#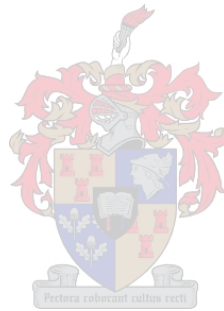 Geographic range, spread and potential distribution of the painted reed frog *Hyperolius marmoratus* in the Western Cape Province, South Africa

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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.

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Date: 20 October 2014

Abstract

Amphibian populations are among the most seriously threatened by global environmental change. Nonetheless, a few species have expanded their ranges to become globally invasive. In southern Africa, several anuran species are undergoing extra-limital range expansion on a regional scale and one of them, the painted reed frog (*Hyperolius marmoratus* Rapp.), is now invasive across the south-western Cape of South Africa. To answer the question of how this tropical, summer-breeding anuran has made such a successful transition into the temperate, winter rainfall region, I investigated several important aspects of the invasion process using a range of approaches from range ecology, physiology and niche modelling. Reconstruction of the painted reed frog's invasion history allowed the date of introduction to be identified as 1997 or early 1998. The novel range was defined as extending from the Tsitsikamma Forest in the east to the Cape Peninsula in the west. Patches and gaps in the range structure and disparate rates of spread indicated that human-assisted jump dispersal and diffusion-based dispersal dominate in different parts of the novel range. A significant gap in the novel range distribution is formed by the Riviersonderend Mountains, a section of the Cape fold mountain range, that acts as a barrier to spread.

To identify physiological range limiters, I investigated the plasticity of key physiological traits that influence thermoregulation, energetics and evaporative water loss. After thermal acclimation at three temperatures commonly encountered in their historical and novel ranges, frogs exhibited a broad thermal tolerance range and higher plasticity in CT_{max} than in CT_{min}. Resting metabolic rates were lowest in cold-acclimated animals, partially supporting the 'colder is better' hypothesis over beneficial acclimation. Active metabolic rates were lowest in warm-acclimated frogs, suggesting compensation for energy conservation. Notably, evaporative water loss was not significantly altered by acclimation in resting or active frogs, demonstrating a lack of plasticity in this trait. Plasticity of thermal tolerance and metabolic rate suggests that painted reed frogs efficiently conserve energy in a range of thermal environments and can withstand seasonal cooling by minimising the costs of resting metabolism. These characteristics could play a beneficial role in the novel range, which has a temporally and spatially variable climate. Together with their significant warming tolerance, they may facilitate spread into more extreme thermal environments north of their current range. On the other hand, the lack of plasticity in water loss rates, combined with reliance on the water-conserving posture to limit evaporative water loss could constrain further expansion to new sites.

To form an integrated picture of the forces facilitating and constraining painted reed frog spread, reciprocal niche modelling was used to investigate the relationship between range shift and niche shift. I tested whether the niche of the painted reed frog has been conserved during recent range expansion or whether spread has been facilitated by a niche shift by using a modelling framework comprising both climatic and landscape variables. Niche models trained in the historical and novel ranges and projected in the reciprocal range revealed that *H. marmoratus* has undergone a niche shift between its historical range in the northern and eastern coastal regions and its novel range in the Mediterranean ecosystems of the south-western Cape. The niche shift corresponds with a climatic gradient towards higher summer aridity and generally lower precipitation in the novel range than the historical range, but is likely mediated by landscape transformation in the form of artificial water bodies that provide additional buffered habitats.

I conclude that the niche shift accompanying range expansion has allowed painted reed frogs to occupy drier and more variable habitats in the novel range, while on a finer scale, access to permanent water bodies in the landscape is limiting. Artificial water bodies provide a key resource supplement for these invasive tropical frogs, which can be recognised as 'urban exploiters'. Unspecialised habitat requirements, rapid spread and significant phenotypic plasticity suggest that they could continue to spread further within the matrix of suitable habitat available to them.

In summary, this study found that availability of water in the landscape and the physiological capabilities of the frogs in relation to water loss are key determinants of the distribution and niche of painted reed frogs in their novel range. The work highlights the importance of broad-scale climatic variables, landscape transformation in the form of artificial water bodies, and synergistic interactions between physiology and behaviour in determining invasion success.

Keywords

Opsomming

Amfibiese populasies is onder die diergroepe wat die ergste deur wêreldwye omgewingsverandering geraak word. Nietemin het 'n paar spesies hul verspreidingsgebiede uitgebrei om wêreldwyd indringers te word. In Suider-Afrika ondergaan verskeie Anura-spesies buitegebiedsuitbreiding op 'n streekskaal. Een van dié spesies, die gestreepte rietpadda (*Hyperolius marmoratus* Rapp.), is nou amptelik 'n indringer deur die hele Suidwes-Kaap-streek van Suid-Afrika. Om te bepaal hoe hierdie tropiese Anura, wat in die somer aanteel, so 'n suksesvolle oorgang na 'n matige winterreënvalstreek kon maak, het hierdie studie ondersoek ingestel na verskillende belangrike aspekte van die indringingsproses deur van verskeie benaderinge, onder meer gebiedsekologie, fisiologie en nismodellering, gebruik te maak. Deur die indringingsgeskiedenis van die gestreepte rietpadda te rekonstrueer, is die eerste aanwesigheid van die spesie in die Suidwes-Kaap-streek tot 1997 of die begin van 1998 teruggevoer. Die nuwe verspreidingsgebied strek van die Tsitsikamma-woud in die ooste tot by die Kaapse Skiereiland in die weste. Kolle en leemtes in die verspreidingstruktuur sowel as ongelyke verspreidingstempo's toon dat menslik gesteunde sprongverspreiding en diffusiegegronde verspreiding die dominante verspreidingsmetodes in verskillende dele van die nuwe gebied was. 'n Beduidende leemte in die nuwe verspreidingsgebied is die Riviersonderend-berge, 'n gedeelte van die Kaapse plooiingsgebergte, wat as 'n versperring vir verspreiding dien.

Om die beperkings op fisiologiese verspreiding te bepaal, is navorsing onderneem oor die plastisiteit in die vernaamste fisiologiese kenmerke wat termoregulering, energetiek en waterverlies deur verdamping beïnvloed. Ná termiese akklimatisasie by drie temperature wat algemeen in die historiese en nuwe verspreidingsgebiede van die spesie voorkom, het die paddas 'n groot termiese toleransiebestek en hoër plastisiteit by CT_{max} as by CT_{min} getoon. Rustende metaboliese tempo's was die laagste by diere wat by lae temperature geakklimatiseer is, wat die 'kouer is beter'-hipotese eerder as voordelige akklimatisasie ondersteun. Aktiewe metaboliese tempo's was die laagste by die paddas wat by hoë temperature geakklimatiseer is, wat weer op kompensasie vir energiebehoud dui. Akklimatisasie het geen beduidende verskil aan waterverlies deur verdamping by rustende of aktiewe paddas gemaak nie, wat 'n gebrek aan plastisiteit in hierdie kenmerk aandui. Plastisiteit in termiese toleransie en metaboliese tempo gee te kenne dat die gestreepte rietpadda in 'n verskeidenheid termiese omgewings energie kan behou, en seisoenale afkoeling kan weerstaan deur die eise van rustende metabolisme te beperk. Hierdie kenmerke kan voordelig wees in die nuwe verspreidingsgebied, wat oor 'n temporeel en ruimtelik veranderlike klimaat beskik. Tesame met 'n beduidende toleransie vir hitte, kan hierdie

eienskappe van die spesie ook verspreiding na meer ekstreme termiese omgewings noord van hul huidige verspreidingsgebied moontlik maak. Tog kan die spesie se gebrek aan plastisiteit in waterverliestempo's, tesame met afhanklikheid van die waterbehoudpostuur om waterverlies deur verdamping te beperk, verdere verspreiding na nuwe terreine bemoeilik.

Om 'n geïntegreerde indruk te vorm van die kragte wat die verspreiding van die gestreepte rietpadda hetsy vergemaklik of beperk, is wederkerige nismodellering onderneem om ondersoek in te stel na die verwantskap tussen gebieds- en nisverskuiwing. Met behulp van 'n modelleringsraamwerk wat uit sowel klimaats- as landskapveranderlikes bestaan, is daar bepaal of die nis van die gestreepte rietpadda gedurende onlangse gebiedsuitbreiding behou is, en of verspreiding eerder deur 'n nisverskuiwing gefasiliteer is. Nismodelle wat aan die hand van die historiese en nuwe verspreidingsgebiede ontwikkel en wederkerig geprojekteer is, bring aan die lig dat *H. marmoratus* 'n nisverskuiwing van die historiese verspreidingsgebied in die noordelike en oostelike kusstreke na die nuwe verspreidingsgebied in die Mediterreense ekosisteme van die Suidwes-Kaap ondergaan het. Die nisverskuiwing val saam met 'n klimaatsneiging na hoër somerdroogheid en 'n algemeen laer reënval in die nuwe verspreidingsgebied in vergelyking met die historiese gebied, maar word waarskynlik bemiddel deur landskaptransformasie in die vorm van kunsmatige watermassas, wat bykomende bufferhabitats voorsien.

Die studie kom tot die gevolgtrekking dat die nisverskuiwing wat met die uitbreiding in verspreidingsgebied gepaardgegaan het, die gestreepte rietpadda in staat stel om in die nuwe gebied in droër en meer veranderlike habitats te oorleef, waar toegang tot permanente watermassas in die landskap op 'n fyner skaal beperk is. Kunsmatige watermassas bied 'n belangrike aanvullende hulpbron vir hierdie tropiese indringerpadda, wat as 'n 'stedelike uitbuiters' bestempel kan word. Ongespesialiseerde habitatvereistes, snelle verspreiding en beduidende fenotipiese plastisiteit gee te kenne dat die spesie, binne die matriks van geskikte habitat tot hul beskikking, selfs verder kan uitbrei.

Ter samevatting bevind die studie dat die beskikbaarheid van water in die landskap en die fisiologiese vermoëns van die padda met betrekking tot waterverlies belangrike bepalers is van die voorkoms en nis van die gestreepte rietpadda in sy nuwe verspreidingsgebied. Die navorsing beklemtoon die belang van klimaatsveranderlikes oor 'n wye skaal, landskaptransformasie in die vorm van kunsmatige watermassas, sowel as sinergistiese wisselwerking tussen fisiologie en gedrag in die bepaling van indringingsukses.

Trefwoorde

Anura; *Hyperoliidae*; biologiese indringing; stedelike uitbuiter; fenotipiese plastisiteit; spesieverspreidingsmodellering; geografiese verspreidingsgebied; gedragstraagheid; nis

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Abbreviations

AIC	Akaike's information criterion
AICc	Akaike's information criterion adjusted for small samples
AMR	active metabolic rate
AOO	area of occupancy
AUC	area under the curve
BRT	boosted regression tree
COUE	centroid shift, overlap, unfilling, expansion (a scheme for organising niche comparisons by Guisan *et al.* 2014)
CT_{max}	critical thermal maximum
CT_{min}	critical thermal minimum
EN	Endangered (IUCN Red List designation)
EOO	extent of occurrence
LC	Least Concern (IUCN Red List designation)
m asl	Metres above sea level
PCA	principal components analysis
R	cutaneous resistance to water loss
RMR	resting metabolic rate
ROC	receiver operating characteristic
WLR	water loss rate
ZIT	zone of intrinsic tolerance

Chapter 1. General introduction

Painted reed frogs outside Stellenbosch.
-33.872°S 18.624°E

1.1   Amphibian invasions globally and in the South African context

Destruction, fragmentation and degradation of natural habitats, especially wetlands and forests, are reducing the viability of amphibian populations worldwide. Emerging diseases have caused catastrophic declines and are interacting with global climate change in complex ways (Daszak *et al.* 1999, 2000; Pounds *et al.* 2006; Seimon *et al.* 2014). Simultaneously, and despite these myriad threats, some amphibian species are expanding their ranges on intercontinental, regional and local scales. In a global analysis of introduced amphibians and reptiles, Kraus (2009) identified 141 introductions of anuran amphibians worldwide, most of which were linked to the pet trade, or travelled as stowaways in cargo or nursery plants (Kraus 2003). The diverse range of vectors and pathways involved in amphibian introductions are growing in magnitude and diversity.

Biological invasions involve the spread of reproductive populations of introduced organisms into new areas some distance from their origin (Richardson *et al.* 2000). Most definitions of invasion agree that only species or populations that have overcome a significant barrier (biogeographic criterion) or have dispersed some minimum distance from their historical range and spread into a novel environment (spread criterion) should be considered invasive (Davis and Thomson 2000; Richardson *et al.* 2000; Daehler 2001). The inclusion of an impact criterion in definitions of invasion is controversial. Under this criterion, organisms must have either positive or negative impacts on some aspect of the receiving environment to be considered invasive (Davis and Thomson 2000, 2001). However, considerable subjectivity and context-dependence is involved in the recognition of such impacts (Pyšek *et al.* 2004; Valéry *et al.* 2008). For instance, rate of establishment and spread may not correlate strongly with severity of impacts (Ricciardi and Cohen 2007), so a species may be highly invasive under one criterion and undistinguished under another. Furthermore, indigenous species that become invasive are not always recognised as such (Guo and Ricklefs 2010), even though the processes involved in extra-regional and extra-limital invasions are similar (Thompson *et al.* 1995; Pyšek et al, 2004; Valéry *et al.* 2008). In this thesis, I use the ecologically-focused definition of Valéry *et al.* (2008) which recognises invasive species as those that undergo rapid spread to become dominant in a novel environment, regardless of the region of origin. I use the terminology of Richardson *et al.* (2000), recognizing that even if negative or positive impacts have not been demonstrated, a range expansion may match the definition of long distance dispersal into and rapid spread within a novel environment.

While amphibians have been introduced to novel locations worldwide, this has resulted in relatively few active invasions. Anurans that are known to have become invasive on a global scale include only a handful of species - the common platanna (or African clawed frog, *Xenopus laevis*) introduced to Europe, South America, North America and various oceanic islands from southern and west Africa; the American bullfrog (*Lithobates catesbeianus*, =*Rana catesbeiana*) introduced to Asia, Europe and extra-limital parts of North America); the coquí frog (*Eleutherodactylus coqui*) introduced to Pacific Islands and the Caribbean; the cane toad (*Rhinella marina*) introduced to Australia, and the Cuban tree frog (*Osteopilus septentrionalis*) introduced to the Caribbean and North America. The impacts of these invasions include, among others, direct and indirect trophic effects and cascades (Doody *et al.* 2009), population declines (Doody *et al.* 2009), novel predator-prey relationships (Boland 2004, Crossland 2000), novel or accelerated disease transmission (Daszak *et al.* 2000, 2004) and social and economic knock-on effects (Beard *et al.* 2009).

Currently, southern Africa has no known extra-regional invasive amphibians (van Rensburg *et al.* 2011; IUCN 2014). The IUCN Invasive Species Specialist Group lists ten invasive anuran species (GISD 2014) none of which are yet present in South Africa, except the common platanna which is indigenous to the sub-continent. However, three indigenous taxa are undergoing extra-limital range expansions within the Western Cape Province: the painted reed frog *Hyperolius marmoratus*; the common platanna *Xenopus laevis*, and the guttural toad *Amietophrynus gutturalis* (Measey and Davies 2011). These extra-limital invasive species ('domestic exotics', see Guo and Ricklefs 2010) all have endemic congeners in the Cape Floristic Region (arum lily frog *H. horstockii* (LC); Cape platanna *X. gilli* and leopard toad *A. pantherinus*). In the case of the reed frogs and platannas, the endemic species and their widespread congeners may have historically occurred in sympatry over parts of their ranges, but on a fine scale they likely inhabited distinct types of water bodies (e.g. temporary black water pans in the case of *X. gilli*; Picker *et al.* 1985).

The potential, though undemonstrated, impacts of these extra-limital amphibians include interference competition and auditory competition at breeding sites (painted reed frog, guttural toad and common platanna), predation on the endemic species and habitat disturbance by the range expanding species (common platanna). Rapid spread of *A. gutturalis* in the Cape Town metropole and the conservation status of *A. pantherinus* (EN) has led to the establishment of a control programme aimed at early eradication of *A. gutturalis* (Measey *et al.* 2014). Similarly, a

control programme is underway for *X. laevis* in protected areas on the Cape Peninsula (Measey *et al.* 2014), with the objective of protecting the habitat of *X. gilli* (EN). The spread of the painted reed frog has been sufficiently rapid and extensive that this species is not regarded as a candidate for physical or chemical control, which has only succeeded in tightly-controlled and spatially-restricted situations (e.g. coquí frogs in Hawai'i - Beachy *et al.* 2011).

In this thesis, I aim to identify the drivers of range change in the painted reed frog by building an explanatory framework for the invasion process based on ecology, physiology and niche modelling. I use approaches from community ecology (theory of range limitation and niche modelling) and physiological ecology (basal and plastic responses) to build an integrated understanding of the range and niche dynamics of the painted reed frog in South Africa over the past *c.* 17 years.

1.2   Range shifts and environmental change

Consonant with climate warming, tropical species ranges may shift to higher latitudes and higher elevations (Hughes 2000; Parmesan and Yohe 2003; Hickling *et al.* 2006) and the onset of phenological events may be earlier (Beebee 1995; Gibbs and Breisch 2001). Perissonotto *et al.* (2011) drew attention to eight insect range expansions in southern Africa (three cetoniine and cerambycid beetles and five lepidopterans), several of which mirror the south-westward range expansion of the painted reed frog. Measey and Davies (2011) noted three range expansions by extra-limital anurans within South Africa that are implicated in potential impacts on congeneric regional endemic species.

It is unlikely that climate is the sole driver of these range expansions, however (see Bradley *et al.* 2010). Other global change processes such as physical transformation of the landscape and creation of novel habitats may be implicated (Ficetola *et al.* 2007; Peacock *et al.* 2007; Roura-Pascual *et al.* 2011). Therefore, the role of landscape change and human agency in species range shifts remains a productive area of research.

Invasive species, by definition, appear to be less limited than native species by their ability to disperse and establish beyond their range margins, and therefore they are useful case studies of the processes that allow populations to overcome range boundaries (Brown *et al.* 1996; Richardson *et al.* 2000). Some invasive species also provide tractable model systems for the study of relationships between key organismal, population-level and environmental variables,

such as range limitations, range structure and size, extinction risk, interspecific competition and community structure (Simberloff 2003). In turn, studies of invasive species range limits can shed light on range limitation more generally.

Measuring the structure of invasive species ranges entails several challenges, however. Invasive species ranges are seldom in equilibrium (Fielding and Bell 1997; Guisan and Zimmerman 2000); niche shifts may occur during the invasion process, making it difficult to clearly describe habitat characteristics in the novel range (Broennimann *et al.* 2007; Broennimann and Guisan 2008), and adaptive responses and phenotypic plasticity interact to increase the complexity of organism-environment interactions (Lee 2002; Ghalambor *et al.* 2007). However, the fundamental process underlying all species range expansions is extra-range dispersal leading to organisms founding populations in novel environments (see Holt and Keitt 2005; Holt *et al.* 2005, Case and Taper 2000; Franklin 2009).

A variety of processes has been shown to contribute to dispersal across range boundaries: genetic variability producing favourable mutations (Kirkpatrick and Barton 1997; Lee 2002); natural selection leading to local adaptation (Bridle and Vines 2007; García-Ramos and Rodríguez 2002; Excoffier and Ray 2008); dispersal ability allowing or preventing colonisation of new habitats beyond the range margins (Zacherl *et al.* 2003; Bridle and Vines 2007), and phenotypic plasticity facilitating persistence in slightly unfavourable habitats (Chown *et al.* 2007; Kellerman *et al.* 2009). These mechanisms interact with two broad patterns of dispersal in the form of diffusion-based spread across range boundaries and long-distance jump dispersal (Skellam 1951; Shigesada *et al.* 1995), and have resulted in a variety of range expansion patterns among colonising organisms (e.g. Measey *et al.* 2007; Le Roux *et al.* 2014).

1.3   Mechanisms of range expansion

To effectively manage invasive species and their impacts, it is necessary to develop tools that can aid in predicting the future extent and rate of range expansion. Invasion biologists often apply correlative approaches, such as climate matching, to predict areas where invasive species might establish and to forecast their spread through novel habitats (Thuiller *et al.* 2005; Richardson and Thuiller 2007). An extension of this approach, reciprocal range modelling, can be used to determine whether the novel environment matches the conditions in the native range, or whether a shift in environmental tolerance has occurred during invasion (Broennimann *et al.* 2007; Broennimann and Guisan 2008).

The addition of mechanistic information, such as physiological or behavioural traits to correlative models can yield more informative results that reveal the causes of and constraints on invasions (Broennimann *et al.* 2007; Broennimann and Guisan 2008; Beale *et al.* 2008; Elith *et al.* 2010). Specifically, if the bioclimatic requirements and natural history of a species can be linked with an understanding of its physiological limits (rates and tolerances) and their level of phenotypic plasticity, a robust explanatory framework can be built for understanding the invasion process (Rödder *et al.* 2009; Seebacher and Franklin 2012; Di Febbraro *et al.* 2014). In this thesis I aim to build a mechanistic framework for understanding the drivers and potential end-points of range expansion in the painted reed frog in South Africa.

1.4   Phenotypic plasticity

Phenotypic plasticity is the capacity to vary the phenotypic expression of a genotype in response to environment stimuli. It occurs within the lifetime of individuals (West-Eberhard 2003), either within a life stage as reversible or irreversible plasticity (Seebacher and Franklin 2011) or across life stages as developmental plasticity (Berrigan and Partridge 1997; Travis *et al.* 1999). Phenotypic plasticity may buffer the selection pressures that follow translocations or climate change and may enhance or hinder evolutionary adaptation (Ghalambor *et al.* 2007; Broennimann *et al.* 2007). In particular, plasticity has been shown to play a pivotal role in the responses of ectotherms to environmental change (Lee *et al.* 2003; Chown *et al.* 2007).

The ability of painted reed frogs to persist in the novel range may be mediated by evolutionary adaptation or phenotypic plasticity. Whilst the role of phenotypic plasticity in the invasion of the cane toad has received much attention (e.g. Seebacher and Alford 2002; Urban *et al.* 2007; Seebacher and Franklin 2011; Overgaard *et al.* 2012), relatively little is known about the degree of plasticity of thermal tolerance and capacity traits in other invasive anurans, particularly in smaller arboreal species such as *H. marmoratus*.

1.5   Aims and key questions

The general aim of this research is to identify the mechanisms behind the range expansion of *H. marmoratus*, by elucidating its invasion history, range dynamics and physiological and behavioural traits in the novel range, their implications for invasiveness and for the potential extent of the invaded range. Using a conceptual model of *H. marmoratus* colonisation, spread and persistence, I investigate several important aspects of the invasion process to answer the

question of how a frog from a tropical biogeographical centre has made such a successful transition into a novel climatic zone. Specifically I aimed to (i) map the distribution in the novel range and measure patterns of invasion; (ii) investigate the role of thermal change in facilitating the spread of this species, and (iii) apply the knowledge acquired in (i) and (ii) to investigate the niche dynamics of the painted reed frog in the novel range.

1.6   Thesis outline

The first part of Chapter 1 has set out the problem statement and context for the thesis. The remainder of this chapter provides general background to the range expansion and the current state of knowledge on the focal species.

Chapter 2 is the biogeographic foundation, and investigates the history and broad patterns of invasion. Specifically, I estimate the extent of occurrence and rate of spread of *H. marmoratus* in its novel range; identify the geographical and temporal origin of range expansion; quantify the internal range structure, and identify environmental correlates of occupancy. In this chapter I also attempt to distinguish among natural diffusion, jump dispersal, and mixed-mode expansion, and to characterise the invasion pattern of *H. marmoratus* in its novel range. The outcomes of this chapter are taken up in the subsequent chapters on phenotypic plasticity and niche modelling.

In Chapter 3, I ask whether physiological traits of painted reed frogs confer an advantage in the novel range, for example through phenotypic plasticity that allows them to respond rapidly to novel conditions. First, I investigate the potential for thermal acclimation to play a role in ameliorating the conditions encountered by frogs in the novel range, through quantifying thermal tolerance, metabolic rates and water loss rates in resting and active animals. Explicit predictions about the direction and shape of the acclimation responses are tested in the strong inference framework of Huey *et al.* (1999), i.e. beneficial acclimation, 'hotter is better', 'colder is better', optimal acclimation temperature and no acclimation response. Chapter 3 also addresses the potential ecological and evolutionary consequences of phenotypic plasticity for the painted reed frog in its novel range.

Chapter 4 uses information on the plasticity of temperature tolerance of painted reed frogs and their habitat characteristics in the novel range to design and test niche models for South Africa. Niche models incorporating variables describing climate and landscape structure are used to

address the question of whether a shift in physiological tolerance or capacity has occurred and to identify its drivers.

Chapter 5 integrates the findings of the individual studies and identifies the implications of the work for the broader fields of ecology and physiology. In this final chapter I round off the discussion with priorities for future research.

1.7 Background information

1.7.1 Regional biogeography

South Africa has a diverse anuran fauna (Measey 2011) in which two distinct faunal assemblages have been identified (Poynton 1964; Alexander *et al.* 2004). The north-east 'tropical' centre exhibits high species richness while the south-west 'Cape' centre has a high level of endemism (Angulo *et al.* 2011). The two centres are interspersed by a broad 'transitional' zone. Very few species span both centres, prompting speculation about whether the break is caused by present climatic conditions or historical processes. The biogeographical break between the tropical and Cape centres clearly coincides with the transition from strong summer rainfall in the north-east, through an aseasonal/bimodal pattern to strong winter rainfall in the south-western extremity (Tyson 1986; Chase and Meadows 2007; Figure 1-1).

The range expansion of *H. marmoratus* from the tropical to Cape centres represents a transition across this biogeographic and climatic boundary, and thus raises several questions for further investigation. Poynton raised the possibility that the distinct fauna of the two biogeographic centres may be an artefact of dispersal:

> "*One possible reason why the tropical margin does not cover the south-western corner of Africa is simply because the current post-Pleistocene tropical fauna has not yet had the time to reach it. This explanation hinges on the dispersal rate of amphibians*, *which is difficult to estimate.*"   Poynton (1964: 231)

The south-western part of South Africa falls into the Cape Floristic Region and is dominated by xeric shrublands of the fynbos biome (Mucina and Rutherford 2006), which differs significantly in structure and nutrient cycling from the rest of the country's sub-tropical thicket, Afro-montane forest, succulent karoo, nama karoo, savanna and grassland biomes. The high levels of endemism and diversification of the Cape flora are largely a result of the interaction between Mediterranean climate, nutrient poor soils and rugged topography where high mountains close to the sea create

steep microclimate gradients. Overlaid on these abiotic gradients are a wide range of biotic interactions such as pollination and seed dispersal mutualisms that drive lineage diversification (Linder 2003; Cowling *et al.* 2009; Linder *et al.* 2010).

The climatic/biogeographic break between the sub-tropical and Cape centres is dynamic (Deacon *et al.* 1992). A biogeographic analysis conducted by Tolley *et al.* (2008) suggested that *H. marmoratus* populations in the south-eastern Cape may be undergoing a natural range expansion as a result of the longer-term trend towards wetter summers in that region and the westward retreat of the winter rainfall zone (Figure 1-1). This would imply that the south-eastern Cape populations of *H. marmoratus* are the leading edge of an ongoing Holocene radiation that is now augmented by jump dispersal, promoting the colonisation of remote as well as nearby areas. The presence of considerable genetic structure within *H. marmoratus* as a whole (Wieczorek *et al.* 2000; Tolley *et al.* 2008) supports this hypothesis and indicates that some genetic differentiation may be possible in response to local conditions.
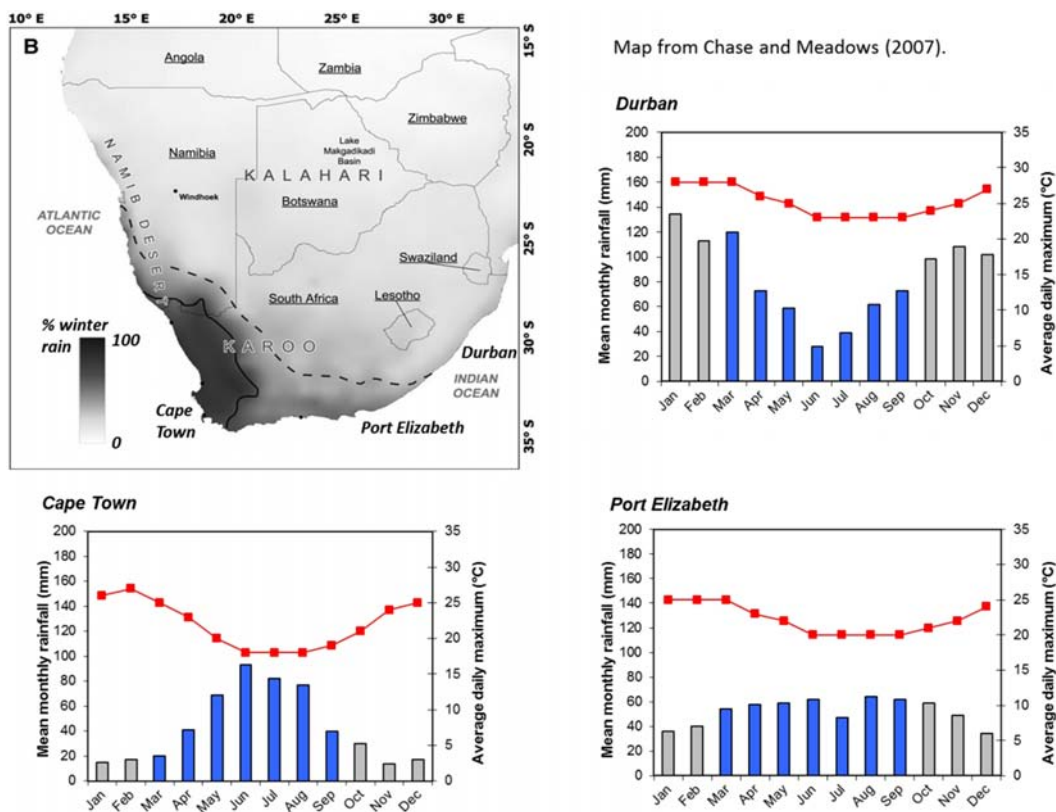
Figure 1-1. Rainfall seasonality of southern Africa, illustrating the climatic distinctiveness of the south-western Cape. Bars represent mean monthly rainfall; line shows average daily temperature. Source: Map reproduced from Chase and Meadows (2007); weather data from South African Weather Service 2008.

Hyperolius marmoratus Rapp 1842, the painted or marbled reed frog, belongs to the largest genus of African frogs (Minter 2004), containing over 200 species, whose distributions are centred on tropical and sub-tropical forests and savannas (Schiøtz 1999). Although many reed frog species appear to be habitat generalists, the genus includes a few habitat specialists such as the grassland specialist *H. pickersgilli* and the tree-hole breeding *H. thomensis*. *Hyperolius marmoratus* has the largest distribution of all Southern African *Hyperolius* species (Channing 2001; Minter *et al.* 2004 b).

Distribution digitised from Channing (2001) at 1 degree square resolution.

0   150   300   600 Kilometres
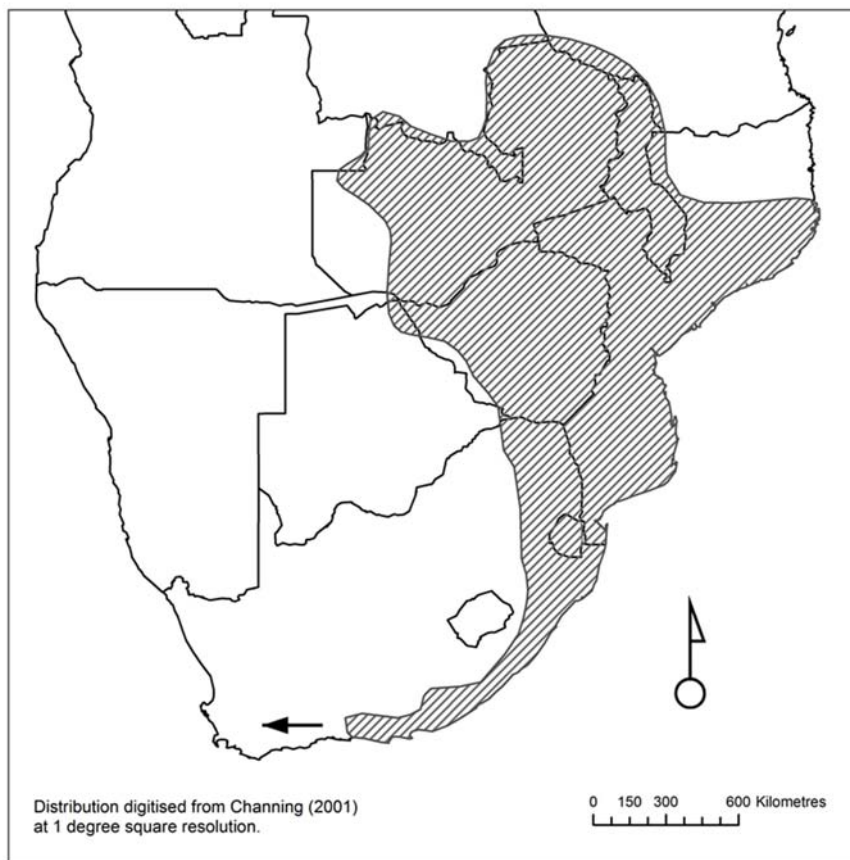
Figure 1-2. Regional range of the painted reed frog, *Hyperolius marmoratus* Rapp, prior to range expansion. Arrow indicates direction of current range expansion in the Western Cape. Projection: Geographic; datum: WGS 84. Source: Map redrawn from Channing (2001).

The painted reed frog is a small (up to 43 mm, Channing 2001: 160), often brightly-coloured, semi-arboreal species that is indigenous to south-eastern Africa (Figure 1-2). The species was described from an unspecified type locality in KwaZulu-Natal Province, South Africa (Frost 2004) and is considered by some authors to be part of the large and widely-distributed superspecies *H. viridiflavus*, which includes many of the reed frogs from central, east and west Africa. Early workers treated all taxa in the *H. viridiflavus* group as sub-species of *H. viridiflavus* (e.g. Schiøtz 1971). However, more recent sources treat *H. marmoratus* as a species

in its own right (Wieczorek *et al.* 2000; Channing 2001), and although substantial genetic structure has been shown to exist within *H. marmoratus* (Grobler and Matlala 1998; Wieczorek *et al.* 2000, 2001; Tolley *et al.* 2008), most researchers agree that the variants are distinguished by dorsal colour pattern only and there is no evidence of cryptic species (Poynton 1964; Poynton and Broadley 1987; Wieczorek *et al.* 2000, 2001; Channing 2001; Bishop 2004; Tolley *et al.* 2008).

Many sub-species or variants of *H. marmoratus* have been described based on dorsal colour pattern, but only three of these occur in South Africa (Passmore and Carruthers 1995; Channing 2001). The sub-species designations given by some authors are not consistent throughout the range; intergrades occur between adjacent pairs (Poynton and Broadley 1987; Lambiris 1988) and the breaks are not supported by genetic evidence (Grobler and Matlala 1998; Tolley *et al.* 2008). For the purposes of this thesis, the three types found in South Africa are referred to as colour morphs of *H. marmoratus*. Where colour morphs need to be distinguished, they are referred to with their sub-species designation or dorsal colour pattern, i.e. 'taeniatus' or striped morph, 'marmoratus' or marbled morph and 'verrucosus' or spotted morph. The striped morph occurs in Limpopo, Mpumalanga and Swaziland, overlapping with the marbled morph in the St. Lucia area; the marbled morph occurs from St. Lucia south to Port Edward, while the spotted morph stretches from southern KwaZulu-Natal to Tsitsikamma in the southern Cape (Lambiris 1988; Channing 2001; Bishop 2004) (see Figure 1-3).

1.7.2 *Historical distribution*

The historical range of *H. marmoratus* in South Africa is relatively well known and has been described by numerous authors (e.g. Power 1934, Fitzsimons 1930, 1937, Loveridge 1941; Poynton 1964; Kannemeyer 1937; Passmore and Carruthers 1979, 1995; Wager 1986; Lambiris 1988; Channing 2001; Bishop 2004). Published records up to 2004 covered large parts of south-eastern Africa, including southern Tanzania and Malawi, Mozambique, eastern Zimbabwe, Swaziland and the eastern and southern portion of South Africa (Channing 2001; Figure 1-2). Within South Africa, the lowveld of Limpopo and Mpumalanga to the central escarpment (Wager 1986; Bishop 2004), the KwaZulu-Natal midlands up to 1 600 m at Ixopo (Bishop 2004), and the eastern coastal belt as far as the Tsitsikamma forest in the south east (Poynton 1964; Lambiris 1988; Bishop 2004) were occupied. The climate of the historical range is sub-tropical in the north and east, but temperate in the south.
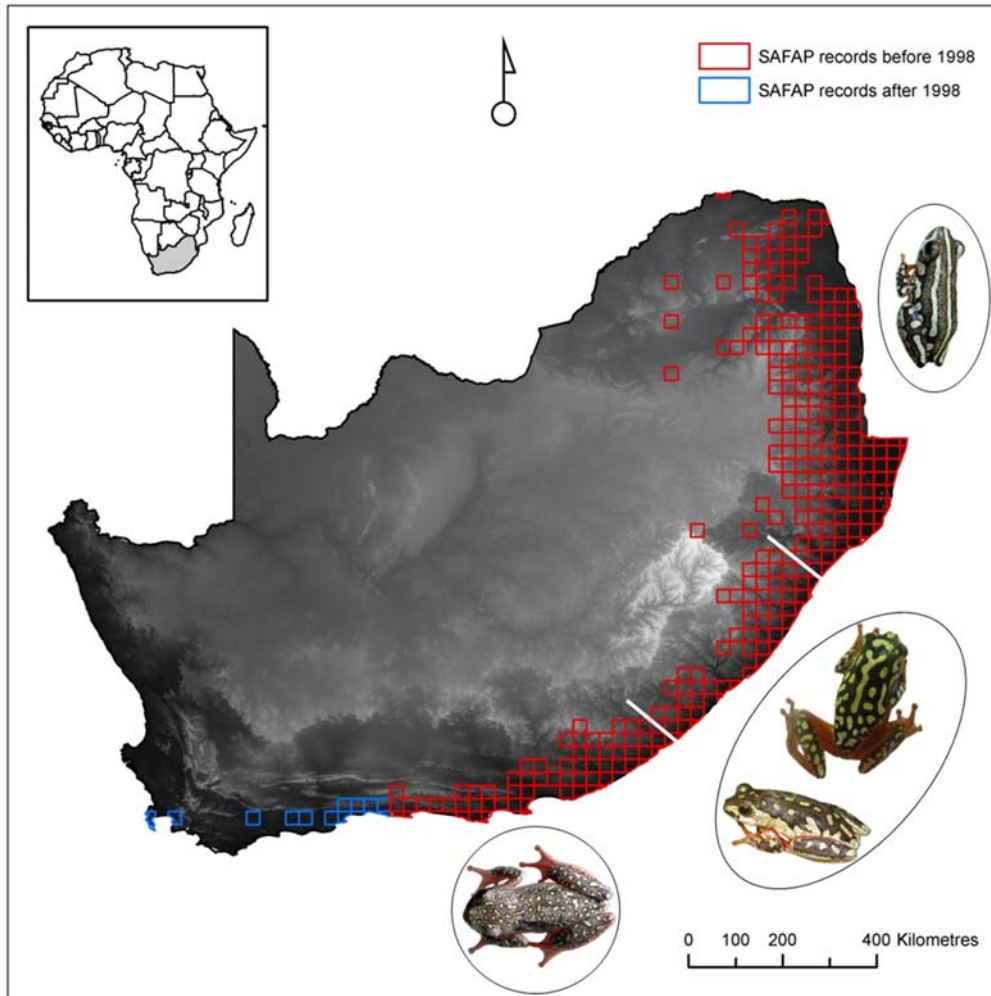
Figure 1-3. Distribution data for *Hyperolius marmoratus* from the South African Frog Atlas. Projection: Geographic; datum: WGS 84. Source: SAFAP database (Minter *et al.* 2004 b). White bars are notional breaks between colour morphs. Isolated records in the central regions are likely to be translocations (Bishop 2004). Grey shading shows elevation above sea level (i.e. lighter colours are higher elevations).

1.7.3   Current distribution

Since 1997, *H. marmoratus* has become established on the Cape peninsula and in inland areas of the Western Cape Province. Bishop (2004) pointed out that *H. marmoratus* occurs in grassland, savanna and forest habitats up to 1600 m asl, and that westerly records in the northern parts of South Africa, as well as Western Cape records (Figure 1-3) were probably translocations. Prior to this study, the full extent of the novel range and the origin of many of the invasive populations were unknown. Recent research on painted reed frogs from four dams in the novel range and 18 sites in the historical range by Tolley *et al.* (2008) showed that multiple introductions had occurred to the Western Cape from different parts of the historical range, within and possibly outside South Africa.

The Southern African Frog Atlas Project (SAFAP) produced the first comprehensive study of the distributions of southern African frogs by collating data from museum and private collections and augmenting this with field work by volunteers and commissioned herpetologists between 1996 and 2003 (Minter *et al.* 2004 a). Examination of the SAFAP database (Minter *et al.* 2004 b) showed that most records of *H. marmoratus* west of 23° E were obtained after 1997. The single exception, a specimen collected from the Cape Flats in 1960 and lodged in the Transvaal Museum, was later found to be an individual of *H. horstockii* (Schlegel, 1837)[1].

1.7.4   Reproductive behaviour

Hyperolius marmoratus breeds in a wide range of shallow, vegetated water bodies, including pans, ponds, dams, vleis, and slow-flowing streams and rivers (Bishop 2004). Breeding choruses of *H. marmoratus* may number hundreds of individual males. Within a breeding site, male frogs occupy and defend exposed calling sites on fringing and emergent vegetation. Calling males emit a "short, loud piercing whistle" (Passmore and Carruthers 1995) from soon after dusk until midnight if weather conditions are suitable (Passmore 1981; Wager 1986; Henzi *et al.* 1995). Calling reaches peak intensity approximately 30 min. after sunset, after which males call continuously for 80 to 236 min. (mean±s.d.: 160±40 min., Passmore *et al. 19*92). After calling, mating and/or oviposition, frogs leave the water body and roost in trees or shrub canopy, but may also be found basking in exposed positions during the day (Passmore and Carruthers 1995). Henzi *et al.* (1995) modelled the environmental variables involved in male and female chorus attendance and concluded that male presence at a breeding site was largely determined by weather conditions on the day and the previous day, specifically, conditions that minimised evaporative water loss. Female presence was strongly governed by the size of the male chorus. It is interesting to note that the body temperatures of both calling males and females may be elevated as much as three degrees above ambient as a result of stored heat from day-time basking (Passmore and Malherbe 1985).

1.7.5   Life history and phenology

The life cycle of *H. marmoratus* is relatively rapid, and reproductive potential is high. Males and females may reach sexual maturity in their first and second seasons respectively (Bishop 2004),

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[1] Specimen examined by SJD at Transvaal Museum on 21/09/2010; museum code TM26714; SAFAP card no. 24026714

and females are able to produce two or three clutches of eggs per season in the wild (Telford and Dyson 1990) and up to 12 in a laboratory-simulated breeding season (Grafe *et al.* 1992). Eggs hatch after five to seven days (Wager 1986), to produce free-swimming, benthonic, herbivorous tadpoles (Passmore and Carruthers 1995; Channing 2001). Metamorphosis takes six to nine weeks (Wager 1986) and metamorphs are relatively large, about 1 cm snout-vent length (Telford and Dyson 1990; Schmuck and Linsenmair 1997).

Painted reed frogs have a prolonged breeding season from September or October to February (Passmore and Carruthers 1995; Bishop 2004), and to early March in parts of the novel range (SJD, unpublished data). This period falls into the wet season in the historical range, but in the novel range, which has a Mediterranean-type climate (Goldblatt and Manning 2002), the species breeds during a dry summer. Thus, the species appears to have overcome a significant natural barrier by expanding its range from summer and aseasonal rainfall regions to a strongly winter rainfall region (Mucina and Rutherford 2006; Chase and Meadows 2007).

Like most pond-breeding amphibians, painted reed frogs use distinct sites for feeding, roosting and breeding, so they must travel some distance from a breeding site to roost or to feed, resulting in diurnal and seasonal movements to and from water bodies of up to hundreds of metres (Bishop 2004). In frogs, both locomotion and vocalisation are energetically expensive (Preest and Pough 1989; Pough *et al. 19*92). Calling is especially costly for males of *H. marmoratus* (Grafe 1996), and this is confirmed by the calling effort expended in a single calling session before mating (number of calls: mean±s.d.: 2137±1866, range 9-7286, Passmore *et al.* 1992). For females, egg production incurs significant energetic costs (Grafe *et al.* 1992). In addition, evaporative water loss is an important variable governing the survival of small-bodied frogs (Tracy *et al.* 2010).

1.7.6   State of knowledge
Apart from *X. laevis*, *H. marmoratus* is undoubtedly the most-studied African amphibian species, with at least 30 papers having been published on *H. marmoratus* (*sensu lato*) between 1985 and 1999. This body of work focussed on two areas: (i) environmental physiology of *H. marmoratus* in relation to the fluctuating conditions in African tropical savannas, e.g. desiccation resistance, aestivation and thermal tolerance (K. Eduard Linsenmair, Philip Withers, Umar Grafe and co-workers); and (ii) the breeding system in relation to sexual selection and mate recognition systems (Neville Passmore and co-workers). However, in the former body of work it is unclear

which species were being worked on because of taxonomic uncertainty in the group as a whole; inconsistent nomenclature used by the writers in relation to superspecies, species and sub-species; unknown origin of animals that were sourced over a very wide study area and often from commercial collectors, and methodological concerns. For example, critical thermal maximum had been measured with a non-standard endpoint (Geise and Linsenmair 1986); critical minimum had not been established, and no active metabolic rates or water loss rates had been measured apart from one study that measured metabolism in calling males (Grafe 1996). Therefore, basic physiological tolerance and rates could not be established unambiguously from the literature for use in niche modelling. Therefore, I conducted replicated experiments to establish these basic physiological parameters for adult frogs including temperature tolerance, energetics and evaporative water loss in frogs acclimated in a range of thermal conditions.

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Chapter 2. Farm dams facilitate amphibian invasion: Extra-limital range expansion of the painted reed frog in South Africa

Painted reed frog habitat near the boundary of the novel range
Groot Brak, 34.037°S 22.200°E

2.1   Introduction

Biological invasions are powerful natural experiments that present ecologists with opportunities to investigate range dynamics in novel environments (Brown and Sax 2004). These dynamics can be understood by quantifying range structure and identifying the factors that contribute to distribution limits (Thomas *et al.* 2004). Since the chronologies of range expansion of invasive species are often recorded, the reconstruction of invasion histories improves our understanding of the process of biological invasion. In particular it is important to be able to identify the causes of range expansion (Didham *et al.* 2005; Parmesan 2006), source populations and entry points (Rollins *et al.* 2011; Ruiz *et al.* 2011), generate hypotheses on the mechanisms of spread, and build predictive models of invasion impacts (Kulhanek *et al.* 2011). Reconstruction of invasion history is thus a precursor to studies of invasion dynamics and the causes, mechanisms and limits of invasion (Andow *et al.* 1990).

Without data on invasion history, recognition of invasiveness of a species may be delayed, hampering appropriate response and control measures (Le Maitre *et al.* 2004; McGeoch *et al.* 2012). Delayed recognition of invasiveness may also occur when indigenous species undergo rapid range expansion outside their historical extent of occurrence (so-called extra-limital species - Spear and Chown 2009), as they are less likely to be acknowledged as invasive than those that are alien to the geographic area of interest (Guo and Ricklefs 2010). Invasive populations of extra-limital species may, however, have impacts very similar in nature and scale to those of extra-regional invasive species (Spear and Chown 2008).

Although the conservation status of amphibians globally is in serious decline (Sodhi *et al.* 2008), there is a handful of instances of amphibian species expanding their ranges and becoming invasive (e.g. Fouquet and Measey 2006; Ficetola *et al.* 2007; Phillips *et al.* 2007). Although there are currently no alien invasive amphibians in South Africa (Minter *et al.* 2004; van Rensburg *et al.* 2011), three local taxa have established extra-limital populations within the country and are expanding their ranges (Measey and Davies 2011). The painted reed frog (*Hyperolius marmoratus* Rapp, Anura: Hyperoliidae) is one such species, with breeding populations having undergone a range expansion of over 500 km in the south-western areas of South Africa early in the last decade (Tolley *et al.* 2008). The congeneric arum lily frog (*H. horstockii*) occupies a much smaller range and is endemic to the Cape Floral Region of the Western and Eastern Cape Provinces (Braack 2004). Historically, the two species' ranges were non-overlapping, except for a small area in the extreme east of the region, but they are now

congruent over large parts. Presence of the two species in the same water bodies could result in competition for calling sites during their overlapping breeding seasons. The rapid extra-limital range expansion of the painted reed frog provides an opportunity to investigate the environmental correlates of a recent range expansion across the majority of its spatial and temporal extent (Boone and Krohn 2000; Sagarin *et al.* 2006).

Ectotherm species ranges are tied closely to temperature and hydrological gradients (Buckley and Jetz 2007). In amphibians this is thought to be due to a tight coupling between thermoregulation and water balance, and the 'leaky boundary' between internal and external environments (Feder 1992), which place limits on the mobility and energy balance of these animals. In consequence, temperature and humidity are generally important determinants of occupancy and abundance in these species (Boone and Krohn 2000; Ficetola *et al.* 2007). The ranges of anuran amphibians, in particular, are expected to be limited by local habitat quality such as vegetation cover, because during prolonged breeding seasons they must periodically leave the water body to feed. Thus they require aquatic and fringing vegetation for cover and foraging opportunities (Wells 1977); fringing vegetation has also been shown to maintain pond-pond and pond-matrix connectivity for dispersal of juvenile amphibians (Joly *et al.* 2001).

Range expansion may occur through long-distance dispersal, diffusion within a local neighbourhood, or by mixed-mode dispersal where both mechanisms operate simultaneously (Shigesada *et al.* 1995). Amphibians accomplish long-distance dispersal naturally, though rarely, via transoceanic voyages (e.g. Measey *et al.* 2007), linear movement along stream corridors (Lowe 2009) and inter-patch dispersal (Smith and Green 2006). Tolley *et al.* (2008) found discordant geographic structure in mitochondrial DNA sequences among breeding populations of painted reed frogs in the novel range, providing clear evidence in support of human-mediated jump dispersal. However, the novel range was not surveyed extensively, and alternative explanations for the current range structure within the novel range are possible. Specifically, establishment and subsequent spread in the novel range may have occurred via (i) human-mediated jump dispersal, for which there is some empirical support (Tolley *et al.* 2008), (ii) natural diffusion-based dispersal or (iii) mixed-mode dispersal in different parts of the novel range.

Here I identify the geographical and temporal origin of the range expansion from adjacent parts of the historical range, quantify the internal range structure and estimate rate of spread, and identify environmental correlates of occupancy in the novel range using a static model of habitat variables (Guisan and Thuiller 2005). I predict that the range limits in the novel range will be explained by climatic variables such as temperature, precipitation and evaporation, and the seasonality of these. I predict that habitat quality, particularly the density and size of water bodies available as breeding habitat and the presence of fringing, emergent and floating vegetation at water bodies, will be important at a local scale (Table 2-1). Finally, I use information on range structure and estimated rate of spread to infer possible mechanisms of dispersal and distinguish among the three modes of range expansion, i.e. natural diffusion across the novel range, expansion entirely by jump dispersal, and expansion by mixed-mode dispersal with different expansion modes in different populations.

2.2   Methods

2.2.1   Study species

The painted reed frog is a widespread pond-breeding species occurring in a range of lentic water body types in savanna and coastal forest, including pans, dams, vleis, and slow-flowing stream and river reaches (Bishop 2004). Little is known about the diet, but several West African *Hyperolius* spp. are generalist arthropod predators, consuming a range of items from six major arachnid and hexapod taxa (Luiselli *et al.* 2004). Adult painted reed frogs are desiccation-resistant (Withers *et al.* 1982), are capable of travelling some distance over land and have a tendency to enter buildings and cargo and to be translocated on fresh produce and nursery plants (Bishop 2004).

The historical range of the species in South Africa extended from the coastal and low-lying areas in the north and east of the country to the central escarpment at approximately 1600 m a.s.l. (Bishop 2004). Passmore and Carruthers (1995) described the south-western boundary of the distribution as the Tsitsikamma Forest, a coastal sub-temperate forest belt extending along the south-eastern coast and ending approximately at the eastern boundary of the Western Cape Province (Figure 2-2).

Table 2-1. Spatial and environmental variables used to explain the distribution of the painted reed frog (*Hyperolius marmoratus*) with published support for their relevance and importance.

Variable	Expected relationship with occupancy	Reasoning / preferred range of values	References
Altitude (m a.s.l., centred)	Threshold effect	Historical range is below 1600 m; expect lower altitudinal range at higher latitudes	Bishop 2004; Parmesan and Yohe 2003
Presence/absence of fringing vegetation at water body	Positive relationship	Sheltered roosting sites preferred, although frequently basks in exposed positions	Passmore and Carruthers 1995; Bishop 2004
Presence/absence of floating and emergent vegetation at water body	Positive relationship	Usually calls from vegetation above the water surface	Passmore and Carruthers 1995; Bishop 2004
Mean annual precipitation (mm)	Positive relationship	Indigenous to tropics and sub-tropics where standing water is required for breeding; chorus attendance is dependent on rainfall	Passmore and Carruthers 1995; Channing 2001; Bishop 2004;Henzi *et al.* 1995
January potential evaporation (mm; A-pan equivalent)	Negative relationship	Indigenous to tropics and sub-tropics; humid micro-habitats; locomotion increases water loss in anurans	Passmore and Carruthers 1995; Channing 2001; Bishop 2004; Preest and Pough 1989
Heat units between April and September (degree days, base 10ºC)	Positive relationship or threshold effect	Threshold number of degree days may be required for dispersal; locomotion increases water loss; highly desiccation-resistant	Preest and Pough 1989; Schmuck and Linsenmair 1997
Water body size (ha)	Threshold effect	Very small water bodies may not be suitable at several life stages as they may dry up altogether during the summer (in winter rainfall regions)	Laan and Verboom 1990
Number of water bodies within 750 m radius	Positive / threshold effect	Usually occurs close to water bodies; call audible over a distance of 400-1000 m	Passmore and Carruthers 1995; SJD unpubl. data (2012).

2.2.2   Overview of methods

I used spatially- and temporally-explicit data collected throughout the period of the range expansion to investigate the range dynamics. I began by pinpointing the spatial and temporal origin of the range expansion from historical records and those collected during this study to define the boundary between the historical and novel ranges. Systematic sampling was then used to collect additional presence and absence records inside the novel range. These were used to map the extent of occupancy and internal range structure. A sub-set of records which had accompanying habitat information was used to identify the environmental correlates of occupancy using a generalised linear model. Annual rate of spread was quantified by identifying the position of the expanding range boundary at the end of each breeding season.

2.2.3   Origin and initiation of range expansion

The geographic and temporal points of introduction into the novel range were identified in relation to the positions and dates of existing records of species occurrence. Point locality data from the South African Frog Atlas Project Database (Minter *et al.* 2004) and the provincial conservation authority CapeNature's Biodiversity Database (Turner 2006) were used to identify the position and date of the first records beyond the recorded historical range boundary at Tsitsikamma National Park headquarters (34°01′18.8″S, 23°53′47.9″E). The Frog Atlas database included records of species presence from all but one quarter degree square in the Western Cape Province, spanning the years 1905 to 2003 (Minter *et al.* 2004); CapeNature's Biodiversity Database consisted of observations of species presence from targeted monitoring and incidental observations collected from 1997 to 2008.

2.2.4   Occupancy sampling

<u>Determination of sampling extent</u>

To define the sampling extent for the study I applied a simple diffusion-based spread model based on water body availability and colonization-extinction processes in 5 km × 5 km grid cells laid over the entire Western Cape Province. In the simulation, frogs were able to move from an occupied cell to one of four neighbouring cells (von Neumann neighbourhood) if $n \geq x+2$, where n and x were the numbers of water bodies in a neighbouring unoccupied cell and the focal occupied cell, respectively. Colonisation and local extinction were modelled stochastically with $\text{col} = 1-e^{-\alpha x}$, where col is the colonisation probability per time step, and $\alpha=0.05$, and $\text{ext} = e^{-\beta x}$ where ext is the extinction probability per time step, and $\beta=0.05$ (see for example Roura-Pascual *et al.* 2009). The modelled range extent at equilibrium (after 440 time-steps) was buffered by 10

km to generate the sampling extent. This defined the maximum potential range of the painted reed frog based only on water body availability and colonization-extinction processes, and covered 39 804 km^2 (1603 grid cells; Figure 2-2).

Detection of breeding populations

Forty grid cells were randomly selected for systematic surveys within the sampling extent. Eight additional cells were visited opportunistically during sampling, giving a total of 48 surveyed grid cells. In each sampled grid cell, two to six water bodies were located by reference to an electronic topographic and water body layer (AfricaTopo Topo GPS maps. www.MadMappers.com 2005) held on a Garmin GPSmap 76CSx global positioning system. Observers visited each water body before dusk to collect habitat data, and returned later the same evening to determine occupancy.

Breeding choruses of painted reed frogs form during the summer breeding season (mid-October to mid-February) and may contain hundreds of males calling from elevated calling sites. The call is a piercing whistle repeated about once per second and is easily audible over 400-1000 m in calm weather (*c*. 104 dB at 50 cm; Passmore 1981). The call is distinct from those of all other frogs in the region. Choruses reach peak intensity approximately 30 min. after sunset, after which calling continues until shortly after midnight (Bishop 2004). Occupancy (presence/absence of a breeding chorus) was determined by auditory surveys of advertisement calls at water bodies. Observations were carried out during the peak nocturnal calling period under suitable calling conditions and lasted at least 10 min. Other studies have employed similar methods to sample anuran species occupancy (e.g. Olson *et al.* 2012).

Due to the conspicuous nature of the species and its tendency to form large choruses, no misidentification occurred (i.e. no false presences). However, to verify the level of false absence in the sampling, I tested whether the length and number of visits affected the outcome of the presence/absence detection. Five potentially suitable sites that were thought to be unoccupied were visited for an extended duration (20h30-22h45) on a single night with suitable calling conditions in February 2007. Painted reed frogs were not heard calling at any of the five test sites, although there were active choruses at nearby monitoring sites on all occasions, indicating that visit duration did not affect detection probability. In the total sample, nine sites were visited repeatedly (twice or more; a total of 27 visits). Only one occupied site was omitted as an

absence, suggesting a detection rate of 96.3% (=26/27) and a low rate of false absence in the dataset (5.8%; i.e. 11 out of the 196 absences could be presences).

2.2.5   Environmental correlates of occupancy

Occupancy and habitat data were collected between October 2006 and November 2008 for 236 water bodies in the novel range. Ninety one percent of the water bodies in the sample were artificial dams, and examination of the topographic data (Chief Directorate Surveys and Mapping 2006) showed a similar high prevalence of artificial water bodies within the sampling extent (88% of mapped water bodies) and the Western Cape Province as a whole (91%). Natural water bodies such as pans, river reaches and seasonally inundated areas were therefore excluded as a category from further analyses of environmental correlates of occupancy because of their infrequent occurrence in the dataset; overall, data for a total of 210 artificial or highly disturbed water bodies were included in the generalised linear model.

Latitude, longitude and altitude of surveyed water bodies were used to perform trend surface analysis (see Appendix 2-1). The following environmental variables were measured at all sampled water bodies: presence/absence of fringing, floating and emergent vegetation in and around the water body, water body type (dam, river, pan or pond), and water body origin (artificial, natural or disturbed but natural in origin) were estimated visually. Altitude was drawn from the Western Cape digital elevation model (Ver. 1.1 2002; 300 m-resolution; Centre for Geographical Analysis, Stellenbosch University); water body size and density (number of water bodies present within a 750 m radius of each site) were calculated from the electronic topographic and water body layer data using ArcView 3.2a and extensions (Environmental Systems Research Institute 1992-2000, Redlands, California, USA; DeLaune 2003; Jenness 2007). Climatic variables (mean annual precipitation, evaporative potential in January and winter degree days) were interpolated $1'$ x $1'$ data extracted from the South African Atlas of Agrohydrology and Climatology (Schulze *et al.* 1997).

In total, seven environmental variables, four of which reflect habitat quality and three, climatic conditions, were used to model variation in occupancy; the reasons for including each explanatory variable are given in Table 2-1, and the descriptive characteristics in Appendix 2-2. Variables with cross-correlations greater than 0.4 were excluded. The dummy variable describing the presence/absence of emergent vegetation was omitted because of its strong correlation with fringing vegetation (Spearman's *rho*=0.413). Variables for water body type and

origin were excluded as most water bodies in the sample were artificially-constructed farm dams (see above).

2.2.6   Modelling approach

A generalized linear model (analysis of covariance with a binary response variable, binomial errors and a logit link function) was used to investigate the relationship between occupancy and the selected explanatory variables. To account for spatial autocorrelation in the occupancy data, the spatial and environmental variables were modelled separately (Legendre and Legendre 1998), and then the minimum adequate spatial and environmental models were concatenated to form a final combined model (hereafter the 'combined model'). The variation explained by the combined model was thus partitioned into (a) non-spatial environmental variation, (b) spatial structuring shared by the environmental data, (c) spatial patterns not shared by the environmental data and (d) unexplained or residual variation.

I used an *a posteriori*, best-fit approach to model selection (Hobbs and Hilborn 2006) using deletion tests with Akaike's Information Criterion (AIC) to assess the effect of removing a given term from the model. Model selection was based on second-order AIC (AIC_c), a measure of relative Kullback-Leibler information content that is suitable for small sample sizes (Anderson and Burnham 2002). All analyses were conducted in R Statistical Software (Ver. 2.8.1; R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria 2010). The effect of term deletion on goodness-of-fit was assessed using χ^2 tests on nested model pairs (Crawley 2007). In all cases the more parsimonious model was selected, unless the effect of term deletion on goodness-of-fit was significant and AIC_c was reduced by term deletion. To further evaluate model performance, inflation and tolerance were calculated for all main effects in the maximal model, and the maximal and combined models were checked for over-dispersion (Diniz-Filho *et al.* 2003). Spatial autocorrelation analysis was conducted to examine the spatial structure of original data (occupancy) and predicted data from the model, and to test for such structure in model residuals. Moran's *I* statistics were calculated for the final combined model in SAM 4.0 (10 distance classes, equal numbers of point pairs; Rangel *et al.* 2006).

The direction and strength of relationships between the explanatory and response variables in the best-fit model were interpreted in the light of the hypotheses posed. Because I did not intend to use the models for predictive purposes at the scale of individual sites, confusion matrix-derived measures of model performance are not presented.

2.2.7   Range structure and extent

To calculate the range extent in the novel range I used extent of occurrence and the alpha hull. The alpha hull is a generalisation of the convex hull (or minimum convex polygon) that minimises the influence of outliers e.g. those caused by false absences (Hui *et al.* 2011). Like more complex kernel methods, the alpha hull converges on the true area occupied as the number of data points increases (Getz and Wilmers 2004; Hui *et al.* 2011). Depending on the value of alpha used, the resulting hull can be used to explore internal range structure and identify the position and extent of gaps and patches within the distribution (Burgman and Fox 2003).

Alpha hulls were drawn and measured in ArcView 3.2a and Quantum GIS (Quantum GIS Development Team, Open Source Geospatial Foundation Project 2010) following the method for threatened species assessments (IUCN Standards and Petitions Subcommittee 2011). Area measurements were made from polygons clipped to the coastline. The alpha values used here (2 and 6) span a range of values used successfully in other studies (e.g. Burgman and Fox 2003; Getz and Wilmers 2004).

Prevalence of painted reed frogs at sampled sites was similar in artificial (26%) and natural water bodies (27%), so records from both types of water bodies were used in the alpha hull analysis; i.e. all occupancy records west of the historical range boundary were included (n=347).

2.2.8   Rate of spread

The invasion of the painted reed frog into and across the Western Cape is known to have progressed chiefly from east to west (Minter *et al.* 2004; Tolley *et al.* 2008), rather than in a radial or semi-circular pattern as in other invasion processes (e.g. Phillips *et al.* 2007 for cane toads, *Rhinella marina* in Australia). Indeed, the study area as a whole is bounded by mountain ranges in the north and the coastal plain in the south, both of which are likely to constrain latitudinal spread since these mountains impose physical barriers to spread on a north-south axis, and interrupt frontal weather systems that produce most of the Western Cape's winter rainfall and low temperatures (Tyson 1986), producing a steep moisture gradient between the coast and the semi-arid inland regions. Therefore, I focused on (i) the rate of expansion along a linear axis measured as longitudinal distance from the identified point of origin of the range expansion and (ii) increase in total area occupied determined from the area of the alpha hull.

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Determinations of rate of spread can be confounded by non-uniform sampling over time and space (Suarez *et al.* 2001). In this study, the majority of sampling effort took place during the Frog Atlas period (1997-2003) and authors' surveys (2006-2009) (Appendix 2-3). To minimise the effects of sampling bias, rate of spread was determined annually as well as for the entire period of range expansion (1997-2009). Longitudinal and areal spread were then plotted against time to estimate minimum rates of spread (since observations of occupancy were made an undetermined length of time after introduction) between 1997 and 2008.

2.3   Results

2.3.1   Origin of range expansion

The range expansion of painted reed frogs in the Western Cape Province began during or shortly before the 1998 breeding season (Appendix 2-3) and originated at or near the western boundary of the Tsitsikamma Forest (NP headquarters 34°01′18.8″S, 23°53′47.9″E) (Figure 2-2). Of 1308 point localities for the species in the Frog Atlas database dating from 1917 to 2003, only 56 lay to the west of the historical range boundary at Tsitsikamma NP and all of these were collected after 1997 (Minter *et al.* 2004). Due to their conspicuous call and behaviour, it is unlikely that breeding populations could have remained undetected for more than one year prior to this putative introduction in date.

2.3.2   Environmental correlates of occupancy

Eight terms were retained in the minimum adequate spatial model after model simplification (Table 2-2). The addition of altitude did not result in significantly improved fit, so this variable was removed from the model. All environmental variables except water body area were retained in the minimum adequate environmental model. However, only January evaporative potential (negative relationship), winter degree days (positive relationship) and fringing vegetation around water bodies (positive relationship) contributed significantly (Table 2-2). Almost two thirds (64%) of variation in occupancy was explained by the combined model (Table 2-2; Appendix 2-4). The spatial variables explained 49% of variation in occupancy and the environmental variables explained 47%.

Table 2-2. Minimum adequate spatial and environmental models, and the combined model. Null deviance for all models=291.12; null d.f.=210; statistically significant model terms are identified by asterisks (p < 0.05); AIC_c: second-order AIC; w_i: Akaike weight of model in full model set. Abbreviations and units: FrVeg: presence/absence of fringing vegetation at water body; FlVeg: presence/absence of floating veg.; JanEvap: January potential evaporation (mm); WinterDD: heat units between April and September (degree days; base=10°C); MAP: mean annual precipitation (mm); WbSize: water body size (ha); Wb750m: number of water bodies within a 750 m radius.

Terms retained	Environmental terms dropped from model	Residual deviance	Residual d.f.	Log likelihood	% deviance explained	AIC_c	w_i
Spatial model: $x* + y* + x^2 + xy* + y^2* + x^2y* + xy^2 + y^3$		149.14	202	-74.572	48.77	165.9	<0.001
Environmental model: FlVeg + FrVeg* + MAP + JanEvap* + WinterDD* + Wb750m	WbSize	154.16	204	-77.017	47.05	166.6	<0.001
Combined model: $y* + x* + y^2* + xy* + xy^2* +$ FrVeg* + FlVeg + JanEvap* + Wb750m	MAP, WinterDD	104.90	201	-52.449	63.97	123.8	0.992

The combined model contained five significant spatial terms up to the third order (Table 2-2). Significant environmental variables were fringing vegetation (positive correlation) and January evaporative potential (negative correlation). Floating vegetation and water body density were retained but were not significant (Table 2-2; Appendix 2-4).

The maximal model had low variance inflation factors (all VIF<4.4 for linear regression of main effects) and there was no over-dispersion in the maximal or final combined models (residual deviance/residual d.f.=0.522 in both cases), which justified the default scaling factor of one and confirmed the adequacy of the combined model. Significant positive spatial autocorrelation in occupancy was present in shorter distance classes (classes 1-4, corresponding to 20 grid cells or 100 km). Negative spatial autocorrelation was present in longer distance classes (8-10, corresponding to 40 grid cells or 200 km) (Figure 2-1). The residuals of the combined model were not significantly autocorrelated in any distance class, demonstrating that spatial autocorrelation had been removed in the modelling process. No periodicity was evident in the correlograms.
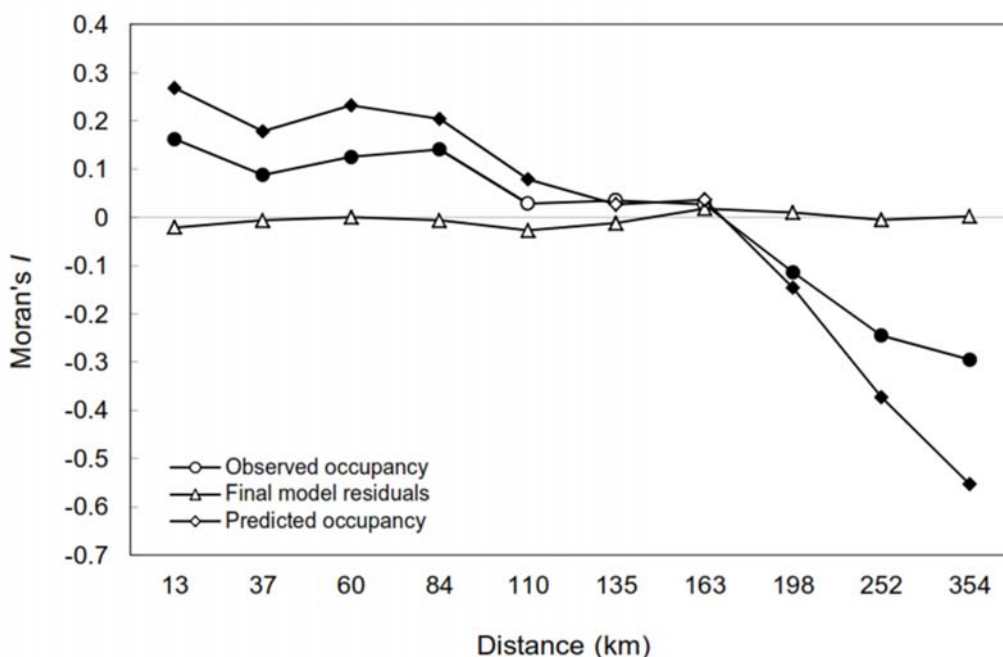
Figure 2-1. Spatial autocorrelation structure in the response variable (occupancy), model residuals, and predicted values from the combined model. Filled markers represent significant Moran's *I* values, open markers non-significant values; α=0.05; significance tested using 199 permutations; 2194 point pairs per distance class. Correlograms for occupancy and predicted values are significant, while that for model residuals is not significant (Bonferroni correction; α=0.05).

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2.3.3   Range structure and extent

The combined dataset of occupancy records from the novel range spanned 12 breeding seasons (1998 to 2009) and contained 151 presence records. The alpha hull (alpha=2) covered 2869.31 km^2 and could be divided into either two or three discrete populations under alpha=6 or alpha=2 respectively (Figure 2-2). Both alpha values resulted in a gap in occupancy in the central part of the novel range of 100 km and 140 km, respectively. Occupied sites lay in a narrow latitudinal band between 33°45′48″ S and 34°11′45″ S, but extended longitudinally from the extreme east (Goukamma Nature Reserve, 22°56′27″ E) to the extreme west of the sampling extent (Cape Peninsula, 18°23′11″ E).

2.3.4   Rate of spread

Both areal and longitudinal spread rates appeared most rapid in the second part of the study period (2004-2009) but were variable throughout 1998-2009 (Figure 2-3). Between 1998 and 1999 the frogs spread westwards from the historical range boundary to the central part of the range (marked 5 on Figure 2-2), a distance of 230 km. During this period, the species occupied 22% of its current novel range extent and was restricted to the area east of 20.4° E. Between 2000 and 2003 there was no further increase in western range limit, though the extent of occurrence increased to 30% of the 2009 range extent (Figure 2-3; Appendix 2-5), suggesting that range expansion was followed by filling of unoccupied parts of the range, rather than continuous expansion of the invasion front. In 2004, the frogs were detected around Cape Town in the extreme south-west of the province, demonstrating a further linear range expansion of about 180 km. This range expansion was also followed by local spread (SJD unpubl. data 2009).
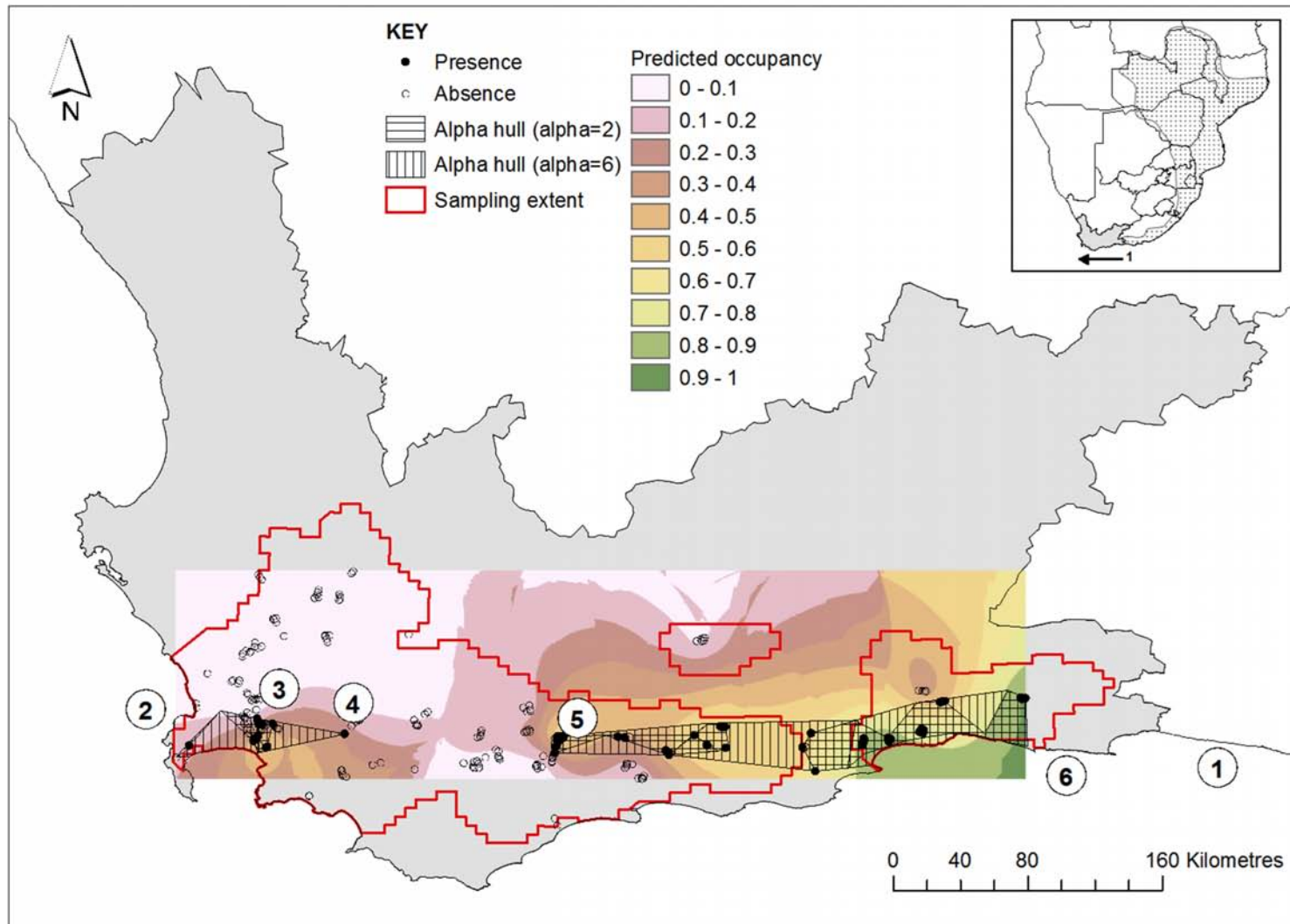
Figure 2-2. The novel range of *Hyperolius marmoratus* Rapp in the Western Cape Province, South Africa. Inset shows the historical range (stippled area; redrawn from Channing 2001; arrow shows general direction of range expansion from the putative origin). Localities: 1 - Tsitsikamma National Park headquarters, 2 - Cape Town, 3 - Stellenbosch, 4 - Villiersdorp, 5 - Swellendam, 6 - Knysna. Map projection: Geographic; datum: WGS 84. Predicted occupancy is a surface generated from the combined model (ArcMap 9.2 ordinary kriging, spherical semi-variogram model, 1 km fixed radius).
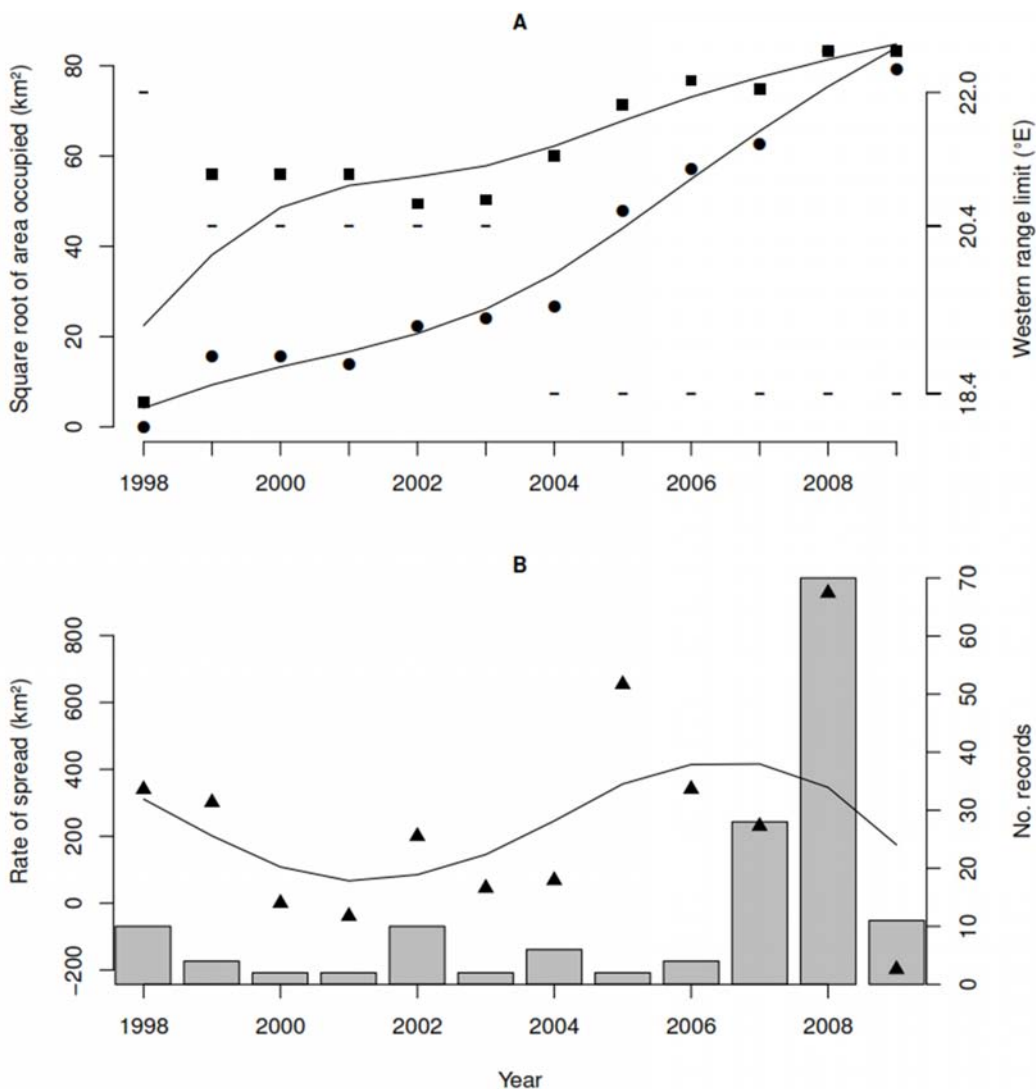
Figure 2-3. Temporal trends in *Hyperolius marmoratus* occupancy in the novel range. A: Minimum area occupied and longitudinal range limit. Area occupied was calculated from annual alpha hulls with alpha=2 (circles) and alpha=6 (squares), with curves fitted by cubic spline. Dashed line depicts the cumulative western range limit since start of invasion. B: Minimum rate of spread and number of presence records used. Rate of spread (triangles) was calculated annually and a cubic spline fitted. Number of records (bars) is the number of presence records in each season. Years refer to the breeding season ending in the specified year.

2.4   Discussion

While the susceptibility of a region to invasion is a combination of organismal, environmental and community characteristics (Richardson and Pyšek 2006), there is strong evidence that anthropogenic disturbance plays a role in facilitating invasion in several important ways. One of these is resource supplementation, as has been demonstrated for Argentine ants (*Linepithema humile*, Menke and Holway 2006) and tropical house geckoes (*Hemidactylus mabouia*, Short and Petren 2011). For pond-breeding amphibians, the presence of additional perennial water bodies

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in the landscape is even more influential, as it directly affects the availability and quality of breeding habitat (Brainwood and Burgin 2009) and increases landscape connectivity by reducing the average distance between neighbouring water bodies or creating corridors that connect them (Ray *et al.* 2002). Farm dams constitute the majority of water bodies in the Western Cape, and the painted reed frog was found in more artificial than natural water bodies (205 vs. 22 water bodies) , albeit at similar prevalence. Together with the importance of summer aridity (January evaporative potential) in the habitat model, this suggests that farm dams have played an important role in the expansion of the species by creating suitable habitat in areas that would not otherwise have provided breeding habitats in summer.

2.4.1   *Determinants of invasion and barriers to spread*

The identification of January evaporative potential and winter degree days as significant correlates of occupancy in the environmental model confirms the prediction that the presence of painted reed frogs is limited by climatic variables. However, winter degree days did not emerge as a significant variable in the combined model, probably because winter temperatures are closely related to other environmental gradients, such as altitude. Fringing vegetation around water bodies was identified as a significant correlate of occupancy in both the environmental and combined models, confirming the assertion that local habitat quality is an effective predictor of species' occupancy at local scales; this is consistent with studies on other anuran species (e.g. Brainwood and Burgin 2009).

To further investigate the constraints on range expansion identified by the generalised linear model, I undertook a post hoc analysis of environmental conditions at water bodies in the widest gap in the range (between 4 and 5 on Figure 2-2) versus those within the range boundary. Climate and local habitat quality in the gap appeared to be less hospitable to painted reed frog breeding populations than those within the range boundary (Table 2-3). For example, water bodies in the gap region had lower mean annual precipitation, higher January evaporative potential, fewer degree days in winter, and lower occurrence of fringing and floating vegetation than those elsewhere in the range, supporting the results of the model.

Table 2-3. Results of post hoc tests of environmental variable values at sites within the range versus its largest intervening gap. Environmental variables retained in the combined model are identified by [#] (not significant) and * (significant at p < 0.05). N=210 for all tests. Abbreviations and units: FrVeg: presence/absence of fringing vegetation at water body; FlVeg: presence/absence of floating veg.; EmVeg: presence/absence of emergent veg.; JanEvap: January potential evaporation (mm); WinterDD: heat units between April and September (degree days; base=10°C); MAP: mean annual precipitation (mm); Alt: altitude (m a.s.l.); WbSize: water body size (ha); Wb750m: number of water bodies within a 750 m radius.

Variable	Value inside range boundary	Value in gap	Test statistic	P-value
Categorical:	Frequency	Frequency	Odds ratio [a]	
Occupancy	53/54	1/54	0.018	<0.001
FrVeg*	106	48	0.317	<0.001
FlVeg[#]	29	8	0.379	0.025
EmVeg	86	47	0.692	0.24
Continuous/discrete:	Mean	Mean	W [b]	
JanEvap*	285.89	304.1	3124.5	<0.001
WinterDD	755.53	681	7002	<0.001
MAP	522.26	474.95	6537	0.002
Alt	194.96	221.81	4434	0.065
WbSize	2.48	2.82	4645.5	0.177
Wb750m[#]	4.2	3.84	5550.5	0.443

[a] Fisher's exact test; [b] Wilcoxon rank sum test.

In contrast, landscape variables such as altitude, water body size and water body density were similar inside and outside the range boundary (Table 2-3). Part of the gap region is covered by the Riviersonderend mountain range that rises to over 1600 m, so it is likely that rugged terrain and generally higher altitude present barriers to spread. In fact, range edges of multiple animal groups are related to biome boundaries (e.g. van Rensburg *et al.* 2004 for southern African birds) and areas of habitat and landscape heterogeneity and topographic complexity (McInnes *et al.* 2009 for the Afrotropics). The lack of a clear relationship between altitude and the range boundary in this study may be due to sparser sampling in the mountainous parts of the study area where water bodies are scarce and sometimes inaccessible to sampling. Available data on terrain rugosity (Schulze *et al.* 1997) were highly correlated with climate, and could not be included in the generalised linear model. Nevertheless, the physiological and ecological requirements of amphibians imply that climate variables are likely to play a larger role in range expansion than terrain rugosity at the scale of this investigation.

2.4.2   Rate of spread

Spread models of invasive species that assume purely diffusion-based dispersal may underestimate actual rates if at least some dispersal is in fact due to jump dispersal (Skellam 1951; Suarez *et al.* 2001; Short and Petren 2011). Such mixed-mode dispersal, as well as environmental heterogeneity, may produce non-linear spread rates (Shigesada *et al.* 1995; Kot *et al.* 1996; Hui *et al.* 2012). Spatially and temporally heterogeneous spread caused both by diffusion and human-mediated jump dispersal is consistent with the Western Cape's spatially and temporally variable topography, rainfall and rainfall seasonality (Schulze *et al.* 1997; Chase and Meadows 2007). The results highlight the coupling of different levels of anthropogenic change with long-distance translocation in driving the spread of invasive anurans, further illustrating the importance of spatially- and temporally-explicit occupancy data for quantifying spread rates and making inferences about dispersal mechanisms.

The most parsimonious explanation of the discrete populations in the novel range is that natural dispersal, or a combination of natural and human-mediated jump dispersal, facilitated spread in the eastern part of the novel range, while human-mediated jump dispersal operated in the west, either from the historical range or from another part of the novel range, across a barrier that could not be breached by natural dispersal, i.e. the region associated with the Riviersonderend mountains. The initial rapid increase of area occupied (1998-1999) and the corresponding rapid change in western range limit (Figure 2-3A) are likely caused by increased sampling effort at the start of the Frog Atlas project. However, it is possible that diffusion-based spread due to increased habitat availability (artificial water bodies) allowed the frogs to disperse westwards during this period. In contrast, the second significant change in western range limit during 2003 or 2004 strongly suggests an independent long-distance dispersal event across the Riviersonderend mountain range. Rate of spread also became more variable in the later years of the range expansion (Figure 2-3B), suggesting that both human-mediated jump dispersal and natural diffusion played a role in the range expansion.

2.4.3   Dispersal modes and mechanisms

Considerable spatial structure was found in the novel range of the painted reed frog. Retention of second- and third-order terms in the trend surface model shows that there are patches and gaps at the scale of the whole study area (Legendre and Legendre 1998). The relatively large proportion of variation shared by the spatial and environmental models (37%) suggests that the observed spatial patterns are driven by either stochastic events and processes or large-scale geographic

gradients. Given the broad longitudinal extent of the sampled area and the east-west trending climatic gradients in the Western Cape (Chase and Meadows 2007), environmental characteristics of sites are likely to be influenced strongly by their position on gradients of rainfall or rainfall seasonality.

The substantial break in continuity in the central part of the range indicates that at least two spatially discrete populations of painted reed frogs exist, separated by a distance of at least 100 km. While there are published (Bishop 2004) and anecdotal reports of adult painted reed frogs being translocated in nursery plants and fresh produce, there is no evidence of natural long distance dispersal such as transport of eggs on the feet of water birds (see also Measey *et al.* 2007). This pattern suggests human-mediated jump dispersal as the most likely mechanism by which painted reed frogs have occupied the western side of the gap. This view is supported by the finding of Tolley *et al.* (2008) that a single identical mitochondrial haplotype existed in frogs from two dams separated by approximately 40 km of mountainous terrain (marked 2 and 3 on Figure 2-2). The identification of one of these sites (3 on Figure 2-2) as a spatial outlier of the range in this study implies that human-mediated jump dispersal occurred between these two sites or that propagules were introduced independently to the two dams from the same source. However, the same haplotype is also present in a third dam in the Cape Town area (Tolley *et al.* 2008), making it highly unlikely that three independent introductions of the same haplotype could have occurred. Thus it is clear from the spread rates and range structure found in this study as well as the identification of barriers to spread, that mixed dispersal modes have contributed to the range expansion of this species. While jump dispersal and diffusion-type spread have operated in the east of the range, jump dispersal alone can account for the rapid expansion to Cape Town in 2003 or early 2004. However, diffusion-based spread is likely to be important at a local scale as jump dispersal cannot account for every introduction to a new water body.

2.5   Conclusion

By including the entire spatial and temporal extent of the recent and ongoing range expansion of the painted reed frog in the Western Cape, I have shown that the species has expanded its range rapidly over little more than a decade to occupy largely artificial habitats across the novel range. However, its distribution in the novel range is limited by summer aridity and winter low temperatures, and associated with the presence of fringing vegetation around water bodies. This extra-limital range expansion shows that a combination of human-mediated jump dispersal and landscape changes, such as the construction of artificial water bodies, allows invasive

amphibians to overcome their historical range limits and barriers to spread. It is likely that spread by natural diffusion and human-mediated events will continue in the Western Cape within the bounds of the climatic and local habitat quality requirements of the species.

In the novel range the painted reed frog appears to fill the role of 'urban exploiter' (Kark *et al.* 2007), being almost restricted to farm dams and ponds in highly modified agricultural and urban areas. It meets several of the criteria for urban exploiter status outlined by Kark *et al.* (2007), having an unspecialised diet, gregarious habits at least during the breeding season, a lack of migratory behaviour and considerable behavioural flexibility. From a conservation perspective, the unspecialised requirements, rapid spread and high local population numbers of the painted reed frog suggests that it may compete with native species, in particular the fynbos endemic arum lily frog. Given that the painted reed frog is currently present in one out of every four dams, the novel range is not saturated, and there is still room for expansion within the network of artificial water bodies available.

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Appendices

Appendix 2-1. Methods - trend surface analysis

This technique removes large-scale spatial patterns from the data, shifting the focus of the analysis to local-scale predictors (Borcard *et al.* 1992, Legendre and Legendre 1998, Diniz-Filho *et al.* 2003).

Spatial variables were centred on their means and all models were forced through the origin, allowing the intercept term to be removed from the model and reducing co-dependence between the terms of the polynomial (Borcard *et al.* 1992; Legendre and Legendre, 1998). The resulting model of spatial structure was the trend surface regression equation

$$f(x,y)=\beta_1 x + \beta_2 y + \beta_3 x^2 + \beta_4 xy + \beta_5 y^2 + \beta_6 x^3 + \beta_7 x^2 y + \beta_8 xy^2 + \beta_9 y^3$$

where x and y are longitude and latitude respectively and β_1 to β_9 are model coefficients (Magnan *et al.* 1994). Once the minimum adequate spatial model had been identified, the centred altitude term was added and the resulting change in goodness-of-fit was assessed.

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Appendix 2-2. Descriptive statistics of explanatory variables used in the models to explain *Hyperolius marmoratus* occupancy. Values in square brackets are frequencies.

Variable	Description	Min.	Median	Mean	Max.
Spatial:					
X	Longitude (°E, centred)	18.39	19.99	20.10	22.93
Y	Latitude (°S, centred)	-34.20	-33.96	-33.90	-33.10
Alt	Altitude (m a.s.l., centred)	1.28	167.80	205.00	1044.00
Habitat quality:					
FrVeg	Presence/absence of fringing vegetation at water body	0 [56]	-	-	1 [154]
FlVeg	Presence/absence of floating vegetation at water body	0 [173]	-	-	1 [37]
EmVeg	Presence/absence of emergent vegetation at water body	0 [77]	-	-	1 [133]
WbSize	Water body size (ha)	0.04	0.65	2.61	104.90
Wb750m	Number of water bodies within a 750 m radius	1	6	6	10
Climate:					
MAP	Mean annual precipitation (mm)	199	485	504	1138
JanEvap	January potential evaporation (mm; A-pan equivalent)	217.0	293.5	293.0	353.0
WinterDD	Heat units between April and September (degree days, base 10ºC)	204	740	727	1045

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Appendix 2-3. Summary of *Hyperolius marmoratus* point locality records used in the study. Years in parenthesis reflect the period over which the records were collected. The quarter degree scale data from the SAFAP Database included one record in the novel range prior to the putative introduction date; the specimen, examined in the hand, proved to be an individual of the sympatric congenor *H. horstockii* (SAFAP card no. 24026714, museum lodging code TM26714, Nov. 1960; examined 23 Sept. 2010 by SJD).

Database	Taxon	Records in database	Records W of Tsitsikamma	Records in novel range	
				Presences	Absences
SAFAP Database	All anuran taxa	24 690 (1905-2003)	4841 (1905-2003)	4200 (1917-2003)	0
SAFAP Database	*H. marmoratus*	1308 (1910-2003)	56 (1992-2003)	20 (1998-2003)	0
CapeNature	*H. marmoratus*	22	22	22 (1997-2006)	0
Authors' surveys:					
- all occupancy records	*H. marmoratus*	305	305	109 (2006-2009)	196 (2006-2009)
- occupancy with habitat data	*H. marmoratus*	210	210	54 (2006-2009)	156 (2006-2009)

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Appendix 2-4. Coefficients of terms retained in the minimum adequate spatial, environmental and combined models.

Variable	Estimate	Std. Error	Z-value	P-value
Spatial model:				
y	-22.18	8.75	-2.54	0.011
x	2.02	0.56	3.59	<0.001
y^2	-212.34	78.86	-2.69	0.007
yx	16.41	7.12	2.30	0.021
x^2	-0.37	0.23	-1.61	0.106
y^3	-318.91	176.71	-1.81	0.071
y^2x	37.55	23.41	1.60	0.109
yx^2	-4.66	1.83	-2.54	0.011
Environmental model:				
FlVeg	0.72	0.45	1.58	0.114
FrVeg	3.72	1.14	3.27	0.001
WinterDD	0.01	0.00	3.46	0.001
MAP	0.00	0.00	1.95	0.051
JanEvap	-0.04	0.01	-6.01	<0.001
Wb750m	0.16	0.08	1.83	0.067
Combined model:				
y	-56.51	17.82	-3.17	0.002
x	2.01	0.47	4.23	<0.001
y^2	-191.80	61.37	-3.13	0.002
yx	22.34	8.28	2.70	0.007
y^2x	76.93	35.28	2.18	0.029
FlVeg	1.00	0.57	1.75	0.080
FrVeg	2.78	1.07	2.59	0.010
JanEvap	-0.03	0.01	-4.63	<0.001
Wb750m	0.21	0.12	1.68	0.092

Appendix 2-5. Details of range expansion by year. Cumulative range limits in each year are represented by closed symbols (circles - western, triangles - eastern, squares - northern, diamonds - southern) and area occupied by open symbols (circles - alpha=2, triangles - alpha=6).
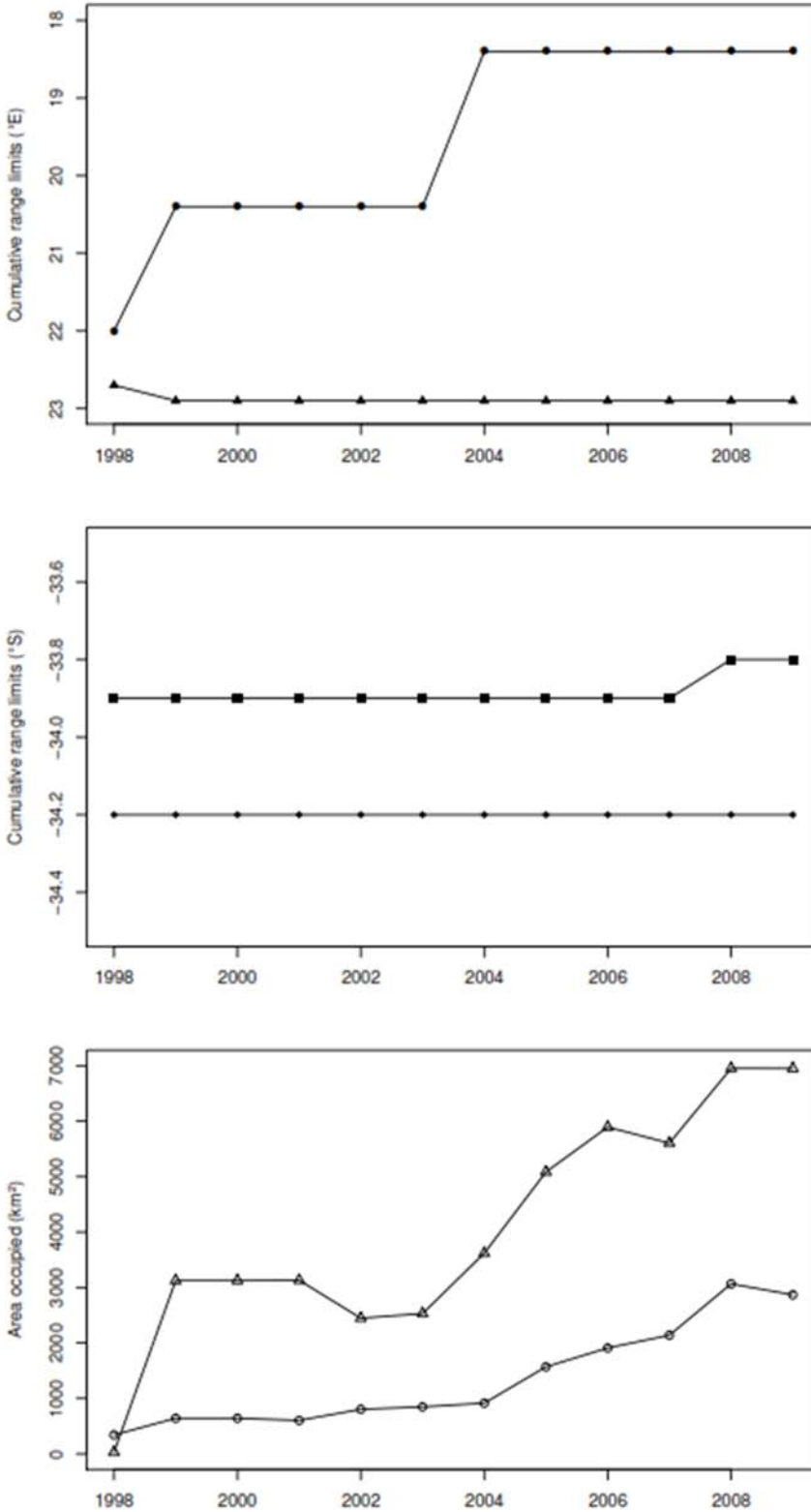
Chapter 3. Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog

In prep. (*Journal of Comparative Physiology B*)

Davies, S. J., McGeoch, M. A. and Clusella-Trullas, S. Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog.

Exposure of the permeable ventral surface during activity
Kenton on Sea, -33.689°S 26.671°E

3.1   Introduction

The capacity of organisms to respond to environmental change through morphological, physiological and behavioural plasticity is pivotal for mitigating the impacts of habitat loss, climate change, biological invasions and epidemics (e.g. Agrawal 2001; Seebacher and Franklin 2012; Cooke *et al.* 2013). Although adaptive evolution can occur in response to environmental change, it is generally slower than the time scale over which anthropogenic change occurs, and requires multiple generations to be fixed. Phenotypic plasticity, the capacity to vary the phenotypic expression of a genotype in response to environmental change, occurs within the lifetime of individuals (West-Eberhard 2003), either within a life stage as reversible or irreversible plasticity (Seebacher and Franklin 2011) or across life stages as developmental plasticity (Berrigan and Partridge 1997; Travis *et al.* 1999). Phenotypic plasticity can therefore play an important role in buffering organisms from selection and competition within generations (Peacor *et al.* 2006, Ghalambor *et al.* 2007), and facilitate their survival and dispersal when colonising new habitats. This is particularly relevant to introduced species and those establishing extra-limital populations (Lennon *et al.* 2001; Chown *et al.* 2007; Engel *et al.* 2011; Hahn *et al.* 2012). Quantifying the level of plasticity in these organisms is essential for improving our capacity to predict environmental change impacts (Westley 2011; Seebacher and Franklin 2012; Cooke *et al.* 2013).

When organisms are introduced into a new region, their establishment and spread requires that they overcome significant abiotic and biotic barriers (Richardson *et al.* 2000; Blackburn *et al.* 2011). The form and extent of phenotypic plasticity show complex patterns in invasive and indigenous animals (Chown *et al.* 2007; Janion *et al.* 2010), but it is clear that plasticity confers some fitness advantages on species occupying new habitats (Cabrera-Guzmán *et al.* 2013; Westley *et al.* 2013). For example, in the cane toad (*Rhinella marina*, =*Bufo marinus*), thermal plasticity such as full compensation of resting metabolic rate (c.f. Feder 1982) and no limitation of exercise performance even at temperatures close to the lethal limit, may be facilitating range expansion in the Australian wet/dry tropics (Seebacher and Franklin 2011; Overgaard *et al.* 2012). McCann *et al.* (2014) reported rapid adjustment of critical thermal minimum in cane toads from the southern margin of the Australian range of the cane toad, and concluded that this capacity might allow them to occupy montane areas.

In view of the predicted effects of climate change on tropical ectotherms (Deutsch *et al.* 2008; Duarte *et al.* 2012), it is important to understand the nature of acclimation responses and the

mechanisms that underlie them. Whole-animal acclimation responses may be mediated by integrated responses of temperature-performance curves of different physiological variables that act to optimize performance (see, for example, Glanville and Seebacher 2006; Rogers *et al.* 2007), or by trade-offs between them that lead to non-optimal solutions (e.g. Woods and Harrison 2002). Determination of levels of plasticity in multiple physiological traits allows the development of mechanistic models for improved prediction of organism responses to environmental change. Studies investigating reversible plasticity of multiple thermal traits in anurans are uncommon and represent only a minority of taxa and functional groups (see Lotshaw 1977 for *Rana* spp.; Carey 1979 for *Bufo boreas*; Rogers *et al.* for *Limnodynastes peronii*; Overgaard *et al.* 2012 and Seebacher and Franklin 2011 for *R. marina*). Arboreal frogs face particular thermal and hydric challenges, as they travel long distances and spend significant lengths of time away from surface water. Body size is one of the variables that influences time to desiccation (Tracy *et al.* 2010), so for small-bodied arboreal frogs the ability to avoid or delay desiccation is pivotal to survival.

In general, plasticity of metabolism is higher in temperate than tropical amphibians (Feder 1978, 1982; Christian *et al.* 1988; Navas 1996; Navas *et al.* 2008), although in some cases this relationship may be related with the altitudinal extent of the species range (Huey 1978; Navas 1996; Navas *et al.* 2008). Brattstrom (1963, 1968) found that amphibian species with small geographic ranges had lower flexibility of temperature tolerance than those with wide ranges. Feder (1978) proposed that salamanders were able to radiate from ancestral temperate areas into the neotropics because of their high thermal plasticity, specifically the capacity to compensate for higher temperatures by lowering metabolic rates and therefore metabolic costs. Therefore, one might expect a sub-tropical amphibian expanding its range in a temperate area to exhibit low plasticity.

The painted reed frog (*Hyperolius marmoratus* Rapp; body mass 1-3 g) has a broad distribution in southern African savannas (Channing 2001; Bishop 2004; Appendix 3-9) and has established breeding populations in the south-western Cape of South Africa since the late 1990s (Bishop 2004; Tolley *et al.* 2008; Davies *et al.* 2013). The novel range is characterised by a temperate, winter-rainfall Mediterranean climate and dry hot summers, in contrast with the largely sub-tropical, summer-rainfall historical range (Tyson 1986). The breeding phenology (timing of reproduction) of the painted reed frog has remained unchanged in the novel range and the peak activity period coincides with the hottest, driest period of the year (SJD, unpublished data).

Correlative models indicate that the cold winters and warm, dry summers of the novel range may limit the frogs' ability to occupy available habitat (Davies *et al.* 2013). This pattern is consistent with amphibian distributions globally (Buckley and Jetz 2007). However, correlative relationships do not necessarily reveal the causes of trait-environment relationships that lead to range limits or niche shifts (Kearney and Porter 2004; Buckley *et al.* 2010). Painted reed frog populations in the novel range provide a useful model system for investigating the magnitude and extent of plasticity in performance traits in order to facilitate prediction of future range expansion of this introduced species.

While most anurans lose water at a rate similar to that of a free water surface (Spotila and Berman 1976), painted reed frogs are highly desiccation-resistant (Geise and Linsenmair 1988; Withers *et al.* 1982). The mechanisms of this resistance are not well understood, but may involve the structure of the dorsal skin (Kobelt and Linsenmair 1986; Lillywhite 2006). In addition, resting frogs adopt a water-conserving posture with limbs tucked under the body and head lowered to be in contact with the substrate. During activity, exposure of the highly permeable ventral and axial skin is associated with high water loss rates (Geise and Linsenmair 1986; Kobelt and Linsenmair, 1986). In addition, painted reed frogs are prolonged breeders whose period and manner of calling is energetically costly (Bishop 2004; Wells 2007); the ability to attend a chorus and call for several consecutive nights increases male mating success, whereas body size and physical condition appear to play secondary roles (Dyson *et al.* 1998; see also Rogers *et al.* 2007; Llusia *et al.* 2013). Depending on the abiotic conditions encountered in the novel range, these morphological and behavioural characteristics could result in limited or enhanced phenotypic plasticity in novel range populations compared to native ones (Lamarque *et al.* 2013).

This study assesses the potential for thermal acclimation of key physiological traits to play a role in buffering the conditions encountered by frogs in the novel range. I hypothesise that the plasticity of critical thermal limits (measured by CT_{min} and CT_{max}), metabolism (resting and active metabolic rates; RMR and AMR) and evaporative water loss (resting and active WLR) may contribute to the ability of this species to occupy new sites. First, I predict that CT_{min} and CT_{max} will adjust through acclimation, with the direction of adjustment tracking the acclimation temperature (i.e. compensation). Second, because metabolic rate represents an energetic cost (Hulbert and Else 2004), selection should lower RMR to allow additional resources to be allocated to capacity functions such as movement to and from nocturnal choruses, egg

production in females, calling and combat in males (Rogers *et al.* 2007; Boratyński and Koteja 2010; Burton *et al.* 2011). Therefore, resting metabolic rate should stay constant or decrease in response to increasing acclimation temperature. I assess this by evaluating explicit predictions about the direction and shape of acclimation responses of RMR and resting WLR within a rigorous experimental framework. Five competing hypotheses are distinguished: beneficial acclimation, 'hotter is better', 'colder is better', optimal acclimation temperature and no acclimation response (after Huey *et al.* 1999; Deere and Chown 2006; Marais and Chown 2008; Kingsolver and Huey 2008, Clusella-Trullas *et al.* 2010). Predictions of the nature and direction of the expected relationships are shown in Appendix 3-8. Third, I expect AMR to be up-regulated in response to cold acclimation treatments, maintaining the capacity of biochemical processes for function and so that animals can undertake the activities required for reproduction, foraging and dispersal. Fourth, due to the high desiccation resistance of resting frogs, I predicted that resting WLR would remain constant over a range of acclimation and test temperatures, but while active, WLR should demonstrate compensatory responses.

3.2 Materials and methods

3.2.1 Collections and acclimation treatments

Adult frogs were collected from three artificial water bodies (dams) in the novel range in the Western Cape Province, South Africa (Durbanville: 33.872°S 18.624°E, Stellenbosch: 33.992°S 18.820°E, Strand: 34.098°S 18.821°E) during the peak reproductive activity period (austral summer). Male and female frogs were captured at night, placed in clear plastic bags and transported to the laboratory where they were transferred to glass aquaria (200 W x 500 L x 350 H mm) within 24 h. Aquaria were assigned to one of three temperature treatments (each treatment contained four aquaria) such that male and female frogs from each collection site were represented equally in each treatment. The temperature treatments are referred to as 'acclimations' hereafter. Aquaria were maintained inside temperature-controlled chambers (LTIE 400 and LTGC 200; LabCon, Maraisburg, South Africa) on a controlled 12 h:12 h L:D cycle for at least 14 days prior to each experiment (mean=42 d min.=15 d max.=85 d).

The temperature inside each aquarium was monitored using iButton temperature loggers (Thermochron; Dallas Semiconductor, Sunnyvale, CA, USA; www.maxim-ic.com). Aquaria contained 9-11 frogs of mixed sexes and sites of origin, *c.* 3 l of water, plants for perches (*Polyganum* sp., *Cyperus* sp., *Papyrus* sp.) and a small area of soil, and were sprayed with dechlorinated water several times daily to maintain high humidity levels. Frogs were fed *ad*

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libitum on captive-bred Mediterranean fruit flies (*Ceratitis capitata*), based on observed feeding rates (5-8 flies per individual per day). Prior to the acclimation treatments, frogs were uniquely marked with Visible Implant Alpha tags (1.2 mm x 2.7 mm) inserted under the skin of an upper hind limb (Northwest Marine Technology Inc., Shaw Island, WA, USA) and the incision sealed with tissue glue (Histoacryl; Braun, Melsungen, Germany). Non-gravid females were used as far as gravidity could be ascertained from external examination. Unavoidably during the peak breeding season, a proportion of the females collected (± 20%) were gravid. These females were not excluded because of the difficulty of finding sufficient females at breeding sites on a given night, and because I wanted to assess the effects of sex on the physiological traits.

Air temperatures were recorded in exposed calling and perching microsites at each of the collection sites for a full year prior to collections (for details see Appendix 3-1). Acclimation treatments (15, 20 and 25°C) were within the range of conditions encountered at field sites. Critical thermal limits, metabolic and water loss rates were measured on two samples of frogs collected in 2010/2011 (referred to as 'year 1') and 2011/2012 ('year 2'). In year 1, resting metabolic rate (RMR) and resting water loss rate (resting WLR) were measured first, followed by CT_{min} and then CT_{max}. In year 2, CT_{min} and CT_{max} were measured first followed by active metabolic rate (AMR) and active WLR. Post-prandial metabolic rates of anurans tend to peak within two days, and smaller body size generates a shorter time to peak (Secor 2009). Therefore, frogs were fasted for 48 h prior to experiments to minimise the effects of specific dynamic action on the experimental results. Animals were blotted with paper towel and weighed before and after each experiment (Toledo AX504, 0.1 mg precision; Mettler, Columbus, OH, USA).

3.2.2 *Critical thermal limits*

Critical thermal limits were determined on 65 frogs in year 1 (15 females, 50 males) and 63 (30 females, 33 males) frogs in year 2, using the dynamic temperature ramp method (Lutterschmidt and Hutchison 1997). Frogs were placed individually in cylindrical chambers (37 mm W x 48 mm H) embedded in a fluid-filled Perspex jacket and connected to a programmable water bath (Grant Gr150; Grant Instruments, Shepreth, UK) containing a 1:1 water-glycol mixture. Chamber apertures were partially closed with acetate film to prevent escape. Two to three frogs were tested at a time and temperatures of four experimental chambers (including an empty chamber) were recorded for the duration of experiments using thermocouples (Type T, 40 gauge) and connected to a data logger (Pico TC-08; Pico Technology, Cambridgeshire, UK). Frogs were allowed to equilibrate in the chambers for 10 min at 20°C prior to temperature ramping down

(CT_{min}) or up (CT_{max}) at a constant rate ($0.5 \pm 0.02°C.min^{-1}$ and $0.4 \pm 0.05°C.min^{-1}$, respectively).

For CT_{min}, frogs were first observed at 20, 15, 10, 9, 8 and 7°C and then turned over their backs every 1°C and from 3°C, every 0.5°C, to check their righting response (Lutterschmidt and Hutchison 1997). CT_{min} was reached when a frog could not right itself. For CT_{max}, frogs were observed at 20, 25, 30 and 35°C degrees and then checked every 1°C. CT_{max} was recorded when frogs were unable to adhere to the chamber wall, fell on their backs and were unable to right themselves. After all trials, the frogs were placed at room temperature to recover for 10 min before being weighed (all individuals fully recovered). Critical thermal limit trials were performed ~50 days after RMR and resting WLR experiments and frogs were allowed to recover for ~16 days in between CT_{min} and CT_{max} trials. Frogs were maintained at their acclimation temperatures between experiments.

3.2.3 *Resting metabolic rate and water loss rate*

Measurements were made on 105 frogs (13 females, 92 males) from the same collection made for year 1 critical thermal limit experiments. Experiments were conducted during the natural resting period (08h00-18h00, Telford and Dyson, 1988), at test temperatures of 15, 20 and 25°C; lights were kept on throughout the experiments. Eight to ten individuals were assayed at a single randomly-selected test temperature on each day; each individual was tested at a single temperature in a randomised block design. Flow-through respirometry was conducted using a LI7000 infrared CO_2 and H_2O analyser (LiCor, Lincoln, NE, USA). VCO_2 was measured in preference to VO_2 because of the greater sensitivity of VCO_2 given the small body size of this frog. The gas analyser, together with the glass cuvette (12.5 ml) and activity detector (AD-1; Sable Systems International, Las Vegas, NV, USA) were placed inside a temperature-controlled chamber. Dry, CO_2-free compressed air (20.9% O_2) was scrubbed with soda lime, silica gel and Drierite (Xenia, OH, USA) and flushed at 100 ml min^{-1} through the cuvette (system response time: 35 seconds for 99% exchange; Lighton 2008) using a two-channel mass flow controller (Sierra Instruments, Monterey, CA, USA). Baseline readings were taken for 10 min before and after each recording to correct for analyser drift. Temperature inside the cuvette was monitored using thermocouples connected to a Pico TC-08 data logger. Each frog was allowed to equilibrate to the targeted cuvette temperature for 10 min prior to recordings, which lasted ~45min, and was weighed before and after each measurement. Frogs settled within 1 minute in the small cuvette and stayed immobile against the curved glass surface.

3.2.4   Active metabolic rate and water loss rate

Experiments were conducted during the main activity period of the frogs (18h00-23h00, Telford and Dyson 1988), using an experimental set-up similar to that described for RMR above, but with a flow rate of 200 ml min^{-1} and a 53 ml glass cuvette (response time: 73 seconds); lights were kept off inside the temperature-controlled chamber. Animals were allowed to equilibrate and rest inside the cuvette for 10 min. Thereafter, the cuvette was rotated at a constant rate by a motor (approx. 15 rpm, 0.023 m s^{-1}). Frogs had to move continuously to maintain an upright position and were prevented from clinging to the side walls of the cuvette by two v-shaped wires placed inside the chamber and controlled by an external magnet on a camshaft (Appendix 3-10). This level of activity was assumed to be higher than voluntary activity and lower than critical activity levels (Walsberg 1986; O'Steen and Bennett 2003), and to relate to dispersal or sustained locomotion (rather than capacity). In pilot trials, animals became exhausted after about 10-12 min., so activity tests were limited to 10 min and monitored with an infra-red webcam (Genius eFace 1325R; Genius, Taipei, Taiwan). Ten male frogs from each acclimation treatment (15°C, 20°C and 25°C) were tested at 15°C, 25°C and 35°C in a full factorial design (n=30). Because repeated exercise trials have been shown to lead to a 'training' effect (reviewed in Davison 1997), individuals were subject to test temperatures in random order and separated by a recovery period of at least seven days (mean ± s.d.: 9 ± 3 days).

3.2.5   Data analysis

All analyses were conducted in R version 2.14.0 (R Development Core Team 2010). Body mass, metabolic rate and water loss rate were log$_{10}$ transformed to improve the normality of the data. However, non-parametric tests were used where necessary, recognising that for smaller sample sizes the assumption of normality may introduce substantial bias into statistical testing (Stewart-Oaten 1995). Unless otherwise stated, all pairwise tests are Wilcoxon rank sum tests with Bonferroni correction for multiple tests. Selected interaction terms of interest were included in initial models and model reduction was carried out using likelihood ratio tests and Akaike weights (Anderson and Burnham 2002). Parameter strengths were assessed using the CAR package in R (Fox and Weisberg 2011). Effect sizes (plasticity) were expressed as the difference between the mean trait values for each acclimation treatment group and the overall mean trait value. Correlations between traits were examined using non-parametric Spearman's rank correlation coefficients (*rho*).

Analysis of covariance (ANCOVA) was used to test for the effect of acclimation (ordered categorical variable) and body mass measured before the experiment (continuous covariate) on critical thermal limits. Because sites are known to have different invasion histories, and introductions are ongoing (Tolley *et al.* 2008; Davies *et al.* 2013), collection site was included as a categorical covariate.

The mean body mass of sampled frogs was 1.3 g (initial weight after collection). In both sampling years, frogs from Durbanville were largest. In year 1 they were significantly larger than Strand frogs (z=-2.810, P=0.005) but not significantly different from Stellenbosch frogs (z=-0.311, P=0.755); in year 2 Durbanville frogs were significantly larger than Stellenbosch frogs (z=-2.431490, P=0.015) but not those from Strand (z=-1.578846, P=0.114). Sex was included as a covariate in models to determine whether there was a mass-independent effect of sex. Females were significantly larger than males (females: 1.6 ± 0.4 g, n=46; males: 1.2 ± 0.3, n=150; W=2925.5, P < 0.001, across all animals collected). Males were larger in the second year than in the first year (year 1: 1.3 ± 0.2 g, year 2: 1.4 ± 0.3 g, W=544, P=0.009) but no inter-annual differences in mass were found for females (year 1: 1.5 ± 0.3 g, year 2: 1.7 ± 0.3 g, W=159, P=0.116). Therefore critical thermal limit data collected in years 1 and 2 were analysed separately. There were no significant differences in body mass among acclimation treatments (15°C: 1.4 ± 0.3 g, 20°C: 1.4 ± 0.3 g, 25°C: 1.4 ± 0.2 g, z ≤ 0, P > 0.1 for all comparisons).

Respirometry data were processed in Expedata ver. 1.0.24 (Sable Systems International, Las Vegas, NV, USA). For resting trials, the portion of each trace with the lowest CO_2 in ppm and H_2O in ppt corresponding to a resting state from the activity detector trace was selected (20 ± 8 min, n=105) and transformed to ml CO_2 h^{-1} and mg H_2O h^{-1} using standard equations (Lighton 2008). ANCOVA was used to test for differences among acclimation treatments, test temperatures, sex and collection site, while adjusting for body mass, on the resting metabolic and water loss rate. I used ordered factors ANOVA with orthogonal polynomial contrasts to identify the specific form and direction of the acclimation response for RMR and resting WLR (Huey *et al.* 1999; Clusella-Trullas *et al.* 2010). Explicit predictions for the direction and shape of acclimation responses of RMR, resting WLR and AMR were tested among five competing hypotheses (Huey *et al.* 1999; Deere and Chown 2006; Marais and Chown 2008; Kingsolver and Huey 2008; Clusella-Trullas *et al.* 2010). Briefly, the 'beneficial acclimation' hypothesis predicts that individuals acclimated to a particular environment (in this case an acclimation temperature) will perform better in that environment than individuals acclimated to other

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environments. In contrast, the optimal acclimation temperature hypothesis predicts that animals acclimated at intermediate temperatures will perform better at that temperature than individuals acclimated at higher or lower (i.e. more extreme) temperatures. 'Hotter is better' and 'colder is better' describe the situations in which individuals acclimated to warmer or cooler temperatures, respectively, have enhanced fitness compared to individuals exposed to other temperatures. 'No acclimation response' predicts no plasticity in the focal trait. These hypotheses lead to the expected relationships shown in Appendix 3-8. Orthogonal contrasts were formulated with the residuals of the metabolic rate-body mass relationship as the response variable, and acclimation treatment (15°C < 20°C < 25°C) and test temperature (15°C < 20°C < 25°C) as ordered factors (Crawley 2007). Sexes were pooled for this analysis. Within-group sample sizes (10 to 13 individuals per acclimation treatment × test temperature group) closely approximated a balanced design.

Because each individual frog was subject to repeated measurements of AMR at different test temperatures, linear mixed-effects models were used with acclimation temperature, test temperature, site and body mass as fixed effects and individual as a random effect. The addition of the random effect significantly improved model fit (Zuur *et al.* 2008; Bates 2010). However, mixed modelling was inappropriate for active WLR data because the addition of the random effect to the model did not significantly improve model fit, so a generalised linear model with Gaussian error structure and identity link function was used to model active WLR.

3.3 Results

3.3.1 *Microsite temperatures at breeding sites*

Microsite temperatures at collection sites in the year preceding the first year of sampling (during which study animals may have metamorphosed) showed that monthly minimum temperatures at Durbanville (mean=7.3°C) were approx. 1°C higher than those at Stellenbosch (6.4°C) or Strand (6.6°C) but this difference was only marginally significant (mixed model t=-2.032; P=0.054; fixed factor: site, random factor: month; see also Appendix 3-1). Mean monthly maximum temperatures did not differ among sites (mean=34.2°C; all t-values less extreme than -1.922; all P > 0.068).

3.3.2 *Critical thermal limits*

CT_{min} ranged from 1.2 ± 1.4°C to 3.1 ± 1.0°C depending on the acclimation treatment applied (Appendix 3-2). Pairwise tests showed that CT_{min} was significantly higher in frogs acclimated at

25°C than in those acclimated at 20°C in both years (year 1: 2.1 ± 0.8 vs. 1.3 ± 0.9°C, P=0.002; year 2: 3.1 ± 1.0 vs. 2.2 ± 0.8°C, P=0.011) and significantly higher than in frogs acclimated at 15°C in year 1 (2.1 ± 0.8 vs. 1.2 ± 1.4°C, P=0.012; Figure 3-1; Table 3-1). The effect size of acclimation temperature on CT_{min} was 0.3°C. In all treatment groups, CT_{min} was lower in year 1 than in year 2 (Figure 3-1, Appendix 3-2). In year 1, frogs from Durbanville had higher CT_{min} than those from Stellenbosch, with Strand being intermediate between the others (Figure 3-1; Table 3-1). There were no consistent inter-site differences in year 2. The linear relationship between CT_{min} and body mass showed that CT_{min} increased with body mass in 15°C- and 20°C-acclimated frogs ($F_{1,39}$=14.200, P < 0.001, $F_{1,42}$=9.879, P=0.003, respectively) but did not change with body mass in 25°C-acclimated frogs ($F_{1,41}$=0.053, P=0.819).

Table 3-1. Best-fit ANCOVA models for CT_{min} and CT_{max} using combined data from year 1 (2010/2011) and year 2 (2011/2012). ACC: acclimation temperature treatment (15, 20 or 25°C). Bold text indicates significant parameters (alpha=0.05). Model results for the separate years are shown in Appendix 3-3.

CT_{min} *

Variable	DF	Type III SS	F value	P value
Intercept	**1**	**32.659**	**31.961**	**<0.001**
ACC	**2**	**16.950**	**8.294**	**<0.001**
Site	**2**	**13.842**	**6.773**	**0.002**
Sex	1	1.624	1.589	0.210
Year	**1**	**27.752**	**27.159**	**<0.001**
Body mass	1	0.328	0.321	0.572
ACC × body mass	**2**	**6.964**	**3.408**	**0.036**
Residuals	118			

CT_{max} #

Variable	DF	Type III SS	F value	P value
Intercept	**1**	**19095.900**	**4249.019**	**<0.001**
ACC	**2**	**52.300**	**5.813**	**0.004**
Site	2	16.500	1.831	0.165
Sex	1	0.500	0.113	0.738
Year	1	0.000	0.010	0.920
Body mass	**1**	**23.700**	**5.283**	**0.023**
ACC × year	**2**	**30.900**	**3.438**	**0.035**
Residuals	118	530.300		

Body mass was $\log_{10}$ transformed prior to analysis. * Overall model multiple R^2=0.4217, $F_{9,118}$=9.561, P<0.001. # Overall model multiple R^2=0.4198, $F_{9,118}$=9.485, P<0.001.

CT_{max} increased with acclimation temperature, but this effect differed between years (acclimation × year interaction, Table 3-1, Figure 3-1). The effect size of acclimation treatment on CT_{max} was 1.3°C and group means ranged from 41.2 ± 2.7°C to 46.4 ± 2.0°C depending on treatment temperature and sampling year (Appendix 3-2). In year 1, 25°C-acclimated frogs had

significantly higher CT_{max} than those acclimated at 15°C, but not 20°C; in year 2, CT_{max} increased significantly with treatment temperature (Figure 3-1; Appendix 3-3). Frogs acclimated at 15°C had higher CT_{max} in year 1 than in year 2, but this was not true for the other treatment groups (Figure 3-1, Appendix 3-2). The body mass effect on CT_{max} was negative in all treatment groups, but was only significant in the 20°C treatment ($F_{1,42}$=7.119, P=0.011).
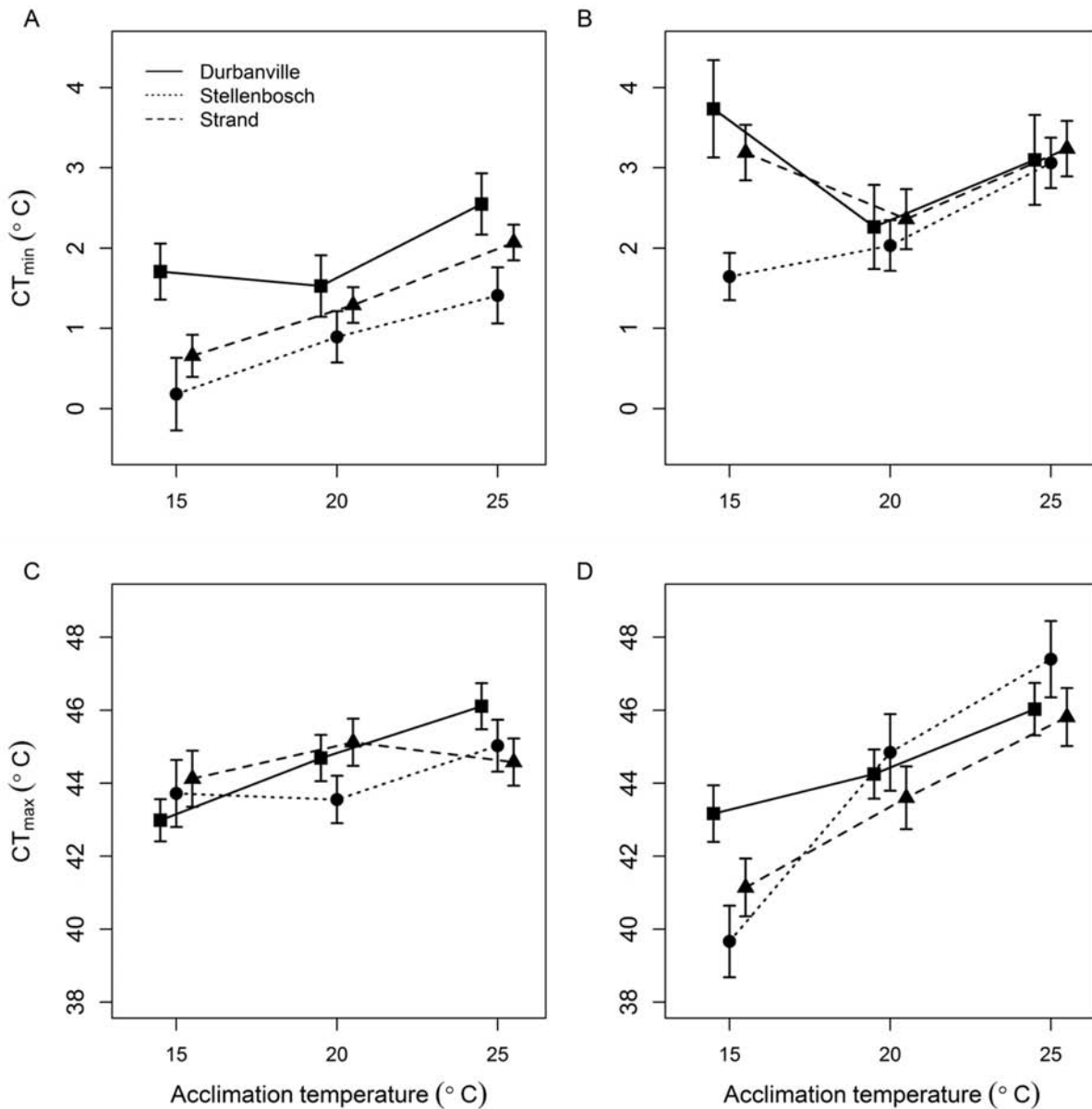
Figure 3-1. Critical thermal minima (CT_{min}) and maxima (CT_{max}) of painted reed frogs from three sites in the novel range tested in two different years. A and C: year 1 (2010/2011), B and D: year 2 (2011/2012). Data are means ± s.e.m.

The mean thermal tolerance range (calculated from absolute differences between CT_{min} and CT_{max}) across all acclimation treatments was 42.1 ± 2.9°C (min.=32.4°C, max.=48.0°C).

Thermal tolerance range increased with treatment temperature ($F_{1,120}$=14.280, P < 0.001) with an effect size of 1.2°C, and decreased with body mass ($F_{1,120}$=13.347, P < 0.001).

3.3.3 *Metabolism and water loss*

During RMR trials, frogs remained inactive with limbs and phalanges tucked under the body, head lowered and gular area in contact with the substrate. This posture has been described as a typical resting and water saving strategy in this species (Geise and Linsenmair 1988). Resting MR was positively related to test temperature and body mass (Figure 3-2 A, Appendix 3-4). 15°C-acclimated frogs had significantly lower RMR than 20°C-acclimated frogs (Appendix 3-4). Orthogonal polynomial contrasts revealed a linear positive relationship between RMR and acclimation treatment; a quadratic increasing relationship between RMR and test temperature, which had the strongest effect on RMR; and a non-significant interaction between acclimation treatment and test temperature (Table 3-2). Overall, the results indicate partial support for 'colder is better' rather than beneficial acclimation (see predictions in Methods).

Table 3-2. Results of ordered factors ANOVA with orthogonal polynomial contrasts on resting metabolic rate of *Hyperolius marmoratus*. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 20 or 25°C). L denotes the linear contrast and Q the quadratic contrast. Bold text indicates significant parameters (alpha=0.05).

Variable	DF	Type III SS	F value	P value
Intercept	1	0.002	0.202	0.654
ACC	**2**	**0.089**	**4.898**	**0.009**
TT	**2**	**1.427**	**78.803**	**<0.001**
ACC × TT	4	0.039	1.065	0.378
Residuals	96	0.869		
	DF	Contrast SS	F value	P value
ACC (L)	**1**	**0.057**	**6.345**	**0.013**
ACC (Q)	1	0.003	0.373	0.543
TT (L)	1	0.014	1.564	0.214
TT (Q)	**1**	**1.405**	**155.162**	**<0.001**
ACC (L) × TT (L)	1	0.006	0.606	0.438
ACC (Q) × TT (L)	1	0.003	0.326	0.569
ACC (L) × TT (Q)	1	<0.001	0.029	0.865
ACC (Q) × TT (Q)	1	0.030	3.301	0.072

Overall model multiple R^2=0.636, $F_{8,96}$=20.96, P<0.001. Metabolic rate was $\log_{10}$ transformed prior to analysis. Contrasts were carried out using the residuals of the metabolic rate-body mass relationship.

Water loss rate did not vary significantly with acclimation treatment or test temperature while frogs were at rest (Figure 3-2, Appendix 3-4). Body mass was the only significant predictor of resting WLR, and the ANCOVA model had limited explanatory power (Appendix 3-4). In

orthogonal polynomial contrasts, neither acclimation treatment, test temperature nor the interaction between them was significant (Appendix 3-5). Resting metabolic rate and resting WLR were correlated in only one of the nine treatment groups (15°C-acclimated frogs tested at 20°C: *rho*=0.664, P=0.031; all other *rho* ≤ 0.51 and P > 0.05).
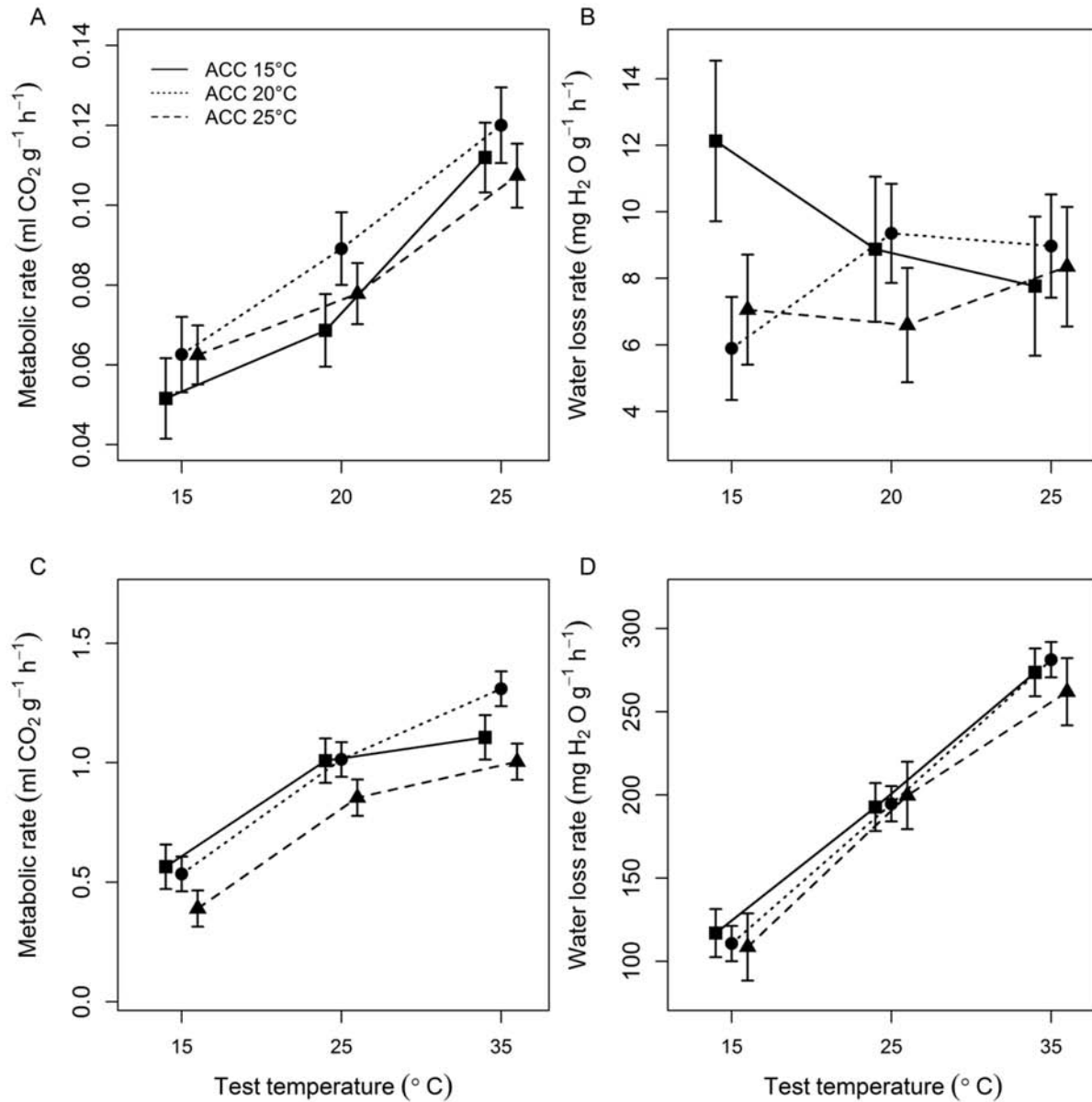
Figure 3-2. Temperature dependence of resting metabolic rate and resting water loss rate of adult painted reed frogs acclimated at 15, 20 and 25°C. Panels A and B show resting rates, panels C and D show active rates. Data are mass-adjusted mean ± s.e.m. Note the different test temperatures used in resting (15, 20 and 25°C) and active experiments (15, 25 and 35°C).

The best fit mixed model of AMR included significant effects of acclimation temperature, test temperature and body mass (Table 3-3). However, only test temperature explained a substantial proportion of the deviance in the data, with higher temperatures resulting in higher metabolic

rates (Table 3-3; Figure 3-2). AMR declined with increased acclimation temperature, with significantly lower rates in 25°C-acclimated frogs than in 15°C- and 20°C-acclimated frogs indicating compensation in this trait (Table 3-3, Figure 3-2). Active WLR did not change with acclimation treatment, but increased strongly with test temperature, and was not affected by body mass (Figure 3-2, Appendix 3-6). Nearly half of the variance in water loss rate was explained by test temperature (Appendix 3-6).

Table 3-3. Results of best-fit linear mixed model fitted to active metabolic rate data of painted reed frogs. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 25 or 35°C). Bold text indicates significant parameters (alpha=0.05).

Parameter	DF (num., den.)	F value	P value	% Deviance explained [a]
Intercept	**1, 58**	**7.553**	**<0.001**	
ACC	**2, 26**	**17.989**	**<0.001**	**5.485**
TT	**2, 58**	**202.268**	**<0.001**	**65.979**
Body mass	**1, 26**	**18.106**	**<0.001**	**2.760**
	Estimate	s.e.m.	T value	P value
Intercept	**-0.254**	**0.030**	**-8.477**	**<0.001**
ACC 20°C	0.026	0.0267	0.975	0.339
ACC 25°C	**-0.100**	**0.026**	**-3.893**	**0.001**
TT 25°C	**0.293**	**0.019**	**15.355**	**<0.001**
TT 35°C	**0.361**	**0.019**	**18.928**	**<0.001**
Body mass	**0.713**	**0.168**	**4.255**	**<0.001**

Body mass and metabolic rate were $\log_{10}$ transformed before analysis. Random effect: 1|indiv. [a] Deviance explained is from the pamer.fnc function in LMERConvenienceFunctions package.

3.4 Discussion

3.4.1 Patterns of plasticity in critical thermal limits

As I predicted, frogs acclimated at warmer temperatures had higher critical thermal limits than did those acclimated at cooler temperatures. The response of CT_{max} to acclimation temperature indicates that plasticity in the upper critical limit might confer a benefit by increasing survival in marginal thermal habitats and extending the fundamental niche (Kearney and Porter 2004; Rogers *et al.* 2007). The higher plasticity of upper than lower critical limits in this species is atypical among ectotherms, in which CT_{max} is usually more constrained than CT_{min} (Snyder and Weathers 1975; Sunday *et al.* 2011; Hoffmann *et al.* 2013). However, this magnitude of plasticity of CT_{max} is in line with data for both widespread and restricted-range bufonid, hylid and ranid species (in which CT_{max} can be adjusted upwards by 4 to 6°C in response to acclimation treatment - Brattstrom 1968), and with a previous study of this species (Geise and Linsenmair 1988).

CT_{min} showed a more complex spatial and temporal response to acclimation treatment than did CT_{max}. Both site and year affected CT_{min}, possibly as a result of developmental plasticity, if higher minimum temperatures experienced during development resulted in higher CT_{min} in adults. Similar effects have been found in some insects (Berrigan and Partridge 1997; Hoffmann and Hewa-Kapuge 2000). Indeed, CT_{min} was highest in frogs from the warmest site, Durbanville. Painted reed frogs reach reproductive maturity after one to two years (Bishop 2004), so spatial variation in environmental temperatures in 2008-2009 may account for variation in adult cold tolerance during the experimental period (2010-2012). An alternative explanation for the observed inter-annual variation in CT_{min} is that critical thermal limits are influenced by genetic variation among populations (Tolley *et al.* 2008).

3.4.2   *Response of metabolism to acclimation treatment and test temperature*

The lower RMR of frogs acclimated at the coldest temperature compared with those from the warmer treatments provides partial support for the 'colder is better' hypothesis, and suggests that these frogs can withstand seasonal cooling by minimising the costs of resting metabolism. This pattern is consistent with the compensation model of the fitness effects of basal metabolism (Boratyński and Koteja 2010; Burton *et al.* 2011) which suggests that lowering resting metabolic rate allows extra resources to be allocated to activity by decreasing the baseline of aerobic metabolism (Killen *et al.* 2007). Indeed, the greatest difference between resting and active CO_2 production in this study was in cold-acclimated frogs tested at the coldest temperature.

Interpreting whether an acclimation response is beneficial or not is contingent on the ecology and behaviour of the organisms being studied (Huey *et al.* 1999) and on the tissues or metabolic pathways involved in acclimation (Rogers *et al.* 2007). I judged the decrease in AMR with exposure to the highest treatment temperature (25°C) to be advantageous, allowing frogs to conserve energy and sustain high levels of activity over the summer, when energetically costly reproductive activities such as chorus attendance and calling in males and egg production in females take place. The down-regulation of AMR in warm-acclimated frogs could also indicate that physiological stress plays a role in the acclimation treatment response at high temperatures. Although 25°C is not a stressful temperature in absolute terms, constant exposure to this temperature over a long period may result in production of heat shock proteins (Bailey and Lazaridou-Dimitriadou 1991; Dong *et al.* 2011; Marshall *et al.* 2011). The increase in AMR with test temperature occurred over the full 15-35°C range, suggesting that the activity of painted reed frogs is not limited by short-term exposure to high environmental temperatures

To inform predictions of dispersal ability in this species, I estimated the vagility of adult *H. marmoratus* in different thermal environments following Hillman *et al.* (2014). Average AMR values of frogs from each acclimation treatment, but tested at 25°C, were used along with published values for respiratory quotient (RQ) and cost of transport (COT). An RQ of 0.74 for calling male *H. marmoratus* determined by Grafe (1996) was used. COT was estimated as 0.002 ml g^{-1} m^{-1} from the value published for a similar-sized North American frog with a similar lifestyle, *Pseudacris crucifer* (=*Hyla crucifer*) tested at 21°C (Gatten *et al.* 1992) as no data are available for *H. marmoratus*. The vagility predicted from these values was 362 m h^{-1}, 368 m h^{-1} and 321 m h^{-1} for frogs acclimated at 15°C, 20°C and 25°C, respectively. This level of vagility is intermediate between predicted values for a ranid (171 m h^{-1}) and a bufonid species (441 m h^{-1}; Hillman *et al.* 2014) and provides a broad indication of the movements that may be possible under optimal conditions for dispersing painted reed frogs.

3.4.3 Lack of plasticity in water loss

Resting frogs maintained a water-conserving posture and had very low rates of evaporative water loss (5.89 mg g^{-1} h^{-1} to 12.13 mg g^{-1} h^{-1}) when measured in dry air. These findings are in accord with the high cutaneous resistance (*R*) known for this species and its close relatives (Geise and Linsenmair 1988; Withers *et al.* 1982). The mechanism behind the high *R* in this species is unknown; it could be related with skin structure, such as the presence of large numbers of iridophores and low vascularisation, but not to skin secretions as in some other anuran species (Withers *et al.* 1982, *c.f.* Shoemaker *et al.* 1989; Gomez *et al.* 2006). The lack of response of resting WLR to test temperature and the sizeable change in WLR between resting and active frogs illustrate the profound effect that behaviour, specifically body posture, has on evaporative water loss in this species. Since maximum environmental temperatures are experienced during the day, frogs need to seek optimal resting microsites when water loss associated with activity is at its highest. Reliance on a particular body posture to minimise water loss may therefore limit mobility and prevent frogs from regulating body temperature precisely (see Tracy 1976; Buttemer 1990; Tracy and Christian 2005). The lack of evidence for acclimation of resting and active WLR suggests that the water-conserving posture may inhibit selection for phenotypic plasticity in water loss in this species, reflecting behavioural inertia in which behaviour hinders rather than drives evolutionary change in water loss rate (Huey *et al.* 2003; Pichegru *et al.* 2010). However, this scenario may be unlikely as the costs associated with the maintenance of a water saving posture should be high in most conditions.

Because of their relatively impermeable dorsal surface (i.e. high cutaneous resistance, $R > 500$ s cm^{-1}; Withers *et al.* 1982), highly desiccation-resistant frogs cannot necessarily use evaporative cooling effectively, and may have body temperatures that are equal to or elevated above ambient (Passmore *et al.* 1985). This is particularly likely to be true if they are small and thus confined within the boundary layer of the substrate (Tracy *et al.* 2010). Under conditions of heat stress, these frogs may make use of evaporative cooling by releasing mucus from skin glands (Geise and Linsenmair 1986; Tracy et al 2008). The presence of this mechanism suggests that a trade-off could exist between evaporative cooling as a thermoregulatory strategy and evolving a high R to minimise water loss. In other words, the lack of plasticity of water loss, combined with the reed frogs' known high R, suggests that desiccation resistance has evolved at the expense of plasticity in R, and that the reduction of R needed to aid evaporative cooling as a thermoregulatory strategy was not a major priority for this species. This may be attributed partly to high availability of optimal thermal habitat within the species' native range, and underscores that absolute environmental tolerance may be traded off against plasticity of tolerance. Such a possibility is not unreasonable, as for example, in ectotherm heat tolerance, trade-offs between elevated basal tolerance and high plasticity of tolerance have been found in interspecific comparisons (e.g. Stillman 2003).

Neither resting nor active *H. marmoratus* showed the sharp or non-linear increase in WLR at high temperatures observed in some water-resistant frogs such as Australian *Litoria bicolor* and *L. xanthomera* (Tracy *et al.* 2008). However, evaporative cooling at temperatures close to CT_{max} was recorded in West African *H. viridflavus nitidulus* ($=H.$ *nitidulus*) (Geise and Linsenmair 1986), a closely related species to *H. marmoratus*. It is possible that I did not observe these changes in the study animals simply because I exposed them to average conditions and not extremes, i.e. test temperatures were not high enough to elicit this response.

The fact that no plastic responses were found following the short-term exposures used in this study could indicate that a longer temperature regime (e.g. seasonal cycle) may be needed to elicit the physiological changes necessary to modify water loss rate such as changes in membrane structures. Geise and Linsenmair (1986) found that *H. viridflavus nitidulus* collected from West African savannas during the dry season ('dry season frogs') had exceptionally low water loss rates while 'wet season frogs' had water loss rates similar to those of non-desiccation resistant species. The water loss rates found in this study of South African frogs were consistent

with the dry season pattern described by Geise and Linsenmair (1986) - maintaining low WLR with no plasticity, and relying on their posture and skin properties to minimise water loss. Therefore, it is possible that the close coupling between desiccation resistance and thermoregulation (Navas *et al.* 2008; Tracy *et al.* 2008, 2010) has dampened the development of plasticity in WLR of painted reed frogs. The mechanisms behind the lack of plasticity (e.g. skin properties) need further investigation in these and other 'waterproof' frogs.

3.5   Conclusion

Examination of the response of key physiological traits to thermal acclimation demonstrated substantial plasticity in thermal tolerance (particularly the upper thermal limit), resting and active metabolism but not in water loss rate at rest or during sub-maximal activity. The results suggest that plasticity of thermal tolerance and metabolism is beneficial. However, the failure to detect plasticity in water loss indicates that *H. marmoratus* may be restricted to areas near permanent water in the summer reproductive season, while the ability to maintain activity during cooler periods may drive increased dispersal in winter. These constraints clearly have the potential to influence future dispersal and inter-pond movement patterns at least in summer in the novel range.

3.6   References

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Appendices

Appendix 3-1. Environmental temperatures (monthly mean, minimum and maximum) measured at the collection sites. Temperature data (°C) was recorded hourly from October 2008 to November 2009, 1 year prior to collection of the first experimental animals, at semi-exposed calling sites ± 1 m above water level among fringing vegetation (iButton Hygrochron temperature and humidity loggers; Dallas Semiconductor, Sunnyvale, CA, USA; www.maxim-ic.com). Values in the header row are mean CT_{min} and CT_{max} for frogs from each site, averaged across all acclimation treatments. See Appendix 3-8 for locations of sites.

	Durbanville (CT_{min} 2.3°C, CT_{max} 44.5°C)			Stellenbosch (CT_{min}1.7°C, CT_{max} 43.9°C)			Strand (CT_{min} 2.2°C, CT_{max} 44.1°C)		
Month	Average	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.
Nov 08	17.5	9.5	34.3	18.2	6.7	34.1	18.6	5.8	39.2
Dec 08	19.4	13.4	31.1	20.8	8.3	37.7	21.2	7.7	39.1
Jan 09	20.1	10.3	38.0	21.0	9.9	33.9	22.2	9.3	40.7
Feb 09	22.8	9.9	43.6	22.3	9.7	38.5	23.8	9.8	46.1
Mar 09	21.4	9.9	46.8	20.4	8.4	43.8	20.8	6.6	47.1
Apr 09	17.6	9.5	38.0	17.5	8.1	39.7	16.3	8.6	31.3
May 09	14.5	7.1	33.3	14.4	6.8	31.3	13.1	6.7	22.6
Jun 09	13.1	3.8	30.4	12.8	3.8	30.5	12.0	4.6	22.0
Jul 09	12.7	2.7	27.5	12.4	2.1	29.6	12.0	4.0	24.6
Aug 09	12.8	3.3	32.2	12.8	3.4	29.2	12.2	5.1	24.8
Sep 09	13.9	2.9	35.9	14.0	2.8	31.9	13.3	3.7	24.3
Oct 09	17.2	5.8	36.9	17.2	6.3	33.4	16.7	7.1	28.7

Appendix 3-2. Comparison of mean values of CT_{min} and CT_{max} (°C) of painted reed frogs tested in each year of the study (year 1 - 2010/2011; year 2 - 2011/2012). Data are mean ± s.d. Bold text indicates significant differences between years (alpha=0.05).

ACC	Year 1		Year 2		Test statistic	P value
	Mean ± s.d.	n	Mean ± s.d.	n	(W)	
CT_{min}						
15	**1.2 ± 1.4 [a]**	**20**	**2.8 ± 1.5 [c, d]**	**21**	**89.5**	**0.002**
20	**1.3 ± 0.9 [a]**	**23**	**2.2 ± 0.8 [c]**	**21**	**84.5**	**<0.001**
25	**2.1 ± 0.8 [b]**	**22**	**3.1 ± 1.0 [d]**	**21**	**107.5**	**0.003**
CT_{max}						
15	**43.4 ± 2.1 [e]**	**20**	**41.2 ± 2.7 [g]**	**21**	**313.5**	**0.007**
20	44.5 ± 2.0 [e, f]	23	44.3 ± 2.5 [h]	21	268.5	0.533
25	45.4 ± 1.6 [f]	22	46.4 ± 2.0 [i]	21	151.0	0.053

Different superscripts indicate significantly different pairs of group means (P < 0.05). All pairwise comparisons are Wilcoxon rank sum test with Bonferroni correction.

Appendix 3-3. Outcomes of ANCOVA models testing for the effects of acclimation treatment, site, sex and body mass on CT_{min} and CT_{max} of painted reed frogs in year 1 (2010/2011) and year 2 (2011/2012). ACC: acclimation temperature (15, 20 or 25°C). Bold text indicates significant parameters (alpha=0.05).

Variable	DF	Type III SS	F value	P value
CT_{min}, year 1 [a]				
Intercept	**1**	**26.455**	**28.995**	**<0.001**
ACC	**2**	**13.923**	**7.630**	**0.001**
Site	**2**	**10.286**	**5.637**	**0.006**
Sex	1	3.204	3.512	0.066
Body mass	1	0.093	0.102	0.751
Residuals	58	52.920		
CT_{min}, year 2 [b]				
Intercept	**1**	**16.316**	**14.463**	**<0.001**
ACC	**2**	**10.819**	**4.795**	**0.012**
Site	2	5.134	2.275	0.113
Sex	1	0.103	0.091	0.764
Body mass	1	0.745	0.661	0.420
ACC × body mass	**2**	**7.627**	**3.381**	**0.041**
Residuals	54	60.919		
CT_{max}, year 1 [c]				
Intercept	**1**	**9972.8**	**2812.728**	**<0.001**
ACC	**2**	**47.3**	**6.675**	**0.002**
Site	2	5.5	0.772	0.467
Sex	1	0.5	0.131	0.718
Body mass	1	6.6	1.862	0.178
Residuals	58	205.6		
CT_{max}, year 2 [d]				
Intercept	**1**	**7006.3**	**1327.574**	**<0.001**
ACC	**2**	**78.4**	**7.432**	**0.001**
Site	2	11.6	1.099	0.340
Sex	1	1.7	0.327	0.570
Body mass	1	11.2	2.122	0.151
ACC × body mass	**2**	**36.8**	**3.486**	**0.038**
Residuals	54	285.0		

Body mass was log_{10} transformed before analysis. Model statistics: [a] CT_{min}, year 1 multiple R^2=0.333, $F_{6,58}$=4.827, P<0.001; [b] CT_{min}, year 2 overall model multiple R^2=0.300, $F_{8,54}$=2.895, P=0.009; [c] CT_{max}, year 1 multiple R^2=0.213, $F_{6,58}$=2.621, P=0.026; [d] CT_{max}, year 2 multiple R^2=0.558, $F_{8,54}$=8.504, P<0.001.

Appendix 3-4. Outcomes of ANCOVA models of the effects of acclimation treatment, test temperature and covariates on resting metabolic and water loss rates of painted reed frogs. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 25 or 25°C). Bold text indicates significant parameters (alpha=0.05).

Resting metabolic rate [a]

Variable	DF	Type III SS	F value	P value
ACC	**2**	**0.088**	**5.014**	**0.009**
TT	**2**	**1.345**	**76.537**	**<0.001**
Site	2	0.017	0.956	0.388
Sex	1	0.001	0.119	0.731
Body mass	**1**	**0.425**	**48.428**	**<0.001**
ACC × sex	2	0.051	2.916	0.059
Residuals	94	0.826		
	Estimate	s.e.m.	T value	P value
Intercept	**-1.094**	**0.019**	**-57.611**	**<0.001**
ACC 20°C	**-0.049**	**0.021**	**-2.325**	**0.022**
ACC 25°C	0.010	0.019	0.536	0.593
TT 20°C	**-0.145**	**0.015**	**-9.726**	**<0.001**
TT 25°C	-0.009	0.014	-0.608	0.545
Site: Stellenbosch	0.023	0.017	1.382	0.170
Site: Strand	-0.012	0.015	-0.809	0.421
Sex	0.008	0.015	0.489	0.626
Body mass	**0.945**	**0.136**	**6.959**	**<0.001**
ACC 20°C × sex	-0.014	0.021	-0.676	0.501
ACC 25°C × sex	-0.033	0.020	-1.668	0.099

Resting water loss rate [b]

Variable	DF	Type III SS	F value	P value
ACC	2	0.099	0.923	0.401
TT	2	0.053	0.498	0.609
Site	2	0.041	0.385	0.681
Sex	1	0.004	0.073	0.787
Body mass	**1**	**0.595**	**11.139**	**0.001**
Residuals	96	5.123		
	Estimate	s.e.m.	T value	P value
Intercept	**0.838**	**0.046**	**18.230**	**<0.001**
ACC 20°C	0.041	0.033	1.225	0.223
ACC 25°C	-0.004	0.032	-0.123	0.903
TT 20°C	-0.020	0.037	-0.557	0.579
TT 25°C	-0.012	0.035	-0.344	0.731
Site: Stellenbosch	-0.036	0.041	-0.877	0.383
Site: Strand	0.017	0.037	0.459	0.647
Sex	-0.010	0.038	-0.271	0.787
Body mass	**1.093**	**0.327**	**3.338**	**0.001**

Body mass, metabolic rate and water loss rate were $\log_{10}$ transformed before analysis. [a] Overall model multiple R^2=0.636, $F_{8,96}$=20.96, P<0.001. [b] Overall model multiple R^2=0.163, $F_{8,96}$=2.341, P=0.024.

Appendix 3-5. Outcomes of ordered factor ANOVA with orthogonal polynomial contrasts on resting water loss rate of painted reed frogs. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 25 or 25°C). L denotes the linear contrast and Q the quadratic contrast. Bold text indicates significant parameters (alpha=0.05).

Variable	DF	Type III SS	F value	P value
Intercept	1	0.002	0.031	0.860
ACC	2	0.119	1.191	0.308
TT	2	0.066	0.666	0.516
ACC × test	4	0.388	1.945	0.109
Residuals	96	4.784		
	DF	Contrast SS	F value	P value
ACC (L)	1	0.001	0.010	0.919
ACC (Q)	1	0.110	2.201	0.141
TT (L)	1	0.001	0.011	0.918
TT (Q)	1	0.081	1.616	0.207
ACC (L) × test (L)	1	0.126	2.524	0.115
ACC (Q) × test (L)	1	0.008	0.160	0.690
ACC (L) × test (Q)	1	0.136	2.726	0.102
ACC (Q) × test (Q)	1	0.118	2.372	0.127

Water loss rate was $\log_{10}$ transformed prior to analysis. Overall model multiple R^2=0.108, $F_{8,96}$=1.452, P=0.185. Contrasts were carried out using the residuals of the water loss rate-body mass relationship; L denotes the linear contrast and Q the quadratic contrast. Bold text indicates significant parameters.

Appendix 3-6. Best-fit generalised least squares model fitted to active water loss rate data of painted reed frogs. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 25 or 35°C). Bold text indicates significant parameters (alpha=0.05).

Parameter	DF (numerator, denominator)	F value	P value	Deviance explained (%) [a]
Null	89			
ACC	2, 87	3.071	0.052	0.859
TT	**2**, **85**	**177.014**	**<0.001**	**49.521**
Site	2, 83	0.617	0.542	0.172
Body mass	2, 82	0.003	0.955	<0.001
	Estimate	s.e.m.	T value	P value
Intercept	**2.162**	**0.034**	**63.814**	**<0.001**
ACC 20°C	0.033	0.021	1.575	0.119
ACC 25°C	-0.018	0.020	-0.861	0.392
TT 25°C	**0.243**	**0.020**	**11.969**	**<0.001**
TT 35°C	**0.376**	**0.020**	**18.557**	**<0.001**
Site: Stellenbosch	-0.004	0.023	-0.184	0.855
Site: Strand	0.018	0.022	0.801	0.425
Body mass	0.009	0.155	0.057	0.955

Body mass and water loss rate were $\log_{10}$ transformed before analysis. [a] Deviance explained is from the pamer.fnc function in R's LMERCONVENIENCEFUNCTIONS package. Bold text indicates significant parameters.

Appendix 3-7. Body mass-adjusted metabolic and water loss rates of resting and active painted reed frogs. ACC: acclimation temperature (15, 20 or 25°C); TT: test temperature treatment (15, 20 or 25°C for resting, and 15, 25 or 35°C for active rates). MR and WL data are mean ± s.d. (alpha=0.05).

Resting

ACC	TT	MR ($ml\ CO_2\ g^{-1}\ h^{-1}$)	WLR ($mg\ H_2O\ g^{-1}\ h^{-1}$)	n
15	15	0.052 ± 0.010	12.131 ± 9.781	9
15	20	0.069 ± 0.014	8.875 ± 5.733	11
15	25	0.112 ± 0.022	7.767 ± 3.355	12
20	15	0.063 ± 0.013	5.894 ± 2.906	12
20	20	0.089 ± 0.030	9.352 ± 5.156	13
20	25	0.120 ± 0.021	8.973 ± 5.127	12
25	15	0.062 ± 0.018	7.060 ± 4.484	13
25	20	0.078 ± 0.011	6.596 ± 4.192	12
25	25	0.107 ± 0.017	8.351 ± 3.140	11

Active

ACC	TT	MR ($ml\ CO_2\ g^{-1}\ h^{-1}$)	x-fold difference (AMR:RMR)	WLR ($mg\ H_2O\ g^{-1}\ h^{-1}$)	x-fold difference (active WLR: resting WLR)	n
15	15	0.565 ± 0.101	11.0	116.933 ± 26.030	9.6	9
15	25	1.008 ± 0.153	9.0	192.698 ± 49.780	24.8	9
15	35	1.106 ± 0.101		273.686 ± 70.667		9
20	15	0.535 ± 0.091	8.5	110.686 ± 13.371	18.8	10
20	25	1.013 ± 0.118	8.4	194.672 ± 35.417	21.7	10
20	35	1.309 ± 0.083		281.304 ± 26.444		10
25	15	0.390 ± 0.041	6.2	108.597 ± 21.297	15.4	11
25	25	0.854 ± 0.144	7.9	199.711 ± 33.456	23.9	11
25	35	1.004 ± 0.329		262.030 ± 94.514		11

Appendix 3-8. Hypothesised significance of the polynomial components of treatment effects (acclimation treatment (ACC) and test temperature (TT)) on resting metabolic rate and water loss rate in painted reed frogs. Models assume that lower RMR and resting WLR enhance fitness via energy and water savings. Derived from Huey *et al.* 1999; Deere and Chown 2006; Marais and Chown 2008; Kingsolver and Huey 2008, Clusella-Trullas *et al.* 2010.

Hypothesis	ACC	TT	Interaction (ACC × TT)
Beneficial acclimation (BAH)			L+/-
Colder is better (CIB)	L+	L+	ns
Hotter is better (HIB)	L-	L-	ns
Optimal acclimation temperature (OAH)	Q+		
No acclimation response	ns	ns	ns

L denotes a linear and Q a quadratic component; +/- denotes the sign of the component; ns=relationship not significant; blank=no prediction. Because low resting metabolic rate is assumed to be beneficial (see text), CIB is indicated by a significant positive trend, and HIB by a negative trend in ACC and TT; BAH would be supported by a significant interaction between ACC and TT and OAH by a quadratic effect of ACC; non-significant trends throughout indicate no trait plasticity; blank cells indicate no particular hypothesis about the relationship.
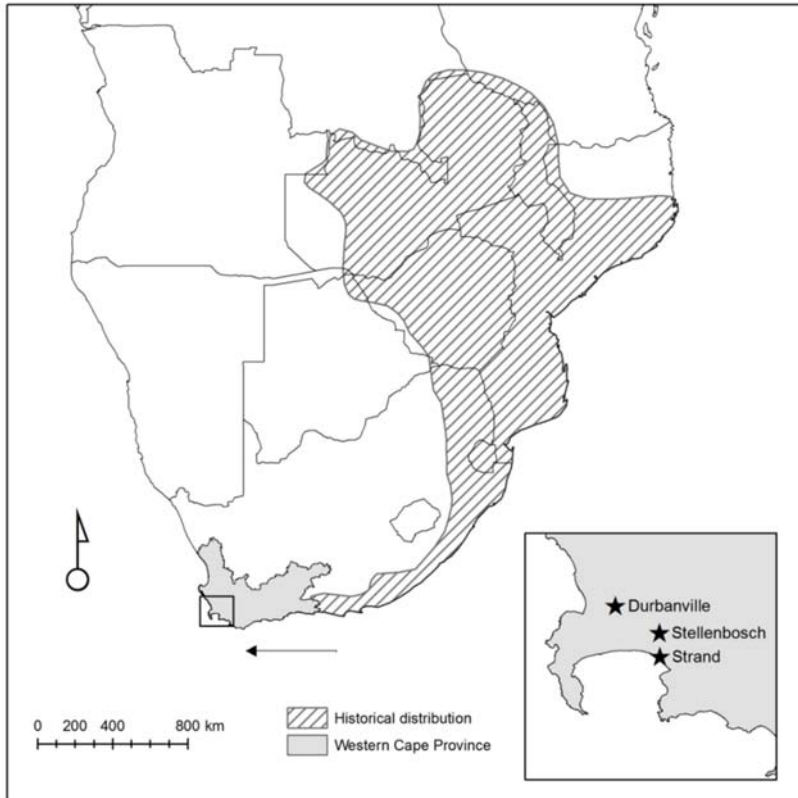
Appendix 3-9. Map of historical distribution digitised from Channing (2001; main panel) and collection sites in the Western Cape Province, South Africa (inset). Arrow shows direction of range expansion since 1997.

Appendix 3-10. Rotating cuvette used to obtain metabolic rate and water loss rates during activity.

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Chapter 4. Painting on a broader canvas: Invasive reed frogs occupy a wider niche in their novel range

In prep. (*Diversity and Distributions*)

Davies, S.J., Hill, M.P., McGeoch, M.A. and Clusella-Trullas, S. Painting on a broader canvas: Invasive reed frogs occupy a wider niche in their novel range.

A typical agricultural/peri-urban landscape in the south-western Cape
-33.900°S 19.213°E

4.1   Introduction

Invasive species are typically added to a community or area by human-mediated introduction and natural dispersal, and before becoming invasive, must survive to establish a reproductive population in the novel environment (Richardson and Pyšek 2006; Blackburn et al. 2011). The processes of establishing reproductive populations (naturalisation) and dispersing into natural areas (invasion) are two in a series of stages that are separated by sequential barriers (Richardson *et al.* 2000; Lodge 1993). Thus, invasion dynamics, like community dynamics, can be shaped by introduction, dispersal, selection and drift (see Vellend 2007).

The niche may be thought of as the set of biotic and abiotic predictors that define the 'space' within which a species can exist, according to its environmental tolerance and physiological capacity. This is the suite of species-environment relationships that defines the potential distribution of a species and allows it to exist and reproduce with population growth >1 (Hutchinson 1957). The realised niche of a species or population lies at the intersection of available, accessible and suitable habitat, and is subject to the limitations imposed by landscape characteristics and structure, dispersal capabilities and barriers to movement. Because of the influence of these factors, and biotic interactions such as competition and predation, the realised niche may map onto the fundamental niche in different ways, with implications for measurement of niche shifts (Guisan *et al.* 2014). For example, the realised niche may exclude certain areas that are included in the fundamental niche if interspecific interactions prevent occupation of certain areas (Pulliam 2000; Tingley *et al.* 2014; Soberón and Nakamura 2009).

The realised and fundamental niches can be modelled using different approaches. Mechanistic models explicitly incorporate the potential or actual demonstrated capacity of organisms to adapt to the environment of the novel range and thus address shifts in the fundamental niche (Kolbe *et al.* 2010; Kearney et al. 2010; Tingley *et al.* 2014). Niche models that use a correlative approach implicitly link organism traits to environmental conditions through an assumed causal relationship based on statistical correlation (i.e. regression) (Kearney and Porter 2009). Differentiating between shifts in the fundamental niche and those in the realised niche is difficult to achieve in a correlative model framework, because correlative models represent the realised niche, inclusive of biotic interactions that modify the fundamental niche (Guisan and Thuiller 2005). Therefore, in a correlative framework, an observed niche shift could be due to several different mechanisms, including a shift in the basal tolerance of the population, a change in biotic interactions (e.g. predator release), altered barriers to dispersal (e.g. enhanced accessibility of habitat through artificial corridors) or local adaptation (see Soberón and Peterson 2005;

Guisan et al. 2014; Tingley *et al.* 2014). Reciprocal niche modelling in a correlative framework can help to identify mismatches in predicted ranges based on the native and invaded range distributions and show where shifts in tolerance may have occurred through plasticity or evolution (Wiens and Graham 2005). The use of predictors based on the physiology, ecology and natural history increases the robustness of model predictions (Wiens and Graham 2005; Rödder *et al.* 2009) and allows the relationship between the fundamental and realised niches to be explored more fully (Tingley *et al.* 2014).

The idea that species niches remain conserved raises many fascinating questions in evolution and ecology. Peterson *et al.* (1999) showed that niches of mammal, bird and butterfly species evolving independently on either side of a dispersal barrier were conserved on an evolutionary time scale. Thus, niche conservation influences the rate and nature of speciation, but may also limit geographic range expansion on a much shorter time scale, influencing species responses when introduced to novel environments or in changing climates and landscapes (reviewed in Wiens and Graham 2005; Guisan *et al.* 2014). Observed niche conservatism may indicate a greater reliance on dispersal than on selection or niche structuring (see Vellend 2010). Distinguishing the roles of dispersal limitation and niche limitation in shaping invasive species ranges is therefore an important area of ecological investigation.

Niche shifts allow species to extend their ranges into novel climatic zones (Broennimann *et al.* 2007; Hill *et al.* 2012, 2013; McCann *et al.* 2014). Climatic niche shift has been found to play a role in the invasiveness of spotted knapweed, *Centaurea maculosa*, an invasive plant that exploits drier habitats in its invaded range than encountered in the native range (Broenniman *et al.* 2007). Hill *et al.* (2012) found evidence of climatic niche shift in the agricultural pest mite *Halotydeus destructor*, and demonstrated in a follow-up study (Hill *et al.* 2013) that temperature tolerance had increased within the invasive populations. Complexity is added by the discovery that niche shifts are not uniform within invasive populations of a given species and that plasticity can evolve rapidly in invasive populations. Kolbe *et al.* (2012) showed that different populations of invasive *Anolis cristatellus* lizards in Florida have either evolved low temperature tolerance or remained unchanged with respect to temperature tolerance over a relatively short period of 35 years.

Interestingly, climatic niche shifts tend to match environmental conditions, and even small changes in environmental conditions relative to the native range can lead to changes in tolerance traits (Kolbe *et al.* 2012). McCann *et al.* (2014) showed that the availability of novel

environments, in this case cold high-altitude habitats, in the invaded range can drive changes in trait means. However, relatively few cases of niche shift have been conclusively demonstrated in most plant and animal groups, and demonstration of niche shift depends on the tools and metrics used, and is highly context-dependent (Guisan *et al.* 2014). Nonetheless, niche models of invasive organisms can be used to evaluate invasion risk and predict future areas of occurrence and spread into suitable habitat, to answer a key question about invasive species ranges (Gallien *et al.* 2010; Di Febbraro *et al.* 2013): is further spread likely in the novel range and if so, what areas may be invaded in future? Niche modelling can also be used to identify undetected areas of occupancy of species that are already introduced (Thuiller *et al.* 2005; Broennimann *et al.* 2007; Higgins and Richardson 2014).

This study aims to test whether the niche of an extra-limital invasive amphibian, the painted reed frog (*Hyperolius marmoratus* Rapp.) has remained conserved during recent range expansion, or whether the spread is facilitated through niche shift. A modelling framework comprising both climatic and landscape-level variables was used to describe the habitat quality of the painted reed frog, drawing from previous work that examined phenotypic plasticity in key physiological traits (Chapter 3) and habitat correlates of occupancy (Chapter 2). The models thus focussed on areas of the historical and novel ranges where the species is likely to be able to survive and reproduce. During the modelling process, the effects of climatic and landscape variables on predictions of habitat suitability were compared.

Data in Chapter 2 suggested that the prevalence of artificial water bodies in the novel range may facilitate range expansion of painted reed frogs, and that likely climatic range limiters were summer dryness and winter cold (Davies *et al.* 2013). On an intercontinental scale, human disturbance and land use can be more important determinants of invasive species ranges than climate (Broennimann *et al.* 2007; Roura-Pascual *et al.* 2011), while on a more localised scale, the presence of abundant water bodies (rivers, dams, pans and ponds) may allow frogs to traverse the landscape using them as 'stepping stones' or invasion hubs (Florance *et al.* 2011). Therefore, prior information suggested that it is important to consider landscape and land use variables such as the presence of artificial water bodies in addition to climatic predictors in niche models of *H. marmoratus*.

4.2   Materials and methods

4.2.1   *Species and range information*

The painted reed frog is a member of the old world tree frogs, and the largest genus of African frogs. This species has invaded the south-western parts of South Africa in a recent range expansion that started in *c.* 1997, moving from a sub-tropical, summer rainfall region to a temperate, Mediterranean-type winter-rainfall region (Bishop 2004; Tolley *et al.* 2008; Chapter 2). Painted reed frogs have broad thermal tolerance, with a critical minimum close to 0°C and critical maximum well above 40°C (Geise and Linsenmair 1988; Chapter 3) consonant with their broad native geographical range across south-eastern Africa (Figure 1-2; Appendix 4-1; see Brattstrom 1968; Gaston and Spicer 2001; Calosi *et al.* 2008).

Published records of the range of *H. marmoratus* up to 2004 covered large parts of south eastern Africa, including southern Tanzania, Malawi, Mozambique, Zimbabwe, Zambia, Swaziland and the eastern and southern portion of South Africa up to the central escarpment (Figure 4-1; Poynton 1964; Wager 1986; Lambiris 1988; Channing 2001; Bishop 2004). Between 1997 and 2004 *H. marmoratus* became established on the Cape peninsula and in inland areas of the Western Cape Province, including the lowlands of the winter rainfall region. In this study, the novel range was defined as the Western Cape Province, excluding its south-easternmost portion around Tsitsikamma and west of 23°E longitude. The historical range covered the remainder of South Africa, i.e. the area east of Tsitsikamma forest. These two range areas are non-overlapping, with distribution records displaying clear spatial and temporal separation, between the historical distribution and the range expansion (see Figure 1-3; Chapter 2).

As the two presence datasets were spatially and temporally independent, it was appropriate to apply the models within a reciprocal framework (see Elith *et al.* 2006; Fitzpatrick *et al.* 2007; Broennimann and Guisan 2008; Rödder *et al.* 2009; Hill *et al.* 2012). Models were trained in the novel or historical range and projected onto the reciprocal range to determine whether *H. marmoratus* occupies similar conditions in its native and invaded ranges within South Africa, and to identify changes in niche parameters between range areas (Fitzpatrick *et al.* 2007; Hill *et al.* 2012).
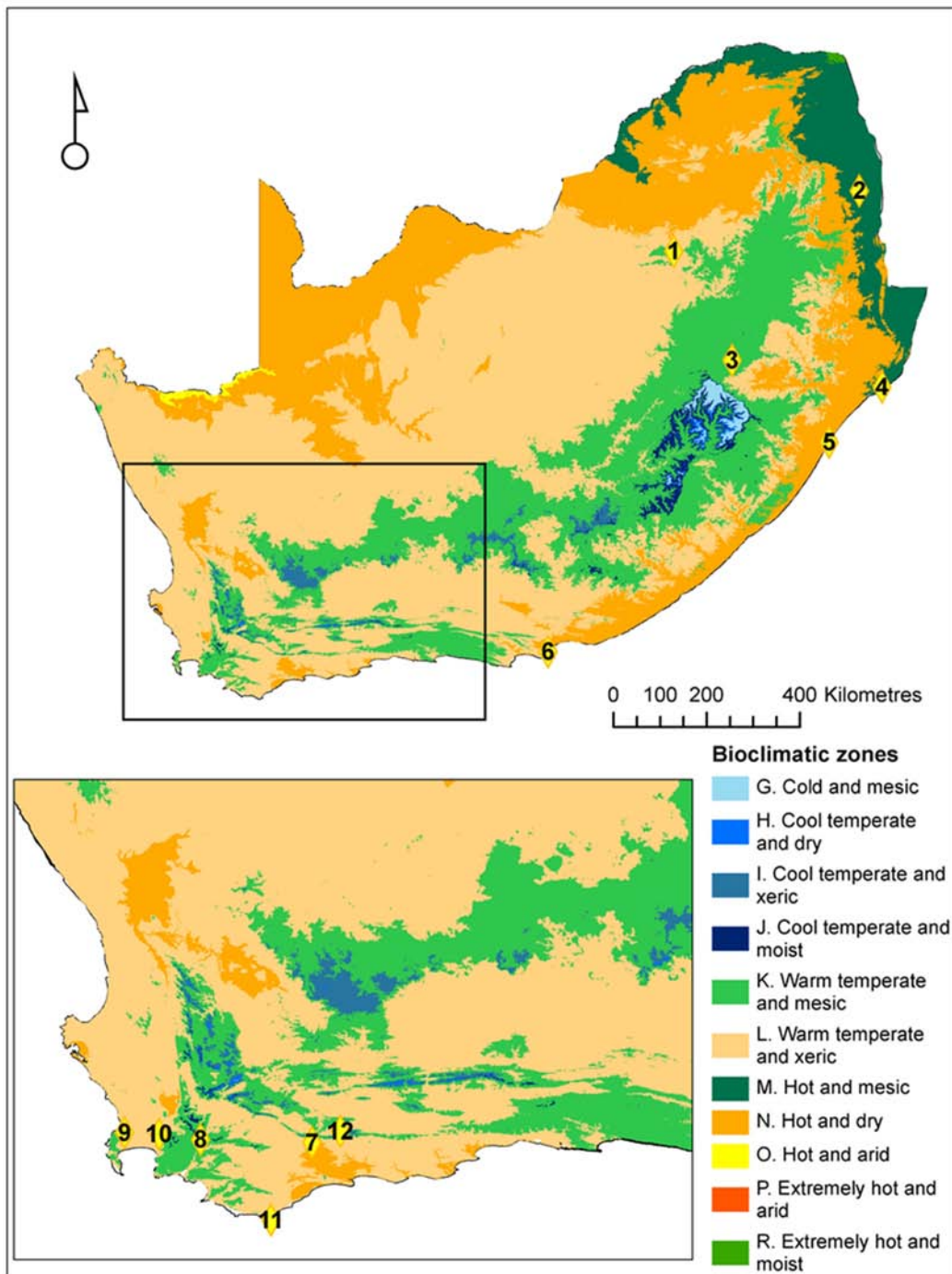
Figure 4-1. Orientation map showing towns and major vegetation zones of South Africa. towns and landmarks: 1: Johannesburg, 2: Skukuza, 3: Harrismith, 4: RichardsBay, 5: Durban, 6: PortElizabeth, 7: Swellendam, 8: Villiersdorp, 9: Cape Town, 10: Stellenbosch, 11: Cape Agulhas, 12: Barrydale.

4.2.2 *Occurrence data for modelling*

Painted reed frog occurrence data were sourced from South African Frog Atlas Project (SAFAP; Minter *et al.* 2004) records collected over the period 1905-2003; CapeNature State of Biodiversity data from 1997 to 2006 (Turner 2006), and systematically and opportunistically sampled occurrences from across the expected novel range by the authors, spanning 2006-2010 (Chapter 2). In total, 151 verified records of *H. marmoratus* occurrence were available for the

novel range and 1221 records for the historical range. Thus, I had access to two spatially and methodologically distinct occurrence datasets, one collected in the historical range prior to the range expansion, and the other collected post-expansion in the novel range (Table 4-1).

Table 4-1. Summary of occurrence data sources and numbers of presence and pseudo-absence records used in niche models of *H. marmoratus*. Data sources: South African Frog Atlas Project (SAFAP, Minter *et al.* 2004); CapeNature state of Biodiversity Database (CNSOB, Turner 2006) and authors' surveys (Chapter 2).

	Historical range	Novel range
Years	1908-2003	1997-2010
No. of presences	1221	151
No. of pseudo-absences:		
SAFAP	1738	28
Random background	834	166
Source	SAFAP	SAFAP, CNSOB, authors' surveys

4.2.3   Pseudo-absence and background data

Pseudo-absence records were generated from distribution data of other nocturnal, summer-breeding taxa in the SAFAP database (pseudo-species - Barbet-Massin *et al.* 2012). Localities where other species had been recorded, but where *H. marmoratus* was not recorded, were taken as pseudo-absences (n=2766 unique localities; see Table 4-1). Considering the conspicuous (call > 100dB; extended breeding season - Passmore and Carruthers 1995), generalist characteristics of *H. marmoratus*, it was justifiable to assume that if breeding populations of *H. marmoratus* had been present in these area for more than one breeding season they would have been detected, and therefore these records simulate absences.

Initially, the models were run with this pseudo-absence dataset, and then, to increase the discrimination and transferability of the models across ranges and ensure that the area available to painted reed frogs was fully represented in models, 1000 additional background points were generated randomly across the thermally accessible areas of South Africa, i.e. areas with July mean minimum temperature > 2.5°C (see section 4.2.9; Appendix 4-3 A). This combined set of pseudo-absences and background points is referred to as the model 'background'. By using a combination of biological reasoning (SAFAP pseudo-absences) and randomly selected environmentally stratified points (using physiological criteria), a model background was produced that accurately represents the area available to painted reed frogs. The background has a similar level and nature of bias to the presence data (Phillips *et al.* 2009; Barbet-Massin *et al.* 2012)

4.2.4   Model interpretation

By analysing the extent and location of prediction errors from the reciprocal models, the ecological distinctiveness of conditions in the historical and novel ranges can be measured. For example, if the historical range model predicts a spatially distinct (or smaller) area of suitable habitat in the novel range than was predicted by the novel model, then frogs in the novel range occupy a different (or broader) set of environmental conditions than frogs in the historical range. This indicates that a niche shift may have occurred during the invasion process. If the putative niche shift can be linked with shift in climatic or other predictor influence in the model and with a shift in the niche centroid (Guisan et al. 2014), the mismatch confirms that a niche shift involving the identified predictors has occurred (but see Rödder and Lötters 2010). In this study, any niche shift detected also corresponds with a separate time period (see Table 4-1), adding confidence to the conclusion. If the suitable habitat predicted by the historical model is larger than that predicted by the novel model, a narrower niche is occupied in the novel range. The latter could be caused by dispersal limitation preventing the occupancy of some suitable habitats or could indicate a shift in environmental tolerance or a narrowing of tolerance.

Interpretation of niche overlap and niche shift

Niche overlap statistics quantify the similarity of two niches in geographical space, as the models are projected before niche overlap is calculated (Warren et al. 2008). In contrast, detecting niche shift using the centroid measure (see Broennimann et al. 2007; Guisan et al. 2014) involves examining the niche in environmental space using an ordination technique such as PCA. If detected, direction of centroid shift can be further analysed by its alignment with one or more of the original environmental predictors.

4.2.5   Climate and landscape predictors

The environmental predictors consisted of variables that are likely to limit painted reed frog distribution: evaporative potential in the driest month of the year (A-pan method; see Chiew and McMahon 1992) and degree days for the three peak winter months (sum of June-August; Chapter 2), and mean annual precipitation, a broad-level range limiter for many amphibians (Wells 2007). A further climatic variable was used, reflecting the daily temperature range in summer (November-February), a measure of metabolic challenge during the breeding season.

Climate data were extracted from the South African Atlas of Agrohydrology and Climatology (interpolated 1′ x 1′ raster data; Schulze *et al*. 1997). All variables had cross-correlations of less

than 0.8. This level of cross-correlation was considered acceptable because the chosen modelling method (section 4.2.6) allows handling of cross-correlated predictors and examination of the effects of the correlations.

In addition to climate, the availability of moist habitats in the landscape is a key resource gradient for amphibians. Water bodies and riparian corridors provide sheltered microhabitats, foraging areas and routes for dispersal through the landscape. Therefore, in addition to the climatic predictors, spatial data from the National Freshwater Ecosystem Priority Areas database (NFEPA; Nel *et al.* 2011) were used to construct a comprehensive data layer describing the density of artificial water bodies (impoundments, pans, ponds, other wetlands) and riparian corridors (rivers) in the landscape. Spatial data on terrain morphology (Kruger 1983, cited in Schulze *et al.* 1997) from the South African Atlas of Agrohydrology and Climatology (Schulze *et al.* 1997) was included as a surrogate for topography as previous habitat modelling (Chapter 2) suggested that mountainous areas and areas of high ruggedness may be inaccessible to painted reed frogs.

4.2.6   *Modelling approach and evaluation*

Models were built using boosted regression trees (BRT; gbm.step function in DISMO package of R [Hijmans *et al.* 2011; R Development Core Team 2012]). Boosted regression trees are ensemble models that use machine learning to gradually refine a postulated relationship between predictors and a response variable (Elith *et al.* 2006, 2008). They allow robust modelling of non-linear relationships and interactions between predictors (e.g. climate variables, which are often highly correlated) and permit correlations between predictors to be examined explicitly. After preliminary parameter exploration, models were built using a learning rate of 0.005, which was low enough to achieve over 1000 trees to optimise the accuracy of the models and their ability to predict the hardest-to-explain occurrences (Elith *et al.* 2008). The default 10-fold cross-validation and 0.5 bag fraction provide a validation technique and introduce stochasticity during the tree-building process, respectively.

Models were constructed with training data from the historical range only ('historical model'), the novel range only ('novel model'), and historical and novel range data together ('all-SA model'). The three models were then projected onto the whole of SA to assess their effectiveness in predicting current painted reed frog distribution.

I examined the effect of including landscape predictors (terrain morphology and density of rivers and artificial wetlands) in addition to climatic predictors in the models by comparing model performance with and without them (gbm.simplify function in DISMO package of R) to ascertain whether their removal from the models resulted in a significant change in deviance. Models were evaluated with commonly-used metrics, including area under the ROC curve, sensitivity and specificity (i.e. ability to correctly predict species presences and absences accurately). The true skill statistic (TSS) was used as a measure of model performance that is independent of the proportion of presences vs. absences in the data (Allouche *et al.* 2006). All model metrics were calculated in the DISMO or PRESENCEABSENCE packages in R (Freeman and Moisen 2008 a, b). In addition, model outcomes were evaluated in the light of available biological information, including data on physiology, tolerance and natural history (see Chapter 3; Rödder *et al.* 2009; Rödder and Lötters 2010).

4.2.7   Modelling extents

The range of the painted reed frog extends over a large area outside South Africa (Channing 2001; Appendix 4-1. Only the southern part of the range of the species was modelled (Figure 4-1), because of the high quality of data available for South Africa, Lesotho and Swaziland. In the northern part of the range (i.e. Zimbabwe, Mozambique, Zambia and Malawi), occurrence records become less reliable due to misidentification and confusion with other members of the *H. viridiflavus* complex at the periphery of its range. Although there are taxonomic issues within the complex as a whole (cryptic species may exist, and undescribed species almost certainly exist - Schiøtz *et al.* 1971; Wieczorek *et al.* 2001), there is no such ambiguity in the South African part of the range and no evidence of cryptic species within *H. marmoratus* (Poynton 1964; Poynton and Broadley 1987; Wieczorek *et al.* 2000, 2001; Channing 2001; Bishop 2004). Species designations within South Africa are therefore regarded as reliable, while records outside South Africa are less reliable and may introduce additional error into the modelling process. Lesotho and Swaziland were included in the models because of their inclusion in the SAFAP database (Minter *et al.* 2004).

The exclusion of the northern part of the range is also justified because climatic conditions in this part of the range become increasingly tropical, with increased precipitation, higher temperatures and lower annual temperature variation (see Appendix 4-1, Appendix 4-2). These conditions most closely match the northern extremity of the historical range in South Africa than they do the novel range. If included in the models, the northern part of the range would likely represent extrapolations beyond the novel range conditions. Therefore, excluding the tropical

part of the range should reduce the breadth of climatic gradients and lead to a conservative assessment of niche shift.

4.2.8   Environmental space available to painted reed frogs

To identify changes in species-environment relationships between the historical and novel ranges, I examined the environmental space occupied by the presence and background data (Rödder *et al.* 2009; Rödder and Lötters 2010). Principal components analysis (PCA) was used to determine independent components of variation across environmental space (Soberón and Nakamura 2009) created by the climatic and water body predictors, and to qualitatively examine the presence data in relation to the model background.

Niche models can be sensitive to the availability and distribution of environmental gradients in the study area (Di Febbraro *et al.* 2013). Model projection outside the training area often entails extrapolation beyond the reference range of one or more environmental predictors, and the results of such predictions are unreliable (Elith *et al.* 2010; 2011). This may be especially important if a niche shift has occurred or is suspected. In addition to identifying changes in climate space through PCA, multivariate environmental similarity surfaces (MESS maps; Elith *et al.* 2010, 2011) were used to measure environmental similarity across each range and permit the identification of non-analog environments that may affect the models (Guisan *et al.* 2014).

4.2.9   Physiological constraints on accessible area

Thermal tolerance data collected on 115 painted reed frogs from the novel range (Chapter 3) were used to construct a mask layer expressing the constraints that may be imposed on the distribution of *H. marmoratus* by their thermal tolerance. This mask was used to define the background in which pseudo-absences were generated for use in modelling (section 4.2.3). To construct the mask, CT_{max} and CT_{min} values of frogs acclimated at 15, 20 and 15°C were used to define the zone of intrinsic tolerance (ZIT; Eme and Bennett 2009; Appendix 4-3) for painted reed frogs. This encapsulates the thermal tolerance 'space' between the highest group mean of CT_{min} and the lowest group mean of CT_{max}, and expresses the intrinsic thermal tolerance of the organism without acclimation effects. For painted reed frogs acclimated between 15 and 25°C, the intrinsic thermal tolerance was from 2.5°C to 42.6°C.

The mean of daily maximum temperatures in the hottest month, January, were inside the zone of intrinsic tolerance (i.e. lower than CT_{max}) throughout South Africa (means of daily max. temp. in January: mean=29.3°C; s.d.=3.0°C; range= 10-35°C; Schulze *et al.* 1997), so the upper bound of

the ZIT did not limit the area accessible to painted reed frogs. The upper bound of intrinsic tolerance therefore represents a 'relaxed' niche variable (Rödder and Lötters 2009, 2010), so this variable was omitted from the mask layer, which was constructed using only the lower bound of the ZIT. Areas with daily minima below 2.5°C were treated as inaccessible for painted reed frogs. Much of the central highland portion of SA fell outside this thermal bound (means of daily min. temp. in July: mean=4.2°C; s.d.=3.6°C; range=-10-13°C; Schulze *et al.* 1997). The mask layer was used to define the model background but also to limit the model predictions to areas with minimum temperatures of 2.5°C or above (Appendix 4-3).

4.2.10 Niche overlap

To gain insight into the similarity or distinctiveness of areas occupied by frogs in the novel and historical ranges, and identify the possibility of a shift having occurred, models were trained with the historical, novel or all-SA dataset, projected to the whole country, and then clipped to the novel range before niche overlap was calculated. Three pair-wise comparisons were made between the three model predictions: (i) novel-historical, contrasting the niche predicted by the historical and novel models, i.e. a reciprocal niche comparison conducted in the novel range, the primary area of interest; (ii) novel-all-SA, and (iii) historical-all-SA, to contrast the predictions of the full dataset against the predictions of the novel range data only and historical range data only, respectively.

Schoener's *D* was used as a measure of niche overlap (Schoener 1974; Warren *et al.* 2008) and calculated in the nicheOverlap function in DISMO package of R. Although Schoener's *D* was originally developed to measure resource partitioning in the context of inter-specific competition, it has been used more recently to quantify niche overlap between populations in geographical space (Warren *et al.* 2008; Hill *et al.* 2012; Di Febbraro *et al.* 2013). Niche overlap metrics (e.g. Schoener's *D*) quantify niche stability in the COUE (centroid, overlap, unfilling, expansion) scheme of Guisan *et al.* (2014), but cannot distinguish between unfilling/filling and expansion/contraction. To detect movement of the niche centroids, PCA and inertia ellipses were used to examine presence data for the two ranges in environmental space.

4.3   Results

4.3.1   Model performance

Overall there was a close match between the model predictions and the actual distribution of painted reed frogs across South Africa. The models had high and consistent AUC, and correctly identified most presences (high specificity) and absences (high sensitivity; see Table 4-2 and

Table 4-3). TSS was high for all models (0 denotes a random guess and 1, a perfect prediction). Therefore the models produced accurate predictions of the current distributions in the novel and historical ranges as well as the combined all-SA dataset.

Table 4-2. Model performance of reciprocal niche models trained in the historical or novel range or all of SA, using climate predictors only or climate and landscape predictors. Models were built using boosted regression trees (BRTs) with a learning rate of 0.005, 10-fold cross-validation and 0.5 bag fraction. TSS: true skill statistic; AUC_{train}: AUC of the model in the training range; AUC_{cv}: AUC values from cross-validation of the model; threshold is the optimal threshold that maximises sensitivity + specificity. Values are mean±s.d.

Model	Threshold	AUC_{train}	Sensitivity	Specificity	TSS
Climate and landscape predictors:					
Historical	0.35	0.984±0.002	0.93±0.01	0.94±0.01	0.87
Novel	0.57	0.999±0.001	0.99±0.01	0.99±0.01	0.98
All-SA	0.37	0.987±0.001	0.94±0.01	0.95±0.01	0.88
Climate predictors only:					
Historical	0.39	0.977±0.00	0.91±0.01	0.93±0.01	0.84
Novel	0.49	0.995±0.00	0.99±0.01	0.95±0.02	0.94
All-SA	0.40	0.979±0.00	0.99±0.01	0.95±0.02	0.94

Table 4-3. Area under the ROC curve for the six reciprocal niche model projections; models were built with both climate and landscape predictors.

Training area	Projection area	AUC_{test}
Historical range	Novel range	0.930
Novel range	Historical range	0.801
All-SA	Historical range	0.984
All-SA	Novel range	0.995
Historical range	All-SA	0.979
Novel range	All-SA	0.991

4.3.2   *Model predictions for the novel range*

The model trained in the historical range showed that only a small portion of the novel range was suitable for painted reed frogs, compared to the other two model predictions (Figure 4-2 A). The areas of predicted habitat fell into three main parts, (i) the eastern part of the novel range, near the boundary with the historical range, with a narrow extension westward along the foothills of the Overberg (Swellendam - see Figure 4-1 for location of landmark); (ii) the western part of the province from the western rim of the Agulhas Plain to Cape Town and northwards into the Cape fold mountains, and (iii) the Cape Peninsula in the extreme south-west. The latter area is largely composed of rugged mountain terrain flanked by a narrow coastal belt and thus provides little

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accessible habitat. These predictions are broadly concordant with the painted reed frog's distribution in that breeding populations are abundant in the east of the region and in the lowlands of the south-western cape, but the Agulhas Plain has never been colonised.

The novel model and the all-SA model made broadly similar predictions when projected to the novel range, but differed slightly in extent of suitable habitat (Figure 4-2 B, C). They predicted broad areas of suitable habitat across the coastal and lowland areas, excluding the Agulhas Plain. Riparian corridors of the largest rivers, which mostly fall in the eastern part of the novel range, were predicted to be suitable for painted reed frogs. These models predicted suitable habitat in the mountainous areas and some areas of occurrence were predicted to the north of Cape Town, which are not in fact occupied by painted reed frogs.

4.3.3   Areas of concordance

Models trained in the historical range and with all SA data gave very similar predictions when projected to the country as a whole, probably because of the large geographical overlap of the training areas (approx. 90%). Areas of concordance between all of the models were: (i) the east coast and north eastern lowveld are suitable for painted reed frogs; (ii) a mid-longitude gap in the central part of the novel range running from west of Swellendam to Villiersdorp was evident in all three model predictions; (iii) none of the models made any predictions in the high-lying central regions of the country, corresponding with the Maloti-Drakensberg mountains (2000-3450 m asl), the Great Karoo escarpment (1200-1700 m asl) and the peaks of the Cape fold Mountains (1400-1700 m asl).

4.3.4   Variable importance

In the historical and all-SA models, diurnal temperature range in the breeding season and degree days in winter had most influence on the model outcomes, while the novel model was driven by diurnal temperature variation during the breeding season and mean annual precipitation (Table 4-4). The water body variables, river and artificial wetland density, had higher influence in the novel model than in either of the other models (16.2% together) but were generally less important than the climatic predictors.

Overleaf: Figure 4-2. Model projections for the three models trained in the historical (A), novel (B) and all-SA (C) ranges. Maps show binary predictions of probability of occurrence generated using the optimal.thresholds function in the PRESENCEABSENCE package of R. Blue areas are those with no model prediction due to lack of occurrence and background data.

A.

Prediction from
historical model
High : 1
Low : 0

No prediction

Presence records

0   100 200   400 Kilometres
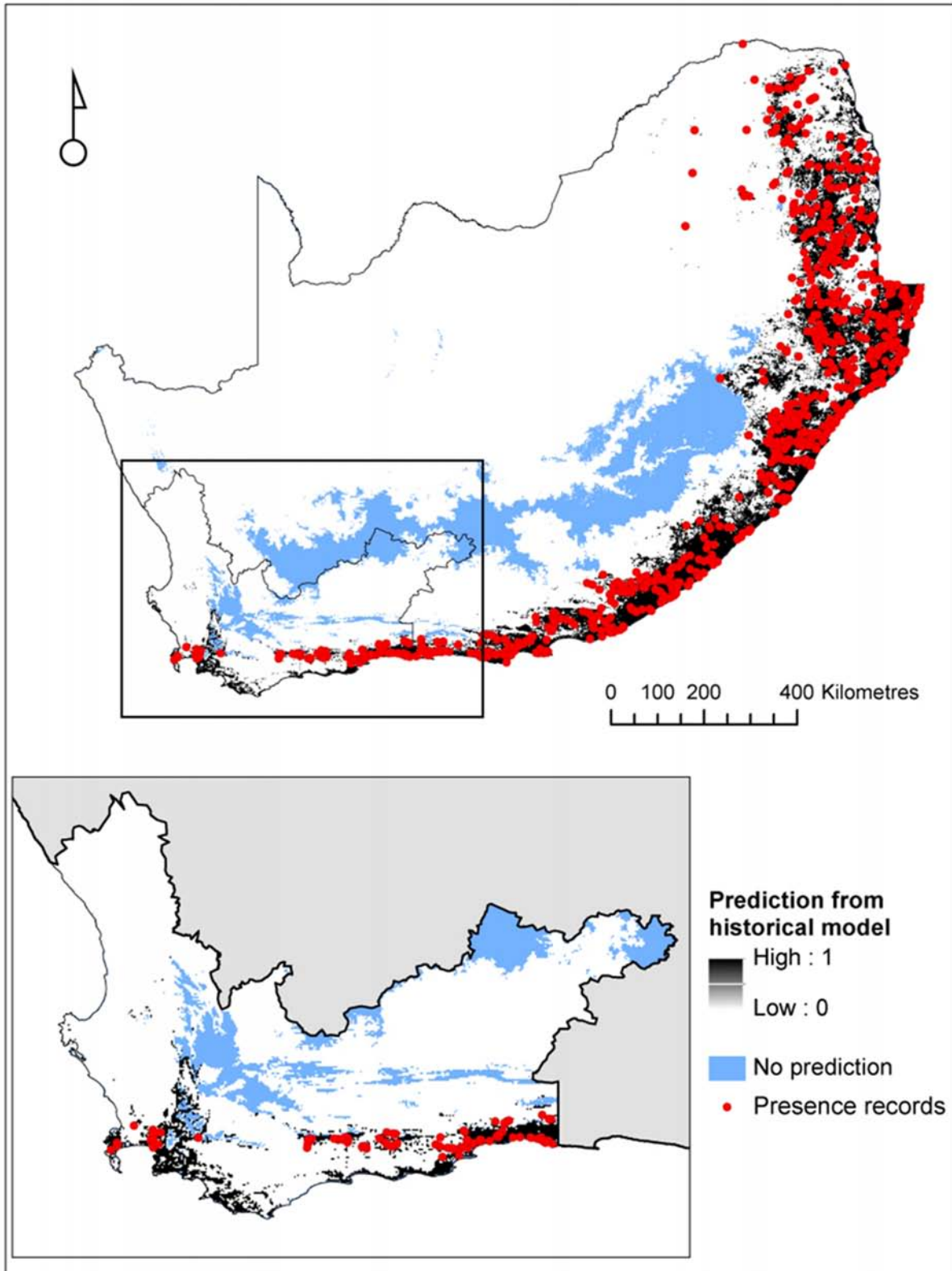
B.
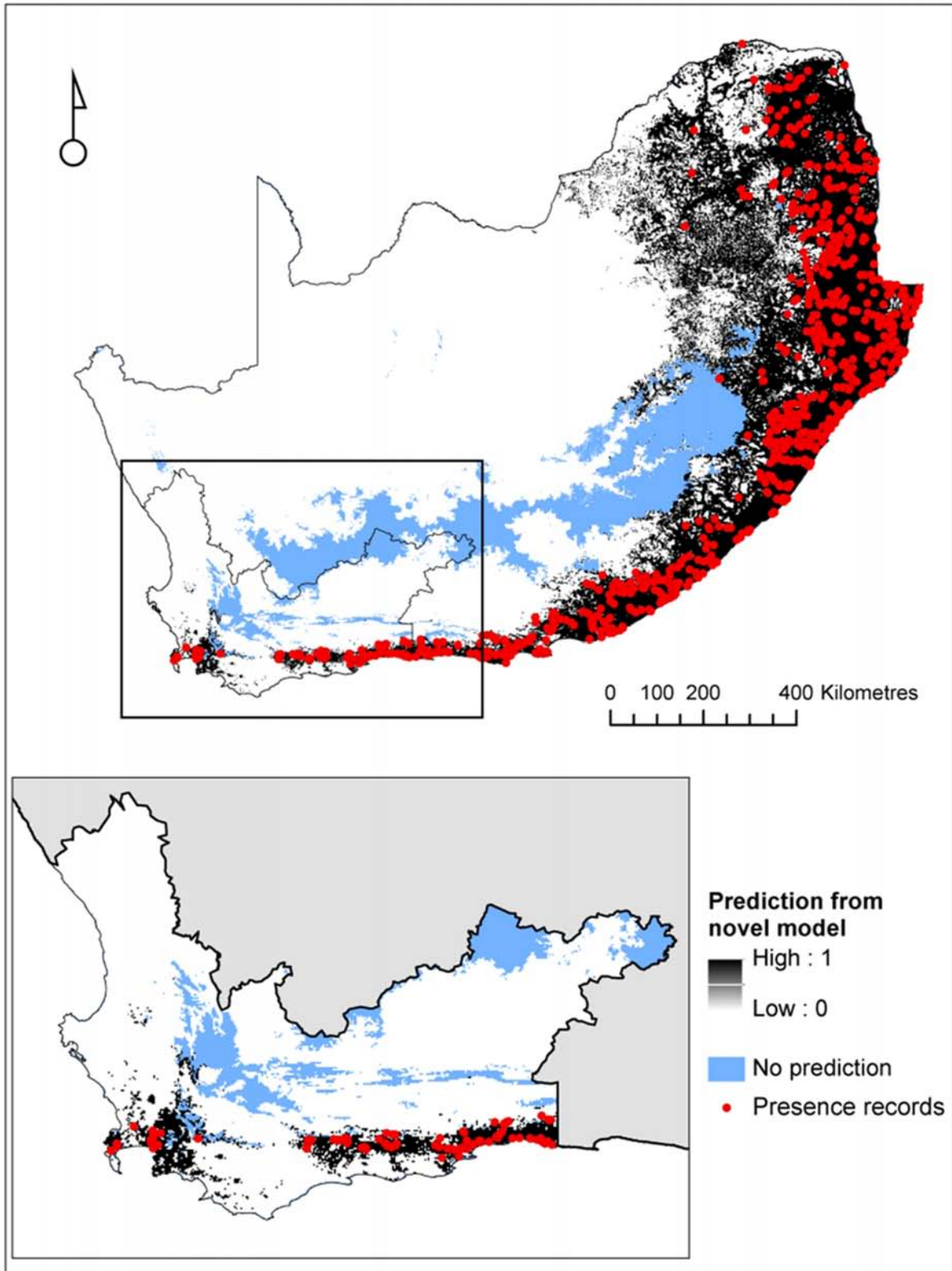
C.

Figure 4-2. Model projections for the three models trained in the historical (A), novel (B) and all-SA (C) ranges. Maps show binary predictions of probability of occurrence generated using the optimal.thresholds function in the PRESENCEABSENCE package of R. Blue areas are those with no model prediction due to lack of occurrence and background data.

4.3.5   Model predictions for the rest of SA

The model trained in the novel range correctly predicted occurrence of painted reed frogs across large areas of the historical range in northern and eastern South Africa. However, the predictions in this area should be interpreted with caution because parts of north-eastern South Africa are extrapolations outside the training range of this model (Figure 4-3). This model also predicted a much broader area of occurrence than did the historical model or all-SA models (Figure 4-2 B), including parts of the high-lying interior and Maloti-Drakensberg mountains.
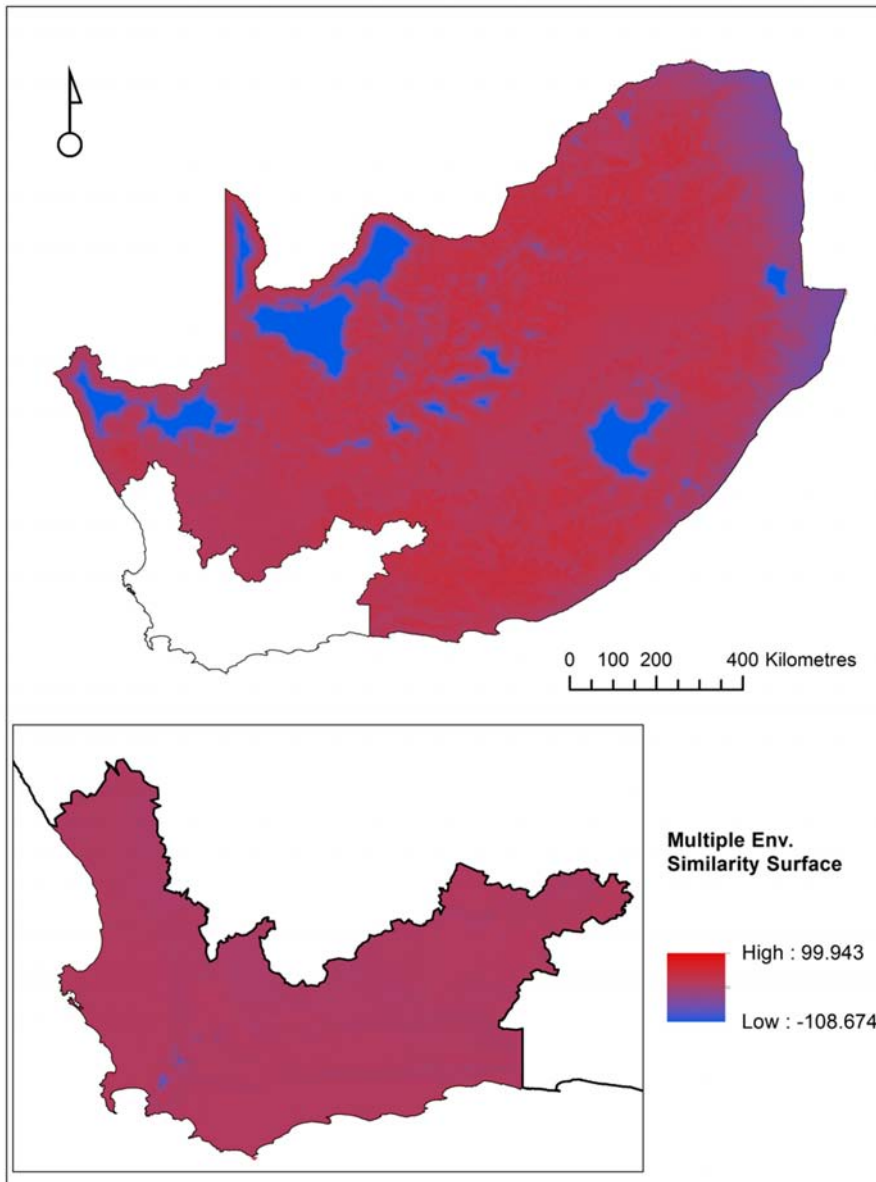
Figure 4-3. Multivariate environmental similarity surfaces (MESS maps) constructed from occurrence records in the novel range (main panel), and historical range (inset). Areas with negative MESS values are areas not represented in the training range of the model (i.e. the reciprocal range).

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Table 4-4. Relative influence of predictors in the boosted regression tree models (BRTs). Values are variable contribution in percentages for models using both climatic and landscape predictors; values in parenthesis are from models using climatic predictors only. The most influential predictor in each model is shown in bold. Asterisk (*) indicates predictors dropped from model when simplified (using gbm.simplify function in DISMO package of R; based on change in deviance when removed).

	Relative influence in BRT models (%)		
Predictor	Historical	Novel	All SA
Evaporation in the driest month	12.6 (16.4)	11.3* (16.0)	16.1 (23.7)
Diurnal temp. range in breeding season	**27.5 (29.7)**	22.3 **(35.2)**	**22.8** (25.6)
Degree days in winter	23.5 (28.6)	10.2 (15.7)	22.6 **(26.6)**
Mean annual precipitation	19.4 (25.3)	**31.5** (33.2)	19.6 (24.0)
Terrain morphology	5.1*	8.6*	7.2*
River density	3.1*	4.4*	3.1*
Artificial wetland density	8.8*	11.8	9.6

Incorporation of additional predictors describing important landscape features such water bodies and topography in the models resulted in more precise, though spatially concordant, predictions of habitat suitability compared with models that used climatic variables only. However, the pattern of variable influence did not change much for individual models, i.e. no change in the historical model and in the novel and all-SA models when the order of the two most influential predictors was swapped around.

4.3.6 Environmental space

The first two PCA axes explained 97% of the variance in the environmental data, and the additional four axes the remaining 3%. Mean annual precipitation (65%) and winter degree days (26%) contributed strongly to component 1, while the same two variables (winter degree days, 70%, and mean annual precipitation, 28%) loaded strongly on component 2 (Appendix 4-4).

The PCA showed the presence and background data clustering across the same environmental space, with most of the presences in the novel range clustering inside the historical presences. This indicates that the environmental space occupied by painted reed frogs in the novel range is similar to that in the historical range. Presence points are clearly within the available background, which covers a larger portion of the environmental space (Figure 4-4). A few novel presence points lie outside the boundary of the 95% probability ellipse around historical presences (Figure 4-4), indicating that some occupied areas in the novel range may have a different combination of environmental conditions from those in the historical range.
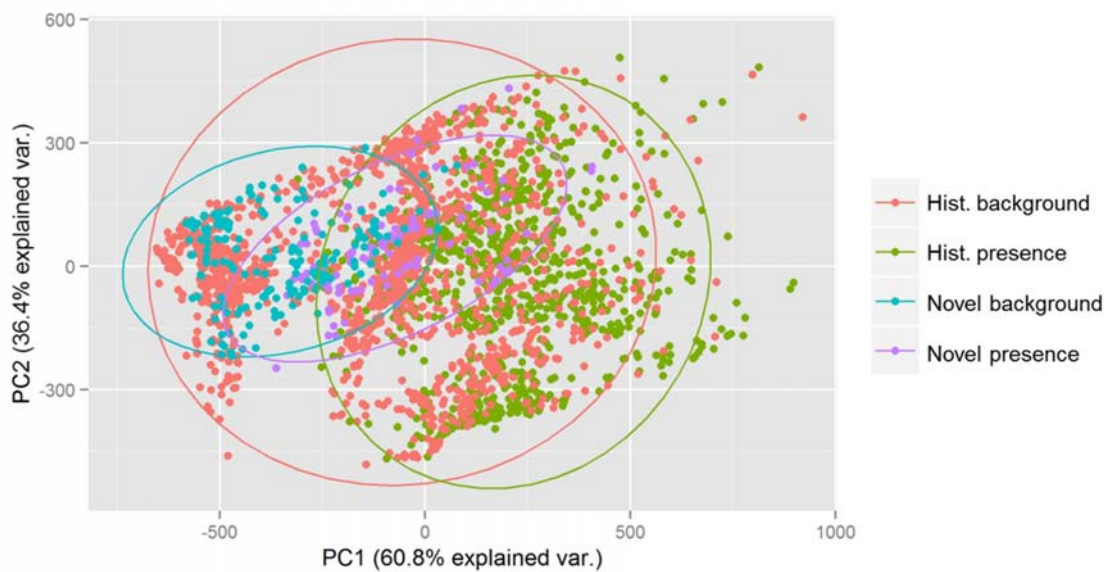
Figure 4-4. Presence points in relation to model background. Points are presence or background points in environmental space defined by the full set of climatic and landscape predictors. Lines are the 95% probability ellipses calculated from the respective presence or background dataset.

The MESS analysis showed that large areas of the historical range lie outside the range of predictor values used to train the novel model (Figure 4-3, main panel). Most of these are outside the model background i.e. outside the lower thermal tolerance of *H. marmoratus*. Within the model background area, the north-eastern part of the historical range has intermediate environmental similarity values. Examination of correlation coefficients revealed that this dissimilarity is driven by degree days in winter, which are higher in this sub-tropical area of eastern Limpopo and northern Zululand than in the temperate Western Cape (also see Appendix 4-2; Appendix 4-1 B and C). In contrast, predictor variables in the novel range are within the range of values of the historical range, with the exception of some high peaks of the Cape fold Mountains (Figure 4-3, inset). Because of the relatively low level of climate dissimilarity in areas within the model background, I did not exclude non-analog areas from the models but took them into account in interpreting the model performance and model predictions.

4.3.7 *Niche overlap*

Niche overlap statistics for the three models projected to the novel range showed that the lowest niche overlap was between the novel and historical models ($D=0.690$). Niche overlap was highest for the novel-all SA comparison ($D=0.854$), and intermediate for historical-all SA comparison ($D=0.764$). Figure 4-5 shows a shift in the niche centroid from the historical to the

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novel niche, corresponding to a shift in environmental space towards areas with higher evaporation and thermal variability.
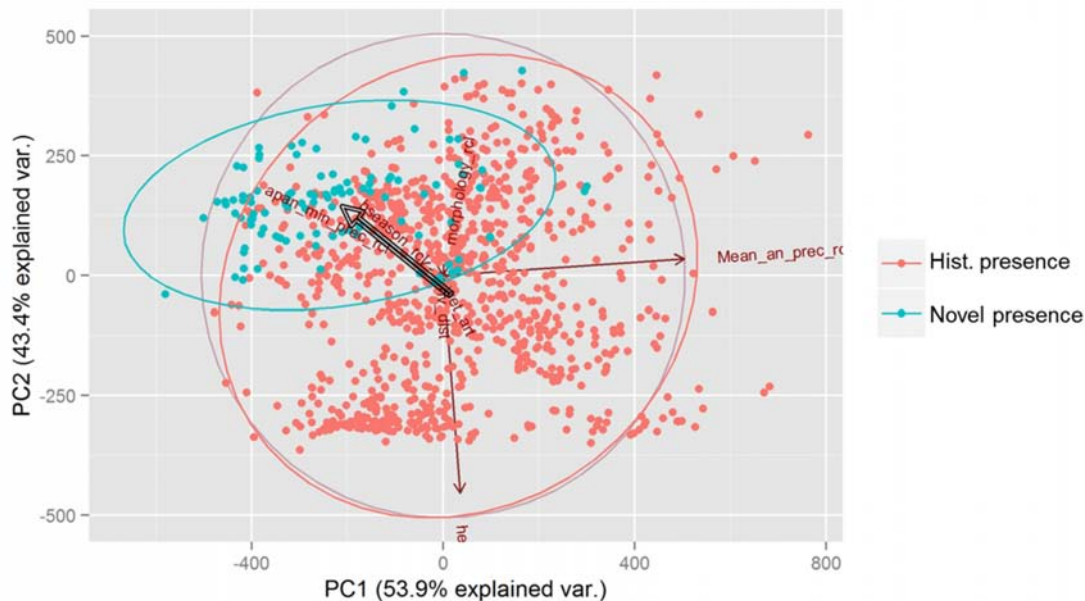
Figure 4-5. Painted reed frog occurrences in the environmental space defined by the full set of climatic and landscape predictors. The black arrow shows the centroid shift between the historical and novel range niches. Lines are 95% probability ellipses calculated from the respective presence dataset (corresponding to 2 standard deviations from the centroid). Maroon arrows show the original environmental predictors in the environmental space (length and direction denotes loading on the respective axis). The maroon circle is the 95% probability circle centred on the combined range (all-SA) in the environmental space.

4.4   Discussion

The range expansion of the painted reed frog has allowed the species to move into climates not found across the historical range. It is likely that landscape level changes (e.g. increase in artificial water bodies) has recently facilitated this expansion into suitable space that was present but previously inaccessible. Spatial predictions differed among the reciprocal range models, with the historical model predicting a smaller, and the novel model predicting a larger, geographical distribution in the novel range than was actually observed from distribution data. The ordination analyses (centroid shift) and patterns of variable importance in the models suggest that there has been a shift in the realised niche of painted reed frogs in the novel range towards increased aridity and temperature extremes (see Figure 4-5). Variables related to water availability, i.e. mean annual precipitation and artificial wetland density, were more important in the novel model than in the historical or all-SA models. On the other hand, the winter thermal environment carried more importance in the historical and all-SA models. Therefore, painted reed frogs

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occupy some habitats in the novel range which they do not occupy in the historical range. This is not because those habitats are not available in the historical range, as the MESS maps show (Figure 4-3).

4.4.1   Niche shift

The reciprocal niche models in this study address the realised niche, and therefore there is no way to accurately distinguish the effects of adaptation after invasion, biotic interactions or the absence of dispersal barriers on the niches measured. All the above factors, in addition to a climatic niche shift, may lead to the appearance of a broader realised niche in the novel range (Guisan *et al.* 2014) as seen here. For example, enhanced dispersal facilitated by artificial water bodies may contribute to the realised niche shift observed here by making areas that would otherwise have been inaccessible available to painted reed frogs.

A shift in the realised niche may also be due to fewer or weaker biotic interactions in the novel range. Tingley *et al.* (2014) found that the fundamental niche of *Rhinella marina* (the cane or marine toad) was unfilled in the native range because of the presence of a congeneric species, *R. schneideri*, (the rococo or Schneider's toad) with which it shares a stable hybrid zone; reciprocal models revealed that together these two congenors fill the fundamental niche of *R. marina* in Australia, emphasizing the role of interspecific interactions in structuring invasive species ranges. The relative lack of congeneric species in the novel range of the painted reed frog (one species, compared with eight species in the historical range) may provide this impetus for *H. marmoratus* by reducing inter-specific competition.

In order for painted reed frogs to evolve a change in their fundamental niche through local adaptation, genetic variation or plasticity, as well as selection is required (Pearman *et al.* 2008). Painted reed frogs in the novel range show plasticity of critical thermal limits, thermal tolerance range and metabolic rates (Chapter 3). One might therefore expect that painted reed frogs have the capacity to shift their fundamental niche and further expand their range in the south-western Cape and in other parts of South Africa. Are frogs in the novel range exposed to greater variety of environmental conditions than those in the historical range that could drive selection for broader physiological tolerance of, for example, dry or extremely hot conditions? A broader set of environmental conditions at novel range occurrence points than historical range occurrence points is suggested by the density plots in Figure 4-6.

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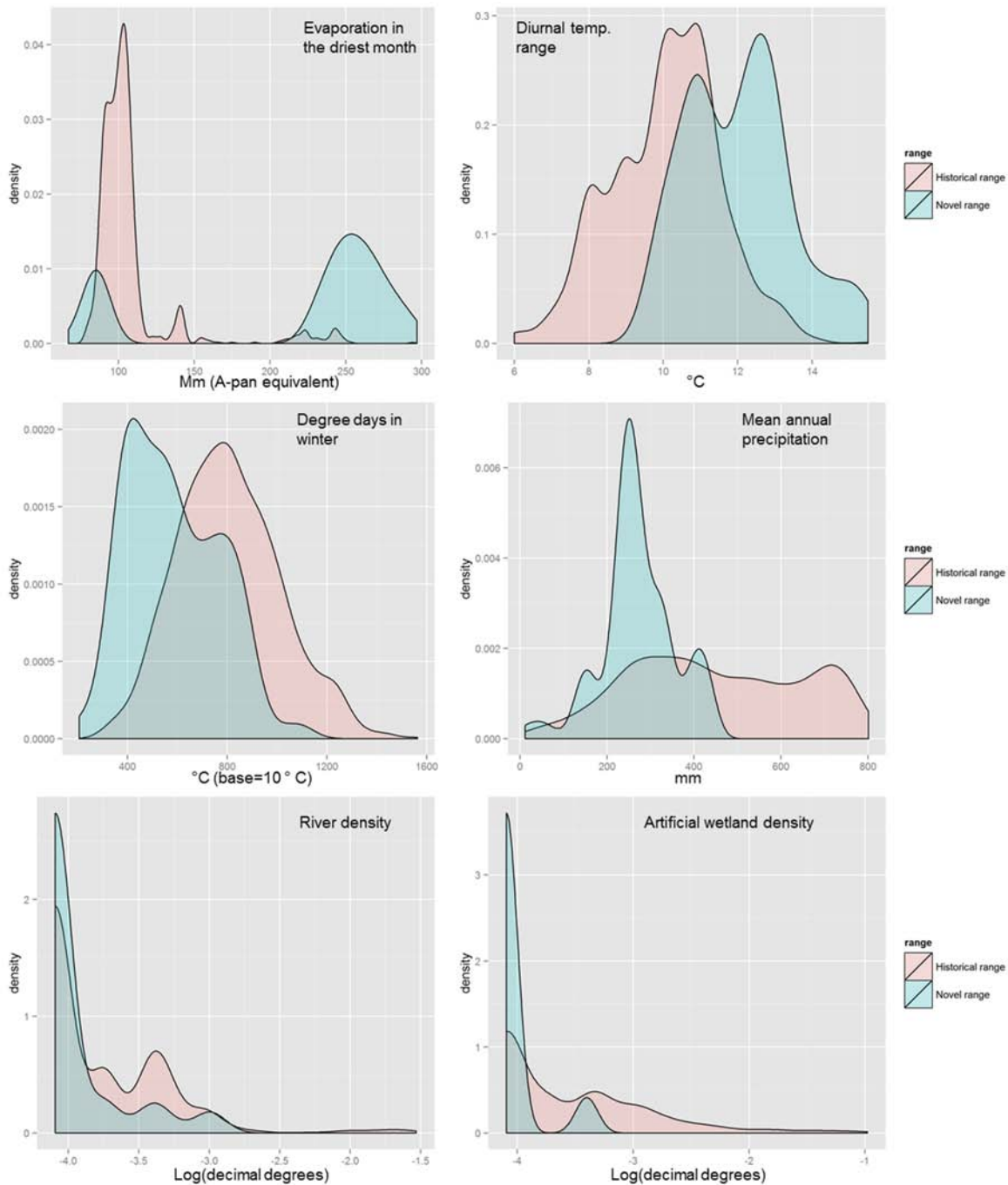
Figure 4-6. Distribution of environmental variable values between historical range and novel range presence points. Plots show the smoothed density function for each range area.

A change in physiological tolerance would indicate a shift in the fundamental niche, which has been found in some invasive plants (Broennimann *et al.* 2007) and ectotherms (Hill *et al.* 2012, 2013; McCann *et al.* 2014). However, painted reed frogs in the novel range are unlikely to undergo a compensatory shift towards increased desiccation resistance as they have little or no plasticity in water loss rates (Chapter 3). Rather, a shift in habitat use or behaviour could allow them to select sheltered microsites around rivers and water bodies to survive dry periods or thermal extremes.

4.4.2 *Niche overlap*

The reciprocal comparison of niches in geographical and environmental space allowed a better understanding of the relationship between distribution and niche and the drivers of changes in both of these patterns (see Soberón and Nakamura 2009). Niche overlap between the historical and novel ranges was relatively low. Inter-study comparisons should be made with caution, due to differences in modelling methods and species distributions, but these values of *D* are higher than those measured for cane toads (*R. marina*) in their native and invaded ranges (Tingley *et al.* 2014) and interspecific comparisons between two *Bufo* species (Denton and Beebee 1994); Hill and Terblanche (2014) found comparable levels of overlap between congeneric invasive fruit flies. This suggests that painted reed frogs are able to select, within their invaded range, areas that are more favorable in terms of tolerance traits.

4.4.3 *Spatial mismatch*

All of the models predicted painted reed frog presence on the Cape Peninsula, despite the small area of obviously suitable habitat in this mountainous area. Populations in this area are restricted to the lower lying inland areas and a few coastal wetlands and tend to be transient. Several breeding sites around Cape Town have shown fluctuating occupancy between years (Krystal A. Tolley, personal communication; SJD unpublished data). This indicates that painted reed frogs may occupy some unsuitable areas in the novel range through source-sink dynamics with re-colonisation of sites through dispersal. Dispersal distances have not been quantified, but Bishop (2004) noted that they travel 'some distance' from breeding sites and often enter buildings; males move several hundred metres from their breeding site each night to roost (Andrew A. Turner, personal communication).

A noticeable mismatch between the historical model and the novel and all-SA models was that the historical model predicted painted reed frogs occurring on the Agulhas Plain, while the other models did not. The Agulhas Plain is a large (*c.* 1500 km^2) coastal flat at the southernmost extreme of the country (Figure 4-1). Many of the wetlands and rivers in this part of the novel range are saline as a consequence of the underlying marine-derived sediments and strong inundation and drying cycles (Russell and Impson 2006). Painted reed frogs may be excluded from this area not by dispersal but by high salinity levels that preclude egg or larval development. Inclusion of water quality (e.g. salinity) and other variables that describe the niche of the larval and egg stages may refine the predictions from these models.

4.4.4   Dispersal

As a result of their non-equilibrium range dynamics, invasive species may not be present in some suitable areas because they have simply not arrived yet or have been prevented from reaching the area by physical barriers such as mountain ranges or by the absence of effective vectors or pathways to accomplish colonisation (Pulliam 2000). Poynton (1964) suggested that the absence of tropical species in the south-western Cape might simply be due to slow post-Pleistocene dispersal rates. These findings suggest an interplay of niche shift and dispersal between water bodies in determining the extent and position of the painted reed frog's novel range.

The capacity for sustained movement of *H. marmoratus* (vagility, *sensu* Hillman *et al.* 2014) has been estimated and is in the region of 300 m h^{-1} (Chapter 3). Although this level of mobility is not particularly high, being intermediate between verified estimates for a ranid and a bufonid (Hillman *et al.* 2014), it suggests considerable capacity for dispersal. Dispersal is likely to be heavily influenced by human-assisted translocation, which is probably frequent given the fact that these frogs can survive long periods without access to free water and can rehydrate rapidly (Geise and Linsenmair 1988).

4.4.5   Future range expansion

Reports of painted reed frog breeding populations in high-elevation inland locations such as Pretoria and Harrismith (Bishop 2004) and a recent record of painted reed frog breeding activity in Barrydale (Leslie R. Minter, personal communication; Figure 4-1) indicate that these frogs are able to persist even in very arid areas if introduced into anthropogenic habitats (Chapter 2). There has been a steep increase in the number of farm dams in the Western Cape from just over 15 000 in 1991 to *c.* 30 000 in 2011 (Siberbauer and King 1991; Chief Directorate Surveys and Mapping 2006; Nel *et al.* 2011). If this trend is replicated in the rest of South Africa, a country-wide range expansion of this species may result from the increase in the artificial water body area.

4.5   Conclusion

This study found evidence of a shift in the realised niche of *H. marmoratus* in South Africa between its historical range in the northern and eastern coastal regions, and its novel range in the Mediterranean ecosystems of the south-western Cape. The niche shift corresponds with a moisture gradient towards increasing summer aridity and generally lower precipitation between the historical and novel ranges, but is likely mediated by landscape transformation in the form of artificial water bodies that provide additional buffered habitats. Thus, range expansion has

allowed painted reed frogs to occupy drier and more variable habitats in the novel range. On a finer scale, access to permanent water in the landscape is limiting as for all amphibians, so artificial water bodies provide a key additional resource for these invasive tropical frogs.

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Appendices
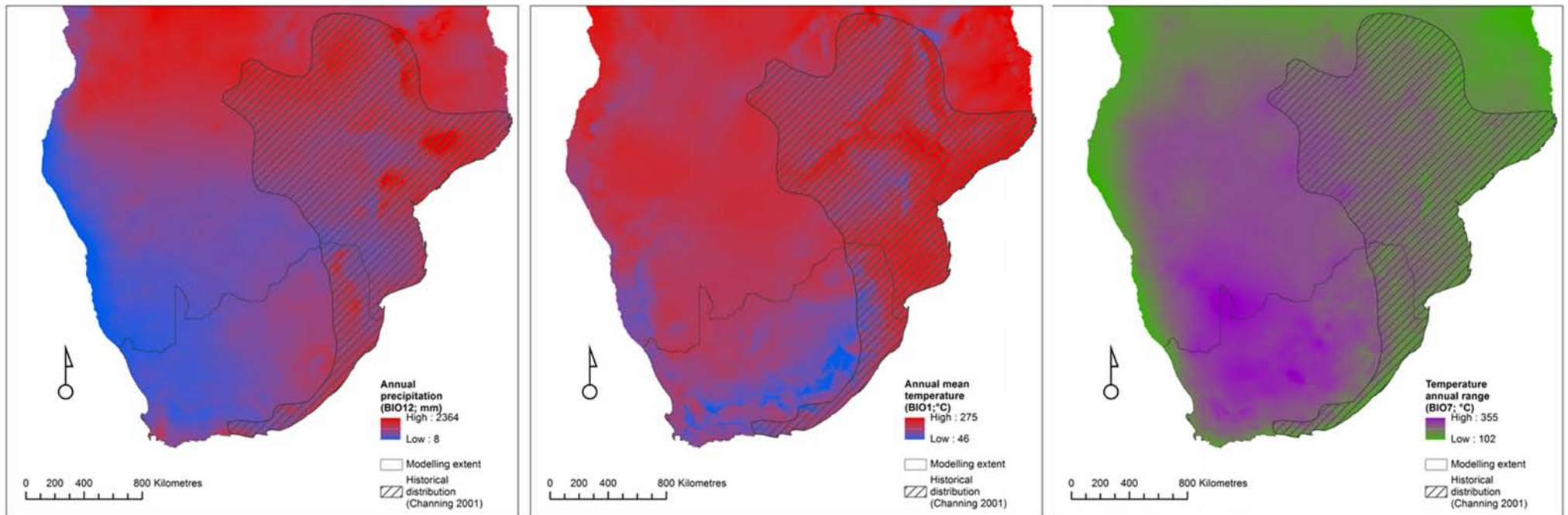
Appendix 4-1. Range of annual precipitation, annual mean temperature and temperature annual range in southern Africa, encompassing the entire *H. marmoratus* range, including the portion outside South Africa which was excluded from the models. Source: Variables BIO12, BIO1 and BIO7 from WorldClim (http://www.worldclim.org/bioclim). Range digitised from Channing (2001).

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Appendix 4-2. Comparison of environmental conditions in the historical and novel ranges. Values are summary statistics for predictor values at presence points.

	Historical presences			Novel presences		
	Mean	Min.	Max.	Mean	Min.	Max.
Evaporation in driest month (mm)	108	77	294	216	67	297
Diurnal temp. range in br. Season (ave. °C)	10	6	16	12	10	16
Degree days in winter (summed °C)	453	18	801	271	12	439
Mean annual precipitation (mm)	807	292	1563	583	207	1089
Terrain morphology (categorical)	n/a					
River density (decimal degrees)	0.015	0.000	0.217	0.010	0.000	0.050
Artificial wetland density (decimal degrees)	0.021	0.000	0.376	0.004	0.000	0.033
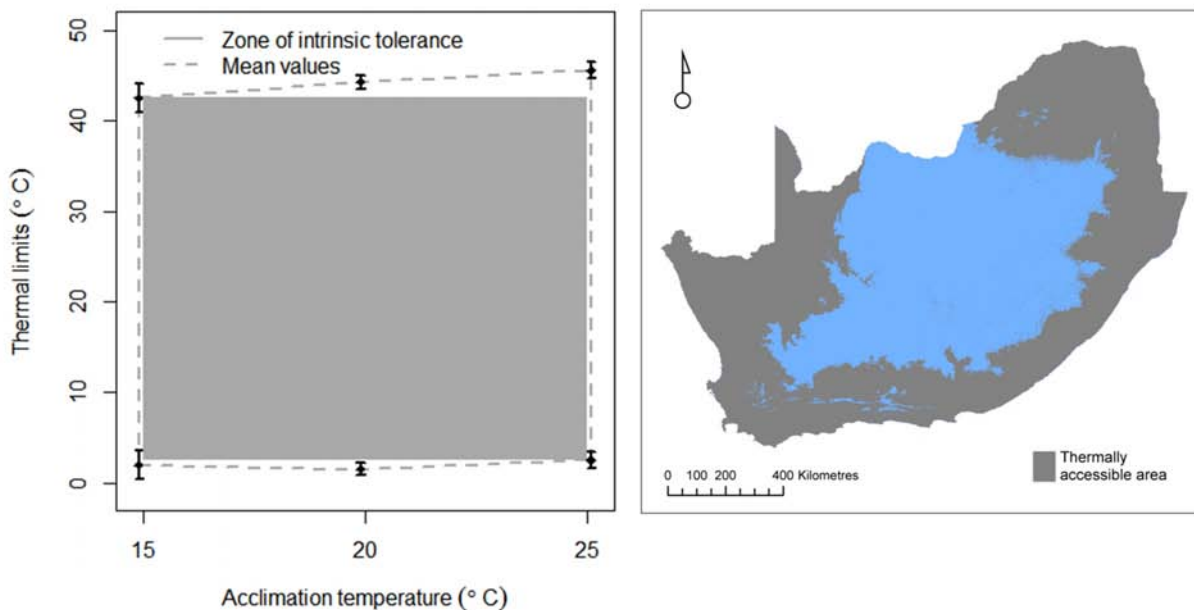
Appendix 4-3. Thermal constraints on adult *Hyperolius marmoratus* in South Africa. A. Zone of intrinsic tolerance (grey area) calculated from critical thermal tolerance tests on 115 frogs from the novel range acclimated at 15, 20 and 25°C (see Chapter 3) using the method of Eme and Bennett (2009). B. Mask delineating the region of South Africa that conforms to the thermal constraint of daily mean minimum temperature in July >1°C (grey area). This mask was used to generate background data for models.

Appendix 4-4. Variable influence in PCA of model occurrence and background data. Values are loadings on PCA axes 1 and 2 for analyses including climatic and landscape variables. Axis 1 explained 60.8% and axis 2, 36.3% of the variance in the data.

	Influence on axis 1	Influence on axis 2
Evaporation in driest month	8.3%	1.4%
Diurnal temp. range in breeding season	0.5%	0.1%
Degree days in winter	26.1%	**69.7%**
Mean annual precipitation	**64.5%**	28.1%
Terrain morphology	0.6%	0.7%
River density	0.0%	0.0%
Artificial wetland density	0.0%	0.0%

Appendix 4-5. Summary information on wetlands and estuaries in the novel and historical ranges and South Africa as a whole. Water body density is higher in the novel range as water bodies are more numerous, though smaller. Data source: National Freshwater Ecosystem Priority Areas (NFEPA; Nel *et al.* 2011).

	Historical range	Novel range	South Africa
Total area (km^2)	1 150 086 (90%)	125 583 (10%)	1 275 669
Density of wetlands (per km^2)	0.314	0.578	0.340
Area of wetlands (km^2 / km^2)	0.022	0.025	0.022
Average size of wetlands (km^2)	0.071	0.044	0.066
Wetland area (km^2):			
Artificial	4 906 (19%)	509 (16%)	5 415 (19%)
Natural	19 675 (76%)	2 150 (67%)	21 826 (75%)
Estuaries	1 144 (4%)	564 (18%)	1 709 (6%)
Total	25 726	3 224	28 950
Number of wetlands:			
Artificial	142 390 (39%)	27 878 (38%)	170 268 (39%)
Natural	218 246 (60%)	44 498 (61%)	262 744 (60%)
Estuaries	638 (<0.5%)	326 (0.5%)	964 (<0.5%)
Total	361 276	72 702	433 978

Chapter 5. Conclusion

Photo: Brummer Olivier

Brown morph males at a breeding chorus in Stellenbosch
-33.995°S 18.772°E

In this thesis I investigated several important aspects of the invasion process, to answer the question of how a frog from a tropical biogeographical centre has made such a successful transition into a novel climatic zone. I found that availability of water in the landscape and the physiological capabilities of the frogs in relation to water loss are key determinants of the invasion.

5.1 Synthesis

5.1.1 Range structure and habitat

Chapter 2 revealed that the novel range structure of the painted reed frog is strongly influenced by patches and gaps in the range, which I inferred were due to human-assisted jump dispersal and barriers to spread. A previous biogeographical analysis of mitochondrial DNA of *H. marmoratus* showed that multiple introductions to the novel range have occurred from different parts of the ancestral range, some of them more than 800 km from the novel range (Tolley *et al.* 2008). The integration of findings from my work with the study of Tolley *et al.* (2008) showed that genetically-uniform populations of *H. marmoratus* were present on either side of a mountain barrier (part of the Cape fold mountain range). This confirmed the idea that the range is structured by anthropogenic processes as well as natural barriers to spread. The identification of habitat correlates of occupancy in Chapter 2 suggested that evaporative potential in the driest month and winter temperatures may be important range limiters for *H. marmoratus*, and these predictors were included in the niche model in Chapter 4.

5.1.2 Plasticity and evolution

Chapter 3 confirmed the close link between thermoregulation and water balance in painted reed frogs. My data did not show thermal acclimation of water loss rates, although there was clear plasticity of resting and active metabolic rate. However, frogs were able to limit water loss behaviourally by using the water-conserving position. This may be a case of behavioural inertia (Huey et al. 2003) or may point to an evolutionary trade-off between evaporative cooling as a thermoregulatory strategy and evolving a high R to minimise water loss (see Tracy 2008).

I could not evaluate the ultimate causes of the lack of water loss plasticity in my experimental design, but they indicate that *H. marmoratus* may be a suitable study organism for further work on this interesting aspect of environmental physiology. Previous studies on *H. marmoratus* have pointed to the special challenges that face arboreal frogs because of the variability of their arboreal environments and distance from standing water (Geise and Linsenmair 1988). Tracy *et*

al. (2008) suggested that in Australian tropical frogs a consequence of elevated skin resistance to water loss may be difficulties with thermoregulation, and indeed Passmore and Malherbe (1985) recorded body temperatures *c*. 3°C above ambient temperature in painted reed frogs. I concluded in Chapter 3 that the water-conserving posture, presumably augmented by selection of sheltered microhabitats, was the only mechanism available to painted reed frogs to limit water loss, supporting the idea that in painted reed frogs the need to thermoregulate has taken precedence over the development of plasticity in water loss rates.

Hyperolius marmoratus is one of the smallest arboreal frogs in which metabolism and water loss have been studied in a rigorous experimental framework. The small size of these frogs means that they are subject to wide fluctuations in body temperature. On the basis of modelled water loss in arboreal frogs, Tracy *et al.* (2010) proposed that large body size increases time to desiccation, and that larger desiccation resistant frogs tend to be arboreal. Therefore *H. marmoratus* and its congenors are unusual cases of very small frogs that are able to exploit arboreal habitats. Further studies of this very tractable experimental subject could address the implications of ectothermy, small body size and arboreal lifestyle for the evolution of desiccation resistance.

5.1.3   *Fundamental knowledge*

Chapter 3 also filled important gaps in basic physiological knowledge of *H. marmoratus*. My experimental data revealed that, in spite of the lack of flexibility of water loss, critical thermal limits show considerable plasticity, and the upper limit responds to acclimation more strongly than does the lower limit. This is unusual among ectotherms, whose CT_{max} is constrained by the relationship between thermally-dependant metabolic processes and environmental temperatures (Hoffmann *et al.* 2012; Hillman *et al.* 2009). *Hyperolius marmoratus* is a tropical to sub-tropical species, and therefore is expected to be living in areas where environmental temperatures are close to its upper critical limit. Whether the plasticity observed in critical thermal limits in novel range frogs has evolved post-invasion, or existed prior to invasion is unknown. A previous study that investigated critical thermal maximum in relation to acclimation treatments in the historical range found some plasticity, but that study cannot be directly compared to this one, as a non-standard experimental end point was used (Geise and Linsenmair 1986).

5.2   Potential for further invasion by *H. marmoratus*

The area currently occupied by painted reed frogs in the novel range is a narrow latitudinal band aligned with east-west trending climatic, ecological and infrastructural zones imposed by the orientation of the coastline and the Cape fold mountain ranges (Goldblatt and Manning 2002; Mucina and Rutherford 2006). The Cape fold mountains impose physical barriers to northward spread, while the coastline imposes a barrier to southward spread. Topography also influences frontal weather systems that produce most of the Western Cape's winter rainfall and low temperatures (Tyson 1986), producing a steep moisture gradient between the coast and the semi-arid interior. These physical features suggest that diffusion-based spread will not proceed in a north or south direction, but the niche model trained in the novel range (Chapter 4) predicted some areas of occurrence in the extreme south-west of the novel range to the north of Cape Town, where the coastline and the mountain range turn and trend north-south (Figure 1-3).

Recently-identified trends towards increasing spring and summer rainfall in parts of South Africa (MacKellar *et al.* 2014) may invoke range expansions in other parts of the range. Significant increases in spring and summer rainfall in the Drakensberg and parts of the Eastern Cape may lead to range expansions in this area. Since the topography of this area is mountainous and bounded by ocean on the east, expansion into the foothills of the Drakensberg is possible where sufficient surface water is available, for example in agricultural areas.

Silberbauer and King (1991) identified over 15 000 artificial farm dams in the Western Cape, representing nearly 90% of water bodies in the province. This number has increased substantially with the National Freshwater Ecosystem Priority Areas database (NFEPA; Nel *et al.* 2011) recording over 27 000, and Chief Directorate Surveys and Mapping (2006) mapping 32 000 artificial water bodies in the province. Despite inevitable differences in enumeration methods, this indicates a substantial increase in artificial wetland habitat in the Western Cape in the past 15 to 20 years.

The Western Cape has relatively few natural permanent water bodies, particularly larger lentic systems (Shaw 1988; Appendix 4-5), but artificial impoundments such as farm dams and irrigation canals are common on agricultural land, adding to the freshwater aquatic habitat available in the landscape. The role of these structures as additional habitat for invasive painted reed frogs was discussed in Chapter 2, and has been shown to be important for other extra-limital and indigenous species (Froneman *et al.* 2001 and Simmons 2011 for birds; Hazell *et al.* 2004

for amphibians; Samways 1989, Apinda-Legnouo *et al.* 2014 and Brainwood and Burgin 2009 for invertebrates). In addition to resource supplementation, these structures may also facilitate the range expansion process by providing stepping stones, corridors (Lobos and Measey 2002 for *Xenopus laevis*) or networks (Woodford *et al.* 2013 for freshwater fish) which increase propagule transfer through the landscape.

Reports of painted reed frog breeding populations in high-elevation inland locations such as Pretoria and Harrismith (Bishop 2004) and a recent record of painted reed frog breeding activity in Barrydale (Leslie R. Minter, personal communication; Figure 4-1) indicate that these frogs are able to persist in very arid areas if introduced into anthropogenic habitats (Chapter 2).

5.2.1 *The influence of environmental change*

Many studies (e.g. Hughes 2000 and Parmesan and Yohe 2003) have drawn attention to the range shifts that accompany climate change. Concurrent with these changes, extra-limital invasions are becoming more common, and can be anticipated to become more problematic as global climate change and landscape change accelerate. These range epxansions could become especially significant if the invading domestic species have demonstrable impacts through, for example, changes in habitat quality, effects on co-occurring species through competition or predation, or transmission of parasites and pathogens. The extent and impact of extra-limital invasive populations are similar to those of extra-regional exotics (Pyšek et al. 2004; Valéry *et al.* 2008). Wall lizards *Podarcis muralis* introduced to north-western Europe from their sub-Mediterranean range competitively displace native lizards and swamp native genotypes at the invaded range edge (Schulte *et al.* 2012). American bullfrogs *Lithobates catesbeianus* in North America (e.g. Both and Grant 2012) and European starlings *Sturnus vulgaris* in Europe (e.g. Keonig 2003) have undergone extra-limital invasions with serious consequences for local biodiversity.

Globally, many amphibians have been affected by or are under threat from climate change and its localised manifestations, as well as chytridiomycosis (Pounds and Crump 1994; Pounds *et al.* 2006). These emerging trends have implications for African tree frogs, one of the largest and most diverse groups of tropical frogs in the world, but they have been almost completely unstudied.

Using the warming tolerance framework of Deutsch et al. (2008), I combined the plasticity of critical thermal limits in *H. marmoratus* with the environmental and micro-site temperatures recorded in this study to calculate the warming tolerance (WT = CT_{max} - T_{hab}) of *H. marmoratus* at three acclimation temperatures. The results show that there is considerable scope for warming tolerance in this species (Figure 5-1), especially if historical patterns of relationship between temperature variability and critical thermal maximum are borne out (Clusella-Trullas *et al.* 2011).
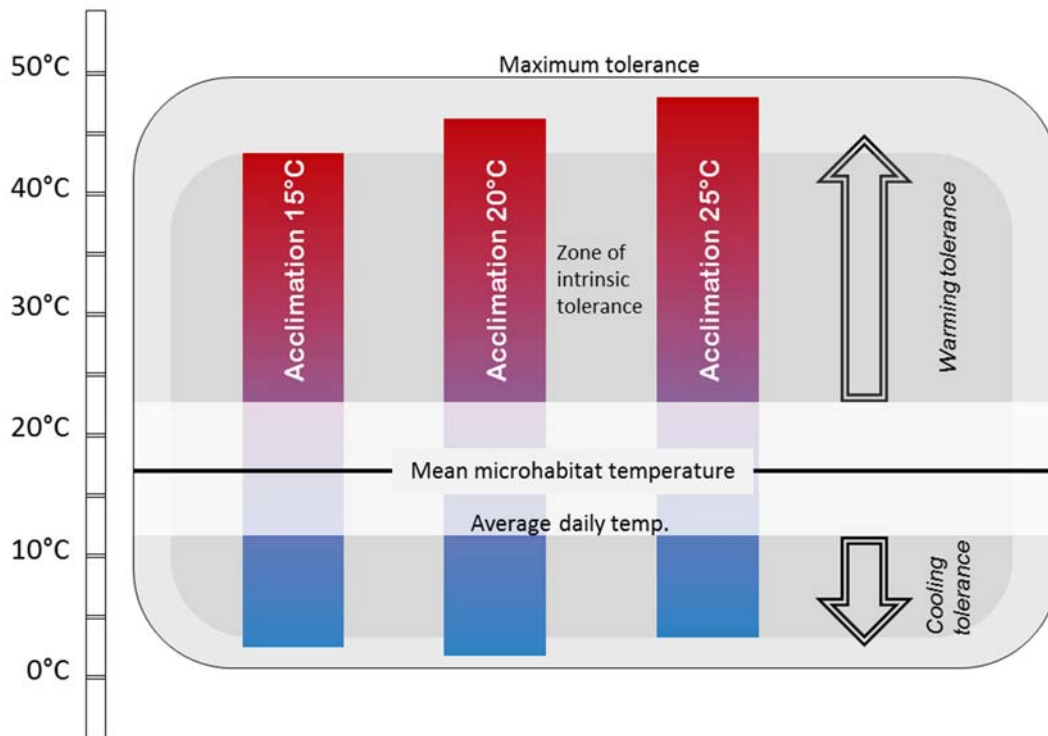
Figure 5-1. Warming tolerance (WT = CT_{max} - T_{hab}) of *Hyperolius marmoratus* estimated from data gathered in Chapter 3. The figure shows the potential for adaptation to changing conditions through phenotypic plasticity (acclimation capacity) or evolution to 'track' environmental change. The grey backgrounds represent the zone of intrinsic tolerance (darker grey) and the absolute maximum tolerance induced by acclimation from experiments in Chapter 3 (lighter grey). The central white block shows the range of long-term average daily temperatures (South African Weather Service, 2008). The mean microhabitat temperature line shows the mean temperature over a full year of monitoring in calling microsites at ten occupied dams (17.3°C; S.J.D., unpublished data)

In addition to the margin of warming tolerance, acclimation of lower critical temperatures suggests that in future painted reed frogs may be able to further adjust to cold winter temperatures and thereby exploit colder habitats. It is unlikely that these new areas would be at higher altitudes, as found for the cane toad (McCann *et al.* 2014), because water bodies are scarce at higher altitudes in the novel range. However, painted reed frogs may be able to move

into agricultural areas north of their present range, and a recent record of their presence in a more arid site, Barrydale (Figure 4-1) supports this idea. Further expansion into the farming areas to the north of the current range would be contingent on the availability of sufficient permanent water in these areas.

5.2.2   *The effects of human disturbance*

The painted reed frog's spread into and within the novel range is strongly influenced by human translocation and its niche is influenced by anthropogenic landscape structures, specifically artificial water bodies such as dams and ponds (Chapter 2 and Chapter 4). Several other invasive species show similar responses to human disturbance regimes. Roura-Pascual *et al.* (2011) found that the distribution of human-modified habitats as well as climatic suitability and biotic resistance played a role in the global distribution of the Argentine ant. The range of the American bullfrog, a globally invasive amphibian, is strongly influenced by hunting pressure in addition to climatic suitability (Ficetola *et al.* 2007). Non-climatic factors have even been used to model spread without the addition of climatic predictors. With (2002) showed how the size and spatial distribution of modelled disturbance, as well as its prevalence across the landscape, influence the spread of invasive species. Kearney *et al.* (2008, 2009) constructed mechanistic models for the cane toad based simply on simulations of water body distributions, showing the powerful effect that human disturbance can have on invasive species spread. Therefore, the identification of the painted reed frog as an urban exploiter can add to knowledge of the relationship between biological invasions and human activities. It may allow potential management actions for *H. marmoratus* to be targeted more realistically within human-modified habitats, and may inform local management strategies for other urban exploiters such as the guttural toad, *Amietophrynus gutturalis* (see Measey *et al.* 2014).

5.3   Value of the study

5.3.1   *Modelling frameworks and data use*

The drivers of painted reed frog invasion identified in this study include climatic and landscape structure variables, and the role of behaviour and microhabitat availability and use. This demonstrates that niche models need to utilise data of different types at multiple scales, including climate variables, information on land use and land cover and detailed habitat quality information. In addition, such models should also take account of behaviour, physiology and interactions between them. This introduces considerable complexity into the modelling process.

However, as most invasions occur, or are more noticeable at inter-continental scale (Guo and Ricklefs 2010), many studies of invasive species niche shift are conducted at large spatial scales and are limited by the range and quality of data available at that scale. In some cases computing power may pose constraints on the resolution of broad-scale models (e.g. Di Febbraro *et al.* 2013). Model systems such as this one, which address extra-limital range expansion at a relatively small spatial scale in a single region provide the opportunity to build and test models at a fine scale.

5.3.2 *Single species studies*

My study addressed the invasion process of a single species in order to identify drivers of invasion in the rapid extra-limital invasion of *H. marmoratus*. I could use distribution data from multiple sources to examine invasion patterns such as rate of spread and range structure and infer invasion mechanisms such as multi-modal spread. An outstanding example of how multidisciplinary studies on a single species can contribute knowledge to influence an entire field of science is the invasion of the cane toad in Australia. Research on *Rhinella marina* has transformed our understanding of vertebrate invasions and advanced the field of invasion biology generally. Studies on this species have moved from basic life history studies (Zug and Zug 1979), through spread models (Urban *et al.* 2007, 2008), population genetics (Estoup *et al.* 2001, 2004) and impact studies (Boland 2004; Doody *et al.* 2006, 2009; Crossland 2000, Crossland *et al.* 2008) to mechanistic and hybrid niche models (Kearney *et al.* 2008; McCann *et al.* 2014), bringing innovative approaches to modelling invasive species generally (Kearney and Porter 2009). The inclusion of information from the native range in studies of Australian *R. marina* has added understanding of the niche dynamics (Urban *et al.* 2007; Tingley *et al.* 2014). As a whole, this body of work has emphasised the dynamic nature of invasions, advanced the field of invasive species modelling and brought the possibility of biological control of this species into the foreground (Shanmuganathan et al. 2010). Urban *et al.* (2007) suggested, based on range models calibrated in the native range, that the cane toad may be limited by abiotic conditions in the invasive range. However, evidence of enhanced dispersal ability (Phillips *et al.* 2006) and cold tolerance (McCann *et al.* 2014) in invasive populations now indicates that cane toads are simply evolving to better exploit the novel environments available to them.

5.4   Pressing knowledge gaps for *H. marmoratus*

5.4.1   Abundance and population structure

Abundance of *H. marmoratus* in the novel range has not been quantified. Traditional capture-mark-recapture studies or spatially explicit methods (SECR, Borchers 2010) would be useful to clarify fine scale population numbers and movements of individuals between habitat patches. The distribution of *H. marmoratus* in its novel range could be a useful model system for examining abundance structure and occupancy between different spatial scales.

5.4.2   Dispersal capacity

Dispersal distances and frequencies for painted reed frogs are unknown, and no other species in the group has been studied. Metamorphs of this species are very large relative to adult size (up to 75% of adult body length, Schiøtz 1971) and may be able to travel long distances soon after emerging from their natal ponds. Quantifying the capacity for dispersal through laboratory and field performance experiments would inform any future studies of niche and range dynamics in these widespread frogs.

5.4.3   Requirements of egg and larval stages

This study addressed the range and niche shift of the painted reed frog through examining the physiology and environmental requirements of the adult life stage. The complex life cycle of aquatic breeding amphibians makes them vulnerable to environmental changes that affect both terrestrial and aquatic life stages (Wells 2007). Egg and larval thermal tolerance, plasticity of development times and thermal tolerance and a variety of other parameters require quantification in this and many other tropical anurans to allow prediction of environmental change effects and invasion potential in these species.

5.5   Horizon scanning

5.5.1   Other *Hyperolius* species

South Africa's amphibian fauna is split into two distinct faunal groups or centres; the north-east centre contains many widespread species which occur widely across southern Africa, whereas the majority of species in the south-west centre are endemic (Poynton 1964; Alexander *et al.* 2004; Measey 2011). While this biogeographic break loosely reflects a climatic (especially thermal) gradient between tropical and temperate zones, very few amphibian species straddle the boundary. Nonetheless, the painted reed frog's range now fully spans this biogeographic break (Minter *et al.* 2004) and it maintains large breeding populations across the novel range.

Hyperolius marmoratus is the most widespread of the nine *Hyperolius* species in South Africa, and most other *Hyperolius* distributions, except that of the arum lily frog *H. horstockii*, appear to be nested within its outer boundary (Minter *et al.* 2004). In a genus of over 120 species, *Hyperolius marmoratus* is the only one that has undergone a rapid range expansion. Over the past five years, two introductions of tinker reed frogs (*H. tuberilinguis*) from north-eastern South Africa to the Cape Town area have been intercepted on fruit purchased from local greengrocers, and which likely originated in the northern part of South Africa over 800 km away. *Hyperolius tuberilinguis* is similar in appearance to *H. marmoratus*, has similar body size and adopts the same water-conserving position, but is not known to have established breeding populations anywhere outside its recorded range (northern and eastern KwaZulu-Natal). However, tinker reed frogs do not have the extremely high desiccation resistance of painted reed frogs (resting water loss rates are about twice as high - Geise and Linsenmair 1988), and uses retreat sites on the ground rather than in exposed positions on plants and buildings. The latter feature indicates that they may be less cold-tolerant than *H. marmoratus*, a proposition that is supported by their strictly sub-tropical range. From this study it is possible to hypothesise that tinker reed frogs will be unable to invade in the south-western Cape because of their higher water loss rates and potential lack of resistance to winter temperatures.

5.6   Implications for invasive species management in South Africa

Regions that are species-rich can be a source of introductions to other regions (Spear and Chown 2009), and the possibility that South Africa may become a source of amphibian invasions for sub-Saharan Africa and other southern ecosystems should be taken seriously (Chown *et al.* 2009). Individual case studies of invasion history, rate of spread and mechanisms driving invasion along with selection of model systems from which generalizable conclusions can be drawn are valuable for expanding the knowledge base for biosecurity programmes.

South Africa has forward-thinking legislation for the prevention and management of biological invasions, but implementation of an integrated biosecurity system for the country is in its infancy (Wilson *et al.* 2013). Further information on ecological systems and human capacity is required to inform decisions, and baseline information on species ranges is essential for informing species lists and regulation implementation (McGeoch *et al.* 2012). Basic physiological information is needed to inform ecological niche modelling for screening programmes. The broad framework used in this study, incorporating range mapping and range structure analysis, rate of spread,

experimental work on physiological rates and tolerance, followed by niche modelling, can be used to accelerate information provision on species subject to risk assessment.

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