole biomass. A decreased availability of alternative food also increased the number of amphibian eggs and tadpoles consumed. They concluded that the longer fish were starved, an increased amount of prey biomass was required to satiate their hunger, and therefore the more biomass they will eat. It is likely that satiation played a similar role in this study, and might also explain why predation intensity was higher in *X. laevis* tadpoles at the early pre-metamorphic stage, in comparison with the free-feeding stages. Fish used in the early-premetamorphic trials were a larger (mean total length 31.0 mm, see Table B7 in Addendum B.) in comparison with fish of the free-feeding stage trials (23.3 mm), therefore a larger amount of tadpole biomass was required to satiate their hunger. If the impact of predation was measured in terms of biomass rather than injury and mortality, it is possible that greater predation effects would have been detected (also noted by Pyke and White, 2000). It is also possible that the boldness to attack was also decreased in mosquitofish when exposed to larger tadpoles, because fish would require increased energy and handling time for incapacitating and consuming larger prey. The role of satiation might also be linked to optimal foraging theory (Krebs and Davies 1989), which is further discussed in the section on the role of alternative prey and tadpole predation.

3.4.3 Eggs of *Tomopterna delalandii*

These experiments showed that there was a dramatic difference in palatability of *T. delalandii* eggs compared to the high palatability of hatchlings and young tadpoles at the free-feeding stage. Different trends concerning the palatability or unpalatability of amphibian larvae are reported in the literature, and the same situation is likewise true for their eggs. In a literature review by Gunzburger and Travis (2005), it was concluded from the metathesis that eggs and hatchlings are the most unpalatable stages of developing amphibians. Zeiber *et al.* (2008) showed that eggs of four North American amphibian species were not consumed by mosquitofish*,* yet their hatchlings were consumed. This same phenomenon was also demonstrated by Reynolds (2009) with five Australian species exposed to *G. holbrooki*, by Drake *et al.* (2014) with *Ambystoma annulatum* and *G. affinis*, and by Smith and Smith (2015) with *Dryophytes versicolor* and *G. affinis*, where hatchlings and young tadpoles were consumed to a much larger degree than eggs, or where eggs were not eaten at all. In contrast, *G. affinis* consumed eggs of North American amphibians (Grubb, 1972), albeit to varying degrees between species, and *G. holbrooki* consumed eggs of the Australian species *O. ornatus* (Komak and Crossland 2000). Any attempts to find trends in palatability according to amphibian families, also yields discrepancies. Reynolds (2009) showed that the eggs of two

Australian Hylid species and three species of the family Myobatrachidae were unpalatable to mosquitofish (*G. holbrooki*). Zeiber *et al.* (2008) also showed that the eggs of four North American species from the families Hylidae, Ranidae, Bufonidae and Ambystomidae were unpalatable to *G. affinis*. Although eggs of these different families have been shown to be unpalatable, eggs from the same five amphibian families have been shown to be consumed by mosquitofish. Eggs of a species of the Myobatrachidae (Komak and Crossland 2000) and two species of the Hylidae (Grubb 1972) were highly palatable when presented to mosquitofish. Eggs of the Ranidae and Bufonidae (Grubb 1972), and Ambystomidae (Drake *et al.* 2014) have also been consumed by mosquitofish, although in low amounts. Therefore species within the same family may display differences in palatability.

There are several factors that determine the palatability of eggs toward predators. The outer jelly coat of an egg may be distasteful (Reynolds 2009), or may be a mechanical barrier to predation (Grubb 1972). Eggs also may not be recognised as prey due to a lack of chemosensory or visual cues (Reynolds 2009), or they are not eaten due to satiation of the fish (Reynolds 2009).

Satiation is a possible reason why the eggs of *T. delalandii* were not eaten. However, other developmental stages in separate trials were eaten despite the fact that these fish experienced the same level of satiation or hunger. All fish across all the trials were starved for two days before being presented with amphibian prey in the one set of trials, and amphibian and invertebrate prey in the other set of trials. In the trials with embryonic stages, the eggs started hatching after 24 h had passed. Therefore fish in the no-alternative prey treatments had been starved for three days before hatchlings were available for predation, but those of fish that had also been presented with the mosquito larvae had only gone without food for 24 h. If satiation played a role, we would also expect a difference in the consumption of eggs or hatchlings between the two treatments, however there was no observable difference. Reynolds (2009) also excluded satiety as a reason for no egg consumption in his study, because fish had been starved for four days prior to trials, and they ate alternative prey when presented to them at the end of experiments. Eggs of *Ambystoma annulata* were consumed in small quantities by *G. affinis* but only after 81 h had passed (Drake *et al.* 2014).

The second possible reason why mosquitofish did not consume eggs of *T. delalandii* is that they probably were not recognised as prey. Smith and Smith (2015) as well as Pyke and White (2000) concluded that the immobility of eggs caused them to be less attractive to mosquitofish in comparison with the movement of hatchlings and tadpoles. Mobility is an important factor in attracting predators, as discussed previously. However, this reason cannot be applied to this study, as some of the mosquitofish were observed to approach and nip at eggs shortly after being presented to them. However, in the case of experiments with the added mosquito larvae, fish were first attracted to the mosquito larvae before inspecting the eggs that were added with them. Although it is possible that these eggs were not as conspicuous as other developmental stages, they still elicited a response from the mosquitofish.

It is probable that the main reason for no predation on eggs is the outer capsule coating of the egg, otherwise known as the jelly coat. This membrane is either chemically unpalatable, or physically impenetrable, which deters fish predators. It was found that the adhesive nature of *T. delalandii* eggs made them difficult to remove from surfaces, and they were especially difficult to separate from one another. Fish were observed nibbling at eggs, but their mouthparts apparently could not penetrate the egg mass. Sometimes small parts of the outer jelly coat could be removed by a fish, but it then showed irritation at pieces of egg capsule sticking to its mouthparts. After a few attempts at seizing an egg, a fish would lose interest. Grubb (1972) reported the same phenomenon, were fish often rejected eggs if they were not able to separate eggs that adhered together. Grubb (1972) and Zeiber *et al.* (2008) both also reported that mosquitofish showed irritation when pieces of the jelly coat became stuck to their mouthparts.

Grubb (1972) noticed that of the seven egg species he observed, those of permanent water breeders were unpalatable. Permanent water breeders have larger egg sizes as well as denser and more impermeable jelly coats, two factors which were correlated with unpalatability. Zeiber *et al.* (2008) also noted that eggs of amphibian species that were unpalatable to mosquitofish were those that were laid either in clusters or strings, and had a firm jelly capsule. In this study, mosquitofish ate the hatchlings but not the casings after eggs had hatched. Further experimental work should be performed in order to elucidate the unpalatability of egg capsules, by removing them from the vitelli of the eggs and then presenting them to mosquitofish. This method will determine whether the casing is the source of unpalatability or the vitellus itself. Werschkul and Christensen (1977) showed that the vitelli were palatable but when enveloped by the jelly coat, the eggs of *Rana spp.* were unpalatable to the bluegill sunfish, *Lepomis macrochirus.* In contrast to this, it has been found in *Bufo marinus* that the vitellus has a larger toxicity than the egg capsule (Hayes *et al.* 2008). Bufonid eggs are known to be unpalatable to predators due to chemical noxiousness, but tadpoles of the family Pyxicephalidae, which are the family of *T. delalandii,* are not known to employ chemical noxiousness as an anti-predator defence mechanism. Although chemical effects cannot be ruled out as a possibility, it is more likely that the jelly coat is used as a mechanical defence tactic to deter predators. The sticky jelly coat has another advantage, in that sediment and detritus adhere easily to the eggs, which camouflage them from predators. It is likely that in South Africa, the single-egg laying amphibian species, with eggs of small sizes (< 3 mm), and with soft and loose capsules (Grubb 1972), are more likely to be preyed upon by mosquitofish.

3.4.4 Palatability and the influence of alternative prey

Although mosquitofish were attracted to mosquito larvae above tadpoles on their immediate addition to the container, the presence of alternative prey had no significant effect on the predation impact of tadpoles by mosquitofish overall. When added to the containers, fish consumed mosquito larvae quickly, and these were normally consumed before tadpoles. Therefore in treatments with an alternative prey, the fish attack response (PRI) towards tadpoles in the first 3 min of the trials was significantly lower than for trials without alternative prey. This pattern was not reflected in the injury or mortality of tadpoles (PII) when tested over a longer period of time. In this case, mosquito larvae did not significantly lower the PII scores, although there were some exceptions for specific developmental stages of certain species. These results are similar to palatability experiments conducted by Reynolds (2009), where *G. holbrooki* also attacked aquatic invertebrates (*Daphnia* and mosquito larvae) above tadpoles of *Ranoidea moorei* in the first few minutes of the trials, but at the end of the study there was no significant difference between the numbers of tadpoles consumed in the presence and absence of alternative prey. Goodsell and Kats (1999) found that mosquitofish preyed on amphibian larvae (*Hyliola regilla*) to the same extent, whether alternative prey was present or not.

The high initial feeding response towards mosquito larvae in the beginning was reflected in the PRI scores. In treatments with alternative prey, there were no significant differences for PRI scores between the three amphibian species. However in treatments without alternative prey there were significant differences between species, with the highest effect size between *S. capensis* and *X. laevis*. The likely reason for this difference is due to the high level of mosquito larvae predation in trials with alternative prey, where there was minimal tadpole predation regardless of species. However, in trials without alternative prey the level of mosquito fish attack was dependent on the type of amphibian species and the developmental stage of the tadpoles. It can also be seen that for species that were palatable according to the high PII scores (*T. delalandii* and *X. laevis*), the presence of mosquito larvae significantly reduced the level of fish response or attack (PRI scores) in comparison with treatments without alternative prey. There was also an overall trend PRI scores were highest in *X. laevis*, then in *T. delalandii*, and then a much lower response exhibited in trails with *S. capensis*. This trend is also reflected in the PII experiments, where *X. laevis* received the highest overall predation intensity, then *T. delalandii*, and was much lower for *S. capensis*.

The results of our study suggest that tadpole palatability was a stronger determining factorof predation, than the role of alternative prey. For instance, both *C. magistratus* and *S. capensis* were generally unpalatable to mosquitofish, regardless of whether there was alternative prey or not, but palatability was high in *T. delalandii* and *X. laevis*, regardless of alternative prey. However, it seems that in the case of palatable species, the presence of alternative prey may significantly lower the initial fish attack response. Alternative prey may also reduce the predation intensity on tadpoles; however in some cases with certain developmental stages the reduction was not significant. The reason for these discrepancies is not entirely clear.

In the trials with alternative prey, it is difficult to determine whether fish were preying on mosquito larvae because they are the prey of choice and more palatable to the fish, or because the fish were reacting to their own hunger levels. It was suggested by Remon *et al.* (2016) that mosquitofish might follow the optimal foraging model, which predicts that predators choose prey that minimize the energy used in foraging and optimize energy intake (Krebs and Davies 1989). When starved, an animal is already deficient in energy. To be energy efficient, an animal would probably seek small, abundant prey items that are easy to capture, instead of using up a lot of energy to incapacitate prey of large sizes that require longer handling times. However, when they are more satiated, and less hungry, they can spend more energy in trying to obtain more energy-dense food, like tadpoles. This may explain why mosquito larvae were eaten first. It may also explain why predation intensity was higher in tadpoles of younger developmental stages than late-stage tadpoles of large size. If mosquitofish follow the optimal foraging model, a hungry fish would exhibit greater boldness to attack smaller tadpoles than larger ones.

This may also explain some discrepancies in the results between developmental stages. For most of the treatments, the presence of alternative prey did not significantly reduce the predation intensity that mosquitofish inflicted on tadpoles. However, if the results are broken down according to developmental stages, we find that mosquito larvae did significantly lower the predation intensity for late pre-metamorphic tadpoles of *T. delalandii* and *X. laevis*, as well as *X. laevis* tadpoles at the free-feeding stage. Reasons for this are not clear, but there may be a link between predation intensity and the size of the tadpoles. It is possible that the larger size of late pre-metamorphic tadpoles, together with alternative prey, had an additive effect on the satiation of the fish and thereby lowered the predation intensity in these tadpoles. This does not explain the lowered predation intensity in the free-feeding stages, although these tadpoles were slightly larger (11.5 mm, see Table B7 in Addendum B) than the free-feeding stage tadpoles of *T. delalandii* (8.5 mm), and also the fish used in the free-feeding stage experiments were relatively smaller (23.3 mm, see Table B7 in Addendum B) than in other trials with *X. laevis* (31.0 mm and 28.3 mm). It is possible that the higher predation intensity that mosquitofish females exerted on tadpoles can also be explained by the larger fish sizes.

3.4.5 The influence of fish sex on tadpole predation

Smith and Smith (2015) showed that fish sex plays a role on tadpole predation, where mosquitofish males exhibited higher feeding rates on tadpoles of *Dryophytes versicolor* than females. These researchers attributed this to the higher degree of aggression that males are known to display towards conspecifics and heterospecifics (Smith and Smith 2015). In this study, fish sex had no overall influence on the PRI scores on tadpoles but did influence the PII scores, where predation intensity on tadpoles was the highest for mosquitofish females. Other studies have also shown that females attack prey at higher levels than males (see Arrington *et al.* 2009; and Blanco *et al.* 2004). It is possible that the higher PII scores that females exerted was due to their increased size, as they probably would have required larger amounts of food to reach a level of satiation. It is also likely that they would require higher energy levels for egg production (Arrington *et al.* 2009). In natural settings, females are often found at higher ratios than males, due to the shorter lifespan of male mosquitofish (Vargas and De Sostoa 1996; Fernández-Delgado 1989). This bias in sex-ratio, with a higher proportion of females, may have an increased negative influence on tadpoles in the natural environment. Further studies are required in order to fully understand the effect of each sex on tadpole predation, preferably using field studies.

3.4.6 Limitations and recommendations

As noted previously, if the impacts on tadpoles were measured in terms of measured biomass instead of classifying injury and mortality, it is possible that more apparent trends would have been detected (also noted by Pyke and White 2000). In this study, tadpoles were grouped according to ontogenic stages because it was suspected that palatability is linked to the changes in chemical make-up that tadpoles undergo with development (Hayes *et al.* 2009). However, it seems that for non-bufonid tadpoles, changes in size play a larger role than changes in body chemistry, which influences predation intensity in tadpoles of different developmental stages, and determines predation intensity in the presence of alternative prey. In order to elucidate the interactions between fish satiation, tadpole biomass and alternative prey, predation trials may be conducted in the laboratory using different abundances of alternative prey, and trials with fish at different levels of starvation. More satiated fish will consume larger portions of tadpole biomass than invertebrate biomass, with the assumption that the optimal foraging model accurately describes mosquitofish behaviour, as mentioned previously. It would also be assumed that tadpoles are more nutritious to fish than invertebrates.

Other reliable methods of determining food preference in mosquitofish are stable isotope analysis or gut-content analysis, as these methods focus on mosquitofish diet in their natural environment, and focus on the ecosystem of interest. However, stable isotope analysis may be the more reliable method of the two. Remon *et al.* (2016) revealed that tadpoles comprised ≈30% of mosquitofish diet using stable isotope analysis, which is in contrast to previous mosquitofish diet analyses using gut-content analysis that showed no evidence of amphibian larvae predation and high quantities of invertebrates that were consumed (Reynolds 2009). It is likely that tadpole flesh and amphibian eggs are not as easily detected in gut content analysis as dead amphibian egg and flesh tissue becomes flaccid very quickly, in comparison with the hard exoskeletons of invertebrates. When literature on gut content analyses was surveyed, it was found that no amphibian tissue was found in the stomachs of mosquitofish (Crivelli and Boy 1987; Specziár 2004; Reynolds 2009). Stable isotope analyses may be a more robust method because the problem of different digestion rates of varied food types is eliminated. They also provide information on a predator’s diet over a longer period of time, where gut-content analysis only provides a snapshot of what was eaten at a specific point in time. Stable isotope analysis may be the way forward in determining the extent of mosquitofish predation on amphibian tadpoles in the wild here in South Africa. However, this has to be combined with an estimation of abundance of each naturally available prey item, in order to determine if the relative percentages of prey items eaten are in proportion with their natural availability, or due to preference for certain prey species even if other food types are present (Remon *et al.* 2016).

3.4.7 The conservation implications of mosquitofish feeding behaviour

The elucidation of the feeding habits of mosquitofish is important when evaluating the ecological risk that they impose on aquatic systems. By determining the preferred prey of the mosquitofish, the prey species that are most at risk can be pinpointed. Also, when prey exhibit more complex life-cycles, such as is displayed in amphibians, it is important to examine mosquitofish predation on different life stages in order to gain a holistic view on the impact within a species. Isolating the impacts makes decision-making in environmental management and conservation more effective and efficient.

The results of this study suggest that tadpole palatability plays a stronger determining role in the overall impact of predation on an amphibian species, than the role of alternative prey. Therefore, the non-bufonid amphibian species are more likely to be impacted by mosquitofish predation than others. Also, the long-term presence of alternative prey had no overall decreasing effect on the predation impact on tadpoles. This suggests that the occurrence of mosquitofish in dams and waterways in South Africa may have serious effects on local amphibian populations, regardless of the presence of mosquito larvae. The broad diets and omnivorous feeding habits of mosquitofish (Kramer *et al.* 1987), and the high rates at which they consume prey (Rehage *et al.* 2005), make them useful as biocontrol agents, but these qualities also make them a highly successful invasive species which are detrimental to local fish, invertebrates and amphibians (Courtenay and Meffe 1989; Rupp 1996). There is generally a bias in opinion towards mosquitofish, where they are either thought of as the ideal mosquito control agent, or as a destructive invader (Pyke 2008; Rupp 1996). This polarization of attitudes has developed since the 1960's (Pyke 2008). Earlier studies at the beginning of the previous century showed that mosquitofish were effective at controlling mosquito populations and they were consumed in high quantities (Meisch 1995). Researchers are positive about this fish especially where there is a need to control mosquitoes in an agricultural setting (such as rice fields), or where there are risks of mosquito-borne diseases. For instance, Miura *et al.* (1984) showed that mosquitofish significantly reduced *Culex tarsus* abundances in rice fields. Cech and Linden (1987) also showed that mosquitofish were more effective at controlling mosquito larvae populations in California, than the native Sacramento blackfish. Another Californian study that was published in the same year (Kramer *et al.* 1987) had contrasting results, when the efficiency of mosquito larvae predation by mosquitofish and the inland silverside was compared. Kramer *et al*. (1987) showed that neither fish species was successful at controlling mosquito populations, because mosquitofish were consuming invertebrate prey other than mosquito larvae.

Other studies have also shown that the invasive fish is generally unreliable at controlling mosquito larvae (Hoy *et al.* 1972; Kramer *et al.* 1987; Bellini *et al.* 1994). Crivelli and Boy (1987) performed gut-content analysis on mosquitofish for over a year, and found no mosquito larvae in their gut contents, even though mosquito larvae were known to be abundant in the area. It has also been found that more recent studies on the efficacy of mosquitofish have a higher statistical reliability than earlier studies that were performed (Pyke, 2008). This adds weight to the evidence that mosquitofish are not beneficial as a mosquito control agent. It has even been suggested that they can increase the abundance of mosquito larvae, through the predation of macroinvertebrates that feed on mosquito larvae, such as dysticids (Blaustein 1992; Hoy *et al.* 1972; Kramer *et al.* 1987). Not only is the evidence of their inefficacy at controlling mosquitoes increasing, but there is also a growing body of literature that shows the ecological impacts caused by mosquitofish are alarming. Certain research shows that mosquitofish not only affect biota at the population level, but they also adversely modify biotic community structures through predation at multiple trophic levels (Hurlbert *et al.* 1972; Shulse *et al.* 2013).

Despite the evidence, the two opposing attitudes toward mosquitofish still occur. The popularity of mosquitofish is demonstrated by how far it has been intentionally spread by people, to the point of where it is now the most widespread invasive freshwater fish in the world (Pyke 2008). It is important that decision-making by environmental managers and conservationists regarding the mosquitofish should not be based on majority opinion but on sound research. If the fish are truly beneficial and effectively controlling mosquito populations, then they are an asset. However, if their use in controlling mosquitoes is at the cost of native biota, then in many cases the cost of their presence in a system outweighs their benefits.

Chapter 4 - Increased group size in mosquitofish (*Gambusia affinis*) amplifies the predation intensity on tadpoles

5.1 Introduction

The Western mosquitofish, *G. affinis* (Baird and Girard 1853), together with its close relative, *G. holbrooki* (Hubs and Lagler 1947), are recognised as virulent invasive species due to their successful colonisation of natural habitats. There are numerous studies that have examined the predation effects of both *G. affinis* and *G. holbrooki* on amphibians (see Tables C1–C3 in Addendum C for the relevant literature). The majority of this research shows that mosquitofish are causing declines in amphibian populations due to their predation on eggs, embryos, and tadpoles (Smith and Smith 2015), and that they also inflict injuries to tadpoles during predation attempts, resulting in sub-lethal effects (Shulse and Semlitsch 2014). Some authors have suggested that predation by mosquitofish may even result in the extirpation of local amphibian populations (Goodsell and Kats, 1999).

Some of these studies are based on field assessments alone (Shulse *et al.* 2013; Shulse and Semlitsch 2014; Pollard *et al.* 2017) or in combination with feeding trials conducted in the laboratory (Morgan and Buttemer 1996; Webb and Joss 1997; Goodsell and Kats 1999; Lawler *et al.* 1999; Reynolds 1999; Segev *et al.* 2009). Field assessments are useful in order to determine impacts in a certain geographical area, but more controlled laboratory trials are often required to elucidate the ecological mechanisms behind the impacts in more detail. They may be used to test the differences in palatability of amphibian eggs or larvae towards various predators, such as fish (Lawler and Hero 1997), aquatic invertebrates (Brodie *et al.*1978; Crossland 1998) or predaceous tadpoles (Heyer *et al.* 1975). They have also been used to examine ontogenic differences in palatability within certain amphibian species to various predators (Brodie *et al.* 1978; Crossland 1998), or their susceptibility to predation due to allometric relationships (Brodie and Formanowicz 1983; Bence and Murdoch 1986).

Feeding trials are generally performed by presenting individual or multiple prey items to a single predator (see Table C4 in Addendum C for citations), and then examining the behaviour of the predator or the number of prey items that it consumes. This experimental design is generally used by studies that examine the predatory habits of mosquitofish, often with a single mosquitofish per replicate (see Table C1 and C2 in Addendum C). A number of these laboratory studies on mosquitofish, although tested without also conducting field assessments, have suggested that the impacts of mosquitofish on amphibians can be detrimental, based on their findings. For instance, *Rana capito* and *Rana sphenocephala* (Gregoire and Gunzburger 2008), *Dryophytes squirellus* and *Gastrophryne carolinensis carolinensis* (Walls *et al.* 2002), *Dryophytes versicolor* (Smith and Smith 2015) and *Opisthodon ornatus* (Komak and Crossland 2000) were all preyed upon heavily by mosquitofish during experimental trials. It is possible however that these laboratory studies misrepresent the true interactions between mosquitofish and prey in natural settings, and therefore the impacts of mosquitofish, by underestimating the social nature of this species.

Mosquitofish are a communal species (Cote *et al.* 2010), and in the natural environment are found in groups. They are often very abundant in the summer months, due to their high fecundity (Pyke 2005), and can attain large group sizes. The success of this invasive species can even be attributed to these high abundances (Parker *et al.* 1999). However, the large densities that mosquitofish can attain may not only directly affect surrounding biota due to their sheer numbers, but group-living also influences the fish themselves, which may indirectly affect other species. Certain behavioural characteristics in group-living animals can be amplified by the presence of conspecifics (Webster and Ward 2010), compared to when the animal is alone. Behavioural characteristics that are particularly affected by group density are aggression and foraging activity (Pintor *et al*. 2009), and boldness (Chapple *et al.* 2012), which are some of the traits that are associated with the success of invasive species (Chapple *et al.* 2012). The use of individual fish in feeding trials is therefore likely to underestimate the impacts of mosquitofish, by eliminating the social context and its influence on fish behaviour.

This was a concern of Linden and Cech (1990), and because of the social nature of mosquitofish they chose to use several fish per replicate in their experiments instead of individual fish. Since then, a few other feeding trials have also used groups of mosquitofish instead of individuals (Table C1 and C2, Addendum C). Of these studies, however, several authors (Webb and Joss 1997; Goodsell and Kats 1999; Pyke and White 2000) used differing predator-prey densities in their feeding trials. Also, Drake *et al*. (2014) chose to use multiple fish due to biomass differences between predators and prey items. Yet the use of groups instead of individual fish in these trials was not necessarily directly motivated by the idea that predatory behaviour is affected by the social context.

Studies with other fish species have shown that the feeding response increases with group size. This was found in three-spined sticklebacks (*Gasterosreus aculeanius*) by Kaitala and Ranta (2006), and walleye pollock (*Theragra chalcogramma*) by Baird *et al*. (1990). Since Linden and Cech (1990) noted that mosquitofish predation on prey may be socially influenced, there has been no feeding trial study on *G. holbrooki* or *G. affinis* that has been conducted to test this hypothesis. Pyke and White (2000) noted that a high mosquitofish density increased the predation on tadpoles, but in this case the tadpole abundance was kept the same and not increased when predator density was increased. By this method, it is difficult to tell whether the increase in predation was due to group influence on behaviour, or simply because of an increase in predators.

This study aimed to determine whether the predation impacts on tadpoles by mosquitofish are increased or diminished when mosquitofish are in groups, in comparison with solitary fish. The predation impact inflicted by mosquitofish was determined by the degree of injuries and mortalities in tadpoles. These feeding trials were conducted based on the general pattern of other common studies (Table C4, Addendum C), using different mosquitofish group sizes, but using the same predator: prey ratio (1: 1) throughout. It was thought that if feeding behaviour or aggression in mosquitofish is more pronounced when they are in groups, it may suggest that the impacts of mosquitofish are even more critical than presumed by previous studies.

5.2 Materials and methods

5.2.1 Collection and maintenance of experimental animals

Mosquitofish were collected on the 17th of December 2016 from an urban stream, the Kuils River, at Durbanville, Cape Town (33°50'46.6"S, 18°40'06.1"E). Procedures for sampling, handling and the maintenance of fish were the same as for Chapters 2 and 3.

Because predation is influenced by prey palatability, and therefore is only useful in a relative sense (Lawler and Hero 1997), two amphibian species were used to compare the effects of mosquitofish: *T. delalandii* and *X. laevis*. These two species were chosen according to availability and accessibility. Tadpoles of *X. laevis* were collected from four different urban localities in Burgundy Estate, Cape Town (temporary stream: 33°50'23.6"S, 18°32'49.9"E; 33°50'27.2"S, 18°32'55.9"E; 33°50'24.1"S, 18°33'06.2"E and ephemeral pool: 33°50'35.2"S, 18°33'14.2"E) on different days in mid-January, 2017. Tadpoles of *T. delalandii* were first reared from eggs that were obtained by capturing *T. delalandii* adults in amplexus by hand, keeping them in captivity overnight, and acquiring the deposited eggs the following morning. Adults were captured on the night of the 12th of October 2016, at a recreational dam at Burgundy Estate, Cape Town (33°50'15.9"S, 18°33'10.4"E). The eggs were then stored in a refrigerator (4° C) to slow down the rate of development. After roughly five days the eggs were removed from the refrigerator and reared until they had reached Gosner stage 25 at room temperature. Water changes were performed regularly, and any unfertilized eggs were removed to prevent rotting and to keep the water fresh. Procedures for the collection, transport, acclimatisation, and maintenance of tadpoles and eggs were the same as for Chapters 2 and 3.

5.2.2 Experimental layout

Experiments commenced on the 19th of December 2016 for *T. delalandii* and on the 18th of January 2017 for *X. laevis.* For each experiment, an increasing number of tadpoles and fish was tested in order to determine if there was an increase in the predation intensity of tadpoles, when the number of fish in a school increased (see Table 4.1). The predator-prey ratio was kept at 1:1 for each treatment. There were at least five replicates for every treatment level performed. See Table 4.1 for a complete outline of all the executed experiments.

Table 4.1. Mean fish and tadpole total lengths for each of three treatments per amphibian species tested.

Amphibian species	**Treatment 1 One fish, one tadpole 5 repetitions**				**Treatment 2 Three fish, three tadpoles 5 repetitions**				**Treatment 3 Five fish, five tadpoles 5 repetitions**			
	Mean fish total length (mm)	**Std. Error**	**Mean tadpole total length (mm)**	**Std. Error**	**Mean fish total length (mm)**	**Std. Error**	**Mean tadpole total length (mm)**	**Std. Error**	**Mean fish total length (mm)**	**Std. Error**	**Mean tadpole total length (mm)**	**Std. Error**
Tomopterna delalandii	27.5	1.3	19.2	0.5	24.6	0.7	17.2	0.6	22.9	0.7	16.9	0.3
Xenopus laevis	27.5	0.7	12.6	0.4	18.1	0.6	14.0	0.5	18.3	0.4	13.7	0.4

The mosquitofish were placed into clean white plastic 5 L containers, filled up with dechlorinated tap water until 2 cm from the brim. Water was dechlorinated by allowing it to stand in an open-top container for at least 12 h before use. The water was not aerated while experiments were running, and the water temperature was measured but not regulated. No structural elements or substrate was provided, as maximum visibility was required to observe the experimental animals. After being released into containers, fish were presented with food pellets. The number of pellets was equal to the number of fish in the container. If each group of fish had not eaten the pellets after the 24 h period, the fish were considered behaviourally incapable of feeding and were not included in the experiment. Fish that had eaten pellets were deprived of food for another 24 h. When the starvation period was complete, each group of fish was presented with tadpoles by pouring them into the 5 L container, with approximately 200 ml water that tadpoles had previously been placed in prior to the experiment. This initiated a fright response in the fish, preventing them from attacking tadpoles immediately as they had been conditioned to take food artificially. Tadpoles were grouped into developmental stages prior to each experiment, according to the normal stages of Gosner (1960) for *T. delalandii* and Nieuwkoop and Faber (1956) for *X. laevis*. Only tadpoles at Gosner stage 25 or Nieuwkoop and Faber stage 45 were used. The total length, snout-to-vent length and body width of each tadpole were also measured (see Table 4.1).

The predation intensity that fish exerted on the tadpoles was measured by the severity of injuries and tadpole mortality. The degree to which tadpole bodies were consumed by fish after being killed was also measured. This is because consumption of prey is also an indication of palatability, regardless of whether that prey is alive or dead. All this was recorded using an injury index and assigning classes/scoring coefficients (coefficient 1 through to 10) to each tadpole each time the experiment was monitored. A total injury score for the monitoring period was given by adding the injury index coefficients of all three tadpoles, and then taking the average. The predation intensity index (PII) classes are as follows:

Class 1 – No observed injury.
Class 2 – Less than 2 mm of the tail removed, tadpole alive.
Class 3 – Between 2 mm and less than ¼ of tail length removed, tadpole alive.
Class 4 – Between ¼ and less than ½ of tail length removed, tadpole alive.
Class 5 – Between ½ and less than ¾ of tail length removed, tadpole alive.
Class 6 – Between ¾ and all of the tail removed, tadpole alive.
Class 7 – Between 0 and ½ of the tail removed, tadpole dead.
Class 8 – Between ½ to all of the tail removed, tadpole dead.
Class 9 – Part of the tadpole head consumed, tadpole dead.
Class 10 – Entire tadpole consumed.

From the first 3 min onwards, predation intensity in tadpoles was monitored at the following intervals: 3 min, 10 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, and thereafter increasing intervals ranging in duration. Trials ended when all the tadpoles had been eaten completely, or if after 48 h it was evident the fish showed no interest in the tadpoles. The maximum duration of the trials was 72 h. Most experiments commenced from late morning to midday.

No control treatments were performed by observing tadpoles in the absence of fish, as tadpole mortality over such a short space of time is generally negligible (Pyke and White 2000). No individual fish or tadpole was used more than once in these experiments, and all experimental fish and any surviving tadpoles were euthanised at the end of each trial. After euthanasia the fish were sexed, and if the sex was indistinguishable the fish was classed as a juvenile. Body size measurements were taken by measuring the total length, body depth, and gape area as in Chapter 3.

5.2.3 Statistical analysis

In order to assess the effect of predation on tadpoles by mosquitofish, a univariate hierarchical linear mixed (HLM) model was used with each repetition as the primary unit of measurement, and with the PII score as the response variable. This model was constructed in SPSS v. 24 (SPSS, Inc.; Chicago, Illinois), with amphibian species, the number of fish, and elapsed time as three fixed effects. Interactions between fixed effects were tested for significance. The subject (experimental repetition) was chosen as the random effect, and a random slope added at the subject level. A restricted maximum likelihood estimation method and an unstructured covariance structure were used. The practical significance of the results was determined by calculating the effect sizes from the differences between means. See Chapter 2 for the formula and the interpretation of the effect sizes.

5.3 Results

The HLM for predation intensity observations showed that fish group size had a significant effect on the PII scores ($F_{(2, 314)} = 14.96$, $p < 0.001$, see Table C5 in Addendum C), even though the predator: prey ratio was kept constant. The mean PII score was relatively low when only one fish was used in the experiments (5.08, SE: 0.24, see Figure 4.1), however PII scores increased significantly when there were either three fish ($d = 1.28$) or five fish ($d = 1.38$) used in the trials. Although mean PII scores were higher in trials with five fish than three fish, there was no significant difference in PII scores between the two groups ($d = 0.11$).

Results from the HLM also showed that there was significant interspecific variation between PII scores ($F_{(1, 21)} = 60.18$, $p < 0.001$). Mean PII scores were significantly higher ($d = 2.24$) in *X. laevis* (8.04, SE: 0.21) than in *T. delalandii* (4.04, SE: 0.30). This is also visually evident when comparing the line graphs of PII scores for *X. laevis* and *T. delalandii* (see Figure 4.2 and Figure 4.3), where steeper gradients for *X. laevis* indicated higher and faster rates of predation intensity than for *T. delalandii.* Tadpole injuries became fatal for tadpoles of *X. laevis* at a quicker rate than for *T. delalandii* (roughly 0.6–1 h and 2.5–25 h respectively, see Figure 4.2 and Figure 4.4). This observation is reflected in the HLM results, where the interaction between species and time was significant ($F_{(10, 309)} = 5.011$, $p < 0.001$). Trials with *T. delalandii* where only solitary fish were used, yielded the lowest PII scores with the highest number of hours to tadpole mortality (≈25 h, Figure 4.2). This was also far higher than time to mortality for groups with five fish (≈2.5 h) or three fish (≈6 h). As time progressed, predation intensity increased. The general trend for *T. delalandii* was regular and steady (Figure 4.2), where PII scores for experiments with single fish were the lowest and where trials with the highest number of fish yielded the highest PII scores throughout. This trend was similar for *X. laevis* up until 0.5 h (see Figure 4.4). At this point the PII scores inflicted by lone fish increased rapidly to become the highest, and PII rates decreased in trials with five fish (Experiment 3) so that they became the lowest. This trend for *X. laevis* continued until roughly 16 h had passed, until the PII scores of all three experiments converged. Of all the three fixed effects (amphibian species, number of fish and time), the *F* statistic revealed that amphibian species had the largest contribution to PII scores ($F_{(1, 21)} = 60.18$, $p < 0.001$), in comparison with low *F* statistic scores for the other fixed effects, in particular the number of fish used ($F_{(2, 314)} = 14.96$, $p < 0.001$, see Addendum B). The HLM also showed that the interaction between species and time was the only two-way interaction between independent variables that was statistically significant, whereas the three-way interaction between all three fixed effects (species, number of fish, and time) was not significant (see Table C5, Addendum C).

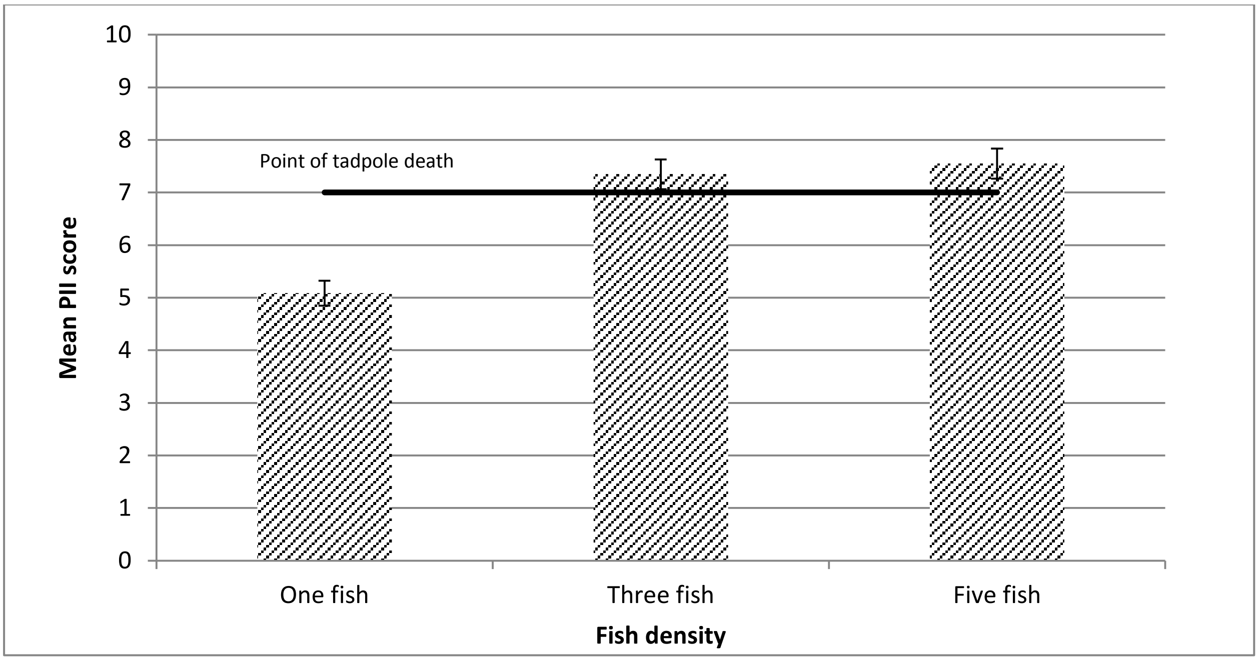

Figure 4.1. Mean PII scores for tadpoles of both *T. delalandii* and *X. laevis* from 0–35 h, exposed to different fish densities. The predator: prey ratio remained constant throughout.

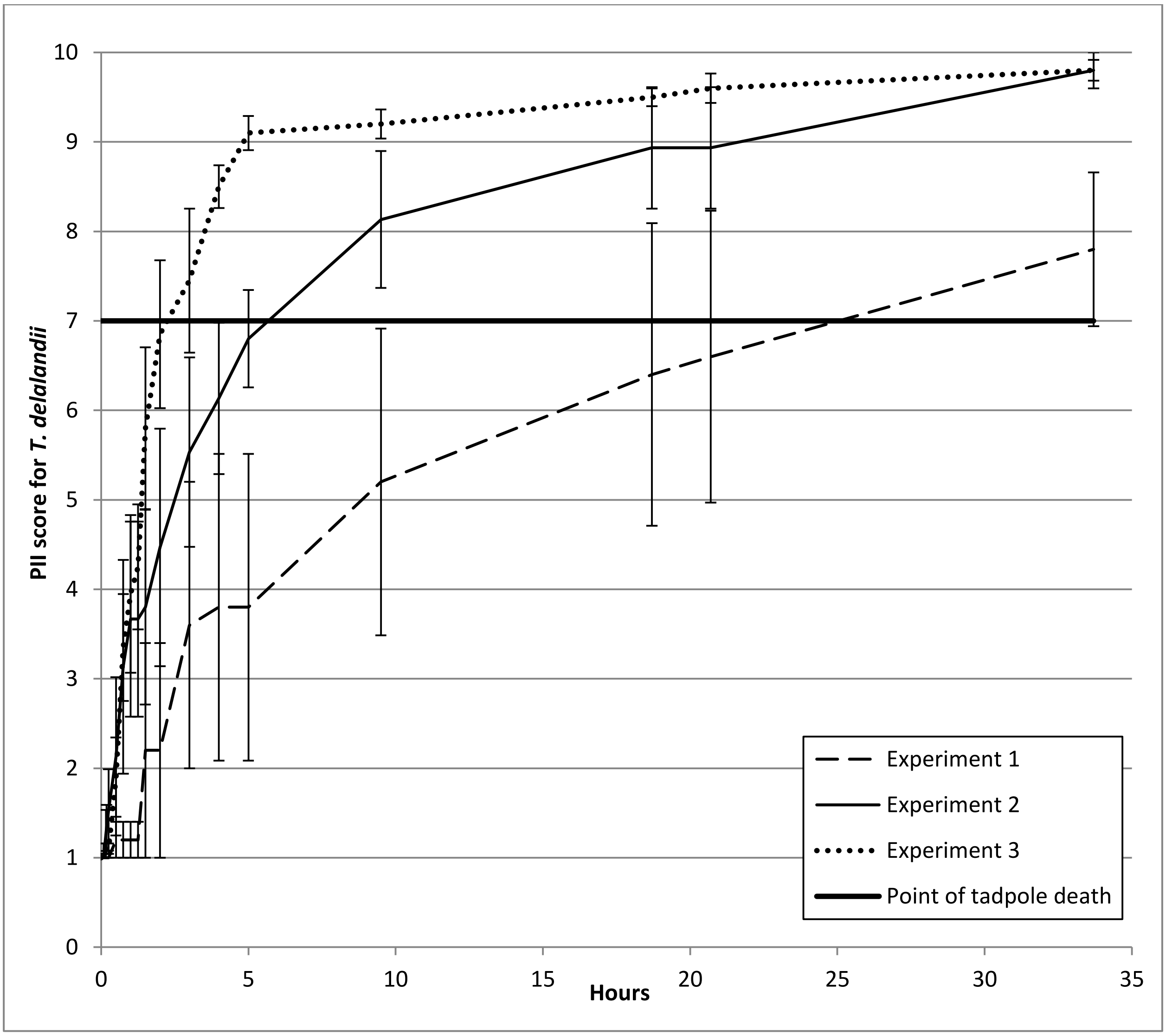

Figure 4.2. PII scores for tadpoles of *T. delalandii* when exposed to one fish (experiment 1), three fish (experiment 2) and five fish (experiment 3) all at the same predator: prey ratio. The solid line (at PII = 7) indicates the threshold where tadpole injuries become fatal. PII scores below the line demonstrate the degree of non-fatal injuries. Scores above the line indicate the degree of tadpole consumption by the fish after tadpole death, where a PII of 10 indicates complete consumption.

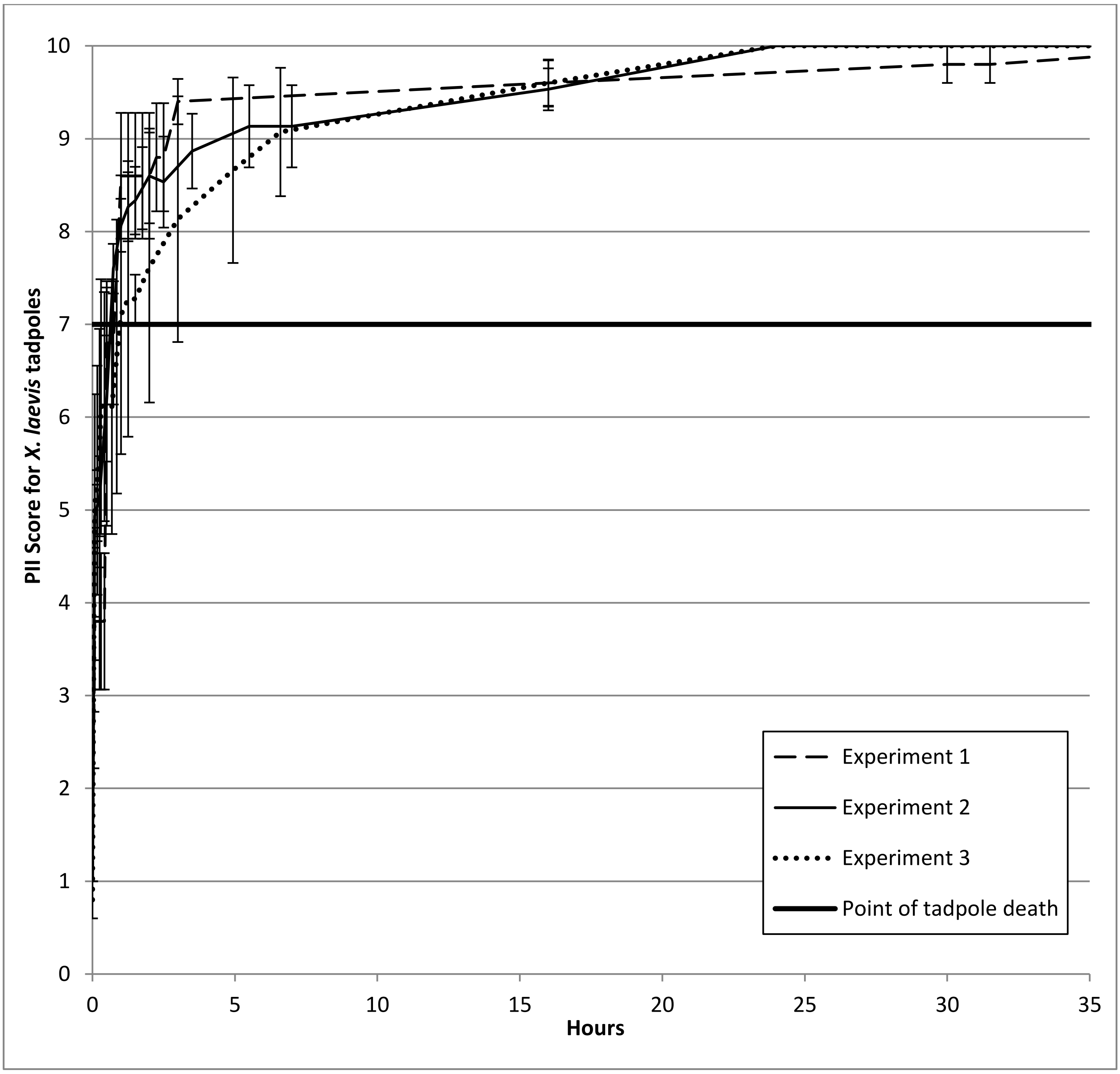

Figure 4.3. PII scores for tadpoles of *X. laevis* when exposed to one fish (experiment 1), three fish (experiment 2 and five (experiment 3) all at the same predator: prey ratio. The solid line (at PII = 7) indicates the threshold where tadpole injuries become fatal. PII scores below the line demonstrate the degree of non-fatal injuries. Scores above the line indicate the degree of tadpole consumption by the fish after tadpole death, where a PII of 10 indicates complete consumption. Please see Figure 4.4 for a higher resolution of the graph at 0–1.5 h.

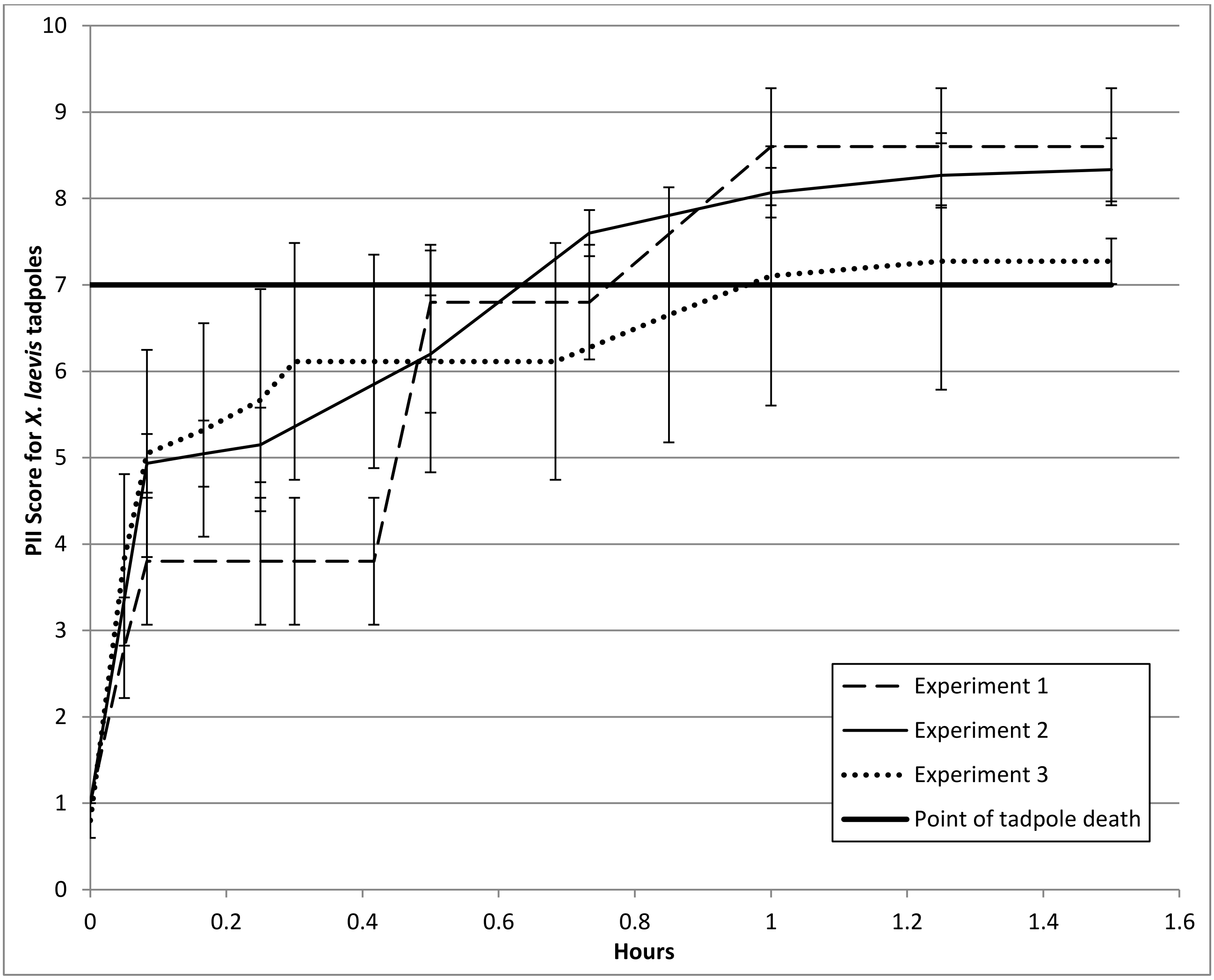

Figure 4.4. A close-up of Figure 4.2 at 0–1.5 h, showing PII scores for tadpoles of *X. laevis* when exposed to one fish (experiment 1), three fish (experiment 2) and five fish (experiment 3).

5.4 Discussion

The overall results of this study show that mosquitofish exert significantly greater predation intensity on tadpoles when the mosquitofish are in groups, compared to solitary fish, even though the predator: prey ratio remains the same. This indicates that predatory behaviour in mosquitofish is affected by social factors. These results parallel the results of other similar studies with other fish species. For instance, Kaitala and Ranta (2006) found that feeding behaviour in three-spined sticklebacks (*Gasterosreus aculeaius*) increased with larger fish schools. Baird *et al*. (1990) also found that feeding response was lowest for solitary juvenile walleye pollock (*Theragra chalcogramma*) that were fed with pellets, but increased when the fish were either in pairs or groups of six. They also found little difference between the feeding response of paired fish and the group or six fish; however the amount of pellets eaten by fish in groups was 3.5 times higher than for isolated fish. This non-linear increase in feeding response with increasing group size was similar to the predation intensity results found in this study.

Trends in predation intensity, according to the fish group size, differed between the two amphibian prey species tested. Predation on tadpoles of *T. delalandii* was clearly associated with group size, whereas results for *X. laevis* were slightly more obscure, although predation intensity on *X. laevis* tadpoles was high regardless of group size. During experiments, fish behaviour was much more aggressive towards tadpoles of *X. laevis* in comparison to *T. delalandii*, regardless of being isolated or in a group, which resulted in tadpoles of *X. laevis* being consumed at a higher rate than those of *T. delalandii*. Other studies have also found that certain amphibian species may be more palatable to mosquitofish in comparison with others (Grubb 1972; Zeiber *et al*. 2008). Bufonids have been found less palatable than ranid species in numerous studies (Preston *et al*. 2012; Shulse and Semlitsch 2014). The unpalatability of bufonids above ranids is largely attributed to their possession of noxious chemical substances in their skin that repel predators (Hayes *et al*., 2009). However, neither *T. delalandii* nor *X. laevis* are known to possess such chemical defences, therefore is unlikely that this caused the differences in predation intensity. It is possible that temperature differences could have altered the feeding responses of the fish, as this physical variable has an influence on animal metabolic rates (Brown *et al.* 2004), particularly poikilotherms. However, water temperatures of experiments with both *T. delalandii* and *X. laevis* were within a similar range (20–24°C).

It is most likely that the differences in predation intensity between the two species were due to allometric effects. The relatively high predation intensity found in the solitary fish that consumed *X. laevis*, could be due to the larger size of these fish, as they averaged 27 mm in total length, whereas the average total lengths of mosquitofish in groups were much smaller (18.1 mm and 18.3 mm). Predation attempts would be easier for large predators as they would require shorter handling times to incapacitate prey (Schröder *et al.* 2016), which may have contributed to the increased predation intensity in solitary fish of trials with *X. laevis.* However, individual fish used in experiments with *T. delalandii* were also larger (27.5 mm), than those fish of experiments with three fish (17.2 mm) or five fish (16.9 mm), yet in this case it did not confound predation intensity results. A possible reason is that tadpoles of *X. laevis* were consistently smaller than those of *T. delalandii* (see Table 4.1). This may have increased attacks on *X. laevis*, as the predator handling time decreases as the prey body size decreases (Schröder *et al.* 2016).

Swimming behaviour of *X. laevis* tadpoles may provide an important cue to predatory fish. The ease at which fish consumed *X. laevis* is probably due to the unique swimming movement of these tadpoles, which are adapted for swimming over large distances in pelagic water (Hoff and Wassersug 1986). It has been found that *X. laevis* hatchlings either move upwards or downwards in the water column when provoked, not horizontally (Roberts *et al.* 2000). It is therefore possible that the shallow experimental containers may have restricted their movement, and facilitated their capture by mosquitofish. The tip of the *X. laevis* tadpole is used as a rudder and constantly flaps from side to side (Hoff and Wassersug 1986), which might also serve as a visual cue and a lure that attracts predators.

From this study it is evident that group membership influences mosquitofish behaviour, which in turn influences the predation impacts on tadpoles. There are three means whereby behaviour in an individual can be influenced socially: either by conformity, by local enhancement, or by social facilitation. Conformity is where an individual observes group members expressing a certain behavioural pattern, and conforms to or mimics it (Webster and Ward 2010). In this case, the active individual(s) performing certain behaviour as models for the observing individual. The likelihood of an observer imitating the model's behaviour increases as the number of performing models around the observer increases (Webster and Ward 2010). An example of this is in a study by Ryer and Olla (1990), where they found that solitary chum salmon (*Oncorhynchus keta*) emulated the behaviour of conspecifics by imitating either the increasing foraging behaviour of feeding groups, or eating less when exposed to non-feeding groups. The second phenomenon of social influence is local enhancement. This is where an individual is influenced by the expression of another's behaviour at a specific locality, drawing the individual's attention to a certain aspect of the environment, and not necessarily imitating the model's behaviour (Thorpe 1956). The third aspect, social facilitation, is where the mere presence of group mates affect the behaviour of an individual animal, regardless of what the group mates are doing (Zajonc 1965). The influence of the group normally has the effect of initiating or amplifying the expression of a certain behavioural action or trait in an individual, such as aggression, activity, foraging behaviour (Webster and Ward 2010). In these cases, social facilitation may be activated by the predator's reduced perception of risk, which may increase boldness, or due to the motivation to compete for prey resources (Ward 2012). Decreased investment in vigilance is another possible initiating factor for social facilitation (Ward 2012).

Unfortunately the design of these experiments did not allow the identification of a single proximate mechanism that was at work. However, it is likely that there was an interaction between all three of these processes. During the trials, isolated fish responded similarly to fish in groups by chasing tadpoles in the first few minutes. Solitary fish that were not responsive at the start remained relatively disinterested, and the time intervals between chases were longer. What was different in group situations was that initially unresponsive fish were often motivated to chase a tadpole when another fish was already chasing it. It was observed that a fleeing tadpole was often chased by more than one fish, which often ignored other tadpoles in the container that were motionless. When a tadpole was incapacitated the mosquitofish would attack and bite at it as a group. It is unclear if the main motivation for observing fish to chase and attack tadpoles was due to the behaviour of supposed focal fish, or by attraction to the fleeing movement of the tadpole. If tadpole movement was the main factor, then conspecifics were enabling other mosquitofish to locate tadpole prey. In this case, the social context may have enabled predation by local enhancement.

However, it is likely that conformity may have played a stronger role than local enhancement. The fish that initiated tadpole attack were often consistently bolder and more aggressive in behaviour. In a study by Burns *et al*. (2012), it was found that certain individuals in groups of eastern mosquitofish (*G. holbrooki*) consistently lead the group in making movement decisions, both in novel and familiar environments. It was also found that this leadership tendency of certain individuals is irrespective of dominance, sex or size, which may suggest that boldness is a part of the personality or behavioural phenotype of an individual. This may explain the consistent unresponsiveness or consistent boldness of certain solitary fish in this study. However, when together in a group, timid fish will observe the behaviour of the focal fish and will have more boldness to also attack (Magnhagen and Bunnefeld 2009). These focal fish may determine the overall behaviour of a shoal, where timid and less aggressive fish conform to the movement decisions and behaviour of leaders (Magnhagen and Bunnefeld 2009). The sum of fish personalities also determines the collective behaviour of the group (Cote *et al.* 2011), however this is not likely to have affected these predation intensity results, as the intercept variance between replicates in the HLM model was not significant (Table C6 in Addendum C). However, all fish were selected from one group of fish from the same natural locality. Therefore the possibility of group personality affects is not ruled out, and may play a significant role when comparing fish groups of different localities, as groups of animals are often heterogeneously structured. It is also possible that social facilitation contributed towards the increased predation intensity when mosquitofish were in groups. Certain behavioural phenotypes in individuals, such as boldness and aggression, may have been amplified by the presence of group mates. Social facilitation is dependent on the fish's perception of risk, which is determined partly by group size as well as information gathered about the fish's environment through exploration (Ward 2012), or vigilance towards predators (Roberts 1996).

In the case of this study, group membership may have increased the boldness of mosquitofish to attack tadpoles and to incur risk by being less attentive to possible dangers. Feeding activity in mosquitofish may also have been influenced by a perception of increased competition (Grand and Dill 1999).

Regardless of the mechanisms that influenced predation intensity, mosquitofish had an overall detrimental effect on the tadpoles tested in this study. Severe injuries were inflicted within a relatively short space of time, resulting in 0% survival. These detrimental impacts of mosquitofish on amphibians are in line with numerous other studies (Goodsell and Kats 1999, Lawler *et al.* 1999; Segev *et al.* 2009; see Table C2 and C3 in the Addendum C for further citations). However, the impacts reported in these assessments may be underestimated, due to the sociality of mosquitofish sometimes being overlooked in the experimental design. Ward (2012) has stated that the means by which group mates influence the behaviour of individuals is a component that is often disregarded in laboratory studies. Behavioural characteristics, which is linked with sociality, is also commonly overlooked in many studies with invasive species (Holway and Suarez 1999; Chapple *et al*. 2012), despite the major contribution that behaviour has on the success of invasives (Pintor *et al*. 2009; Chapple *et al*. 2012). Given the findings of this study, it is suggested that future research on mosquitofish impacts should incorporate multiple fish instead of only using individuals, in order to increase the accuracy of results. Ward (2012) has stated that most social behaviour studies only use a few animals per group (two to three fish) with a maximum group size of six individuals. However, Ward (2012) also argues that such studies should use groups of different sizes, and at a larger scale. Given the small group sizes used in this study (three to five fish), and compared with the large shoal sizes of mosquitofish in the natural environment, the influence of sociality on predation intensity should be further examined. The use of larger numbers of animals may sometimes be limited by ethical considerations; however increasing the accuracy of invasive species evaluations may be critical and of higher priority when their impacts are severe. Ecological invasions bring about extensive economic and ecological losses, and understanding the mechanisms that drive these are important in order to prevent and manage them.

Chapter 5 - Mosquitofish (*Gambusia affinis*) occurrence in the greater Cape Town area: a literature review and preliminary field survey

6.1 Introduction

The Cape Floristic Region (CFR), one of the world's most remarkable biodiversity hotspots, and an area that is considered to possess a 'globally outstanding' biological distinctiveness (Thieme *et al*. 2005). The CFR region stretches along Cape Fold Mountain Region, from Vanrhynsdorp in the southern part of the West Coast, and up until Port Elizabeth in the east. It is a relatively small area compared to the other five floral kingdoms of the world (Cowling *et al*. 1992), but despite the CFR's small size, it has the highest species richness (Cowling *et al*. 1992). It is also recognised as a World Heritage site and one of the world's 25 biodiversity hotspots (Myers 2000) supporting 20% of the African continent's plant species with a very high concentration of endemics (Goldblatt and Manning 2002). Although the plant kingdom and terrestrial component of the CFR have received a lot of attention, the aquatic systems of this area are equally valuable and unique. The rivers of this area are largely geographically isolated, which has contributed to the high endemism of aquatic species in these areas (Tweddle *et al*. 2009). For example, 63% of the aquatic invertebrate species are endemic to the region, and represent 33% of all South Africa's aquatic invertebrates (Wishart and Day 2002), while 60% of native freshwater fish species in the CFR are either Endangered or Critically Endangered (Tweddle *et al*. 2009).

The greater Cape Town area (GCTA) is located in this biodiversity hotspot, and shares the valuable and unique biological distinctiveness of the CFR. It has an area of 2, 460 km² (Holmes *et al*. 2012), and the region encompasses the entire Cape Peninsula, the South Western Coastal Belt up to Atlantis and Malmesbury in the north, and to the Hottentots Holland Mountains in the east (see Figure 5.1). Although it makes up only 3% of the CFR, it is considered a 'hotspot of hotspots' (Wood *et al*. 1994) and represents a third of the CFR's plant species (Rebello *et al*. 2011). It has at least 112 endemic animal taxa that represent 47 families, which have restricted habitats limited to Table Mountain and the South Peninsula (Brown and Magoba 2009). It also has unique and threatened marine and freshwater ecosystems (Holmes *et al*. 2012), with a 307 km coastline (Holmes *et al*. 2012), and 16 estuaries, 13 of which are critically endangered (Van Niekerk and Turpie 2012). One of these estuaries is an Important Bird Area and two of these estuaries are significant fish nursery areas (Van Niekerk and Turpie 2012). The GCTA also possesses numerous wetlands of conservation importance. However, habitat destruction is one of the primary threats to the city's biodiversity, due to increased urbanisation (Holmes *et al*. 2012), which is driven by increasing population growth. Cape Town is one of South Africa's largest cities by population, and in 2010 the number of people living in this area was 3.7 million, a number which is rapidly increasing every year (Holmes *et al*. 2012).

Another primary factor that jeopardizes the city's natural ecosystems is alien species (Holmes *et al*. 2012). Although plant invaders are the main threat, invasive fauna also compromise the environmental integrity. Not only Cape Town, but the entire CFR has the highest number of invasive animal species in South Africa (Picker and Griffiths 2011), making it the most invaded area in the country (Wilson *et al*. 2014). A particular problem for the aquatic ecosystems of the CFR is invasive fish (Marr *et al*. 2013). For instance, Cape Town has eight indigenous fish species (Holmes *et al*. 2012), but also eight alien and extralimital invasives (Brown and Magoba 2009). The reason for this high number of fish invaders may partly be due to the GCTA being the central point in South Africa where fish introductions and invasive fish hatcheries began. From 1900, alien fish were bred at Jonkershoek hatchery in Stellenbosch and then deliberately stocked in rivers and dams, primarily for angling, and the stocking process continued up until the mid-1980s (Brown and Magoba 2009).

One of the fish that were introduced in the GCTA in 1936 (De Moor and Burton 1988), is the western mosquitofish, *G. affinis*. Since its introduction eight decades ago, the mosquitofish has become widespread in South Africa (Van Rensburg *et al*. 2011). Although small and seemingly innocuous, there is increasing evidence that mosquitofish adversely affect different aquatic biota at the population level, due to their broad diets and high feeding rates (Meffe 1985; Gamradt and Kats 1996; Howe *et al*. 1997; Goodsell and Kats 1999, Rehage *et al*. 2005), and can impact entire communities (Hurlbert *et al*. 1972; Shulse *et al*. 2013). However, impacts of most invasive fish species in South Africa are generally not well understood (Lowe *et al*. 2008), and there have been no studies that study the impacts of mosquitofish in the country. There is, however, strong evidence that they are likely to affect local biota. For instance, Ellender and Weyl (2014) evaluated the invasion status of alien and extralimital fish species in South Africa according to criteria proposed by Blackburn *et al*. (2011). Their results showed that the mosquitofish was a 'fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence' (Ellender and Weyl 2014). Furthermore, Marr *et al*. (2017) recently did a trait-based risk assessment using the Fish Invasiveness Screening Kit (FISK; Copp *et al*. 2005) for each invasive fish in South Africa. Their results showed scores for mosquitofish were above the medium to high-risk threshold, indicating that mosquitofish are high-risk invasives for South Africa, in terms of their biogeography, history, biology, and ecology (Marr *et al*. 2017).

In order to manage and limit the impacts and spread of invasive fish, a comprehensive record of all the invasive species present is needed (Wilson *et al*. 2014), as well as an understanding of their mechanisms of invasion, and their distributions (Thieme *et al*. 2005). The identification of established distributions is also useful for modelling future invasion, as introduction successes at certain localities can be used to predict alien fish establishment in other areas (Moyle and Marchetti 2006). However, little of this information is available for most alien fish in South Africa (Ellender and Weyl 2014). For distribution data that do exist for certain species, there is a lack of resolution and also many inconsistencies (Ellender and Weyl 2014). For instance, Skelton (2001) documents their distribution in South Africa as scattered and isolated, with only two point distributions for the Western Cape, yet Marr *et al*. (2013) stated that mosquitofish occur in nearly a quarter (23%) of the catchments in the Western Cape, although the localities were not specified. It is clear that more information on the drainage-basin scale distributions of invasive fish is required (Ellender and Weyl 2014; Wilson *et al*. 2014).

The purpose of this study was to consolidate the available information regarding mosquitofish distribution in the greater Cape Town area (GCTA) by way of a literature review, and to supplement this information with a preliminary field survey. This combined information would be used to determine a list of areas that require further assessment for mosquitofish occurrence. Localities within the GCTA that were prioritised for evaluation during the literature review and field survey were conservation areas, as the majority of these protect endemic species and threatened ecosystems. The other areas that were prioritized were the major river systems of the GCTA. Rivers are important systems of connectivity, and unlike endorheic water bodies, their connectivity increases the likelihood of alien species being transferred from infested river reaches to non-infested sites. This can lead to the invasion of an entire river network, depending on in-stream barriers and the topography of the area.

The data would also be used to evaluate the threat that mosquitofish pose on the amphibians of the GCTA. It is reported that invasive fish species affect 37% of all South African frogs (Measey 2011), however it is unclear to what extent mosquitofish play a role in comparison with other exotics. The highest number of South Africa’s endemic amphibians species occur in the Western Cape (Minter *et al*. 2004; see Table 5.1 for a species list), with the highest concentration of Red Data Listed amphibian species occurring within this area (Stuart *et al*. 2008, Table 5.1). Mosquitofish may threaten the persistence of amphibians in this area, as studies abroad show that this fish species is causing declines in amphibian populations (Smith and Smith 2015), and may even cause amphibian extinctions in localised areas (Goodsell and Kats 1999). Therefore distribution data acquired from the literature review and field survey in this study would also be used to determine where mosquitofish occur in sympatry with threatened amphibians, and identify local amphibian species that are most at risk.

Table 5.1. Amphibians of the GCTA (Minter *et al*. 2004) and their IUCN conservation status (IUCN 2017).

Scientific name	**Common name**	**IUCN STATUS (IUCN 2017)**
Amietia fuscigula	Cape River Frog	Least Concern
Arthroleptella lightfooti	Cape Peninsula Moss Frog	Near Threatened
Breviceps gibbosus	Cape Rain Frog	Near Threatened
Breviceps montanus	Cape Mountain Frog	Least Concern
Breviceps rosei	Sand Rain Frog	Least Concern
Cacosternum aggestum	Klipheuwel Dainty Frog	Least Concern
Cacosternum capense	Cape Dainty Frog	Near Threatened
Cacosternum platys	Flat Dainty Frog	Near Threatened
Capensibufo deceptus	Deception Peak Mountain Toadlet	Data Deficient
Capensibufo magistratus	Landdroskop Mountain Toadlet	Data Deficient
Capensibufo rosei	Rose's Mountain Toadlet	Critically Endangered
Capensibufo selenophos	Moonlight Mountain Toadlet	Data Deficient
Heleophryne rosei	Table Mountain Ghost Frog	Critically Endangered
Hyperolius horstockii	Arum Lily Frog	Least Concern
Hyperolius marmoratus	Painted Reed Frog	Least Concern
Microbatrachella capensis	Micro Frog	Critically Endangered
Sclerophrys capensis	Raucous Toad	Least Concern
Sclerophrys gutturalis	Guttural Toad	Invasive
Sclerophrys pantherina	Western Leopard Toad	Endangered
Semnodactylus wealii	Rattling Frog	Least Concern
Strongylopus bonaespei	Banded Stream Frog	Least Concern
Strongylopus grayii	Clicking Stream Frog	Least Concern
Tomopterna delalandii	Cape Sand Frog	Least Concern
Vandijkophrynus angusticeps	Cape Sand Toad	Least Concern
Xenopus gilli	Cape Platanna	Endangered
Xenopus laevis	Common Platanna	Least Concern

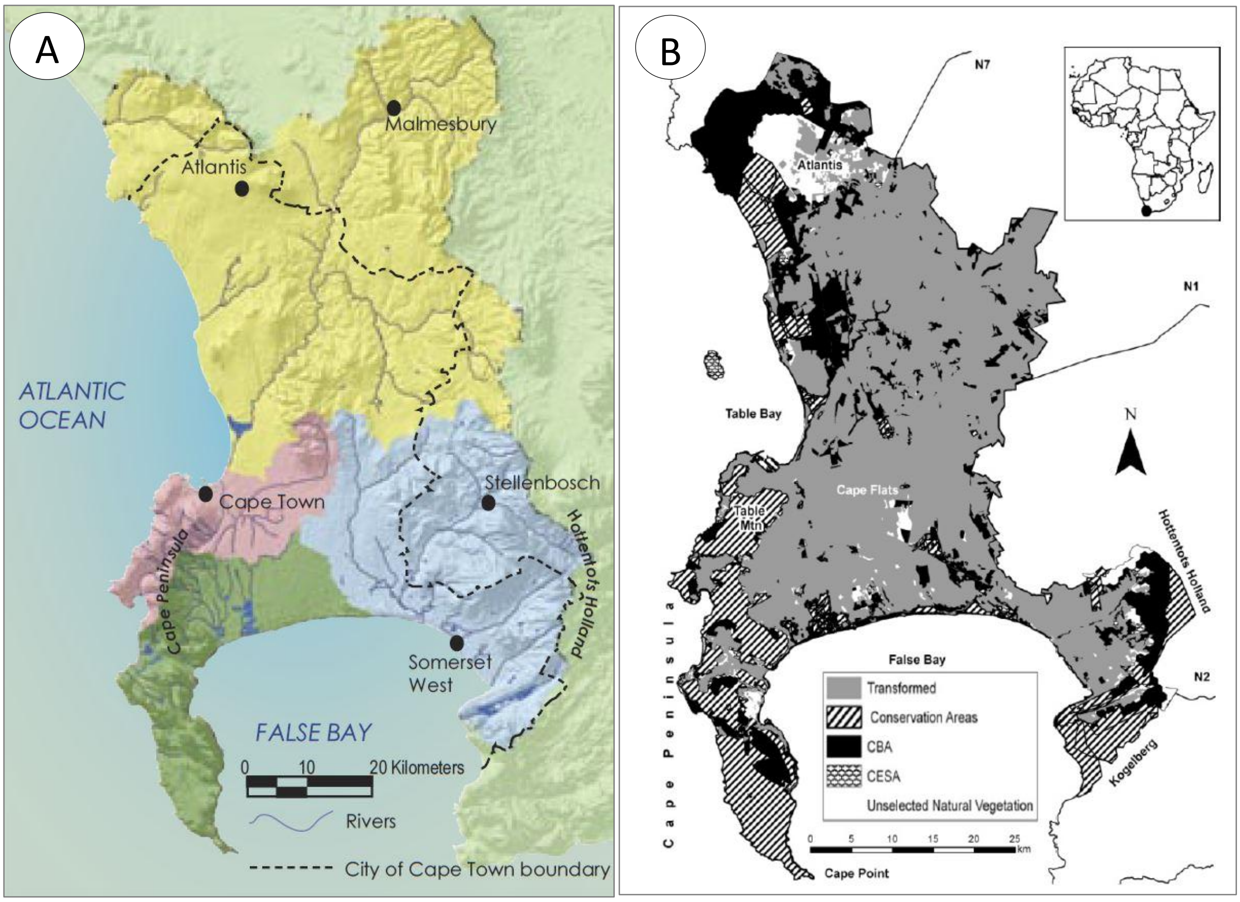

Figure 5.1. Maps of the greater Cape Town area (GCTA). (A) Map showing the four regions of the GCTA, with the area in yellow the northern region, pink the central region, green the southern region, and blue the eastern region (Source: RHP 2005). (B) Map showing the BioNet prioritization areas for the City of Cape Town (Source: Holmes *et al*. 2012). CBA = critical biodiversity areas, and CESA = critical ecological support areas.

6.2 Materials and methods

6.2.1 Literature survey

In order to collect information regarding the distribution of mosquitofish in the GCTA, a systematic literature review was performed. Due to the paucity of scientific publications regarding mosquitofish in the Western Cape Province, and in South Africa in general, the literature examined was expanded to various forms of grey literature. Species collection records from the South African Institute for Aquatic Biodiversity (SAIAB) and species occurrence records from national and global bioinformatics databases were also included. Electronic databases that were examined for information on mosquitofish occurrence were the Global Biodiversity Information Facility (GBIF), the Encyclopaedia of Life (EOL), the Global Invasive Species Database (GISD), which is managed by the Invasive Species Specialist Group

(ISSG) of the IUCN Species Survival Commission; FishMAP, a database compiled by the Animal Demography Unit (ADU) of the University of Cape Town; and iSpot, a database which is affiliated with the South African National Biodiversity Institute (SANBI). The Africa Biodiversity Management (ABM) database was also examined for occurrence records of mosquitofish, which is an electronic archive used by conservation managers of various protected areas in South Africa.

Theses and dissertations on fish surveys in the Western Cape were also searched for in the repositories of Stellenbosch University, the University of the Western Cape, the University of Cape Town, and the North-West University. When information regarding mosquitofish appeared in a Masters dissertation or Ph.D. thesis, but also appeared in a peer-reviewed paper that was later published, only the publication is cited. Reports and articles on mosquitofish in the Western Cape were also searched for in the electronic libraries and repositories of the Water Research Commission (WRC), Western Cape Government, the City of Cape Town (CoCT), Cape Nature, SANParks, SAIAB, SANBI, the Department of Water Affairs and Forestry (DWAF), the Department of Water and Sanitation (DWS), Department of Agriculture, Forestry and Fisheries (DAFF), the Department of Environmental Affairs (DEA), the Council for Scientific and Industrial Research (CSIR), and the DST-NRF Centre of Excellence for Invasion Biology (CIB).

Mosquitofish occurrence data was also acquired from the national survey by the River Health Programme (RHP), of the DWS in 2007 (Kleynhans *et al*. 2007). The RHP used this data to compile a reference fish frequency of occurrence (FROC) list, which is necessary in order to calculate the fish response assessment index (FRAI) for rivers, one of the indices used by the DWS to assess the ecological integrity of rivers in South Africa.

Online reports and species lists within environmental impact assessments of environmental consulting agencies in Cape Town and Somerset West were also assessed for occurrence records of mosquitofish, such as the Freshwater Research Centre, DH Environmental Consulting, and the Southern Waters Ecological Research and Consultancy. When technical reports and scientific publications did not mention mosquitofish occurrence in species lists or in fish surveys, they were recorded as absent from a certain locality. When there were several reports of mosquitofish by the same authors and from the same localities but of different years, only one occurrence was documented. River systems where there were no records for mosquitofish during ichthyofaunal surveys were noted. Literature was examined for records of mosquitofish occurrences at both point locations and entire river systems or dams. The data acquired was sorted according to mosquitofish occurrence within the four major areas of the

GCTA: north, central, south, and east (see Figure 5.1). Data were also grouped according to the river systems of the GCTA (Table 5.3), and according to mosquitofish occurrence or absence in conservation areas (Table 5.4). For an in-depth description of the hydrological landscape of the GCTA, see Addendum D in the Appendix.

6.2.2 Field survey

Due to the large area of the GCTA, potential areas for assessment had to be determined within the already prioritized conservation areas and main river system areas. In order to determine possible areas for evaluation within the conservation sites, the hydrological areas of each nature reserve in the GCTA (see Table 5.4) first had to be evaluated, as not all aquatic habitats can sustain mosquitofish populations and allow them to establish. These areas were examined using the online SANBI BGIS tools for Georeferencing® (Powrie, 2015), and by acquiring information on the hydrological landscape using the wetland layer of the City of Cape Town's Biodiversity Network (CoCT BioNet) dataset. This dataset contains information that describes the vast majority of wetlands within the GCTA (see a detailed description of this dataset in the following section). Conservation areas that had no surface water were excluded from the list, and when wetland areas were described as seeps (unless permanently inundated) or seasonal wetlands; the area was considered low-risk and excluded. A list of possible water bodies where mosquitofish could occur was compiled, and the survey targeted these areas where accessible (see Table 5.4). There were 34 conservation areas where mosquitofish could potentially occur based on water body type, from a total of 46 conservation areas within the GCTA. Stewardship sites and private nature reserves that do not form part of the CoCT BioNet were not included in this list.

A survey of the existing mosquitofish population in and around the GCTA was performed by visiting lotic and lentic water bodies during the summer months, from December 2016 to March 2017, while temporary water bodies were dry. This further enabled the exclusion of ephemeral sites, where mosquitofish are not likely to occur. Absence or presence of mosquitofish was checked by dip-netting at about ten sites within a water body, depending on its area. Mosquitofish prefer shallow and well-vegetated areas (Pyke 2005), therefore areas with this habitat structure within a river or large dam were first targeted. Mosquitofish abundance was also documented by counting adults per sweep of the dip net (2.5 mm mesh). Water pH and TDS was also measured, and the habitat structure documented. Nets, buckets, and boots were disinfected with a bleach solution after each sampling effort in order to prevent the spread of pathogens. Where land-owner permission could not be attained timeously for sampling, but where public access was granted, the presence of mosquitofish was visually assessed and photographs were taken, a method used for data acquisition by the databases of FishMAP of the ADU, SANBI's iSpot, and ABM.

6.2.3 Mapping and analysis of mosquitofish distribution

The localities of mosquitofish occurrences obtained from the literature review and field survey were mapped using the online SANBI tools for Georeferencing (Powrie 2015), specifically using the CoCT BioNet dataset. These GIS tools use ESRI's ArcView 3.3 and are viewed via the SANBI BGIS map viewer portal. The distribution of mosquitofish was noted for each nature reserve and the amount of information available per conservation area was analysed. Conservation areas known to be inhabited by IUCN threatened amphibians (Table 5.4) as well as recorded to inhabit mosquitofish, were identified. The occurrences of threatened amphibians within nature reserves were evaluated according to Minter *et al*. (2004), Rebelo *et al.* (2004), Gibbs *et al*. (2011), Kellerman (2011), and Retief (2011). Nature reserves that did not report the presence of mosquitofish were listed as conservation areas that required further evaluation.

The data from the literature review and field survey were also grouped according to the main river systems of the GCTA. Main river systems were determined according to their tributaries and connectivity (Table 5.2). Mosquitofish occurrences were excluded from this data when the locality was not hydrologically linked with a river system. There were 19 main river systems encompassing the four regions of the GCTA, listed in Table 5.2. The Buffels and Modder Rivers in the northern region of the GCTA were not listed because they are ephemeral, and therefore mosquitofish are not likely to occur in these watercourses. Nature reserves and large wetland areas that are hydrologically associated with the main river systems are also listed in Table 5.2.

Table 5.2. The main rivers of the GCTA and their tributaries. Ephemeral rivers, namely the Buffels and Modder Rivers, are not included. The main wetland areas and associated nature reserves are listed according to the main river system to which they are linked.

GCTA region	Main river system	Main tributaries	Hydrologically connected wetlands and dams	Hydrologically connected conservation areas
North	Diep	Riebeeck, Mosselbank	Uitkamp wetlands, Rietvlei, Zoarvlei	Rietvlei (TBNR), Zoarvlei (TBNR), Milnerton Lagoon (TBNR), Uitkamp Wetland Nature Reserve (tributary headwaters)
	Silverstroom	–	–	Coastal Corridor (West Coast)
	Sout	–	–	–
Central	Hout Bay	–	–	Table Mountain National Park (headwaters)
	Salt	Elsieskraal, Liesbeeck, Black, Vygekraal, Kromboom, Jakkalsvlei, Blomvlei	Magic Forest, Doordekraal Dam, Cool Runnings Quarry (Elsieskraal); Raapenberg Bird Sanctuary (Liesbeeck)	Kirstenbosch Botanical Gardens (tributary headwaters), Raapenberg Bird Sanctuary
South	Sand	Diep, Keysers, Westlake, Grootboschkloof	–	Zandvlei Estuary Nature Reserve
	Zeekoe	Big Lotus, Little Lotus	Princessvlei, Little Princessvlei, Rondevlei, Zeekoeivlei, Strandfontein WWTW, Edith Stevens Wetlands	Princessvlei and Little Princessvlei (FBNR), Rondevlei (FBNR), Zeekoeivlei (FNBR), Strandfontein WWTW (FBNR), Edith Stevens Nature Reserve
	Silvermine	–	Silvermine Dam, Silvermine Wetlands	Silvermine Wetland Nature Reserve, Table Mountain National Park (upper reaches)
	Bokramspruit	–	–	Table Mountain National Park (headwaters and section of lower reaches)
	Elsje	–	Glencairn Wetlands	Glencairn Wetland Nature Reserve, Table Mountain National Park (headwaters)
	Schusters	–	Schustersvlei	Table Mountain National Park
	Klawervlei	–	Lewis Gay and Kleinplaas Dams	–
	Hout	Klaasjagters, Krom	–	Table Mountain National Park
	Buffels	–	–	Table Mountain National Park
East	Eerste	Blaauwklippen, Bonte, Jonkershoek, Plankenbrug, Moddergat	Theewaterskloof Dam, Kleinplaas Dam	–
	Kuils	Bottelary	Driftsands Wetlands, Mfuleni Wetlands, Nooiensfonteinvlei, Dreamworld Wetlands	Driftsands Nature Reserve
	Lourens	Disa Stream	–	Helderberg Nature Reserve (tributary headwaters)
	Sir Lowry's Pass	–	–	Hottentots Holland Nature Reserve (headwaters)
	Steenbras	–	–	Hottentots Holland Nature Reserve

Mosquitofish occurrence data was also evaluated according to the conservation status of the wetland where the fish were found. The conservation value of each relevant water body type was determined using the online CoCT BioNet dataset, and acquired specifically from the wetland layer. This information was originally compiled by specialist consultants, evaluated in order to prioritize the conservation of Cape Town's wetland areas. This was performed by way of several wetland assessments that occurred through several phases (see Ewart-Smith *et al*. 2008). The wetland areas were located, categorised, and prioritised in 2009 (Snaddon *et al*. 2009; Snaddon and Day 2009). Each wetland type was classified according to the National Wetland Classification System (Ollis *et al*. 2009), and prioritized according to several ranking criteria, such as connectivity, size, sensitivity, and naturalness (see Snaddon and Day 2009). Once assigned a rank, wetlands were either placed in the Critical Biodiversity Area (CBA), Critical Ecological Support Area (CESA), or Other Ecological Support Area (OESA) category. Further subcategories for the CBA, ESA and OESA categories were determined according to the quartile that the original wetland ranking score fell in (Snaddon and Day, 2009). See Table 5.3 for a breakdown of the classification of CBA, CESA and OESA wetlands. Since 2009, wetlands that have been destroyed or modified have been either removed from the dataset or reclassified, and the data are updated on a continuous basis (Holmes and Pugnalin 2016). See Figure 5.1 for a map of the GCTA and the BioNet prioritization areas for both wetlands and terrestrial areas (Holmes *et al*. 2012).

Table 5.3. Prioritisation of Cape Town wetlands according to Snaddon and Day (2009). Ranks were assigned according to the quartile of total scores for all wetlands within the area. Rank 1 indicates the highest scoring quartile, and rank 4 as the lowest quartile.

Prioritization category	**Wetland classification**
Critical Biodiversity Area 1 (CBA1)	Rank 1 of natural or semi-natural wetlands. Rank 1 of estuaries.
Critical Biodiversity Area 2 (CBA2)	Rank 2 of natural or semi-natural wetlands. Ranks 2, 3, and 4 of all estuaries.
Critical Ecological Support Areas (CESA)	Rank 1 of artificial wetlands. Rank 3 of natural or semi-natural wetlands.
Other Ecological Support Areas (OESA)	Ranks 2, 3, and 4 of artificial wetlands. Rank 4 of natural or semi-natural wetlands.

6.3 Results

6.3.1 Literature review

An exhaustive literature review revealed that there were very few peer-reviewed articles on mosquitofish distribution for the GCTA, the CFR, or within the wider area of the Western Cape Province. One study on the Diep River estuary (Viskitch 2016) reported mosquitofish occurrence in this area, and three other publications included mosquitofish in fish species checklists for the Zandvlei estuary (Morant 1991; Clark *et al*. 1994; Quick and Harding 1994). There are several technical reports and publications written in the previous century that record mosquitofish introduction and establishment in water bodies in the GCTA (see Musil and MacDonald 2007), but this literature is largely inaccessible. De Moor and Bruton (1988) also give one older record for mosquitofish occurrence in the GCTA, at Princessvlei (1965), and a few other locations within the Western Cape, which are the same records held by the SAIAB database. There were only three other publications that document the occurrence of mosquitofish in the Western Cape, which were all ichthyofaunal studies performed on the Wilderness Lakes System (Olds 2011, 2016; Sloterdijk *et al*. 2014). The majority of other publications that report mosquitofish in the GCTA, or within the wider area of the Western Cape Province, do not specifically mention localities of occurrence (Ellender and Weyl 2014; Marr *et al*. 2012, 2013, 2017). There was also no mention of the occurrence of mosquitofish when other aliens were discussed in a recent literature review on the freshwater fishes of the Cape Fold aquatic ecoregion (Ellender *et al*. 2017), although this paper primarily focussed on indigenous species.

The review of grey literature and online databases yielded much more information concerning the distribution of mosquitofish, with 42 data points. Of these, 31 sources gave the GPS coordinates where mosquitofish occurred (see Table D1, Addendum D). The rest of the literature sources only named the river or conservation area where mosquitofish were found (Table D2, Addendum D).

Of the combined data points from both publications and grey literature, the earliest record of established populations of mosquitofish in the GCTA was for the year 1965. The largest portion of occurrences for mosquitofish has been reported in the last two decades (82.62%), with the majority taking place in the years 2000–2009 (47.8% of the total). The majority of these data points can be attributed to the large-scale monitoring programme of the RHP that occurred in 2007 (Kleynhans *et al*. 2007). There is a decline in occurrences reported for the last decade (2010–2017), which is 34.8% of the total descriptions that have been reported since 1965.

The data from the literature review per GCTA region are given in a later paragraph in combination with results from the field survey.

6.3.2 Physical survey

During the months of December 2016 to March 2017, a total of 30 locations were surveyed for the presence of mosquitofish (see Figure 5.2). Kirstenbosch Botanical Gardens and Kenilworth Racecourse Conservation Area were each considered a single locality, but four different water bodies were assessed at Kirstenbosch Botanical Gardens and three water bodies were surveyed at Kenilworth Racecourse Conservation Area, making up a total of 35 geographical points surveyed (see Table D3, Addendum D). Mosquitofish were present in 50%, and absent in 40%, of the 30 locations. The remaining 10% of sites were considered data deficient because, at the time of assessment, these areas had dried up due to drought conditions. Of the sites where mosquitofish were surveyed, the pH ranged from 8.0 – 9.5, whilst TDS ranged from 70 –16 200 ppm. The altitudes that mosquitofish ranged were from sea-level to 225 metres above sea level. The results from the field survey per GCTA region is given in combination with the literature review results, in Section 6.3.3 below.

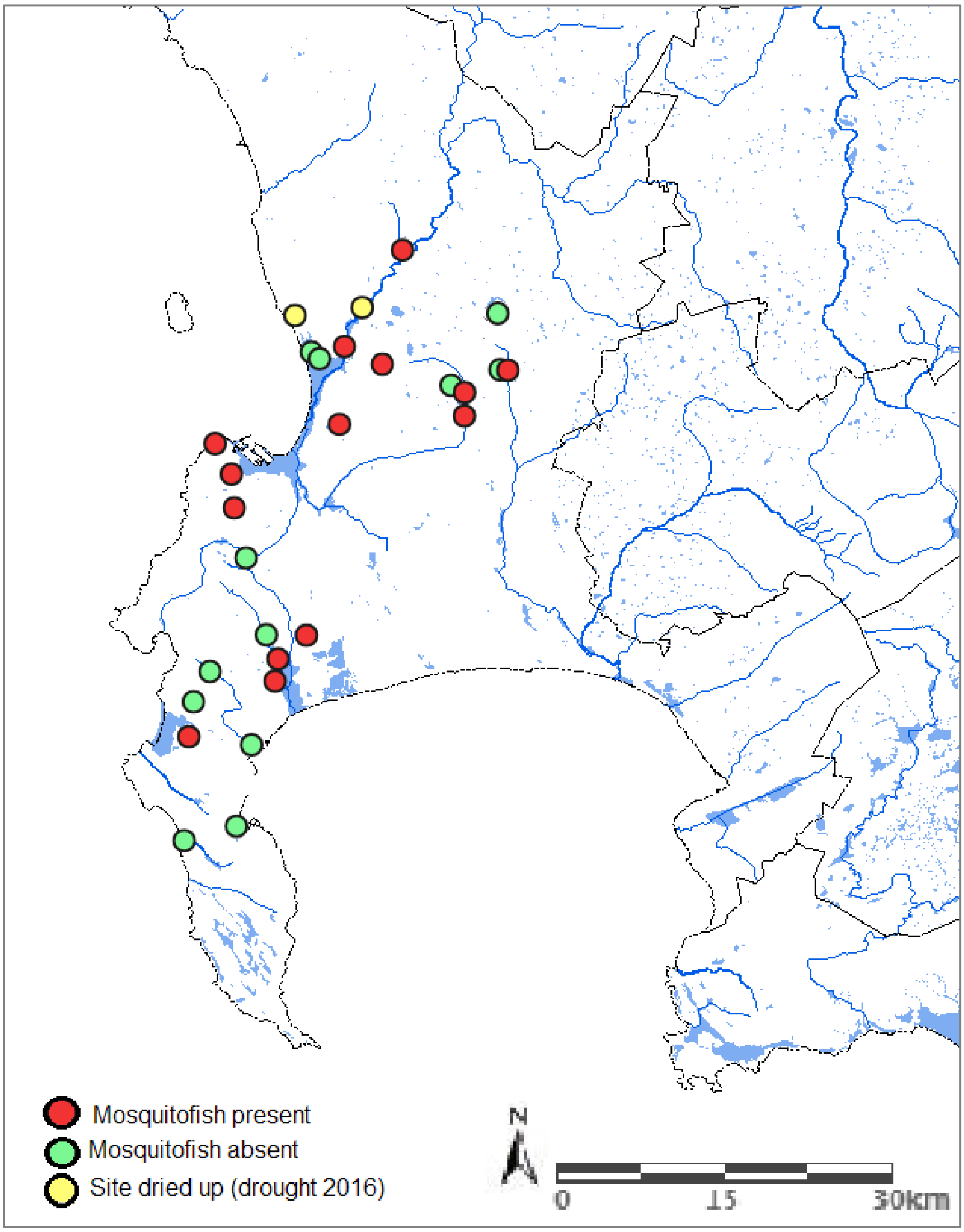

Figure 5.2. Distribution of data points from the field survey for mosquitofish occurrence in the GCTA from January 2016 to March 2017.

6.3.3 Mosquitofish occurrence within the different regions of the GCTA

6.3.3.1 The northern region

The second greatest portion of data points documenting mosquitofish presence in the literature review was for the northern region of the GCTA (30.4%). The majority of these occurrences are from the Table Bay Nature Reserve (TBNR), spanning from 2003–2014, with mosquitofish recorded at all the water bodies of the area: Diep River, Rietvlei, Zoarvlei, Milnerton lagoon/Diep River estuary, except for the Milnerton Racecourse section of the TBNR. Data from the literature review also showed that the majority of the Diep River is inhabited by mosquitofish: from the headwaters up north at Kalbaskraal Station (Kleynhans *et al*. 2007) to its estuary at Milnerton. Mosquitofish were also found in the northern region of the Diep River near Philadelphia, at its confluence with the Mosselbank River during the present field survey. The fish was also present in the floodplain of the Diep River, before it enters the TBNR, however no fish were present at Rietvlei. This was most probably due to toxic cyanobacterial blooms that were present at the time (March 2017), which had flared up due to the drought-induced reduction in water levels.

Mosquitofish were also recorded in the Riebeeks River by Kleynhans *et al*. (2007), one of the northernmost tributaries of the Diep River. Another northern river where mosquitofish have been located is the Sout River (Kleynhans *et al*. 2007), near Melkbosstrand. There were no literature records confirming mosquitofish presence or absence in the Silverstroom River, and this area was also not surveyed.

Although fish were found at the Diep River/Mosselbank confluence during the survey, no mosquitofish were found at the headwaters of the Mosselbank, at Uitkamp Wetland Nature Reserve, as the area had also dried up due to the drought. The dam at Botterblom Nature Reserve in Durbanville was also devoid of mosquitofish, but other aliens, *M. salmoides* and *C. carpio*, were present. Mosquitofish were also found at a suburban dam in Burgundy Estate, Milnerton.

Mosquitofish occurrences have been documented for three of the 10 conservation sites in the northern region which possess aquatic habitat that has the potential for mosquitofish establishment. All of these sites were the smaller protected reserves of the larger TBNR that were previously mentioned. Of the remaining seven sites, mosquitofish were absent from one nature reserve (the Botterblom Nature Reserve mentioned previously). The remaining six nature reserves require further assessment: the Dassenberg Coastal Catchment Partnership Reserve, Koeberg Nature Reserve, Blaauwberg Nature Reserve, Witzands Aquifer Nature Reserve, Uitkamp Wetland Nature Reserve, and the Milnerton Racecourse section of the TBNR. Two conservation sites in the northern region, the Bothasig Fynbos section of the TBNR and the Durbanville Nature Reserve, are not likely to have mosquitofish present.

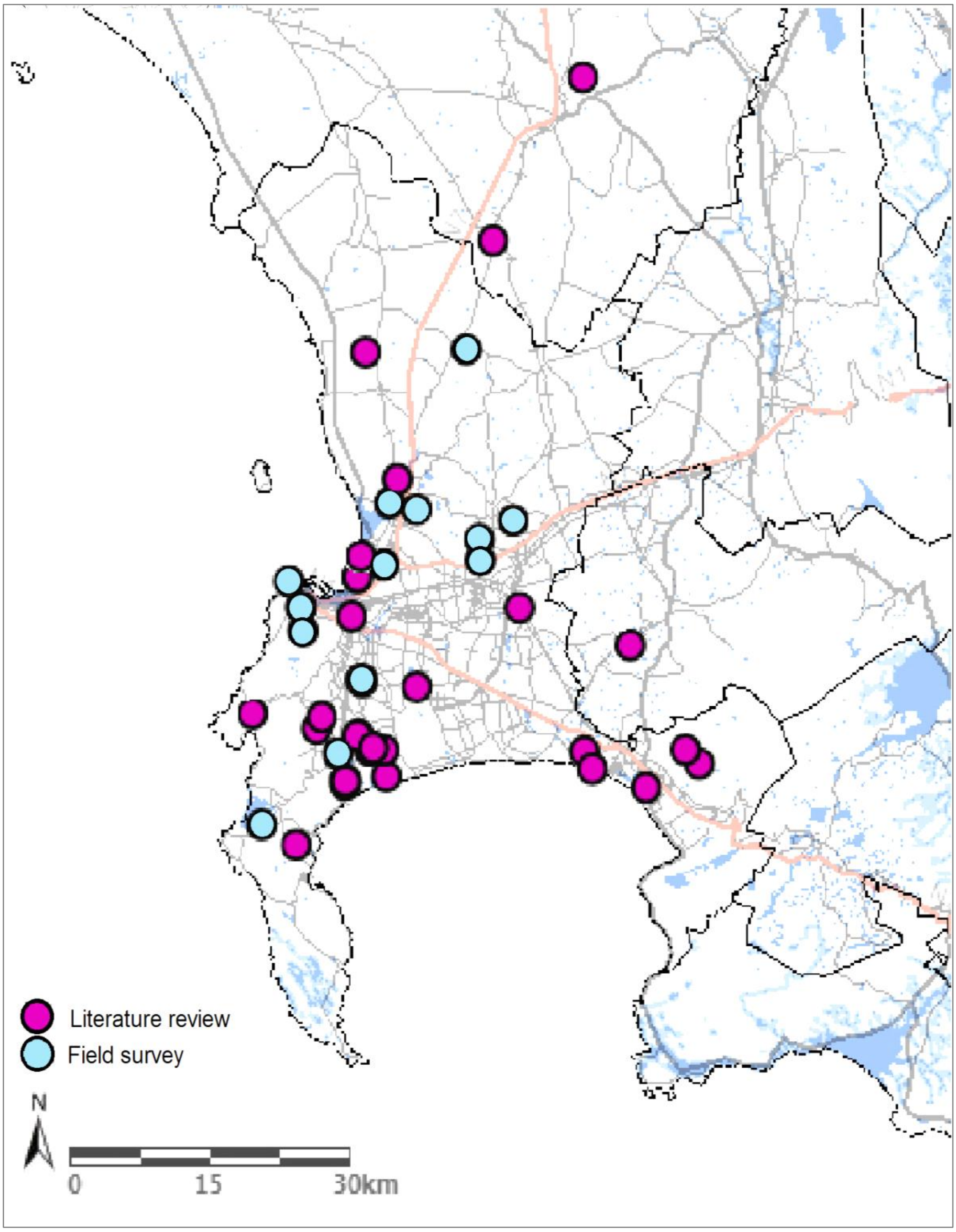

Figure 5.3. Distribution of *G. affinis* in the greater Cape Town area (GCTA) according to occurrences obtained from the literature review (1965–2017) and the field survey (Dec 2016–March 2017).

6.3.3.2 The central region

The literature review yielded relatively few mosquitofish occurrences for this region. There were two records for the Liesbeeck River: one at Hartleyvale, near Raapenberg Bird Sanctuary (Brown and Magoba 2009), and another record from the SAIAB data at Observatory (2010).

Mosquitofish were also recorded in the Hout Bay River by the RHP (2003). In contrast, the field survey yielded more mosquitofish occurrences for the central region. Mosquitofish were found in high numbers at Green Point Urban Park, in the wetland system of the Biodiversity Garden. Mosquitofish were also found in the three artificial ponds of the Company's Garden City Park in the Cape Town city centre, although these are small artificial ponds in a highly modified environment and are of little conservation value. They were, however, found in other areas of conservation concern in the central region. One of these was at Intaka Island in Century City, and also in a spring-fed mountain pond in Deer Park, north of Table Mountain. The latter locality is however not protected by the Table Mountain National Park. Kirstenbosch Botanical Gardens was also surveyed, but no mosquitofish were found at the four localities evaluated in this area.

Although the Elsieskraal River in the central region was not surveyed, two exorheic dams that are linked with the Elsieskraal were evaluated and mosquitofish were found at both localities (see Table D3, Addendum D). Another exorheic dam linked with the Elsieskraal, located at Magic Forest Park in Bellville, was evaluated and no mosquitofish were found here. However, the sites at this locality that were accessible for sampling were not the preferred habitat of mosquitofish, whilst the shallower vegetated areas were inaccessible. It is therefore possible that these results are not reliable. None of the other canalised rivers forming part of the Black/Liesbeeck/Elsieskraal/Sout river system were surveyed.

There are seven conservation sites in the central region that are part of the CoCT BioNet (Table 5.4). Of these, mosquitofish occurrence is not likely at the Rondebosch Common and the Plattekloof Natural Heritage site due to unsuitable or absent aquatic habitat. Of the remaining five potential sites for infestation, mosquitofish were present at two sites and recorded absent from one. There were no data for the Tygerburg Nature Reserve and Driftsands Nature Reserve.

6.3.3.3 The southern region

The majority of mosquitofish occurrences found in the literature review were mainly from this region of the GCTA (39.1%), of which most of the data points were from the False Bay Nature Reserve (FBNR), and the Zandvlei Estuary Nature Reserve (see Table D1 and D2, Addendum D for sources). The dates for mosquitofish occurrence in the FBNR and the Zandvlei estuary span from 1970 to 2014, and 1994 to 2011 respectively. There were also other reports of mosquitofish occurrences in the Keysers River (Kleynhans *et al*. 2007) and the Grootboschkloof

River (iSpot 2017) which are tributaries of the Sand River that feeds the Zandvlei estuary. During the field survey, mosquitofish were also found in the Keysers River, downstream from the site of occurrence recorded in the literature study (Kleynhans *et al*. 2007), and at the Zandvlei estuary. The literature review recorded mosquitofish occurrences for most of the wetland areas of the FBNR: Rondevlei (1970–2014), Zeekoeivlei (1970), and the Strandfontein Birding Section (2012). There was also a record for mosquitofish occurrence at Princessvlei (1965), which was also confirmed by the survey. There was also a literature record for the Elsje River that flows through Glencairn Wetland Nature Reserve (Kleynhans *et al*. 2007), but no mosquitofish were found during this study at a site roughly 1.5 km downstream. Localities inhabited by mosquitofish that were not found in the literature survey were the Wildevoëlvlei wetlands at Kommetjie, and at Kenilworth Racecourse Conservation Area. However, at the latter conservation area the mosquitofish was only found in one of the three dams at the reserve.

Mosquitofish were absent from the majority of water bodies surveyed on the Southern Peninsula: Silvermine Dam, Klawersvlei River at Simons Town, and the Schusters River at Scarborough. All these areas are protected by the Table Mountain Nature Reserve. No mosquitofish were found at Die Oog Conservation area in Bergvliet. There were no records for mosquitofish absence or presence for the Bokramspruit River in Noordhoek, or for the Buffels, Hout, Klaasjagters and Krom rivers of the Cape Point section of Table Mountain National Park.

Of the 14 conservation sites in the southern region of the GCTA, there is only one area where mosquitofish are not likely to occur, namely Meadowridge Common. Overall mosquitofish were present at eight of the 13 possible conservation sites, recorded absent from two localities, and three areas still need to be assessed: De Hel Nature Area, and Pelican Park and the Coastal Corridor section of the FBNR (Table 5.4).

6.3.3.4 The eastern region

The majority of mosquitofish occurrence data obtained from the literature review, for the eastern region of the GCTA, is from the Lourens River (see Table D1 and D2 in Addendum D for sources) and dated from 1997 to 2008. Mosquitofish were located at the upper reaches of the Lourens River at Vergelegen Wine Estate, as well as the lower reaches near the coast at Strand (Kleynhans *et al*. 2007).

Mosquitofish have been located in the middle reaches of the Eerste River, another eastern river, at its confluence with the Blou Klip River (Kleynhans *et al*. 2007), as well at the Eerste

River Estuary in Macassar. There are two dates of mosquitofish occurrence for the estuary, one in 1988 (SAIAB collection) and another in 2007 (Kleynhans *et al*. 2007). The Kuils River is also infested with mosquitofish. Fish were found at its headwaters at Eversdal during this field survey, and there is also a record for its occurrence in the middle reaches in the suburb of Kuils River (Kleynhans *et al*. 2007). The Kuils River joins with the Eerste before the Eerste River Estuary, and as they have been found here it can be reasoned that they are also likely to be present in the lower reaches of the Kuils. No mosquitofish distribution data were found for the Sir Lowry's or Steenbras Rivers, and neither were these rivers surveyed.

Of all the 12 conservation areas in the eastern region, there are only five areas that have the potential for mosquitofish establishment (see Table 5.4). Of these five areas, mosquitofish have been recorded in two areas, namely the Helderberg Nature Reserve and Edith Stevens Wetland Reserve as mentioned previously. There were two records for mosquitofish occurrence in the Helderberg Nature Reserve in Somerset West, for 2008 and 2011 (Wittridge 2011; GBIF 2017). There are however no data for the Silverboomkloof section of the Helderberg Nature Reserve, the Wolfgat Nature Reserve, and the Hottentots Holland Nature Reserves.

Table 5.4. Results of the literature review and field survey for mosquitofish (*G. affinis*) occurrence, grouped according to the conservation areas of the GCTA. These areas exclude stewardship sites and private reserves not listed in the City of Cape Town Biodiversity Network. Areas that were surveyed, but that had dried up due to the drought, were classed as 'no data' sites. Presence of amphibians was found according to Minter *et al*. (2004), Rebelo *et al*. (2004), Gibbs *et al*. (2011), Retief (2011), and Kellerman (2011).

Area	Conservation area	Custodian	City of Cape Town Biodiversity Network Classification	Literature review results (Tables D1 and D2, see Appendix)	Field survey results (Table D3, see Appendix)	Combined occurrence data	Threatened amphibian species known to occur in area
North	Dassenberg Coastal Catchment Partnership	Multiple custodians	CESA exorheic non-vegetated in-channel dam, CBA 2 natural/semi-natural wetland	No data			–
North	Koeberg Nature Reserve	Eskom	CBA2 littoral endorheic depression, permanently inundated	No data			–
North	Witzands Aquifer Nature Reserve	City of Cape Town	CBA2 and CESA endorheic depression littoral	No data			–
North	Blaauwberg Nature Reserve	City of Cape Town	CBA1, endorheic depression littoral	No data			–
North	TBNR: Diep River and Fynbos Corridor	City of Cape Town	CBA1 river and inundated wetland	*G. affinis* present	*G. affinis* present	*G. affinis* present	–
North	Bothasig Fynbos Nature Reserve	City of Cape Town	Water body absent	*G. affinis* unlikely to occur, water body absent			–
North	TBNR: Milnerton Racecourse Nature Reserve	City of Cape Town	CBA1 littoral exorheic depression	No data			–
North	TBNR: Rietvlei Wetland Reserve	City of Cape Town	CBA1 wetland and estuary, Diep River floodplain	*G. affinis* present	*G. affinis* absent due to blue-green algae	*G. affinis* likely to occur	*Cacosternum capense, Sclerophrys pantherina, Breviceps gibbosus*
North	TBNR: Zoarvlei wetlands	City of Cape Town	CESA exorheic depression	*G. affinis* present	No data	*G. affinis* present	–
North	Durbanville Nature Reserve	City of Cape Town	CBA 1 seasonally inundated endorheic depression	*G. affinis* unlikely to occur, water body unsuitable			–
North	Uitkamp Wetlands	City of Cape Town	CBA 1 valley floor wetland	No data			*Breviceps gibbosus, Cacosternum capense*
North	Botterblom Nature Reserve	City of Cape Town	OSEA endorheic depression, permanently inundated	No data	*G. affinis* absent	*G. affinis* absent	–

Table 5.4 Cont. Results of the literature review and field survey for mosquitofish (*G. affinis*) occurrence, according to the conservation areas of the GCTA.

Central	Tygerburg Nature Reserve	City of Cape Town	OSEA endorheic depression. CBA 1 and CBA 2 seeps	No data			–
Central	Driftsands Nature Reserve	Cape Nature	CBA 2 Floodplain wetland, CESA exorheic wetland, OESA endorheic depression	No data			–
Central	Plattekloof Natural Heritage Site	Eskom	CBA 1 natural and semi-natural seep	*G. affinis* unlikely to occur			–
Central	Rondebosch Common	City of Cape Town	CBA 1 and 2 seasonal endorheic depression	*G. affinis* unlikely to occur			*Sclerophrys pantherina*
Central	Two Rivers Urban Park/ Raapenberg Bird Sanctuary	City of Cape Town	CBA 2 and CESA permanently inundated wetland	*G. affinis* present	No data	*G. affinis* present	*Sclerophrys pantherina*
Central	Kirstenbosch Botanical Gardens	SANBI	CBA 2 reservoir, unspecified streams and ponds	No data	*G. affinis* absent	*G. affinis* absent	*Arthroleptella lightfooti, Heleophryne rosei*
Central	Intaka Island	Private	OESA endorheic littoral storm water pond, permanently inundated	No data	*G. affinis* present	*G. affinis* present	–
South	Kenilworth Racecourse Conservation Area	City of Cape Town	CBA 1 and permanently inundated OESA storm water ponds, excavation pit	No data	*G. affinis* present	*G. affinis* present	*Microbatrachella capensis*
South	Princessvlei and Little Princessvlei	City of Cape Town	CBA 2 (princess) and CESA (little princess) permanent limnetic exorheic depressions	*G. affinis* present	*G. affinis* present	*G. affinis* present	*Sclerophrys pantherina*
South	FBNR: Rondevlei	City of Cape Town	CBA 1 and CBA 2 exorheic depression natural to semi-natural, limnetic	*G. affinis* present	No data	*G. affinis* present	*Sclerophrys pantherina*
South	FBNR: Zeekoeivlei	City of Cape Town	CESA exorheic permanently inundated limnetic depression	*G. affinis* present	No data	*G. affinis* present	*Sclerophrys pantherina*
South	FBNR: Strandfontein Birding Section	City of Cape Town	CESA: Cape Flats Sewage Works. Endorheic depression littoral	*G. affinis* present	No data	*G. affinis* present	*Sclerophrys pantherina*
South	FBNR: Pelican Park	City of Cape Town	CBA1 littoral endorheic depression.	No data			–
South	FBNR: Coastal corridor section	City of Cape Town	CBA1, CBA 2, OESA and CESA WWTW	No data			–
South	Zandvlei Estuary Nature Reserve	City of Cape Town	CBA1 permanently open estuary and wetlands	*G. affinis* present	*G. affinis* present	*G. affinis* present	*Sclerophrys pantherina, Breviceps gibbosus*
South	De Hel Nature Area	City of Cape Town	CESA permanently saturated seep	No data			*Sclerophrys pantherina*

Table 5.4 Cont. Results of the literature review and field survey for mosquitofish (*G. affinis*) occurrence, according to the conservation areas of the GCTA.

South	Meadowridge Common	City of Cape Town	No water body	*G. affinis* unlikely to occur			–
South	Die Oog Conservation Area	City of Cape Town	CESA littoral exorheic depression, and CESA seep	No data	*G. affinis* absent	*G. affinis* absent	*Sclerophrys pantherina*
South	Lower Silvermine Wetlands	City of Cape Town	CBA 1 permanently inundated river floodplain	*G. affinis* absent	*G. affinis* absent	*G. affinis* absent	*Sclerophrys pantherina*
South	Glencairn Wetland Nature Reserve	City of Cape Town	CESA permanently inundated littoral exorheic depression	*G. affinis* present	*G. affinis* absent	Present upstream, absent downstream	*Sclerophrys pantherina*
South	Table Mountain National Park (Wildevoëlvlei)	SAN Parks	Multiple types: CESA reservoirs, CBA 1 and CBA 2 seasonal seeps, CESA exorheic depressions	No data	*G. affinis* present	*G. affinis* present	*Arthroleptella lightfooti, Breviceps gibbosus, Capensibufo rosei, Heleophryne rosei, Sclerophrys pantherina, Xenopus gilli*
East	Edith Stevens Wetland Park	City of Cape Town	CBA 1 seep, CESA and CBA 2 permanently inundated endorheic depression	*G. affinis* present	No data	*G. affinis* present	*Sclerophrys pantherina*
East	Bracken Nature Reserve	City of Cape Town	No water body	*G. affinis* unlikely to occur			–
East	Haasendal Nature Reserve	City of Cape Town	No water body	*G. affinis* unlikely to occur			–
East	Penhill Nature Reserve	City of Cape Town	CBA 2 seeps	*G. affinis* unlikely to occur			–
East	Macassar Dunes	City of Cape Town	CBA 1 seep from detention pond	*G. affinis* unlikely to occur			–
East	Harmony Flats Nature Reserve	City of Cape Town	CBA seep	*G. affinis* unlikely to occur			–
East	Jack Muller/ Danie Uys Park	City of Cape Town	No water body	*G. affinis* unlikely to occur			–
East	Cape Flats Nature Reserve	University of the Western Cape	Multiple CBA 2 seasonal endorheic depression	*G. affinis* unlikely to occur			–
East	Wolfgat Nature Reserve	City of Cape Town	CBA 2 littoral endorheic depression	No data			–
East	Helderberg Nature Reserve: Silverboomkloof section	City of Cape Town	Wetland not assessed	No data			*Arthroleptella landdrosia, Arthroleptella villiersi*
East	Hottentots Holland Nature Reserve	Cape Nature	Multiple types: OESA reservoirs, CBA 1 and CESA seasonal seeps, CBA1 permanently inundated wetland	No data			*Capensibufo magistratus, Capensibufo deceptus, Capensibufo selenophos*
East	Helderberg Nature Reserve	City of Cape Town	CBA 1 seep and CESA limnetic endorheic depression	*G. affinis* present	No data	*G. affinis* present	*Arthroleptella landdrosia, Arthroleptella villiersi*

6.3.4 Overall results for conservation areas

Of the 33 conservation areas in the GCTA that possess water bodies suitable for mosquitofish, a record of mosquitofish presence and absence could be established for 19 of these areas (Table 5.4), with mosquitofish present in 16 of the conservation areas (48.5%). Mosquitofish were recorded absent in three of these areas, namely Kirstenbosch Botanical Gardens, Die Oog Conservation Area, and the Lower Silvermine Wetlands. The remaining 14 of the 33 sites still need to be evaluated (48.5%, see Figure 5.4). Of the total 45 conservation areas in the GCTA, which possess both suitable and unsuitable areas for mosquitofish, 20 of these protect IUCN threatened amphibians. Mosquitofish occur in 12 of these nature reserves (60%). Four nature reserves inhabited by amphibians of conservation concern still need to be evaluated for mosquitofish occurrence, namely the Hottentots Holland Nature Reserve, the Silverboomkloof section of the Helderberg Nature Reserve, De Hel Nature Area, and Uitkamp Wetland Nature Reserve.

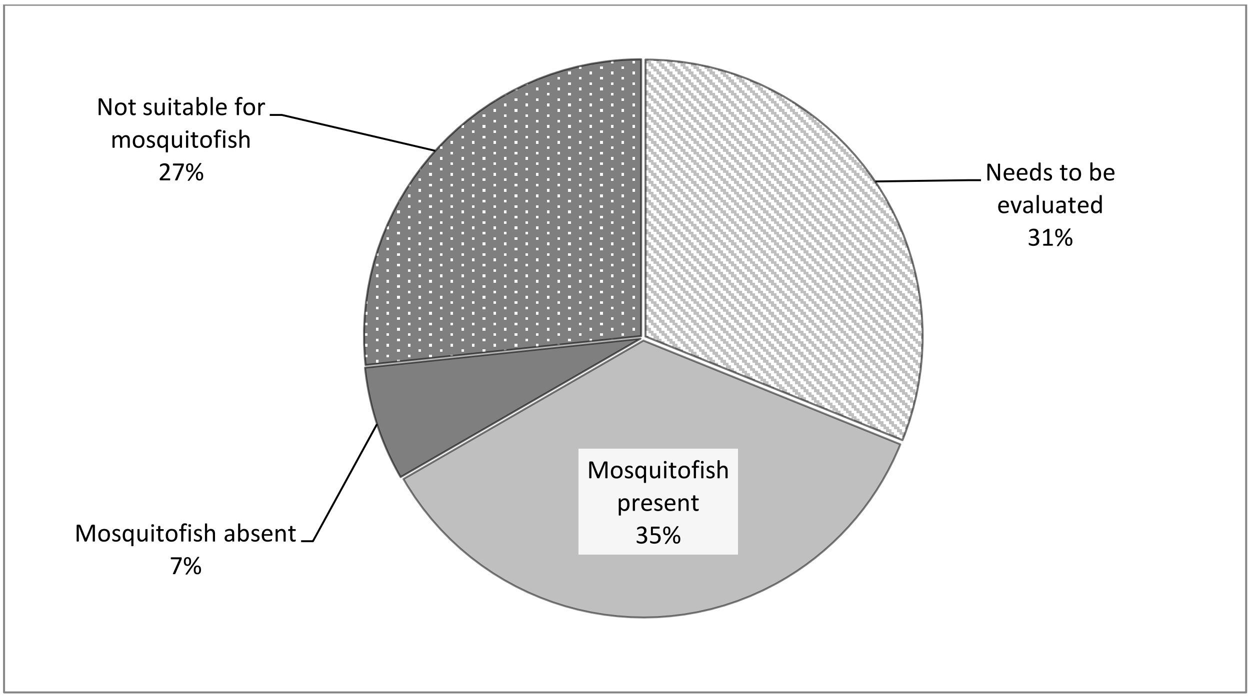

Figure 5.4. Conservation areas of the GCTA that form part of the CoCT Biodiversity Network (Holmes *et al*. 2012), and mosquitofish occurrence within these areas, based on the literature review and field survey. Areas not suitable for mosquitofish habitation are either seasonal water bodies or where surface water is absent from the conservation site.

6.3.5 Overall results for main river systems

There were 13 records for mosquitofish occurrence in the Diep River and its tributaries in the northern region, which is the highest record for all main river systems of the GCTA. There were also many reported occurrences for the Sand River and its tributaries (11 records) and for the Zeekoe system (nine records) in the southern region of the GCTA. Mosquitofish occurrences were documented for the Lourens River in the eastern region, with a total of six records. There were also records for the Eerste and Kuils Rivers which have very large catchment areas. No information was available for the Silverstroom River in the north, the Silvermine, Bokramspruit, Krom and Schusters Rivers in the south, and for the Sir Lowry's and Steenbras Rivers in the east.

6.3.6 Aquatic habitat types inhabited by mosquitofish

Of the locations of mosquitofish occurrence obtained from the literature review and field survey, a large portion (41%) was riverine habitat (See Figure 5.5). A similarly large portion of the locations inhabited by mosquitofish were limnetic depressions (41%), 13% of which were endorheic, in other words not connected to a watercourse, and 28% exorheic, standing waters hydrologically linked with a river system. Estuaries made up 8% of the occurrences, and the remaining habitat types were littoral exorheic depressions (5%), littoral endorheic depressions (2%), and seeps (3%). Roughly half of the mosquitofish occurrences were recorded from lentic waters (51%), with the other half from lotic water bodies, namely rivers and estuaries.

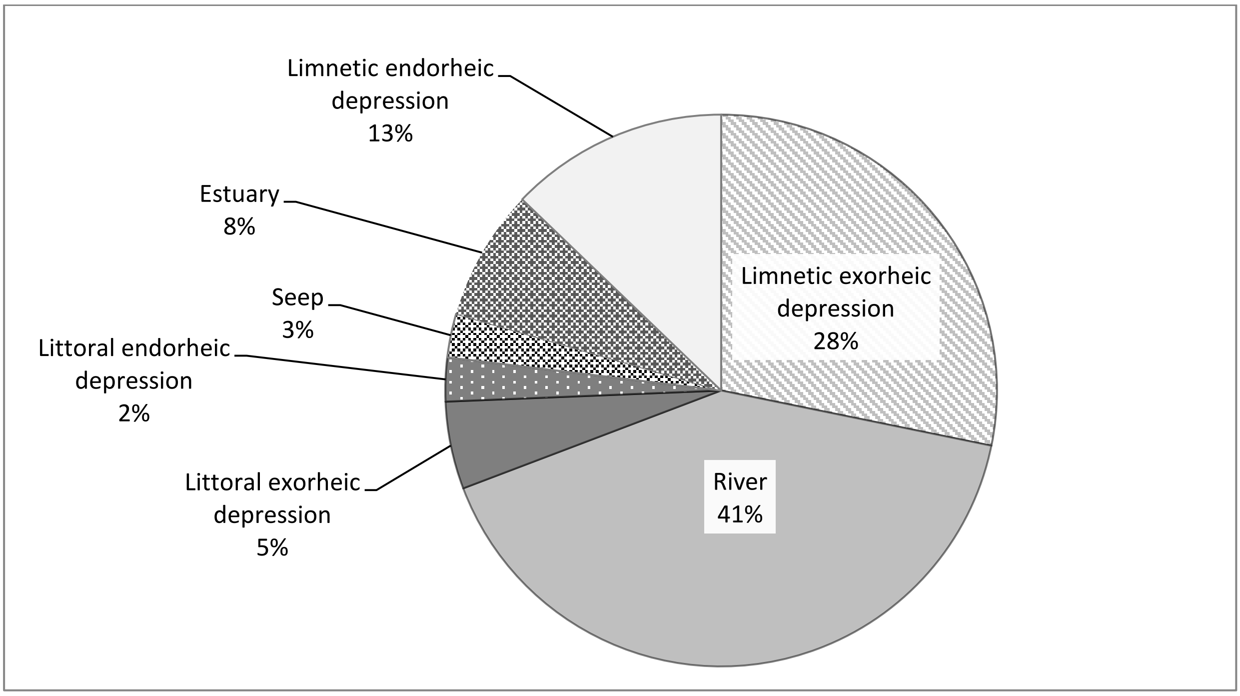

Figure 5.5. Habitat types of the mosquitofish occurrences obtained from the literature review and the field survey.

6.3.7 Conservation status of wetlands and rivers inhabited by mosquitofish

Mosquitofish inhabited a wide range of habitat types with varying conservation priorities that were prioritised by the CoCT BioNet. The majority of these occurrences were at locations given a CESA status (34%). The rest were CBA 1 (29%), OESA (26%) and lastly CBA2 (11%).

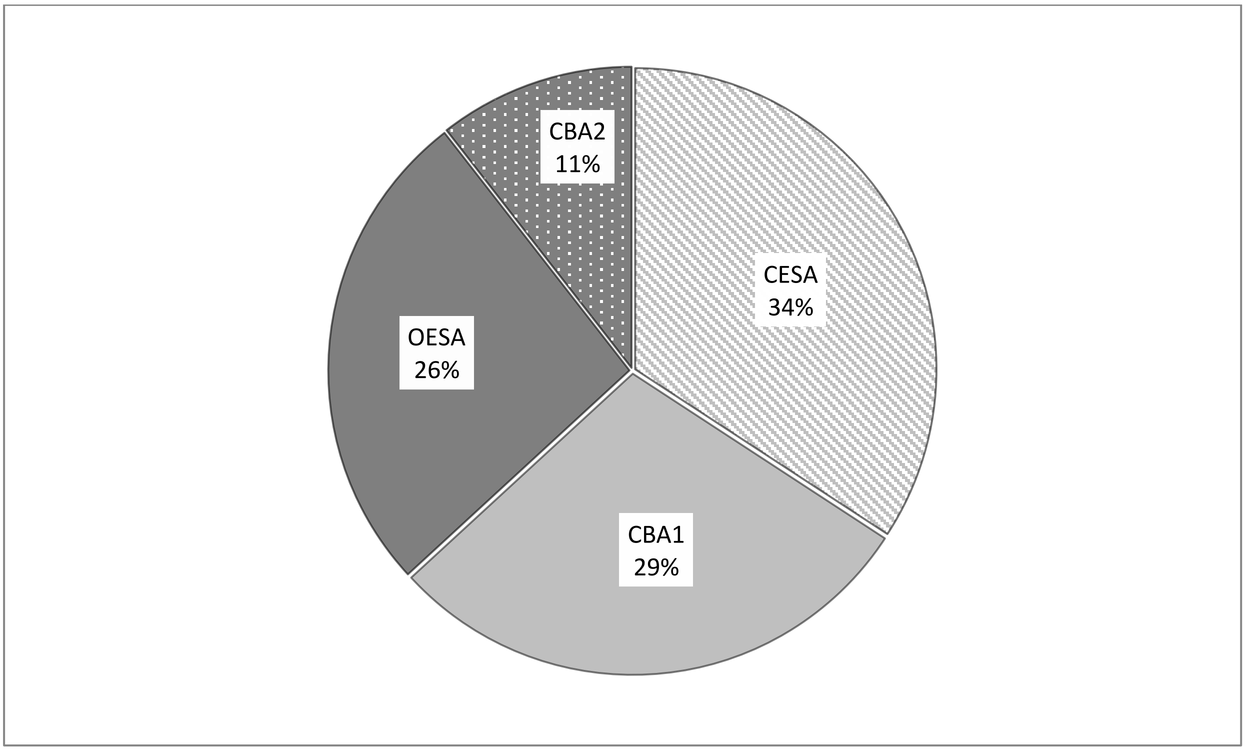

Figure 5.6. Conservation status of wetlands and rivers inhabited by mosquitofish, from data obtained from the literature survey and field analysis.

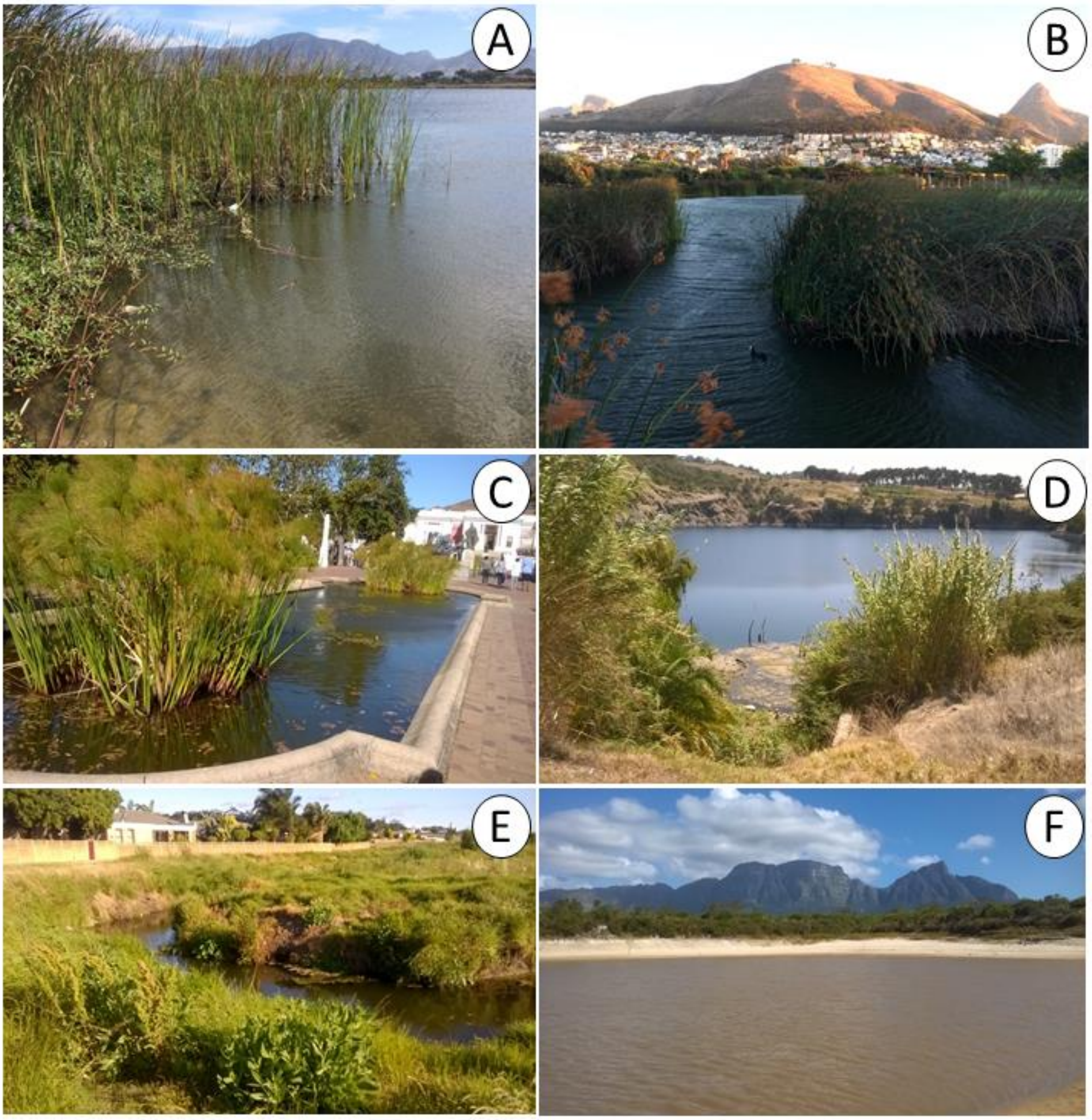

Figure 5.7. Photos of a few mosquitofish-infested areas in the GCTA. (A) Princessvlei in the southern suburbs. (B) Biodiversity Garden at the Green Point Urban Park. (C) Ponds at the Company's Garden, Gardens. (D) Quarry in Bellville. (E) Headwaters of the Kuils River, Eversdal. (F). Kenilworth Racecourse Conservation Area. Photos by Roxanne Conradie.

6.4 Discussion

The GCTA has a diverse aquatic landscape and possesses a myriad of riverine networks and urban streams that traverse the area (Brown and Magoba 2009). The Diep, Eerste, Kuils, and Salt River Systems are networks that encompass the largest lengths and areas in comparison to other GCTA river systems. Records of mosquitofish presence in these large networks, as well as in other systems (Else, Hout Bay, Lourens, Sand, Sout, and Zeekoe), is of concern as it demonstrates their wide occurrence over the entire GCTA. Other river systems that have not been evaluated for mosquitofish presence (Bokramspruit, Krom, Schusters, Silvermine, Silverstroom, Sir Lowry, and Steenbras) have generally shorter lengths with high velocities and steep gradients. Mosquitofish generally prefer slow-flowing, lowland streams (Pyke 2008), with higher temperatures (Lee *et al*. 2017), therefore their establishment within rivers of this type, particularly those of the Southern Peninsula, is unlikely. This may explain why mosquitofish introduced into the Silvermine River in 1941 was not successful, one of few failed introductions in the area (Brown and Magoba 2009). It is likely that the headwaters of the Diep, Eerste, Hout Bay, Liesbeeck and Lourens Rivers are also not suitable for mosquitofish establishment. However, mosquitofish have been found to cause amphibian declines in mediterranean-type mountain streams in California (Goodsell and Kats 1999), therefore their occupation of these headwater zones cannot completely be ruled out and should be examined.

The larger river systems of the GCTA pass through areas that have largely been modified for agriculture, such as the upper reaches of the Diep, Eerste and Kuils; or modified for urban development (i.e. the low-land zones of the Diep, Eerste, Kuils, Lourens, and Sand Rivers). All of these rivers receive effluent water from waste water treatment works (WWTW; Brown and Magoba 2009). Nutrient enrichment from these sources is likely to contribute to macrophyte growth, which creates the ideal habitat for mosquitofish (Pyke 2008; Lee *et al*. 2017). In addition, the heat-island effect of the urban environment is also likely to allow mosquitofish establishment through the increase of water temperature (Stranko *et al*. 2008). A large portion of these rivers and their low-land zones are at sea-level, and their gentle gradients and low velocities are also likely to contribute to the increased temperatures of these waters.

Mosquitofish occurred in 16 of the 19 evaluated nature reserves areas which protect the endemic biodiversity of the GCTA, and although a large portion of conservation areas (14 sites) still need to be evaluated, this is concerning. For instance, mosquitofish were present in the majority of water bodies of the False Bay Nature Reserve: Rondevlei, Zeekoeivlei and the Strandfontein Birding Section, areas which are associated with the Zeekoe System. This invasive fish was also present at most of the water bodies that are protected by the Table Bay Nature Reserve: Rietvlei, Zoarvlei, and the Diep River Estuary. Mosquitofish occurrences have also been documented from the Zandvlei Estuary Nature Reserve, Kenilworth Racecourse Conservation Area, Intaka Island, Helderberg Nature Reserve, Edith Stevens Nature Reserve, Glencairn Wetland Reserve, and also the Liesbeeck River which is connected hydrologically with the Raapenberg Bird Sanctuary. Considering that many of these areas protect amphibians of conservation priority, mosquitofish may threaten amphibian species in these areas. This is further discussed below.

6.4.1 Mosquitofish distribution and threatened amphibian species of the GCTA

Threatened amphibians are protected by 20 of the 45 conservation areas in the GCTA. Of these 20 areas, that harbour amphibians of conservation concern, mosquitofish have been reported absent from three of these areas (see Table 5.4), but occurrences have been documented at 12 of these nature reserves, making up 60% of the reserves that contain threatened amphibians. Of the five remaining nature reserves, one is unsuitable for mosquitofish establishment, namely Rondebosch Common, and four areas still need to be evaluated for mosquitofish occurrence: the Hottentots Holland Nature Reserve, the Silverboomkloof section of the Helderberg Nature Reserve, De Hel Nature Area, and Uitkamp Wetland Nature Reserve.

An overlapping distribution of mosquitofish and threatened amphibians in the GCTA may be of concern, as this fish has been reported to prey heavily on amphibian eggs and larvae (Shulse and Semlitsch 2014, Smith and Smith 2015). Intensive predation levels cause sublethal effects and high mortality rates in amphibian populations, and may result in local extinctions (Goodsell and Kats 1999). However, the life histories and habitat preferences of certain amphibian taxa of the GCTA may isolate them from mosquitofish, making interaction improbable, even though both occur within the same area.

For instance, *Arthroleptella lightfooti* lays its eggs on damp soil or beneath moss, or at the bases of grass tussocks (Minter *et al*. 2004), therefore the likelihood of mosquitofish affecting this species is improbable. The same can be said for *Breviceps gibbosus,* as these are terrestrial breeders and lay their eggs in nest chambers which they construct under the soil surface (Minter *et al*. 2004). *Capensibufo spp.* tend to breed in small, shallow temporary pools during the rainy season (Minter *et al*. 2004), which is unsuitable habitat for mosquitofish as these require permanent waters to survive. Although habitat alteration may force temporary breeders to utilize permanent waters for breeding, this effect is unlikely for species of the *Capensibufo* genus, as these amphibians inhabit mountainous areas where habitat alteration for agriculture or urban development is improbable. The likelihood of *Heleophryne rosei* being threatened by mosquitofish is also small, as they normally inhabit mountain streams of Table Mountain at altitudes higher than 260 metres above sea-level. Although mosquitofish were found at a mountain pond north of Table Mountain during this field survey, at an altitude of roughly 225 m, *H. rosei* occurrences have not been recorded on the northern slopes of the mountain for the last few decades. Although recorded historically from Platteklip Gorge, a northern stream, they were not found in this area in a survey in 1980 (Boycott and De Villiers 1986), nor in the late 1990's (De Villiers 1997). It is reported that they generally prefer the eastern and southern slopes of Table Mountain that receive more rainfall (Minter *et al*. 2004). Consequently, unless mosquitofish inhabit other areas on the mountain, it is not likely that this species poses a threat to *H. rosei.*

The mosquitofish was also found in one of the dams at the Kenilworth Racecourse Conservation Area, renowned for its protection of the critically endangered *Microbatrachella capensis*. However, only one of the three dams on the reserve was inhabited, and not at the breeding site of *M. capensis.* This breeding site is composed of temporary wetlands, which do not retain water at the end of the dry season (S. Memami pers. comm.). Therefore, it seems improbable that mosquitofish would be a threat to this *M. capensis* population, although careful monitoring of the situation is recommended. This is the only surviving population of *M. capensis* within the GCTA, while other populations also occur at Betty's Bay, Kleinmond, and Ahulhus/Gansbaai (Minter *et al*. 2004). Although *M. capensis* mainly inhabits temporary wetlands, some of the reported breeding sites outside the GCTA do not dry up (Minter *et al*. 2004), which may be possible areas for mosquitofish establishment. Such sites should be evaluated and monitored.

Cacosternum capense is a temporary water breeder, which potentially eliminates the risk of mosquitofish threatening the species. However, 90% of its breeding sites are in modified habitats (Minter *et al*. 2004). As such land-use changes alter the aquatic landscape; drained and dammed areas may restrict their breeding areas, forcing them to utilize permanent water bodies. Therefore, mosquitofish may be a threat to this species, especially in the non-protected agricultural areas north of the GCTA where they are known to occur. This distribution is also within the catchment area of the Diep River, of which several mosquitofish occurrences have been reported at the headwaters and low-lying areas (Table D1), as well as the greater Berg River catchment area, of which mosquitofish occurrences have also been reported (Kleynhans *et al*. 2007). Nature Reserves in the GCTA that conserve this species are the Uitkamp Wetland Nature Reserve, of which mosquitofish occurrence is uncertain, and Rietvlei of the TBNR, where mosquitofish have been recorded in the past.

Xenopus gilli populations within the GCTA only occur at the Cape Point section of the Table Mountain National Park, where they inhabit seepages that have been excavated to form waterholes (Minter *et al*. 2004). These specific ponds have been reported to possess no threats to *X. gilli* (Minter *et al*. 2004), so it would seem that *X. gilli* populations at Cape Point would not be threatened by mosquitofish, unless they were illegally introduced into these areas. However, a few *X. gilli* populations also occur outside of the GCTA, and threats due to invasive fish would need to be evaluated for these populations.

By eliminating amphibians whose habitat preferences and life-histories do not coincide with the aquatic habitat required by mosquitofish, there is only one remaining IUCN threatened amphibian species that seems most at immediate risk to mosquitofish, namely *Sclerophrys pantherina*. Of the 13 conservation areas within the GCTA where this endangered amphibian is known to breed, mosquitofish have been recorded at 10 of these areas, which comprises 77% of the reserves that protect their breeding sites in the GCTA (see Table 5.4), namely Rondevlei, Zeekoeivlei and the Strandfontein Birding Sections of the FBNR, Princessvlei, Zandvlei Estuary Nature Reserve, Edith Stevens Wetland Park, Glencairn Wetland Nature Reserve, the Liesbeeck River at the Two Rivers Urban Park/Raapenberg Bird Sanctuary, and the Rietvlei section of the TBNR. These areas are composed of permanent water bodies, an aquatic habitat type that is generally preferred by *S. pantherina* for breeding. Future impact studies on *S. pantherina* should therefore be prioritized. Mosquitofish populations occurring in sympatry with *S. pantherina* at other breeding sites outside of the GCTA, from Betty’s Bay to the Pearly Beach (Minter *et al*. 2004), should also be evaluated.

6.4.2 Mosquitofish distribution and non-threatened amphibian species within the GCTA

Although only one of the 11 IUCN threatened amphibian species in the GCTA is likely to be at risk to mosquitofish predation, there are six other amphibian species in the GCTA that breed in aquatic habitat types that also support mosquitofish establishment, and predation by this fish species is likely where they co-occur. These are *Amietia fuscigula, Hyperolius horstockii, Sclerophrys capensis*, *Semnodactylus wealii, Strongylopus grayii,* and *Vandijkophrynus angusticeps.* These species all breed in a variety of aquatic habitat types, ranging from ponds, marshes, vleis, dams, or slow-flowing streams. These amphibians are not listed as threatened species and generally have widespread distributions, although some species have distributions limited to the Western Cape or occur in scattered regions within the province. For instance, *S. wealii* and *H. horstockii* only have a few localised distributions along coastal regions in the

Western Cape (Minter *et al*. 2004), although *S. wealii* does also occur in other areas of South Africa as well. *Vandijkophrynus angusticeps* is endemic to the Western Cape (Minter *et al*. 2004), while *T. delalandii, A. fuscigula,* and *S. capensis* have widespread distributions throughout South Africa (Minter *et al*. 2004). Further validation of the predation on *V. angusticeps* and *S. capensis* is necessary however, as tadpoles of bufonid species have been found to be unpalatable to fish predators in Chapters 2 and 3, and therefore may not be threatened by mosquitofish. The same also applies to the tadpoles of *S. pantherina*. However, due to the findings of Chapter 2, it is possible that mosquitofish may still cause significant harm to bufonid species, although not to the same degree as non-bufonids.

Mosquitofish are not able to affect amphibian species that use shallow seasonal pools for breeding, such as *Cacosternum aggestum*, *Cacosternum platys,* and *Strongylopus bonaespei.* However, as urbanisation and agricultural practises alter the aquatic landscape through the draining and damming of wetlands, these temporary breeders may be left with little suitable habitat and be forced to breed in permanent waters, as such modifications of aquatic habitat are also usually accompanied by invasive fish introductions (Hamer *et al*. 2002).

The GCTA is also inhabited by three extralimital amphibian species, namely *Hyperolius marmoratus, Sclerophrys gutturalis* and *X. laevis* (De Villiers 2006), which also share the aquatic habitat types utilised by mosquitofish (Minter *et al*. 2004). Fish predation on these three amphibian species may be beneficial for controlling their distributions, and may also have a positive effect on other native taxa. Through the reduction of these dominant species within an amphibian community, the reduction of interspecific competition may enable the proliferation of the native species (Walls *et al*. 2002). However, the reverse is also possible, and it is more likely that mosquitofish will increase the invasive potential of these extralimitals through differential predation of the local species. The endangered *S. pantherina* is known to be threatened by the invasive congeneric species, *S. gutturalis* (Measey *et al*. 2017), where they co-occur in Noordhoek and Constantia in the southern region of the GCTA. Further additive effects of *S. gutturalis* on *S. pantherina* due to mosquitofish predation should be considered in future studies.

6.4.3 Probable impacts on other biota in the GCTA

The western mosquitofish has caused declines of at least three native poecilid fish species in the USA (Meffe 1985; Laha and Mattingly 2007; Schumann *et al*. 2015), while its close relative, *G. holbrooki,* has been linked to the decline of a fish species in Australia, namely *Pseudomugil signifer* (Howe *et al*. 1987). Predation and aggression towards local fish species seem to be the primary stressors caused by mosquitofish (Laha and Mattingly 2007), through the display of antagonistic behaviour with biting, chasing and fin-ripping of natives (Sutton *et al*. 2013). Although only two indigenous freshwater species currently occupy the rivers and permanent wetlands of the GCTA, they share certain water bodies where mosquitofish have been recorded. The Cape galaxias (*Galaxias zebratus*), is generally widespread within the GCTA, although endemic to the CFR. Genetically unique populations of this species that may be threatened by mosquitofish are the *G. zebratus* populations that inhabit the Diep, Lourens and Hout Bay Rivers (Wishart *et al*. 2006). The Cape kurper (*Sandelia capensis*) is not a widespread species, with populations only occurring in the lower reaches of the Hout Bay, Diep and Eerste Rivers (Brown and Magoba 2009), all systems where mosquitofish have also been recorded. A third native fish species, the Berg River redfin (*Pseudobarbus burgi*), previously inhabited the Eerste River but is now locally extinct (Brown and Magoba 2009). The high endemicity and restricted geographical distribution of these species increase their vulnerability to the predatory impacts and competition of invasive fish.

Mosquitofish may also influence the estuarine biota of the GCTA, as this alien fish has been recorded in four estuaries of the area (the Diep River, Wildevoëlvlei, the Eerste River, and Zandvlei estuaries). Mosquitofish are also likely to inhabit the estuaries of the Hout Bay, Elsje, Lourens, and Sout rivers as the fish has been found in the lower reaches of these watercourses. Diadromous fishes are sometimes found in the lower reaches of rivers in the GCTA, such as the freshwater mullet (*Myxus capensis*), longfin eel (*Anguilla mossambica*) and estuarine round herring (*Gilchristella aestuaria*) (Brown and Magoba 2009). These fish may be affected when the lower reaches of rivers and the headwaters of functional estuaries are infested with mosquitofish. In particular, the Diep River and Wildevoëlvlei estuaries are two important nurseries for estuarine-dependent marine fish (Van Niekerk and Turpie 2012), areas known to be inhabited by mosquitofish as mentioned previously. However, very little is understood about invasive fish species in South Africa’s estuaries and their impacts on estuarine biota (Van Niekerk and Turpie 2012).

Little is also known about the invertebrate fauna of the GCTA (Holmes *et al*. 2012). However, high levels of endemic invertebrate species have been found on the South Peninsula (Picker and Samways 1996). Endemism in the invertebrate assemblages studied tend to decrease with altitude (Picker and Samways 1996), therefore it is likely that the majority of endemic species will not be affected by mosquitofish in the lowland areas. It is likely however, that mosquitofish will influence the aquatic invertebrates where they occur, due to their broad diets and high feeding rates (Rehage *et al*. 2005).

6.4.4 Main implications of this study

The results of this study, namely the literature review and field survey, provide information that contributes to the knowledge base of the distribution of the mosquitofish in the GCTA, which also makes up part of the CFR. However, further work needs to be done on their abundances and specific distributions within local watersheds, which is necessary in order to manage current invasions and predict future dispersion. Although the literature review and field survey yielded 61 records for mosquitofish occurrence from different localities within the GCTA, only four of these records were from peer-reviewed publications. This indicates a paucity of published information regarding the distribution of this species. There are also discrepancies in information concerning their general distribution. For instance, Brown and Magoba (2009) stated that mosquitofish are widespread within the dams and rivers of Cape Town, while Skelton (2001) indicated mosquitofish presence at only one point location towards the northern region of the GCTA. It is probable that their distribution within the GCTA, and possibly within the entire Western Cape, is underestimated due to a lack of records for this invasive species. This discrepancy in distribution data for the mosquitofish is a common problem for alien fish species in South Africa (Ellender and Weyl 2014). With a major focus on other alien fish taxa, particularly *Micropterus spp.* (Ellender and Weyl 2014), and with the previous opinion that their impact on native biota was minimal (de Moor and Bruton 1988), it is likely that the invasive potential and impact of this small poecilid fish have been largely disregarded and therefore research on this species has been neglected.

This literature review and preliminary field survey also indicate that mosquitofish occur in numerous conservation areas and in most of the large river systems of the GCTA, however additional sites within the area still need to be evaluated. Although conclusions cannot be made from distribution data, the wide occurrence of mosquitofish may threaten certain amphibian species within the GCTA, namely the IUCN endangered *S. pantherina*, and six other amphibians that are not listed as threatened according to IUCN criteria. Even if mosquitofish do not jeopardize the existence of these last six species, local extirpations of these amphibians should be prevented due to the ecosystem services that they provide. For instance, tadpoles greatly influence periphyton and algal community structure and decrease their biomass (Altig *et al*. 2007), factors which may boost the resilience of standing waters to eutrophication. Tadpoles are also temporary nitrogen sinks, which contribute to the nitrogen cycling process (Seale 1980), another factor which reduces the total suspended organic nitrogen in the water and may decrease eutrophication. Tadpoles are also an important food source for many consumer groups (Regester *et al*. 2006), and fulfill an important function within the food webs of ecosystems. Adult amphibians also put invertebrate populations in check (Beard *et al*.

2003), which may prevent exploding pest populations in agricultural and urban areas. The consumption of invertebrates also contributes to the nitrogen cycle, as the form of nitrogen in frog waste is more soluble than invertebrate waste (Beard *et al*. 2003). These are only a few of the ecosystem services that amphibians provide (see Hocking and Babbitt 2014, for a review on this topic), which are all services required in urban and agricultural environments, such as those that make up the GCTA. Proper ecosystem functioning is also necessary for the support of other biota, which will be necessary if the area is to maintain its rich and endemic biodiversity.

The acquired data provided by this study may be useful for future research and for conservation managers within the GCTA, as it provides a theoretical framework that identifies amphibian species that are most at risk of mosquitofish predation and that should be prioritized in future impact studies. This study also identifies areas that require further assessment for the presence of this invader.

CHAPTER 6 – SUMMATIVE DISCUSSION

The results of this study are summarised below, in the context of the study aims that were formulated in Chapter 1. The findings of this study demonstrate that mosquitofish have the potential to severely impact local amphibian populations, but that the predator-prey relationship remains complex, rendering the outcome in nature of an invasion on local communities hard to predict. Suggestions for mosquitofish management are given at the end of the chapter.

7.1 Main findings of this study

7.1.1 Aim 1: Determine the impact of mosquitofish on amphibian species of the Western Cape.

In the mesocosm trials (Chapter 2), mosquitofish completely wiped out tadpole sub-populations of *S. grayii* and *T. delalandii* within 1–2 weeks, despite the provision of alternate food for the fish and refuge for the tadpoles. Mortality was not significant for *S. pantherina* but the level of injuries was. This indicates that mosquitofish have the potential to severely impact amphibian populations through the direct effects of predation. However, indirect effects may also influence tadpoles, as the mesocosm trials revealed that surviving tadpoles experienced retarded development and growth. These detrimental physiological effects were associated with inactivity and stress in tadpoles, and refuge use and decreased foraging were strongly correlated with the level of injuries and mortalities within the tadpole sub-population.

It was found in the predation trials (Chapter 3 and 4) that mosquitofish are capable of consuming larger tadpoles that exceed their gape size by repeated biting until the prey is incapacitated. Predation intensity is also amplified when mosquitofish are in groups (Chapter 4), which increases the rate of morbid effects in tadpoles. The fact that the mosquitofish is not a gape-limited predator and that predation is socially facilitated, increases its potential to attack a wider range of prey sizes and with increased fervour.

7.1.2 Aim 2: Investigate whether certain amphibian species are more susceptible to mosquitofish predation than others.

Mesocosm trials (Chapter 2) showed that tadpole mortality and injury was higher in non-bufonid species (*T. delalandii* and *S. grayii*), than for a bufonid species (*S. pantherina*). There was also marked interspecific variation in the predation trials of Chapter 3, where injuries were so few in trials with the bufonids *S. capensis* and *C. magistratus*, to the point of being negligible, while the effects of predation where clearly evident across all treatments with the non-bufonid species *X. laevis* and *T. delalandii*. This indicates non-bufonid species are more palatable to mosquitofish than bufonid species, and predation effects are most destructive for the palatable species. However, results from Chapter 2, 3 and 4 also show interspecific variation in predation between the palatable non-bufonid tadpoles, although to a much lesser extent than to the unpalatable bufonids. Tadpole species was also a stronger determinant of mosquitofish predation than ontogeny (Chapter 3), or the absence or presence of alternative prey (Chapter 4).

7.1.3 Aim 3: Investigate whether certain developmental stages of amphibians within species are more susceptible to mosquitofish predation than others.

Predation trials in Chapter 3 revealed different patterns for ontogenic variations in palatability between bufonid and non-bufonid species. For the bufonid species (*C. magistratus* and *S. capensis*) there was no ontogenic variation, with all stages unpalatable towards mosquitofish. For the non-bufonid species (*T. delalandii* and *X. laevis*) smaller developmental stages (hatchling, free feeding, and early premetamorphic stages) were more palatable towards mosquitofish than larger developmental stages. However, mosquitofish predation on tadpoles of larger developmental stages was still significant, with a high degree of tadpole injuries and mortalities. This shows that although fish display a greater feeding response to smaller tadpoles, large tadpoles can still be impacted by mosquitofish predation to a significant degree, and that the mosquitofish is not a gape-limited predator.

7.1.4 Aim 4: Determine whether the presence of invertebrate prey alters the impacts of mosquitofish on amphibians.

Predation trials in Chapter 3 showed that the presence of alternative invertebrate prey had no significant effect on the overall predation impact that mosquitofish exerted on tadpoles. This suggests that mosquitofish impacts on amphibians will not be lessened by the presence of mosquito larvae or other invertebrate prey in natural settings. These results also show that the use of mosquitofish as a bio-control agent is likely to be ineffective, and rather disadvantage non-target natural biota.

7.1.5 Aim 5: Determine whether mosquitofish predation is amplified when they are in groups, in comparison with solitary fish.

Predation trials in Chapter 4 showed that mosquitofish exert significantly greater predation intensity on tadpoles when they are in groups, compared to solitary fish, even though the predator: prey ratio remains the same. This indicates that predatory behaviour in mosquitofish is affected by social factors, and should be considered in the experimental plan of impact studies with this species. It is likely that previous research on mosquitofish, whose findings demonstrate the detrimental impacts on amphibians, are in reality even more severe, because the sociality of the species was overlooked in these studies.

7.1.6 Aim 6: Determine where mosquitofish occur in sympatry with threatened amphibians, in order to determine the species most at risk in the greater Cape Town area of the Western Cape.

The literature review and field survey of Chapter 5 revealed that mosquitofish are widely distributed throughout the greater Cape Town area, and occur in many nature reserves that protect threatened amphibians. However, the only IUCN threatened species that occupies the same habitat type as mosquitofish is *S. pantherina*. Other amphibians currently listed as Least Concern (IUCN 2017) that may also be at risk of local declines or extirpations due to co-habitation with mosquitofish are *A. fuscigula*, *H. horstockii*, *S. capensis*, *S. wealii*, *S. grayii*, and *V. angusticeps*. However, due to the findings of Chapter 2 and 3, it is unlikely that the bufonid species (*S. capensis*, *S. pantherina*, and *V. angusticeps*) will be as palatable to mosquitofish as the non-bufonid species, and are not likely to be as impacted as the non-bufonids. However, in Chapter 2 it was found that mosquitofish exerted a significant amount of injury on tadpoles of *S. pantherina*, which could negatively impact the species.

7.2 Mosquitofish management

Due to the large predation effects recorded in the various amphibian species tested in Chapters 2–4, it is recommended that mosquitofish not be stocked in urban or rural ponds, dams and waterways. The spread of mosquitofish should also be prevented, and if possible they should be removed from areas where they are already established. However, managing alien fish invasions in South Africa is a challenging task (see Woodford *et al.* 2017). Therefore the above-mentioned interventions should be prioritised per area according to the framework developed by Kimberg *et al*. (2014). This decision support toolkit was developed in order to maximise the success of planned conservation interventions (see Figure 6.1), and is designed to make the best use of available human and financial resources, and prevent resistance from stakeholders that have conflicting interests over invasive fish (Woodford *et al*. 2017). Suggestions for mosquitofish management using this decision framework are discussed briefly below, according to practical implementation suggestions by Woodford *et al*. (2017).

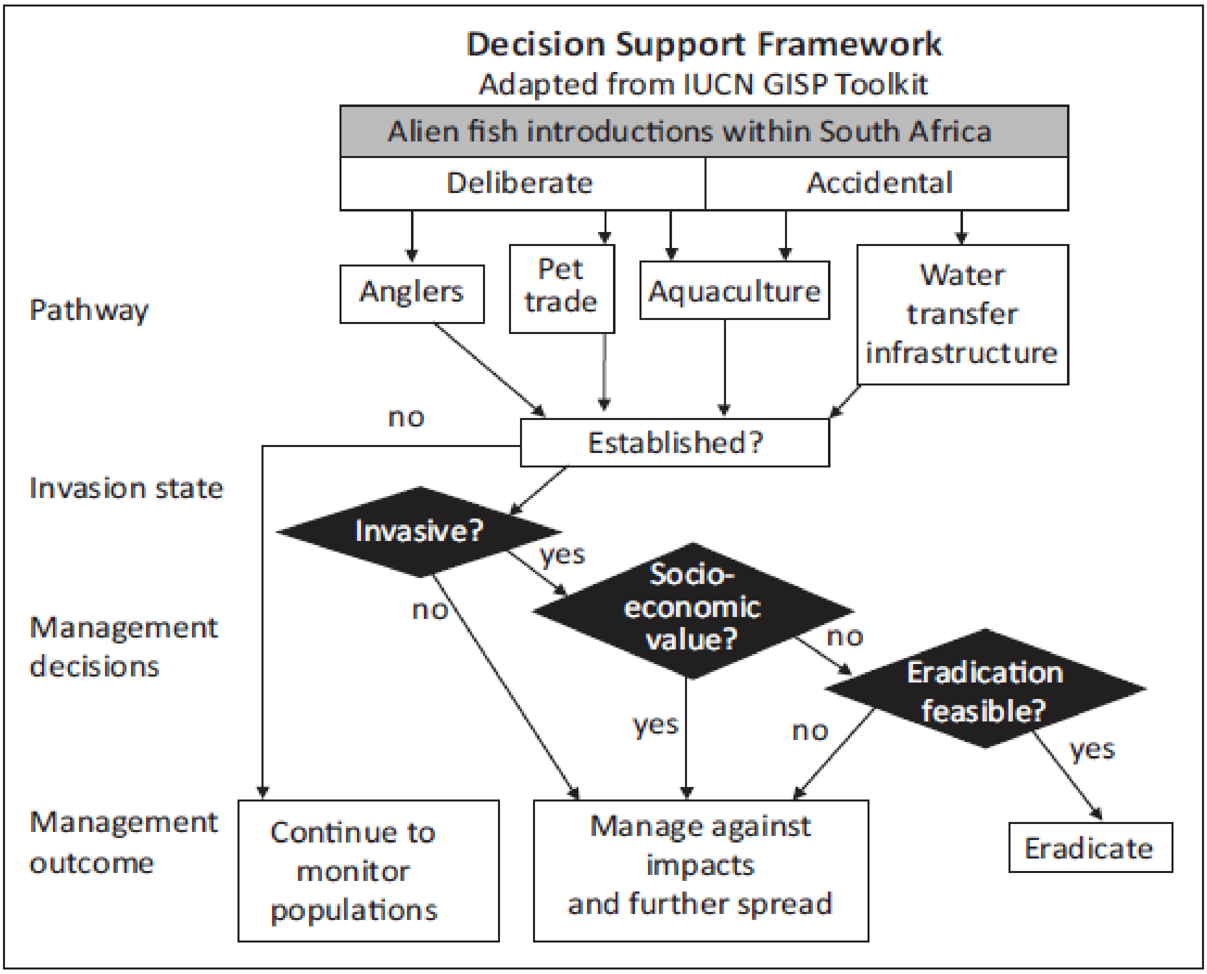

Figure 6.1. Decision-tree formulated by Kimberg *et al*. (2014) in order to manage alien fish invasions in South Africa.

The first step of the decision tree by Kimberg *et al*. (2014) is to evaluate whether the alien species is established (see Figure 6.1). This has already been confirmed for mosquitofish, as Ellender and Weyl (2014) evaluated the invasion status of alien and extralimital fish species in South Africa according to criteria proposed by Blackburn *et al*. (2011), and affirmed that this was a fully invasive and established species. This information also already answers the second question, 'Is the species invasive?' (Figure 6.1). Furthermore, a trait-based risk assessment by Marr *et al*. (2017), using the FISK mentioned previously in Chapter 5 (FISK; Copp *et al*. 2005) showed that mosquitofish are high-risk invasives for South Africa, which also affirms the second question.

Thirdly, it must be determined whether the invasive species has socio-economic value (Figure 6.1), as this determines whether it is a 'conflict species' so that management can be applied accordingly. Since the implementation of NEMBA in 2004, disputes have emerged between policy-makers and stakeholders over certain invasive fish (see Woodford *et al*. 2017), which hold up the implementation of conservation interventions for these species (Woodford *et al*. 2017). However, in a study by Zengeya *et al.* (2017), the mosquitofish was not listed as one of the five conflict-generating invasive fish species in South Africa. Fish were only placed into this category when they had both a high socio-economic benefit and a high impact on the environment. The 'harmful' category is best suited to mosquitofish: species that do not have a socio-economic benefit and high impact on the environment (Zengeya *et al*. 2017).

This is followed by the next question according to the decision tree criteria by Kimberg *et al*. (2014): 'Is it feasible to eradicate them?' Due to the widespread distribution of mosquitofish, eradication would require great logistical effort and is likely to be costly. Such efforts should first be prioritised to the protected areas where the effects of eradication are likely to pay off the most (Woodford *et al*. 2017). Eradication is likely to be worthwhile however, as it has been shown that the removal of mosquitofish in wetlands improves breeding in amphibians remarkably (Shulse *et al.* 2013; Pollard *et al.* 2017). Although controversial, mosquitofish eradication is possible through the application of rotenone. Fairly recent studies by Dalu *et al*. (2015) and Woodford *et al*. (2013) have shown that rotenone did not have a negative impact on local taxa when applied to the Rondegat River in the Cedarberg, a region within the CFR. See Weyl *et al*. (2016) for a guide on application procedures in a South African context.

For other areas where eradication is not possible, it is recommended that the next option according to the decision-support tool is implemented: impact mitigation and the prevention of dispersal. Impacts can be reduced through aquatic habitat enhancement and restoring the ecological integrity of aquatic systems. This increases the biotic resistance of natural fauna (Carpenter and Cottingham 1997) and reduces the amount of desirable habitat for mosquitofish. It is suggested by Lee *et al*. (2017) that the impacts of mosquitofish can be mitigated by the restoration of riparian vegetation along waterways, thereby influencing in-stream characteristics such as temperature, flow velocity and macrophyte growth, which are all determinants of mosquitofish abundance (Lee *et al*. 2017). In order to restore riparian habitat, a few suggestions are that urban waterways be lined with gabions instead of concrete, that alien vegetation be removed, and that the nutrient loads in effluent waste water discharged into watersheds be reduced.

Besides mitigating the impacts of mosquitofish, their spread must be prevented. Previous modes of dispersal were deliberate introduction for bio-control and as a fodder fish for bass (De Moor and Bruton 1988). Ellender and Weyl (2014) suggest that the vector pathway by which mosquitofish spread is no longer through bio-control, because invasion risks are better understood. However, Ellender and Weyl (2014) were referring to knowledge within the scientific community. Unfortunately, public attitudes towards mosquitofish that are not based on sound research are likely to still contribute to the illegal spread of the species via the 'bio-control agent' pathway. This is not through the purposeful release by government agencies as was practiced in the past, but via the pet trade. A simple internet search yields numerous advertisements for mosquitofish, for a moderate price. Such web-based advertisements over exaggerate the benefits of mosquito control as a marketing technique, despite the fact that it is illegal to sell this fish in the country. This incorrect promotion of mosquitofish is also reflected in a few aquarist forums and hobbyist sites on the internet. It is likely that in small artificial systems, such as in garden ponds, the hardiness and high feeding rates of mosquitofish will make it an effective agent at controlling mosquitoes. However, tolerating the possession and sale of this species increases the risk that it will be ignorantly or accidentally introduced into aquatic systems occupied by amphibians and other biota. Although the use of mosquitofish as a bio-control agent has often been justified in malaria-stricken areas, there is no sense in allowing its persistence in the Western Cape for this reason, as this region has no known mosquito-borne diseases that affect humans. Even if it were so, there is increasing evidence that mosquitofish are not as effective at controlling mosquitoes as previously thought, indicating that there is no sensible reason for promoting its use for bio-control. Laird (1988) stated nearly three decades ago: *"Mosquitofish are far too aggressive and predatory to be indiscriminately spread throughout the world without recognition of dangers to native biota. An international ban on their use as a control agent is biologically appropriate and warranted."*

In South Africa, the National Environmental Management: Biodiversity Act (RSA 2004) recognises the mosquitofish as an invasive species and currently it is listed as Category 1b for protected areas, Category 2 for the breeding of fish stock for zoos and animal breeders, and listed as Category 3 for all other areas (RSA 2014). Legislation for Categories 1b and 2 are more strict and prohibitive than for Category 3, where the latter allows the possession or presence of mosquitofish in already established areas, in order that its eradication is not a legal obligation. However, the breeding, buying, and selling of mosquitofish is prohibited according to Category 3 legislature, which makes the previously mentioned activities of selling fish and advertising over the internet illegal. However, there is a great divide between policy and practice in South Africa (Holmes *et al*. 2012; Woodford *et al*. 2017), with only loose regulation within the pet trade (Wilson *et al.* 2014). The trade of mosquitofish requires more stringent regulation and enforcement in order to curb the spread of this species. The misconceptions surrounding mosquitofish should also be addressed through education and public awareness.

The injudicious spread of mosquitofish is already reflected by the wide distribution of the fish in the urban capital of the Western Cape, the city of Cape Town. Such urban environments will have to maximise measures that increase the ecological integrity of green spaces and aquatic systems if endemic species are to be saved and ecosystem services maintained. This is an especially large and challenging problem for the management staff of the City of Cape Town, as this large city lies right on a biodiversity hotspot with very high conservation importance and low biotic resistance. Unfortunately, various problems in the jurisdiction of this city hinder conservation management at lower levels of government (Holmes *et al*. 2012), which is also a problem for the implementation of management strategies for invasive fish in the entire country as a whole (Woodford *et al*. 2017). Imperilled species and ecosystems will not be saved by short-term reactionary solutions that treat symptoms but not causes (Carpenter and Cottingham 1997). Instead, the urgent engagement of all stakeholders and spheres of government is required to develop integrated, long-term solutions by addressing the systemic causes that threaten the persistence of natural biota. The management of invasive species also requires a strong knowledge base, which is currently insubstantial for South Africa (Ellender and Weyl 2014). Further research is required on the distribution, dispersal pathways, establishment process, and impacts that this invasive fish has on amphibians, as well as other native species.

7.3 Conclusion

Although similar studies have been conducted abroad, this is the first study in South Africa to show that the mosquitofish has a negative impact on local amphibians. Several important aspects were identified, for instance that the mosquitofish has severe destructive potential, but also that several species are more susceptible to endangerment than others. These and the other findings of this research can be used to target certain aspects that require further study, such as specific amphibian species at risk of invasive fish predation. The acquired information may also be useful to policymakers and environmental managers for decision-making.

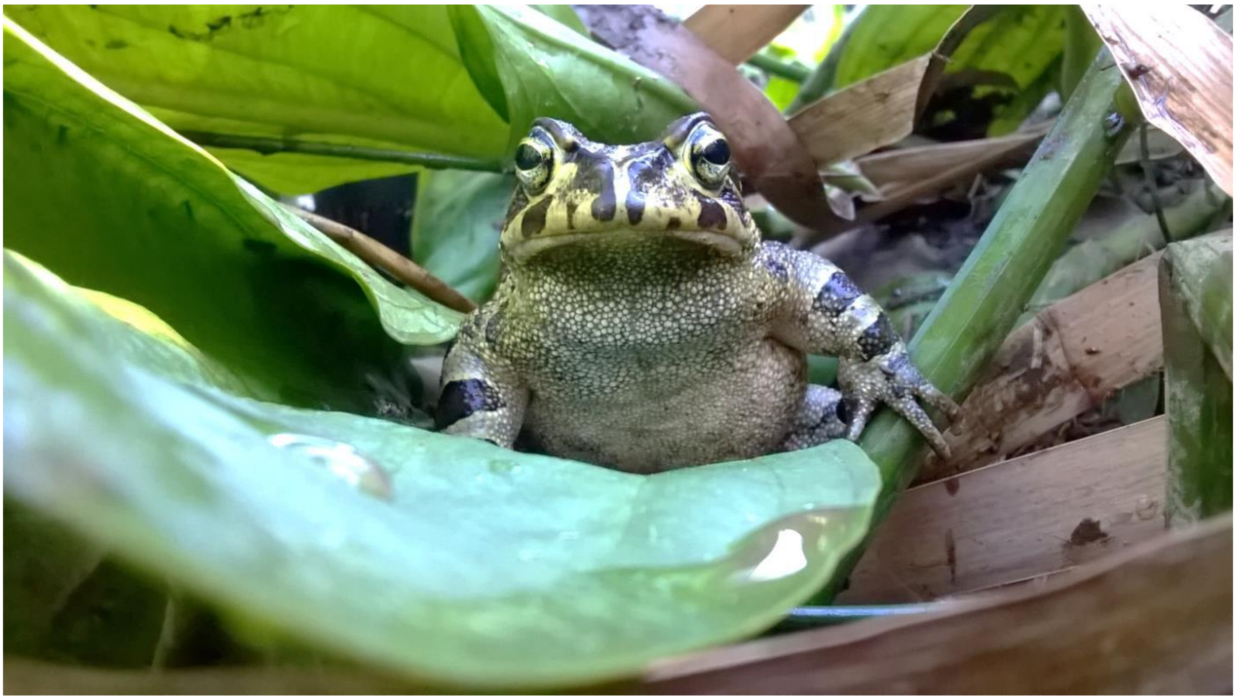

Figure 6.2. The Western Leopard Toad, *Sclerophrys pantherina.* Photo by Roxanne Conradie.

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APPENDIX

Addendum A: Data tables from Chapter 2

Table A1. Stocking densities and their literature sources.

Density (L.fish^{-1})	Fish per 150 L	Predator to prey ratio	Reference
13.6	11	n/a	Sutton *et al.* 2013
10.8	15	n/a	Lydeard and Belk 1993
16	9	n/a	Westhoff *et al.* 2012
16.6	9	n/a	Cech and Linden 1987
11.3	13.3	1 to 2	Segev *et al.* 2009
18.9	7.93	1 to 2	Stanback 2010
48.3	3.1	1 to 3.6	Lawler *et al.* 1999

Table A2. Type III tests of fixed effects for model intercepts.

HLM Model	Numerator df	Denominator df	*F*	Sig.
Total length	1	11.5	10011.1	<0.0001
Body width	1	11.3	6109.3	<0.0001
Gosner stage	1	9.8	95339.8	<0.0001
Length: width ratio	1	20.7	15368.1	<0.0001
Injury	1	11.3	7995.8	<0.0001
% Tadpoles in shelter	1	4.1	125.9	<0.0001
Tadpole abundance	1	5.5	4116.1	<0.0001
Foraging tadpoles	1	48	14.2	<0.0001

Table A3. Covariance parameters and Type III test results for each univariate model, with 'species' as fixed effect.

Species	**Residual estimate/ Mean square Error**	**MSE significance**	**MSE SE**	**Intercept variance**	**Intercept variance significance**	**Intercept variance SE**	**Type III test: Numerator DF**	**Type III test: Denominator DF**	**Type III test: F**	**Type III test: Significance**
Total length	3.10	<0.0001	0.12	0.06	0.20	0.05	2	1284.3	160.7	<0.0001
Body width	0.47	<0.0001	0.02	0.01	0.17	0.01	2	1289.5	938.5	<0.0001
Gosner stage	0.81	<0.0001	0.03	0.03	0.13	0.02	2	1304.6	255.4	<0.0001
Length: width ratio	0.21	<0.0001	0.01	0.00	0.37	0.00	2	1306.7	440.9	<0.0001
Injury	0.02	<0.0001	0.00	0.00	0.14	0.00	2	1297.9	300.4	<0.0001
% Tadpoles in shelter	96.80	<0.0001	4.46	34.45	0.17	24.83	1	944.0	909.2	<0.0001
Tadpole abundance	3.38	<0.0001	0.46	0.81	0.18	0.60	2	114.3	134.0	<0.0001

Table A4. Covariance parameters and Type III test results for each univariate model, with 'group' as fixed effect.

Group	**Residual estimate/Mean square Error**	**MSE significance**	**MSE SE**	**Intercept variance**	**Intercept variance significance**	**Intercept variance SE**	**Type III test: Numerator DF**	**Type III test: Denominator DF**	**Type III test: F**	**Type III test: Significance**
Total length	3.09	<0.0001	0.12	0.06	0.20	0.05	1	115.6	48.8	<0.0001
Body width	0.46	<0.0001	0.02	0.01	0.17	0.01	1	102.5	0.1	0.699
Gosner stage	0.81	<0.0001	0.03	0.03	0.13	0.02	1	64.2	4.4	0.040
Length: width ratio	0.21	<0.0001	0.01	0.00	0.37	0.00	1	302.0	30.3	<0.0001
Injury	0.02	<0.0001	0.00	0.00	0.14	0.00	1	89.3	304.2	<0.0001
% Tadpoles in shelter	96.80	<0.0001	4.46	34.45	0.17	24.83	1	4.1	38.0	0.003
Tadpole abundance	3.38	<0.0001	0.46	0.81	0.18	0.60	1	21.6	645.0	<0.0001
Foraging tadpoles	0.71	<0.0001	0.14	–	–	–	1	48	8.6	0.005

Table A5. Covariance parameters and Type III test results for each univariate model, with 'day' as fixed effect.

Day	**Residual estimate/ Mean square Error**	**MSE significance**	**MSE SE**	**Intercept variance**	**Intercept variance significance**	**Intercept variance SE**	**Type III test: Numerator DF**	**Type III test: Denominator DF**	**Type III test: F**	**Type III test: Significance**
Total length	3.09	<0.0001	0.12	0.06	0.20	0.05	6	1354.8	70.1	<0.0001
Body width	0.47	<0.0001	0.02	0.01	0.17	0.01	6	1354.7	395.9	<0.0001
Gosner stage	0.81	<0.0001	0.03	0.03	0.13	0.02	6	1354.3	163.6	<0.0001
Length: width ratio	0.21	<0.0001	0.01	0.00	0.37	0.00	6	1355.5	191.9	<0.0001
Injury	0.02	<0.0001	0.00	0.00	0.14	0.00	6	1354.7	165.1	<0.0001
% Tadpoles in shelter	96.80	<0.0001	4.46	34.45	0.17	24.83	4	944.2	1086.1	<0.0001

Table A6. Covariance parameters and Type III test results for each univariate model, with 'group' and 'day' interactions as fixed effect.

Group*Day	**Residual estimate/ Mean square Error**	**MSE significance**	**MSE SE**	**Intercept variance**	**Intercept variance significance**	**Intercept variance SE**	**Type III test: Numerator DF**	**Type III test: Denominator DF**	**Type III test: F**	**Type III test: Significance**
Total length	3.10	<0.0001	0.12	0.06	0.20	0.05	5	1355.2	56.3	<0.0001
Body width	0.47	<0.0001	0.02	0.01	0.17	0.01	5	1355.1	12.6	<0.0001
Gosner stage	0.81	<0.0001	0.03	0.03	0.13	0.02	5	1354.7	37.8	<0.0001
Length: width ratio	0.21	<0.0001	0.00	0.00	0.37	0.00	5	1355.7	13.8	<0.0001
Injury	0.02	<0.0001	0.00	0.00	0.14	0.00	5	1355.1	198.1	<0.0001
% Tadpoles in shelter	96.80	<0.0001	4.46	34.45	0.17	24.83	4	944.2	441.2	<0.0001
Tadpole abundance	3.38	<0.0001	0.46	0.81	0.18	0.60	15	110.2	45.5	<0.0001
Foraging tadpoles	0.71	<0.0001	0.14	–	–	–	11	48	1.4	0.216

Table A7. Covariance parameters and Type III test results for each univariate model, with 'species' and 'group' interactions as fixed effect.

Species* Group	Residual estimate/ Mean square Error	MSE significance	MSE SE	Intercept variance	Intercept variance significance	Intercept variance SE	Type III test: Numerator DF	Type III test: Denominator DF	Type III test: F	Type III test: Significance
Total length	3.09	<0.0001	0.12	0.06	0.20	0.05	2	46.0	189.4	<0.0001
Body width	0.47	<0.0001	0.02	0.01	0.17	0.01	2	45.0	42.1	<0.0001
Gosner stage	0.81	<0.0001	0.03	0.03	0.13	0.02	2	36.2	81.9	<0.0001
Length: width ratio	0.21	<0.0001	0.01	0.00	0.37	0.00	2	77.6	14.2	<0.0001
Injury	0.02	<0.0001	0.00	0.00	0.14	0.00	2	43.9	300.4	<0.0001
% Tadpoles in shelter	96.80	<0.0001	4.46	34.46	0.17	24.83	4	944.2	441.2	<0.0001
Tadpole abundance	3.38	<0.0001	0.46	0.81	0.18	0.60	2	15.2	31.5	<0.0001

Table A8. Covariance parameters and Type III test results for the tadpole abundance model, with 'species', 'group' and 'day' interactions as fixed effect.

Species* Group *Day	Residual estimate/Mean square Error	MSE significance	MSE SE	Intercept variance	Intercept variance significance	Intercept variance SE	Type III test: Numerator DF	Type III test: Denominator DF	Type III test: F	Type III test: Significance
Tadpole abundance	3.38	<0.0001	0.45	0.81	0.18	0.60	7	110.2	15.0	<0.0001

Addendum B: Data sheets of Chapter 3

Table B1. Results of the HLM analysis with four factors (species, developmental stage, alternative prey and time), their interactions, and their influence on PII scores. Significant *p* values are marked (*).

Type III Tests of Fixed Effects				
Source	**Numerator df**	**Denominator df**	**F**	**Sig. (*p*-value)**
Intercept	1	111.9	719.6	0.000*
Amphibian species	3	118.6	39.8	0.000*
Developmental stage	5	137.0	34.3	0.000*
Amphibian species * Developmental stage	3	132.1	12.8	0.000*
Alternative prey	1	115.7	12.3	0.001*
Amphibian species * Alternative prey	3	132.0	8.5	0.000*
Developmental stage * Alternative prey	4	160.7	14.7	0.000*
Amphibian species * Developmental stage * Alternative prey	2	133.9	0.7	0.500
Time interval	23	1914.3	108.1	0.000*
Amphibian species * Time interval	48	1912.0	12.0	0.000*
Developmental stage * Time interval	79	1913.4	5.1	0.000*
Alternative prey *Time interval	23	1911.6	2.7	0.000*
Amphibian species * Developmental stage * Time interval	26	1913.2	3.8	0.000*
Amphibian species * Alternative prey *Time interval	31	1911.6	3.0	0.000*
Developmental stage * Alternative prey * Time interval	38	1912.9	3.3	0.000*
Amphibian species * Developmental stage * Alternative prey * Time interval	14	1913.1	2.4	0.003*

Table B2. Covariance parameters of the above HLM analysis for PII scores.

Estimates of Covariance Parameters						
Parameter	**Estimate**	**Std. Error**	**Wald Z**	**Sig. (*p*-value)**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Residual	1.49	0.05	30.89	0.000	1.40	1.59
Intercept variance	0.91	0.14	6.57	0.000	0.68	1.23

Table B3. Results of the three-way ANOVA with three factors (species, developmental stage and alternative prey), their interactions, and their influence on PRI scores. Significant *p* values are marked (*).

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig. (*p*-value)**
Amphibian species	27.22	2	13.61	17.2	0.000*
Developmental stage	14.24	5	2.85	3.6	0.005*
Alternative prey	12.65	1	12.65	15.9	0.000*
Amphibian species * Developmental stage	7.71	3	2.57	3.2	0.025*
Amphibian species * Alternative prey	5.88	2	2.94	3.7	0.028*
Developmental stage * Alternative prey	1.87	4	0.47	0.6	0.671
Amphibian species * Developmental stage *Alternative prey	0.33	2	0.17	0.2	0.813

Table B4. Results of the HLM analysis with the two independent variables, fish sex and time, and their influence on PII scores. Significant *p* values are marked (*).

Type III Tests of Fixed Effects				
Source	**Numerator df**	**Denominator df**	**F**	**Sig. (*p*-value)**
Intercept	1	139.3	89.5	0.000*
Time interval	23	2150.4	29.2	0.000*
Fish sex	3	314.3	19.7	0.000*
Time interval * Fish sex	56	2148.7	3.2	0.000*

Table B5. Covariance parameters of the HLM analysis for fish sex and PII scores.

Estimates of Covariance Parameters						
Parameter	**Estimate**	**Std. Error**	**Wald Z**	**Sig. (*p*-value)**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Residual	3.78	0.12	32.70	0.000	3.56	4.01
Intercept variance	4.43	0.61	7.21	0.000	3.37	5.81

Table B6. Results of the univariate ANOVA with PRI score as dependent variable and fish sex as independent variable. Significant *p* values are marked (*).

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig. (*p*-value)**
Sclerophrys capensis	0.28	2	0.14	0.9	0.405
Tomopterna delalandii	3.08	2	1.54	1.8	0.179
Xenopus laevis	12.13	2	6.06	3.4	0.043*

Table B7. Mean tadpole and fish lengths according to trial type.

Species	**Developmental stage**	**Mean tadpole total length**	**Std. Error**	**Mean fish total length**	**Std. Error**
Capensibufo magistratus	Late pro-metamorphosis	18.2	0.36	23.6	0.39
Sclerophrys capensis	Free feeding stage	12.0	0.36	26.0	0.71
	Late pre-metamorphosis	17.6	0.36	25.6	0.72
	Late pro-metamorphosis	25.2	0.33	26.3	0.76
Tomopterna delalandii	Hatchling stage	5.5	0.34	30.7	0.89
	Free feeding stage	8.5	0.36	26.3	0.72
	Late pre-metamorphosis	23.8	0.34	24.3	0.93
	Late pro-metamorphosis	32.6	0.34	23.8	1.5
Xenopus laevis	Free feeding stage	11.5	0.26	23.3	0.64
	Early pre-metamorphosis	20.5	0.29	31.0	1.9
	Late pre-metamorphosis	32.2	0.36	28.8	1.5

Addendum C: Reference list for literature review of Chapter 4

Table C1. Feeding trials used to examine predatory behaviour of either *Gambusia affinis* or *Gambusia holbrooki*.

Author(s)	Predators in feeding trial	Number of predators	Prey type	Prey species	Purpose of research
Bence and Murdoch 1986	*Gambusia spp.*	1	Invertebrate	*Daphnia spp.*	Examine prey size selection
Drake *et al*. 2014	*G. affinis; Notophthalmus viridescens louisianensis* (amphibian); Dragonfly larvae; *Rana sphenocephala*(amphibian)	3	Amphibian	*Ambystoma annulatum*	Differences in predation on *A. annulatum* by different predators
Gregoire and Gunzburger 2008	*G. holbrooki*	1	Amphibian	*Rana capito*	To test impacts of mosquitofish and differential predation
				Rana sphenocephala	
Grubb 1972	*G. affinis*	1	Amphibian	*Acris crepitans crepitans*	Test differential predation
				Incilius valliceps	
				Gastrophryne olivacea olivacea	
				Dryophytes chrysoscelis	
				Pseudacris clarkii	
				Scaphiopus couchii rectifrenis	
				Rana pipiens	
Komak and Crossland 2000	*G. holbrooki*	1	Amphibian	*Opisthodon ornatus*	To test impacts of mosquitofish and differential predation
				Bufo marinus	
Linden and Cech 1990	*G. affinis*	12	Invertebrate	*Anopheles, Culex, Daphnia, Cenocorixa, Hyalella, Buenoa spp.*	Differential predation effects amongst inverts
Pyke and White 2000	*G. holbrooki*	5 to 10	Amphibian	*Ranoidea aurea*	To test impacts of mosquitofish
				Pengilleyia peronii	
Segev *et al*. 2016	*G. affinis*; *Barbus paludinosus* (fish); *Pseudocrenilabrus philander* (fish)	1	Invertebrate	*Culex spp.*	Examine predation on eggs and larvae
Smith and Smith 2015	*G. affinis*	1	Amphibian	*Dryophytes versicolor*	To test impacts of mosquitofish
Walls *et al*. 2002	*G. affinis*	1	Amphibian	*Gastrophryne carolinensis carolinensis*	Differences in susceptibility to predation
				Dryophytes squirellus	
Zeiber *et al*. 2008	*G. affinis*	2	Amphibian	*Rana sylvatica*	To test impacts of mosquitofish predation
				Bufo americanus charlesmithi	
				Ambystoma tigrinum	
				Pseudacris triseriata	

Table C2. Studies that utilized feeding trials and field observations to examine the predatory behaviour of mosquitofish (*G. affinis* or *G. holbrooki*) in order to test their impacts on amphibians.

Author(s)	Experimental design	Predators	Number of predators	Amphibian prey species
Goodsell and Kats 1999	Feeding trial and field observations	*G. affinis*	2	*Hyliola regilla*
Lawler *et al*. 1999	Feeding trial and mesocosms	*G. affinis*	1	*Aurorana draytonii*
Morgan and Buttemer 1996	Feeding trial and field assessment	*G. holbrooki*	5	*Ranoidea aurea*
				Rawlinsonia dentata
Reynolds 2009	Feeding trial and field assessment	*G. holbrooki*	6	*Crinia insignifera*
				Crinia affinis affinis
				Coggerdonia adelaidensis
				Ranoidea moorei
Webb and Joss 1997	Feeding trial and field assessment	*G. holbrooki*	5 and 10	*Pengilleyia peronii*
				Ranidella varius

Table C3. Studies that used large-scale mesocosm trials (or experimental wetlands) and/or field assessments to examine predatory behaviour either *G. affinis* or *G. holbrooki,* in order to test the impacts on amphibians.

Author(s)	Experimental design	Predators	Amphibian prey species
Lawler *et al*. 1999	Field assessment	*G. affinis*	*Aurorana draytonii*
Pollard *et al*. 2017	Field assessment	*G. holbrooki*	*Ranoidea aurea*
Shulse and Semlitsch 2014	Experimental wetlands	*G. affinis*	*Rana catesbeiana*
			Rana clamitans melanota
			Rana areolata circulosa
			Rana blairi
			Rana sphenocephala
Shulse *et al*. 2013	Experimental wetlands	*G. affinis*	*Dryophytes versicolor*
			Dryophytes chrysoscelis
			Pseudacris maculata
			Rana clamitans melanota
Stanback *et al*. 2010	Mesocosm experiment	*G. holbrooki*	*Pseadacris feriarum*
Segev *et al*. 2009	Mesocosm experiment and field assessment	*G. affinis*	*Salamandra semenovi*

Table C4. Studies that used feeding trials to examine the interactions between a predator species and prey species.

Author(s)	Predator	Experiment type	Number of predators	Amphibian prey species	Purpose of research
Adams *et al.* 2011	*Lepomis cyanellus*(fish) *Lepomis macrochirus* (fish) *Procambarus nigrocinctus* (crayfish) Anax junius (dragonfly larvae)	Feeding trial	1	*Rana sphenocephala*	Relative palatability of tadpoles toward different predators
				Rana catesbeiana	
				Rana clamitans melanota	
				Bufo woodhousii	
				Bufo nebulifer	
				Dryophytes versicolor	
				Dryophytes cinereus	
				Pseudacris crucifer	
				Pseudacris fouquettei	
				Acris crepitans crepitans	
				Gastrophryne carolinensis carolinensis	
				Scaphiopus hurterii	
Brodie and Formanowicz 1987	*Notophthalmus viridescens viridescens* (amphibian) *Anax junius* (dragonfly larvae) *Belostoma spp.* (water bugs)	Feeding trial	1	*Pseudacris crucifer*	Differences in susceptibility to predation
				Bufo americanus charlesmithi	
				Rana sylvatica	
Brodie and Formanowicz 1983	*Dytiscus verticalis* (diving beetle) *Anax junius* (dragonfly larvae) *Lethocerus americanus* (giant water bugs) *Ambystoma jeffersonianum* *Notophthalmus viridescens viridescens* (amphibian)	Feeding trial	1	*Rana sylvatica*	Susceptibility of predation due to size
				Rana clamitans melanota	
				Rana palustris	
				Pseudacris crucifer	
Brodie *et al.* 1978	*Dytiscus verticalis* (diving beetle) *Lethocerus americanus* (giant water bugs)	Feeding trial	1	*Bufo americanus charlesmithi*	Palatability of tadpoles, ontogenic development of noxiousness
				Pseudacris crucifer	
Crossland 1998	*Lethocerus insulanus* (Belostomatids) *Hydaticus vittatus* (diving beetle) *Cherax quadricarinatus* (crayfish)	Feeding trial	1	*Bufo marinus*	Palatability of tadpoles to invertebrate predators, ontogenic development of noxiousness
Crossland 2001	*Lates calcarifer* (fish)	Feeding trial	1	*Bufo marinus*	Fish avoidance of toxic tadpoles
	Hephaestus fuliginosus (fish)				

Table C4 cont.: Studies that used feeding trials to examine the interactions between a predator species and prey species.

Author(s)	Predator	Experiment type	Number of predators	Amphibian prey species	Purpose of research
Formanowicz and Brodie 1982	*Cambarus diogenes* (crayfish) *Orconectes propinquus* (crayfish) *Dytiscus verticalis* (diving beetle) *Lethocerus americanus* (giant water bugs)	Feeding trial	1	*Notophthalmus viridescens viridescens*	Differences in susceptibility to predation
				Rana clamitans melanota	
				Rana sylvatica	
				Rana palustris	
				Bufo americanus charlesmithi	
				Pseudacris crucifer	
				Ambystoma maculatum	
				Rana catesbeiana	
Heyer *et al.* 1975	*Leptodactylus pentadactylus* (amphibian)	Feeding trial	1	*Bufo marinus*	To test palatability of tadpoles
				Physalaemus pustulosus ruthveni	
Lawler and Hero 1997	*Lates calcarifer* (fish)	Feeding trial	1	*Bufo marinus*	To test palatability of tadpoles
				Opisthodon ornatus	
Light 1969	*Salmo clarkia* (fish) *Ambystoma gracile* (amphibian) *Gasterosteus aculeatus* (fish)	Feeding trial	Groups	*Rana catesbeiana*	To test palatability of eggs
				Aurorana aurora	
				Rana pipiens	
				Bufo boreas halophilus	
				Rana clamitans melanota	
				Hyliola regilla	
Manteifel and Reshetnikov 2002	*Perccottus glenii* (fish)	Feeding trial	1	*Rana temporaria*	To test palatability of tadpoles
				Bufo bufo bufo	
				Rana arvalis	
Voris and Bacon 1966	*Lepomis macrochirus* (fish)	Feeding trial	1	*Bufo americanus charlesmithi*	To test palatability of tadpoles
				Pseudacris triseriata	
Walters 1975	*Ambystoma maculatum* (amphibian) *Ambystoma jeffersonianum* (amphibian) *Ambystoma opacum* (amphibian) *Notophthalmus viridescens viridescens* (amphibian)	Feeding trial	1	*Dryophytes versicolor*	Differences in susceptibility to predation
				Pseudacris triseriata	
				Ambystoma maculatum	
				Ambystoma jeffersonianum	
				Ambystoma opacum	
				Notophthalmus viridescens viridescens	
				Rana pipiens	
				Rana sylvatica	
				Bufo americanus charlesmithi	
				Rana catesbeiana	
				Rana clamitans melanota	

Table C5. Results of the HLM analysis with three fixed effects (amphibian species, school size, and time), their interactions, and their influence on PII scores. Significant *p* values are marked (*).

Type III Tests of Fixed Effects				
Source	**Numerator df**	**Denominator df**	**F**	**Sig.** **(*p*-value)**
Intercept	1.0	9.	779.6	0.000*
Amphibian species	1.0	20.6	60.2	0.000*
School size	2.0	313.7	15.0	0.000*
Elapsed time	136.0	307.0	8.4	0.000*
Amphibian species * School size	2.0	311.5	0.93	0.395
Amphibian species * Elapsed time	10.0	309.4	5.0	0.000*
School size * Elapsed time	53.0	309.2	0.77	0.875
Amphibian species * School size * Elapsed time	7.0	309.8	0.70	0.673

Table C6. Covariance parameters of the above HLM analysis for PII scores.

Estimates of Covariance Parameters						
Parameter	**Estimate**	**Std. Error**	**Wald Z**	**Sig.**	**95% Confidence Interval**	
				(*p*-value)	**Lower Bound**	**Upper Bound**
Residual	2.78	0.22	12.42	0.000	2.37	3.25
Intercept variance	0.41	0.24	1.73	0.084	0.13	1.28

Addendum D: Supplementary information for Chapter 5

D1. Background information on the hydrology of the Greater Cape Town Area (GCTA)

The hydrological region of the Cape Floristic Region (CFR) area is called the Cape Fold aquatic ecoregion, which is synonymous with the CFR (Ellender *et al*. 2017). This is a region composed of mediterranean-type systems to the west, and temperate coastal systems to the east, mainly with relatively short coastal rivers that drain the Cape Fold Mountains. The CFR was divided into eight biogeographic zones, based on habitat discontinuities between the river systems: (1) Olifants, (2) Berg, (3) western Agulhas, (4) Breede and adjacent rivers, (5) Gouritz, (6) south coastal rivers, (7) Gamtoos and adjacent rivers, and (8) Swartkops, Sundays and adjacent rivers (Skelton, 1980). The areas that are managed by the Western Cape Government are more broadly divided into four Water Management Areas (WMAs): the (1) Olifants/Doorn, (2) Berg, (3) Breede, and (4) Gouritz.

The GCTA hydrological area lies within the Berg biogeographic zone or WMA. This area has a mediterranean climate, which is due to the frontal systems that move eastward over the Southern Ocean, which bring rains in the winter (April to September). The frontal systems are weaker and tend to move more south in the summer, which causes a predominantly winter-rainfall region with strong winds (Brown and Magoba 2009). This influences the flow regime of the rivers: they are mostly seasonal, with the strongest flow in the winter.

In order to simplify the description of surface hydrology the GCTA, it can be divided into four geographic areas: the northern, central, southern, and eastern areas (RHP 2005). This entire region encompasses the entire Cape Peninsula, the South Western Coastal Belt up until Atlantis and Malmesbury in the north, and to the Hottentots Holland Mountains in the east (Figure 5.1).

D.1.1 The northern region of Cape Town

The northern region has the largest catchment area of 1087 km² (RHP 2005). Major rivers of the north are the Diep River, which rises in the Riebeeck-Kasteel and Perdeberg mountains, and flows westward and enters the sea at Table View. Major tributaries that join with the Diep from the eastern side of its headwaters are the Klapmuts, Kalbaskraal, Mosselbank and Vissershok Rivers (Figure D1). The Diep River used to be connected to a major wetland further south at Table View, Rietvlei, but it now empties into the sea only by the Milnerton Lagoon. Rietvlei and the Milnerton Lagoon are collectively known as the Diep River estuary (Brown and Magoba 2009) and are protected by the TBNR. The Diep River system receives treated waste water at two locations: the Mosselbank from the Kraaifontein WWTW, and the Milnerton Lagoon from the Potsdam WWTW Works (Brown and Magoba 2009). Other smaller rivers in the northern region of Cape Town that are not part of the Diep system are the Buffels, Modder, Silverstroom and Sout Rivers, which all drain westward into the ocean. Both the Modder and Buffels rivers are ephemeral.

D.1.2 The central region of Cape Town

This is the smallest catchment area of the Cape Town region, with an area of 327 km² (RHP 2005). Major rivers of the central region are the Hout Bay River and the Salt/Black/Liesbeeck/Elsieskraal River system (Figure D2 and D3). The Hout Bay River rises from various tributaries in Table Mountain, primarily the Disa and Main Disa Streams (Brown and Magoba 2009), and flows southwards through the Orange Kloof. After exiting the Kloof, smaller streams join the Hout Bay River, the main ones being the Baviaanskloof River and the Bokkermanskloof Stream, before the river enters the sea via the Hout Bay Estuary. This estuary is a small and temporarily open system.

Salt/Black/Liesbeeck/Elsieskraal River system is mainly canalised and predominantly surrounded by urban development, with exceptions of the headwaters of the Elsieskraal and the Liesbeeck Rivers. The Liesbeeck drains the eastern slopes of Table Mountain and originates at Kirstenbosch Botanical Gardens. The Liesbeeck flows in a north-easterly direction and joins with the Black River, the latter of which has its headwaters in Arderne Gardens. The confluence that these two rivers form gives rise to the Salt River, which covers only a few kilometres of land before draining into Table Bay at Paarden Eiland. A wetland that was once connected to the Salt River is Zoarvlei, which now only receives water from urban runoff and groundwater intrusion (Brown and Magoba, 2009). Another wetland area in this river system is the Raapenberg Bird Sanctuary, situated on the Liesbeeck before its confluence with the Black River.

There are also side canals and tributaries that feed the Black River before it converges with the Liesbeeck. The main tributary is the Elsieskraal River, which begins in the Tygerberg Hills on the farm Altydgedacht, and flows in a south-westerly direction before joining with the Black River. Other side canals that feed the Black River include the Kromboom, Vygekraal, Bokmakierie, Jakkalsvlei, Kalksteenfontein and Blomvlei Canals. Most of these were once vleis or watercourses, but have now been canalised in order to carry the runoff from developed areas on the western side of the Cape Flats, such as Langa, Athlone, and Epping Industria. The Black River receives sewage effluent from Athlone and Borcherds Quarry WWTW (RHP 2005).

A large wetland area that lies within the central region of Cape Town, but that is not connected with the major rivers, is Intaka Island, which was once known as Blouvlei. It now forms part of the artificially constructed canals of the Century City complex, and its main source of water is partially treated effluent water from the Potsdam WWTW (Brown and Magoba 2009). The Green Point Urban Park contains another wetland area that is located in central region of the GCTA. This wetland is primarily fed with water that flows off Table Mountain, from a group of springs in Oranjezicht. This water is not suitable for human use and is diverted to Green Point through storm water drains (Brown and Magoba 2009).

D.1.3. The southern region of Cape Town

This area is has a catchment area of 471 km² (RHP 2005), and is predominantly made up of short rivers and wetland systems that drain the South Peninsula. The area spans from Mitchell's Plain of the Cape Flats in the east, to Constantia Berg in the north, and Cape Point in the south. On the eastern side of the Peninsula, there are two main hydrological areas: rivers and wetlands that neighbouring and connected to Zeekoeivlei, and those linked with Zandvlei.

There are three rivers that flow into Zandvlei: the Keysers, the Sand and the Westlake Rivers (Figure D5). Much of the lengths of these are canalised (Brown and Magoba 2009). The Keysers River has the largest catchment area of the three rivers that feed Zandvlei, and has three tributaries of its own: the Spaanschemat, Grootboschkloof and the Prinseskasteel Rivers. These all arise in Constantiaberg, flowing in a south-westerly direction, and join to form a confluence in Tokai as the Keysers. The second tributary of Zandvlei, the Sand River, is the northernmost of the three. It first begins as the Diep River and originates at Wynberg Hill, and flows in a southward direction to a small wetland, Little Princessvlei. Once it has passed the vlei, it is no longer called the Diep River but the Sand. The third river that flows into Zandvlei is the Steenberg or Westlake River. It is the southernmost river of the three and flows in an easterly direction, which arises in Steenberg and flows into Kirstenhof wetlands before entering Zandvlei. The latter is a large estuarine system which is composed of three parts: the Main Vlei, the Marina da Gama canal system, and the Westlake Wetlands (Brown and Magoba 2009). Capricorn Lake, an artificially constructed water body within Capricorn Park, lies east of Marina da Gama.

Zeekoeivlei is a large wetland in the suburban area of Grassy Park, and has a surface area of 2.56 km² (Brown and Magoba 2009). It receives water runoff by way of the Big Lotus and Little Lotus canals that drain the urban areas around Landsdowne, Gugulethu, and Nyanga (see Figure D5). Other wetlands in the same area are Rondevlei, which lies west of Zeekoeivlei and is smaller in size, and Princessvlei, which is the smallest of the three and that is connected to Rondevlei by a watercourse. Both Rondevlei and Zeekoeivlei have the same outlet to the sea, a canal called the Zeekoe. The Zeekoe outlet canal also receives water by way of effluent from the Strandfontein WWTW (Brown and Magoba, 2009), another large wetland area between Zeekoeivlei and the coastline. Although artificial constructed for waste water management, the Strandfontein wetland area has become an important area for bird conservation and together with Zeekoeivlei and Rondevlei, forms part of the FBNR. Another section of the FBNR is the Pelican Park Wetlands, situated to the east of Zeekoeivlei and Rondevlei. Edith Stevens Nature Reserve is another protected area in the region and lies north of the Phillipi horticultural area. This wetland receives water from the Big Lotus canal (RHP 2005).

Further south of the FBNR and Zandvlei estuary, there are several additional eastwardly flowing rivers that drain into False Bay (Figure D6). The Silvermine River rises in the Steenberg Mountains, enters a coastal plain known as the Silvermine Wetlands, and then reaches the sea at Clovelly. The second is the Elsje River, which has parts the Red Hill plateau as its catchment area and runs downstream into the Glencairn Vlei before reaching the sea. The Klawersvlei River also arises from the top of Red Hill, and has two storage dams on its course at the top of the plateau, the Jackson and Rawson Dams. It flows rapidly down the plateau, forming the Admiral’s Waterfall, and drains into the sea at Simon’s Town. Additional storage dams on the mountain plateaus of the Southern Peninsula are the Alexandra, De Villiers, Hely-Hutchinson, Victoria, and Woodhead reservoirs on top of Table Mountain, Silvermine Dam on the Steenberg Plateau, and the Lewis Gay and Kleinplaas dams on the Red Hill plateau above Simon’s Town.

On the western side of the Peninsula, there are numerous connected wetlands in the Noordhoek Valley basin (Figure D7). These are the Papkuilsvlei to the north, Lake Michelle on the western side of the valley, Louw’s Vlei at the centre, and the twin Wildevoël vleis and the Wildevoël WWTW to the south. On the western side, the Noordhoek backshore lagoons lie along the Noordhoek beach, with the southern lagoon being fed by the Wildevoël Vlei Wetlands, which receives its main water from the WWTW. The two main rivers in this area are the Brookwood Stream, positioned north of the Noordhoek wetlands and which flows into Papkuilsvlei; and Bokramspruit, which is not connected to the Noordhoek wetland area but arises above Ocean View south of the wetlands and flows directly into the sea.

The southernmost region of the Peninsula is largely protected by the Cape Point Nature Reserve. As a result, most of these rivers in this area are in a natural state (RHP 2005). The Schusters River drains the Red Hill and Wildeschutzbrand areas, and flows westward to meet the sea just south of Scarborough. Further south, the Krom and the Klaasjagters Rivers converge to form the Hout River, which drains towards the west of Cape Point. On the eastern side of Cape Point, the Buffels River drains into the False Bay.

D.1.4. The eastern region of Cape Town

This area is the second largest catchment area, with a size of 588 km². Main rivers of the eastern part of Cape Town are the Eerste, Kuils, Lourens, Sir Lowry's Pass, and the Steenbras.

The Eerste River is primarily fed by three main tributaries (Figure D8). The first is the Jonkershoek, which originates in the Hottentots Holland Mountain Reserve, and flows through the Jonkershoek valley and past Stellenbosch (RHP 2005). Other sources call the Jonkershoek River the Eerste River (Brown and Magoba 2009). The Kleinplaas Dam at Jonkershoek is on its headwaters, and is linked with the Theewaterskloof Dam by an interbasin water transfer scheme. Just southeast of Stellenbosch, the Jonkershoek/Eerste River forms a confluence with the Plankenberg and the Krom Rivers that drain the northern region of Stellenbosch. Further south of the river, other tributaries flow into it from the eastern side, namely the Blou Klip and Bonte Rivers, which drain the western slopes of Stellenbosch Mountain, before it empties into the False Bay near Macassar. Unfortunately the water quality of the Eerste River is deteriorated, due to the more turbid water it receives from Theewaterskloof Dam by the Berg-Riviersonderend interbasin transfer scheme (RHP 2005), and also due to effluent that is discharged at both its headwaters and the low land zones (Brown and Magoba 2009). These effluent sources are from the Stellenbosch and Macassar WWTW, respectively. Just before entering the sea, the Eerste River is met with the Kuils River that flows from the west, and the Moddergat River from the west.

The Kuils River begins near Durbanville and flows southward through the Cape Flats before reaching the Eerste River at Macassar (see Figure D8). Its only main tributary is the Bottelary River, which flows from the direction of Stellenbosch through agricultural land before meeting the Kuils River under the Van Riebeeck Road Bridge in the suburb Kuils River. Wetlands that are associated with the Kuils River in the Cape Flats are the Driftsands Nature Reserve, Mfuleni wetlands, Nooiensfonteinvlei, and the Dreamworld Wetlands. The Kuils River also receives effluent from the Bellville, Kuils River, Scottsdene, Zandvliet, Mfuleni and SA Infantry Battalion WWTW (Brown and Magoba 2009).

The Lourens River arises from the Hottentots Holland Mountain Range and flows in a south-westerly direction through Somerset West. Just before emptying into the sea it forms a small estuary west of the Strand. A relatively large water body in this area is Paardevlei, which lies west of the Lourens River, and is connected to it by way of Melcksloot stream. The Sir Lowry's Pass River also rises in the Hottentot Holland Mountain Range at Moordenaarskop, and then flows in a south-westerly direction to the sea at Gordon's Bay. A diversion canal that branches off from the Sir Lowry's River, 2 km upstream of the mouth, was built in 1993 for flood alleviation (Brown and Magoba 2009). A third river that also has its catchment in the Hottentots Holland Mountain Range is the Steenbras. Two major storage dams on this river, that supply Cape Town with water, are the Upper and Lower Steenbras Dams. These are linked with the Rockview Dam by way of the Palmiet Pumped Storage Scheme (RHP 2005). Downstream from the Lower Steenbras Dam, the river flows through the Steenbras River Gorge before reaching the sea.

D.2. Maps of the greater Cape Town area

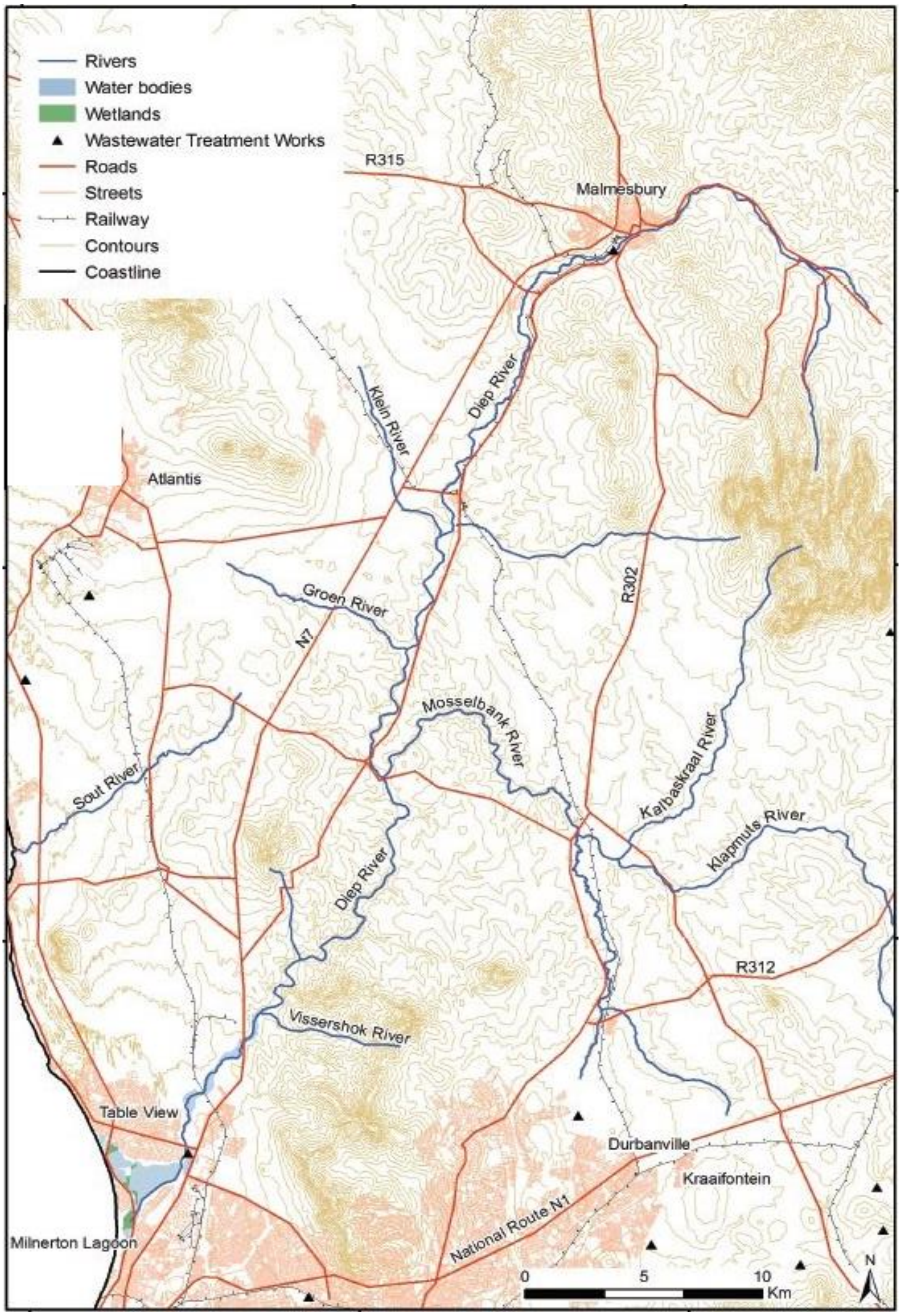

Figure D1. Map of the northern region of the greater Cape Town area, showing the Diep River and its tributaries (Source: Brown and Magoba 2009).

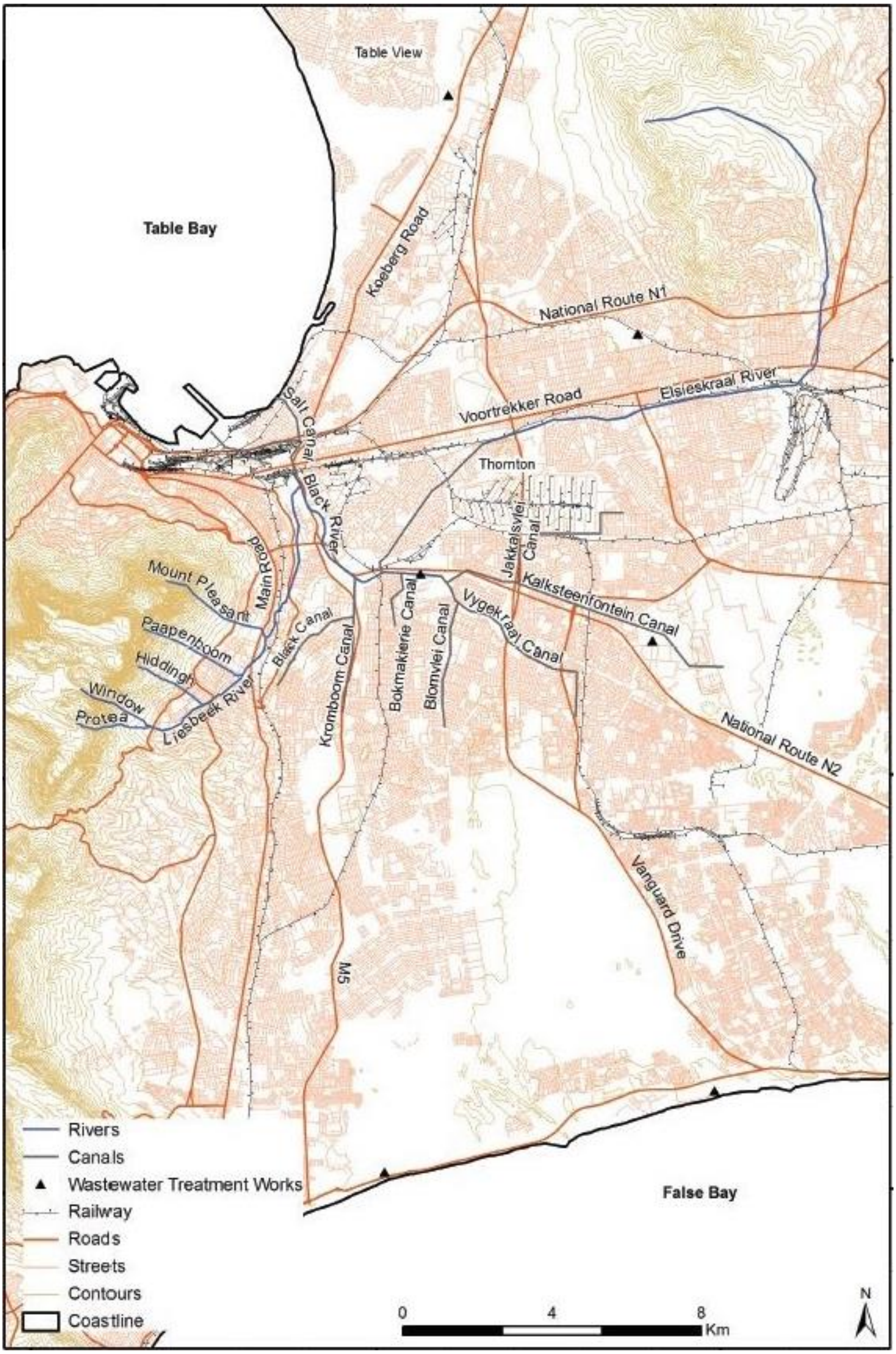

Figure D2. Map of the central region of the greater Cape Town area, with the Liesbeeck, Black, Elsieskraal, and Swart Rivers and their tributaries (Source: Brown and Magoba 2009).

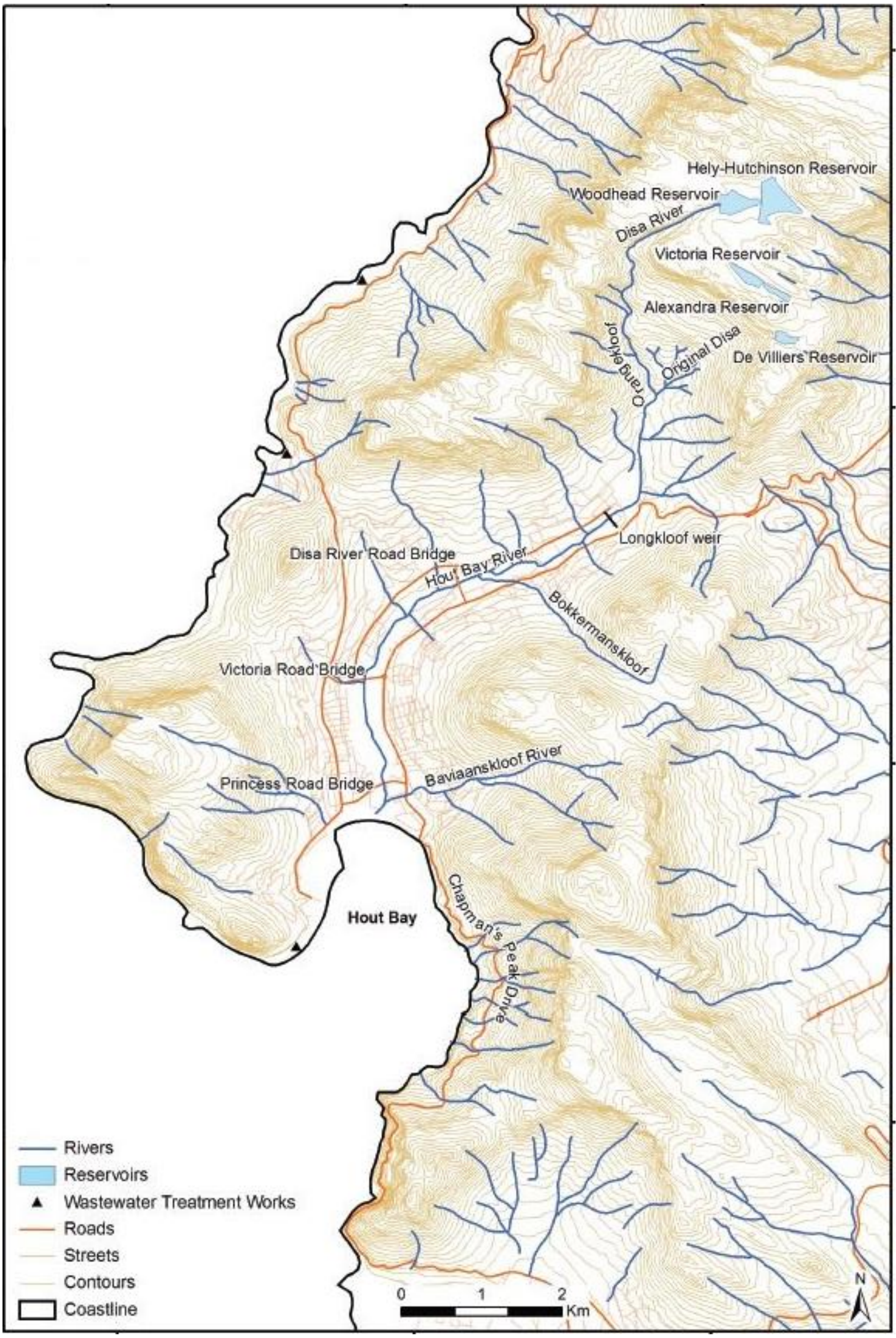

Figure D3. Map of the Hout Bay River and its tributaries, located within the central region of the greater Cape Town area (Source: Brown and Magoba 2009).

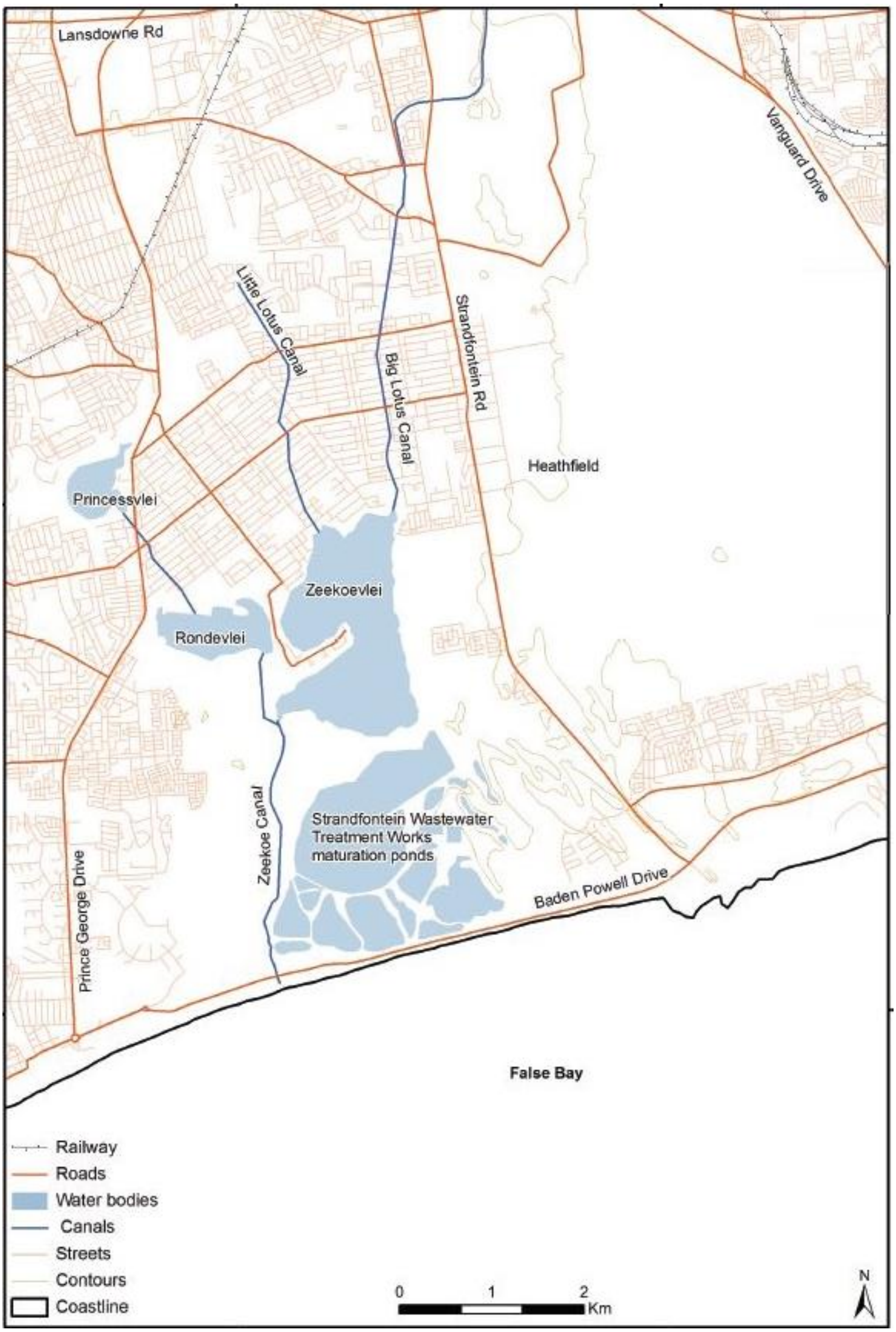

Figure D4. Map of the Zeekoe system in the southern region of the greater Cape Town area, with Princessvlei, Rondevlei, Zeekoeivlei, and the Strandfontein waste water treatment works (Source: Brown and Magoba 2009).

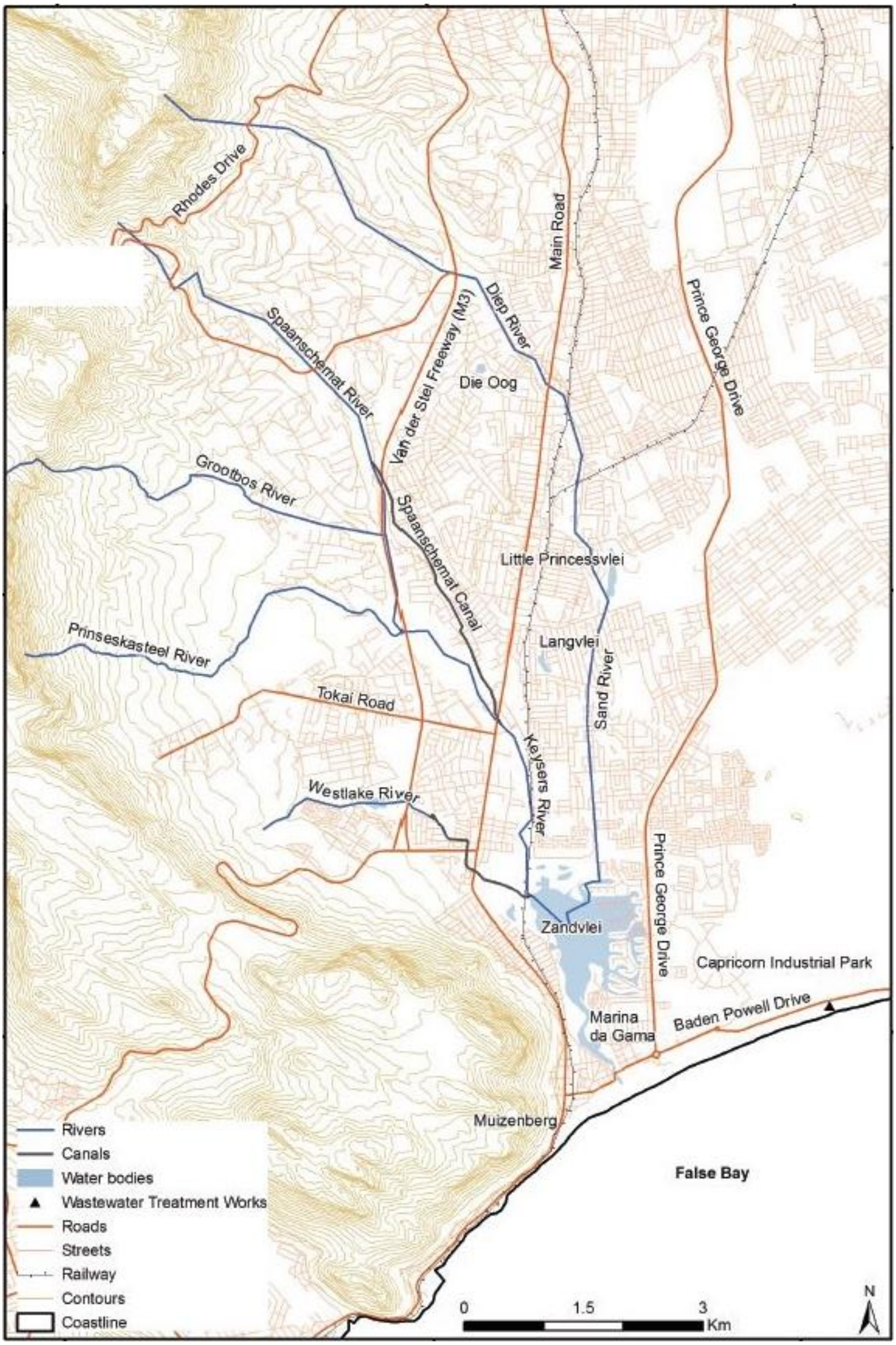

Figure D5. Map of the Sand River system in the southern region of the greater Cape Town area, with the Keysers, Westlake and Prinseskasteel Rivers, and the Zandvlei estuary (Source: Brown and Magoba 2009).

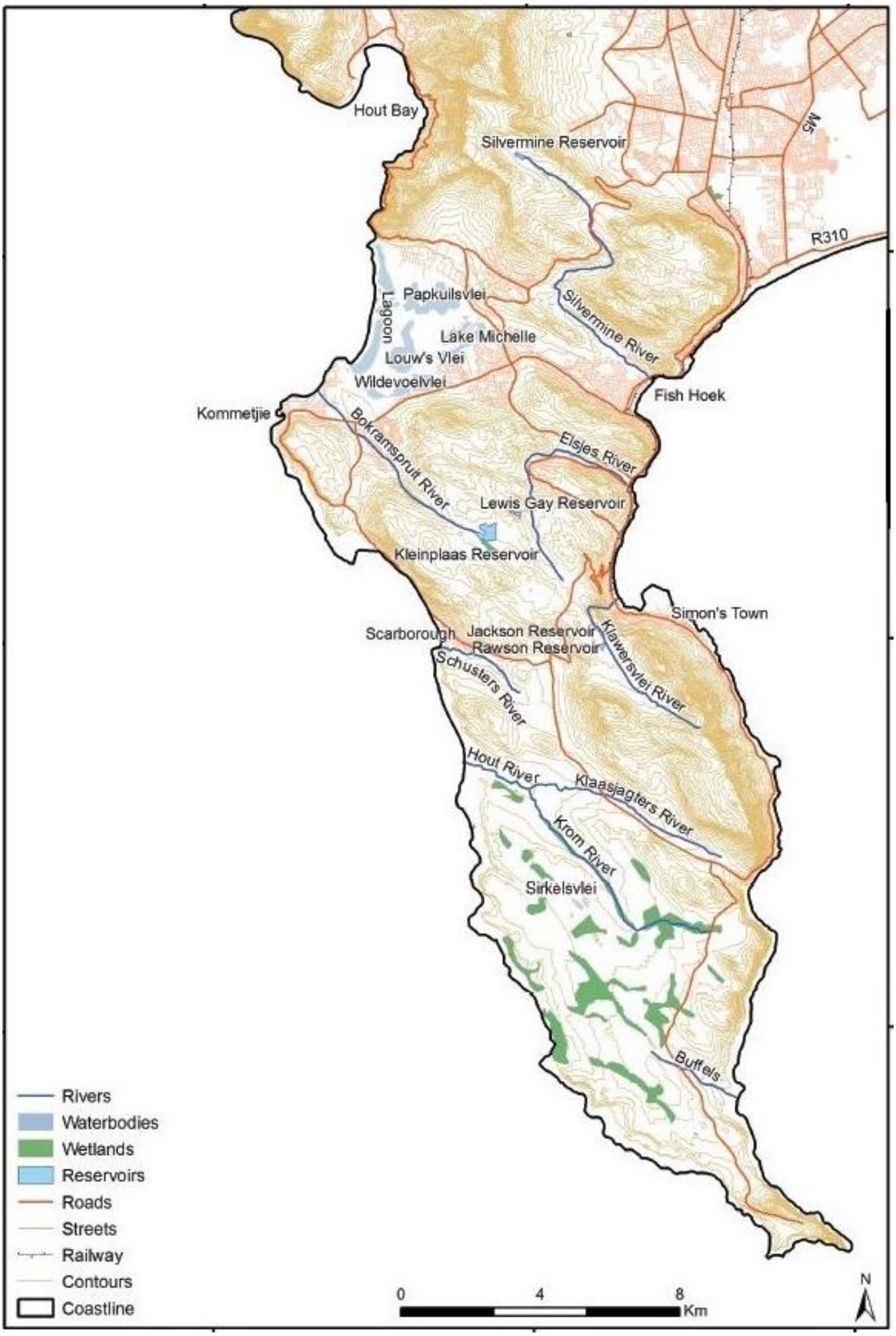

Figure D6. Map of the South Peninsula of the greater Cape Town area. The southernmost rivers from the Schusters River are all associated with the Cape Point section of the Table Mountain National Park (Source: Brown and Magoba 2009).

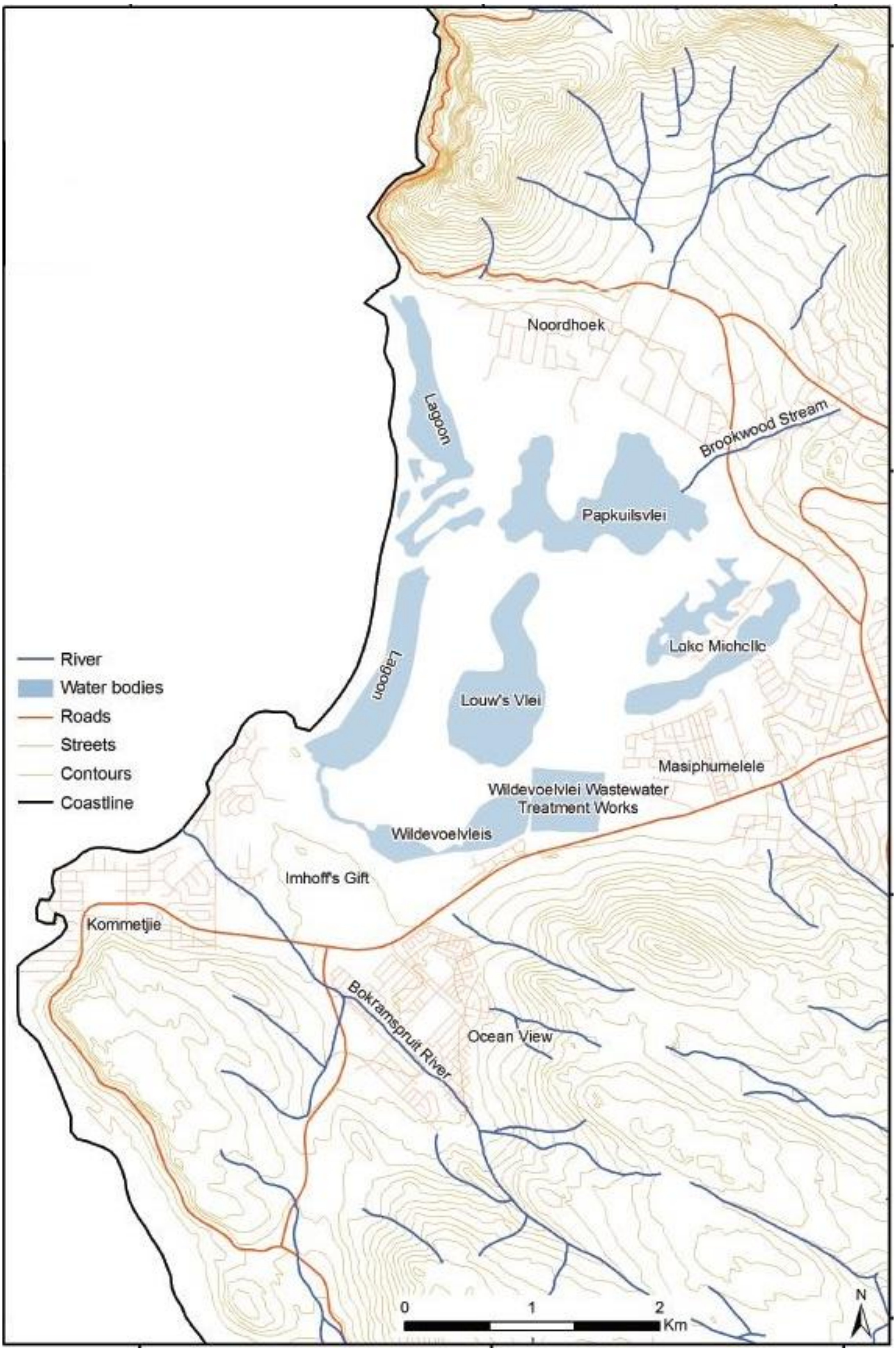

Figure D7. A close-up map of the Noordhoek Wetlands and the Bokramspruit River, located on the western side of the South Peninsula (see Figure D6 for larger map) (Source: Brown and Magoba 2009).

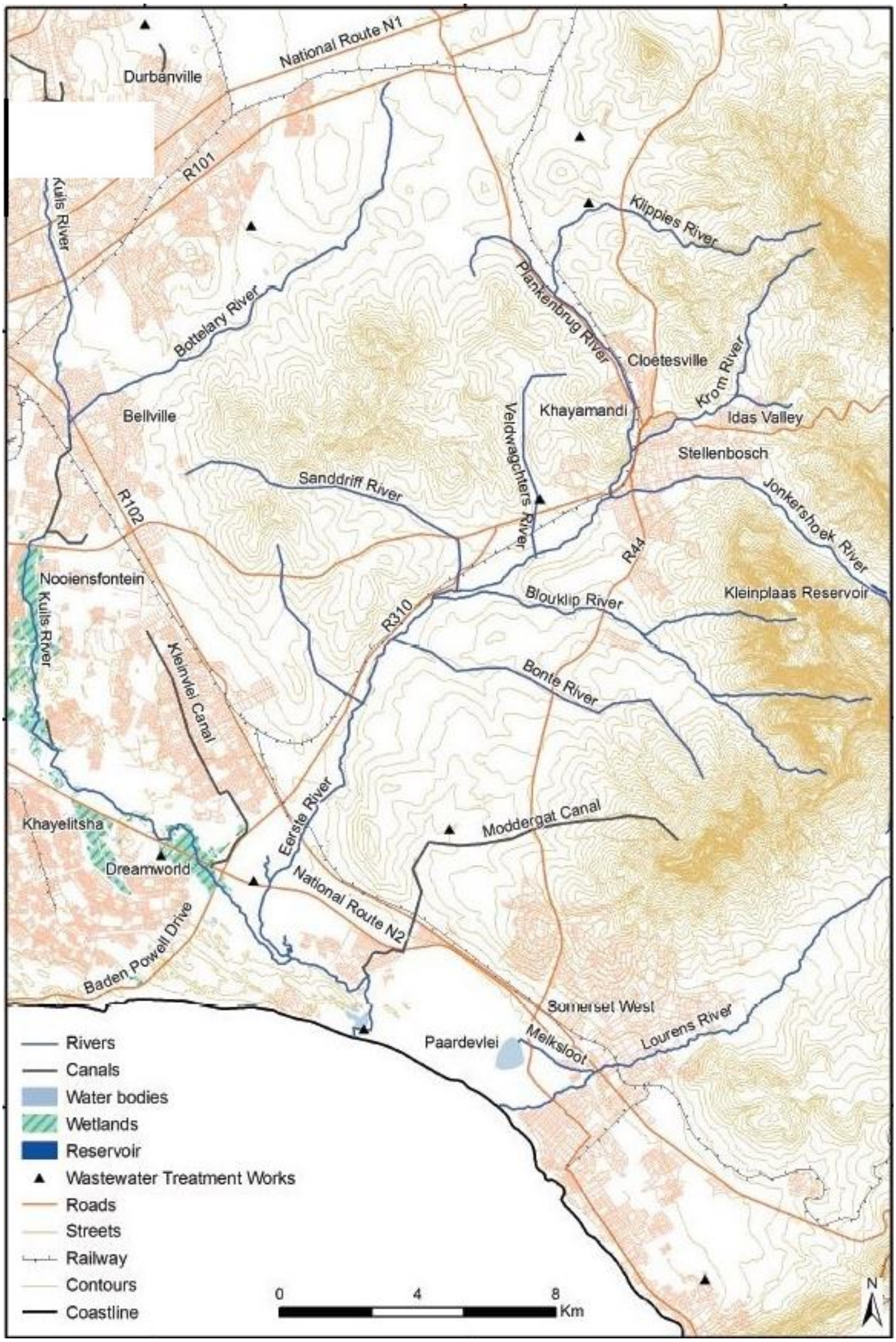

Figure D8. Map of the eastern region of the greater Cape Town area, showing the Kuils, Eerste and Lourens Rivers and their tributaries (Source: Brown and Magoba 2009).

D. 3. Data points from the literature review of Chapter 5

Table D1. Locations obtained in the literature review for occurrences of *Gambusia affinis* in the Greater Cape Town Area (GCTA).

GCTA region	Locality	Conservation area	Year	Source	Type of data	River system	City of Cape Town Biodiversity Network Classification	Latitude	Longitude
North	Melkbosstrand agricultural land	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Sout River	Not specified	-33.6918	18.4901
North	Malmesbury agricultural land	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Riebeeks River	Not specified	-33.4284	18.7554
North	Milnerton rural area	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Diep River	CBA1 floodplain	-33.8089	18.5313
North	Kalbaskraal station	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Diep River	Not specified	-33.5824	18.645
North	Rietvlei	TBNR	2004	ABM 2017	Species sighting	Diep River	CBA1 limnetic exorheic depression	-33.8432	18.4790
North	Rietvlei	TBNR	2014	ABM 2017	Species sighting	Diep River	CBA1 limnetic exorheic depression	-33.8432	18.4790
North	Zoarvlei	TBNR	2008	ABM 2017	Species sighting	N/A	CESA exorheic permanently inundated limnetic depression	-33.8994	18.4828
North	Zoarvlei	TBNR	2011	ABM 2017	Species sighting	N/A	CESA exorheic permanently inundated limnetic depression	-33.8994	18.4828
North	Milnerton Lagoon	TBNR	2014	Viskitch *et al*. 2016	Publication	Diep River	CBA1 Estuary	-33.89	18.48
Central	Observatory	N/A	2010	BGIF 2007 (SAIAB collection)	Species collection	Liesbeeck River	OESA littoral exorheic permanently inundated depression	-33.9366	18.4758

Table D1 cont. Locations obtained in the literature review for occurrences of *Gambusia affinis* in the Greater Cape Town Area (GCTA).

GCTA region	Locality	Conservation area	Year	Source	Type of data	River system	City of Cape Town Biodiversity Network Classification	Latitude	Longitude
South	Princessvlei	N/A	1965	BGIF 2007 (SAIAB collection)	Species collection	N/A	CBA 2 permanent limnetic exorheic depression	-34.0494	18.4598
South	Rondevlei	FBNR	1970	ABM 2017	Species sighting	N/A	CBA 1 limnetic exorheic depression	-34.0629	18.4977
South	Rondevlei	FBNR	2014	ABM 2017	Species sighting	N/A	CBA 1 limnetic exorheic depression	-34.0604	18.5016
South	Rondevlei	FBNR	2004	BGIF 2007 (SAIAB collection)	Species collection	N/A	CBA 1 limnetic exorheic depression	-34.0588	18.5002
South	Rondevlei	FBNR	2008	ABM 2017	Species sighting	N/A	CBA 1 limnetic exorheic depression	-34.0606	18.5021
South	Strandfontein Birding Section	FBNR	2012	ABM 2017	Species sighting	N/A	CESA: Cape Flats Sewage WWTW. Endorheic depression littoral	-34.0855	18.5193
South	Zeekoeivlei	FBNR	1970	ABM 2017	Species sighting	N/A	CESA exorheic permanently inundated limnetic depression	-34.0639	18.5158
South	Glencairn	Glencairn Wetland Nature Reserve	2010	BGIF 2007 (SAIAB collection)	Species collection	Elsje River	CESA permanently inundated littoral exorheic depression	-34.1500	18.4100
South	Zandvlei	Zandvlei Estuary Nature Reserve	1998	ABM 2017	Species sighting	Sand River	CBA1 permanently open estuary and wetlands	-34.0896	18.4689
South	Zandvlei	Zandvlei Estuary Nature Reserve	2009	ABM 2017	Species sighting	Sand River	CBA1 permanently open estuary and wetlands	-34.0896	18.4689
South	Zandvlei	Zandvlei Estuary Nature Reserve	2011	ABM 2017	Species sighting	Sand River	CBA1 permanently open estuary and wetlands	-34.0896	18.4689
South	Constantia	N/A	2017	iSpot 2017	Species sighting	Grootboschkloof River	CESA seasonally inundated floodplain wetland	-34.0427	18.4351
South	Spaanschemat River Rd, Belle Constantia	N/A	<2007	Kleynhans *et al.* 2007	Database for FROC	Keysers River	CESA permanently saturated valley-floor wetland	-34.0313	18.4400

Table D1 cont. Locations obtained in the literature review for occurrences of *Gambusia affinis* in the Greater Cape Town Area (GCTA).

GCTA region	Locality	Conservation area	Year	Source	Type of data	River system	City of Cape Town Biodiversity Network Classification	Latitude	Longitude
East	Edith Stevens Nature Reserve	N/A	2017	ABM 2017	Species sighting	N/A	CBA 1 seep	-34.0031	18.5536
East	Spier Wine Estate, Stellenbosch	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Eerste and Blou Klip River confluence	None specified	-33.9688	18.7893
East	Macassar	N/A	1988	BGIF 2007 (SAIAB collection)	Species collection	Eerste River	CBA 1 permanently open estuary	-34.0847	18.7622
East	Nooiensfontein Rd, Kuils River	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Kuils River	None specified	-33.9293	18.6755
East	Macassar	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Eerste River	CBA 1 Floodplain wetland	-34.0647	18.7531
East	Vergelegen Wine Estate	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Lourens River	None specified	-34.0750	18.8890
East	Broadway Boulevard, The Palms	N/A	<2007	Kleynhans *et al*. 2007	Database for FROC	Lourens River	CESA floodplain wetland	-34.0981	18.8272
East	Upper Pond at Helderberg Nature Reserve	Helderberg Nature Reserve	2008	BGIF 2007 (SAIAB collection)	Species collection	Tributary of Lourens River	CESA limnetic endorheic depression	-34.0622	18.8733

Table D2. Locations obtained in the literature review for occurrences of mosquitofish in the GCTA, which did not provide GPS point locations.

Year	GCTA region	River system	Conservation area	Literature Type	Source
2003	North	Diep River	N/A	DWAF Report	RHP 2003
2007	North	Diep River estuary	TBNR	C.A.P.E. Report	Turpie and Clarke 2007
2011	North	Diep River system	TBNR	CoCT Integrated Management Plan	Retief 2011
2003	Central	Hout Bay River	N/A	DWAF Report	RHP 2003
Before 2009	Central	Liesbeeck River, Hartleyvale	Near Raapenberg Bird Sanctuary	WRC Report	Brown and Magoba 2009
1991	South	Sand River	Zandvlei Estuary Nature Reserve	Publication	Morant 1991
1994	South	Sand River	Zandvlei Estuary Nature Reserve	Publication	Quick and Harding 1994
1994	South	Sand River	Zandvlei Estuary Nature Reserve	Publication	Clark *et al*. 1994
2011	South	N/A	FBNR	CoCT Integrated Management Plan	Kahn 2011
2011	South	N/A	Zandvlei Estuary Nature Reserve	CoCT Integrated Management Plan	Gibbs *et al*. 2011
1997	East	Lourens River	N/A	River assessment for Somerset West municipality	Tharme *et al*. 1997
2003	East	Lourens River	N/A	DWAF Report	RHP 2003
2011	East	Lourens River system	Helderberg Nature Reserve	CoCT Integrated Management Plan	Wittridge 2011

D.4. Data points from the literature survey of Chapter 5

Table D3. Locations surveyed for *Gambusia affinis* in the greater Cape Town area, in Dec 2016 and March 2017.

GCTA region	Locality	Conservation area	River system	City of Cape Town Biodiversity Network Classification	Latitude	Longitude	*G. affinis* occurrence
North	Botterblom Dam	Botterblom Nature Reserve	N/A	OSEA endorheic depression	-33.8061	18.6572	Absent
North	281 Blaauwberg Rd, Tableview	N/A	Diep River	CBA1 river and inundated wetland	-33.8325	18.5193	Present
North	Burgundy Estate Dam	N/A	N/A	CESA endorheic depression	-33.8363	18.5524	Present
North	Diep River, Malibongwe Drive and Sand down Rd	N/A	Diep River	CBA1 floodplain	-33.8008	18.536	Dried up
North	Diep River, Philadelphia Rd, Malmesbury Farms	N/A	Mosselbank and Diep River	Not specified (River)	-33.6921	18.6096	Present
North	Drainage canal, Sail St, Blaauwbergstrand	N/A	N/A	OESA endorheic littoral storm water pond, permanently inundated	-33.8046	18.4788	Dried up
North	Rietvlei	Table Bay Nature Reserve	Diep River	CBA1 limnetic exorheic depression	-33.8368	18.4927	Absent
North	Uitkamp Wetlands, Durbanville	Uitkamp Wetland Nature Reserve	Mosselbank	CBA 1 valley floor wetland	-33.8168	18.6400	Dried up
Central	Green Point Urban Park	Green Point Urban Park	N/A	Not specified (wetland)	-33.9048	18.4021	Present
Central	Colonel's bird bath, Kirstenbosch	Kirstenbosch Botanical Gardens	N/A	Not specified (Pond)	-33.9835	18.429	Absent
Central	Main pond, Kirstenbosch	Kirstenbosch Botanical Gardens	N/A	Not specified (Pond)	-33.9888	18.4319	Absent
Central	Old dam, nursery stream, Kirstenbosch	Kirstenbosch Botanical Gardens	Nursery stream	Not specified (In-stream pond)	-33.9879	18.4289	Absent
Central	Otter pond, Kirstenbosch	Kirstenbosch Botanical Gardens	N/A	Not specified (Pond)	-33.9879	18.4289	Absent
Central	The Company's Garden, CBD	N/A	N/A	Not specified (Pond)	-33.9286	18.4166	Present
Central	Intaka Island (Blouvlei), Century City	Private	N/A	OESA endorheic littoral storm water pond/wetland	-33.8877	18.5142	Present
Central	Deer Park, Oranjezicht	Table Mountain National Park	Platteklip stream	Not specified (In-stream pond)	-33.9499	18.4183	Present

Table D3 cont. Locations surveyed for *Gambusia affinis* in the greater Cape Town area, in Dec 2016 and March 2017.

GCTA region	**Locality**	**Conservation area**	**River system**	**City of Cape Town Biodiversity Network Classification**	**Latitude**	**Longitude**	***G. affinis* occurrence**
Central	Cool Runnings Quarry, Bellville	N/A	Elsieskraal River	OESA limnetic exorheic depression	-33.884	18.6291	Present
Central	Doordekraal Dam, Bellville	N/A	Elsieskraal River	OESA limnetic exorheic depression	-33.8649	18.6284	Present
Central	Majik Forest, Bellville	N/A	Elsieskraal River	OESA exorheic permanently inundated depression	-33.86	18.6162	Absent
South	Die Oog, Bergvliet	Die Oog Conservation Area	N/A	CESA littoral exorheic depression	-34.0482	18.4475	Absent
South	Glencairn Wetland	Glencairn Wetlands Nature Reserve	Elsje River	CESA permanently inundated littoral exorheic depression	-34.1579	18.4281	Absent
South	Dam 1, inside racecourse, Kenilworth	Kenilworth Racecourse Conservation Area	N/A	CBA 1 limnetic endorheic depression	-33.9998	18.4837	Present
South	Dam 2 (outside racecourse), Kenilworth	Kenilworth Racecourse Conservation Area	N/A	OESA limnetic endorheic depression	-33.9959	18.4884	Absent
South	Dam 3 (Irrigation dam), Kenilworth	Kenilworth Racecourse Conservation Area	N/A	OESA limnetic endorheic depression	-33.9969	18.4869	Absent
South	Clovelly wetlands	Lower Silvermine Wetlands Nature Reserve	Silvermine river	CBA 1 permanently inundated river floodplain	-34.1312	18.4362	Absent
South	Admirals Waterfall, Barnard St, Simon’s Town	N/A	Klawersvlei River	Not specified	-34.1916	18.4198	Absent
South	Cape Point Vineyards, Noordhoek	N/A	N/A	OESA limnetic exorheic depression	-34.0942	18.387	Absent
South	Keysers River, Tokai	N/A	Keysers	CESA floodplain wetland	-34.065	18.4593	Present
South	Princessvlei	N/A	N/A	CBA 2 permanent limnetic exorheic depression	-34.0476	18.4852	Present
South	Schusters River, Scarborough	N/A	Schusters River	CBA 1 floodplain wetland	-34.2024	18.3746	Absent
South	Wildevoëlvlei Estuary, Kommetjie	Table Mountain National Park	N/A	CESA limnetic exorheic depression	-34.1372	18.3564	Present
South	Silvermine Dam, Silvermine Rd	Table Mountain National Park	Silvermine River	CESA limnetic exorheic depression	-34.0742	18.3963	Absent
South	Zandvlei Estuary	Zandvlei Nature Reserve	Sand River	CBA1 permanently open estuary and wetlands	-34.0922	18.4675	Present
East	Fairtrees Road, Durbanville	N/A	Kuils River	CESA valley bottom wetland	-33.8463	18.6683	Present
East	Sonstraal Dam, Durbanville	N/A	Kuils River	OESA exorheic depression	-33.8481	18.6595	Absent