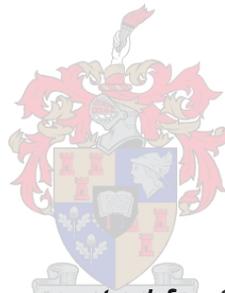


**Exploring the invasion of the guttural toad
Sclerophrys gutturalis in Cape Town through a
multidisciplinary approach**

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*Dissertation presented for the degree of
Doctor of Philosophy in the Faculty of Science
at Stellenbosch University*

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Co-supervisor: Dr Sarah J. Davies

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Giovanni Vimercati

Date: March 2017

Abstract

Invasive populations of amphibians may have considerable ecological and socio-economic impacts; reconstructing their invasion dynamics is essential to perform adaptive management. Investigating these populations is also an opportunity to address eco-evolutionary questions; it helps to improve our comprehension of biological systems and define in greater detail invasion potential. This study explores the invasion of the guttural toad *Sclerophrys gutturalis* in Cape Town through a multidisciplinary approach that integrates physiology, evolutionary biology, ecological modelling and environmental economics.

The species is domestic exotic in South Africa, being native in most of the country but not in Cape Town, where an invasive population established in 2000. Although an extirpation program (started in 2010) removed some thousand adults, tadpoles and eggs until 2016, the population is still spreading. Invasion dynamics emerging from traits of the invader and characteristics of the invaded landscape are unknown. Additionally, efficacy and efficiency of the current mode of removal as well as the possibility to implement more effective extirpation strategies have not been investigated. Since the winter rainfall environment of Cape Town is drier and colder than that of the source population (Durban), especially during the summer breeding season, the species' ability to spread is remarkable. Currently it is not clear how the abiotic conditions of Cape Town constrain this species and whether invasive toads adaptively respond to reduce phenotypic mismatch in the novel environment.

Firstly, I built an age structured model that can be utilized to simulate population dynamics of invasive pond-breeding anurans. The model follows a metapopulation approach and simulates change in survival and dispersal behaviour as a function of age. It also integrates dispersal with landscape complexity through least cost path modelling to depict functional connectivity across the pond network. Then I applied the model to my case study; parameterization was conducted through field and laboratory surveys, a literature review and data collected during the extirpation. I found a lag phase in both demographic and spatial dynamics. Also, I found that the spatial spread fits an accelerating trend that causes the complete invasion of the network in six years. Such dynamics match field observations and confirmed patterns previously detected in other invaders characterized by high dispersal abilities.

The age structured model was further employed to explore efficacy and efficiency of the current management. I investigated how a scenario incorporating the demographic effects of the current removal differs from a no-extirpation scenario. I also asked which limitations might impede the management from being successful and whether alternative strategies

may determine better results. I found that the current management does not sufficiently take into consideration non-linear population dynamics and it reduces the efficiency; moreover the removal started during the spread phase of the invasion. Spatial limitations linked to the social dimension of the landscape severely reduce efficacy of the current removal; other management countermeasures such as control or containment should thus be considered.

To explore how the species phenotype is constrained in the invaded environment during the breeding period and whether invasive toads underwent any adaptive response, I performed a comparison between the invasive population of Cape Town and the native source population of Durban. Field data and physiological traits such as evaporative water loss, water uptake, sensitivity of locomotion to desiccation and critical thermal minimum were collected. In accordance to the more desiccating and colder environment of Cape Town, invasive guttural toads responded physiologically and behaviourally on short time scale (less than two decades) to reduce sensitivity to lower conditions of hydration and temperature. The species is still constrained in the novel environment but its invasion potential is higher than I could infer from the source population.

To confirm that the colder environment of Cape Town constrains invasive toads also during the non-breeding season, I investigated post-breeding energy storage in populations from Cape Town (high latitude), Durban (intermediate latitude), Mauritius and Reunion (low latitude) where the species is also invasive. Although post-breeding energy storage should be high (capital breeding strategy) at high latitudes and low (income breeding strategy) at low latitudes, guttural toads unexpectedly shifted energy storage strategy from capital to income breeding when introduced from lower to higher latitude. The invaded environment is therefore less severe during the non-breeding season; winter rainfall promotes, and does not reduce, toads' activity.

In summary, I showed that the invasion success of the guttural toad in Cape Town may be attributable to several factors such as initial lag that delayed management, accelerating spread, rapid adaptive response and less severe non-breeding season. The spatial dimension of the invaded landscape strongly limited the efficacy of the current management program. My work has relevant management implications; it shows that the invasion potential of the species is already higher than that I could infer from the source population and only tackling social limitations could have promoted effective extirpation.

Opsomming

Inringer amfibiese diere kan ernstige ekologiese en sosio-ekonomiese impak hê; dit is dus noodsaaklik om hul indringe dinamika te verstaan om voorkomende of toepaslike bestuur toe te pas. Navorsing op hierdie diere is ook 'n geleentheid om eko-evolutionêre vrae aan te spreek; dit help om ons begrip van biologiese sisteme te verbeter en indringe potensiaal van die spesie te definieer. Hierdie studie ondersoek die indringing van die gorrelskurwepadda, *Sclerophrys gutturalis*, in Kaapstad deur 'n multi-dissiplinêre benadering wat fisiologie, evolutionêre biologie, ekologiese modellering en omgewingsekonomie integreer.

Die gorrelskurwepadda is 'n plaaslike eksotiese spesie in Suid-Afrika: dit is inheems in meeste dele van die land, maar nie in Kaapstad nie. 'n Inringer bevolking van paddas was in 2000 in Kaapstad gevind. Hoewel 'n uitwissing program (wat begin het in 2010) so paar duisend volwassenes, paddavissies en eiers verwyder het tot en met 2016, is die populasie steeds besig om te versprei. Indringe dinamika van hierdie spesies, wat bepaal word deur eienskappe van die inringer en eienskappe van die ingeneemde landskap, is onbekend. Daarbenewens, die effektiwiteit van die huidige modus van verwydering asook die moontlikheid om meer effektiewe uitwissing strategieë te implimenteer was nog nooit ondersoek en bepaal nie. Alhoewel die winterreengebied van Kaapstad kouer en droer is as die somerreengebied van Durban, is die vermoë van die spesie om in die somer broeiseisoen uit te broei en te versprei, merkwaardig. Tans is dit nie duidelik hoe die abiotiese toestande van Kaapstad hierdie spesies inperk nie en of die inringer skurwepaddas aanpassings maak om die fenotipiese wanaanpassing te verminder in hulle nuwe omgewing.

Eerstens het ek 'n ouderdoms-gestruktureerde model gebou wat gebruik kan word om bevolkingsdinamika van inringer- dambroeiende skurwepaddas na te boots. Die model volg 'n metabevolking benadering en simuleer verandering in oorlewing en verspreiding gedrag as 'n funksie van ouderdom. Dit integreer ook die komplekse landskap verspreiding deur middel van die kort-pad-model, om die funksionele verbindings tussen die dam netwerke uit te beeld. Ek het die model toegepas op my spesifieke studie; parameters vir die model was bepaal deur informasie wat gevind was tydens veld en laboratorium ondersoeke, 'n literatuuroorsig en data wat ingesamel was tydens die uitwissing van skurwepaddas. Ek het gevind dat daar 'n waf fase is in beide demografiese en ruimtelike dinamika. Ek het ook gevind dat die ruimtelike verspreiding 'n versnelde tendens tot gevolg het wat die volledige indringing van die damnetwerk binne die volgende ses jaar gaan veroorsaak. Hierdie patrone onderskryf veldwaarnemings en bevestig patrone wat voorheen waargeneem was in ander indringers wat gekenmerk is deur hoë verspreiding vermoëns.

Die ouderdoms-gestruktureerde model word verder gebruik om die doeltreffendheid en effektiwiteit van die huidige bestuur te ondersoek. Ek het getoets hoe 'n scenario waar die demografiese gevolge van die huidige verwydering van skurwepaddas verskil van 'n scenario waar geen uitwissing van die skurwepaddas plaasvind nie. Ek het ook ondersoek watter spesifieke beperkings daar in plek is wat die suksesvolle bestuur kan belemmer-en of alternatiewe strategieë beter resultate kan behaal. Ek het gevind dat die huidige bestuur nie die “nie-lineêre” bevolking dinamika genoeg in ag neem nie en dat dit die doeltreffendheid van die bestuur verminder. Ek het gevind dat die verwydering van die skurwepaddas begin het gedurende die verspreiding fase van die indringing. Ruimtelike beperkings, wat verband hou met die sosiale dimensie van die landskap beperk die huidige verwydering van die skurwepaddas geweldig; ander bestuur teenmaatreëls vir die beheer of inperking moet dus gevind word.

Om te ondersoek hoe die fenotipe van die skurwepaddas in die binne gedringde omgewing beperk word tydens die broeiseison en of-indringer skurwepaddas aangepas het, het ek 'n vergelyking getref tussen die indringer bevolking van Kaapstad en die plaaslike bevolkingsbron van Durban. Velddata en fisiologiese eienskappe soos waterverlies deur verdamping, wateropname, bewegings as gevolg van sensitiwiteit teen uitdroging en kritieke minimum temperature is van albei skurwepaddabevolgings ingesamel. Ek het gevind dat in die koue, droe klimaat van Kaapstad, die gorrelskurwepaddas hul gedrag en fisiologie in n kort tydperk (van minder as twee dekades) aangepas het om hul sensitiwiteitsvlakke teen laer temperature en laer humiditeit, te verminder. Alhoewel die spesie nog beperk is in die Kaapstad omgewing, is hul indringing-potensiaal hoer as wat ek uit die populasiebron kon aflei.

Om te bevestig dat die kouer omgewing van Kaapstad die indringer skurwepaddas ook beperk tydens die res van die jaar wanneer hulle nie broei nie, het ek gekyk na die energie berging van die skurwepaddas nadat hulle gebroei het. Ek het dit toegepas op paddas van Kaapstad (hoë breedtegraad), Durban (intermediêre breedtegraad), Mauritius en Reunion (lae breedtegraad) waar die spesies ook indringend is. Alhoewel die energie berging hoog behoort te wees (kapitaal-broei strategie) by hoë breedteliggings en laag behoort te wees (inkomste-broei strategie) by lae breedtegrade, het die gorrelskurwepaddas onverwags hulle energie-bergings strategie verskuif van kapitaal tot inkomste-broei wanneer hulle van laer na hoër breedtegraad verander het. Die omgewing wat binne gedring is deur die gorrelskurwepaddas, Kaapstad, is nie so erg vir die skurwepaddas wanneer hulle nie in hulle broeiseisoen is nie; winterreënval bevorder die aktiwiteit van skurwepaddas.

Om op te som, ek het gewys dat die suksesvolle indringing van die gorrelskurwepadda in Kaapstad-toegeskryf kan word aan verskeie faktore soos die aanvanklike trae groei in van die skurwepadda populasie wat veroorsaak het dat die beheer van die skurwepaddas nie vinnig genoeg gebeur het nie, die versnelde verspreiding van die skurwepadda, die vinnige aanpasbaarheid en die matige nie-broei seisoen. Die ruimtelike dimensie van die binnegevallende landskap het die doeltreffendheid van die huidige bestuur program sterk beperk. Die ruimtelike landskap en omgewing wat die skurwepaddas ingedring het, bemoeilik die doeltreffende toepassing van die huidige bestuursprogram. Die resultate van my werk het verskeie bestuurs implikasies. Dit het getoon dat die indringings potensiaal van die spesifieke spesie hoër is as wat ek aanvanklik kon aflei uit die populasiebron. Dit het ook gewys dat die skurwepaddas meer suksesvol verwyder kon word as dit nie was vir die sosiale beperkings nie.

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Figure 4.4: Relationship between scaled fat body mass and scaled mass index (SMI) obtained from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban, Mauritius and Reunion populations. The linear regression is depicted separately for each population when a significant correlation between the two variables was detected (Table 4.3).

General Introduction

The current breakdown of biogeographical barriers due to globalization and international trade is causing an unusual reshuffling of species ranges (Rosenzweig 2001). The number and diversity of organisms currently introduced to areas outside those in which they historically evolved are without precedence (Ricciardi 2007). Moreover, such human-mediated introductions occur at spatial and temporal scales that are several orders of magnitude higher than natural events of dispersal. This led some authors to use the term “Homogocene” to describe the ongoing process of biogeographic homogenization on the Earth (Rosenzweig 2001). Individuals introduced into novel areas generally establish populations characterized by limited ranges and negligible impact (simply defined as alien). When these populations disperse and reproduce into areas distant from the original point of introduction and/or cause severe consequences to the environment, they are classified as invasive (Pyšek et al. 2004; Valéry et al. 2009).

Biological invasions are one of the main drivers of global change, having negative impacts on the natural world through habitat and ecosystem alteration, loss of biodiversity and spread of parasites and pathogens (Simberloff et al. 2013, Hulme 2014, Tittensor et al. 2014). Moreover their detrimental consequences on human health and economic systems are today largely documented (Olson 2006, Pyšek and Richardson 2010). It follows that considerable effort through management countermeasures is necessary to minimize effects of invasive populations and limit their spread into new areas. Since management involves actions characterized by disparate aims and costs such as detection, eradication or containment, the optimal strategy should be chosen through a cost-benefit evaluation (Meyerson et al. 2007, Epanchin-Niell et al. 2014). This evaluation should preferentially take into account characteristics of the invaded landscape and traits of the invader; experience shows that ignoring case-specific peculiarities of a biological invasion can hamper our capacity to implement effective countermeasures (Epanchin-Niell et al. 2014). Any ongoing management process should thus be carefully monitored for evaluating success probability and improving its efficacy through adaptive management strategies. This is particularly important during the first incursion of a biological invasion (Van Wilgen et al. 2014); i.e. when the limited spatial extent of the invaded area makes successful management still economically feasible (Epanchin-Niell et al. 2014, Van Wilgen et al. 2014).

The importance to study invasive populations goes far beyond implementing effective countermeasures and improving management efficacy. Since 1800s naturalists, ecologists and evolutionary biologists used alien and invasive species to obtain insights into ecology and evolution (Sax et al. 2007, Sexton et al. 2009). Joseph Grinnell was the first to talk about them

as “experiments in nature” (1919) and after one century, invasive and introduced taxa are now recognized not only as socio-economic and biological issues but also as an opportunity to address some basic eco-evolutionary questions (Lee 2002; Huey et al. 2005; Strauss et al. 2006; Zenni and Nunez 2013). According to Sax et al. (2007) biological invasions are helpful for basic research because they: i) provide unplanned experiments across large spatial and temporal scales through which it is possible to collect unique information that is complementary to planned manipulative experiments; ii) allow observing evolutionary and ecological processes in real time; iii) allow examining the rate of these processes; iv) provide information that would often be deemed unethical to collect in a planned experiment. Outcomes emerging from such investigation not only increase our comprehension of eco-evolutionary phenomena but can also generate insights for management; for example, local adaptation in an invasive species can increase invasion potential, consequently hampering our capacity to effectively respond (Broennimann et al. 2007, Kolbe 2010). Conversely maladaptive behaviour (Ward-Fear et al. 2009), or sub-optimal phenotypes observed in some invasive populations established in a new environment may facilitate their removal or control (Clarke et al. 2016, Phillips et al. 2016).

As invasive phenomena become more frequent across the world, the scientific knowledge generated by invasion biology constantly increases: authors estimated that in this last decade biological invasions have received, with the exception of climate change, more attention than any other ecological and conservational topic (Lockwood et al. 2013). However, scientific attention is not equally distributed across different taxonomic groups but is concentrated mostly on terrestrial invasions of plants, mammals and insects (Kraus 2009). This unbalanced treatment is justifiable because these taxa may create severe effects on natural ecosystems and human societies; however, it may lead to underestimate or put aside invasive potential and impact of several other less-studied species.

Amphibians are among those taxa whose introduction has largely been ignored, especially in the past (Kraus 2009). This is surprising because their worldwide decline is dramatic (Wake and Vredenburg 2008) with about 30% of all species listed by IUCN as threatened (Vie' et al. 2009), and one of the most critical threats to their conservation is the introduction of other amphibians (Blaustein et al. 2011). Globally, there are relatively few well-known amphibian invaders, such as, the cane toad *Rhinella marina*, the American bullfrog *Lithobates catesbeianus* and the African clawed frog *Xenopus laevis*. These species invaded areas significantly distant from their native range (i.e. different continents) and had noticeable impact at both individual and community level (Kumschick et al. in prep). Kraus (2009) sourced 104 species that established alien populations across the globe (Kraus 2009) and a number of them are already having ecological and socio-economic impacts (Measey et al. 2016).

In South Africa, there are only three invasive species of amphibians and all of them are domestic exotics, i.e. invaded areas within national boundaries (Guo & Ricklefs 2010). These are: the painted reed frog *Hyperolius marmoratus*, the African clawed frog *Xenopus laevis* and the guttural toad *Sclerophrys gutturalis*. Although in the case of the first two species, studies have been carried out for investigating different aspects of the invasion (Measey and Channing 2003, Tolley et al. 2008, Measey et al. 2012, Davies et al. 2013 and 2015), literature about the guttural toad is mostly anecdotal (Measey and Davies 2011).

The guttural toad *Sclerophrys gutturalis* is a common African bufonid naturally distributed in across central and southern Africa (du Preez et al. 2004). The species is tolerant to different altitudes (from sea-level to about 1800 m) and latitudes (from the equator to 30° S). It inhabits disparate vegetation types like Savannah, Grassland and Thicket biomes (du Preez et al. 2004) and due to a highly synanthropic behaviour, it is not uncommon to find these toads in peri-urban areas. The guttural toad is native in most of South Africa but not in Cape Town, where an invasive population has established in 2000. It was likely introduced as eggs or tadpoles with a consignment of aquatic plants (De Villiers 2006) from KwaZulu-Natal (Telford 2015). Since its first detection in Constantia, the occurrence of this species raised concerns about its potential impact on the conservation of the endemic and IUCN Endangered western leopard toad *Sclerophrys pantherina* whose range overlaps partially with the guttural toad introduced area (De Villiers 2006). During the adult phase, the two species could compete for food resources; conversely competition during the larval phase should be minimal given that their breeding seasons do not temporally overlap in Cape Town (western leopard toad, winter; guttural toad, summer). Additionally, the guttural toad could have an indirect impact on the western leopard toad acting as a vector and host for both native and introduced parasites. However direct and indirect impacts of this species on its congeneric still have to be tested in the field. Following the recognition of the invasion, the City of Cape Town (CoCT) implemented an ongoing extirpation program since 2010 that removed about 5000 post-metamorphic individuals and many thousands of tadpoles and eggs. However, the population is still in expansion, and to date it is unknown to what extent demographic and spatial invasion dynamics are affected by traits of the species and characteristics of the invaded landscape. Similarly, both long-term effectiveness and efficiency of the current mode of removal have never been quantified and the possibility to implement other more effective extirpation strategies or alternative management actions is unknown.

The ability of the species to establish and spread in Cape Town is surprising; most of endemic amphibians that occur here and more generally in the Cape Floristic Region breed in winter to exploit favourable conditions of water availability (Branch and Harrison 2004). Conversely the guttural toad naturally inhabits summer rainfall areas of central and southern Africa

characterized by tropical and subtropical climates, where it adaptively synchronizes reproduction timing with precipitation pattern (du Preez et al. 2004). Invasive guttural toads still breed in summer despite in Cape Town this season is notably drier than that characterizing the environment of the source population. Currently, it is not clear how the abiotic conditions of Cape Town constrain the physiology of the species and whether invasive toads adaptively respond to reduce potential phenotypic mismatch in the novel environment. Similarly it is not clear to what extent the colder environment of Cape Town may limit the toads' activity during the winter non-breeding season.

The first aim of the thesis is to reconstruct demographic and spatial dynamics of the invasive population of guttural toad in Cape Town emerging from the interplay between traits of the invader and characteristics of the invaded landscape. This aim is specifically addressed in chapter one through the construction of a model that integrates age structured and least cost path approaches to simulate population dynamics of invasive pond-breeding anurans. The age structured model is successively utilized in the second chapter to explore efficacy and efficiency of the current management program and the possibility to adopt other more effective strategies. The thesis aims also to explore to what extent the species phenotype is constrained in the invaded environment during the breeding period and whether invasive toads underwent a response that in less than two decades could have increased their fitness and, more generally, invasion potential. Field surveys and laboratory trials reported in chapter three address this aim and provide also evolutionary and management insights. Lastly, chapter four adopts energy storage analysis to investigate whether and to what extent the invaded environment of Cape Town limits the activity of the invasive toads during the non-breeding season. The main goal of the thesis is to identify which factors make this invasion particularly successful despite the sustained extirpation program and the establishment of the guttural toad into a novel environment. This will be helpful to propose adaptive management countermeasures and address eco-evolutionary questions.

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Chapter one: Integrating age structured and least cost path models to disentangle invasion dynamics of a pond-breeding anuran

1.1 Introduction

The study of amphibian population dynamics and their drivers is essential from a conservation perspective. Amphibia are the most threatened group of vertebrates (Stuart et al. 2004, Wake and Vredeburg 2008), where several native populations are currently declining across the globe (Houlahan et al. 2000, Green 2003) and some populations have already headed toward extinction (Wake and Vredeburg 2008, Howard and Bickford 2014). This trend is mainly caused by anthropogenic activities such as land-use change, greenhouse gas emissions and accidental introductions of pathogens and invasive species (Blaustein and Kiesecker 2002, Collins and Storer 2003, Grant et al. 2016). Amphibians themselves can be invasive (Kraus et al. 2009) and their introduction and establishment are predicted to increase in the coming years as a consequence of globalization and international trade (Kraus and Campbell 2002, Reed and Kraus 2010). Since ecological and social-economic impact of these invasive populations can be severe (Measey et al. 2016), it is important to reconstruct their demography and spatial dynamics in order to predict invasion potential and perform adaptive management.

Demographic and spatial invasion dynamics inferred by field surveys or mathematical models indicate recursive patterns across taxa and regions (Essl et al. 2012, Larkin 2012; Van Wilgen et al. 2014, Hui and Richardson 2017); however traits of the invader and characteristics of the invaded environment may significantly influence timing and modes of such dynamics (Jongejans et al. 2011, Larkin 2012, Hastings et al. 2015, Roques et al. 2016, Hui and Richardson 2017). For example, at the onset of an invasion, most alien populations show a lag phase consisting of a low number of invasive individuals and/or invaded patches (Crooks and Soulé 1999, Crooks 2005, Essl et al. 2012). The lag duration may however range between three and hundreds of generations with factors such as propagule pressure or population growth rate often hypothesized to play a role (Schreiber and Lloyd-Smith 2009, Larkin 2012, Aagaard and Lockwood 2014). Similarly the phase of spatial spread may be considerably variable, where it may fit an accelerating and sigmoid, or a linear and decelerating relationship (Crooks 2005, Aikio et al. 2010; Kelly et al. 2014). Long range dispersal events, environmental heterogeneity or evolutionary phenomena may all contribute to such variation (Higgins and Richardson 1999, Schreiber and Lloyd-Smith 2009, Jongejans et al. 2011, Marco et al. 2011). Since predicting timing and modes of an invasion may have an important role to respond quickly through effective countermeasures (Higgins and Richardson

1999), complexity of invasive dynamics should never be underestimated. Each invasion should preferentially be modelled by incorporating species-specific characteristics and environmental features (Schreiber and Lloyd-Smith 2009, Roques et al. 2016).

Most amphibian populations are not homogeneously distributed across the landscape; instead they occur at greater densities in or around habitat patches that allow or facilitate survival and reproduction, such as wetlands and water bodies (Marsh and Trenham 2001). Therefore their dynamics, especially in case of pond-breeding species, can be profitably visualized through a metapopulation “ponds-as-patches” approach (Marsh and Trenham 2001) where: i) each breeding site is considered a single discrete habitat patch that exchanges individuals with other analogous patches (Skelly 2001, Smith and Green 2005); ii) the number of individuals at each pond is exclusively due the birth/death rate within pond and the exchange rate among ponds (Marsh and Trenham 2001, Pontoppidan and Nachman 2013). Classic metapopulation models require explicitly incorporating discrete and stochastic events of extinction and recolonization within patches (Marsh and Trenham 2001). However, a metapopulation approach is still valid when such events are not incorporated, for example to visualize how temporal and spatial dynamics of amphibian populations vary according to environmental factors (Skelly et al. 1999). Reproduction and survival in and around a pond may be affected among others by pond size, occurrence of predators and/or competitors, abundance of trophic resources or pollutants (Skelly 2001, Van Buskirk 2005, Hamer and Parris 2013). Similarly, exchange rate among ponds may vary as a function of pond-pond distance, availability of ponds, habitat and landscape heterogeneity and species vagility (Decout et al. 2012, Willson and Hopkins 2013, Hillman et al. 2014).

The capacity to incorporate this variation is essential in our effort to model population dynamics; but this may be particularly challenging considering that in most amphibians each individual passes through different life stages (e.g. larval, metamorphic, adult) which ontogenetically alter physiology and behaviour. Age structured models are a powerful approach to depict this complexity because they incorporate changes in survival and reproduction as a function of age (Caswell et al. 2003, Govindarajulu et al. 2005). Such a bottom-up approach explores emergent properties of a population by modelling interactions within (e.g. competition) and among (e.g. cannibalism) discrete age classes (Gamelon et al. 2016). Age structured models also allow application of differential dispersal dynamics to each age class by reconstructing how virtual organisms disperse across the landscape according to their life stage (Neubert and Caswell 2000, Steiner et al. 2014). Dispersal is generally affected by the interplay between landscape complexity and species-specific vagility (Hillman et al. 2014) linked to physiological and behavioural traits. An effective way to simulate such interplay is least-cost path modelling, where functional connectivity across a landscape is

modelled, combining the cost for an individual to move between habitat patches and detailed information about the landscape itself (Adriansen et al. 2003). Since landscape complexity may strongly affect efforts to model amphibian populations (Ficetola and De Bernardi 2004, Willson and Hopkins 2011), the incorporation of least-cost distance modelling into an age-structured approach seems essential to simulate among-patch dynamics.

In this chapter, I describe a novel model that integrates age structured and least-cost path approaches to reconstruct population dynamics of invasive pond-breeding anurans. The model is applied to my case study, the ongoing invasion of guttural toads *Sclerophrys gutturalis* in Cape Town, South Africa. Field data collected during management attempts, laboratory surveys and a literature review were employed to parameterize the model. Considering both demographic and spatial dynamics of the invasive population, I explore: i) occurrence and duration of lag phase; ii) whether the spatial spread fits an accelerating or a linear trend; iii) to what extent these dynamics match field observations. Additionally, I estimate sensitivity of the proposed model to demographic and behavioural traits. I conclude by discussing future implementations of the model to forecast amphibian invasive dynamics and test alternative management countermeasures.

1.2 Materials and methods

1.2.1 Case Study

The guttural toad *Sclerophrys gutturalis* is domestic exotic in South Africa (Measey and Davies 2011) being native in most of the country but not in Cape Town, where an invasive population has recently established. The invaded area is characterized by a peri-urban landscape which provides numerous suitable breeding sites, namely artificial ponds, for the toads (Figure 1.1). The invasion is occurring within the range of the congeneric species western leopard toad *Sclerophrys pantherina*, currently listed as Endangered by the IUCN (SAFRoG & IUCN SSC-ASG 2010) and endemic to two restricted areas of south-western South Africa (Measey and Tolley 2011). Moreover, invasions of toads in particular are known to have relevant environmental and economic impacts (Measey et al. 2016). Following the recognition of the invasion, the City of Cape Town (CoCT) started a sustained extirpation program (i.e. eradication at local scale, Panetta 2007) in 2010, but despite the removal of more than 5000 post-metamorphic individuals and many thousands of tadpoles and eggs (Measey et al. in press) the invasive population is still in expansion.

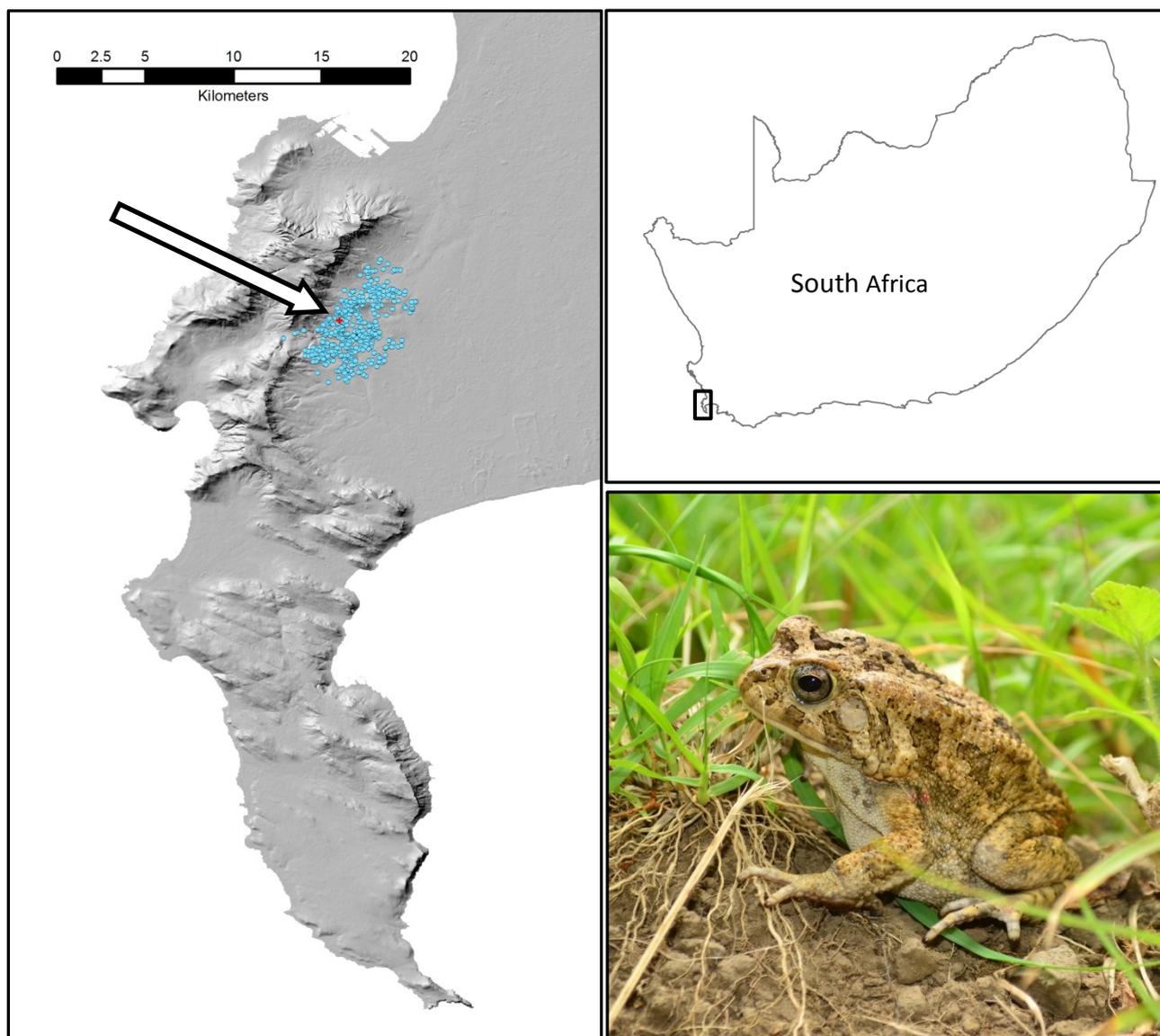


Figure 2.1: Spatial layer (provided by Stellenbosch University, Digital Elevation Model - SUDEM- 2016 Edition) showing the ponds located in Constantia and surroundings ($34^{\circ}01'53''\text{S}$, $18^{\circ}25'06''\text{E}$) through aerial imaging (in blue) and the pond where the guttural toad *Sclerophrys gutturalis* (see photo) where first observed in the season 2000/2001 (in red, see white arrow).

1.2.2 Model description

I follow the ODD (Overview, Design concepts, Details) protocol of Grimm et al. (2006) to describe the age structured model. Although the protocol was initially conceptualized to describe individual based models, it can help to delineate any bottom-up simulation and complex model by systematically isolating model components and facilitating their description (Grimm et al. 2006 and 2010). Since the least-cost path model is nested within the age structured model, its description is reported in the sub-model section below (see section

1.2.2.7.3). The age structured model is implemented in Mathematica version 10. (Wolfram Research 2015).

1.2.2.1 Purpose

The purpose of the model is to simulate guttural toad population dynamics in the pond network of the invaded area that emerge from species specific life-history traits, density-dependent survival and dispersal behaviour.

1.2.2.2 Entities, state variables and scales

The model is an age structured model of integrodifference equations where each pond utilized by adults to breed represents a population with a detailed life-cycle. The modelled entities are the ponds. Each pond works as a source or sink according to life-history stage specific demography and dispersal behaviour of its individuals. Each pond is characterized by three state variables: number of individuals present for each life-history stage (egg, tadpole, metamorph, juvenile, adult), pond location (x- and y- coordinates) and pond size. Discrete life-history stages of the guttural toad in Cape Town are defined in section 1.2.2.3 and depicted in Figure 1.2. The number of individuals in a pond is affected by within-pond demographic dynamics and inter-pond dispersal dynamics. Inter-pond connectivity is described below in the section 1.2.2.7.3 as a function of Euclidean distance and least-cost path distance. At the first model step, the number of individuals present in all ponds is zero (i.e. empty ponds) with the exception of the pond in which the guttural toad was first detected (Figure 1.1 and section 1.2.2.5).

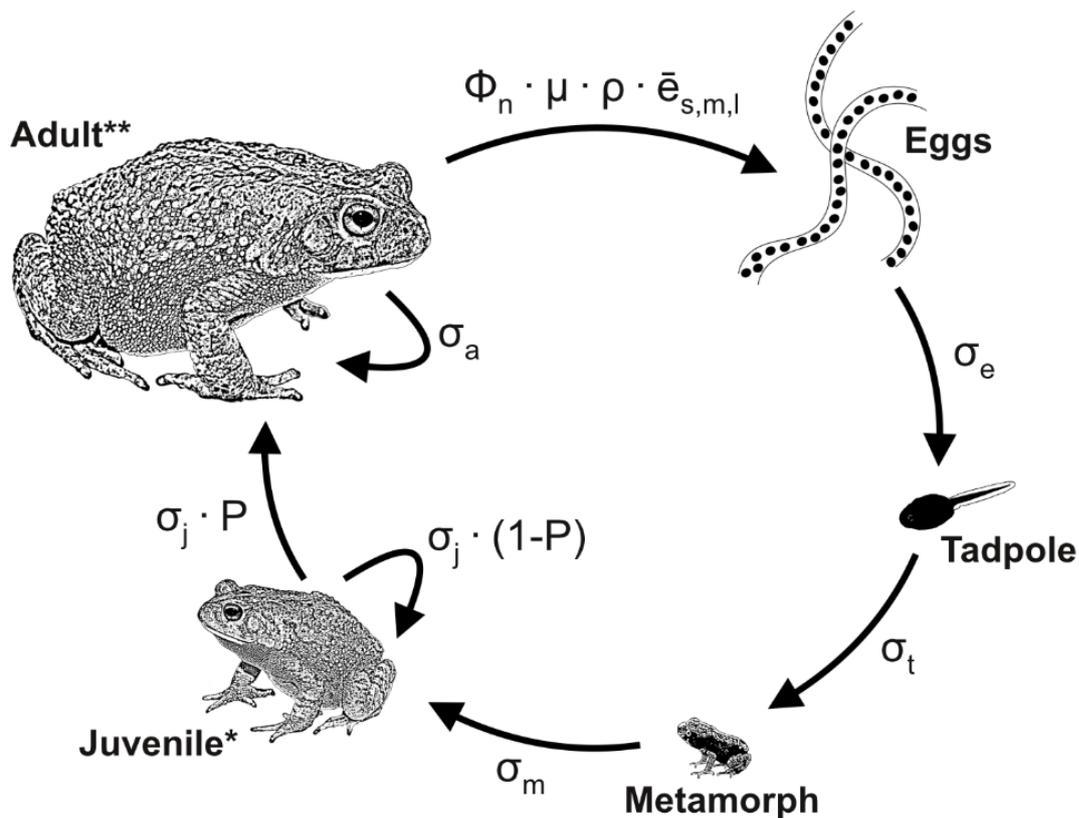


Figure 1.2: Life-cycle of the guttural toad *Sclerophrys gutturalis* in Cape Town. Egg deposition starts in late spring (October-November) and the total eggs number per female is determined by the clutch size (ϕn), the number of clutch per year (μ), the sex-ratio (ρ) and the probability to lay eggs in a pond according to the pond size ($\bar{e}_{s,m,l}$). Tadpoles hatch from eggs after one week with the probability σ_e and survive to metamorphosis after 4-5 weeks with the probability σ_t . σ_t is a function of the initial density of tadpoles in the pond as described by equation (1). Metamorphs over-winter and emerge the next spring as juveniles with the probability σ_m . σ_m is a function of the initial density of metamorphs in the pond edge area described by equation (4). After one year, juveniles survive with a probability σ_j and mature with a probability P . The annual adult survival is σ_a * and ** represent respectively dispersal of juveniles (no philopatry) as described by the equation (7) and of adults (no site fidelity) as described by equation (8).

To record the geographic coordinates of all potential breeding sites (ponds) within the invaded range in 2015 of the toads plus a 1.5 km wide buffer (Figure 1.1), I used aerial images provided by the City of Cape Town (<http://maps.capetown.gov.za/isisiv/>). The effectiveness of the aerial imaging survey to locate toad breeding sites was confirmed by the fact that through this method I located approximately ninety-five percent of ponds already recorded during the extirpation process. I also broadly classified ponds according to size in order to incorporate tadpole and metamorph density-dependence survival into the model. Small (2.5m^2), medium (25m^2) and large (250m^2) ponds represent fountains, garden ponds and small artificial lakes respectively.

1.2.2.3 Process overview and scheduling

In the model, a one-time step corresponds to one year. Within each time step, different life phases of an individual are processed according to the guttural toad life-cycle depicted in Figure 1.2; the cycle has been defined following the amphibian movement ecology frameworks proposed by Sinsch (2014) and Pittman et al. (2014) and adapted to the invasive population of Cape Town through field observations (see section 1.2.2.7.1 for details about each life-history stage). Each individual proceeds sequentially through egg, larval and metamorph stages until the juvenile stage in one step according to demographic dynamics (see section 1.2.2.7.1). The same individual turns into an adult in one more step according to its maturing probability. The model runs for thirty steps in total. Only individuals at juvenile and adult stage can disperse across the pond network according to dispersal dynamics (see section 1.2.2.7.2) and only adults can breed.

1.2.2.4 Design concept

1.2.2.4.1 Emergence of system level phenomena

Total number of adults in the population and their spatial distribution emerge for each year from individuals that survive, disperse and breed across the pond network.

1.2.2.4.2 Sensing

Individuals that disperse do not selectively target ponds with low density of conspecifics. However they preferentially move toward nearer ponds according to the dispersal kernel. Moreover pond nearness takes into account functional connectivity calculated through least-cost modelling (see section 1.2.2.7.3). Toads are assumed to know differential costs of locomotion across elements they encounter in the landscape and adaptively target ponds according to the least-cost path configuration. Individuals are also assumed to know their age in order for them to apply different age-specific dispersal behaviour.

1.2.2.4.3 Interaction

Individuals competitively interact as tadpoles and metamorphs in a pond according to the number of conspecifics at the same stage and pond size. Between-stage interactions (e.g. adult cannibalism on metamorphs) are not incorporated in my model.

1.2.2.4.4 Stochasticity

Stochasticity is not incorporated in my model. All life-history traits are set to constant values. The dispersal kernel derives from a probability distribution estimated through a mark-recapture study (Smith and Green 2006). Landscape features and their costs on locomotion are

modelled deterministically. Environmental stochasticity has not been incorporated in my model as the pond dynamics, i.e. temperature and biomass fluctuations are largely unknown and the climate is approximately homogenous across the arena given its small spatial scale.

1.2.2.4.5 Observation

The model outputs the number of individuals per each pond separately for each life-history stage. So I obtain for each year the total number of adult over time and the spatial distribution of the invaded population calculated in ArcGIS as the minimum convex polygon MCP in km² described by the ponds with at least one adult. The total number of adults and their spatial distribution are the auxiliary variables (i.e. “variables containing information that is deduced from low-level entities”, see Grimm et al. 2006).

1.2.2.5 Initialization

In the case of the invasive population of guttural toad in Cape Town, about ten males were heard for the first time in 2000 (De Villiers 2006) around a large pond at a known site in Constantia. However field observations on this species in Cape Town and Durban showed also that within a chorus some males do not call and this is known to be density dependent (Leary et al. 2005). Thus the model was initialized with 40 adults (i.e. propagule size) on that specific pond in the season 2000/2001 (2001 hereafter), considering the sex ratio to be 1:1. All the other ponds were assumed to be empty at the first step in order to simulate a colonization scenario.

1.2.2.6 Input data

The list of ponds, the size of each pond (2.5 m², 25 m², 250 m² for small, medium and large ponds respectively, section 1.2.2.2) and the Euclidean and least-cost path distance (see section 1.2.2.7.3) are read from external files.

1.2.2.7 Submodels

1.2.2.7.1 Demographic dynamics

I set egg production per female using the clutch size (ϕ_n) adjusted by the annual clutch number (μ), the adult sex ratio (ρ) and the probability of laying eggs in a pond estimated in the field ($\bar{e}_{s,m,l}$). In eggs, the probability of hatching successfully is σ_e whereas the tadpole survival (σ_t) is a function of the larval density of the pond. The tadpole density of the pond is a function of pond area ($A_{s,m,l}$) and the total initial number of tadpoles (T_i):

$$\sigma_t = \frac{\sigma_{tmax}}{(1+d\left(\frac{T_i c}{A_{s,m,l}}\right))^{\gamma}} \quad (1)$$

where σ_{tmax} is the highest larval survival without density-dependence, d is the density-dependent coefficient (m^2 / number of tadpoles), c is to indicate that for a given female that breeds in a pond there is no competition between the tadpoles of the first clutch and the tadpoles of the second clutch and γ is the density-dependence exponent with:

$$T_i = \phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \quad (2)$$

The rationale for equation (1) is reported in Appendix 1.A.1

Being the total initial number of metamorphs (M_i)

$$M_i = \phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \sigma_t \quad (3)$$

the survival of metamorphs (σ_m) is expressed as the ratio between the final density of metamorphs and their initial density where:

$$\sigma_m = 1 - \left(\frac{((\phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \sigma_t / E_{s,m,l})^2 / 2.76)^{0.623}}{\phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \sigma_t / E_{s,m,l}} \right) \quad (4)$$

with $E_{s,m,l}$ representing the pond edge area and σ_m that has to be ≥ 0 .

The rationale for equation (4) is reported in Appendix 1.A.2

The number of metamorphs that survive and emerge as juveniles the following spring (J_i) is expressed by:

$$J_i = \phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \sigma_t \sigma_m \quad (5)$$

The survival of juveniles after one year is σ_j whereas the probability to mature is P . So the initial adult number (N_i) is:

$$N_i = \phi_n \sigma_e \mu \rho \bar{e}_{s,m,l} \sigma_t \sigma_m \sigma_j P \quad (6)$$

with each adult having a probability of survival to the following year expressed by σ_a .

1.2.2.7.2 Dispersal dynamics

I implemented the life-cycle of the guttural toad in the pond network of Cape Town (Figure 1.1). The movement from a starting pond to a destination pond is always due to the dispersal of juveniles and adults; eggs, tadpoles and metamorphs are constrained to stay within or around the natal pond. As a consequence of these different dispersal strategies, the number of metamorphs in a pond i at time t ($met [i,t]$) is determined by the number of eggs in the pond ($egg[i,t]$), and the survival of eggs (σ_e) and tadpoles (σ_t) whereas the number of juveniles ($juv [i,t]$) is expressed by

$$juv [i, t] = \sum_{j=1}^{415} mj[i, j] (met[j, t] \sigma_m + juv[j, t - 1] \sigma_j (1 - P)) \quad (7)$$

and the number of adults is expressed by

$$adu [i, t] = \sum_{j=1}^{415} (mj[i, j] \sigma_j P * juv[j, t - 1] + m[i, j] \sigma_a adu[j, t - 1]) \quad (8)$$

where $mj[i,j]$ and $m[i,j]$ represent the juvenile and the adult movement matrix, respectively.

However, not all juveniles and adults disperse toward a new pond; indeed some individuals of a population can show philopatry and site fidelity (Pittman et al. 2014, Sinsch 2014).

Philopatric juveniles assume a stationary behaviour around the natal breeding site (see “philopatry” in Sinsch 2014) whereas non-philopatric ones disperse around the natal pond in order to look for a future alternative breeding site. Thus the juvenile movement matrix ($mj[i,j]$) is

$$mj[i, j] = (1 - \phi) \text{kernel}[c_{ij}/\text{norm}] \quad (9)$$

where ϕ is the probability for juveniles to remain in the same pond (i.e. to perform philopatry) whereas the kernel expresses the probability for juveniles to move from pond i to pond j as a function of: 1) the dispersal kernel defined by Smith and Green (2006) and 2) the distance between the two ponds defined by the distance matrix c (see below). Most of the adults select the same breeding site after one year (see “site fidelity” in Sinsch 2014) whereas the remnants target a novel site. Thus the adult movement matrix ($m[i,j]$) is

$$m[i,j] = (1 - \text{fid}) \text{kernel}[c_{ij}/\text{norm}] \quad (10)$$

where fid is the probability for adults to select the same pond one year after breeding (i.e. to perform site fidelity).

Following Smith and Green (2006), I use the same dispersal kernel and the same distance matrix for both adults and juveniles. I sourced the mark-recapture data reported in Smith and Green (2006) for adult dispersal in the North American toad, *Anaxyrus fowleri* to estimate the kernel described by an inverse power law as reported below:

$$\text{kernel}[c_{ij}] = 4.1651[c_{ij}]^{-0.884} \quad (11)$$

The kernel outputs the probability to move from pond i to pond j according to the distance matrix c and is normalized by a factor norm (= 3.8003 in the landscape network).

1.2.2.7.3 Least cost path model

The geographic coordinates of the ponds identified through aerial images were converted into shape-files and utilized to obtain two distance matrices through: 1) the Euclidean Distance Tool in the ArcGIS Spatial Analyst Toolbox, which expresses the ordinary distance between two ponds (Euclidean matrix later in the text); 2) the ModelBuilder function in ArcGIS which expresses the least-cost path distance between two ponds (LCP Matrix hereafter). The tool created runs sequentially in 4 steps : i) the Weighted Overlay tool, in order to overlay the components of a referenced raster using a common scale and to weight each according to its importance; ii) the CostDistance tool, in order to calculate the least cumulative cost distance for each cell to the nearest source over a cost surface; iii) the Cost Path tool, in order to calculate the least-cost path from one pond to another; iv) the Raster to Polyline tool, in order to convert the path raster to a polyline in meters. To automate the least-cost path calculation

for all the ponds, I used a script in Python for ArcGIS (Appendix 1.B). The referenced raster has to incorporate the spatial elements of the landscape that cause different costs of locomotion. From a toad's perspective the landscape is mostly characterized by private grassy gardens (where the ponds are located), each surrounded by walls and connected by streets (see Appendix 1.A.3). As a consequence of this simplified structure, I built a raster with 5 m resolution characterized by three features (i.e. grass, wall and street) through overlaying a cadastral spatial layer file on a roads spatial layer of Cape Town. Then I simulated different cost scenarios by assigning different costs to each feature through the Weighted Overlay tool; I successively scored the alternative paths for five pairs of ponds in light of my experience on the species locomotion. The most successful configuration of costs (Appendix 1.A) was utilized in the LCP matrix construction for all the pairs of ponds.

1.2.2.7.4 Parameterization

Most demographic and dispersal parameters of the model were obtained by a literature review. When species-specific parameters were not available, I used parameters collected on similar bufonid species (Table 1.1). However clutch size, annual clutch number and probability to lay eggs in small, medium and large ponds were specifically estimated for the guttural toad population of Cape Town (Appendix 1.A.4).

Table 1.1: Model parameters. Parameters with asterisk represent guttural toad *Sclerophrys gutturalis* species-specific information collected through laboratory and field surveys on the Cape Town population or a literature review on the species. Parameters without asterisk represent information collected from the literature on similar bufonid species.

Parameter	Baseline Values	Source
*Clutch size (ϕ_n)	13000	See appendix 1.A.4
*Annual clutch number (μ)	2	See appendix 1.A.4
Adult sex ratio (ρ)	0.5	Assumption
*Probability to lay eggs in small, medium and large ponds respectively ($\bar{e}_{s,m,l}$)	0.06 0.4 0.22	See appendix 1.A.4
Egg survival (σ_e)	0.7	Blaustein et al. 1994; Biek et al. 2002
Maximum larval survival (σ_{tmax})	0.8	Vonesh and De la Cruz 2002
Density-dependent coeff. (d)	0.007	Vonesh and De la Cruz 2002
Annual proportion of competing tadpoles from the same female	0.5	Assumption

(c)

*Pond area of small, medium and large ponds respectively ($A_{s,m,l}$)	2.5 m ² 25 m ² 250 m ²	Estimated in the CT invaded area through aerial images
Density-dependent exponent (γ)	1	Vonesh and De la Cruz 2002, see appendix 1.A.1
*Pond-edge area within a radius of 5 meters ($E_{s,m,l}$)	106.4 m ² 166 m ² 357.7 m ²	Calculated using the pond area $A_{s,m,l}$, see appendix 1.A.2
Juvenile survival (σ_j)	0.2	Lampo and De Leo 1998, Vonesh and De la Cruz 2002, Biek et al. 2002
Maturing probability (P)	0.25	Vonesh and De la Cruz 2002
Adult survival (σ_a)	0.6	Vonesh and De la Cruz 2002, Biek et al. 2002
Juvenile probability to show philopatry (ϕ)	0.66	Assumption
Adult probability to show site fidelity (fid)	0.8	Assumption

All demographic and behavioural parameters are set at their baseline value reported in Table 1.1 (but see sensitivity analysis below) with the exception of the parameter h . Given the initial pond, the dispersal kernel and the distance matrix, every pond has a specific probability to receive adult toads, with ponds closer to the initial ponds having a higher probability of being colonized. The parameter h regulates which ponds can be used by toads to breed according to their density; in other words, it acts as a threshold with ponds that can be successfully deployed to lay eggs only when their probability of having toads is equal to or larger than h . In other words, h regulates how fast the invasive spread occurs within the pond network with high values of h resulting in slow spread and low values of h resulting in rapid spread. Since I cannot know with certainty the demography of the invasive population at the time the extirpation program started, I assume for simplicity that the number of adults removed during the first eradication season (≈ 700) is representative of the adult population. Although this assumption is optimistic, it does not necessarily imply that this removal would have allowed extirpating the population in one year; most of individuals from other life-history stages (e.g. juveniles and metamorphs) were not removed during extirpation (Vimercati in prep.) and this could have caused adult recruitment in the successive years.

During the parameterization process, I ran the model with values of h ranging between 0 and 1 (increment = 0.005) and compared through t -tests the model outputs for adults (i.e. number of adults predicted by the model per pond) with the eradication data collected in 2011, using both Euclidean and LCP matrices. All outputs obtained using the Euclidean matrix were significantly different ($p < 0.05$) from the eradication data. On the contrary, using the LCP matrix I found that the output obtained with $h = 0.07$ matched best the eradication data ($t = 0.0795$, $p = 0.937$). Thus, I parameterized the model with the LCP matrix using this value of h and removed the Euclidean distance from further analyses.

1.2.3 Simulation experiments

Firstly I simulate the population dynamics using the parameterized model. Secondly, in order to provide insights into which survival and dispersal parameters have the highest impact on invasive population dynamics, I perform sensitivity analysis by decreasing each parameter by 10% from 1 to 0 and keeping all the other parameters at their baseline values. The parameters to be tested were: egg survival; maximum larval survival; juvenile survival; adult survival; maturing probability; site fidelity; philopatry. I also test model sensitivity to propagule size initializing the model with different sizes according to the geometric sequence “5, 10, 20, 40, 80, 160”. This sequence has been chosen in order to test realistic propagule pressures. Lastly I run the parameterized model using the Euclidean matrix instead of the LCP matrix for the baseline propagule size in order to explore to what extent the effect of the least-cost path modelling. Then I ran the parameterized model again for all sensitivity scenarios. The total number of adults over time matches a logistic curve in 63 out of 70 scenarios (and when it does not, the population collapses). Therefore, the Self-Starting NIs Logistic Model in R software (package *stats* version 3.4.0, R Development Core Team 2014) has been used to estimate “Asym” (i.e. the upper asymptote), “xdim” (the x value at the inflection point of the curve) and “scal” (a numeric scale parameter describing the growth rate) for each scenario. Sensitivity analysis is commonly deployed in population modelling to estimate adult demography at the equilibrium (i.e. the upper asymptote); however I also aim to investigate to what extent each parameter affects timing and modes of this invasion. The x value at the inflection point of the logistic curve (i.e. “xdim”) indicates when the adult population demography reaches 50% of its maximum value at equilibrium and is a proxy of the lag phase. Conversely the numeric scale parameter (i.e. “scal”) indicates how fast the population grows after the lag phase.

1.3. Results

1.3.1 Invasion dynamics

The model predicted for the adult population of guttural toads in Cape Town a logistic demographic dynamic (Figure 1.3) showing sequentially: i) a lag (2001-2010); ii) an expansion (2011-2013); and iii) a dominance phase (2014-2030). The lag phase is defined by the years at which the adult population is $\leq 5\%$ of the difference between the upper and the lower asymptote whereas the saturation phase is defined by the years at which the corresponding demographic values are above the 95% of the difference between the upper and the lower asymptote.

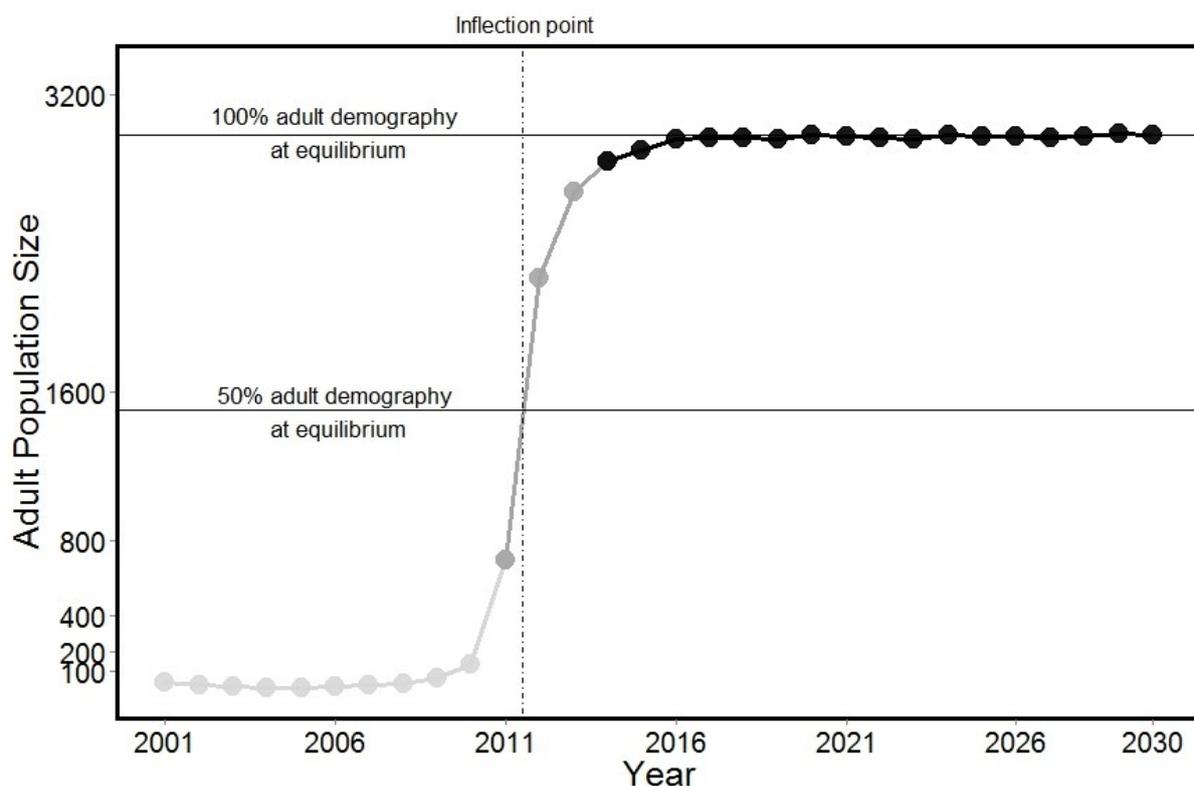


Figure 1.3: The demographic population dynamic of the guttural toad *Sclerophrys gutturalis* in Cape Town forecasted by the age structured model follows a logistic curve described by three different stages (lag, expansion and dominance; in pale grey, dark grey and black respectively). The inflection point represents the point with the highest growth rate (i.e. where the curve reaches 50% adult demography at equilibrium) whereas the 100 % adult demography at equilibrium is defined by the upper asymptote.

The spatial dynamics of the invasive population shows a similar trend with: i) a lag phase, during which toads do not spread across the pond network (2001-2005, Figure 1.4(a)); ii) a

spread phase, during which toads invade new ponds every year (2006 -2012, Figure 1.4(b-f)); and iii) a dominance phase, during which all the ponds are invaded (2013-2030, Figure 1.4(g-i)). It suggests that the guttural toads in Cape Town started to spread across the pond network five years before their demographic expansion. I also identified a linear relationship between the log of the total number of adults and the log of the invaded area (Appendix 1.D).

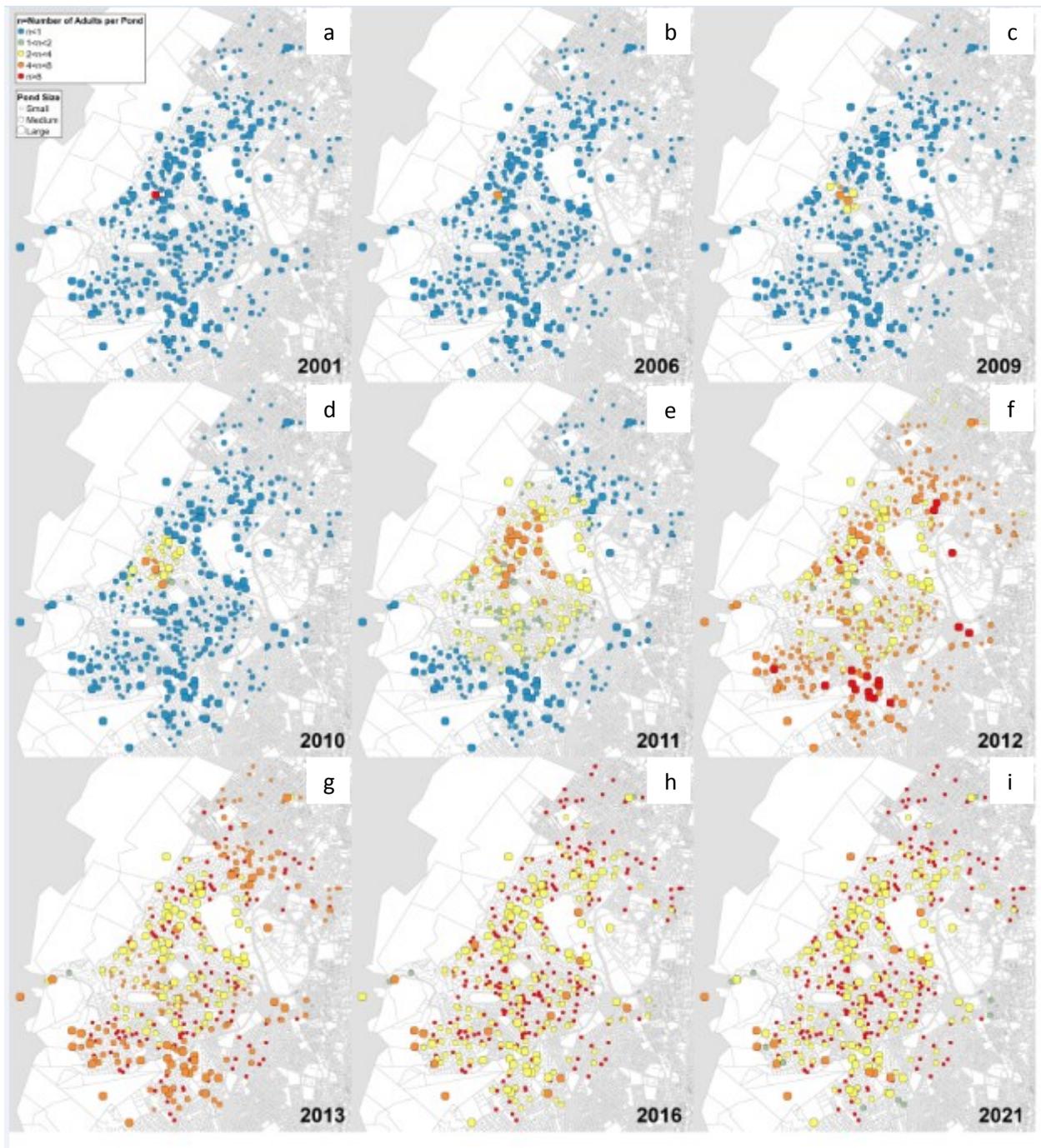


Figure 1.4 (colour should be used for this figure in print): Spatial layers showing the spatial dynamics of the guttural toad *Sclerophrys gutturalis* in Cape Town across years as forecasted by the age structured model. Colours represent different number of individuals in each pond

(blue, less than one individual; green, between one and two individuals; yellow, between two and four individuals; orange, between four and eight individuals; red, more than eight individuals).

The lag phase I detected is mainly due to the interaction between the dispersal kernel and density dependent survival. The kernel sourced from Smith and Green (2005) is characterized by a leptokurtic probability distribution that determines the incorporation of rare long distance dispersal events. Thus, just after the introduction, the majority of individuals do not disperse across the pond network and the few which do disperse are not able to breed. This is confirmed by the fact that the spatial spread of the population is delayed until 2006 and starts five years before the demographic expansion (2011). It also suggests that during those years (2006-2011) the density of the invaders is lower than the initial density. Additionally, at the beginning of the invasion many adults bred in the same pond as a consequence of the reduced initial dispersal, with density-dependent survival of tadpoles and metamorphs that caused low recruitment and negative growth rate in the first five years.

The spread/expansion phase shows an accelerating and sigmoidal trend, and is determined by the same mechanisms expressed previously at a different scale of space and time. When the density at the leading edge reaches the threshold, allowing reproduction, the population grows faster and faster (Appendix 1.C) because the low density at the periphery determines high survival of tadpoles and eggs (Figure 1.4(c-e)). This is confirmed by the linear relationship I detected between the number of toads and the area they invaded; since both these auxiliary variables are expressed on a log scale (Appendix 1.C), the spatial spread of the invaded area grows exponentially with the number of adults. Although I plotted the relationship between total number of toads and invaded area in an XY graph for simplicity, neither of these variables should be considered independent, as during the spread they tend to reinforce one to another.

Although a systematic survey on the invasion of the guttural toads across years does not exist for the period before the eradication (pre 2011), some scattered information collected about the invaded area help to qualitatively compare the outputs of my model against field observations. A lag phase was indeed detected after the first species detection in Cape Town (season 2000-2001), with toads heard calling in less the 2km² in the following years (De Villiers 2006, Measey and Davies 2011); however my model predicted smaller invaded areas during this phase. Conversely, the model forecasted for 2011 an invaded area larger than the area estimated through eradication in the same year and more compatible with that estimated in the season 2014-2015 (9.72 km², 3.51 km² and 7.56km² respectively). Lastly, the model

predicted the invasion of the entire arena (about 27.11 km²) by 2012 (Figure 1.4(f)), whereas guttural toads are still known to invade new portions of the arena every year (Measey et al. in press). To summarize, my model showed a longer lag phase and a shorter exponential phase than those observed in the field.

1.3.2 Sensitivity analysis

In the model, adult demography at equilibrium increased exponentially with juvenile and adult survival, and linearly with maturing probability (Appendix 1.E(c-e)). Juvenile survival had the highest effect; for example, a survival four times higher than the baseline value in juveniles led to a number of adults approximately ten times higher (Appendix 1.E(c)). Conversely, modelled adult demography was robust to variation in pre-metamorphic demographic traits (e.g. egg survival, Appendix 1.E(a)) and behavioural traits (e.g. site fidelity, Appendix 1.E(f)). Variations in propagule size and different approaches to model pond-pond distance did not affect the total number of adults at equilibrium. (Appendix 1.D(g,h)). Similarly, population growth was sensitive to juvenile and adult survival but robust to all other demographic and behavioural traits (Appendix 1.F). Conversely timing of invasion, expressed as inflection point, was sensitive to propagule size (Appendix 1.G(h)), with smaller sizes that led to inflection points occurring in later years (i.e. longer lag phase) and bigger sizes that led to points occurring in earlier years (i.e. shorter lag phase). For instance, had the introduction at the initial pond been a propagule size four times bigger (160 individuals instead of 40), it would have resulted in a lag phase seven years shorter. The Euclidean distance matrix led to an earlier inflection point than that obtained using least cost modelling (Appendix 1.G(h)). Lower values of site fidelity and higher values of maturing probability anticipate the inflection point whereas all the other traits do not show a coherent pattern (Appendix 1.G). Lastly I observed that extremely high values of site fidelity and philopatry and extremely low values of post-metamorphic survival led the population to collapse before invading the arena (Appendix 1.E,1.F,1.G).

1.4 Discussion

For the guttural toad invasion of Cape Town my model forecasts demographic and spatial dynamics that are characterized by lag phases and accelerating spread. The spatial spread starts five years earlier than the demographic expansion, suggesting a low density of invasive individuals at the beginning of the invasion. Such dynamics noticeably match real field observations and confirmed what was previously detected in other invasive populations (Crooks 2005, Aikio et al. 2010, Essl et al. 2012). However, sensitivity analysis also suggests that it would be preferable to quantify parameters such as propagule size or post-metamorphic

survival in the field. Small oscillations of these parameters may have important consequences on our capacity to effectively reconstruct and predict amphibian invasions.

Lag times have been frequently detected at the onset of a biological invasion across different taxa (Crooks 2005, Aagaard and Lockwood 2014; van Sittert & Measey 2016) with multiple mechanisms hypothesized to play a role such as Allee effects (Courchamp et al. 1999, Stephen and Sutherland 1999), spatial heterogeneity (Schreiber and Lloyd-Smith 2009) and population growth trajectories (Pyšek and Hulme 2005). For example, exponential growth trajectories are intrinsically expected to generate lags because at the onset of any invasion the number of individuals and/or invaded areas is necessarily low (Crooks 2005). However in my model the lag phase deviated from an exponential growth and showed a prolonged trend suggesting the occurrence of more complex processes. Mounting evidence shows this deviation in ecological models (Aikio et al. 2010) where factors such as evolution or competition were hypothesized to prolong lags (Crooks 2005). Intriguingly the relaxation of competitive interactions caused by lower density at the periphery should cause an abrupt end of the lag phase (Crooks 2005, Marco et al. 2010), similar to what I observed in my model when invasive toads breed at the invasion front. Notably a prolonged lag and the low density of individuals during the first years of the spatial spread could have delayed the first detection of the population (season 2000/2001) and/or management reactions (season 2010/2011) until the successive spread phase (Epanchin-Niell et al. 2012). The sensitivity analysis also showed that bigger propagule sizes determine shorter lag times. Authors have suggested propagule size as a predictor of establishment and invasion success (Hayes and Barry 2007) and although in my model a small propagule size (five individuals) invasion after a very long lag phase (Appendix 1.F), it did not account for demographic and environmental stochasticity. Stochastic effects can potentially eliminate a small established population before the onset of the spread phase; therefore, a shorter lag phase due to a higher propagule pressure could *per se* reduce the occurrence of these effects and promote a higher probability of invasion. From a management perspective it implies that removing a subset of individuals during a lag phase might not only postpone the invasive spread but also promote a crash of the established population (Crook 2005).

In my model the lag is followed by accelerating spread; this is in accordance to what was predicted by Kot et al. (1996), Neubert and Caswell (2000) and Neubert and Parker (2004) in case of models based on integrodifference equations and characterized by fat-tailed (leptokurtic) kernels. Although authors suggest that minimal variations in the shape of dispersal kernel could generate different invasion speeds (Kot et al. 1996, Caswell et al. 2003, Phillips et al. 2008), the choice to incorporate long-distance dispersal events seems

appropriate. *Sclerophrys gutturalis* has been anecdotally observed (GV pers. obs.) to show long-distance dispersal events in Cape Town (>1 km) as in many species of toads (Smith and Green 2005) and performance trials on *S. gutturalis* showed that adult individuals may have locomotive endurance up to 1 km /per night (see chapter three.). An accelerating spread was confirmed in several other biological invasions across taxa and regions (Hastings et al. 2005, Arim et al. 2006) and it is a recursive pattern in species characterized by long dispersal events (Mundt et al. 2009; Kelly et al. 2014). Moreover, the kernel I used was estimated through mark-recapture methods in the North American toad, *Anaxyrus fowleri* (Smith and Green, 2006) compatible for size, ecology and breeding strategy with the guttural toad (du Preez et al. 2004).

Interestingly I observed that the population growth rate was particularly sensitive to variation in post-metamorphic survival (Appendix 1.E), suggesting that high survival at life-history stages with no density dependence may cause faster spreads. Since invasive individuals may have survival significantly higher than native individuals (e.g. due to enemy release, DeWalt et al. 2004, Lakeman-Fraser and Ewers 2013), it would be wise to estimate this life-history trait during invasion; small differences in its estimation may have large effects on modelling invasive dynamics of pond-breeding anurans (Lampo and De Leo 1998). It also implies that perturbations occurring later in the life-cycle have bigger consequences on amphibian populations, as other theoretical studies on amphibians have suggested (Vonesh and de la Cruz 2002, Govindarajulu et al. 2005, Beaty and Salice 2013, see chapter two). This is also in accordance to the high sensitivity of adult demography to variation in the post-metamorphic demographic traits I observed (Appendix 1.E). Density of invasive individuals is known to be positively correlated to their impact on ecosystems and native populations (Yokimizo 2009); therefore, I suggest that if management aims to reduce the impact of the guttural toad, it could mainly target removal of adults and juveniles in order to maximize success (Beaty and Salice 2013). I call, however, for more theoretical and field investigations on this aspect because complex non-linear dynamics have been observed in other models (Govindarajulu et al. 2005).

Although these invasion dynamics are qualitatively in accordance with field observations, this model is not free of limitations. Firstly, I set that the dispersal behaviour is mainly due to the interaction between the dispersal kernel and LCP distance. My LCP calculation incorporates an element of realism (i.e. two ponds are distant each other not only because of their geographic position but also because of their connectivity linked to the toad dispersal preferences); however quality of breeding sites, density dependent dispersal, habitat predictability and climate matching between native and invasive area may all interfere with toad dispersal behaviour (Ficetola and De Bernardi 2004, Smith and Green 2005, Cayuela et al. 2016). Also, the use of demographic parameters estimated in other toads, given the

scarcity of literature on the guttural toad, is a source of uncertainty, although my review suggests the parameters used are within bufonid interspecific variation (Table 1.1).

Lastly, limitations are associated with the field data I used to parameterize the models. For example, I cannot exclude with certainty that when the guttural toad was first detected in 2000, some toads had already colonized a few more ponds. In that case my parameterization would overestimate the species' capacity to spread across the pond network, resulting in a faster spread across the arena. Similarly, the spatial eradication data collected in 2011 could be inaccurate because it was observed that the eradicators only systematically targeted the invasion front just after 2013 (Sara Davies pers. obs.). Therefore, the spatial extent of the invaded area in 2011 and 2012 could also have been underestimated.

Despite these limitations, my approach appears particularly promising to further explore demographic and spatial dynamics of pond-breeding invasive anurans from a management perspective (Vimercati et al. in prep). Since in my model I can regulate survival of virtual individuals at different life-stages for any target pond, it would be possible to simulate different management strategies that remove individuals from the population and then forecast their effects on population dynamics (see chapter two). Such strategies can be simulated not only across space (e.g. removal of individuals from some specific ponds) and time (e.g. removal of individuals in some specific years) but also across the whole life-cycle. The age structured approach allows for example simulating removal of: i) eggs and tadpoles at the invasion front using chemical traps (Crossland and Shine 2011); ii) adults and juveniles by hand or mechanical traps (Schwarzkopf and Alford 2007) during the exponential phase; iii) toads at any life-stage but only in a subset of target ponds. Since these strategies may have different time and labour costs, their impacts on population dynamics may be compared and the most effective strategy selected through a cost-benefit approach.

To conclude, I suggest that the model described here may help not only to reconstruct invasion dynamics of pond-breeding anurans by the integration of the invader and characteristics of the invaded landscape; it can be further deployed to gain insights on management decision making (Caplat et al. 2012 and Addison et al. 2013).

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Appendix 1.A

Appendix 1.A.1

Equation (1) derives from the model proposed by Hassel (1975) to incorporate the effect of intraspecific competition on tadpole survival and follows the parameter estimation reported in Vonesh and de la Cruz (2002). The density-dependence coefficient d acts as a scaling parameter, whereas the density-dependence exponent γ regulates the relationship between the number of metamorphs (here M_i) and the initial number of tadpoles (T_i). Although this

relationship can be linear ($\gamma = 0$), weakly density dependent ($0 < \gamma < 1$), compensatory ($\gamma = 1$) or overcompensatory ($\gamma > 1$), we decided to set it as compensatory following the literature reviewed by Vonesh and de la Cruz (2002) for the family Bufonidae. We used surface area to estimate density-dependent survival following Vonesh and de la Cruz (2002) and Tejedo and Reques (1994), as tadpoles of *S. gutturalis* are browsers of superficial algae (JM pers. obs.). We eliminated competition between the tadpoles of the first clutch and those of the second clutch by incorporating the annual percentage of competing tadpoles from the same female (c) into equation (1).

Appendix 1.A.2

Equation (4) derives from the manipulative study conducted by Harper and Semlistch (2007) in *Anaxyrus americanus*. The authors used enclosures of 2 m² to manipulate metamorph density and detected density-dependent survival after one year. We observed in the field that most metamorphs can be detected within 1 m from the pond edge and that all of them were found within 5 m. This spatial distribution around the pond edge may be due to dehydration sensitivity of toads at this life-history stage as largely hypothesized in the literature (see pre-departure phase in Pittman et al. 2014) and confirmed by field studies (Child et al. 2008, a,b). Since in Cape Town metamorphosis takes place during the dry, hot summer, I suggest that the dehydration stress of metamorphs should be high, strongly limiting their dispersal from the natal pond. Thus we calculated the pond edge area within a radius of 5 meters ($E_{s,m,l}$) and used it to estimate the initial density of metamorphs (during the pre-departure phase). It should be noted that equation (4) describes a theta-logistic growth model in which the first year survival of metamorphs is strongly over-compensatory, i.e. with lower survival at very low and very high density and higher survival at intermediate density.

Appendix 1.A.3

We observed that all ponds of the arena are located in private properties; moreover field surveys showed guttural toads tend to use paved streets for moving from one property to another as detected in radiotracking studies on the western leopard toad in Cape Town (JM pers. obs.) and in other bufonids (Brown et al. 2006), conversely avoiding habitats with dense vegetation. Toad locomotion on paved surfaces is also more effective (faster speed) than on vegetated and grassy habitat (GV pers. obs.) as already detected in invasive cane toads *Rhinella marina* (Brown et al. 2006). Conversely toads were strongly limited in their movement by the walls that surround each property, using fenced-gates preferentially to move from the street to get inside a property. Lastly, green corridors and water channels are not preferentially used by toads to disperse, contrary to what has been observed in other species of invasive frogs (Peterson et al. 2013).

Appendix 1.A.4

In the laboratory, dissection of 15 females captured and euthanized during the extirpation followed by removal of their ovaries allowed estimation of clutch size (i.e. number of eggs per clutch per female) through subsampling. Additionally, field surveys showed that in Cape Town the breeding season occurs between October and February (instead of between August and March as in the South Africa native range, du Preez et al. 2004), thus restricting annual number of clutches to two instead of three. Lastly, probability to lay eggs was calculated separately for each pond size category (small, medium, large) as the probability to detect eggs and tadpoles in a pond where the presence of females was already confirmed around the same pond during the extirpation process. For example the high probability to lay eggs in a medium pond (0.4) means that eggs and tadpoles were detected in four out of ten medium ponds where at least a female was detected.

Appendix 1.B: Python code utilized to automate the calculation of the LCP matrix. “All_points” is the shape.file with all the ponds whereas “points folder” is the folder containing all the shape.files of the pond coordinates

```
import arcgisscripting, os, sys, string, math, traceback, shutil, subprocess
from time import localtime, strftime
```

```
import arcpy
from arcpy import env
from arcpy.sa import *
```

```
arcpy = arcgisscripting.create(10.1)
env.overwriteOutput = True
arcpy.CheckOutExtension("Spatial")
arcpy.SetProduct("ArcInfo")
```

```
class GetOutOfLoop( Exception ):
    pass
```

```
# Directories (Input)
TEMP_LOC # Temporary storage folder for execution.
WEIGHT_RASTER # Weighted raster.
POINTS # Points folder
LOG # Log file for debugging purposes
OUTPUT_LOC # Output folder directory.
```

```
env.workspace = TEMP_LOC
env.scratchWorkspace = TEMP_LOC
env.snapRaster = WEIGHT_RASTER

# Checks the extension of a given file
def checkExtension(fileName, extensions):
    length = len(fileName)
    lowercase = fileName.lower()

    # If the string length is less than 3 character, it has no extension
    if length < 3:
        return False
    else:
        for extension in extensions:
            found = lowercase.find(extension, length - 4, length)

            # Extension found, return true
            if found != -1:
                return True
        # Extension not found, return false
        return False

# Writes a message to the arcMap console and a log file
def writeMessage(message):
    arcpy.AddMessage(message)

    msg = open(LOG, "a")
    msg.write(message + "\n")
    msg.close()

try:
    writeMessage("START: Giovanni distance...")

    env.workspace = POINTS
    list_points = arcpy.ListFeatureClasses()
    env.workspace = TEMP_LOC
```

```

#merged_points = OUTPUT_LOC + "All_points.shp"

for start_point in list_points:
    writeMessage("Point file found: " + start_point)
    pointFile_dir = POINTS + start_point
    temp_final_file = TEMP_LOC + "temp_current_merge.shp"
    final_file = OUTPUT_LOC + start_point.replace("-", "_")
    exist_final = arcpy.Exists(final_file)

    if not exist_final:
        arcpy.RepairGeometry_management(pointFile_dir)

        temp_costDis = TEMP_LOC + "costDis"
        temp_backlink = TEMP_LOC + "backlink"
        raster_costDistance = CostDistance(pointFile_dir, WEIGHT_RASTER, "#",
temp_backlink)
        raster_costDistance.save(temp_costDis)

    for destination_point in list_points:
        destination_final_file = OUTPUT_LOC + destination_point.replace("-", "_")
        exist_destination = arcpy.Exists(destination_final_file)
        if not exist_destination:
            if start_point not in destination_point:
                destination_dir = POINTS + destination_point
                writeMessage("Destination point: " + destination_point)

                arcpy.RepairGeometry_management(destination_dir)

                temp_extracted = TEMP_LOC + "temp_extracted.shp"
                arcpy.CopyFeatures_management(destination_dir, temp_extracted)

                writeMessage("Adding fields...")
                arcpy.AddField(temp_extracted, "Start", "TEXT")
                arcpy.AddField(temp_extracted, "Destinatio", "TEXT")
                arcpy.CalculateField_management(temp_extracted, "Start", "\"" +
start_point.replace(".shp", "") + "\"")

```

```

        arcpy.CalculateField_management(temp_extracted, "Destinatio", "\"" +
destination_point.replace(".shp", "") + "\"")

    arcpy.DeleteField_management(temp_extracted, ["ERF_number"])

    writeMessage("Cost path...")
    temp_costPath = TEMP_LOC + "costPath"
    raster_costPath = CostPath(destination_dir, temp_costDis, temp_backlink)
    raster_costPath.save(temp_costPath)

    writeMessage("Raster to polyline...")
    temp_polyline = TEMP_LOC + "polyline.shp"
    arcpy.RasterToPolyline_conversion(temp_costPath, temp_polyline, "ZERO", 0,
"SIMPLIFY", "Value")
    arcpy.MakeFeatureLayer_management(temp_polyline, "polyline")

    writeMessage("Calculate length...")
    arcpy.AddField_management("polyline", "Length", "FLOAT")
    arcpy.CalculateField_management("polyline", "Length", "!shape.length!",
"PYTHON")
    temp_statsTable = TEMP_LOC + "statsTable"
    arcpy.Statistics_analysis("polyline", temp_statsTable, [{"Length", "SUM"}])

    arcpy.JoinField_management(temp_extracted, "FID", temp_statsTable, "FID",
["SUM_LENGTH"])

##         exist = arcpy.Exists(merged_points)
##         if not exist:
##             writeMessage("Final merge file does not exist, creating...")
##             arcpy.CopyFeatures_management(temp_extracted, merged_points)
##         else:
##             writeMessage("Final merge file does exist, appending...")
##             arcpy.Append_management([temp_extracted], merged_points,
"NO_TEST")

    exist = arcpy.Exists(temp_final_file)
    if not exist:
        writeMessage("Single merge file does not exist, creating...")
        #arcpy.CopyFeatures_management(temp_extracted, temp_final_file)

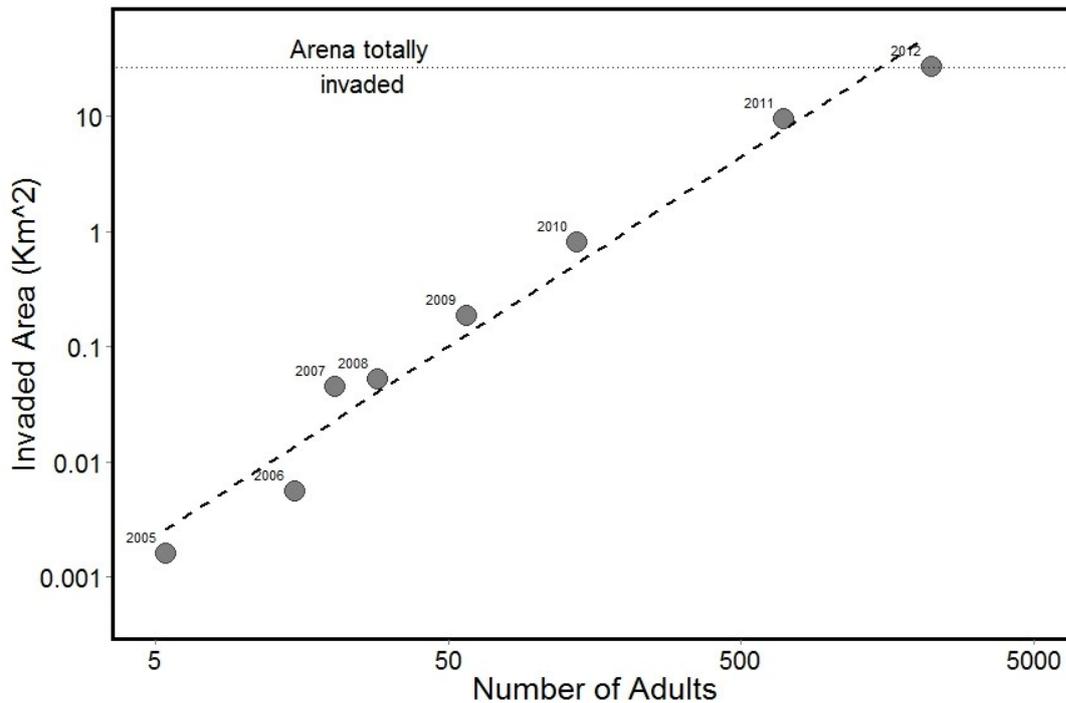
```

```
        arcpy.FeatureClassToFeatureClass_conversion(temp_extracted,
TEMP_LOC, "temp_current_merge.shp")
    else:
        writeMessage("Single merge file does exist, appending...")
        arcpy.Append_management([temp_extracted], temp_final_file, "NO_TEST")
    else:
        writeMessage("Destination point output file already exists, ignoring execution...")

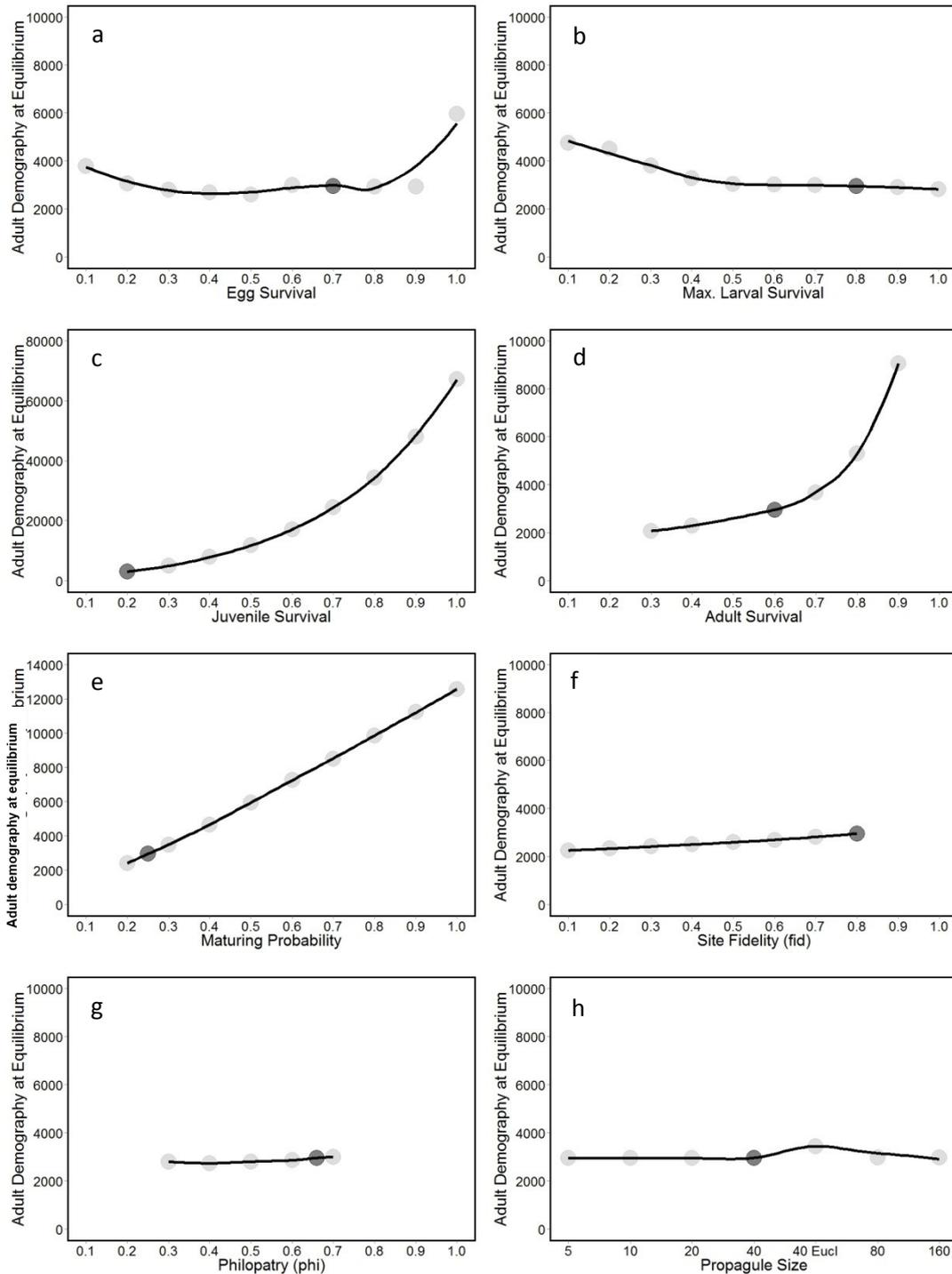
writeMessage("Creating the final file: " + final_file)
exist = arcpy.Exists(temp_final_file)
if exist:
    arcpy.CopyFeatures_management(temp_final_file, final_file)
    arcpy.Delete_management(temp_final_file)
    #arcpy.DeleteFeatures_management(temp_final_file)
    #break
else:
    writeMessage("Output file already exists, ignoring execution...")

writeMessage("SUCCESS: Script finished!")

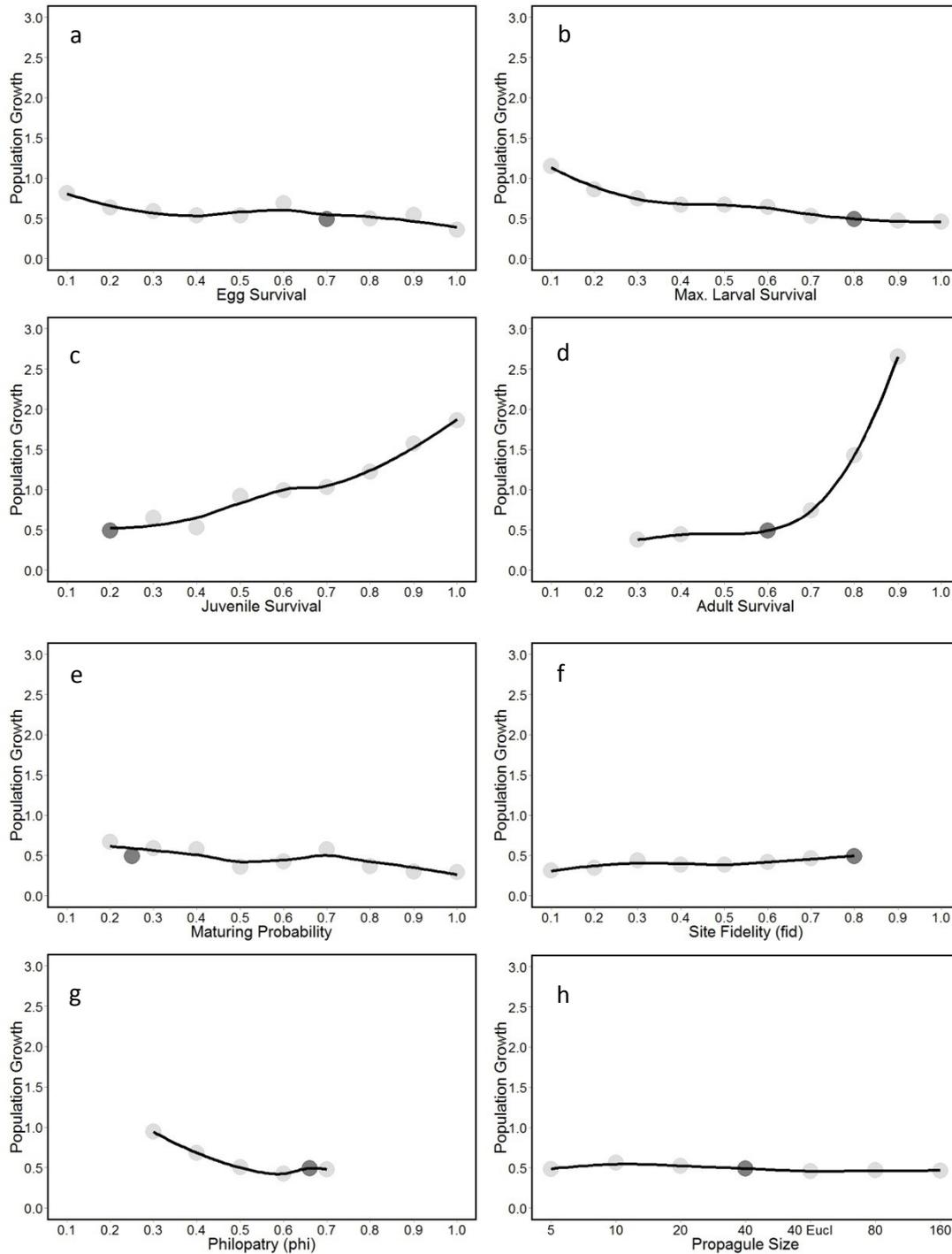
except GetOutOfLoop:
    pass
```

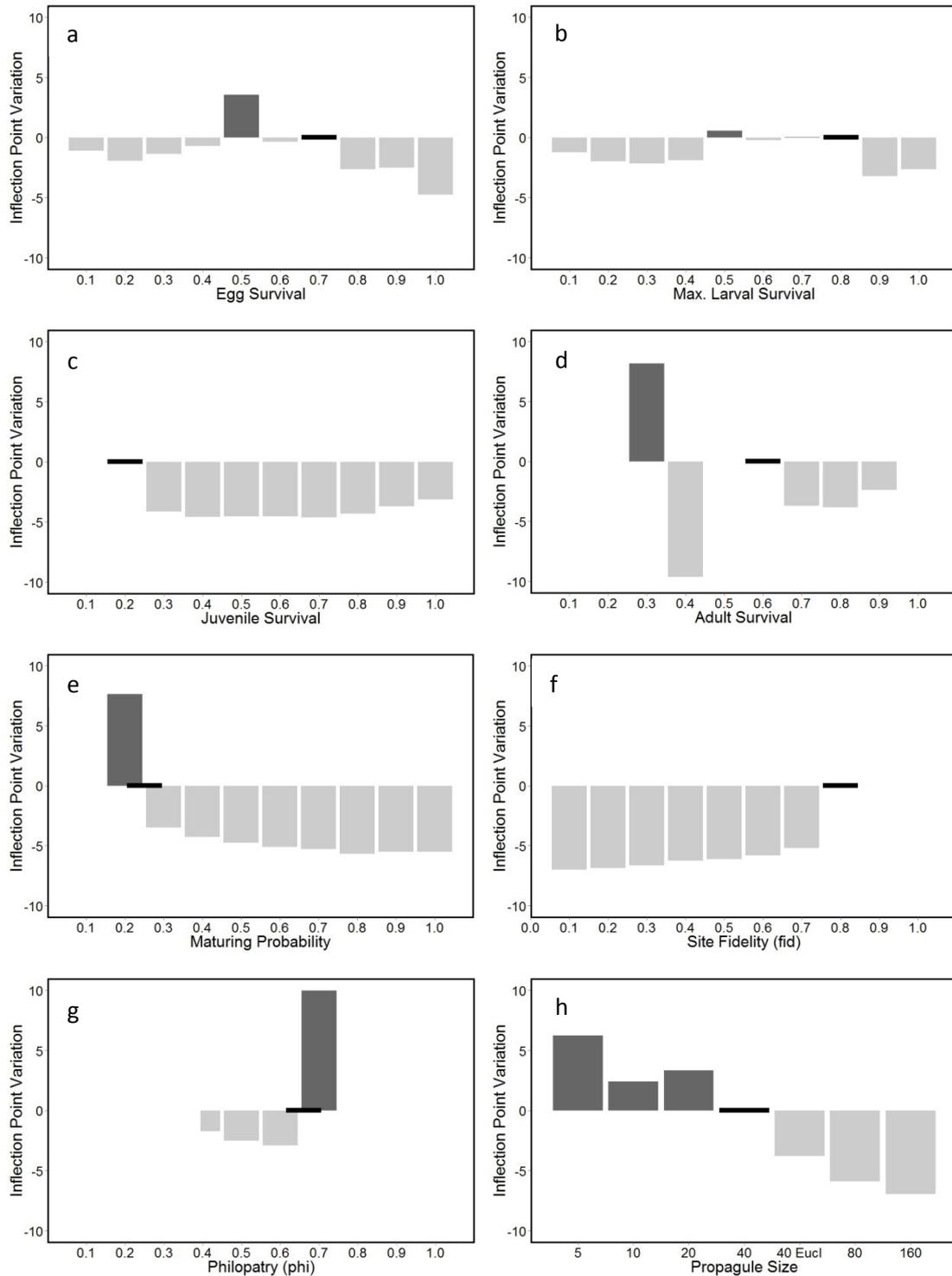
Appendix 1.D: Log-Log graph showing the linear regression (dashed line) between the number of adult guttural toad *Sclerophrys gutturalis* in the population and the area invaded forecasted by the age structured model. Note that from 2001 to 2005 the adults utilize only the initial pond whereas from 2012 to 2030 the adults exploit all the ponds in the arena; thus only the years involved in the spatial spread (2005-2012) are reported here.



Appendix 1.E: Sensitivity of adult demography at equilibrium to variations in demographic and dispersal traits. Each trait was varied by 10 % between 0.1 and 1 while fixing all other traits to the baseline values reported in Table 1. The only exception was propagule size that varied following the geometric sequence reported in the text plus the result we obtained using the Euclidean matrix. Dark dots represent adult demography of the model we parameterized using the baseline value reported in Table 1; pale dots represent adult demography simulated varying each traits; absence of dots represents population collapse.



Appendix 1.F: Sensitivity of population growth to variations in demographic and dispersal traits. Each trait was varied by 10 % between 0.1 and 1 while fixing all other traits to the baseline values reported in Table 1.1. The only exception was propagule size that varied following the geometric sequence reported in the text plus the result we obtained using the Euclidean matrix. Dark dots represent adult demography of the model we parameterized using the baseline value reported in Table 1.1; pale dots represent adult demography simulated varying each traits; absence of dots represents population collapse.



Appendix 1.G: Sensitivity of inflections points to variations in demographic and dispersal traits. Each trait was varied by 10 % between 0.1 and 1 while fixing all other traits to the baseline values reported in Table 1.1. The only exception was propagule size that varied following the geometric sequence reported in the text. Black line represents the inflection point of the parameterized model; dark grey bars mean inflection point occurring in later years than that inflection point of the parameterized model; pale grey bars represent inflection points occurring in earlier years than the inflection point of the parameterized model.

Chapter two. Efficacy or efficiency? Strengths, limitations and hydra effect in the management of the invasive guttural toad in Cape Town

2.1 Introduction

Biological invasions are one of the main drivers of global change, having detrimental consequences on the natural world through habitat and ecosystem alteration, biodiversity loss and spread of new parasites and pathogens (Simberloff et al. 2013, Hulme 2014, Tittensor et al. 2014). Furthermore their negative impacts on human health and economic systems are today conspicuously documented (Olson and Roy 2006, Pyšek and Richardson 2010). It follows that a considerable management effort is required to limit the spread of invasive taxa and minimize their effects once established. Eradication of invasive populations is often considered unfeasible and thus a promotion of managing invaded novel ecosystems (Hobbs et al. 2006); however invasive populations have in the past been successfully targeted and eradicated across the globe (Simberloff 2009, Keitt et al. 2011). Eradication is defined as "the removal of every potentially reproducing individual of a species or the reduction of their population density below sustainable levels" (Myers et al. 2000). Although many interacting factors can contribute to successful eradication (Myers et al. 2000), a leading role is played by both the temporal scale at which the management effort is sustained (timing) and the planning of such effort (modes, Simberloff 2003, Finnoff et al. 2005, Mehta et al. 2007).

Invasive populations are by definition characterized by non-equilibrium time-space dynamics (Pyšek and Hulme 2005, Baker and Bode 2016); performing a prompt and effective removal is therefore optimal in light of a cost-benefit evaluation (Meyers et al. 2000, Epanchin-Niell et al. 2014). A prolonged and less effective mode of eradication might only slow down the invasive spread, be ineffective in the long term and raise the costs of future management (Kettenring and Adams 2011). Also it could subtract economic and social resources from other management activities such as detection or containment (Olson and Roy 2005, Mehta et al. 2007, Bogich et al. 2008, Epanchin-Niell et al. 2012, Chadès et al. 2011, Holden et al. 2016). Most invasive populations are distinctively characterized by an initial lag phase, when eradication is still a realistic goal, followed by an expansion and a dominance phases (see chapter one and Van Wilgen 2014 and Epanchin-Niell and Liebhold 2015), when high management costs may make maintenance the only feasible option (but see Simberloff 2009). However, the temporal transition between these phases may often be defined only *a posteriori* (Simberloff 2003), adding uncertainty to the effort to select the optimal management strategy (Januchowski-Hartley et al. 2011, Moore et al. 2011, Epanchin-Niell et al. 2014).

Even when eradication is promptly chosen among different management options, a further step is needed to quantify and adaptively improve both its efficacy (capacity to accomplish the goal) and efficiency (capacity to function with the least waste of resources); at this scope it is necessary to consider biology and autoecology of the target species and environmental and socio-economic features of the invaded area. If for instance our aim is to eradicate invasive species characterized by complex life-history stages (e.g. aquatic larvae vs. aerial adults or pelagic larvae vs. sessile adults), we should explicitly consider demographic structure, behaviour and survival at each different stage (Shea et al. 2006, Ramula et al. 2008, Morris et al. 2010, Pichancourt et al. 2012, Beaty and Salice 2013). This is particularly important when: i) the capacity to detect and remove invasive individuals as well as the costs linked to their removal vary as a function of the life stage (Buhle et al. 2005, Hastings et al. 2006, Pichancourt et al. 2012); ii) density dependent survival or other intra- and inter-stage interactions determine non-linear dynamics that make some management strategies ineffective or even detrimental (Govindarajulu et al. 2005, Pardini et al. 2009, Gornish and James 2016).

Testing efficacy of eradication and proposing more successful actions require also taking into account environmental and socio-economics limitations linked to the invaded landscape (Steel et al. 2014, Coutts et al. 2011). For instance removal of invaders may be particularly problematic when the landscape is fragmented in several subunits (e.g. in high land-use landscapes such as urban or rural areas); the high number of agents involved in the management or their differential perceptions (Carrasco et al. 2012) may impede managing the entire landscape as a whole and implement a coordinated action. In some cases, however, such fragmentation could represent an opportunity to control invasive populations; for example unsuitable habitat sites may be employed as barriers to control spread, a situation particularly favourable in species that have localized dispersal (With 2004). The invaded landscape may be also characterized by conflicts of views and interests among stakeholders (e.g. urban areas, Foster and Sandberg 2004, Warren 2007) or public opposition to some management practices (Verbrugge et al. 2013, Gaertner et al. 2016); these limitations may be tackled through adequate legislation or sustained awareness campaigns, even though often the necessity to rapidly respond to a new invader makes these efforts ineffective. Experience shows that underestimating these social and economic dimensions may hamper our capacity to react to invasive populations (Botham et al. 2009, Mackenzie and Larson 2010) and propose realistic alternative management strategies (Gaertner et al. 2016).

Complexity of non-equilibrium time-space dynamics (Pyšek and Hulme 2005) as well as environmental and social features of the invaded area may be profitably integrated through an ecological modelling approach (Caplat et al. 2012, Cuddington et al. 2013, Wood et al. 2015).

This allows scientists, managers and policy-makers to formulate predictions concerning efficacy and efficiency of a specific eradication program and time required to be successful. Modelling also allows exploring the existence of limitations that can hamper program success and proposing alternative strategies to withstand or bypass those limitations. Here I deploy an age-structured model designed to reconstruct guttural toad invasion dynamics in a peri-urban area of Cape Town and parameterized with field data and field surveys (see chapter one) to simulate the effects of alternative modes of removal. The model forecasts how demographic and spatial dynamics of the invasive population vary in response to different modes of removal at different life history stages or at different spatial scales.

The guttural toad is domestic exotic in South Africa (Measey and Davies 2011) being native in most of the country but not in Cape Town, where an invasive population has established in 2000 (de Villiers 2006). Since then, guttural toads were observed to use artificial ponds for breeding and invade new ponds every year. In 2010 the City of Cape Town contracted a private company to perform an extirpation (i.e. eradication at local scale, Panetta 2007) by opportunistically removing toads at any life stage (adult, juvenile, metamorph, tadpole and egg) from garden ponds, public open spaces and roadways. The removal from the ponds was particularly arduous because they were all located in private properties not always accessible to the eradicators. The management effort has been prolonged with no interruption until 2015 in order both to avoid the invasive spread of the population and promote its total extirpation. However in 2016 the species has still been observed to actively invade Cape Town despite the high number of toads removed across years.

Up to date, individuals from different life history stages were removed from the accessible properties exclusively as a function of eradicators' capacity to visually detect and remove them by hand. In other words, no life history stage has been preferentially targeted for eradication considering how its removal may affect invasive dynamics and the cost linked to such removal. Additionally, it is not clear whether and how management actions performed only on the accessible ponds may significantly affect the invasive population.

These uncertainties raise questions on: i) the long term efficacy of the current mode of removal; ii) whether alternative modes might have higher efficiency and efficacy iii) to what extent the efficacy of a management strategy performed exclusively on accessible properties differs from a strategy that implements removal in all properties.

To address these questions, I use the age structure model proposed in chapter one to explore how the scenario incorporating the demographic effects of the current extirpation differs from a no-extirpation (baseline) scenario where the invasive population dynamics is exclusively

determined by density dependence dynamics, life history traits and dispersal (see chapter one). Secondly, I ask which limitations might impede the extirpation from being successful and whether and to what extent alternative strategies may determine better results. To address this, I simulate and compare multiple extirpation scenarios by postulating different modes of removal, such as extirpating individuals at different life history stages or at different spatial scales.

2.2 Materials and methods

2.2.1 Guttural toad in Cape Town and extirpation

In the 2000-2001 season about 10 males were heard for the first time calling from a large artificial pond in the Constantia Valley (De Villiers 2006), a low density high income residential area of Cape Town characterized by peri-urban landscape. Since establishment, the species has been observed using artificial ponds for reproduction in less than 2 km² around the site of the first detection. Some tens of individuals plus eggs and tadpoles were removed in the following six years but the effort was not sustained. In 2009, after the decision of the CAPE Invasive Alien Animal Working Group (CAPE-IAA) to map the guttural toad invasive range, it was determined that the species was occupying an area of approximately 5 km² (Measey et al. in press).

In 2010 the City of Cape Town contracted a private company to remove the invasive individuals. The removal was challenging because the capacity of the eradicators to detect and remove individuals at some life stages (e.g. tadpoles or juveniles) was limited, especially in certain weather conditions (e.g. during cold windy nights). Moreover, most of the breeding sites are garden ponds located on private properties and some owners were not willing to allow access, especially at night (Jonathan Bell, pers. comm.). Lastly, a fixed number of properties could be visited effectively in each season and each visit had to be pre-arranged with the owners. The extirpation process was conducted between October and February (i.e. the breeding season of the invasive guttural toads in Cape Town) five nights per week for a total of about 110 nights. Since the eradicators could survey not more than two properties per night and the total number of accessible properties was 128, each accessible pond was on average visited only in two different nights every 60 days across the whole breeding season.

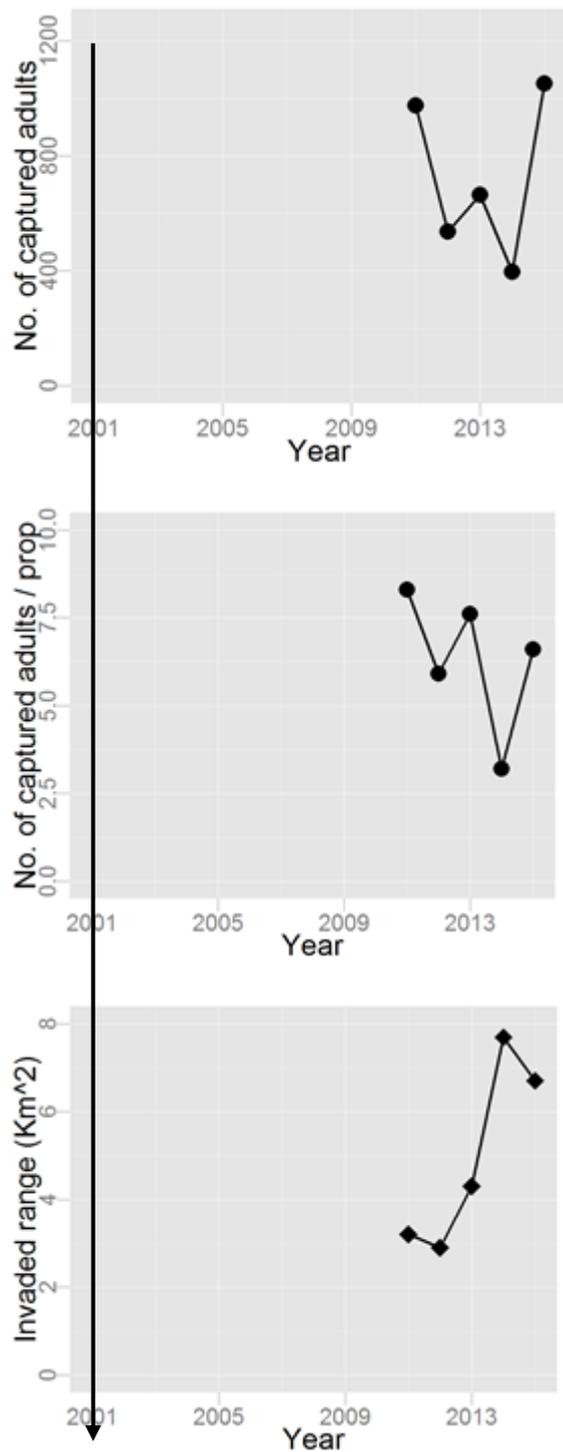


Figure 2.1 Total number of adult guttural toads *Sclerophrys gutturalis* captured during the ongoing extirpation process in Cape Town started in the season 2010/2011 (a); mean number of adults captured visiting each property during the extirpation process (b) and invaded range estimated by captures (c). The vertical arrow indicates the first detection of the species in Cape Town.

2.2.2 Model description

I used an age structured model of integrodifference equations where each pond utilized by toads to breed represents a population with a detailed life cycle (see chapter one). The ponds (i.e. the entities of the model) work as a source-sink network (metapopulation) according to life history stage specific demography and dispersal behaviour of their individuals. Ponds were located using aerial images and their functional connectivity was calculated through a least-cost pathway approach, i.e. combining the cost for an individual to move between habitat-patches and detailed information of landscape (see Chapter one). The guttural toad population dynamics emerges in the pond network of the invaded areas from species-specific life history traits, density-dependent survival and dispersal and reproductive behaviour. The model was parameterized using field data and field surveys and runs 30 steps (i.e. 30 years, from 2001 to 2030); in each step the different life phases of an individual are processed following the species life cycle in the invaded area.

2.2.3 Eradication strategy simulations

Each pond is considered a discrete entity; it allows simulating different modes of removal by altering survival of virtual individuals across time, space and life-stage.

2.2.3.1 Time

All the simulated modes of removal were initialized from 2011, i.e. when the eradication program was first implemented in Cape Town., and interrupted in 2020; this enabled me to explore the population capacity to recover after ten years with no eradication (until 2030).

2.2.3.2 Space

Firstly, removal of individuals only from the accessible ponds was simulated (see Figure 2.3). The model forecasted for 2011 an invaded area larger than the area estimated by the eradicators in 2011 and more compatible with that estimated in 2014 (9.8 km², 3.5 km² and 7.6 km² respectively; see also section 1.3.1 in Chapter one). Due to the invasive spread some ponds were targeted for eradication (and became accessible as a consequence of eradicators' request) only after 2011; thus I assumed for simplicity that the all ponds accessible in 2014 (n=128) were already available for eradication in 2011. It allowed me to simulate a removal of individuals constant across space and time and take into account the invaded area predicted by the model for 2011.

Secondly, removal of individuals from all ponds was simulated to explore to what extent access restriction influenced capacity to eradicate the invasive population.

2.2.3.3 Life-stage

The accessible ponds were first utilized to simulate the effects on population dynamics and invasive spread of the current mode of removal (S_1). Since this mode does not preferentially target any specific life stage, I assumed for simplicity that the proportion of individuals removed derives from the interplay between removal capacity by eradicators and spatial and temporal occurrence of invasive individuals in the property at each visit. For instance the proportion of tadpoles that can be removed is low because individuals at this stage are difficult to spot and capture (e.g. by netting) and they stay in the pond for only 4-5 weeks before metamorphosing. Conversely the proportion of adults removed from a property should be high because individuals at this stage are easy to spot and remove and they congregate to breed in or around a pond for most of the breeding season. The proportions of individuals that are removed for each stage according to the current mode of removal (S_1) and their rationale are reported in Table 2.1. Although these proportions may not be exact, field survey and field data plus consultation with eradicators showed they are realistic (see last column in Table 2.1 for an explanation).

2.2.3.4 Efficacy evaluation

The scenario obtained by simulating the current mode of removal (S_1) was compared with the no-extirpation baseline scenario S_0 (see chapter one) to explore the effects of the current extirpation program on both demographic and spatial dynamics of the invasive population.

Following an analogous approach, I postulated and tested the demographic effect of alternative extirpation strategies that remove proportions of individuals at different life history stages and at different spatial scales. The scenario S_2 consists of a mode which removes a large proportion of tadpoles and eggs from the accessible properties; conversely the scenario S_3 targets only adults in these ponds. Therefore, the scenarios S_1 , S_2 and S_3 can be utilized to explore the effects of concentrating the effort of removal on different life-history stages. The same modes of removal (i.e. current mode, removal of only tadpoles and eggs, removal of only adults) were also simulated in all ponds with the scenarios S_4 , S_5 and S_6 respectively. A scenario based on the removal of most of adults, tadpoles and eggs from all ponds was simulated (S_7). These scenarios (S_4 - S_7) were thus used to explore the effect of forcing more access to different properties. The alternative modes of removal are summarized together with their rationale in Table 2.2. Additionally, modes that remove differential proportions of eggs and tadpoles from the accessible ponds were simulated to explore the opportunity to allocate more resources in removing pre-metamorphic individuals.

2.2.3.5 Efficiency evaluation

Since I aimed to evaluate not only the efficacy (i.e. capacity to extirpate the invasive population) of these different modes of removal but also their efficiency (capacity to function minimizing costs), I broadly quantified through field surveys the time (h) spent by eradicators to target each life stage during the current mode of removal. At each visit: 1, 0.25 and 0.5 hours were allocated to remove adults and juveniles (T_{aj}), metamorphs (T_m) and tadpoles and eggs (T_{te}), respectively. Although this is an estimation, it is based on my repeated shadowing of the contractors in the field. For instance the removal of adults was more time consuming because it required survey of the entire extent of the property by walking; conversely the removal of metamorphs was faster because they are spatially distributed exclusively around the pond edge (see Chapter One). Removal of adults and juveniles was considered here as a single unit because eradicators targeted contemporary individuals from both these age classes within the same interval of space and time (same for tadpoles and eggs). Then I used these costs to estimate the total time (T) needed by eradicators for each mode of removal (see Table 2.2) following the formula:

$$(T_{aj} + T_m + T_{te}) \times 2 \times N_p = T \quad (1)$$

where the time spent to remove individuals for each stage in one property was multiplied by two (i.e. the number of visits per year) and the number of properties that were visited (N_p). Note that all calculations are based on a single person visiting each property in the evening, but that health and safety standards in different countries may make the minimum number of people higher. If this is the case, the time required would not decrease, but efficiency would need to be adjusted upwards.

Table 2.1: Proportions of guttural toads *Sclerophrys gutturalis* removed from each pond accessible to the eradicators in Cape Town according to the current mode of removal and simulated in the age structured model with the scenario S₁. Each proportion was estimated for each life history stage taking into consideration: removing capacity by eradicators; spatial occurrence of the toads in the property visited by eradicators, temporal occurrence of the toads in the property visited by eradicators; evidences collected from field data and surveys.

Life history stages	Proportion of individuals removed	Removing capacity by eradicators	Spatial occurrence	Temporal occurrence	Evidences from field data and field surveys
Adults	0.8	High. Most males and females can be easily spotted by eradicators in and around the pond because of the large size (Snout to Vent Length SVL, >45mm) and breeding behaviour (e.g. calling in males).	High. Most males and females congregate in and around the pond during the reproductive season.	Medium. Most males call and stay in and around the pond during the whole reproductive period. Females stay in and around the pond only until termination of egg laying.	Most of the post-metamorphic individuals captured during the extirpation were adults (70%). The number of adults removed in a pond at first visit was on average significantly higher than the number of adults detected at second visit.
Juveniles	0.05	Low. Juveniles are difficult to spot because of the small size (15mm<SVL<45mm) and the absence of breeding behaviour.	Low. Juveniles do not congregate in or around the pond but are more equally distributed across the invaded area.	Low. Juveniles do not congregate in or around the pond during the breeding season.	Only 30% of post-metamorphic individuals captured during the extirpation were juveniles. However the model reported in chapter one and other similar models (e.g. Beatty and Salice 2013) forecasted a number of juveniles between three and ten times higher than the number of adults.

M e t a m o r p h s	0.25	Low. Metamorphs are extremely difficult to spot because of the small size (SVL<15mm). Also, their high density around the pond makes the removal labour-consuming.	High. Metamorphs stay around the pond edge (within a radius of 5 m) for some weeks after metamorphosis.	Medium. Metamorphs stay around the pond for some weeks only after metamorphosis.	Most of metamorphs (90%) were removed during the second part of the breeding season (middle December-February). In many cases the eradicators did not manage to remove a large proportion of metamorphs around the pond.
T a d p o l e s	0.25	Low. Tadpoles are extremely difficult to spot and remove by netting in the pond, especially during the night.	High. Tadpoles stay in the pond although their removal is more difficult in large ponds.	Medium. Tadpoles stay in the pond for 4-5 weeks before metamorphosis.	Tadpoles were removed across the entire breeding season. In many cases the eradicators did not manage to remove a large proportion of tadpoles in the pond.
E g g s	0.05	Low. Eggs are extremely difficult to spot and remove by netting in the pond, especially during the night.	High. Eggs stay in the pond although their removal is more difficult in large ponds.	Low. Eggs stay in the pond for only 5-7 days before hatching.	Eggs were detected and removed much less frequently than tadpoles during the extirpation program.

Table 2.2: Proportions of guttural toads *Sclerophrys gutturalis* removed from each pond according to different modes of removal and simulated in the age structured model with scenarios S_0 - S_7 . S_0 represents a baseline scenario without eradication (see chapter one), S_1 represents the scenario obtained simulating the current mode of removal (see Table 1) whereas S_2 , S_3 , S_4 , S_5 and S_6 and S_7 represent hypothetical scenarios obtained simulating alternative modes of removal. For each mode of removal, the number of pond accessible for eradication, rationale and total time necessary to perform the removal in one year (T) are also reported.

Scenarios simulating different extirpation strategies	Proportion of individuals removed					Number of ponds visited (N_p) / Total number of ponds	Rationale	Total time T (h) needed to eradicators to remove individuals while visiting properties as expressed by the formula (1): $(T_{aj} + T_m + T_{te}) \times 2 \times N_p = T$
	Adults	Juv.	Met.	Tad.	Eggs			
S_0	0	0	0	0	0	0/415	No-extirpation	$(0+0+0) \times 0 \times 0 = 0$
S_1	0.8	0.05	0.25	0.25	0.05	128/415	Estimated current mode of removal (See Table 1)	$(1+0.25+0.5) \times 2 \times 128 = 448$
S_2	0	0	0	0.8	0.8	128/415	Mode that removes most eggs and tadpoles from accessible ponds	$(0+0+1.5) \times 2 \times 128 = 384$
S_3	0.8	0	0	0	0	128/415	Mode that removes by hand and/or by trap most adults from accessible ponds	$(1+0+0) \times 2 \times 128 = 256$
S_4	0.8	0.05	0.25	0.25	0.05	415/415	Estimated current mode of removal implemented in all ponds	$(1+0.25+0.5) \times 2 \times 415 = 1453$
S_5	0	0	0	0.8	0.8	415/415	Mode that removes most eggs and tadpoles from all ponds	$(0+0+1.5) \times 2 \times 415 = 1245$
S_6	0.8	0	0	0	0	415/415	Mode that removes by hand and/or by trap most adults from all ponds	$(1+0+0) \times 2 \times 415 = 830$
S_7	0.95	0	0	0.8	0.8	415/415	Mode that removes most adults, eggs and tadpoles from all ponds	$(2+0+1.5) \times 2 \times 415 = 2905$

1550 **2.3 Results**

1551 ***2.3.1 Efficacy of the current mode of removal***

1552 The current extirpation strategy (S_1) led to a reduction of about 30% of the estimated adult
1553 population size when compared with the no-extirpation baseline scenario (S_0 , Figure 2.2).
1554 However it did not allow extirpation of the population, which conversely recovered after
1555 interrupting the removal (i.e. 2020); the saturation point was reached again within two
1556 years. Also, no effect due to this removal strategy was detected on the spatial invasion
1557 dynamics (Figure 2.3) that mirrored the logistic trend of the adult population
1558 demographics, with a lag, a spread and a dominance phase respectively (see chapter
1559 one). Notably the model suggests that the eradication process started during the spread
1560 phase of the invasion (Figure 2.3).

1561 ***2.3.2 Efficiency of the current mode of removal***

1562 A result approximately analogous to S_1 was obtained performing the current removal only
1563 on adults (S_3 , Figure 2.3); it implies that the removal of pre-metamorphic individuals
1564 implemented in the current modes of removal does not have any noticeable effect on the
1565 population demography but raises the costs (i.e. reduces efficacy) (Table 2.2, T in S_1 vs.
1566 S_3). Rather, the exclusive removal of most tadpoles and eggs caused a higher adult
1567 population size (S_2 , Figure 2.4); more eggs and tadpoles were removed, larger adult
1568 population size was, with the removal of 70% of tadpoles and eggs that determined a
1569 demographic fluctuation among years instead of a constant adult equilibrium (Figure 2.4).
1570 This suggests that exclusive removal of individuals at these stages is highly detrimental
1571 because it simultaneously increases the adult population size as well as management
1572 costs (Table 2.2, T in S_0 vs. S_2).

1573 ***2.3.3 Efficacy and efficiency of alternative modes of removal in all the ponds***

1574 Applying the current mode of removal to all the ponds, I obtained a much more consistent
1575 reduction (-80%) of the population size (S_4 , Figure 2.2). It implies that the limited number
1576 of ponds accessible for management represents a critical constrain to the capacity to
1577 control the invasive population. However this effort appeared ineffective enough to
1578 extirpate the invasive population after 10 years or to significantly reduce the invaded area.
1579 Again, approximately the same output was obtained targeting only adults in all ponds (S_6 ,
1580 Figure 2.2) whereas the partial removal of tadpoles and eggs led to a higher population
1581 size (S_5 , Figure 2.2) and a full invasion of the area. Only the removal of 95% of adults and
1582 80% of tadpoles and eggs led to the successful extirpation before 2030, with an invasive

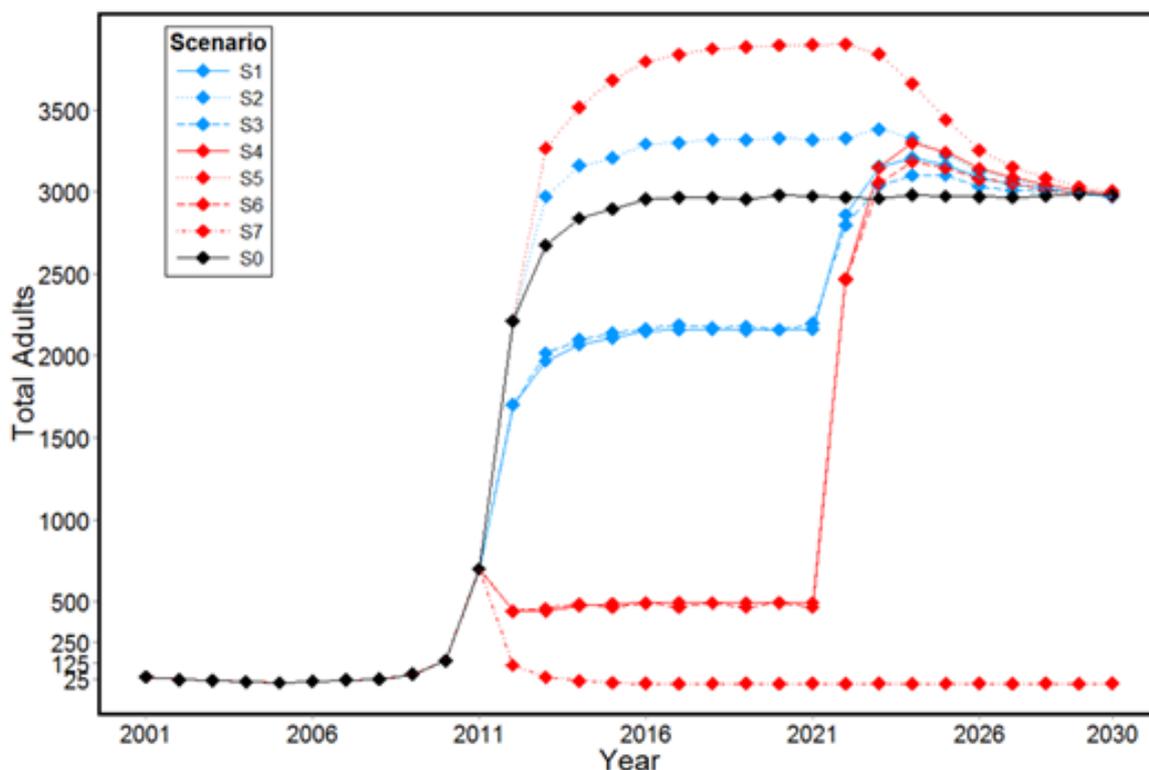
1583 population that is not able to recover and no pond colonized after ten years (S_7 , Figure
 1584 2.2). However this mode of removal is about six times more costly than the current mode
 1585 (Table 2.2, T in S_7 vs. S_1).

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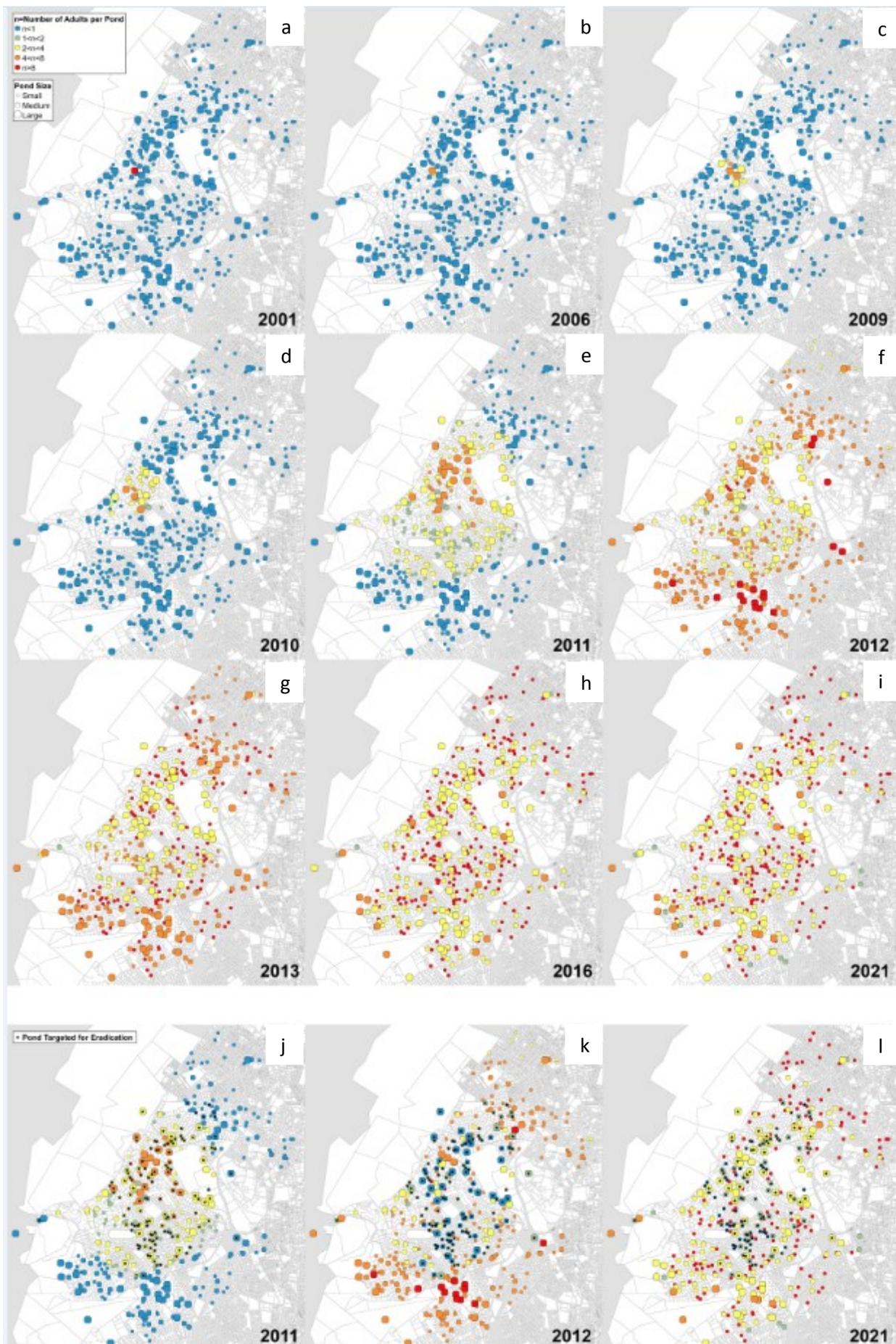
1591

1592 **Figure 2.2:** Adult population size of a population of guttural toad *Sclerophrys gutturalis* in
 1593 Cape Town estimated by an age structured model that simulates the different hypothetical
 1594 modes of removal listed in Table 2.1. Colours (blue, red and black) indicate number of
 1595 targeted ponds (accessible ponds, all ponds and none of them respectively) whereas line
 1596 types (solid, dotted, dashed and dot-dash) indicate which life stages are removed in case
 1597 of extirpation (all of them, eggs + tadpoles, adults, eggs + tadpoles + adults respectively).
 1598 Management is simulated to start in 2011 and to be interrupted in 2020 after which the
 1599 invasive population is allowed to run for a further 10 years until 2030.

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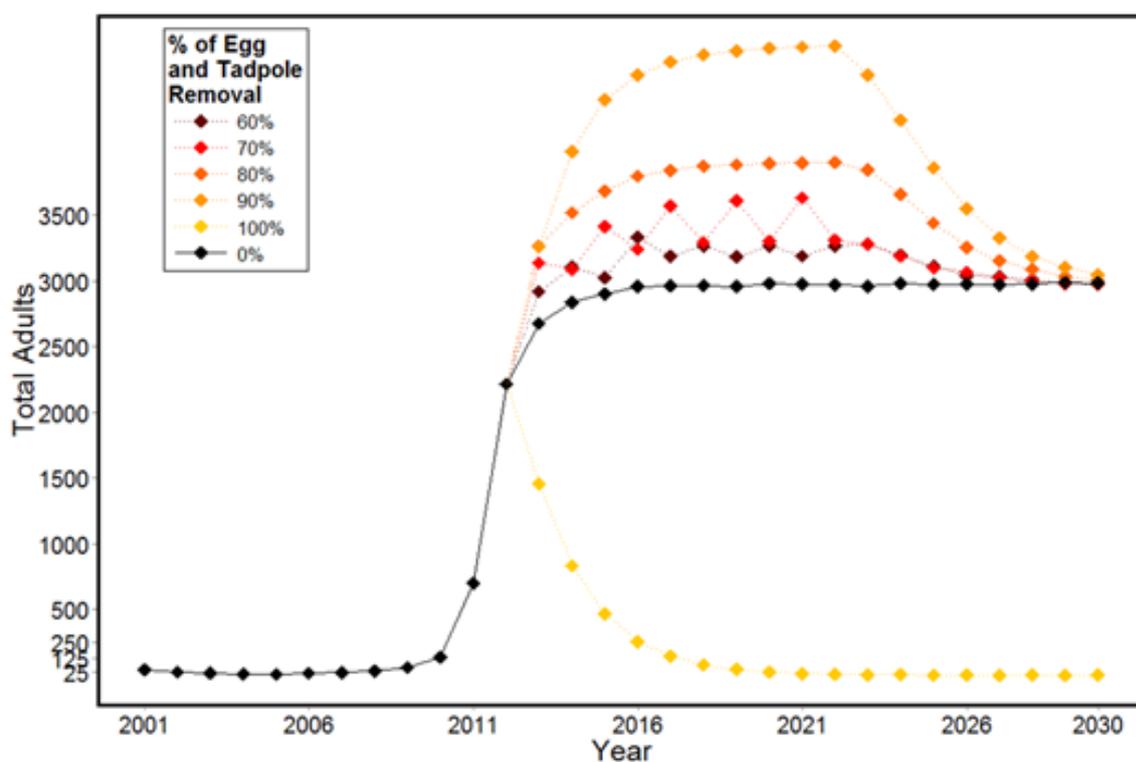
1604 **Figure 2.3:** Spatial layers showing the spatial dynamics of the guttural toad *Sclerophrys*
 1605 *gutturalis* in Cape Town across years as estimated by an age structured model that
 1606 simulates different modes of removal. Colours represent different number of individuals
 1607 predicted by the model (blue, less than one individual; green, between one and two
 1608 individuals; yellow, between two and four individuals; orange, between four and eight
 1609 individuals; red, more than eight individuals) whereas black dots represented accessible
 1610 ponds targeted in the current mode of removal. Figures 2.2 (a-i) maps represent the
 1611 baseline scenario (S_0) whereas figures 2.2(j-l) represent the scenario resulting from the
 1612 current mode of removal (S_1) as estimated in table 2.1.

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1618 **Figure 2.4:** Adult population size of a population of the guttural toad *Sclerophrys*
 1619 *gutturalis* in Cape Town estimated by an age structured model that simulates differential removal of
 1620 eggs and tadpoles. Colours indicate different percentages of removal. Management is
 1621 simulated to start in 2011 and to be interrupted in 2020 after which the invasive population
 1622 is allowed to run for a further 10 years until 2030.

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1625 **2.4 Discussion**

1626 In this chapter, I show that the efficacy of the current mode of removal is highly limited; it
1627 does not allow eradication of the invasive guttural toad population and impedes its spread
1628 across the invaded area. This is confirmed by field observations, where the population
1629 was known in 2015 to thrive and spread across Cape Town despite consistent removal of
1630 individuals since 2011. I also show that the current mode of removal does not sufficiently
1631 take into consideration non-linear population dynamics linked to density dependence and
1632 this reduces its efficiency. Modes of removal that explicitly consider these dynamics could
1633 increase management efficiency but are not sufficient to extirpate the invasive population.
1634 Only modes of removal that tackle spatial limitations (access restriction) by explicitly
1635 recognizing the social dimension of the landscape could promote a successful extirpation
1636 of the population, even though a conspicuous management effort would have been
1637 required to accomplish this goal. Rather, other more feasible management targets such as
1638 control or containment may still be conducted in the invaded area, specifically through the
1639 removal of adults.

1640 I observed that the efficiency of the current management decreases by removing eggs
1641 and tadpoles; their removal does not noticeably affect the population demography but
1642 rather subtracts resources (i.e. time) to other modes of removal (e.g. of adults). In other
1643 words such time consuming removal does not provide any significant demographic benefit
1644 (S_1 vs. S_3) and results detrimental when applied at higher intensity (Figure 2.4). This
1645 positive effect of mortality at population-level, also defined the “hydra effect” (Abrams
1646 2009), has been detected in many other age and stage -structured population models as
1647 well as in empirical studies (Govindarajulu et al. 2005, Hilker and Liz 2013, Schröder et al.
1648 2014) and may be due to many interacting demographic factors (excellently summarized
1649 in Schröder et al. 2014). Two of them, mortality preceding density dependence and
1650 overcompensating density dependence between stages, are explicitly incorporated in my
1651 model. The higher mortality of eggs and tadpoles caused by removal precedes density
1652 dependence survival in tadpoles and metamorphs; thus, the immediate demographic
1653 benefit of this mode of removal is neutralized by the higher survival occurring upstream in
1654 the life-cycle. The positive effect of mortality preceding density dependence seems quite
1655 common in structured populations (Abrams 2009, Schröder et al. 2014) and should be
1656 more often considered in management planning (Zipkin et al. 2009, Turner et al. 2016) for
1657 instance implementing removal only after density dependence phenomena (but see Hilker
1658 and Liz 2013).

1659 The model shows also that density dependence in tadpoles is followed by density
1660 dependence in metamorphs causing a relaxation of the density-dependent bottleneck; as
1661 a consequence, a higher equilibrium density is reached (Schröder et al. 2014). Intriguingly
1662 this could also explain why during the saturation phase I observed a significant difference
1663 in terms of adult density among ponds characterized by different size (see chapter one);
1664 small ponds were counter-intuitively characterized by a higher number of adults than
1665 medium and large ponds (Figure 2.3). An indication comes from the number of individuals
1666 observed in the ponds at different life stages forecasted by the model. During the
1667 saturation phase, small ponds are characterized by low numbers of eggs, tadpoles and
1668 metamorphs; however the situation is totally reversed in juveniles, suggesting that
1669 metamorph density dependence survival occurring at the pond edge has a much more
1670 severe regulatory effect in large and medium ponds. Interestingly this pattern was not
1671 observed during the expansion phase (Figure 2.3), therefore limiting the possibility to
1672 perform a management strategy during the invasive spread that maximizes the removal of
1673 adults by targeting ponds with a specific size.

1674 The occurrence of a strong positive mortality at population-level implies that any
1675 management action on the guttural toad in Cape Town should target eggs and tadpoles
1676 only when it is possible to perform their full removal (Figure 2.4) for example through
1677 draining periodically a pond or using chemicals. The lack of selectivity of all these
1678 techniques however limits their utilization in the field because they may cause collateral
1679 negative effects on native or non-harmful alien populations of anurans, fish and
1680 invertebrates. A recent study by Clarke et al. (2016) showed the possibility to deploy in
1681 water bodies invaded by the cane toad *Rhinella marina* species-specific chemicals that
1682 inhibit the development of conspecific embryos; however it is not clear yet to what extent
1683 the same technique can be utilized in other species.

1684 Conversely juveniles are more evenly distributed across space and time (Pittman et al.
1685 2014); it may also explain the considerable difference between the juvenile/adult ratio
1686 estimated by extirpation captures and the ratio forecasted by the model (1:3 and 10:1
1687 respectively). Moreover it is noteworthy that in my study an equal removal (-80%) of
1688 juveniles instead of adults from the accessible ponds determines a less severe effect on
1689 the population demography (-20% VS -30% respectively). This contradicts what was
1690 observed by Govindarajulu et al. (2005) modelling the management of the invasive
1691 American bullfrog *Lithobates catesbeianus*, where an equal removal of juveniles was
1692 more effective than removing adults. Adult bullfrogs however may cannibalize juveniles
1693 and this behaviour was explicitly incorporated in the model; conversely toads rarely ingest
1694 other anurans (Measey et al. 2015) and it makes the demographic impact of such

1695 intraspecific interaction negligible in the guttural toad. Given the low capacity to detect and
1696 remove juveniles (Table 2.1), I do not advocate utilization of a mode of removal that
1697 specifically targets individuals at this life stage.

1698 Above I showed that taking into consideration complex population dynamics of stage-
1699 structured invasive species could have helped to improve management efficiency.
1700 However my study also suggests that the main obstacle to the successful extirpation of
1701 this population in Cape Town was not caused by suboptimal removal strategies but rather
1702 by spatial limitations linked to the social dimension of the landscape. The complex peri-
1703 urban landscape did not allow monitoring the invaded area as a whole, being fragmented
1704 into about 3000 private properties. The high number did not allow removing invasive
1705 individuals from the whole area and it called into question the necessity to maximize
1706 management success targeting a few specific sites. Such a goal has been partially
1707 addressed by eradicators visiting properties with ponds during the breeding season. My
1708 simulations showed however that the number of ponds accessible for management was
1709 not sufficient to limit the spread of the species across the area but only to reduce locally
1710 the density (Figure 2.2). Inaccessible ponds are utilized by the toads as invasion hubs at a
1711 small scale to spread across the area (Florance et al. 2011) making the eradication
1712 ineffective. Only a minority (about 15 %) of the ponds I mapped through aerial imaging
1713 (see chapter one) were not targeted for removal because they were totally unknown to the
1714 eradicators. Conversely for most of the mapped ponds, the toad presence was locally
1715 confirmed by hearing the breeding call, but the eradicators failed to get access from the
1716 owners of the private property containing the pond. For example, some owners were
1717 pleased to have frogs in their gardens and disagreed with the removal, whereas others
1718 simply did not reply to phone calls or printed information about the toad removal
1719 programme. Thus, the lack of legislation on invasive populations of guttural toad
1720 established out of the South African native range impeded the removal of many
1721 individuals until 2015, when it was listed under Chapter 5 of the National Environmental
1722 Management: Biodiversity Act (NEM:BA, 2004) and recognized as an invasive species
1723 that must be controlled (Category 1b, Measey et al. in press). My study highlights that the
1724 availability of invasive species regulations earlier in the process (e.g. in 2011), would have
1725 promoted more effective management countermeasures. This underlines the fact that
1726 management actions in urban areas should also be accompanied by adequate legislation
1727 and communication in order to tackle social limitations.

1728 Additionally, I showed that not only modes but also times of management effort may play
1729 a crucial role to remove or control invasive species. All the simulated management
1730 strategies (with the exception of S_7 which removes most adults, tadpoles and eggs in all

1731 ponds; Figure 2.1) failed to crash the population, i.e. to reach the density threshold that
1732 impedes to the population to recover (Simberloff and Gibbons 2004). Also, only three of
1733 them (S_4 , S_6 and S_7) were able to limit the spread of the species across the area to a
1734 smaller subset of ponds (data not shown) suggesting that despite large demographic
1735 reduction, some individuals are equally able to reach the most peripheral ponds and carry
1736 on the invasion (similar to what was observed for instance by Facon and David 2006 in
1737 invasive populations of freshwater snails). Since the management was started during the
1738 spread phase of the invasion, I argue that an extirpation process conducted before this
1739 phase could be fully effective both to crash the population and limit its spread, as already
1740 suggested for other invasions (Pluess et al. 2012, Baker and Bode 2016). More generally,
1741 a rapid response seems particularly important in species characterized by high dispersal
1742 capacities (Pichancourt et al. 2012, Panetta and Cacho 2014) such as many bufonids
1743 (Smith & Green 2005). Whether rare long distance dispersal events enabled some
1744 individuals to colonize ponds outside the managed area even before the eradication,
1745 these individuals could theoretically remain undetected, or not reproductive, for years
1746 before actively contributing to the invasion (see chapter one and Blossey 1999, Hulme
1747 2006, Harvey et al. 2009).

1748 In light of my work the extirpation of the guttural toad in Cape Town appears at this stage
1749 highly improbable; however other more feasible management targets may still be
1750 successfully performed. Since impact of invasive populations on native communities is
1751 predicted to increase both linearly and non-linearly as a function of invader abundance
1752 (Yokomizo et al. 2009, Elgersma and Ehrenfeld 2011, Thomsen et al. 2011, Jackson et al.
1753 2015), a steady active removal of adult individuals may theoretically reduce the negative
1754 effects of the guttural toad in the invaded area. Also, the hiatus in large ponds I observed
1755 at the periphery of the area (Figure 2.3) could be used as a soft barrier against which to
1756 conduct removal and control the invasive population. However, I suggest that field studies
1757 should be conducted in Cape Town to estimate the real impact of this species on native
1758 communities both at individual and landscape scale (Latkza et al. 2016). Additionally, a
1759 sustained removal may reduce the occurrence probability of invasive bridgehead effect
1760 (Lombaert et al. 2010); the presence in the invade area of plant nurseries and the peri-
1761 urban landscape may voluntarily or accidentally determine an acceleration of the invasive
1762 spread and/or an incursion of the species into new territories (Measey et al. in press). For
1763 instance the guttural toad was episodically heard calling in 2016 from another area of
1764 Cape Town (Noordhoek, 10 km distant from the current invasive range) (Measey et al. in
1765 press), raising concern about the possibility of a new invasion event. Although the
1766 introduction pathway by which the toads arrived in this new area is unknown, one might

1767 expect that a lower density of guttural toads in Cape Town should necessarily reduce *per*
 1768 *se* the probability of other potential translocations.

1769 The recent observation of the guttural toad in Noordhoek flags the possibility of a new
 1770 invasive outbreak in Cape Town and calls the necessity to perform a rapid and consistent
 1771 removal of guttural toads at the new location. This chapter shows that not only the
 1772 rapidness of the response but also the capacity to remove adults for the private properties
 1773 will influence its efficacy; the new legislation enacted in 2015 could play a pivotal role at
 1774 this aim. This lesson should be applied to manage other invasive populations such as the
 1775 invasive population of Asian common toad *Duttaphrynus melanostictus* recently detected
 1776 in an urban area of Madagascar. While I agree with Kolby (2014) and Andreone (2014,
 1777 but see Mecke et al. 2014) who suggested that this population should be targeted for
 1778 eradication as soon as possible, my study shows that not taking into account biology and
 1779 autecology of the species or not tackling social constraints through adequate legislation
 1780 may strongly hamper the management success and raise costs. More generally, I show
 1781 that it would be particularly wise to test through ecological modelling both efficacy and
 1782 efficiency of any intended management program; indeed these two components can
 1783 indicate different strengths and limitations of management and be essential to identify
 1784 alternative more successful strategies.

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2036 **Chapter three: Never underestimate your opponent: rapid adaptive response**
2037 **reduces phenotypic mismatch in a recent amphibian invader**

2038

2039 **3.1 Introduction**

2040 The mean phenotype of a population should generally maximize the fitness of its
2041 individuals in the given environment (Lande 1976); such maximization emerges from the
2042 interplay between environmental pressures and mechanisms of genetic and non-genetic
2043 inheritance occurring at evolutionary time scales (Lande and Shannon 1996, Laland et al.
2044 2015). Invasive populations at incursion stage (van Wilgen et al. 2014) may, however, be
2045 an exception to this generalization. At the onset of an invasion process, ecological
2046 conditions are often dissimilar from those the invaders adapted to (Richardson and Pyšek
2047 2006); therefore, a mismatch between the current phenotypes and the optimal phenotype
2048 that could emerge in the invaded environment may occur (Novak et al. 2007, Prentis et al.
2049 2008, Hendry et al. 2011). This may lead the invasive population to adaptively respond to
2050 the novel environmental conditions to reduce the mismatch (Lee 2002, Whitney and
2051 Gabler 2008, Hendry et al. 2011, Foster 2013). Although any adaptive response improves
2052 fitness of the invaders, it should not necessarily be linked to genetic changes (see
2053 “adaptation” in Hendry et al. 2011.); non-evolutionary and evolutionary mechanisms such
2054 as environmental induced plasticity (Strauss et al. 2006, Ghalambor et al. 2007, Sultan
2055 2000, Foster 2013, Sih 2013), maternal effect (Fox et al. 1997, Dyer et al. 2010, Monty et
2056 al. 2013) or epigenetic changes (Moran and Alexander 2014) can also contribute to
2057 reduce phenotypic mismatch. These mechanisms act also on a much shorter time scale
2058 than that requires by classic adaptation (also in case of contemporary evolution, Carroll
2059 2007); phenotypic plasticity for instance occurs within one generation and numerous
2060 authors have highlighted its importance as a driver of invasion success (Richards et al.
2061 2006, Ghalambor et al. 2007, Davidson et al. 2011, Forsman 2014).

2062 The importance to explore adaptive responses that reduce phenotypic mismatch in
2063 established invasive populations during their initial incursion goes, however, far beyond
2064 exploring eco-evolutionary phenomena. Such populations may have considerable impacts
2065 on native species and communities (Simberloff et al. 2013) and significant efforts should
2066 thus be directed toward promptly predicting future invasive distributions and obtaining
2067 insights for prioritization and control (Broennimann et al. 2007). Authors showed that
2068 ignoring the occurrence of contemporary adaptive responses may be misleading in our
2069 aim to forecast invasion potential (Broennimann et al. 2007). For example, models that

2070 predict invasion potential of a species from the niche-space estimated in the native
2071 distribution may fail to robustly estimate the invaded range because they do not take into
2072 account adaptive responses that minimize phenotypic mismatches (Kolbe 2010).

2073 If we aim to investigate the occurrence of phenotypic response in an invasive population,
2074 a first step is to explore species phenotype between native and invaded areas (Van
2075 Kleunen et al. 2010; Bossdorf et al. 2005; Zalewski and Bartoszewicz 2012), defined by
2076 Hierro et al. (2005) as “biogeographical intraspecific comparison”. The next step is to
2077 compare the eventual phenotypic response with other similar adaptive responses
2078 detected in closely related populations or species. Although this does not allow
2079 conclusively distinguishing for instance between adaptive phenotypic plasticity and local
2080 adaptation (Strauss et al. 2006, Van Kleunen et al. 2010), it helps explore to what extent
2081 the invaders present novel characteristics that confer higher fitness in the new
2082 environment.

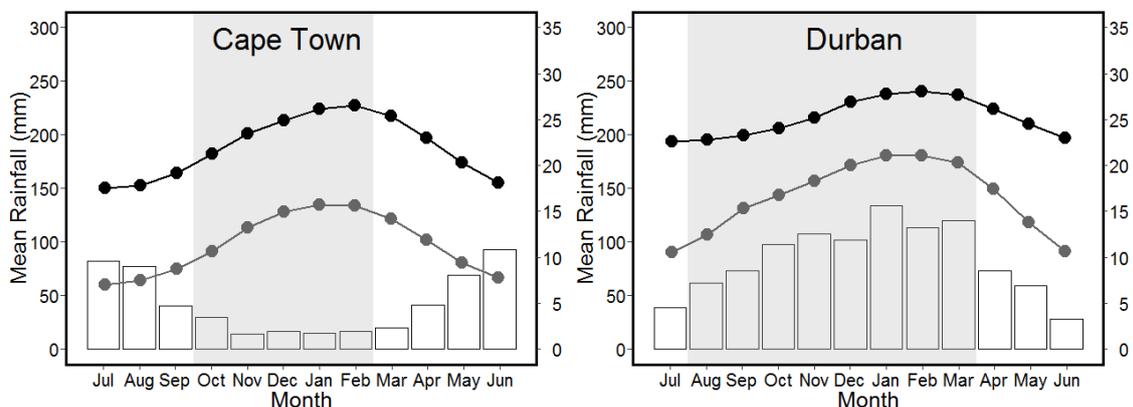
2083 Toads represent an excellent model for seeking rapid phenotypic response in new
2084 environmental contexts. They have evolved a range expansion phenotype which has
2085 allowed them to attain a cosmopolitan distribution and invade disparate environments (van
2086 Bocxlaer et al. 2010) in a relative short time (Pramuk 2006). Furthermore, the deliberate
2087 introduction and subsequent invasion by the cane toad *Rhinella marina* in more than 40
2088 countries across the globe has spawned an extensive literature on the physiological and
2089 behavioural response of these toads to novel environmental challenges (Lever 2001,
2090 Rollins et al. 2015 in Australia). Also, toad invasions are known to have a
2091 disproportionate environmental and economic impacts when compared to those of other
2092 invasive amphibians (Shine 2010, Measey et al. 2016) and recent observations of
2093 bufonids accidentally moved outside their native range (Kolby 2014, Measey et al. in
2094 press) showed the need to dedicate more studies to their capacity to adaptively respond
2095 to novel environments.

2096 Toads, and more generally amphibians, are particularly vulnerable to dehydration; as
2097 many physiological performances such as locomotion are severely affected by individual
2098 hydration state (Beuchat et al. 1984, Prest and Pough 1989), this group evolved several
2099 adaptations to regulate water exchange (McClanahan and Baldwin 1969, Parson et al.
2100 1993, Prates and Navas 2003). However, the sensitivity of physiology to hydration state
2101 differs among species and populations, with amphibians that evolved in drier
2102 environments generally outperforming those from wetter environments when dehydrated
2103 (Beuchet et al. 2014, Tingley et al. 2012). Although the capacity to cope adaptively with
2104 challenges imposed by drier environmental conditions may emerge evolutionary time

2105 scale (Toledo and Jared 1993), it is not clear to what extent adaptive responses can
 2106 rapidly reduce a phenotypic mismatch caused by introduction of a sub-optimal genotype
 2107 into a new environment. To address this question, I use an invasive population of guttural
 2108 toad *Sclerophrys gutturalis* recently established (in 2000) in Cape Town, South Africa, and
 2109 a native population of the same species from Durban; the native population has been
 2110 chosen because hypothesized to be the original source of the invaders by genetic
 2111 evidences (Telford 2015).

2112 The guttural toad naturally inhabits tropical and subtropical southern Africa, where it
 2113 adaptively synchronizes reproduction with rainfall in order to exploit favourable conditions
 2114 of temperature and water availability (du Preez et al. 2004). Despite the winter rainfall
 2115 climate regime of Cape Town, with summers notably drier than those characterizing the
 2116 native environment of the source population, invasive toads still breed in the hotter, drier
 2117 months of the year. The invasion is facilitated by the occurrence in the invaded area of
 2118 numerous artificial ponds (see chapter one and two) and the synanthropic behaviour of
 2119 the species (Measey et al. in press); however it is not clear whether and how the
 2120 phenotype of the guttural toad has rapidly responded to an environment theoretically
 2121 much drier than that of the source population.

2122



2123

2124 **Figure 3.1** Mean monthly rainfall (bars), maximum temperature (black dots and line) and
 2125 minimum temperature (grey dots and line) in Cape Town and Durban. Shaded area
 2126 represents the breeding season of the guttural toad in each location. Climate data sourced
 2127 from: the World Meteorological Organization, <http://public.wmo.int/>

2128 Firstly, I explore to what extent invasive individuals are exposed to more severe
 2129 desiccating conditions than native conspecifics. At this scope, I measured toads' field
 2130 hydration level respectively in both Cape Town and Durban during the reproductive
 2131 period. Secondly, I performed an intraspecific biogeographical comparison on the

2132 physiological phenotype of the guttural toad at the two populations. Rates of evaporative
2133 water loss (EWL), rates of water uptake (WU) and sensitivity of locomotor performance to
2134 hydration state were measured in the laboratory. Since Cape Town is not only drier but
2135 also colder than Durban, Critical thermal minima (CT_{min} s) and interaction between CT_{min}
2136 and hydration state were additionally estimated. I selected these physiological traits
2137 because they have consequences on fitness and they were commonly investigated in
2138 amphibians to measure phenotypic response to local environmental conditions across
2139 populations (Tingley et al. 2012, McCann et al. 2014). Also, such traits are particularly
2140 helpful to define species niche-space and forecast invasion potential (Kolby et al. 2010;
2141 Tingley et al. 2012). Assuming that invasive individuals of Cape Town are exposed to drier
2142 and colder environmental conditions than conspecifics from Durban, I hypothesize the
2143 occurrence of a rapid adaptive response that reduces phenotypic mismatch of the
2144 invaders in the novel environment.

2145 **3.2 Materials and methods**

2146 **3.2.1 Study species and locations**

2147 The guttural toad *Sclerophrys gutturalis* is a common African bufonid naturally distributed
2148 in across central and southern Africa (du Preez et al. 2004). The species is tolerant to
2149 different altitudes (from sea-level to about 1800 m) and latitudes (from the equator to 30°
2150 S). It inhabits disparate vegetation types like Savannah, Grassland and Thicket biomes
2151 (du Preez et al. 2004) and due to a highly synanthropic behaviour, it is not uncommon to
2152 find these toads in peri-urban areas. The guttural toad is a domestic exotic in South Africa
2153 (Measey and Davies 2011) being native in most of the country but not in the Western
2154 Cape, where an invasive population established in Cape Town since 2000 (De Villiers
2155 2006).

2156 Adult toads were collected within an area of 10 km² both in Cape Town (South Africa, 87
2157 m.a.s.l., 34° 0'54"S, 18°25'51"E), where the population is still expanding every year (see
2158 chapter one), and in Durban (75 m a.s.l., 29°47'16"S, 31° 1'47"E), KwaZulu-Natal, where
2159 the species is native. The two populations share the same altitude and a very similar peri-
2160 urban landscape characterized by many artificial ponds which toads use to breed (see
2161 chapter one). Ethics clearance was obtained from Stellenbosch University Animal Ethics
2162 Committee (Protocol Number U-ACUD14-00112) and collections in the native area
2163 (Durban) occurred under permission from KZN Wildlife (Permit Number OP553/2015)

2164 **3.2.2 Hydration state in the field**

2165 In January and February 2016, 35 and 43 adult toads were captured respectively in Cape
2166 Town and Durban after dark every three days for a total of two weeks. For each individual,
2167 I recorded air temperature and relative humidity (using a pocket weather meter, AZ-8910
2168 5 in 1, AZ Instrument Corp ©) and cloacal body temperature (using a thermal probe
2169 connected to a digital thermometer, CHY 507, CHY Firemate ©). I also measured empty-
2170 bladder body mass (Tracy et al. 2014) with a portable balance (± 0.01 g, WTB 2000,
2171 Radwag ©) and then placed each toad separately in a plastic container filled to a depth of
2172 20 mm with water in order to promote hydration. The body mass was measured again
2173 every 15 minutes until it did not change after two consecutive measurements; this was
2174 considered the fully hydrated body mass (Tracy et al. 2014). The hydration state was
2175 calculated as the percentage between the initial body mass against the fully hydrated
2176 body mass. Since some toads defecated during the experiment, I removed them from the
2177 analysis.

2178 **3.2.3 Phenotypic traits tested in the laboratory**

2179 To test animals in the laboratory, adult toads (sex ratio 1:1) were collected in the field
2180 between December 2015 and February 2016 and housed in tanks with water and shelter
2181 *ad libitum* on a natural photoperiod (12:12) and fed mealworms twice a week. Collection of
2182 preliminary data in the field allowed setting temperature and relative humidity of the room
2183 constant at 23°C ($\pm 2^\circ\text{C}$) and 70% ($\pm 5\%$) respectively. Animals were not fed for three days
2184 before any experiment in order to avoid the effects of specific dynamic action (Secor and
2185 Faulker 2002) and defecation on the dehydration results. Individuals tested in the
2186 laboratory are different from those used to estimate hydration state in the field.

2187 **3.2.3.1 Evaporative water loss and water uptake**

2188 Evaporative water loss (EWL) and water uptake (WU) were tested in 22 toads from Cape
2189 Town and 20 toads from Durban. Animals were placed in individual plastic containers
2190 filled to a depth of 20 mm with water for one hour and left inside an acclimated room with
2191 controlled temperature (23°C) and relative humidity (65%), to ensure that they were fully
2192 hydrated before the tests. Then each toad was blotted with paper tissue, its bladder
2193 emptied by gently pressing the abdomen, and body mass was recorded (± 0.01 g, WTB
2194 2000, Radwag). This body mass was considered the standard mass (hydration state of
2195 100%).

2196 Each toad was subsequently placed inside a plastic wind tunnel (diameter=0.25 m;
2197 length=1 m) equipped with an electric fan that created an air-flow, and weighed (± 0.01 g)
2198 at intervals of 15 minutes. The EWL trial stopped when the toad reached 80% of its
2199 standard body mass. Body mass was converted into surface area using the empirical
2200 equation derived by McClanahan and Baldwin (1969) and assuming that the water
2201 conserving posture exposes two thirds of the total surface area to the air (Withers et al.
2202 1984). The rate of EWL from each individual was calculated from the regression of the
2203 total surface area (cm^2) against time (min). Since EWL may covary with that proportion of
2204 time spent performing water conserving posture, I inspected toad posture in the plastic
2205 tube every 15 minutes following Tingley et al. (2012).

2206 After the EWL test, the dehydrated toads (hydration state of 80%) were placed in an
2207 individual plastic container filled with water to a depth of 10 mm, blotted with a paper towel
2208 and weighed (± 0.01 g) at each 5 min for one hour. Since smaller individuals reach full
2209 hydration state earlier, the experiment was stopped when body mass did not change after
2210 two consecutive weighings. Rate of water uptake (WU) was calculated from the
2211 regression of the body mass (g) against time (min) following Titon et al. (2010).

2212 **3.2.3.2 Sensitivity of locomotor performance to hydration state**

2213 The effect of hydration on performance was tested on 22 toads from Cape Town and 20
2214 toads from Durban. To determine whether hydration status influenced locomotor
2215 performance, toads at three hydration states (100%, 90% and 80% of fully hydrated mass;
2216 ten toads in Cape Town and eight toads in Durban for each treatment) were tested on an
2217 indoor circular racetrack (4.1 m) using a rubber grip mat as a substrate (Tingley et al
2218 2012) at constant temperature and humidity (23°C and 70%, respectively). Differential
2219 hydration states were obtained using the same protocol described in the previous
2220 sections. Since at both sampling locations toads are active after sunset, I performed
2221 performance trials during night.

2222 Each toad was individually placed in the racetrack and stimulated to hop by gently tapping
2223 it on the back with a stick. For each toad I counted the number of laps it performed until it
2224 did not voluntarily hop for 60 seconds (i.e. exhaustion). Pilot trials showed shorter times of
2225 hopping refusal (e.g. refusing to hop for 20 seconds) did not give a reliable indication of
2226 the toad tiredness (i.e. some toads recovered and were able to hop again for more than
2227 30 minutes with no pauses after stopping for about 30 seconds). For each lap in the
2228 racetrack, I also recorded time taken and number of hops until exhaustion. Since two 80%
2229 dehydrated individuals from Cape Town showed anomalous behaviour once placed in the

2230 racetrack (i.e. they did not hop for the first 45 seconds of the experiment and refused to
2231 hop again after about 30 seconds), I removed them from the analysis. Each individual was
2232 euthanized straight after the experiment using a 1 gl^{-1} solution of tricaine methane
2233 sulfonate (MS 222) for twenty minutes; snout-vent length (SVL) and tibia lengths were
2234 measured using a digital caliper in order to explore a possible role of morphology on
2235 hopping ability (Phillips & Shine 2006).

2236 **3.2.3.3 Critical thermal minimum and interaction with hydration state**

2237 CT_{\min} was tested in 30 toads from Cape Town and 20 toads from Durban. One hour
2238 before the experiment, individuals were placed in individual plastic containers filled to a
2239 depth of 20 mm with water and kept inside a climate room (23°C) and relative humidity
2240 (65%), to ensure that they were fully hydrated before the tests (hydration state of 100%).
2241 Then, each toad was weighed and individually placed in a metal chamber (80 mm L x 100
2242 mm W x 150 mm H) submerged into fluid-filled Perspex jacket and connected to a water
2243 bath (Grant Gr150; Grant Instruments, Shepreth, UK) containing a 1:1 water–glycol
2244 mixture at 0°C. The aperture of the chamber was closed with acetate film to avoid escape
2245 and to maintain the targeted temperature and high humidity (~100% RH) within the
2246 chambers. Every two minutes the toad was turned on its back in order to test the righting
2247 reflex (Spellerberg 1972); in addition, cloacal body temperature was collected with a
2248 thermal probe. The experiment was repeated until the toad was unable to right itself for 15
2249 seconds (Kolbe et al. 2010); the CT_{\min} was considered the highest body temperature at
2250 which the toad first lost its righting response (Sanabria et al. 2012). Although I followed
2251 the protocol adopted by Kolbe et al. (2010) and McCann et al. (2014) to estimate CT_{\min} in
2252 toads, I utilized a water bath at 0 °C instead of a cooler box with ice in order to
2253 standardize the chamber temperature and reduce the variation in cooling rate among
2254 individuals.

2255 In order to test to what extent the hydration state affects the CT_{\min} , the same toads
2256 previously tested for CT_{\min} were placed one week later into a plastic wind tunnel (see
2257 above) and dehydrated until they reached 80% of their standard body mass. After that, I
2258 repeated the CT_{\min} experiment as described above. Since each individual was tested for
2259 CT_{\min} at two different hydration states, I performed repeated measures ANCOVA with
2260 cooling rate as the covariate and population, hydration state and their interactions as
2261 effects.

2262 **3.2.4 Data analysis**

2263 To investigate differences in field hydration state between the two populations, I
2264 performed a Kruskal-Wallis test. A Spearman correlation test was also performed between
2265 the hydration state and air temperature, body temperature and relative humidity to
2266 investigate their effect on individual hydration state.

2267 As water loss can be affected by water conserving posture, differences between the two
2268 populations in term of EWL were explored using ANCOVA with the proportion of time
2269 spent in water conserving posture used as a covariate following Tingley et al. (2012).
2270 Differences in terms of WU between the two populations were evaluated by a t-test. To
2271 explore the occurrence of a correlation between EWL and WU and population of origin, I
2272 performed ANCOVA with WU as a response variable.

2273 I used ANOVA to investigate effects of population and hydration state on total endurance
2274 (m), total speed ($\text{m}\cdot\text{s}^{-1}$), distance covered in the first ten minutes (m) and number of hops
2275 per meter. Also I performed ANOVA to study the effect of the same independent variable
2276 on total endurance, total speed and distance covered in the first ten minutes, all
2277 expressed in SVLs (body lengths). Both SVL and tibia length do not differ between
2278 populations and among treatments, suggesting that these morphological traits do not
2279 explain eventual variations observed in our groups (Tingley et al. 2012, McCann 2014).

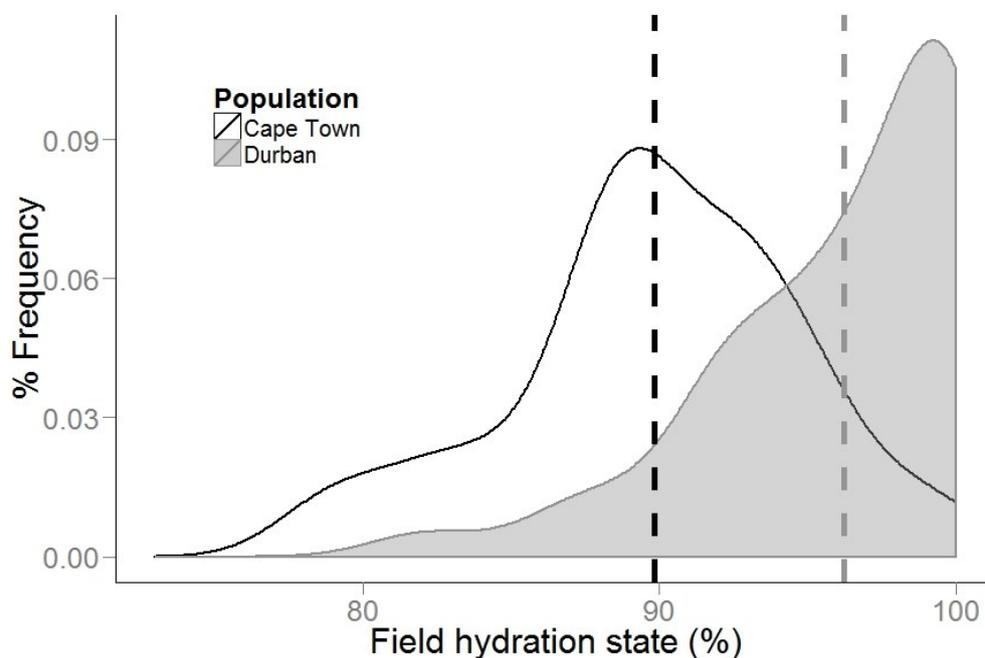
2280 To explore differences between the two populations in term of CT_{min} in fully hydrated toads
2281 (100% hydration state) I used ANCOVA with body mass and cooling rate as covariates.
2282 Since mass was not significant as a covariate, it was removed from the successive
2283 analyses (Kolbe et al. 2010, McCann et al. 2014). Since each individual was tested for
2284 CT_{min} at the two different hydration states, I used repeated measure MANCOVA with
2285 population and hydration state as factors and cooling rate as covariate in order to test the
2286 effect of hydration state on CT_{min} .

2287 **3.3 Results**

2288 **3.3.1 Do Cape Town individuals experience drier environmental conditions than** 2289 **conspecifics from Durban?**

2290 On capture from the field, Cape Town toads showed an hydration state significantly lower
2291 than that from the toads of Durban (Cape Town = 89.9%; Durban = 96.2%, Kruskal-Wallis
2292 , $\chi^2 = 27.7509$, $p < 0.0001$, Figure 3.2). In general, hydration state was positively

2293 correlated with relative humidity ($\rho=0.67$, $p<0.0001$) but not correlated with air or cloacal
 2294 body temperature.



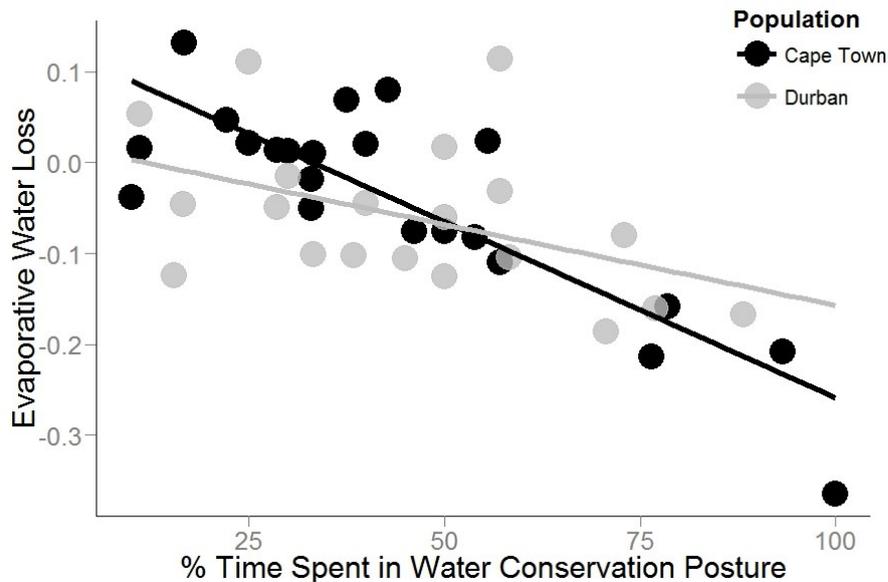
2295

2296 **Figure 3.2** Smoothed frequency functions obtained considering field hydration states of
 2297 guttural toads *Sclerophrys gutturalis* from Cape Town (black and white) and Durban (grey)
 2298 populations. The field hydration state is expressed as a percentage of the field empty
 2299 bladder body mass against the fully hydrated body mass measured in the field. Vertical
 2300 lines represent means.

2301 **3.3.2 Do the two populations differ in terms of evaporative water loss and water** 2302 **uptake?**

2303 The two populations did not differ in terms of evaporative water loss ($F_{1,38} = 0.35$, $p =$
 2304 0.558) once corrected for the time spent in water conserving posture (that also does not
 2305 differ between Cape Town and Durban). However, an interaction between time spent in
 2306 water conserving posture and population was detected ($F_{1,38} = 4.62$, $P < 0.05$); toads from
 2307 Cape Town were more efficient than those from Durban to minimize water loss through
 2308 postural adjustments (Fig.3.3; in Cape Town, $r = -0.84$, $P < 0.0001$; in Durban, $r = -0.45$, $P <$
 2309 0.05).

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2313 **Figure 3.3** Linear regression of evaporative water loss ($\text{mg cm}^{-2} \text{min}^{-1}$) on percentage of
 2314 time spent in water conserving posture in guttural toads *Sclerophrys gutturalis* from Cape
 2315 Town (black dots and line, $r = -0.84$, $P < 0.0001$) and Durban (grey dots and line, $r = -0.45$,
 2316 $P < 0.05$) populations. Note y-axis is on logarithmic scale.

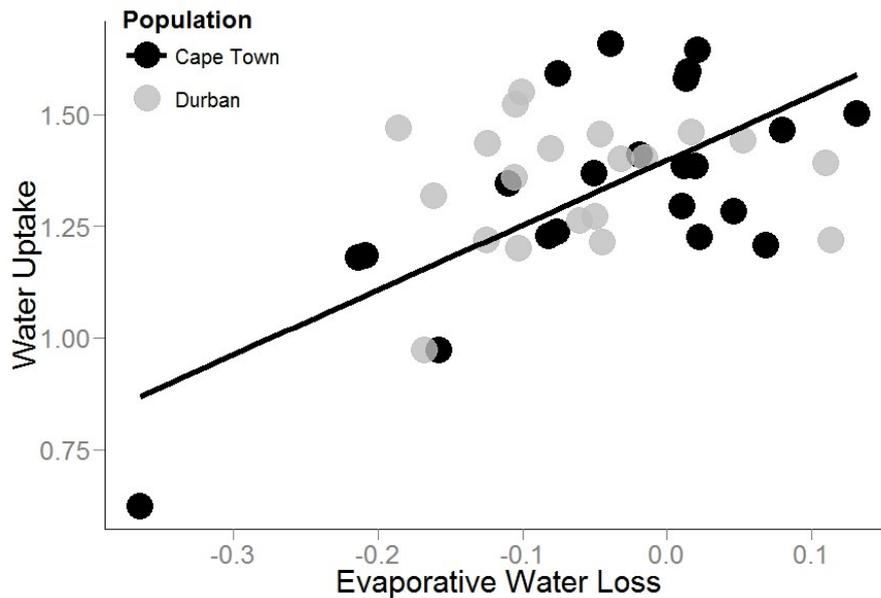
2317 The two populations did not differ in term of WU ($t_{34.7} = 0.37$, $p = 0.72$). Globally (among all
 2318 individuals from both populations), evaporative water loss positively correlated with water
 2319 uptake rate ($F_{1,38} = 16.55$, $p < 0.001$); toads that lost water faster also gained water more
 2320 rapidly. However, this relationship showed a population effect ($F_{1,38} = 5.53$, $p < 0.05$); EWL
 2321 predicted WU in the Cape Town population ($r = 0.69$, $P < 0.001$) but not in the Durban
 2322 population ($r = 0.11$, $P = 0.6$; Fig. 3.4). One individual from the Cape Town population lost
 2323 and gained water much more slowly than all other conspecifics (outlier in Fig. 3.4);
 2324 however its removal from the analysis does not qualitatively alter the correlation between
 2325 EWL and WU detected in Cape Town ($r = 0.47$, $P < 0.05$).

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2331 **Fig. 3.4** Linear regression of water uptake on evaporative water loss in guttural toads
 2332 *Sceloporphys gutturalis* from Cape Town (black dots and line, $r = 0.69$, $P < 0.001$) and
 2333 Durban (grey dots and line, $r = 0.11$, $P = 0.6$) populations. In Cape Town the correlation
 2334 between the two variables was significant also after removing the outlier showing the
 2335 lowest EWL and WU. Conversely no significant correlation was detected in Durban. Note
 2336 x- and y-axis are on logarithmic scale.

2337

2338

2339 **3.3.3 Do the two populations differ in terms of sensitivity of locomotor performance** 2340 **to hydration state?**

2341 An interactive effect between hydration state and population on total endurance (Figure
 2342 3.5(a)) and distance covered in the first ten minutes (Figure 3.5(c)) was notable but not
 2343 statistically significant (Table 3.1); invasive toads outcompeted native toads when
 2344 dehydrated (90% and 80%) but not when fully hydrated (Figure 3.5(a-c)). Globally,
 2345 hydration state affected total endurance (Figure 3.5(a)), total speed (Figure 3.5(b)) and
 2346 distance covered in the first ten minutes (Figure 3.5(c)), with fully hydrated toads (100%)
 2347 outperforming those that were partially dehydrated (90% and 80%; Table 3.1). A
 2348 population effect was detected in endurance, with Cape Town individuals that were able to
 2349 cover longer distances than toads from Durban. Analogous results were obtained when
 2350 the response variables were expressed in SVL (body lengths) instead of meters
 2351 (Appendix 3).

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2355 **Table 3.1** Effects of population and hydration state on total endurance, total speed,
 2356 distance covered in the first ten minutes and number of hops per meter in guttural toads
 2357 *Sclerophrys gutturalis* from Cape Town and Durban populations. Significant differences
 2358 ($P < 0.05$) are highlighted in bold

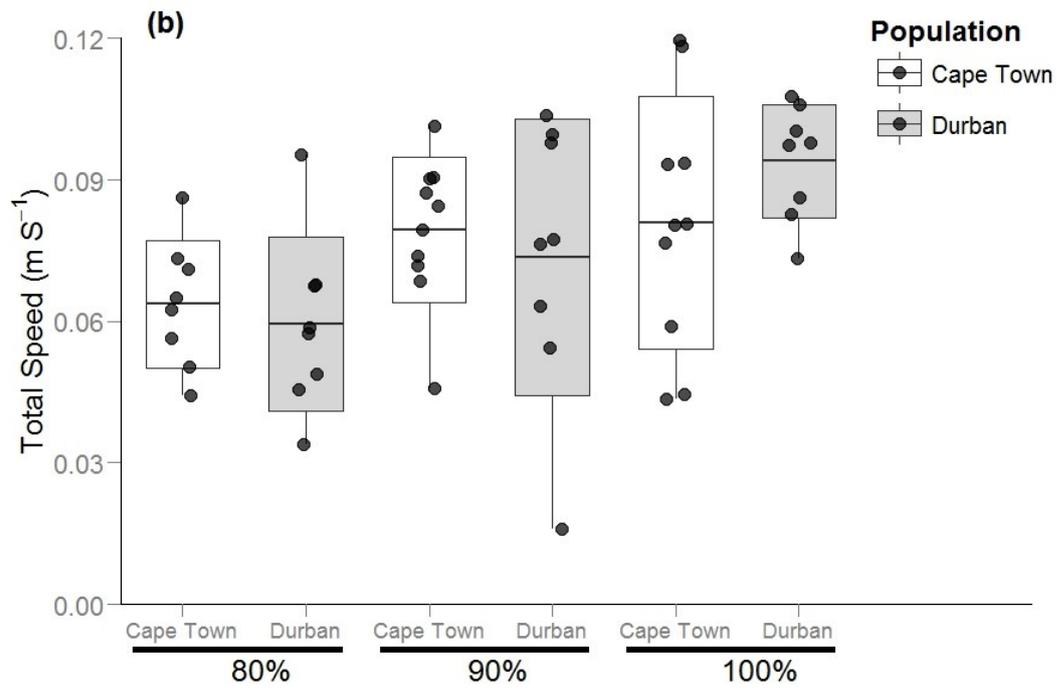
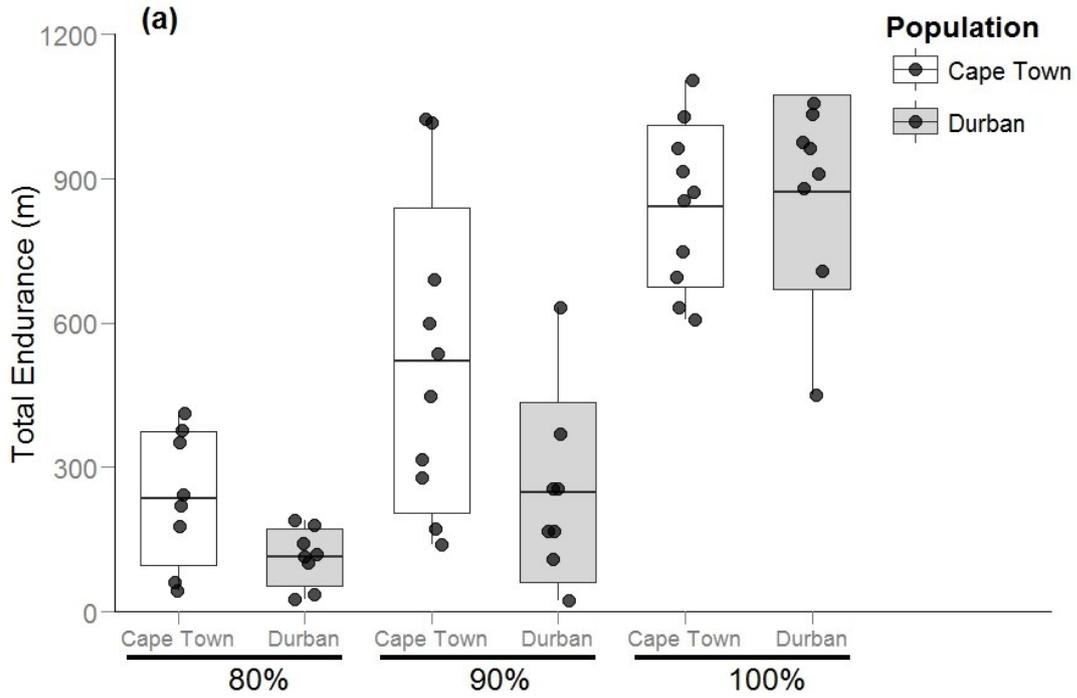
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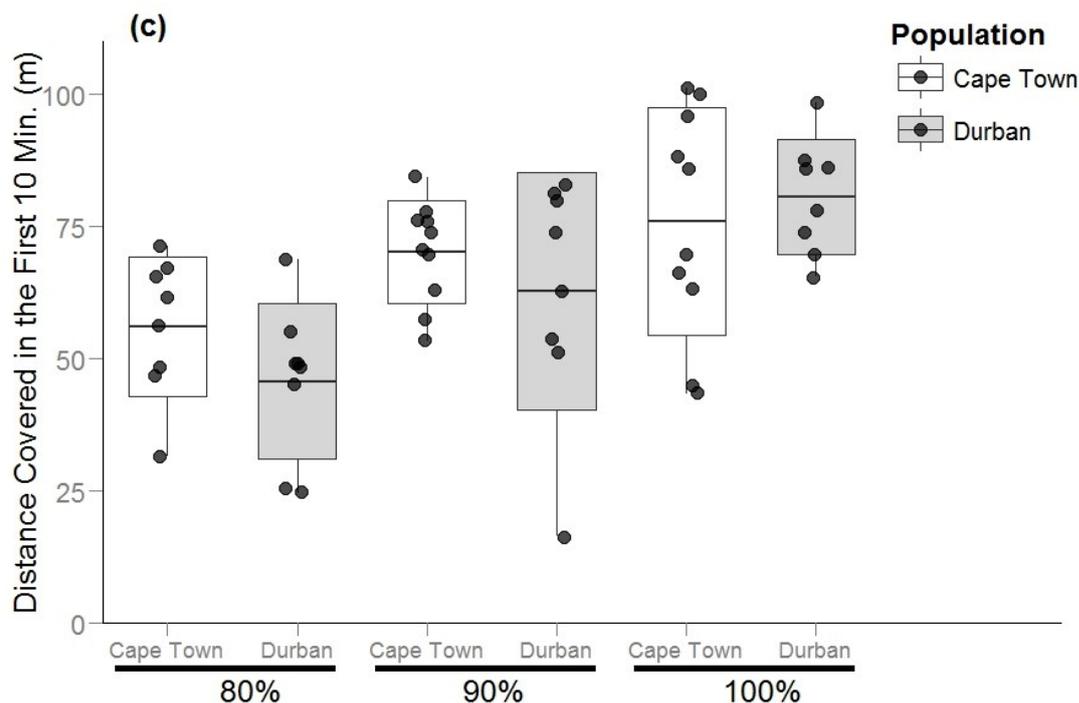
Variable	Statistic test	<i>P</i>
Total endurance (m)		
Population	$F_{1,46} = \mathbf{9.16}$	$P < \mathbf{0.01}$
Hydration state	$F_{2,46} = \mathbf{31.19}$	$P < \mathbf{0.0001}$
Population : hydration state	$F_{2,46} = 2.18$	$P = 0.124$
Total speed (m s^{-1})		
Population	$F_{1,46} = 0.723$	$P = 0.127$
Hydration state	$F_{2,46} = \mathbf{4.15}$	$P < \mathbf{0.05}$
Population : hydration state	$F_{2,46} = 1.44$	$P = 0.25$
Distance covered in the first ten min (m)		
Population	$F_{1,46} = 1.95$	$P = 0.169$
Hydration state	$F_{2,46} = \mathbf{8.17}$	$P < \mathbf{0.001}$
Population : hydration state	$F_{2,46} = 1.36$	$P = 0.27$
Number of hops per meter		
Population	$F_{1,46} = 0.24$	$P = 0.627$
Hydration state	$F_{2,46} = 1.10$	$P = 0.340$
Population : hydration state	$F_{2,46} = 0.32$	$P = 0.730$

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2367 **Figure 3.5** Effect of hydration state on total endurance (a), total speed (b) and distance
 2368 covered in the first ten minutes (c) of guttural toads *Sclerophrys gutturalis* from Cape
 2369 Town (white boxes) and Durban (grey boxes) populations. Boxes represent means and
 2370 standard deviations (\pm SD); whiskers extend to maxima and minima. Dark and pale dots
 2371 represent males and females.

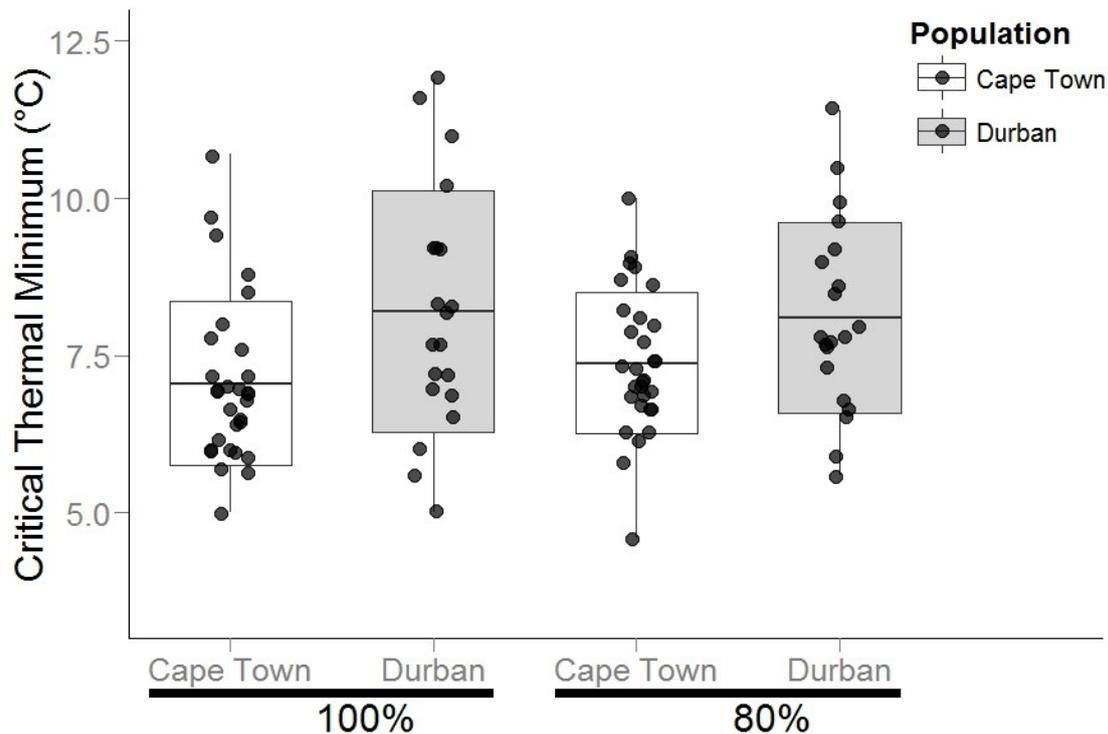
2372 **3.3.4 Do the two populations differ in terms of critical thermal minimum and in term**
 2373 **of sensitivity of critical thermal minimum to hydration state?**

2374 When fully hydrated, toads from Cape Town showed a CT_{min} lower than Durban
 2375 conspecifics (Cape Town = 7.1 °C, Durban = 8.2 °C, $F_{1,46} = 3.95$, $p < 0.05$; Fig.6). Cooling
 2376 rate significantly affected CT_{min} ($F_{1,46} = 10.93$, $p < 0.001$) while an interaction effect between
 2377 cooling rate and population was also observed ($F_{1,46} = 7.77$, $p < 0.01$).

2378 The two populations did not differ from each other in terms of CT_{min} when tested across
 2379 the two hydration states ($F_{1,46} = 1.66$, $P = 0.10$; Fig.6). However hydration state did not
 2380 affect the CT_{min} ($F_{1,46} = 0.33$, $P = 0.75$) while cooling rate had a significant effect ($F_{1,46} =$
 2381 2.66 , $P < 0.05$).

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2385 **Fig. 3.6** Critical thermal minimum of guttural toads *Sclerophrys gutturalis* from Cape Town
 2386 (white boxes) and Durban (grey boxes) populations at the two different hydration states.
 2387 Note that the same toads were tested when fully hydrated (100% body mass) and after
 2388 one week, when dehydrated up to 80% of their body mass. Boxes represent means and
 2389 standard deviations (\pm SD); whiskers extend to maxima and minima

2390 3.4 Discussion

2391 My study shows that invasive guttural toads underwent in less than two decades an
 2392 adaptive response that reduces phenotypic mismatch in the invaded area. In accordance
 2393 to the more desiccating and colder environment of Cape Town, the invasive population
 2394 responded physiologically and behaviorally to reduce sensitivity to lower conditions of
 2395 hydration and temperature. Such a response indicates that invasive guttural toads have
 2396 already a higher potential to invade Cape Town, and more generally drier environments,
 2397 than I could infer from the source population.

2398 In the breeding season, Cape Town individuals were exposed to lower relative humidity
 2399 than Durban conspecifics (Cape Town = 65.7%; Durban = 87.0%), leading to lower field
 2400 hydration states in the invaded area (Cape Town = 89.9%; Durban = 96.2%). Although ten
 2401 individuals out of 43 were collected in Durban during a single rainy night and all these
 2402 toads were fully hydrated (100%), their removal from the analysis did not consistently
 2403 change the mean field hydration state of the population (95.1%). Firstly, this observation
 2404 implies that the lower relative humidity in Cape Town was sufficient *per se* to cause a
 2405 difference in hydration state between the two populations. Secondly, it suggests that such
 2406 hydration state difference may be even larger, given that both precipitation and frequency

2407 of rainy days are significantly higher in Durban than Cape Town during the breeding
2408 season (Fig. 1; monthly average precipitation days between October and February
2409 according to the World Meteorological Organization, <http://public.wmo.int/>: Durban = 14.8;
2410 Cape Town = 6).

2411 Amphibian physiological performance is negatively affected by a decrease in hydration
2412 level (Prest and Pough 1989, Titon et al. 2010). As consequence, I predicted that
2413 populations invading environments characterized by more desiccating conditions should
2414 respond by regulating water exchange more effectively and/or developing lower
2415 physiological sensitivity to dehydration. My findings partially confirm this prediction and
2416 show that adaptive responses reducing phenotypic mismatch in the invaded environment
2417 may emerge on a short time scale (less than two decades).

2418 Although the two populations did not globally differ in term of evaporative water loss,
2419 individuals from Cape Town minimized more effectively water loss through water
2420 conserving posture (Figure 3.3); this suggests that behavioral modifications could partially
2421 compensate physiological constrains (e.g. skin permeability) into a novel environment
2422 (Snell-Rood 2013, Sol et al 2013). Changes of behaviour have been detected in other
2423 invasive species exposed to environmental conditions different from those they adapted to
2424 not necessarily among generations (Pizzatto et al. 2008, Alford et al. 2009 in frogs; Liebl
2425 and Martin 2012 in birds) but also within generation through behavioral plasticity (Terkel
2426 1995 in mammals, Price et al. 2008 in birds, Weiss 2010 and Grason and Miner 2012 in
2427 invertebrates, Pettit et al. 2016 in frogs). Such plasticity may facilitate invasion and is a
2428 predictor of invasion success across taxa (Wright et al. 2010, Amiel et al. 2011, Foster
2429 2013, Sih 2013). Mitchel and Bergman (2016) noted that in the green frog *Lithobates*
2430 *clamitans* modes of water conserving posture can slightly differ according to the position
2431 of head and limbs, something I observed in the experiments on guttural toads. Invasive
2432 individuals may have learned to adjust their posture to perform a more effective water
2433 conserving behavior in a more challenging environment.

2434 Once corrected for water conserving posture, the rate at which water is lost through
2435 evaporation depends mainly on skin structure (Navas et al. 2004). Several studies
2436 detected a counter-intuitive pattern within species; due to thinner and smoother skin,
2437 individuals inhabiting semi-arid environments possess lower cutaneous resistance than
2438 individuals from mesic environments (Navas et al. 2004, Prates and Navas 2009, Tingley
2439 2012). Thinner or smoother skin causes not only a faster evaporative water loss but also a
2440 faster water uptake. Authors hypothesized that in a drier environment the advantage of
2441 facilitating water absorption may exceed the advantage of minimizing water loss (Warburg
2442 1964, Prates and Navas 2009, Tingley et al. 2012). My study confirmed that in guttural

2443 toads the EWL is correlated with WU but I did not detect any differences in these variables
2444 between native and invasive populations. Thus it is unlikely that significant adaptation of
2445 skin structure has occurred in the Cape Town population. Although the recent
2446 establishment of the invasive population could not have allowed this skin adaptation, the
2447 behavioral plasticity observed in water-conserving posture could also minimize selective
2448 pressures toward a higher cutaneous resistance (Auld et al. 2009, Relyea 2002, Davies et
2449 al. 2015). It also provides an alternative explanation for why local adaptation should favor
2450 faster water uptake over lower water loss (Warburg 1964, Prates and Navas 2009, Tingley
2451 et al. 2012).

2452 Intriguingly EWL and WU were positively correlated in the population exposed to drier
2453 conditions (Cape Town), while no such correlation was detected in native Durban (Fig 4).
2454 Exactly the same result was observed by Tingley et al. (2012) when comparing invasive
2455 cane toads *Rhinella marina* from two invasive areas: a semi-arid and a mesic
2456 environment. It suggests that toads inhabiting drier environments seem more effective in
2457 counterbalancing a faster water loss through a faster uptake. Testing this hypothesis
2458 across populations and species exposed to differential hydric regimes seems ripe for
2459 further investigations.

2460 As previously reported in other species of amphibians (Titon et al. 2010, Tingley et al.
2461 2012), guttural toad locomotor performance is affected by hydration state (Table 3.1); this
2462 implies that the capacity of the species to disperse and migrate across Cape Town is
2463 significantly constrained by abiotic conditions of the novel environment, at least during the
2464 hot, dry, summer breeding season. In other words, the desiccating conditions of Cape
2465 Town make the phenotype of the species sub-optimal in the invaded area thus causing a
2466 phenotypic mismatch. Despite this, the invasive population is currently spreading, where
2467 toads colonize new ponds every year through leading edge dispersal (Measey et al. in
2468 press). The spread is relatively slow: since the first detection, the guttural toad invaded an
2469 area of 10 km² and the invasion front advanced about 2 km (see chapter two) in the last
2470 five years. Many factors such as the constant removal of the toads by the eradication or
2471 the high availability of breeding sites in Cape Town (see chapter two) may contribute to
2472 this limited spread. However my laboratory trials showed that the phenotypic mismatch
2473 may play a role *per se*; I suggest that mark-recapture studies performed in both
2474 populations may be highly informative and may link physiological constraints to the actual
2475 dispersal of the species in the field.

2476 My study also shows that Cape Town individuals notably, but not significantly,
2477 outperformed Durban conspecifics when dehydrated; for example endurance of invasive
2478 toads is about twice that of native toads at both dehydration treatments (Figure 3.5(a)).

2479 Conversely, native toads partially outperformed invasive toads when fully hydrated (Figure
2480 3.5(a-c)). It suggests not only that the phenotypic mismatch caused by the introduction of
2481 a sub-optimal genotype into the novel environment has been already reduced in the
2482 invasive population but also that this reduction could have caused a trade-off (Futuyama
2483 and Moreno 1988). Although at this stage I cannot conclusively disentangle which
2484 mechanisms are responsible to reduce the phenotypic mismatch (e.g. local adaptation vs.
2485 phenotypic plasticity, see Rollins et al. 2015), my findings possess both evolutionary and
2486 conservation implications. A mismatch reduction could first of all signal the onset of a
2487 genetic change (Lee 2002, Strauss et al. 2006, Whitney and Gabler 2008); it may be due
2488 to a classic mechanism of natural selection (Lande 1976), for example whether individuals
2489 less sensitive to dehydration have higher fitness than conspecifics due to reaching
2490 breeding sites faster (i.e. local adaptation). Alternatively, it may be due to phenomena
2491 linked to spatial disequilibrium dynamics such as spatial selection (Shine et al. 2011), for
2492 example whether individuals less sensitive to dehydration disperse farther than
2493 conspecifics and exploit low density areas. Environmental induced plasticity (Sultan 2000)
2494 or founder effect could have also reduced phenotypic mismatch, although the second
2495 mechanism seems highly improbable considering the clear adaptive significance of the
2496 reduction. Controlled translocation or common-garden experiments may help to
2497 investigate whether this rapid response has an evolutionary component or it derives from
2498 phenotypic plasticity (Sultan 2000, Moloney et al. 2009, Van Kleunen et al. 2011, Monty et
2499 al. 2013). As local adaptation may reduce physiological sensitivity to dehydration over
2500 time and accelerate the invasion spread, this investigation could have additional
2501 management implications; our capacity to effectively adopt management
2502 countermeasures could in the future not only be hampered by the social dimension of the
2503 invaded landscape (see chapter two) but also by an improved capacity of the species to
2504 disperse across the novel environment. More generally my study suggests that a model
2505 aiming to incorporate locomotion to forecast invasion potential should incorporate the
2506 lower sensitivity of Cape Town toads to dehydration (Tingley et al. 2014); ignoring this
2507 rapid response could significantly underestimate invasion potential (Whitney and Gabler
2508 2008, Kolbe et al. 2010).

2509 Lastly, I found that when fully hydrated Cape Town toads had significantly lower CT_{min}
2510 than Durban conspecifics; also this difference was still noticeable although not statistically
2511 significant when the same toads were experimentally dehydrated. Cape Town is generally
2512 colder than Durban (Figure 3.1); furthermore the mean July minimum temperature is
2513 notably lower in Cape Town than Durban (7° C and 10.5° C respectively) and this
2514 temperature is known to be a reliable proxy of CT_{min} for toads in the southern Hemisphere
2515 (Kolbe et al. 2010). As CT_{min} and thermal tolerance are highly plastic in amphibian

2516 physiology (Kolbe et al. 2010, McCann et al. 2014), the difference between the two
2517 populations may likely be due to thermal acclimation (i.e. phenotypic plasticity) on a short
2518 time scale. This rapid phenotypic response may be adaptive in Cape Town prolonging the
2519 activity of invasive toads in the coldest months of the year (McCann et al. 2014).
2520 Interestingly CT_{min} recorded in guttural toads and their relationship with the mean July
2521 minimum temperature of Cape Town and Durban are compatible with the values detected
2522 in cane toads *Rhinella marina* invading areas of Australia across a broad latitudinal and
2523 longitudinal range (Table 1 in Kolbe et al. 2010). As the guttural toad inhabits areas
2524 characterized by disparate latitudes and elevations across central and southern Africa,
2525 more studies should be conducted on this species to investigate to what extent its thermal
2526 tolerance is fine-tuned by environmental conditions.

2527 In both the population of Cape Town and Durban, CT_{min} differed only slightly between
2528 hydrated and dehydrated toads (Fig. 6) suggesting that the experimental protocol is robust
2529 to variation in hydration state. Authors showed that estimation of thermal tolerance may
2530 be significantly affected by methodological context such as rate of temperature change
2531 (Terblanche et al. 2007, Chown et al. 2009); also Rezende et al. (2011) noticed that such
2532 change may alter desiccation in insects thus having collateral additive effects on CT_{max}
2533 estimate. Since evaporation proceeds faster at higher temperatures, it would be highly
2534 informative in the future to estimate CT_{max} between hydrated and dehydrated individuals
2535 among populations using a protocol similar to that I used here for CT_{min} .

2536 In summary, I showed that invasive guttural toads underwent an adaptive response that
2537 reduces the difference (i.e. mismatch) between their actual phenotypes and the
2538 phenotypes that “would be best suited in the invaded environment” (Hendry et al. 2011).
2539 Since adaptive responses should rarely occur at no cost (Ghalambor et al., 2007, Hendry
2540 et al. 2011), more studies should be conducted to estimate to what extent the mismatch
2541 reduction detected in Cape Town is detrimental under native environmental conditions.
2542 Individuals of Cape Town for example notably underperformed native conspecifics when
2543 fully hydrated, where my study also showed that full hydration state can be reached
2544 significantly more often in the native area. Alternatively, the release of other selective
2545 pressures in the invasive population (e.g. due to the absence of predators) could have
2546 promoted negative selection toward defensive traits and saved some resources that can
2547 be redirected toward reproduction or dispersal (Burton et al. 2010; Philips et al. 2010). For
2548 example, guttural toads lost some of their parasites (i.e. enemy release) once introduced
2549 from the native source population to Cape Town (Kruger et al. in prep.); estimating
2550 whether and to what extent this parasite release facilitated the occurrence of adaptive
2551 responses in the invaded area could deserve further investigations. I also showed that this

2552 response does not necessarily require long time scales to occur and instead can be
 2553 detected during the initial phase of an invasion. Although more studies are required to
 2554 distinguish between quick genetic-epigenetics adaptation, phenotypic plasticity and
 2555 founder effects/genetic drift, the consequences of this response should not been
 2556 underestimated (Whitney and Gabler 2008). It may allow a species to survive, breed and
 2557 expand into environments theoretically inhospitable and hamper our capacity to predict
 2558 invasion potential and/or adopt management countermeasures.

2559

2560 3.5 References

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2826 **Appendix 3.A.** Effects of population and hydration state on total endurance expressed in
 2827 SVL, total speed expressed in SVL and distance covered in the first ten minute expressed
 2828 in SVL in guttural toads *Sclerophrys gutturalis* from Cape Town and Durban populations.
 2829 Significant differences ($P < 0.05$) are highlighted in bold

Variable	Statistic test	<i>P</i>
Total endurance (SVL)		
Population	$F_{1,46} = 8.34$	$P < 0.01$
Hydration state	$F_{2,46} = 30.48$	$P < 0.0001$
Population : hydration state	$F_{2,46} = 2.03$	$P = 0.143$
Total speed (SVL s ⁻¹)		
Population	$F_{1,46} = 0.058$	$P = 0.81$
Hydration state	$F_{2,46} = 4.376$	$P < 0.05$
Population : hydration state	$F_{2,46} = 1.22$	$P = 0.30$
Distance covered in the first 10 min (SVL)		
Population	$F_{1,46} = 1.32$	$P = 0.257$
Hydration state	$F_{2,46} = 7.45$	$P < 0.01$
Population : hydration state	$F_{2,46} = 1.00$	$P = 0.377$

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2842 **Chapter four. High latitude does not always mean high capital. Unexpected**
2843 **shift toward income-breeding in a subtropical anuran invading higher**
2844 **latitude.**

2845

2846 **4.1 Introduction**

2847 Life-history traits represent differential investments of acquired energy in growth,
2848 survivorship and reproduction that maximize individual fitness (Stearns 1976 and 1977).
2849 Due to allocation trade-offs, organisms should present the combination of traits that
2850 promotes optimal fitness in specific environmental circumstances (Stearns 1976). At an
2851 individual level, energy reserves are an essential driver of fitness maximization because
2852 they allow partially buffering environmental fluctuations to optimize reproduction, growth
2853 and survival (Shine and Brown 2008). Since unfavorable conditions (e.g. of temperature
2854 or food availability) frequently restrict breeding activity to a specific time of the year,
2855 natural and sexual selection should promote the exploitation of previously-stored energy
2856 to fuel reproduction as soon as the environmental conditions are suitable. This could allow
2857 breeding early in the season to maximize individual fitness (Verhulst et al. 2008) or have
2858 sufficient energy reserves to perform demanding activities such as gametogenesis
2859 (Tejedo 1992, Warne et al. 2012), courtship (Abrahams 1993) or combat (Arak 1983) .

2860 When the energy acquired from the environment (i.e. exogenous source) is not
2861 immediately allocated to maintenance or reproductive investment, it constitutes a capital
2862 that can be stored until later stages. Species that use this capital (i.e. endogenous source)
2863 to fuel reproduction have been defined as capital breeders (Drent and Daan 1980, Bonnet
2864 et al. 1998). It differentiates them from those that promptly exploit exogenous energy for
2865 reproduction, conversely known as income breeders. These two categories are not
2866 intended to be absolute, but rather represent the extremes of a continuum of intermediate
2867 strategies (Warne et al. 2008). It follows that each species or population will adopt a
2868 specific strategy along the continuum in accordance to the cost-benefit trade-off resulting
2869 from intrinsic physiological capacity and extrinsic environmental conditions (Houston et al.
2870 2007). In ectoderms, the low cost to store energy (Bonnet et al. 2002, Houston et al. 2007,
2871 Warne et al. 2012) and the role of thermal seasonality in restricting breeding activity
2872 (Warne et al. 2012; Plot et al. 2013), make it particularly advantageous to rely on
2873 previously-stored “capital” (Warne et al. 2012; Plot et al. 2013). However, a conspicuous
2874 array of strategies has been observed at the intraspecific level, with populations
2875 characterized by shorter breeding seasons and/or exposed to lower temperatures (e.g. for
2876 effect of latitude or altitude) that were more capital in fish (McBride et al. 2015), frogs

2877 (Jönsson et al. 2009, Chen et al. 2013), snakes (Gregory 2006) and copepods (Sainmont
2878 et al. 2014).

2879 In terrestrial ectotherms, temperature should be the main driver of energy storage across
2880 latitude (Bonnet et al. 1998). However, the role of other variables such as availability of
2881 water or trophic resources has been understudied. Amphibians represent an excellent
2882 model to fill this gap, given the importance that water has in many aspects of their biology
2883 such as survival, reproduction, behaviour and life-history strategy (Spotila et al. 1992;
2884 Hillman et al. 2009). Although frogs can partially store energy in the form of protein and
2885 carbohydrates, the most important source of stored energy is lipids from fat bodies
2886 (Jönsson et al. 2009, Chen et al. 2011). Fat bodies allow storage and mobilization of lipids
2887 in order to address functions associated with reproduction and maintenance (Girish and
2888 Saidapur 2000, Jackson and Ultsch 2010) and numerous studies have suggested a fine-
2889 tuned response of fat bodies to local environmental conditions (Jorgensens et al. 1986)
2890 and to individual health and reproductive status (Girish and Saidapur 2000). Energy may
2891 also be stored in the liver, as lipids and glycogen, as well as in somatic tissues, where
2892 storage can be at least partially quantified through measurement of lean body mass
2893 (Jönsson et al. 2009). Since energy storage should have consequences on body mass,
2894 authors also proposed that a body condition index correcting for body size may be utilized
2895 as a proxy of energy storage (see scaled mass index in Peig and Green 2010 and
2896 McCracken et al. 2012), although the validity of this assumption across populations of
2897 amphibians has rarely been tested.

2898 Invasive populations, especially those established in environments that differ strongly from
2899 those of the historical or native range, represent an invaluable source of information to
2900 test eco-evolutionary hypotheses (Hierro et al. 2005, Van Kleunen 2010). When the
2901 establishment of such populations is very recent, they may also help to explore whether
2902 new environmental conditions represent a challenge to the species' phenotype before
2903 adaptations reduce eventual mismatches (Brown et al. 2011, Hendry 2011). It is
2904 reasonable to expect that when a frog is introduced to a more severe environment, its
2905 energy storage strategy should shift toward capital-breeding (Ejsmond et al. 2015): this
2906 because the cost of building energy storage is counter-balanced by the benefit of using
2907 that supplementary source of energy during or after periods of restricted activity (non-
2908 breeding season; Varpe et al. 2009). Notably, Brown et al. (2011) observed that cane
2909 toads *Rhinella marina* invading subtropical Australia, which is significantly more seasonal
2910 than the native range, showed a capital breeding strategy, with fat bodies heaviest during
2911 the wet-dry transition and lightest during the dry-wet transition.

2912 In this chapter, I investigate post-breeding energy storage in a generalist African anuran,
2913 the guttural toad *Sclerophrys gutturalis*. This species naturally inhabits summer-rainfall
2914 areas of central and southern Africa characterized by tropical and subtropical climates and
2915 its reproductive timing mostly mirrors precipitation patterns. Guttural toads were
2916 introduced to Mauritius and Reunion islands about one hundred years ago (Cheke 2010)
2917 and, more recently, a peri-urban area of Cape Town, in South Africa's Western Cape
2918 Province (De Villiers 2006). The founders of these populations, all currently invasive, are
2919 known to come from KwaZulu-Natal, South Africa, by genetics analyses (Telford 2015).
2920 Therefore, the guttural toad has moved to latitudes significantly lower (Mauritius and
2921 Reunion) and higher (Cape Town) than its point of origin. Since Cape Town is
2922 characterized by a mediterranean (i.e. winter-rainfall) climate (see chapter three), the
2923 invasive population also successfully established despite a seasonal pattern of water
2924 availability notably different from that of the native distribution.

2925 I targeted all three invasive populations and a native population from Durban, KwaZulu-
2926 Natal (see chapter three). This native population was chosen because it inhabits a low
2927 altitude human-modified habitat similar to those of Cape Town, Mauritius and Reunion,
2928 and it is thought to be close to the original sources of each invasive population (Telford
2929 2015). This allowed me to compare populations occurring in areas that differ in latitude
2930 and seasonal rainfall pattern but not in habitat.

2931 Assuming that reproductive strategy should shift from income towards capital breeding
2932 when the severity of the non-breeding season increases, I examined how post-breeding
2933 energy storage varies when the species is introduced at lower and at higher latitudes. I
2934 additionally asked to what extent energy storage patterns agree: i) between sexes; ii)
2935 among different organs functionally involved in energy storage (fat bodies, liver, lean body
2936 mass); and iii) with the scaled mass index. In accordance with what was observed for
2937 temperate species, I expected the populations at lower latitudes adopt an income
2938 breeding strategy (i.e. lower energy storage) and the population at higher latitude (Cape
2939 Town) to adopt capital breeding (i.e. higher energy storage) while I expected the native
2940 population (Durban) to be intermediate. I also expected a positive correlation between
2941 pairs of energy storage organs and between these organs and the scaled mass index
2942 because animals should use all the structures available to achieve storage capacity.

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2944 **4.2 Materials and methods**

2945 **4.2.1 Study species**

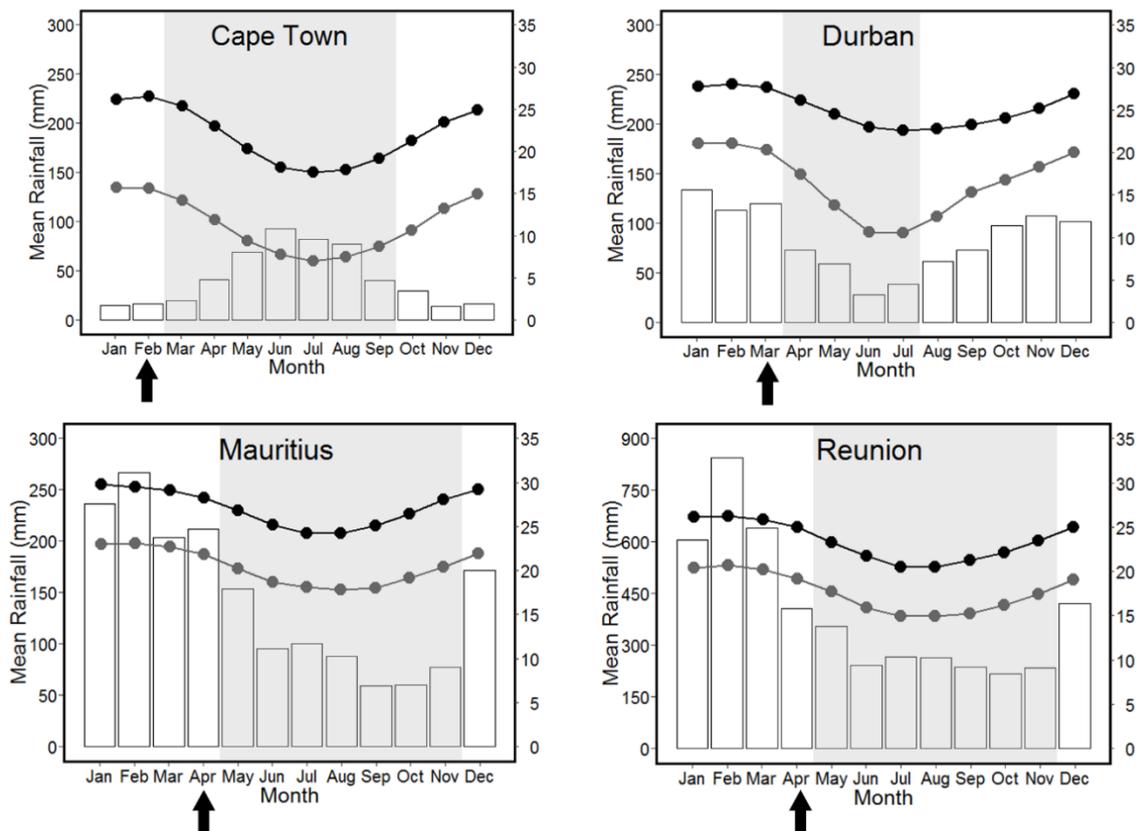
2946 The guttural toad *Sclerophrys gutturalis* is a common African bufonid naturally distributed
2947 across central and southern Africa (du Preez et al. 2004). The species is tolerant of
2948 different altitudes (from sea-level to about 1800 m) and latitudes (from the equator to 30°
2949 S). It inhabits a range of vegetation types in the Savannah, Grassland and Thicket biomes
2950 (du Preez et al. 2004) and due to a highly synanthropic behaviour, it is not uncommon to
2951 find these toads in peri-urban areas. The guttural toad became invasive in Mauritius
2952 (1922) and Reunion (1927) islands where it was introduced from KwaZulu-Natal South
2953 Africa (Telford 2015) as a biological control agent against mosquitoes (Cheke 2010). The
2954 species is also a domestic exotic in South Africa (Measey and Davies 2011) as it is native
2955 in most of the country but not in the Western Cape, where an invasive population has
2956 established in Cape Town since the end of the 1990s (De Villiers 2006). It was likely
2957 introduced as eggs or tadpoles with a consignment of aquatic plants (De Villiers 2006)
2958 from KwaZulu-Natal (Telford 2015).

2959 **4.2.2 Study localities and climate**

2960 I selected one native and three invasive populations for this study. The native population
2961 inhabits a peri-urban area of Durban, South Africa (75 m a.s.l., 29°47'16"S, 31°01'46"E)
2962 where the species breeds from August to March (Figure 4.1). The climate is classified as
2963 humid-subtropical, being characterized by hot-humid summers and mild-dry winters
2964 (Figure 4.1). The two invasive insular populations were sampled in a peri-urban area of
2965 Mahebourg, Mauritius (20 m a.s.l. 20°25'11"S, 57°42'07"E) and an agricultural area of
2966 Saint-Benoît, Reunion (518 m a.s.l., 21°05'48"S, 55°39'16"E). Since both these insular
2967 populations are located on the eastern side of the islands, they are subjected to a tropical
2968 climate with hot-humid summers and relatively mild, dry winters (Figure 4.1). However, the
2969 mid-altitude site of Reunion has about three times more rainfall than the low-altitude site
2970 of Mauritius (Figure 4.1). Since in Reunion the sampling site was located at an altitude of
2971 about 500 m, I chose the closest weather station located at approximately the same
2972 altitude (Bellevue Bras Panon uplands, 480 m a.s.l., 8 km from the sampling locality). In
2973 both the populations of Mauritius and Reunion the species breeds from December to April
2974 (Figure 4.1). The South African invasive population inhabits a peri-urban area of Cape
2975 Town (South Africa, 87 m a.s.l., 34° 0'53"S, 18°25'50"E) where the species breeds from
2976 October to February. The climate is classified as mediterranean, being characterized by
2977 hot-dry summers and mild-wet winters (Figure 4.1).

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2981 **Figure 4.1:** Mean monthly rainfall (bars), maximum temperature (black dots and line) and
 2982 minimum temperature (grey dots and line) at the four locations of Cape Town, Durban,
 2983 Mauritius and Reunion where guttural toads *Sclerophrys gutturalis* were sampled. For
 2984 each location, black arrow represents sampling period and shaded area represents the
 2985 non-breeding season of the guttural toad. Note that the Reunion location has
 2986 approximately three times more rainfall than that of Mauritius. Climate data sourced from:
 2987 the World Meteorological Organization, <http://public.wmo.int/>, for Cape Town and Durban;
 2988 the Mauritius Meteorological Services, <http://metervice.intnet.mu/>, for Mauritius; from
 2989 Météo-France, <http://www.meteofrance.com/>, for Reunion.

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2991 4.2.3 Data collection

2992 Adult toads were collected in each locality at the end of the breeding season. At each
 2993 sampling site, individuals were opportunistically captured by hand after sunset within an
 2994 area of approximately 10 km². Each toad was euthanized straight after capture by
 2995 immersion in a 1 gl⁻¹ solution of tricaine methane sulfonate (MS222) for twenty minutes,

2996 and then frozen in labelled plastic bags until dissection. Ethics clearance was obtained
 2997 from Stellenbosch University Animal Ethics Committee (Protocol Number U-ACUD14-
 2998 00112) and collections in the native area (Durban) occurred under permission from KZN
 2999 Wildlife (Permit Number OP553/2015). In the laboratory, after defrosting each specimen at
 3000 ambient temperature, all animals were weighed using a balance (± 0.01 g, FA 304 T,
 3001 Avery Berkel) and their SVL (snout to vent length, i.e. the straight-line distance from the
 3002 posterior cloacal margin to the snout tip) was measured using a digital caliper (± 0.01 mm).
 3003 The mass of fat bodies, liver and gonads was weighed (± 0.001 g, FA 304 T, Avery Berkel)
 3004 after dissection of each organ; tissues were patted dry with a paper tissue before
 3005 weighing. Lastly, individuals were fully eviscerated and weighed to obtain lean body mass.

3006 **4.2.4 Data analysis**

3007 Since body organs scale allometrically with body size and mass (Gould 1966) and
 3008 preliminary analyses showed high body size variability among the target populations
 3009 (Appendix 4), I calculated scaled mass index for fat bodies, liver and lean body mass
 3010 following the equation proposed by Peig and Green (2009):

$$3011 \quad M = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}} \quad (1)$$

3012 where M_i and L_i represent respectively organ mass and body length (SVL) of the individual
 3013 i and L_0 represents an arbitrary value (e.g. average SVL within population). I calculated L_0
 3014 from SVL of all individuals collected in the four populations ($L_0 = 66.50$ mm, $n = 156$). The
 3015 exponent b_{SMA} represents the slope of the standardised major axis (SMA) regression on
 3016 \ln -transformed mass and length and was calculated through *lmodel2* package in R
 3017 (Legendre 2014; R Development Core Team 2014) using data from all populations
 3018 separately for each organ (Table 4.1; $N = 153$). Since some individuals showed no fat
 3019 bodies, I removed them from analysis to estimate the exponent b_{SMA} in this organ.

3020 Since in females presence of eggs at different stages of development determines
 3021 supplementary variability in body mass measurements and prevents effective comparison
 3022 between sexes (Withers et al. 2001), I obtained the gonad free mass (BM_{gf}) for each
 3023 individual and then I used the equation of Peig and Green (2009) with BM_{gf} as M_i to
 3024 calculate the scaled mass index (SMI).

3025 Scaled organ mass for fat bodies, liver and lean body and the scaled mass index were
 3026 analysed through ANOVA to explore the effect of population and sex; Tukey's post hoc
 3027 tests were used to explore pairwise differences between populations (Table 1). I
 3028 performed Kruskal-Wallis tests instead of ANOVA where the assumptions of normality

3029 (evaluated through Kolomogorov-Smirnov test) and homoscedasticity (through Levene's
3030 test) were violated.

3031 Lastly, Spearman's rank correlation test was used to investigate the relationship between
3032 organs involved in energy storage and the scaled mass index considering all individuals.
3033 In order to explore a possible population effect, a non-parametric ANCOVA was also
3034 performed through the *SM* package in R (Bowman and Azzalini 2005) with population as a
3035 factor; where a significant effect was detected, the Spearman's rank correlation test was
3036 repeated separately for each population.

3037 All analyses and visualizations were all performed using R version 3.1.2 (R Development
3038 Core Team 2014).

3039 **4.3 Results**

3040 ***4.3.1 Does post-breeding energy storage increase from lower to higher latitudes?***

3041 Contrary to my expectations, post-breeding energy storage expressed by fat bodies and
3042 liver mass was higher at lower latitude and lower at higher latitude. Cape Town individuals
3043 had scaled fat bodies masses significantly lighter than those from Durban, Mauritius and
3044 Reunion (Table 4.1 and Figure 4.2(a)). No significant difference in mass of fat bodies was
3045 detected between Durban, Mauritius and Reunion, although individuals from Reunion had
3046 notably, but not significantly, heavier fat bodies (Table 4.1 and Figure 4.2(a)). Similarly
3047 Reunion individuals had liver mass significantly heavier than those from Durban, Mauritius
3048 and Cape Town, which did not significantly differ from each other. The Cape Town
3049 population had the lowest liver mass detected in my study (Table 4.1 and Figure 4.2(b)).
3050 Masses of fat bodies and liver did not differ between males and females across the study
3051 (Table 4.1, Figure 4.2(a,b)).

3052 Consistent with my expectations, scaled lean body mass increased from lower to higher
3053 latitude, with Cape Town toads being significantly heavier than those from Mauritius and
3054 Reunion but not significantly different from Durban toads (Table 4.1, Figure 4.2(c)). Scaled
3055 mass index was quite homogeneous across the populations, where only the individuals
3056 from Cape Town had a significantly heavier mass than those from Mauritius (Table 4.1,
3057 Figure 4.2 (d)). Lean body mass and scaled mass index differed between sexes, with
3058 males being heavier than females (Table 4.1, Figure 4.2 (c,d)).

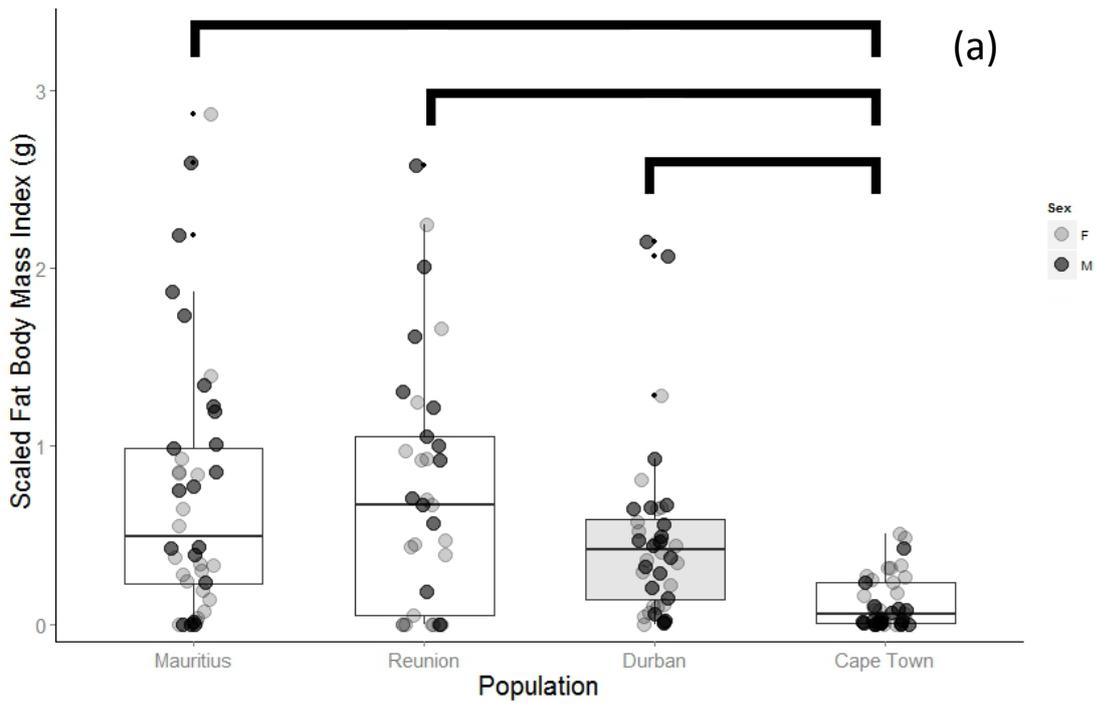
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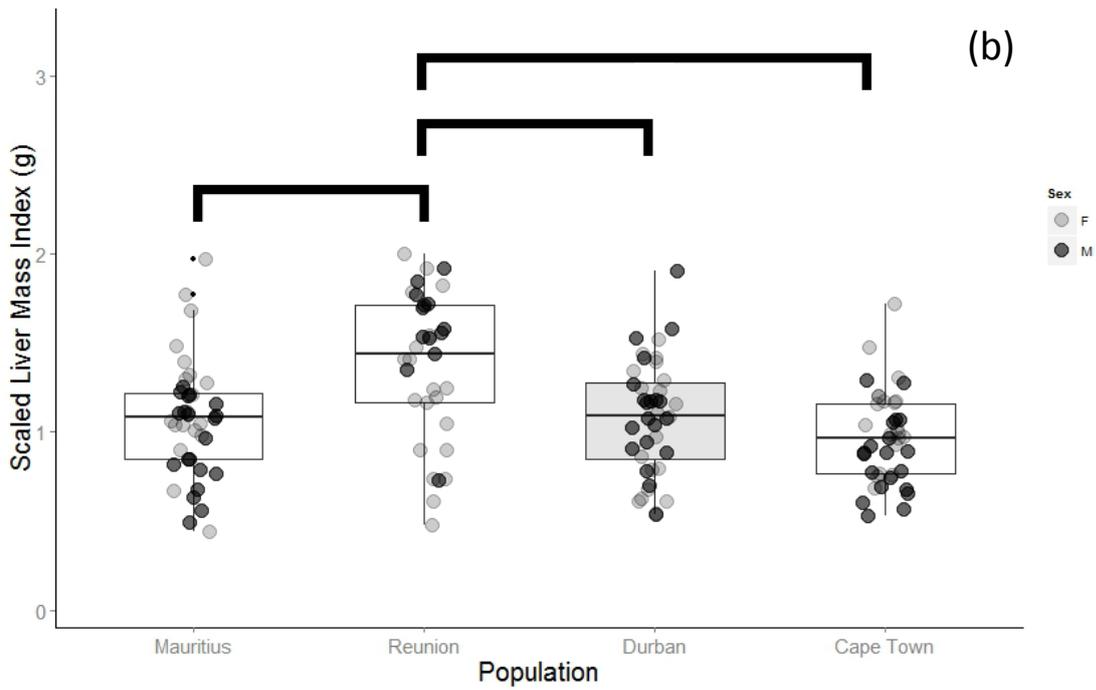
3061 **Table 4.1:** Exponent b_{SMA} estimated for each organ and results of ANOVA (or Kruskal-
 3062 Wallis) on scaled organ mass and scaled mass index obtained from guttural toads
 3063 *Sceloporus gutturalis* in Cape Town, Durban, Mauritius and Reunion populations. Results
 3064 of Tukey post hoc multiple test comparing the populations are also given. Significant
 3065 differences ($P < 0.05$) are highlighted in bold.

Organ	b_{SMA}	Statistic test	Tukey's post hoc test	
Fat Bodies	7.501	Population	Durban : Cape Town	P < 0.001
			Durban : Mauritius	P = 0.781
			Durban : Reunion	P = 0.838
			Cape Town : Mauritius	P < 0.0001
		Sex	Cape Town : Reunion	P < 0.0001
			Mauritius : Reunion	P = 0.999
		$X^2_3 = 30.5$		
		P < 0.001		
Liver	3.647	Population	Durban : Cape Town	P = 0.404
			Durban : Mauritius	P = 0.998
			Durban : Reunion	P < 0.01
			Cape Town : Mauritius	P = 0.503
		Sex	Cape Town : Reunion	P < 0.0001
			Mauritius : Reunion	P < 0.001
		$F_{3,148} = 9.58$		
		P < 0.001		
Lean Body (evisc)	3.282	Population	Durban : Cape Town	P = 0.057
			Durban : Mauritius	P = 0.156
			Durban : Reunion	P = 0.815
			Cape Town : Mauritius	P < 0.0001
		Sex	Cape Town : Reunion	P < 0.01
			Mauritius : Reunion	P = 0.678
		$F_{3,148} = 7.72$		
		P < 0.0001		
Scaled Mass Index (Gonad free Body Mass)	3.28	Population	Durban : Cape Town	P = 0.155
			Durban : Mauritius	P = 0.760
			Durban : Reunion	P = 0.962
			Cape Town : Mauritius	P < 0.01
		Sex	Cape Town : Reunion	P = 0.430
			Mauritius : Reunion	P = 0.489
		$F_{3,148} = 3.33$		
		P < 0.05		
		$F_{1,148} = 6.92$		
		P < 0.01		

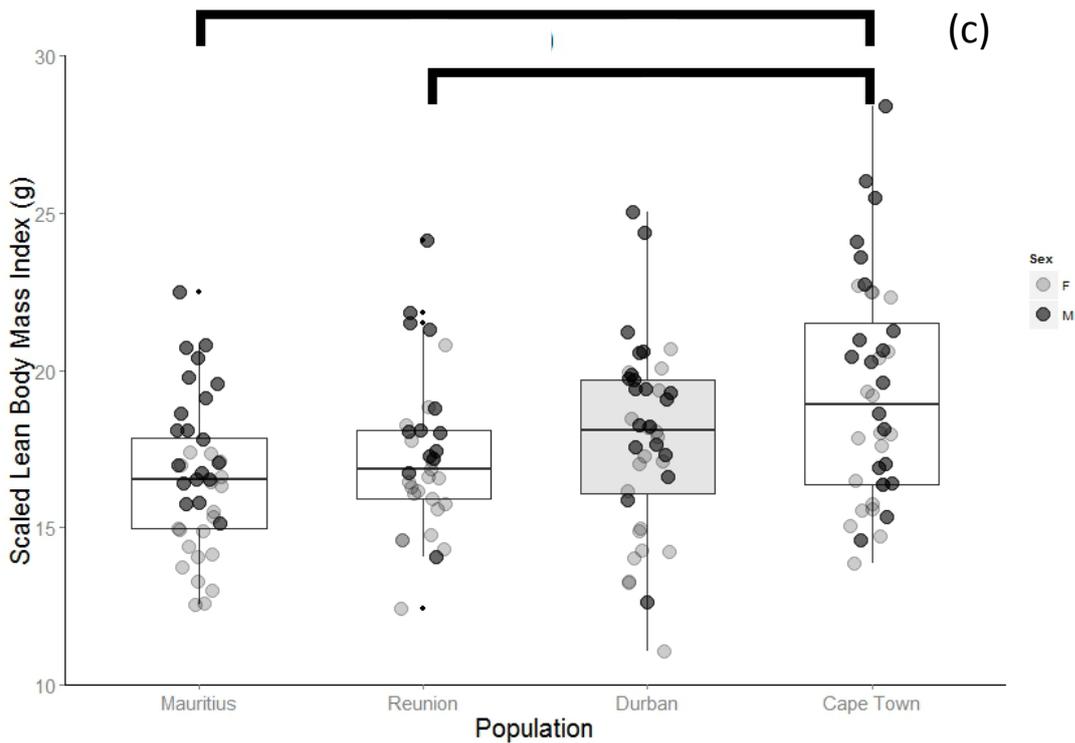
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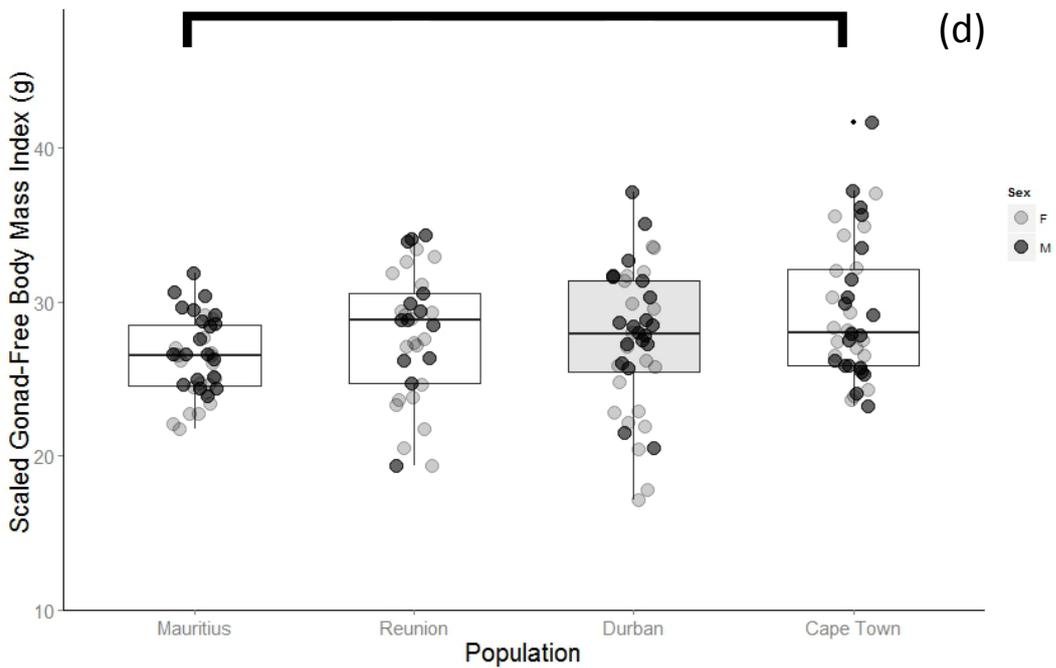


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3072 **Figure 4.2:** Scaled mass for fat bodies (a), liver (b), lean body (c) and scaled mass index
 3073 (d) obtained from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban, Mauritius
 3074 and Reunion populations. Boxes represent means and standard deviations (\pm SD);
 3075 whiskers extend to maxima and minima; dark and pale dots represent males and females.
 3076 Brackets indicate significant differences between populations as identified by Tukey's
 3077 post-hoc test (Table 4.1). Note that the populations are ordered in terms of latitude from
 3078 North to South.

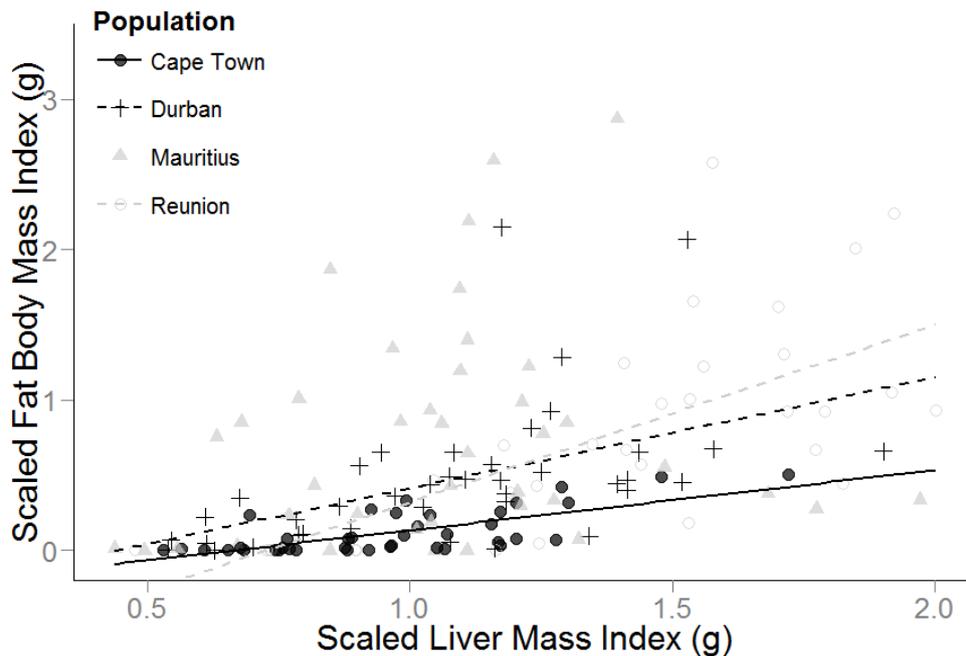
3079 **4.3.2 Does post-breeding energy storage agree between different organs?**

3080 Post-breeding energy storage co-varied between fat bodies, liver and lean body mass at
 3081 individual level, with fat bodies and liver that had the highest positive correlation (Table
 3082 4.2). However the relationship between body structures varied significantly among
 3083 populations (Table 4.2); I observed that the scaled mass of fat bodies grew faster than the
 3084 scaled mass of liver and scaled lean body mass moving from higher to lower latitudes (i.e.
 3085 populations of Mauritius and Reunion invest proportionally more in fat bodies than those
 3086 of Durban and Cape Town; see Figure 4.3 for fat bodies over liver).

3087 **Table 4.2:** Relationship between pairs of energy storage organs (fat bodies, liver and lean
 3088 body mass) obtained from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban,
 3089 Mauritius and Reunion populations and expressed through Spearman correlation
 3090 coefficients (ρ); effect of population on this relationship estimated through non-
 3091 parametric ANCOVA is also reported. Significant differences ($P < 0.05$) are highlighted in
 3092 bold.

	Liver		Lean Body	
	$\rho = 0.63$	Cape Town	$\rho = 0.21$	Cape Town
	$P < 0.0001$	$\rho = 0.71$	$P < 0.01$	$\rho = -0.01$
		$P < 0.0001$		$P = 0.99$
	Population effect	Durban	Population effect	Durban
	(non-parametric	$\rho = 0.67$	(non-parametric	$\rho = 0.43$
	ANCOVA)	$P < 0.0001$	ANCOVA)	$P < 0.01$
Fat Bodies	$h = 0.125$	Mauritius	$h = 1.121$	Mauritius
	$P < 0.001$	$\rho = 0.25$	$P < 0.0001$	$\rho = 0.52$
		$P = 0.127$		$P < 0.001$
		Reunion		Reunion
		$\rho = 0.78$		$\rho = 0.58$
		$P < 0.0001$		$P < 0.001$
	$\rho = 0.21$	Cape Town		
	$P < 0.01$	$\rho = 0.18$		
		$P = 0.27$		
	Population effect	Durban		
	(non-parametric	$\rho = 0.46$		
	ANCOVA)	$P < 0.01$		
Lean Body	$h = 1.121$	Mauritius		
	$P < 0.001$	$\rho = 0.04$		
		$P = 0.81$		
		Reunion		
		$\rho = 0.59$		
		$P < 0.001$		

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3096 **Figure 4.3:** Relationship between scaled fat body mass and scaled liver mass obtained
 3097 from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban, Mauritius and Reunion
 3098 populations. The linear regression is depicted separately for each population when a
 3099 significant correlation between the two variables was detected (Table 4.2).

3100

3101 **4.3.3 Does post-breeding energy storage agree with the scaled mass index?**

3102 The scaled mass index predicted post-breeding energy storage expressed by fat bodies,
 3103 liver and lean body mass; the highest positive correlation was detected between scaled
 3104 mass index and lean body mass (Table 4.3). However the relationship between scaled
 3105 mass index and organs varied significantly among populations (Table 4.3); I observed that
 3106 the scaled mass of fat bodies and liver grew faster over the scaled mass index moving
 3107 from higher to lower latitudes (i.e. populations of Mauritius and Reunion invest
 3108 proportionally more in fat bodies and liver than those of Durban and Cape Town) (see
 3109 Figure 4.4 for fat bodies over scaled mass index).

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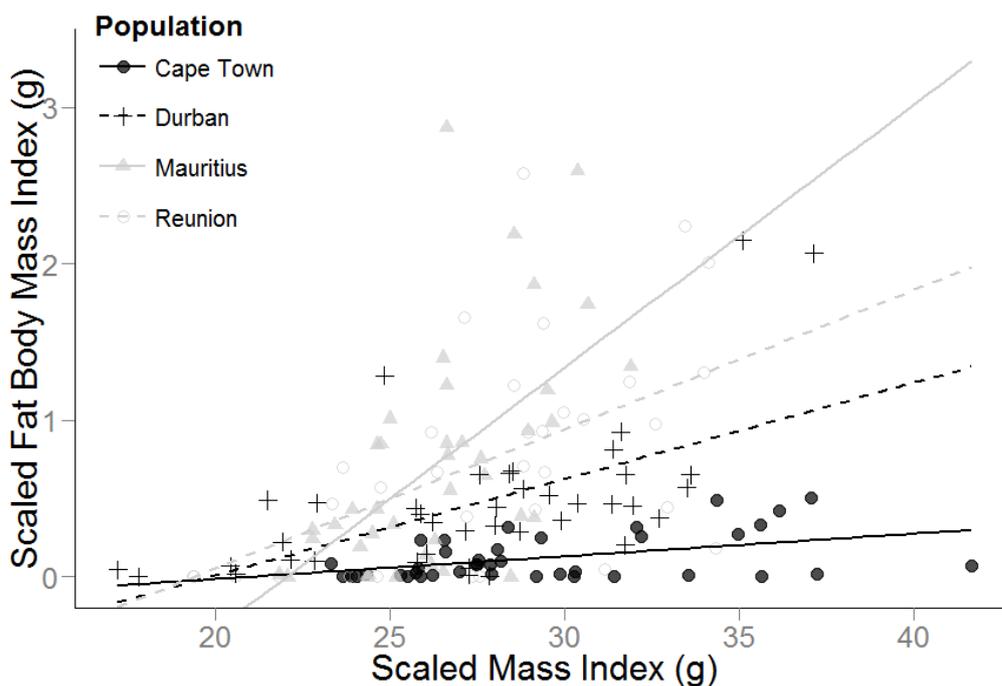
3113

3114 **Table 4.3:** Relationship between pairs of energy storage organs (fat bodies, liver and lean
 3115 body mass) and scaled mass index obtained from guttural toads *Sclerophrys gutturalis* in
 3116 Cape Town, Durban, Mauritius and Reunion populations and expressed through
 3117 Spearman correlation coefficients (ρ); effect of population on this relationship estimated
 3118 through non-parametric ANCOVA is also reported. Significant differences ($P < 0.05$) are
 3119 highlighted in bold.

		Scaled Mass Index	
Fat Bodies		$\rho = 0.39$ $P < 0.0001$	Cape Town $\rho = 0.41$, $P < 0.01$
			Durban
	Population effect (non-parametric ANCOVA)		$\rho = 0.61$, $P < 0.0001$
			Mauritius
			$\rho = 0.62$, $P < 0.0001$
			Reunion
		$h = 0.136$ $P < 0.0001$	$\rho = 0.57$, $P < 0.001$
Liver		$\rho = 0.46$ $P < 0.0001$	Cape Town $\rho = 0.58$, $P < 0.001$
			Durban
	Population effect (non-parametric ANCOVA)		$\rho = 0.56$, $P < 0.0001$
			Mauritius
			$\rho = 0.183$, $P = 0.256$
			Reunion
		$h = 0.136$ $P < 0.0001$	$\rho = 0.66$, $P < 0.0001$
Lean Body		$\rho = 0.81$ $P < 0.0001$	Cape Town $\rho = 0.83$, $P < 0.0001$
			Durban
	Population effect (non-parametric ANCOVA)		$\rho = 0.87$, $P < 0.0001$
			Mauritius
			$\rho = 0.74$, $P < 0.0001$
			Reunion
		$h = 1.35$ $P < 0.05$	$\rho = 0.72$, $P < 0.0001$

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3122

3123 **Figure 4.4:** Relationship between scaled fat body mass and scaled mass index (SMI)
 3124 obtained from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban, Mauritius and
 3125 Reunion populations. The linear regression is depicted separately for each population
 3126 when a significant correlation between the two variables was detected (Table 4.3).

3127

3128 4.4 Discussion

3129 My study shows that guttural toads at the onset of the non-breeding season stored less
 3130 energy at high latitude and more energy at low latitude; once introduced into novel
 3131 environmental contexts, the species shifted its energy storage strategy toward income
 3132 breeding from lower to higher latitude. It contradicts what was observed in temperate
 3133 species of amphibians, where high and low latitude promoted respectively capital and
 3134 income breeding strategies. I propose that environmental variables such as availability of
 3135 water and food resources may affect energy storage to a greater extent than temperature
 3136 in tropical-subtropical species and environments.

3137 The Cape Town guttural toad population showed the most extreme income breeding
 3138 strategy (i.e. it had the lowest post-breeding energy storage in term of scaled mass of fat
 3139 bodies and liver) despite inhabiting the coldest and driest location of the study (Figure
 3140 4.1). Although this climate should theoretically determine a more severe post-breeding
 3141 season when compared to Durban, Mauritius and Reunion, some indications suggest that
 3142 it may not be the case. Guttural toads from Cape Town have a very low critical thermal
 3143 minimum (about 7 °C, see chapter three) and are physiologically less sensitive to
 3144 dehydration than native conspecifics (see chapter three). Furthermore, in Cape Town the

3145 reproductive season is followed by a period characterized by increasing rainfall, which
3146 should promote, and not suppress, toad activity. Lastly, seasonal fluctuations on insect
3147 biomass in fynbos habitats (Cape Town area) are much lower than those of subtropical
3148 habitats (Schlettwein 1984) and some authors even showed that in the Fynbos, arthropod
3149 abundance associated to endemic plants is significantly higher in the cold-wetter autumn
3150 (March-May) and winter (June-August) (Coetzee 1989; Roets et al. 2006). The wet-cold
3151 winters of Cape Town may suppress reproduction but not activity, with invasive toads still
3152 able to forage and grow during at least the first part of the non-breeding period (Figure
3153 4.1). Testing this hypothesis in the field or investigating its eventual consequences for
3154 growth trajectories seem ripe for further investigations. Also, investigating yearly
3155 fluctuations on arthropod abundance between the invaded area of Cape Town and the
3156 native area of Durban (e.g., using pitfall-traps) could help to elucidate to what extent food
3157 availability may affect energy storage in the guttural toad.

3158 Conversely, the breeding period is followed by decreasing rainfall in the populations of
3159 Mauritius, Reunion and, to a lesser extent, Durban; it triggers a capital strategy at these
3160 locations to overwinter and fuel the reproduction in the following spring (Stephens et al.
3161 2014). Firstly, the wet-dry climate transition occurring at the end of the reproductive
3162 season should cause a lower availability of arthropods and other invertebrates (Lowman
3163 1982, Frost 1987, Frith and Frith 1985, Kai and Corlett 2002), reducing the food intake for
3164 the toads. Secondly, both activity and locomotion of amphibians are physiologically
3165 constrained by water availability (Hillman 1987, Titon et al. 2010) and in chapter three I
3166 showed that the negative effect of dehydration on locomotion is more severe in Durban
3167 toads than in Cape Town conspecifics. Reduced winter activity of both toads and their
3168 prey due to more severe conditions of temperature and water availability promotes a
3169 capital breeding strategy through which individuals allocate energy acquired in fat bodies
3170 and liver. Intriguingly this strategy is also notable in Durban despite the short non-
3171 breeding season; it suggests a strong selective pressure toward storing energy as a
3172 response to increasing environmental severity (Stephens et al. 2014).

3173 Additionally, my work detected a supplementary role of liver in storing energy and
3174 confirms effectiveness to use the scaled mass index, but not the lean body mass, as a
3175 proxy of energy storage in amphibians.

3176 The high positive correlation between fat bodies and liver (Table 4.2, Figure 4.3) at the
3177 individual level partially mirrored the concordance I detected in these organs at population
3178 level (Figure 4.2(a) vs. Figure 4.2(b)), where Reunion showed the heaviest scaled organ
3179 masses and Cape Town the lightest ones. Although it confirms the additional role of liver
3180 to store lipids observed in temperate species of anurans (Lu et al. 2008, Jönsson 2009,

3181 Chen et al. 2011 and 2013), I advise caution to interpret such intraspecific variation as
3182 exclusively due to differential energy storage patterns (Jönsson et al 2009, Lu et al. 2008,
3183 Chen et al. 2011). Liver has multiple storage functions in amphibian physiology being a
3184 source of glycogen (Christiansen and Penney, 1973, Fournier and Guderley 1993), blood
3185 (Frangioniand and Borgioli 1994) and proteins (Whiters et al. 2001); moreover its
3186 composition of organic macromolecules is influenced by multiple environmental variables
3187 such as anoxia (Jackson and Ultsch 2010) and soil water potential (McClanahan 1972).
3188 In this sense Whiters et al. (2001) suggested that at least at interspecific level, liver mass
3189 may be correlated to body shape, habitat and environmental variables (e.g. maximum
3190 rainfall). The heavy liver mass observed in Reunion (Figure 4.2(b)) may also have
3191 alternative adaptive functions rather than being involved in energy storage; for instance it
3192 could facilitate ureagenesis and burrowing in a wetter and more seasonal environment
3193 (Withers et al. 2001). I call for more studies aiming to compare mass and composition of
3194 liver at intraspecific level and suggest this could be performed especially by sampling
3195 invasive populations; from a conservation perspective the removal of their individuals
3196 should not have any negative consequence on the surrounding environment.

3197 A weak relationship between energy storage organs and lean body mass at individual and
3198 population level was detected in other studies (Jönsson et al. 2009) and may be linked to
3199 a multiplicity of factors that affect lean body mass measurement in amphibians. Assuming
3200 mobilization of lipids to muscles in order to fuel locomotion and activity, a higher lean body
3201 mass in more income-breeding populations is expected (Jonson et al. 2009, Chen et al.
3202 2013) and it agrees with my observations in Cape Town (Figure 4.2(c)). However, masses
3203 of other organs such as muscles or skeleton contribute to lean body mass and they can
3204 be subject to sexual selection, thus partially impeding effective comparison across
3205 different populations. My study, for example, shows that lean body mass of males was
3206 significantly heavier than that of females (Table 4.1 and Figure 4.2(c)), a difference that
3207 may be explained in light of sexual selection favouring males with a heavier skeleton and
3208 muscles (Jönsson et al. 2009). Breeding males compete indirectly to grasp and hold
3209 females or directly through intrasexual agonistic behaviour (Shine 1979, Woodward 1982,
3210 Sullivan 1992); additionally the female choice should be directed toward mates showing
3211 costly displays (Sullivan and Kwiatkowski, 2007). Given these confounding factors, I
3212 suggest that the differences I detected among populations in terms of lean body mass are
3213 less valuable to get insights on energy storage.

3214 Lastly, in my study the scaled mass index predicted energy storage among individuals
3215 (Table 4.3 and Figure 4.4) as previously observed in small mammals, birds and snakes
3216 (Peig and Green 2009). However the lack of a clear correspondence between fat bodies

3217 and scaled mass index at population level (Figure 4.2(a) vs. Figure 4.2(d)) implies that in
3218 anurans this index alone may not be sufficient to estimate energy storage among
3219 populations; other organs such as lean body could indeed mask energy patterns (see
3220 previous paragraph). Also my findings indicate it would be preferable, when data on fat
3221 bodies and liver are available, to always apply the scaling method (eq. 1) as suggested by
3222 Peig and Gren (2009) not only on body mass but also on organ masses (McCracken et al.
3223 2012). Correcting for the allometric relationship between mass and body size is
3224 particularly important to effectively compare organs between sexes and among
3225 populations that strongly differ in size (Peig and Green 2010) as in this study (Appendix
3226 4).

3227 In my studies I exclusively focused on post-breeding energy storage; however future
3228 studies regarding energy reserves in the guttural toad should additionally investigate this
3229 life-history trait at the onset of the breeding season. Scattered data on fat bodies and liver
3230 of guttural toads collected in Cape Town (n=15) and Durban (n=10) at the beginning of the
3231 breeding season show that energy storage does not differ between the two populations;
3232 however the small sample size and the absence of data on pre-breeding energy storage
3233 from Mauritius and Reunion does not allow a direct comparison among the four
3234 populations. Minor or no differences linked to energy storage are expected among
3235 populations at the onset of the breeding season because most of the resources should
3236 have been depleted during inactivity (Jönsson et al. 2009, Chen et al. 2013). However, I
3237 suggest that only an extensive sampling effort performed also at the onset of the breeding
3238 season will help to clarify how much energy is stored and subsequently consumed in
3239 populations that adopt a capital-breeding strategy (e.g. Durban).

3240 Invasive populations are frequently investigated to quantify their capacity to establish and
3241 thrive in areas considered more severe from those of their native range (Brown et al.
3242 2011). My study however shows that during the non-breeding season invasive guttural
3243 toads from Cape Town are exposed to environmental conditions less severe than those
3244 they encounter in the native source population of Durban. The cold-wet winters of Cape
3245 Town represent an opportunity rather than a limitation to allocate energy to growth instead
3246 of reproduction, where the availability of permanent artificial water bodies allows the
3247 guttural toad to breed in the hot-dry summers (see chapter one). The species was
3248 behaviourally and physiologically pre-adapted to cope with the novel environmental
3249 conditions where its establishment and spread were partially facilitated by the peri-urban
3250 area it invaded. Conversely chapter three showed that during the breeding season, the
3251 environment Cape Town is more desiccating (i.e. more severe) than that of Durban, and
3252 that this likely promoted adaptive responses minimizing phenotypic mismatch.

3253 Environmental conditions such as temperature, moisture and food availability may thus
3254 cause selective pressures that act differentially not only across functional traits but also
3255 across seasons; the lack of approaches integrating physiological and life-history traits and
3256 exploring their fitness contributions can hamper our capacity to explore adaptive evolution.
3257 In Cape Town, for example, selective pressures exerted by the desiccating environment
3258 should act only during the breeding season where their effect should be mostly neutral
3259 during the non-breeding season. Invasive toads are exposed to an environment that is
3260 likely less severe than the native area in terms of moisture and food availability; this could
3261 significantly counterbalance the reduction of activity expected in an ectothermic species
3262 introduced from a sub-tropical (e.g. Durban) to a temperate (Cape Town) area.
3263 Additionally the lower CT_{min} detected in Cape Town (see chapter three) shows that the
3264 invasive population should prolong activity at lower thermal conditions than the native
3265 source population; this should simultaneously shorten the inactivity period of the species
3266 and favour a more income breeding strategy. In light of my findings, two aspects seem
3267 particularly profitable for future investigations. Most of the frogs (toads included) inhabiting
3268 the Cape Town area breed in winter; exploring whether they adopt capital breeding (e.g. t
3269 withstand dry summers) or rather income breeding is common in such environment could
3270 provide important insights on how they adapted to a winter rainfall climate regime.
3271 Furthermore, at a global scale, other invasive amphibian populations established in
3272 environments characterized by seasonal water availability patterns notably different from
3273 those of the native distribution. Examples are the American Bullfrog *Lithobates*
3274 *catesbeianus* introduced in the tropics, the Japanese wrinkled frog *Glandirana rugosa*
3275 introduced in Hawaii and the Mediterranean painted frog *Discoglossus pictus* introduced
3276 in France and Spain (Lever 2003, Kraus 2009). These populations may provide insights
3277 about how acquired energy is invested in growth, survivorship and reproduction according
3278 to environmental specific circumstances.

3279

3280 4.5 References

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Appendix 4: Body size (SVL), body mass, scaled mass index (SMI), organ masses (% of body mass) and scaled mass for each organ obtained from guttural toads *Sclerophrys gutturalis* in Cape Town, Durban, Mauritius and Reunion. Data are presented separately for each population and sex.

Population	Sex	n	SVL (mm)	BM _{gf} (g)	SMI (g)	Liver mass (%)	Scaled Liver Mass Index (g)	Fat Bodies Mass (%)	Scaled Fat Bodies Index (g)	Lean Body Mass (%)	Scaled Lean Body Mass (g)
Cape Town	F	20	89.57±1.80	77.54±3.87	29.15±0.89	4.095±0.171	1.072±0.057	1.891±0.295	0.185±0.035	0.625±0.009	18.218±0.637
	M	20	72.51±1.49	38.86±1.87	29.50±1.12	2.998±0.124	0.86±0.048	0.295±0.106	0.056±0.023	0.690±0.013	20.335±0.852
Durban	F	20	79.03±1.70	46.94±3.38	26.23±1.12	4.176±0.223	1.036±0.072	2.834±0.482	0.395±0.07	0.635±0.012	16.517±0.594
	M	20	68.39±1.40	31.75±1.81	28.78±0.89	3.979±0.251	1.127±0.071	1.940±0.365	0.549±0.132	0.665±0.012	19.124±0.61
Mauritius	F	20	61.16±1.29	19.64±1.17	25.36±0.48	4.535±0.291	1.191±0.08	1.275±0.216	0.563±0.147	0.595±0.010	15.084±0.357
	M	20	49.01±1.38	10.47±0.95	27.40±0.54	3.072±0.160	0.947±0.054	0.936±0.188	0.901±0.17	0.662±0.011	18.133±0.448
Reunion	F	20	54.81±1.6	14.51±0.82	27.27±0.94	4.144±0.238	1.24±0.098	0.695±0.152	0.556±0.141	0.605±0.0154	16.297±0.404
	M	13	53.35±1.11	14.15±1.09	28.85±1.1.6	5.012±0.235	1.568±0.083	1.366±0.202	1.065±0.197	0.654±0.014	18.811±0.743

3411 **Thesis conclusion**

3412 In this PhD thesis I showed that the invasion success of the guttural toad in Cape Town may
3413 be due to some factors linked to demographic and spatial dynamics such as initial lag that
3414 delayed management response and accelerating spread that caused rapid invasion of the
3415 landscape. Rapid adaptive response that reduced phenotypic mismatch in the novel
3416 environmental context and novel environmental conditions facilitating activity of invasive
3417 individuals during the non-breeding season could have also increased the invasion success.
3418 I found also that the spatial dimension of the invaded landscape strongly hampered the
3419 efficacy of the current management program.

3420 The model described in chapter one integrates age structured with least cost path
3421 approaches and incorporates the most recent frameworks on amphibian movement ecology
3422 (Pittman et al. 2014, Sinsch 2014) with density dependent survival at pre-metamorphic
3423 stages. Although specifically applied to this case study, its approach is more general and
3424 can be employed not only to explore invasive amphibian populations but also invasive
3425 dynamics of other species characterized by age structured populations. The lag phase
3426 followed by and accelerating spread predicted by the model match real field observations
3427 and confirmed what was previously detected in other invasive populations. These dynamics
3428 could have delayed both detection of the invasive population in Cape Town and
3429 implementation of management countermeasures.

3430 Chapter two showed that the efficacy of the current mode of removal is highly limited; it does
3431 not allow eradication of the invasive guttural toad population or impedes its spread across
3432 the invaded area. The current mode of removal does not sufficiently take into consideration
3433 non-linear population dynamics linked to density dependence such as the hydra-effect (i.e.
3434 positive mortality) thus reducing efficiency. Management countermeasures that explicitly
3435 consider these dynamics may increase management efficiency but are not sufficient to
3436 extirpate the invasive population. Only modes of removal that tackle spatial limitations
3437 caused by access restriction could promote a successful extirpation of the population;
3438 however a conspicuous management effort would have been required to accomplish this
3439 goal. It suggests that management actions in urban areas should also be accompanied by
3440 adequate legislation and communication to tackle social limitations. Other more feasible
3441 management targets such as control or containment may still be conducted in the invaded
3442 area, specifically through the removal of adults; it could reduce the potential impact of the
3443 invasive population.

3444 Chapter three shows that guttural toads invading Cape Town underwent in less than two
3445 decades an adaptive response that reduces phenotypic mismatch in the invaded area. In
3446 accordance to the more desiccating and colder environment, the invasive population
3447 responded physiologically and behaviourally to reduce sensitivity to lower conditions of
3448 hydration and temperature. Although other authors observed similar patterns for example in
3449 the cane toad *Rhinella marina* invading Australia, the response observed here occurred on a
3450 time scale notably shorter. More studies should be conducted to disentangle whether this
3451 difference is due to evolutionary (e.g. local adaptation) or non-evolutionary (e.g. phenotypic
3452 plasticity) phenomena. However, my study clearly shows that invasive guttural toads have
3453 already a higher potential to invade Cape Town, and more generally drier environments,
3454 than I could infer from the source population.

3455 Chapter three clearly shows that during the breeding season, the environment of Cape Town
3456 is significantly more desiccating than that of Durban; however this pattern seems reversed
3457 during the non-breeding season. Chapter four showed that Cape Town guttural toads stored
3458 less energy than conspecifics occurring at lower altitude and it should be explained with a
3459 less severe non-breeding season in this invasive population. Firstly, it could imply that in
3460 tropical-subtropical amphibians, rainfall and moisture could be more important than
3461 temperature in regulating energy storage and activity patterns. Secondly, I suggest that in
3462 some invasions the novel environment may constrain and facilitate the invasive population in
3463 different periods/seasons across the year. Testing this in other species, not necessarily
3464 amphibians, seems ripe for further investigations.

3465 In conclusion, I showed that the invasion success of the guttural toad in Cape Town may be
3466 attributable to several factors such as initial lag that delayed management, accelerating
3467 spread, rapid adaptive response and less severe non-breeding season. The spatial
3468 dimension of the invaded landscape strongly limited the efficacy of the current management
3469 program. My work has relevant management implications; it shows that the invasion
3470 potential of the species is already higher than that I could infer from the source population
3471 and only tackling social limitations could have promoted effective extirpation. Moreover each
3472 chapter provides an original approach to investigate not only the invasive population of the
3473 guttural toad in Cape Town, but more generally invasive amphibian population by utilizing
3474 different disciplines.

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