Conservation biology of the giant bullfrog, *Pyxicephalus adspersus* (Tschudi, 1838)

by

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Conservation biology of the giant bullfrog, *Pyxicephalus adspersus* (Tschudi, 1838)

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Abstract

The giant bullfrog, *Pyxicephalus adspersus*, is a large, explosive-breeding anuran from southern Africa, which spends most of the year buried in a state of torpor. In South Africa this species is considered to be Near-Threatened by habitat loss and other factors, especially in the densely human populated Gauteng Province. The aim of this thesis was to obtain essential outstanding information about the ecology of *P. adspersus* to contribute towards improved conservation management of this species.

A model was used to predict the geographic range of *P. adspersus* in southern Africa, and recent land cover data were used to determine the amount of suitable habitat remaining for this species in Gauteng. As a step towards identifying *P. adspersus* conservation management units, genetic structure and gene flow for populations from 23 localities in Gauteng and seven additional localities in the north-eastern interior of South Africa was quantified using 708 base pairs of the mitochondrial gene cytochrome *b*. To investigate the unpredictable activity and unknown spatial habitat requirements of *P. adspersus*, a population's spawning and non-breeding activity was monitored, and the movements of 70 adult frogs were radio- or spool-tracked during five summers at a site in Diepsloot, Gauteng. Using skeletohronology, the age distribution of breeding *P. adspersus* at this and two other peri-urban sites near Johannesburg, Gauteng, was examined.

Bioclimatic conditions were predicted to be suitable for *P. adspersus* in the temperate to semi-arid interior, but not the low-lying eastern subtropical and arid western sides of southern Africa. Limited genetic data suggested that *P. adspersus* was common in the north-eastern interior of South Africa, and that populations in the Free State Province represent an evolutionary significant unit of this species. In central Gauteng, where *P. adspersus* may have declined by > 90%, populations < 20 km apart exhibited significant genetic differentiation, possibly as a result of genetic drift. At Diepsloot, both annual numbers of spawning events and numbers of spawning males were positively correlated with rainfall, although other meteorological variables also affected the activity of *P. adspersus*. Radio- or spool-tracked frogs showed high fidelity to their breeding site and burrows, which were situated up to 1 km away from the water. Male *P. adspersus* probably live ≥ 20 years in the wild, but at some peri-urban breeding sites adult life expectancy and body size may be declining.

The geographic range of *P. adspersus* was predicted to be slightly smaller than that reported by other authors, and deserves phylogeographic validation. The main conservation priority for *P. adspersus* in South Africa should be the protection of terrestrial habitat for adult foraging and aestivation around, and for juvenile dispersal and gene flow between, breeding sites. In Gauteng, the conservation of a *P. adspersus* meta-population is critical, and could most likely be achieved in the northern region of this province. Populations in the Free State Province deserve improved protection given their reported genetic uniqueness. At local spatial scales specific threats (e.g. pollution) should be ameliorated, and long-term monitoring should be implemented to detect real population trends.

Keywords amphibian, Anura, biology, Gauteng, grassland, Pyxicephalidae, southern Africa, threatened, wetland

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Disclaimer

This dissertation was written as a collection of five manuscripts to simplify the process of publication of the results, as recommended by the Department of Zoology and Entomology. There has consequently been duplication of some information between the chapters. Chapters 2, 3 and 4 have been published and are, respectively, cited below. Chapters 5 and 6 might be submitted for peer-review in the future.

Chapter 2

Yetman, C.A. & J.W.H. Ferguson (2011a). Spawning and non-breeding activity of adult giant bullfrogs (*Pyxicephalus adspersus*). *African Journal of Herpetology* **60:** 13-29.

Chapter 3

Yetman, C.A. & J.W.H. Ferguson (2011b). Conservation implications of spatial habitat use by adult giant bullfrogs (*Pyxicephalus adspersus*). *Journal of Herpetology* **45:** 56-62.

Chapter 4

Yetman, C.A., P. Mokonoto & J.W.H. Ferguson (2012). Conservation implications of the age/size distribution of giant bullfrogs (*Pyxicephalus adspersus*) at three peri-urban breeding sites. *Herpetological Journal* **22:** 23-32.

Declaration

I, Caroline Angela Yetman declare that the thesis, which I hereby submit for the degree Ph.D. (Zoology) at the University of Pretoria, is my own work and has not been submitted by me for a degree at this or any other tertiary institution.

Caroline A. Yetman: ____________________________ Date: __________________________

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Chapter 1

Introduction

Global decline of amphibians

Concern about widespread amphibian population declines emerged in 1989 at the First World Congress of Herpetology (Stuart *et al*. 2004). Research has shown, however, that declines had already begun in Australia, and in north and central America during the 1970s (Czechura & Ingram 1990; Drost & Fellers 1996; Burrowes *et al*. 2004). Although amphibian populations are known to fluctuate dramatically in response to variable weather conditions, individual survival and reproductive success (Pechmann & Wilbur 1994; Marsh 2001), probalistic models indicate that the declines have been significantly more severe and extensive than expected under normal fluctuationg demographic conditions (Pounds *et al*. 1997).

To properly assess the situation, the Global Amphibian Assessment was driven by the IUCN (World Conservation Union) to obtain data on the distribution, abundance, population trends and threats of the 5 743 amphibian species that were recognized at that time (Stuart *et al*. 2004). The Assessment revealed that almost one third (32.5%) of the evaluated species was globally threatened and that amphibians were, therefore, the most threatened of the five Vertebrate classes (Stuart *et al*. 2004). Moreover, since 22.5% of the species were considered Data Deficient, the actual proportion of threatened amphibians was almost certainly larger. The most common and widespread threat was habitat loss (Stuart *et al*. 2008), although many amphibians suffered severe enigmatic declines possibly related to the virulent fungal disease chytridiomycosis, climate change, increased UV-B radiation or synergistic effects of different threats (Pounds *et al*. 2006).

Conservation status of South African anurans

South Africa boasts an amazing variety of anurans of which 43% are endemic (Measey 2011). In 2004, 117 anuran species were recognized in the country of which 20 (17%) and 8 (7%) were considered globally threatened or Data Deficient, respectively (Minter *et al*. 2004). In 2010, the number of South African anurans recognized increased to 118, of which 17 (14%) are considered globally threatened and none are Data Deficient (Measey 2011). Habitat loss is currently the greatest threat to South African anurans, of which 50% are affected by agriculture and aquaculture, 37% by invasive organisms (mainly alien plants), 33% by residential and commercial development, 26% by modification of natural systems (e.g. fire) and 15% by pollution (Measey 2011).

Although considered Least Concern globally (Measey 2011; IUCN 2011), since 2001 the giant bullfrog, *Pyxicephalus adspersus*, has been regarded as Near-Threatened in South Africa (Minter *et al.* 2004) due to estimated population declines of between 50 and 80% (Harrison *et al.* 2001). These declines were observed mainly in South Africa's Gauteng Province, and habitat loss has been considered the greatest threat to this species, although road traffic and harvesting for human consumption have also significantly affected certain populations (Harrison *et al.* 2001; Cook 2002; Minter *et al.* 2004). In Swaziland, where *P. adspersus* appeared to occur marginally, populations have alledgedly gone extinct (Boycott 2001).

The aim of this thesis was to obtain essential outstanding information about the ecology of *P. adspersus* to contribute towards improved conservation management of this species. A brief description of what was known about *P. adspersus* prior to this research follows.

Biology of *Pyxicephalus adspersus*

Pyxicephalus adspersus (Tschudi, 1838) is the largest amphibian in southern Africa (Channing 2001) and one of the largest anurans in the world (Mattison 1992). Adult *Pyxicephalus adspersus* are also known for their aggressive disposition and tendancy to bite using the canine-like projections on their lower jaw. An additional unique feature of *P. adspersus* is the pronounced, reversed sexual size dimorphism of adults (males: ~ 400-1000 g; females: ~ 90-300 g; Cook 1996), which otherwise have a uniform olive-green dorsum with elevated longitudinal skin ridges, and a white to yellow ventral surface (Carruthers 2001).

Pyxicephalus adspersus inhabits mainly grassland and open savanna in parts of east and across most of southern Africa, including all nine provinces of South Africa (Channing 2001; Clauss & Clauss 2002; Minter *et al.* 2004). Individuals spend most of the year in a state of torpor buried underground, where they produce a cocoon comprising multiple layers of sloughed skin (Parry & Cavill 1978). Cocoon formation may reduce evaporative water loss by ≥ 50% (Loveridge & Crayé 1979), and reduction in metabolic rate can facilitate a 75% decline in the rate of oxygen consumption of *P. adspersus* in torpor (Loveridge & Withers 1981). This enables individual *P. adspersus* to survive underground for typically 6-8 months of the year where conditions are dry during winter.

During summer, adult *P. adspersus* emerge after heavy rainfall to breed explosively in shallow, seasonal wetlands with emergent grassy vegetation (Balinsky & Balinsky 1954; Grobler 1972; Cook 1996). Males produce a soft, low-pitched "whoop" (Channing *et al.* 1994) and exhibit different size-related breeding behaviours (Cook 1996). The largest males aggressively defend territories where conditions appear to be most favourable for oviposition and tadpole development. Medium-size males congregate in groups around territorial males, jostling continuously for territorial space and females. The smallest (satellite) males wait in anticipation of opportunistic matings on the periphery of the spawning activity.

Adult females show preference for mating with territorial males (Cook 1996), moving towards them with only their eyes above the water (Channing 2001). During amplexus, females stretch their legs to raise their vent above the water, thereby facilitating fertilization of their ova from the sperm of their mate (Balinsky & Balinsky 1954). Couples spend approximately 3-29 minutes in amplexus (Channing *et al.* 1994), during which time a female may deposit 1 000-6 000 eggs (Cook 1996). The fertilized eggs are deposited with those of other females in a dominant male's territory, and within 36 hours small, black tadpoles hatch from the eggs (Du Preez & Cook 2004).

Some territorial males remain with offspring to defend them against predators, and may excavate a channel with their legs to regulate the depth and temperature of water for offspring (Kok *et al.* 1989; Cook *et al.* 2001). *P. adspersus* tadpoles congregate in massive schools at the water's edge, and become increasingly grey in colour as they reach up to 71 mm in length (Channing 2001). Tadpole metamorphosis is usually completed in 18-33 days depending on water temperature and food availability (Van Wyk *et al.* 1992; Channing 2001). Juvenile *P. adspersus* have a white ventral surface and a mottled green and black dorsum with a conspicuous bright-green vertebral stripe (Carruthers 2001).

Relevance and objectives of this thesis

This thesis was designed to investigate several poorly understood aspects of the ecology of *P. adspersus*, which would contribute towards improved conservation management of this species in South Africa, and in particular, Gauteng Province. The main motivation and objectives of the different research chapters comprising this thesis follow.

Chapter 2

The activity of *P. adspersus* is sporadic and difficult to predict, and consequently, populations have been poorly monitored. To better understand the activity of this species for improved monitoring and conservation management of populations, the objectives of this study were to:

- quantify the spawning and non-breeding activity of adult *P. adspersus* during > 1 season.
- determine whether adult male and female *P. adspersus* exhibit different patterns of diurnal or nocturnal activity.
- examine the activity of adult *P. adspersus* in relation to meteorological variables.
- infer consequences of the results on the conservation management of *P. adspersus*.

Chapter 3

Nothing is known about terrestrial habitat use by *P. adspersus*, which has important implications for *in situ* conservation management of populations. To better understand the spatial habitat requirements of this species, the objectives of this study were to:

- investigate the movements of adult *P. adspersus* within and between seasons.
- determine whether adult male and female *P. adspersus* exhibit different spatial patterns of habitat use.
- establish whether there is any relationship between spatial habitat use and the body size or body condition of same-sex adult *P. adspersus*..

- infer consequences of the results on the conservation management of *P. adspersus*.

Chapter 4

Nothing is known about the age of wild *P. adspersus* although age at maturity, and longevity, have important conservation implications for threatened species. To better understand this fundamental life history aspect of *P. adspersus*, the objectives of this study were to:

- estimate the age range of wild, breeding *P. adspersus* males and females using skeletochronology.
- examine the relationship between body size and age in adult *P. adspersus.*
- compare the age, body size and body condition of adult *P. adspersus* at different breeding sites.
- infer consequences of the results on the conservation management of *P. adspersus*.

Chapter 5

Habitat loss has fragmented and isolated many *P. adspersus* populations in Gauteng Province and elsewhere in South Africa. Yet nothing is known about this species' population genetic structure and gene flow. To gain a better understanding of the past connectedness, present viability and appropriate future conservation management of *P. adspersus*, the objectives of this study were to:

- quantify *P. adspersus* population genetic structure and gene flow in Gauteng Province.
- quantify *P. adspersus* population genetic structure and gene flow in other parts of South Africa, where possible.
- identify genetically unique and, therefore, important *P. adspersus* populations in the study area.
- infer consequences of the results on the conservation management of *P. adspersus.*

Chapter 6

The geographic range of *P. adspersus* has been difficult to accurately assess due to the sporadic activity and superficial morphological similarity of species in this genus. In South Africa's Gauteng Province, where considerable habitat transformation has occurred, there is an urgent need to identify remaining patches of natural habitat for proactive and effective conservation of *P. adspersus.* The objectives of this study were, therefore, to:

- predict the potential geographic range of *P. adspersus* in southern Africa using a species distribution model.
- estimate the amount of suitable habitat for *P. adspersus* remaining in Gauteng Province, South Africa using recent high resolution land cover data.
- infer consequences of the results on the conservation management of *P. adspersus*.

Chapter 2

Spawning and non-breeding activity of adult giant bullfrogs (*Pyxicephalus adspersus*) *

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Abstract.—Populations of the Near-Threatened giant bullfrog (*Pyxicephalus adspersus*) have been poorly monitored due to the unpredictable appearance of this species aboveground. To better understand the activity of *P. adspersus* we quantified spawning by a population during five summers, and the activity of twenty adult frogs radio-tracked at the same site ca. twice weekly during the first three summers. In addition we examined animal activity, and population spawning in relation to meteorological variables, day of season, and moon light. During the six-month summer period, males and females, respectively, spent 10 ± 7 and 3 ± 2 days at water, and 22 ± 5 and 13 ± 5 nights active. Greater proportions of radio-tracked animals moved overland, and/or foraged at night, around full moon, after heavy rainfall, when cooler, and less windy conditions prevailed. More animals were found at water, or on land during the day, and population spawning was more likely, earlier in summer, following heavier rainfall. Spawning occurred most frequently, in descending order, during December, January, and November, and was triggered by 40 ± 16 mm rain in 24 h. Spawning events lasted 2 ± 1 days, but were prolonged around full moon. Numbers of males at spawning events varied between 30 and 500 males, and were positively correlated with the previous day's rainfall. Annually 6 ± 1 spawning events occurred, and numbers of annual spawning events were positively correlated with total summer rainfall. Results of this study show that single counts of spawning adults will often result in gross underestimates of population size, and therefore, long-term adult counts are necessary to detect real population trends. Due to the unpredictable activity of adults, however, it may be more practical to monitor, within certain areas, the number of aquatic sites where breeding occurs, for improved conservation management of *P. adspersus*.

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Introduction

Although photoperiod and the lunar cycle provide predictable cues that amphibians may use to synchronize their behaviour (Fitzgerald & Bider 1974; Both *et al*. 2008; Canavero & Arim 2009; Grant *et al*. 2009), the activity of many species is ultimately dictated by unpredictable climatic variables because of their permeable skin and ectothermic bodies (Duellman & Trueb 1994). Among these, precipitation tends to exert the greatest influence (Bulger *et al*. 2003; Lemckert & Brassil 2003), but temperature (Howard 1978; Van Gelder *et al*. 1986), humidity (Bellis 1965; Bartelt *et al*. 2004), wind (Penman *et al*. 2006; Philips *et al*. 2007), light intensity/cloud cover (Jameson 1955; Blankenhorn 1972), and barometric pressure (Blankenhorn 1972) can also significantly affect amphibian activity. Amphibians in dry and/or cold environments are most limited by suitable warm, wet conditions, making it difficult to predict their behaviour or even their presence (e.g., Bulger *et al*. 2003; Bartelt *et al*. 2004; Goldberg & Schwalbe 2004). This can represent a serious challenge for effective conservation management of threatened amphibian taxa (e.g., Penman *et al*. 2006).

Giant bullfrogs (*Pyxicephalus adspersus*) are large frogs (males: ~ 400-1 000 g; females: ~ 90-300 g; Cook 1996) inhabiting arid to subtropical grassland and savannah in southern Africa (Channing 2001). Individuals remain underground in a state of torpor except in summer when triggered by heavy rain to spawn explosively in shallow, ephemeral water bodies (Balinsky & Balinsky 1954; Grobler 1972). Little is known about the non-breeding biology of this species apart from its diet, and burrowing physiology (Loveridge & Withers 1981; Van Aardt 1992; Du Preez & Cook 2004; Secor 2005). Currently, *P.*

adspersus is listed as Near-Threatened in South Africa (Minter *et al*. 2004; IUCN 2008), where habitat loss and other factors have led to local population declines of 50-80%, particularly in Gauteng Province (Harrison *et al*. 2001). Unfortunately, due to the highly unpredictable activity of *P. adspersus,* the species has been subject to limited field-based research and monitoring (Jacobsen 1989; Kok *et al*. 1989; Van Wyk *et al*. 1992; Cook 1996). This has hindered accurate assessment of the size, and breeding success, of important historical populations (e.g., at Glen Austin and Bullfrog pans near Johannesburg). The resulting lack of demographic information for the species has impeded assessment of its conservation status, and provides a weak baseline for evaluation of efforts to protect threatened populations.

In the present study we aimed to examine the spawning and non-breeding activity of adult *P. adspersus* in relation to meteorological variables, to contribute towards improved detection, monitoring, and conservation management of this species. Specific objectives of the study were to:

- Quantify the annual spawning, and non-breeding activity of adult *P. adspersus*.
- Determine whether males and females exhibit different patterns of diurnal or nocturnal activity.
- Examine the diurnal, nocturnal, and spawning activity of adult *P. adspersus* in relation to meteorological variables, day of season, and moon light.
- Infer consequences of the results on the conservation management of *P. adspersus*.

Materials and Methods

Study site

The study site was located in Diepsloot, Gauteng Province, South Africa (2528CC: 25º56'23.59"S, 28º01'21.88"E; 1 427-72 m a.s.l.), and comprised ~ 100 ha of degraded Egoli Granite Grassland (Mucina *et al*. 2005). Local bullfrogs bred in three small (~ 0.2-0.9 ha), shallow (< 2 m deep), human-created, seasonal dams (named Dams 1, 2 and 3), situated in the southern section of the site. The site is representative of many areas where *P. adspersus* faces encroaching urbanization on remaining fragments of undeveloped habitat. We collected data on *P. adspersus* at the site between 1 October and 31 March in the seasons 2003/2004, 2004/2005, 2005/2006, 2006/2007, and 2007/2008 - hereafter referred to as Summers 1-5.

Radio-tracking

In Summers 1-3, we haphazardly selected 10 males and 20 females for radio-tracking from adult *P. adspersus* caught for a separate mark-recapture study. We captured frogs by hand or with a hand-held net during spawning events in Dams 1 and 2 (*P. adspersus* did not favour Dam 3). Animals were each implanted off-site with a radio-transmitter by a veterinary surgeon, monitored overnight, and released the next day at their capture sites. The transmitters and surgical protocol are described in Yetman & Ferguson (2011b, [Chapter 3]).

Tracking was conducted between 06h00 and 18h00 on average twice per week, with additional surveys undertaken in response to rainfall events. A four-element Yagi antenna and AVM LA12 receiver (AVM Instruments, Champagne, Illinois) were used to detect radio-tagged frogs. Between three and 16 animals were tracked on a day. When a frog was located, we recorded its geographic coordinates (with a global positioning system, accurate to 4 m), and the date, time, and behaviour observations. An animal's behaviour was classified as: buried (inside an open or closed burrow); present at a spawning event (regardless of whether an animal mated); guarding offspring (resting < 1 m from or aggressively defending eggs or tadpoles); resting at water (when an animal was found in or < 1 m from a dam or other inundated depression, and spawning or parental care of offspring was not observed); or resting on land (resting aboveground > 1 m from water, usually under vegetation). Data on the diurnal activity of radio-tracked animals were obtained for 129 days during Summers 1, 2, and 3.

Evidence of nocturnal frog activity

No nocturnal radio-tracking was performed because the area was considered unsafe at night. However, in Summer 3 we deduced the nocturnal emergence of animals from their burrows when the radio-tracked location of individuals changed overnight, or when a small stone placed on the plug sealing an animal's burrow had disappeared or been displaced off the plug between consecutive days of tracking. Pearson (1955) used chalk dust for the same purpose, but in our study stones were used as less conspicuous markers to prevent animals from being harvested for human consumption (Du Preez & Cook

2004). Data on the nocturnal activity of radio-tracked animals were obtained for 31 nights in Summer 3.

Monitoring of spawning

All aspects of breeding by *P. adspersus* take place at the edge of ephemeral wetlands where the water is most shallow and warm, and thus favourable for tadpole development (Cook 1996). We therefore inspected the edge of the seasonal dams on foot, usually every day that animals with radio-transmitters were tracked in Summers 1-3, and after rainfall events of 20 mm or more rain at the study site in Summers 4-5. Evidence of spawning by *P. adspersus* included observation of calling, fighting and/or mating adults, eggs, tadpoles, and/or newly-metamorphosed froglets – which were clearly visible at the water's edge and are highly diagnostic (Du Preez & Carruthers 2009).

When a spawning event was observed, males at Dams 1-3 were counted in groups of approximately 10 individuals, if possible at different times of the day for each day that adults were seen calling, fighting and/or mating. Thus the largest count of males, and the duration of each observed spawning event was determined. Females were not counted because their smaller size and inconspicuous behaviour (Channing *et al.* 1994; Cook 1996) made them difficult to detect.

When eggs, tadpoles or newly-metamorphosed froglets were found without spawning having been observed, we estimated the date of spawning from the development of offspring (Gosner 1960; Van Wyk *et al.* 1992; Haas 1999) and measurements of daily rainfall from Diepsloot Nature Reserve. Hence, for Summers 2-5, we estimated the annual number of spawning events as the sum of all spawning events that were observed or revealed by eggs, tadpoles or froglets. The total number of spawning events in Summer 1 could not be determined since the study site was found in late mid-summer (January 2004).

Meteorological data

For periods when *P. adspersus* spawned at the study site, we obtained measurements of daily rainfall from a local resident who lived < 100 m from the dams. These data were supplemented with rainfall measurements taken at 07h00 daily in gauges at five different localities in Diepsloot Nature Reserve, which lies ~ 6 km west of the study site (25°55'36.35"S, 27°57'56.09"E). We treated 1 October as the first day of season (DS), and used a lunar calendar (South African Astronomical Observatory) to determine the number of days since full moon on specific dates. Days since full moon were assigned to one of eight lunar phases, ranked from 0-4 depending on the amount of moon light (ML; Table 1, adapted from Grant *et al*. 2009).

We obtained daily measurements of maximum and minimum atmospheric temperature (MaxT and MinT in °C), relative humidity (RH in %), wind speed (WS in m s^{-1}), and atmospheric pressure (P in hPa) from a weather station in Irene, situated ~ 19 km east of the study site (25°54'36.00"S, 28°12'36.00"E). Missing temperature data in November and December 2005, and October 2006 were substituted with daily MaxT and MinT measurements from a weather station in Kempton Park (situated ~ 31 km south-east of the study site; 26°08'60.00"S, 28°13'48.00"E). These measurements were corrected by 0.4-3.0 °C depending on the mean monthly difference in each variable between the two weather stations during Summers 1-3.

Statistical analyses

Statistical tests were performed in Statistica 7.0 (© StatSoft, Inc. 1984-2004 Tulsa). Animals found present at a spawning event, guarding offspring, resting at water, or resting on land during radio-tracking, were considered active by day. Nocturnal activity was deduced from overnight change in an animal's radio-tracked location, or overnight displacement of the stone placed on an animal's burrow. Mann-Whitney *U* tests were performed to assess whether the sexes differed in the mean number of dates during summer that individuals were buried or active by day, and/or buried or active at night. Behavioural data were used from six males and seven females that were successfully radio-tracked in the day through Summers 2 and/or 3; and three males and seven females for which data on their nocturnal activity were obtained through Summer 3. Data presented are mean ± standard deviation.

We used generalized linear models (GLZs) to investigate animal activity, and population spawning in relation to meteorological variables. Diurnal or nocturnal animal activity was measured as the daily diurnal or nocturnal "active" (arc sine transformed) proportion of animals that were successfully radio-tracked on 129 days in Summers 1-3, or 31 days in Summer 3, respectively. Population spawning was measured as a binomial variable on 743 days in Summers 1-5. Diurnal animal activity and population spawning were examined in relation to study year, the amount of previous day's rainfall (PDR), MaxT, MinT, mean day time RH, WS, and P (measured at 08h00 and 14h00), and DS. Nocturnal animal activity was examined in relation to the amount of daily rainfall (DR), MaxT, MinT, night time RH, WS, and P (measured at 20h00), DS, and ML.

We tested the predictor variables for multicollinearity using multiple regressions, and excluded predictors with a Variance Inflation Factor (VIF) > 2, and/or a Tolerance (T) value < 0.5. We therefore excluded RH (VIF = 3.1; $T = 0.3$) from the models of diurnal animal activity, DS (VIF = 2.4; $T = 0.4$), and MaxT (VIF = 2.3; $T = 0.4$) from the models of nocturnal animal activity; and RH (VIF = 3.6; $T = 0.3$) from the models of population spawning. VIF and T values for the remaining predictor variables in the models are shown in Table 2. We assessed the utility of models of animal activity or population spawning based on a normal or binomial distribution, and an identity or logit link function, respectively. We corrected values of Akaike's Information Criterion (AIC) that were generated in Statistica, for over-dispersion of the data in all models (QAIC), and for small sample size in the models of diurnal or nocturnal animal activity (QAIC$_c$; Symonds & Moussalli 2010). For model averaging we used models with a corrected AIC value that differed by < 2 from that of the "best" model of population spawning, or diurnal or nocturnal animal activity (Symonds & Moussalli 2010). The importance of a specific predictor was estimated as the sum of the Akaike weights (Σw_i) of all models of diurnal or nocturnal animal activity, or population spawning, which included that specific predictor (Symonds & Moussalli 2010).

We used a logistic regression with binomial errors to more accurately describe the relationship between PDR and population spawning, measured on 743 days in Summers 1-5. The duration of spawning events, or numbers of spawning males could not be examined in relation to meteorological variables using GLZs or step-wise multiple regressions, due to a small sample size (n = 10 observed spawning events), multicollinearity, and skewed variable distributions. Therefore, Spearman Rank correlations were used instead. Due to a small sample size (n = 4 summers), a Kendall Tau correlation was performed to determine whether annual numbers of spawning events were related to total summer rainfall through Summers 2-5. This method is more sensitive to some types of dependence, and deals better with ties between variables than the Spearman method (Kendall 1938).

Results

Tracking

Of the 30 transmitter-implanted animals, 10 males and 12 females were tracked post-release. Eight females were lost from the start, and one female died and another was lost for uncertain reasons shortly after release. The 10 males and 10 remaining females were each tracked to at least one burrow. Of these 20 animals, three females and two males were tracked for several days or weeks. Seven females and four males were tracked for a complete summer, and four males were tracked during two or three successive summers. We removed transmitters from five animals, but most animals were lost when their transmitters expired.

Male versus female activity

Radio-tracked males were found on significantly more days in summer at water (10 ± 7 days, range: 4-18 days, $n = 6$ males tracked through Summer 2 and/or 3), than females (3 ± 2 days, range: 2-6 days, $n = 7$ females tracked through Summer 3, Mann-Whitney $U = 5.0$, $P = 0.02$). More specifically, males were found in summer on 4 ± 2 days at a spawning event (range: 3-6 days, $n = 6$ males), 1 ± 1 days guarding offspring (range: 0-2 days, $n = 4$ males), and 5 ± 5 days resting at water (range: 0-11 days, $n = 5$ males). Females were found in summer on significantly fewer days at a spawning event (2 ± 1 days, range: 2-3 days, $n = 7$ females, $U = 6.0$, $P = 0.03$), and on 1 ± 1 days resting at water (range: 0-3 days, $n = 2$ females, $U = 8.5$, $P = 0.07$). Only males were found in summer, on 2 ± 1 days resting on land (range: 0-4 days, $n = 5$). In Summer 3, males were active on more nights (22 ± 5 nights, range: 16-26 nights, $n = 3$ males) than females (13 ± 5 nights, range: 9-20 nights, $n = 7$ females, $U = 2.0$, $P = 0.05$). Overall, males were active in the day and/or night on a greater cumulative number of dates in Summer 3 (27 ± 10 dates, range: 17-37 dates, $n = 3$ males), than females (14 ± 5 dates, range: 9-23 dates, $n = 7$ females, $U = 1.5$, $P = 0.04$).

Activity in relation to meteorological variables

The relative importance (Σw_i) of each predictor of diurnal or nocturnal animal activity is shown in Table 3. Model averaging involved 62 models of diurnal animal activity, and 14 models of nocturnal animal activity. The coefficient estimate (β) and standard error (SE) obtained from model averaging, for the relationship between each predictor variable, and daily diurnal or nocturnal "active" proportions of successfully radio-tracked animals, is shown in Table 3.

PDR and DS were the first and second most important predictors in all 127 models of diurnal animal activity, and were included in 100% and 52% of the 62 "best candidate" models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that more animals were diurnally active (at water or on land) earlier in summer, and following heavier rainfall. MinT, night time WS, DR, and ML were, in descending order, the most important predictors in all 31 models of nocturnal animal activity, and were, respectively, included in 100%, 86%, 71%, and 57% of the 14 "best candidate" models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that more animals were nocturnally active (i.e., moved overland, and/or foraged) around full moon, after heavy rainfall, when cooler, and less windy conditions prevailed.

Spawning in relation to meteorological variables

Daily rainfall in Diepsloot, and *P. adspersus* spawning at the study site in Summers 1-5, are depicted in Figures 1a-e, respectively. The logistic regression between PDR and population spawning, measured on 743 days in Summers 1-5, is shown in Fig. 1f. The coefficient estimate, SE, and *P* value was, respectively, 0.121, 0.014, and < 0.001 for PDR, and -4.254, 0.301, and < 0.001 for the regression intercept. The mean amount of rain recorded 24, 48 or 72 h prior to 25 spawning events that were observed, or revealed by offspring, was 40 ± 16 mm rain (min. = 20 mm), 45 ± 20 mm (min. = 22 mm), and 51 ± 25 mm (min. = 22 mm), respectively. Spawning occurred most frequently, in decreasing order, during December, January, and November (Fig. 2). The mean maximum count of males at 10 observed spawning events was 162 ± 148 males (range: 30-500 males), and the mean duration of 15 spawning events, of which 12 were observed and three were revealed by observation of one- or two-day old eggs, was 2 ± 1 days (range: 1-4 days).

The relative importance (Σw_i), coefficient estimate (β), and standard error (SE) of each predictor of population spawning, is shown in Table 3. Model averaging was based on 64 models. PDR and DS were, respectively, the first and second most important predictors in all 743 models of population spawning, and were included in 100% and 50% of the 64 "best candidate" models used for model averaging (Table 3). The coefficient estimates (Table 3) indicate that population spawning was more likely earlier in summer, and following heavier rain.

The Spearman Rank correlation between the duration of spawning events, or numbers of spawning males, and each of 11 different predictor variables, is shown in Table 4. The duration of spawning events was positively correlated with ML ($P = 0.05$), and the number of males at spawning events was positively correlated with the amount of previous day's rainfall ($P = 0.04$). Annually, there were 6 ± 1 spawning events (range: 4-7 spawning events, $n = 4$ summers), and estimated annual numbers of spawning events during Summers 2-5 were positively correlated with total summer rainfall (Kendall $T = 1.0$, $P = 0.04$, Fig. 3).

Discussion

Male versus female activity

Pyxicephalus adspersus is a difficult species to observe. Study animals cumulatively spent a month or less active during the six-month summer period. Other burrowing anurans inhabiting mesic or arid habitats exhibit similarly brief activity. For example, eastern spadefoots (*Scaphiopus holbrooki*) in Gainesville, Florida (Pearson 1955), and shoemaker frogs (*Neobatrachus sutor*) and orange-crowned toadlets (*Pseudophryne occidentalis*) in Western Australia (Thompson *et al.* 2003) spent, respectively, ca. 29 nights, and up to four days on 9 and 17 occasions, active in summer. During their brief activity, male *P. adspersus* that were radio-tracked for more than one season, evidently caught sufficient prey to sustain their large bulk through winter. This was possible, despite the degraded habitat, because *P. adspersus* preyed on termites, which were abundant on the site (CAY pers. obs.).

Certain activity differences between male and female *P. adspersus* might be due to their pronounced dimorphism in body size (Hayes & Licht 1992). Females avoid being terrestrially active during the day possibly because their greater surface-to-volume ratio increases their vulnerability to desiccation (Peters 1983). Males spend a greater number of nights active in summer because they visit breeding habitat more often, and because they probably need to spend more time foraging than females to satisfy their greater absolute metabolic requirements (Branch 1976; Paukstis & Reinbold 1984). Similarly, Ovaska (1992) claimed that females of the burrowing frog *Eleutherodactylus johnstonei*, moved longer distances than males overland, due to their larger size and greater energy requirements. In contrast, similar-sized (~ 50 g) male and female *Hoplobatrachus occipitalis* in a West African savanna, exhibited no difference in their timing of migrations to breeding sites, activity and home range areas, and growth during summer (Spieler & Linsenmair 1998).

Due to the more costly and limited production of ova relative to sperm (Van Beurden 1979; Reading 1988; Blem 1992), females of many anurans only spawn 1-3 times in a season, whereas males visit most spawning events to increase their reproductive success (e.g., Perrill & Daniel 1983; Loman & Madsen 1986; Sinsch 1988a; Barandun *et al.* 1997). *P. adspersus* appears to fit the same trend. If adult *P. adspersus* are present for only one day of a spawning event, females and males would, respectively, attend two (33%) and four (67%) of six mean spawning events per annum. Thus, assuming *P. adspersus* populations have an adult sex ratio of 1:1, spawning events will usually appear male-biased in this species, as observed by Cook (1996).

Human disturbance at the Diepsloot dams accounts for why at least one radio-tracked male abandoned offspring (CAY pers. obs.), and likely explains why males spent one day on average in summer guarding offspring. In contrast, 109 males at Glen Austin Pan remained with their offspring for 21 ± 9.8 days (Cook *et al.* 2001).

Activity in relation to meteorological variables

Radio-tracked males and females, respectively, spent 40 and 67% of their diurnal summer activity attending spawning events. Therefore, as with population spawning, a greater number of animals were active in the day after heavy rain, earlier in summer. Adult *P. adspersus* visit breeding sites after heavier rain because the risk of desiccation during overland migration between their burrows and breeding sites (Sjögren-Gulve 1998; Schwarzkopf & Alford 2002), and risk of evaporation and mortality of offspring at breeding sites (Kok *et al.* 1989; Cook *et al.* 2001) is probably reduced. Adults visit breeding sites, and spawn earlier in summer because adults may attend more spawning events per annum, adults and newly-metamorphosed froglets may accumulate larger energy reserves prior to winter, and/or the abundance of predators at breeding sites may increase over summer.

On nights with less wind, and/or increased moon light, greater proportions of animals were active because it was possibly easier to catch prey, and/or detect predators (Fitzgerald & Bider 1974; Penman *et al*. 2006). The influence of the moon or wind on anuran activity has been considered in only a few studies. For example, Penman *et al*. (2006) showed that the activity of *Heleioporus australiacus* was related to rainfall, as well as temperature, humidity, and wind strength. A meta-analysis by Grant *et al*. (2009) revealed that various European amphibians exhibit mass migration and spawning around full moon. Some anurans are, however, terrestrially less active during full moon possibly because the risk of detection by predators is greater, and/or important prey is less active at this time (Church 1960; Ferguson 1960; Fitzgerald & Bider 1974; Goldberg & Schwalbe 2004).

Spawning in relation to meteorological variables

Pyxicephalus adspersus often spawns after ca. 50 mm or more rainfall (Grobler 1972; Cook 1996; Du Preez & Cook 2004), but not always. At the Diepsloot site a minimum of 20 mm rain in 24 h could trigger spawning, which is equal to that reported for *P. adspersus* in the Free State Province (Kok *et al*. 1989), and for other explosive-breeding, burrowing anurans in mesic or arid habitats, e.g., *S. holbrooki* (Gosner & Black 1955). Although *P. adspersus* spawns readily in October, population spawning appears to be most common in November, December, and January (e.g., Balinsky 1969; Kok *et al*. 1989; Van Wyk *et al*. 1992; Cook 1996). This occurred at the study site because in October the artificial dams often remained dry, and by February and March, females, at least, had probably spent most or all of their ova.

Greater moon light prolonged spawning at the study site even though *P. adspersus* spawns predominantly during day light (Balinsky 1969; Channing *et al*. 1994). This was possibly because moon light facilitated foraging at night around the artificial dams, which enabled males to call, fight, and/or spawn over a longer period. We noticed that spawning was also protracted when the weather was cool, windy, and/or overcast (CAY pers. obs.). However, due to a small sample size perhaps, no significant relationship emerged between spawning duration, and temperature, or WS. Greater numbers of males attended spawning events triggered by heavier rain because this possibly reduced the risk of desiccation during overland migration (Sjögren-Gulve 1998; Schwarzkopf & Alford 2002), and/or improved the probability of successful reproduction, particularly for young, weak, less experienced, and/or distantly buried males.

Cook *et al.* (2001) reported that *P. adspersus* breeds, on average, once in four years at a particular site. In contrast, *P. adspersus* at the Diepsloot study site spawned, on average, six times each summer. The reproductive success of *P. adspersus* may differ between large, natural pans, and small, artificial dams, but many spawning events probably go unnoticed due to the unpredictable, sporadic, and brief activity of this species. Therefore, *P. adspersus* could experience greater reproductive success than might be assumed. However, cohorts of larval *P. adspersus* often suffer high mortality caused by desiccation, and predation (Cook *et al.* 2001), and at high larval densities there is increased competition for food (Cook 1996), cannibalism (Grobler 1972), and possibly spread of disease.

Conservation implications

Significant differences in the temporal and spatial use of aquatic and terrestrial habitat by adult male and female *P. adspersus* have important conservation implications for the species. Males spend significantly more time at breeding sites than females, and consequently, are more vulnerable to harvesting for human consumption, which is common in Limpopo Province (Du Preez & Cook 2004). Increased mortality of males at breeding sites could adversely affect tadpole survival and juvenile recruitment due to paternal care of offspring in *P. adspersus* (Cook *et al*. 2001). In contrast, females spend virtually their entire lives in burrows situated significantly farther from breeding sites than those of males (Yetman & Ferguson 2011b [Chapter 3]). Females are consequently more vulnerable to encroaching land transformation (Du Preez & Cook 2004). *Pyxicephalus adspersus* therefore requires effective protection of both aquatic and terrestrial habitat.

For estimation of *P. adspersus* population sizes, single counts of spawning adults are grossly inadequate because they will frequently result in severe underestimates. This was evident at our study site, where only a small proportion (10-50%) of the highest male count (i.e., 500 males in January of Summer 3) was present at most spawning events. Adult male *P. adspersus* remain relatively close to their breeding site within and among years (Yetman & Ferguson 2011b, [Chapter 3]); therefore, frog movements are unlikely to account for the observed variation in numbers of males at spawning events. The great variation in numbers of spawning males at the Diepsloot dams obscured any trend in population size. Long term adult counts (exceeding 10 or 20 years; see Meyer *et al*. 2010) will, therefore, be necessary to detect real trends in the size of *P. adspersus* breeding populations. However, due to the unpredictable and brief activity of adults, it may be more practical to monitor, within certain areas, the number of aquatic sites where *P. adspersus* breeding occurs (Joseph *et al*. 2006). In this way large-scale trends can be detected for improved conservation assessment and management of *P. adspersus* at regional, provincial and national scales (Lindenmayer & Likens 2010).

Acknowledgements

We sincerely thank Jimmy, Alice and Mark Yetman, and Johan Lötter for their patient and generous assistance with field work; Wendy Duncan for measuring local rainfall, reporting frog activity at the dams, and kindly assisting with field work; Roger Wood and Diepsloot Nature Reserve for providing additional rainfall data; the South African Weather Service for providing the bulk of the meteorological data; the Gauteng Department of Agriculture and Rural Development for issuing permits 1 240 and 1 296 to CAY; and Mike Perry, who introduced us to the site. The study was funded through the Endangered Wildlife Trust by Rand Merchant Bank, the Pretoria East branch of the South African Hunter's and Game Conservation Association, Arrow Bulk Marketing, Cellar Rats Wine Club, Bill Flynn and Diaz Films. We thank Michael Bates, Frank Lemckert, and three anonymous reviewers for valuable comments on previous drafts of this manuscript.

Table 1. Assignment of days since full moon (DFM) to one of eight lunar phases, ranked from 0-4 depending on the amount of moon light (adapted from Grant *et al*. 2009).

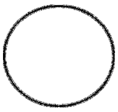
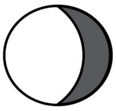
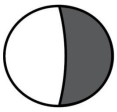
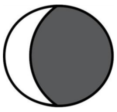
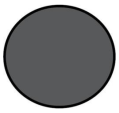
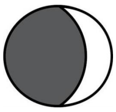
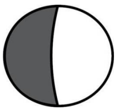
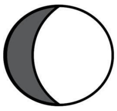
	Full moon	Waning gibbous	3rd quarter	Waning crescent	New moon	Waxing crescent	1st quarter	Waxing gibbous
Lunar phase	1 	2 	3 	4 	5 	6 	7 	8 
DFM	28, 29, 0, 1	2, 3, 4, 5	6, 7, 8	9, 10, 11, 12	13, 14, 15, 16	17, 18, 19, 20	21, 22, 23	24, 25, 26, 27
Rank	4	3	2	1	0	1	2	3

Table 2. Variance Inflation Factor (VIF) and Tolerance (*T*) values for predictor variables used in generalized linear models of the diurnal, nocturnal, or spawning activity of *Pyxicephalus adspersus* at the Diepsloot study site. The values were obtained from multiple regressions performed after certain variables with a VIF > 2 and/or $T < 0.5$, were excluded (excl.) to reduce multicollinearity.

	Diurnal activity		Nocturnal activity		Spawning	
	VIF	*T*	VIF	*T*	VIF	*T*
Study year	1.2	0.8	-	-	1.0	1.0
Day of season	1.4	0.7	excl.	excl.	1.2	0.8
Previous day's rain	1.3	0.8	-	-	1.2	0.8
Daily rain	-	-	1.2	0.8	-	-
Max. temperature	1.3	0.8	excl.	excl.	1.5	0.7
Min. temperature	1.3	0.8	1.7	0.6	1.4	0.7
Wind speed	1.1	0.9	1.1	0.9	1.2	0.9
Relative humidity	excl.	excl.	1.4	0.7	excl.	excl.
Atmospheric pressure	1.1	0.9	1.5	0.7	1.2	0.8
Moon light	-	-	1.2	0.8	1.0	1.0

Table 3. Percent (%) inclusion, relative importance (Σw_i), coefficient estimate (β), and standard error (SE) of predictor variables used in generalized linear models of the diurnal, nocturnal, or spawning activity of *Pyxicephalus adspersus* at the Diepsloot study site. Percent inclusion pertains to the 62, 14, or 64 “best” models of diurnal, nocturnal, or spawning activity used for model averaging. Σw_i represents the sum of the Akaike weights of every diurnal, nocturnal, or spawning activity model (max. n = 127, 31, or 743 models, respectively) that involved a specific predictor.

	Diurnal animal activity				Nocturnal animal activity				Population spawning			
	%	Σw_i	β	SE	%	Σw_i	β	SE	%	Σw_i	β	SE
Intercept	-	-	0.672	0.003	-	-	3.687	0.078	-	-	54.446	0.122
Study year	48	0.46	-0.012	$4x10^{-5}$	-	-	-	-	50	0.49	-0.056	$2x10^{-4}$
Day of season	52	0.58	-0.001	$2x10^{-6}$	-	-	-	-	50	0.60	-0.012	$2x10^{-5}$
Previous day’s rain	100	0.87	0.009	$5x10^{-6}$	-	-	-	-	100	0.93	0.122	$6x10^{-5}$
Daily rain	-	-	-	-	71	0.63	0.006	$9x10^{-5}$	-	-	-	-
Max. temperature	50	0.51	-0.005	$1x10^{-5}$	-	-	-	-	50	0.50	-0.007	$7x10^{-5}$
Min. temperature	50	0.54	-0.016	$3x10^{-5}$	100	0.76	-0.061	$5x10^{-4}$	50	0.50	0.054	$2x10^{-4}$
Wind speed	48	0.45	$3x10^{-5}$	$7x10^{-6}$	86	0.63	-0.061	$7x10^{-4}$	50	0.49	-0.014	$9x10^{-5}$
Relative humidity	-	-	-	-	43	0.47	$7x10^{-4}$	$2x10^{-5}$	-	-	-	-
Atmospheric pressure	48	0.45	$-4x10^{-5}$	$3x10^{-6}$	36	0.47	-0.003	$9x10^{-5}$	50	0.51	-0.068	$1x10^{-4}$
Moon light	-	-	-	-	57	0.54	0.043	$9x10^{-4}$	-	-	-	-

Table 4. Spearman Rank correlation between the duration of, or maximum number of males counted at, 10 observed *Pyxicephalus adspersus* spawning events, and various meteorological variables in Diepsloot. Not significant = n.s., * $P < 0.05$.

	Spawning duration		Max. no. of males	
	r_s	P	r_s	P
Study year	-0.52	n.s.	0.12	n.s.
Day of season[1]	-0.10	n.s.	0.19	n.s.
Previous day's rain[1]	0.03	n.s.	0.65	*
Total rain[2]	0.43	n.s.	-0.11	n.s.
Max. temperature[3]	0.02	n.s.	-0.18	n.s.
Min. temperature[3]	0.04	n.s.	0.04	n.s.
Wind speed[3]	0.28	n.s.	0.21	n.s.
Relative humidity[3]	-0.27	n.s.	-0.02	n.s.
Atmospheric pressure[3]	-0.08	n.s.	0.41	n.s.
Moon light[1]	0.64	*	0.06	n.s.
Max. no. of males[2]	0.34	n.s.	-	-
Duration of spawning	-	-	0.34	n.s.

[1] For the first day of spawning.

[2] Over the full duration of spawning.

[3] Mean of daily values during spawning.

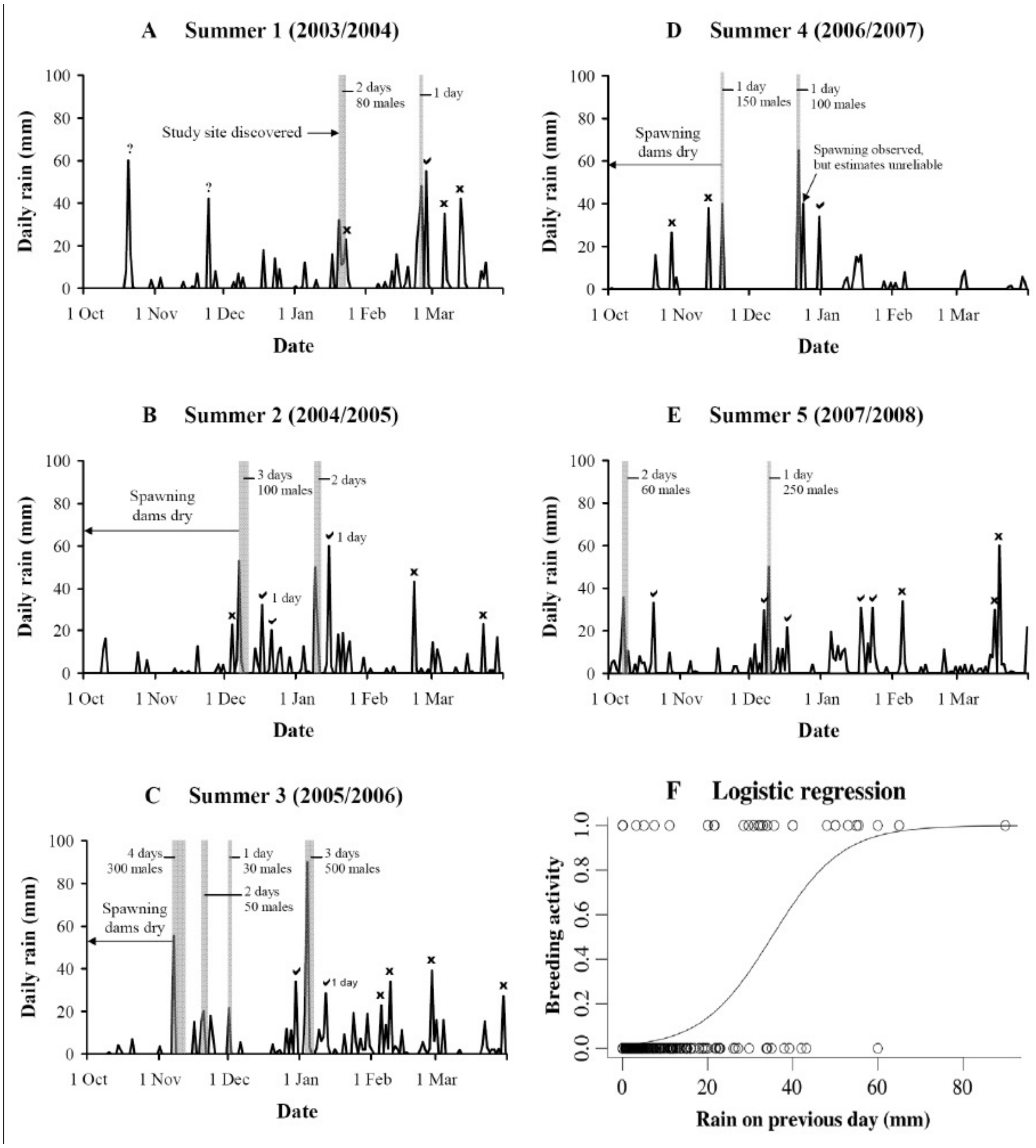


Figure 1. Daily rainfall in Diepsloot, Gauteng Province, South Africa, and known *Pyxicephalus adspersus* spawning activity at the study site during Summers 1-5, shown in A-E, respectively. Spawning events represented by vertical bars were observed. Those indicated by ✓ were revealed by the presence of eggs, tadpoles, and/or froglets. **X** indicates when spawning did not occur after heavy rain. (F) Logistic regression between the amount of previous day's rainfall, and *P. adspersus* spawning at the site, measured on 743 days in Summers 1-5.

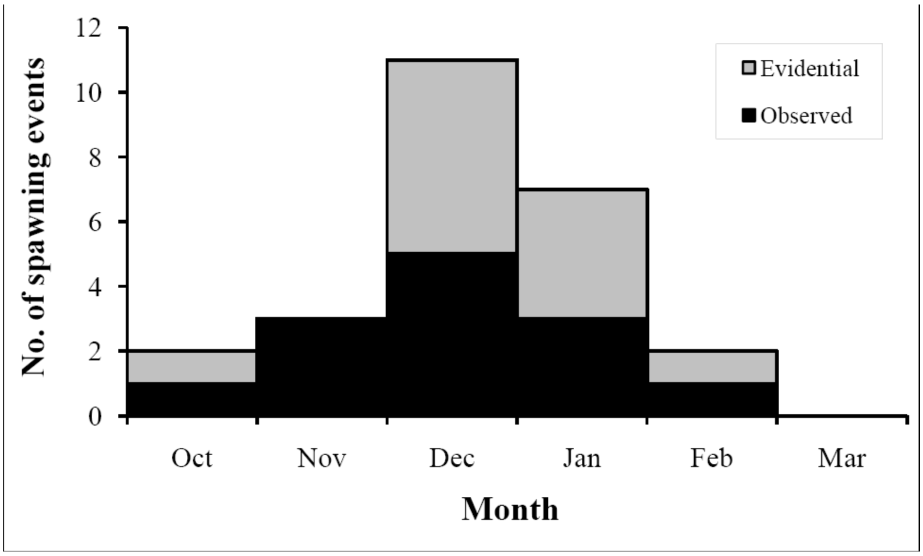


Figure 2. The number of recorded *Pyxicephalus adspersus* spawning events at the Diepsloot study site in different months during Summers 1-5. Thirteen spawning events were observed, and 12 were revealed by the presence of eggs, tadpoles and/or froglets.

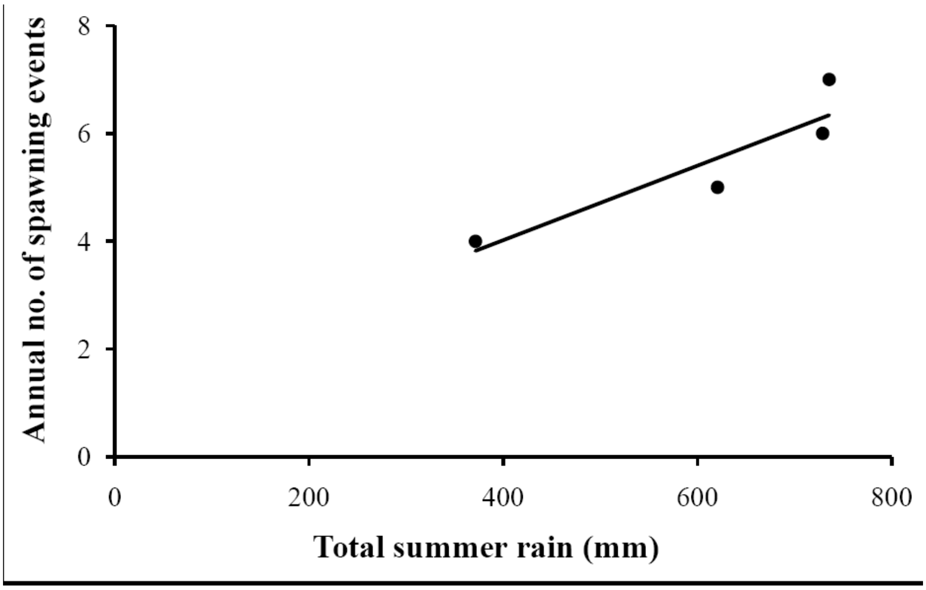


Figure 3. Kendall Tau correlation between total summer rainfall, and annual estimated numbers of *Pyxicephalus adspersus* spawning events during four summers at the Diepsloot study site.

Chapter 3

Conservation implications of spatial habitat use by adult giant bullfrogs (*Pyxicephalus adspersus*) *

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Abstract.—In South Africa, particularly Gauteng Province, populations of the large, explosive-breeding giant bullfrog (*Pyxicephalus adspersus*) are suffering increasing habitat loss due to encroaching urbanization. To investigate the spatial habitat requirements of this regionally threatened species, 70 adult frogs were radio- or spool-tracked during five summers around a peri-urban breeding site. Male and female *P. adspersus* moved a maximum overnight distance of 350 m when returning to their burrows post-spawning. On average animals of either sex used one long-term burrow (LTB) in a summer. Four males each used a single LTB or burrowing area for two or three consecutive summers. The LTBs of females were situated almost four times further (mean = 446.8 m) from the seasonal dams where spawning occurred, than those of males (mean = 131.0 m). Female body condition was significantly positively correlated with distance of their burrows from the seasonal dams ($r_s = 0.77$). Limited evidence indicated

that adult *P. adspersus* probably forage mostly within 20 m of their burrows. To protect the LTBs of all radio-tracked animals a 950-1 000 m wide buffer would be necessary around the seasonal dams. Since adult *P. adspersus* appear philopatric, juvenile dispersal is predicted to be largely responsible for gene flow among populations.

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Introduction

The growing number of threatened amphibian species (Houlahan *et al*. 2000; Stuart *et al.* 2004) and greater sophistication of equipment and methods to track wildlife (e.g., Sampson & Delgiudice 2006; Wikelski *et al.* 2007) have led to increased research on amphibian terrestrial habitat use. Several authors (Semlitsch 2000; Semlitsch & Bodie 2003; Lemckert 2004; Smith & Green 2005) have reviewed this expanding body of information to derive general recommendations for improved *in situ* conservation of amphibians and their habitat. The majority of studies upon which these recommendations have been based were performed on species living in temperate areas within the Nearctic and Palearctic Ecozones (e.g., Appendix 1 of Semlitsch & Bodie 2003; Tables 1-6 of Lemckert 2004; Table 4 of Smith & Green 2005). Relatively few analogous studies have been conducted in more tropical or arid locations and/or within the southern hemisphere (e.g., Woolbright 1985; Miaud *et al.* 2000; Goldberg & Schwalbe 2004). Spieler & Linsenmair (1998) performed the only published study on movements and habitat use of an amphibian in Africa to date. There is therefore, a clear need for additional information on terrestrial habitat use by amphibians living in hot environments and/or in Africa.

The giant bullfrog (*Pyxicephalus adspersus*) is a large, explosive-breeding anuran that inhabits arid to sub-tropical grassland and savanna throughout most of southern Africa, and further north into southern Angola and Kenya (Channing 2001; Minter *et al.* 2004). Although listed as Least Concern globally (IUCN 2008), since 2001 *P. adspersus* has been considered Near-Threatened in South Africa (Harrison *et al.* 2001; Minter *et al.* 2004). Here, the most severe and widespread threat to the species is habitat loss caused by urban development and agriculture (Harrison *et al.* 2001; Minter *et al.* 2004). Nothing is known about terrestrial habitat use by *P. adspersus*, except that individuals spend most of the year buried underground in a state of torpor (Parry & Cavill 1978; Loveridge & Withers 1981). In areas where there is increasing loss of terrestrial habitat, however, population declines suggest that *P. adspersus* excavate burrows at significant distances from breeding sites (e.g., Glen Austin and Bullfrog pans in suburban Johannesburg; Cook 2002; Slater-Jones, unpubl. data).

Many amphibians, especially anurans, travel hundreds of meters or several kilometers between breeding sites and terrestrial habitats used for foraging and/or overwintering, and/or among breeding sites (e.g., Table 4 of Smith & Green 2005). Without access to suitable terrestrial habitats individuals would be unable to complete their life cycles, and populations would eventually fail to persist (Semlitsch & Bodie 2003; Trenham & Shaffer 2005). Unfortunately, general recommendations for *in situ* protection of anurans are unlikely to adequately protect all populations, because there is great variation in distances moved by individuals within and among species (e.g., Fig. 2 of Lemckert 2004; Fig. 3 of Smith & Green 2005; Fig. 2 of Smith & Green 2006). Therefore, studies on the spatial habitat requirements of threatened taxa remain crucial (Lemckert 2004).

We examined spatial habitat use by adult *P. adspersus* within and among seasons, and in relation to their sex, size, or body condition. Adult males may exceed one kilogram but typically weigh between 400 and 800 g, whereas adult females usually weigh between 90 and 300 g (Cook 1996). Therefore differences in spatial habitat use between the sexes could be expected (e.g., Ovaska 1992). Adult male *P. adspersus* also exhibit three distinct size-related mating tactics, including territorial, non-territorial, and satellite behavior (Cook 1996). In some species same-sex but different-sized individuals have been found to exhibit different patterns of spatial habitat use, apparently reducing their intra-specific and intra-sexual competition for resources (e.g., Martof 1953; Berven & Grudzien 1990; Ponséro & Joly 1998). Where there is less competition especially for food, animals are likely to exhibit improved body condition, which could in turn increase their reproductive success (e.g., through clutch size). Therefore relationships between habitat use and body condition might have implications for *in situ* protection of threatened amphibian populations (e.g., Ponséro & Joly 1998).

Materials and Methods

Study Site

We concentrated field work in a ~ 100 ha triangular tract of degraded Egoli Granite Grassland (Mucina *et al.* 2005) located in Diepsloot, Gauteng Province, South Africa (25º56'23.59"S, 28º01'21.88"E, 1 427-72 m a.s.l., Fig. 3). The site is representative of many areas in Gauteng, and elsewhere in South Africa, where *P. adspersus* face encroaching urbanization on remaining fragments of undeveloped habitat. A busy tarred road and a dirt road bordered the site to the west and east, respectively. West of the tarred road was low-cost housing. Agricultural small-holdings and vacant grassland surrounded the rest of the site. Three small (~ 0.2-0.9 ha), shallow (< 2 m deep), seasonal, human-created dams (named Dams 1, 2, and 3) near the south-eastern edge of the site provided habitat used by > 500 Giant bullfrogs for breeding (Yetman, unpubl. data).

General sampling

We caught study animals during spawning events by hand or with a hand held net at or near Dam 1 or 2. Dam 3 was much less favored by *P. adspersus*. An electronic balance and steel tape-measure were used to measure each animal's body mass (to the nearest gram) and snout-vent length (SVL; to the nearest millimeter), respectively. During mark-recapture research male *P. adspersus* weighing under 200 g have not been encountered at spawning events (Cook 1996; Yetman, unpubl. data). We therefore classified animals in this study lighter than 200 g and shorter than 120 mm as females.

Radio-tracking

We performed radio-tracking between 1 October and 31 March in each of three seasons 2003/2004, 2004/2005, and 2005/2006 (hereafter referred to as Summers 1-3). A total of 10 males and 20 females was released with radio-transmitters over these periods. A veterinary surgeon anaesthetized and implanted animals with transmitters. A 15-25 mm longitudinal incision was cut through the ventral abdominal skin and muscle, roughly 10-30 mm left or right of the ventral abdominal vein. We used different types and sizes of radio-transmitters (Biotrack, Ltd, United Kingdom) depending on frog body mass. TW31 (15 g or 18 g) transmitters were implanted into males; PIP22 (5 g) and PIP21 (1-4 g) transmitters were inserted into females. A transmitter never exceeded 5% of an animal's body mass. Abdominal musculature was closed in a simple continuous pattern; skin was closed with several horizontal mattress sutures. Study animals awoke within 15-40 min and resumed fully alert behavior 1-3 h post-surgery. Animals were kept in their containers overnight to allow further recovery before being released where they had been captured.

We used a locally-designed four-element Yagi antenna mounted on a 4.3 m pole, and an AVM LA12 receiver (AVM Instruments, Champagne, Illinois) to track animals when weather conditions promoted Giant bullfrog activity, or otherwise on average twice per week, between 06h00 and 18h00. We did not perform nocturnal radio-tracking because the area was considered unsafe at night. When a radio-tracked (RT) animal was located, we recorded the date, time, the frog's behavior, and geographic co-ordinates measured with a global positioning system (GPS) unit. We lost eight females from the start, but successfully tracked all 10 males and 10 of the 12 remaining females to at least one burrow (Table 1). We tracked seven females and four males through a complete summer, and four males during two or three successive summers. We lost most animals when their transmitters expired.

Spool-tracking

To investigate the movements and habitat use of adult *P. adspersus* at a finer spatial resolution, we performed spool-tracking (Heyer, 1994) between 1 October and 31 March in each of two seasons 2006/2007 and 2007/2008 (hereafter referred to as Summers 4 and 5). A total of 26 males and 14 females was released with spools over these periods.

The white, cocoon-shaped polyester spools (Nm120/2 10W: ~ 4.5 g and ~ 330 m; Danfield, Ltd, United Kingdom) weighed less than 5% of the body mass of each animal. Because attempts to fit animals with a waistband or harness failed, and because adult *P. adspersus* are strong enough to break a spool's thread and could be lost without having a waistband or harness removed, we attached spools with cyanoacrylate glue (Henkel International, Düsseldorf, Germany). Prior to field work we allowed a strip of silicone (Permoseal, Ltd, South Africa), applied along the length of each spool, to set. We cleaned an animal's dorsum with water, and dabbed it dry with disposable toweling before applying a small patch of glue behind the animal's head. The patch was left to dry for a few minutes to create a suitable attachment site. For attachment, we applied glue to the silicone strip of a spool, which was held in place on the dorsum of an animal for a few minutes. Each animal was kept in a separate container for 30 min prior to release at its capture point. Here, we tied the loose end of an animal's thread to a flagged stake.

Released animals exhibited no or very limited escape behavior (at most a few hops, usually < 1 m, across land or into water). We therefore measured thread trails from the flagged stakes onwards. We recorded the movements of spool-tracked (ST) animals daily, post-release, using a GPS unit to save the co-ordinates along trails every 2-6 m, and whenever the direction of a trail changed markedly. Thread was collected as it was followed. We lost three males from the start, but tracked 37 animals for 1-2 days, of which five males and four females were each tracked to one burrow (Table 1). Animals were lost when their spools detached prematurely, or when their thread trails snapped, ended, or became badly entangled with other thread.

Data analysis

We measured animal movements and spatial habitat use using Hawth's Analysis Tools 3.26 (© 2002-06) in ArcMap 9.2 (© ESRI, Inc. 1999-2006), and performed statistical tests in Statistica 7.0 (© StatSoft, Inc. 1984-2004 Tulsa). All shapefiles were projected using transverse Mercator; spheroid: WGS 1984, with a central meridian of 29. Numbers of relocation points obtained for RT males during more than one summer were sufficient to construct minimum convex polygons (MCPs) representing individual seasonal "home ranges." The proportion of each male's unique relocation points contained within each season's MCP was calculated to assess individual spatial use of habitat among summers. We performed Spearman Rank correlations to investigate linear relationships with body mass, SVL, or body condition (= body mass/SVL) of study animals. Mann-Whitney U tests were performed to investigate differences between the sexes. Data presented are mean ± standard deviation (SD), except where otherwise indicated. The level of significance for all tests was $P = 0.05$. Multiple tests were subjected to Bonferroni correction.

Results

Overnight movement

Study animals moved mostly at night. During daytime radio-tracking study animals were almost always buried or at the seasonal dams. Only 19 (2%) of 976 location fixes obtained for all RT animals represented instances where males were found aboveground during daytime away from water (resting under vegetation). The only daytime overland movements observed were on 6 December 2004 and 6 November 2005 when respectively, 53 mm and 56 mm of rain fell in under an hour during the afternoon, prompting *P. adspersus* to emerge from their burrows and move rapidly to the seasonal dams.

Animals spent a day or two at spawning events, and four RT males were found guarding offspring on one or two days each in summer. Females, as well as those males that did not remain at the seasonal dams to guard offspring, returned directly to their burrows (Fig. 1, 2). ST males and females exhibited little difference in the distances calculated between their actual post-mating movement away from the seasonal dams and the measured straight line distances between the end points (males: 21.5 ± 24.9%, range = 0.9-65.7%, $n = 6$; females: 18.4 ± 11.8%, range = 4.7-42.7%, $n = 9$; Mann Whitney $U = 23.00$, $df = 4$, $P = 0.64$). The maximum known distance moved overnight post-spawning was similar for males (350.5 m) and females (350.4 m).

Spool-tracking revealed that animals readily: used human-created foot paths when returning to their burrows; travelled along the gravel road on the eastern boundary of the site, particularly in furrows on the road side where rain water was channeled; and moved through an electrified fence if it was necessary to reach their burrow (Fig. 1, 2).

Since spools were found to only last a night or two, and because nocturnal radio-tracking was not done, limited data on overnight foraging of adult male and female *P. adspersus* were obtained. We directly observed adult *P. adspersus* foraging in the evening at the water's edge and within 20 m of the seasonal dams after several spawning events. An ST male was observed foraging under house lights within 10 m of his burrow (Fig. 1), and a concentration of short, criss-crossing tracks left by another ST male indirectly indicate that this frog foraged within 12 m of his burrow before retiring underground (Fig. 1). The recorded movement of an ST female around a termite mound is perhaps indicative of foraging (Fig. 2).

Seasonal movement

A burrow used by an animal for less than two weeks in a summer was classified as "temporary." Burrows used for longer periods were classified as "long-term" burrows (LTBs). Temporary burrows were used briefly, for example, by females en route to LTBs (Fig. 3). Empty burrows of either type had an elliptical entrance, and a volume just large enough to contain the inflated body of an adult male or female *P. adspersus* ca. 150 mm beneath the ground surface. Burrows retained their form, and animals exhibited high burrow fidelity. The number of LTBs used per summer by males (1.1 ± 0.2 burrows, range = 1.0-1.5 burrows, $n = 9$) and females (1.1 ± 0.3 burrows, range = 1.0-2.0 burrows, $n = 9$) did not differ significantly ($U = 33.00$, $df = 7$, $P = 0.51$). An electrified fence and vehicle traffic on the tarred road on the western boundary of the site also did not deter two RT males from repeatedly returning to their burrows (Fig. 4).

LTBs of animals were measured from the center (not the edge) of the nearest seasonal dam (CNSD) because each dam's surface area was small (and changed continually in response to weather) relative to large, natural breeding sites (e.g., Glen Austin [~ 9 ha] and Bullfrog [~ 81 ha] pans). LTBs of females were situated almost four times further from the seasonal dams (446.8 ± 295.4 m, range = 119.1-902.7 m, $n = 9$) than those of males (131.0 ± 105.2 m, range = 33.3-329.8 m, $n = 9$, $U = 11.00$, $df = 7$, $P = 0.009$). The latter result remained significant following a Bonferroni correction.

Movement among years

Spatial use of habitat by four RT males during two or three successive summers suggests animals had a strong preference and good memory for specific areas (Fig. 5). The proportion of unique relocation points contained within the minimum convex polygons constructed around each summer's relocation points was 83.1 ± 14.0% (range of 68.8-100.0%) for all four males across all summers. These animals used between one and two (1.3 ± 0.2, range = 1.0-1.5, $n = 4$) burrows per summer, and LTBs used consecutively by three of the four males within and/or among summers, were separated by 28.9 ± 21.5 m (range = 16.4-53.7 m, $n = 3$) (Fig. 5a, b, d). One male used the same burrow for three summers (Fig. 5c) and appeared to use the same burrow for the following two seasons (the burrow was occupied, but we could not determine for certain if it was the same male). Only one animal was known to return (after a period of 53 days) to a LTB (Fig. 5b). During winter, males with functional transmitters ($n = 7$) did not change position.

Body size and condition

The Spearman rank correlation between body mass, SVL or body condition, and the number of LTBs used in a summer by males ($n = 9$) was $r_s = 0.51$ ($P = 0.17$), $r_s = 0.63$ ($P = 0.07$), and $r_s = 0.34$ ($P = 0.37$), respectively. The correlation between body mass, SVL or body condition, and the number of LTBs used in a summer by females ($n = 9$) was $r_s = 0.55$ ($P = 0.13$), $r_s = 0.55$ ($P = 0.13$), and $r_s = 0.41$ ($P = 0.27$), respectively. There were no significant correlations between male ($n = 9$) body mass ($r_s = -0.13$, $P = 0.74$), SVL ($r_s = -0.08$, $P = 0.84$), or body condition ($r_s = 0.10$, $P = 0.79$), or female ($n = 9$) body mass ($r_s = 0.52$, $P = 0.16$) or SVL ($r_s = -0.02$, $P = 0.97$), with distance of their LTBs measured from the CNSD. Only female body condition and distance of their LTBs measured from the CNSD were significantly correlated ($r_s = 0.77$, $P = 0.02$, $n = 9$).

Discussion

Overnight movement

Like many pond-breeding anurans (e.g., Sinsch 1988b, 1990; Sjögren-Gulve 1998; Schwarzkopf & Alford 2002), *P. adspersus* are largely nocturnal, and move directly to and from a breeding site. This reduces both their risk of desiccation, and predation by diurnally-active birds that are major predators on this species (Channing 2001). Footpaths and the nearby dirt road were likely used by study animals because there was less vegetation to hamper their movement; channeled rain water run-off provided more moisture; and/or prey and predators would have been more visible. Similar behavior has been reported for other anurans (Moore 1954; Sinsch 1988b; Mazerolle 2005).

Pyxicephalus adspersus moved a maximum known overnight distance that is similar to that reported for other pond-breeding anurans: 200-500 m (e.g., Gittins *et al.* 1980; Carpenter & Gillingham 1987; Miaud *et al.* 2000; Bulger *et al.* 2003). It was, however, shorter than expected considering that animal size and movement capacity are predicted to scale positively (Peters 1983), and that a smaller, pond-breeding anuran species (*Hoplobatrachus occipitalis*) inhabiting savanna habitat in West Africa, covers 1.4 km in a night (Spieler & Linsenmair 1998).

Limited evidence suggested that adult *P. adspersus* forage up to 20 m from their burrows. This behavior is analogous to the concentrated foraging of many anurans around burrows or "forms" (i.e., small patches of ground cleared for resting) situated within a terrestrial summer home range or non-breeding "activity area" (e.g., Dole 1965; Lamoureux *et al.* 2002; Penman *et al.* 2008).

Movement within and among seasons

Pyxicephalus adspersus shows strong site fidelity to their non-breeding habitats with both adult males and females being highly faithful to their LTBs over time. Strong fidelity to specific terrestrial sites has been reported for various anurans (e.g., Kelleher & Tester 1969; Haapanen 1970; Penman *et al.* 2008; Pitman *et al.* 2008), and may increase familiarization of individual anurans with surrounding habitat features for improved predator avoidance and foraging efficiency (Bellis 1965). *Pyxicephalus adspersus* commonly prey on termites (Channing 2001), therefore, familiarity of individuals with the location of termite colonies around their burrows could be beneficial.

As predicted by their extreme dimorphism in size, adult males and females exhibited a clear difference in spatial habitat use. Results were, however, contrary to the expectation that males would migrate further to satisfy their greater absolute energy needs. The LTBs of females were situated almost four times further from the seasonal dams compared to those of males. Greater spatial concentration of males around breeding sites appears to be common among pond-breeding anurans (e.g., Pilliod *et al.* 2002; Regosin *et al.* 2005; Bull 2006; Johnson *et al.* 2007), although the trend is not ubiquitous (e.g., Lemckert & Brassil 2003; Smith & Green 2006; Kovar *et al.* 2009). We argue that resource-competition (Austin *et al.* 2003; Palo *et al.* 2004) provides the best explanation for the biased spatial distribution of non-breeding adult male and female *P. adspersus* around breeding sites. By remaining close to breeding habitat males might increase their chances of securing a territory, obtaining mates, and rearing offspring where aquatic habitat conditions are most favorable for tadpole development. By moving further from breeding habitat females might reduce their competition for food (e.g., termites and other invertebrates) with the high abundance of much larger males that are also able to consume larger prey items (e.g., birds, anurans and snakes; Channing 2001) near the water.

Body size and condition

Body mass and SVL appear to be poor predictors of spatial habitat use by *P. adspersus*, as is the case for other anuran species (e.g., Bellis 1965; Miaud *et al.* 2000; Bulger *et al.* 2003; Lemckert 2004). Body condition instead might be a more useful predictor (e.g., Sztatecsny & Schabetsberger 2005). In this study, a positive relationship was found between female body condition and distance of their LTBs from the seasonal dams. Not only may individuals move further if they have a better body condition; in doing so they might benefit from reduced interspecific competition for food.

Conservation implications

Semlitsch & Bodie (2003) determined that 32 amphibian (including 19 anuran) species utilized terrestrial habitat within a mean range of 159-290 m from aquatic breeding habitat. Lemckert (2004) established that the mean distance moved away from breeding habitat to terrestrial sites was approximately 300 m for bufonids, hylids, and ranids, treated separately or combined (*n* = 28). Therefore, a 300 m wide buffer could be expected to protect roughly half the members of an "average" pond-breeding anuran population (Lemckert 2004). At the Diepsloot site a 300 m wide buffer measured from the center of the three seasonal dams would include the LTBs of 67% (*n* = 12) of RT animals that used one or more LTBs (*n* = 18) (Fig. 6). However, since the LTBs of females were almost four times further than those of males, a 300 m wide buffer would include the LTBs of 89% of males (*n* = 9) but only 44% of females (*n* = 9) that used one or more LTBs. Moreover, LTBs of females in the best body condition were situated furthest from the seasonal dams. Since body condition can influence survival and reproductive success in anurans (Reading 2007), a 300 m or even a 500 m wide buffer around the seasonal dams would exclude the LTBs of females that were potentially most fecund.

To protect the LTBs of all study animals, a 950-1 000 m wide buffer would be necessary around the Diepsloot seasonal dams. Assuming that results of this study are representative of spatial habitat use by *P. adspersus* at other localities, buffer zones 500-1 000 m wide will be necessary to protect most adults buried around breeding sites. If these are larger than the Diepsloot seasonal dams, buffer zones should be measured from the periphery (not the center) of spawning sites. Similarly large protective buffers have been proposed for other pond-breeding anurans (e.g., Richter *et al.* 2001).

If there was more natural habitat around the Diepsloot site, would animals have moved further from the seasonal dams? Walls around properties clearly constrained animal movements, and *P. adspersus* probably cannot bury into ferricrete (i.e., sand and gravel cemented into a hard mass by iron oxide), exposed by topsoil erosion immediately west of Dam 1, and between Dams 1 and 3. *P. adspersus* crossing nearby roads were likely to get hit by traffic, and were therefore perhaps less likely to be caught or successfully tracked. Hence animal movements extended mainly north of the seasonal dams. Most adult *P. adspersus* on the site, however, probably remained within a kilometer of the seasonal dams. Although the species could move up to ~ 1.5-2 km north and north-east of the dams, the furthest animal relocation was 903 m north-east of the Dam 1. Yet a population genetics study (Yetman& Ferguson, unpubl. data, [Chapter 5]) based on *P. adspersus* sampled from Diepsloot and other localities mostly in Gauteng Province revealed significant (historical) gene flow between populations up to 20-100 km apart. We therefore suggest that like other pond-breeding anurans (e.g., Dole 1971; Schroeder 1976; Sinsch 1997; Berven & Grudzien 1990), *P. adspersus* adults are generally philopatric to their breeding sites, and utilize terrestrial habitat most often within a kilometer radius thereof; whereas immatures typically disperse, facilitating gene flow between neighbouring populations.

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Table 1. Measures of success with radio- and spool-tracking of adult *Pyxicephalus adspersus* in Diepsloot, Gauteng Province, South Africa.

	Radio-tracking		Spool-tracking	
No. of animals	♂	♀	♂	♀
Released	10	20	26	14
Tracked:	10	12	23	14
through < 1 summer	2	5	23	14
through 1 summer	4	7	-	-
through > 1 summer	4	-	-	-
to ≥ 1 burrows	10	10	5	4
Lost	6	8	15	10
Died	1	2	0	0
Device removed or shed	3	2	8	4

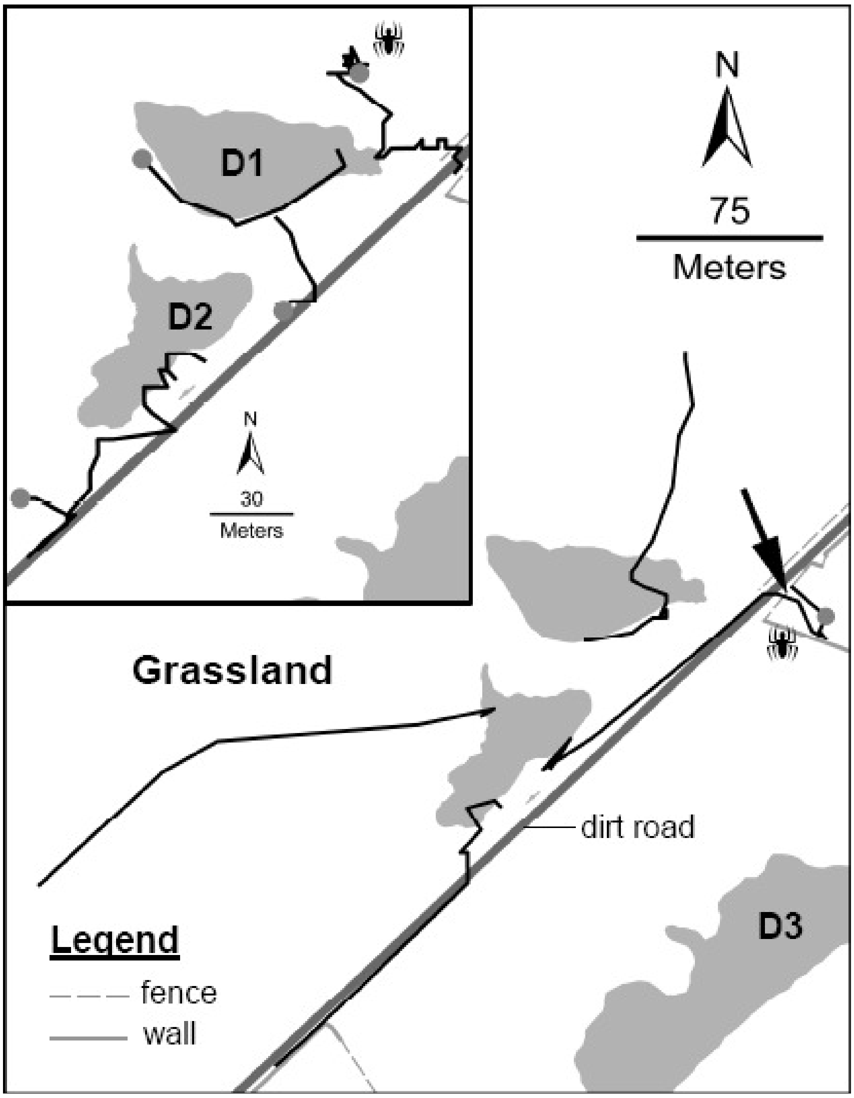

Figure 1. Overnight movements by spool-tracked adult male *Pyxicephalus adspersus* (*n* = 8) that returned to their burrows (●) after spawning activity at three seasonal dams (D1, 2, and 3). The black arrow indicates where an animal moved though an electrified fence. 🕷 = where foraging by an animal was observed, or inferred from its movements.

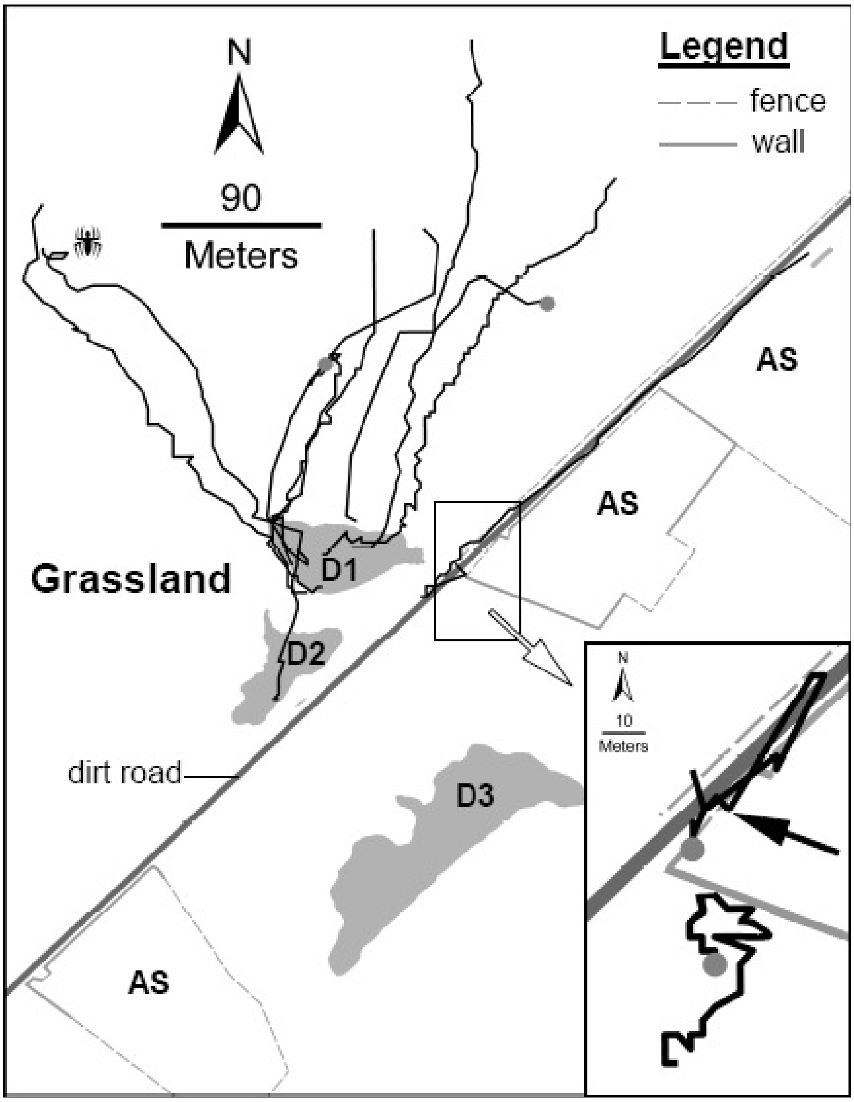

Figure 2. Overnight movements by spool-tracked adult female *Pyxicephalus adspersus* (*n* = 11) that returned to their burrows (●) after spawning activity at three seasonal dams (D1, 2, and 3). The black arrow indicates where an animal moved though an electrified fence. 🕷 = where foraging by an animal was inferred from its movement around a termite mound. AS = agricultural small-holding.

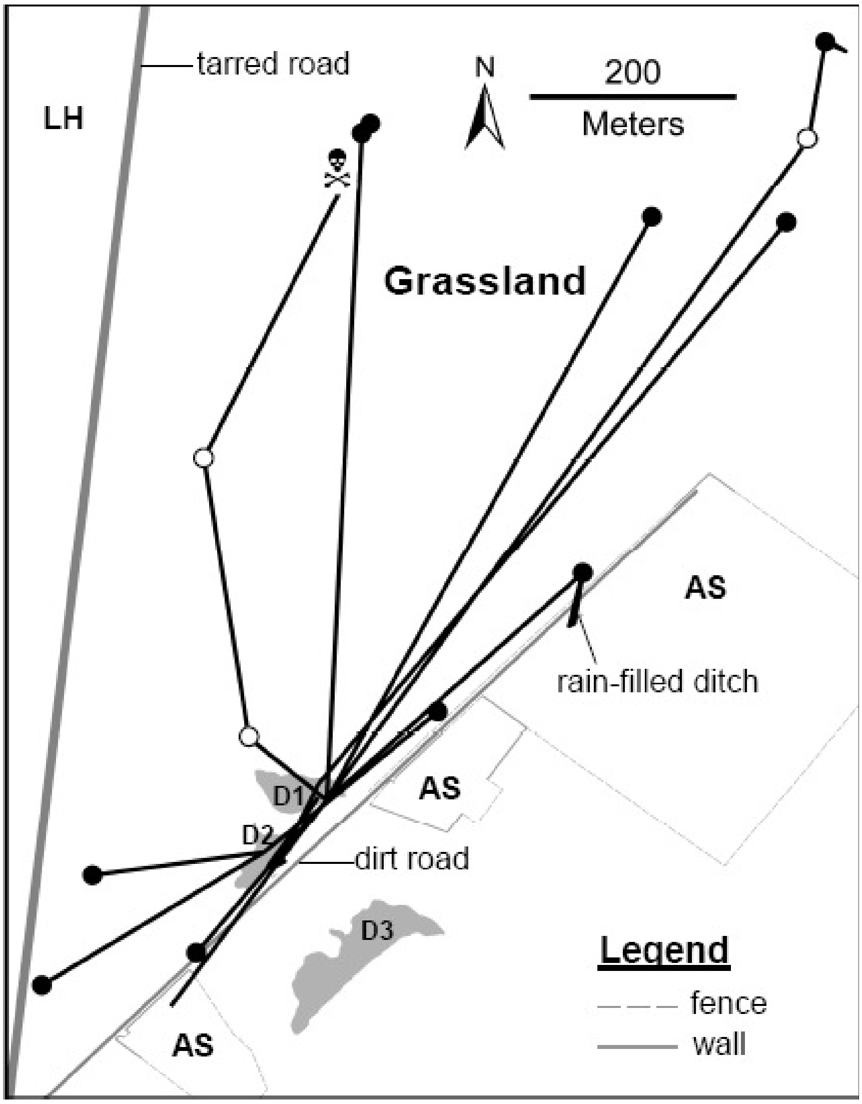

Figure 3. Within-season movements by radio-tracked adult female *Pyxicephalus adspersus* (*n* = 11) between seasonal dams (D1, 2 and 3) and their burrows. ○ = "temporary" burrows used for less than two weeks. ● = "long-term" burrows used for more than two weeks. ☠ = where an animal died for uncertain reasons. AS = agricultural small-holding. LH = low-cost housing.

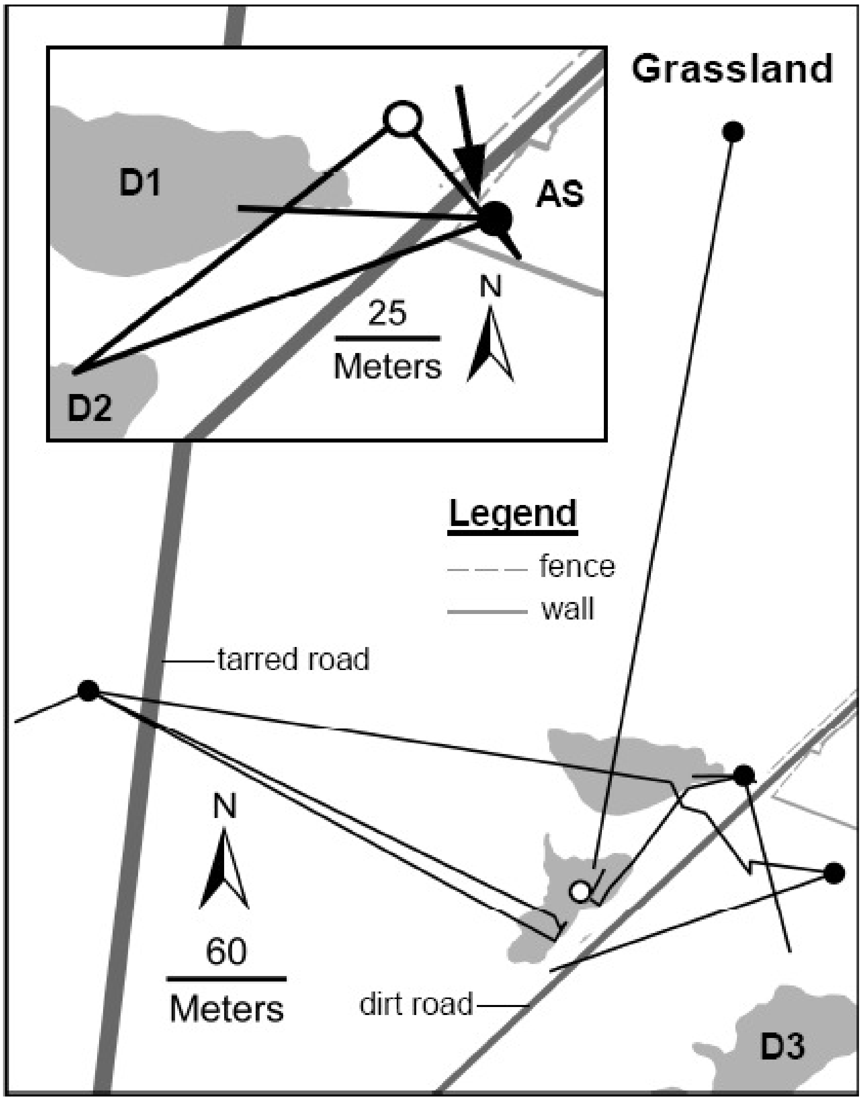

Figure 4. Within-season movements by adult male *Pyxicephalus adspersus* (*n* = 6) radio-tracked (for a maximum of one full summer) between seasonal dams (D1, 2 and 3) and their burrows. ○ = "temporary" burrows used for less than two weeks. ● = "long-term" burrows used for more than two weeks. The black arrow indicates where an animal moved though an electrified fence. AS = agricultural small-holding.

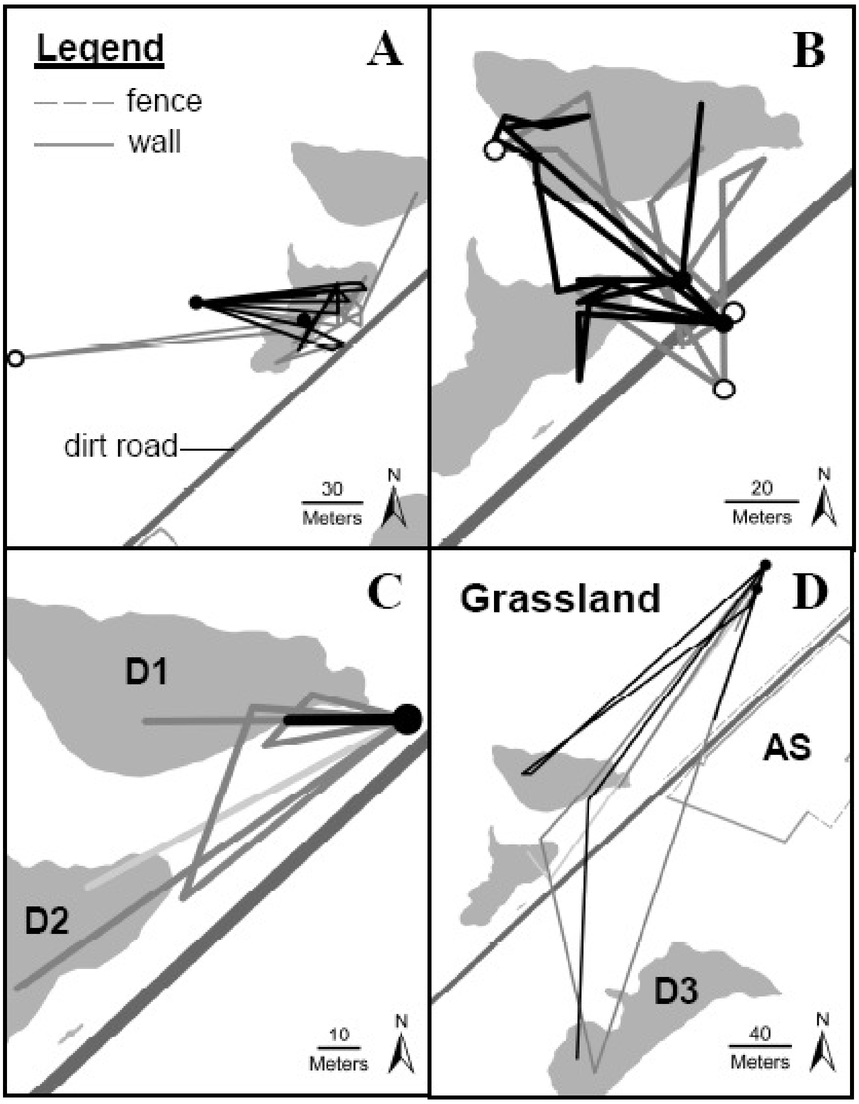

Figure 5. Between-season movements by adult male *Pyxicephalus adspersus* radio-tracked between seasonal dams (D1, 2 and 3) and their burrows, during: a-b) two summers (*n* = 2 males); or c-d) three summers (*n* = 2 males). ○ = "temporary" burrows used for less than two weeks. ● = "long-term" burrows used for more than two weeks. AS = agricultural small-holding.

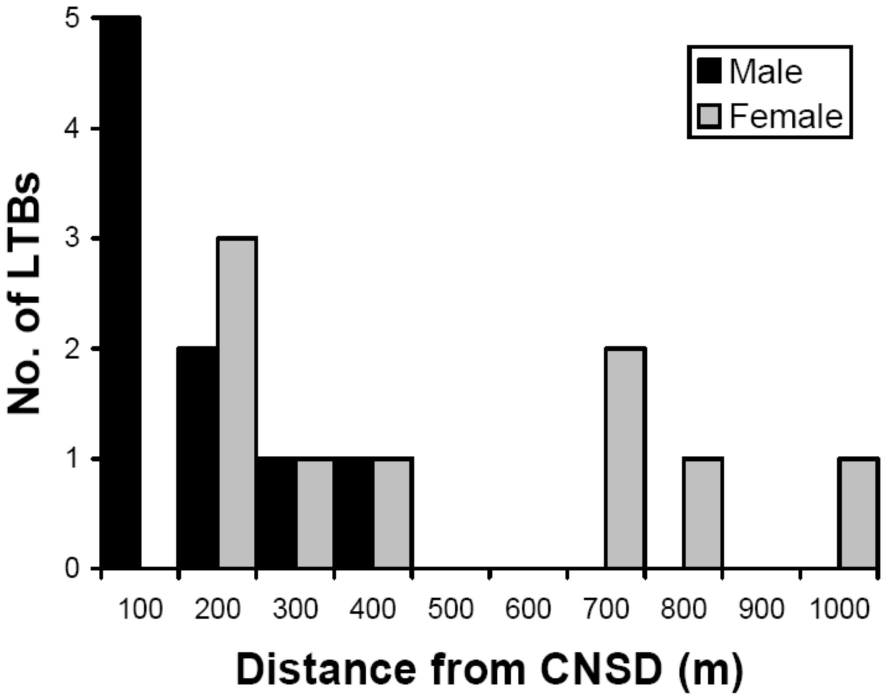

Figure 6. Number of “long-term” burrows (LTBs; i.e., used for more than two weeks) of radio-tracked adult male or female *Pyxicephalus adspersus* as a function of distance from the center of nearest seasonal dam (CNSD) on the site. The LTBs of most males (i.e., five of $n = 9$ males) were located within 100 m, whereas those of all females ($n = 9$) were found beyond 200 m from the CNSD.

Chapter 4

Conservation implications of the age/size distribution of giant bullfrogs (*Pyxicephalus adspersus*) at three peri-urban breeding sites *

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Abstract.—Nothing is known about the age of wild giant bullfrogs (*Pyxicephalus adspersus*); yet this information has important conservation implications for this regionally threatened species. We quantified and compared the age, body size, and body condition of adult male and female *P. adspersus* caught during spawning events at peri-urban breeding sites in Diepsloot, and at Glen Austin and Bullfrog pans in Gauteng Province, South Africa. Age was estimated from lines of arrested growth (LAG) counted in cross-sections of animal phalanges. Males and females from all three sites possessed 6 ± 2 (max. 16) and 4 ± 1 (max. 11) LAG, respectively, suggesting shorter female longevity. Individuals with < 3 LAG were not encountered at the breeding sites, implying that newly metamorphosed *P. adspersus* require at least three years to reach sexual maturity. There was no significant difference in the LAG counts of same-sex animals between the three sites. However, mean male snout-vent length, mass, and body condition was greatest at Glen Austin Pan, and lowest at Bullfrog Pan. The latter is possibly explained by chemical contamination of Bullfrog Pan from an adjacent disused landfill. At Glen Austin Pan males and females sampled in 2004-06 for this study were significantly shorter than those sampled at the same site in 1992-93 for a different study. Our results suggest that male *P. adspersus* may live for 20 years or more in the wild, but at some peri-urban breeding sites adult life expectancy is declining. Juvenile *P. adspersus* are most threatened by terrestrial habitat transformation because they take ≥ 3 years to mature, during which period they may move great distances from their natal site. Differences in the size and condition of *P. adspersus* between the study sites, suggests that the species requires site-specific management in addition to conservation at larger spatial scales.

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Introduction

In temperate, mid-latitude environments anurans require from several months to three years to reach sexual maturity (e.g. Zug & Zug 1979; Tsiora & Kyriakopoulou-Sklavounou 2002) while at high latitudes or altitudes anurans may take four or more years to mature (e.g. Metter 1967; Matthews & Miaud 2007). Many anurans live less than 10 years (e.g. Measey 2001; Esteban *et al.* 2004; Guarino & Erismis 2008), but larger species and/or captive individuals can exceed this, e.g. wild and captive *Rana catesbeiana* reached 10 and 16 years, respectively (Oliver 1955; Goin & Goin 1962) and wild and captive *Bufo marinus* reached 15 and 40 years, respectively (Tyler 1975). Age at maturity and longevity have important conservation implications for threatened anurans. As age at maturation increases, generation time increases and population resilience decreases (Duellman & Trueb 1994). Older females are generally larger than younger females, and therefore produce larger and more clutches during a breeding season (Howard 1978; Reading & Clarke 1995; Reading 2007). Although there have been numerous studies on anuran age (see Tables 2-9 in Duellman & Trueb 1994; Table 1 in Monnet & Cherry 2002), very limited data have been obtained for mainland Africa species, e.g. *Amietophrynus pardalis* (Cherry & Françillon-Vieillot 1992), *R. saharica* (Esteban *et al.* 1999; Meddeb *et al.* 2007) and *Xenopus laevis* (Measey 2001).

The giant bullfrog (*Pyxicephalus adspersus*) is one of the largest extant anurans (Du Preez & Cook 2004) and is exceptional in that males (~ 400-1 000 g) can weigh up to ten times more than females (~ 90-300 g; Cook 1996). The species is widely distributed across the grassland and savanna regions of southern Africa (Channing 2001) but is regarded as Near-Threatened in South Africa due to habitat loss and other factors (Harrison *et al.* 2001; Minter *et al.* 2004). Due to the very brief and unpredictable appearance of *P. adspersus* aboveground during summer (Yetman & Ferguson 2011a, [Chapter 2]), little systematic field-based research and monitoring has been performed on this species (Jacobsen 1989; Kok *et al.* 1989; Van Wyk *et al.* 1992; Cook 1996). The resulting lack of demographic information has impeded the assessment of the conservation status of *P. adspersus*, and provides a weak baseline for evaluation of efforts to protect threatened populations. Captive *P. adspersus* have reportedly reached an estimated 45 years of age (Channing 2001) but nothing is known about the age of wild specimens. Juvenile *P. adspersus* are expected to require several years to mature given the large body size of adults (Peters 1983); therefore populations may have slow generation turnover and low resilience to perturbations in density.

Skeletochronology is a commonly-used technique for aging anurans (Halliday & Verrell 1988) and has been applied to a wide variety of species from arid (Rogers & Harvey 1994; Tessa *et al.* 2007), temperate (Miaud *et al.* 1999; Guarino *et al.* 2003), subtropical, and even tropical environments (Morrison *et al.* 2004; Lai *et al.* 2005). The technique involves analysis of concentric lines of arrested growth (LAG) visible in cross-sections of animal long bones and phalanges (Castanet & Smirina 1990). LAG are deposited during periods of animal inactivity and are therefore pronounced in species with highly seasonal activity. Adult *P. adspersus* are active after heavy rainfall for cumulatively less than a month between October and March (Yetman & Ferguson 2011a, [Chapter 2]), and were therefore considered appropriate subjects for phalangeal skeletochronology, which precludes the need to sacrifice whole animals. Using this method we aimed to estimate the age of wild, adult *P. adspersus* to provide important life history and demographic information for improved conservation management of this species. In particular we wanted to determine whether *P. adspersus* at different breeding sites differed significantly in age, body size or body condition, and therefore, require site-specific conservation management. Specific objectives of this study were to:

- estimate the age range of wild, breeding *P. adspersus* males and females using skeletochronology.
- examine the relationship between body size and age in adult *P. adspersus.*
- compare the age, body size and body condition of adult *P. adspersus* at different breeding sites.
- infer consequences of the results on the conservation management of *P. adspersus*.

Materials and Methods

Study sites

Adult *P. adspersus* were caught by hand or with a hand-held net at spawning events between 1 October and 31 March in the 2003/2004, 2004/2005 and 2005/2006 summer seasons at three peri-urban, seasonal breeding sites in Gauteng Province, South Africa. At the Diepsloot site (25°56'23.59"S, 28º01'21.88"E) *P. adspersus* bred in three small (~ 0.2-0.9 ha) artificial dams where there was a relatively high level of human activity. Glen Austin Pan (25°58'36.00"S, 28º09'58.33"E; ~ 9 ha) and Bullfrog Pan (26º08'22.35"S, 28º18'51.10"E; ~ 81 ha) were proclaimed bird sanctuaries that gave limited protection for the historically large resident populations of *P. adspersus* (Cook 1996; Slater-Jones 1996). The three sites (separated by 15-36 km) had very similar climates and remaining natural grassland (Mucina *et al.* 2005) relative to the variety of habitats that *P. adspersus* inhabits in southern Africa. The agricultural small-holdings surrounding each site were becoming increasingly urbanized.

Field work

Study animals were handled in a manner complying with the "Guidelines for Use of Live Amphibians and Reptiles in Field Research" (Society for the Study of Amphibians and Reptiles, the American Society of Ichthyologists and Herpetologists, and The Herpetologists' League). We used an electronic balance (accurate to 1 gram) and steel tape-measure (accurate to 1 millimeter) to measure each animal's body mass and snout-vent length (SVL), respectively. Animal body condition was estimated as mass/SVL (Schulte-Hostedde *et al.* 2005). A sterilized bone cutter or wire clipper was used to clip the two most distal phalanges from the second (i.e. longest) toe on the right hind limb of each animal. Toe clips were stored in separate vials containing 70% ethanol, and animals were released near their point of capture.

Histology

To analyze animal body size in relation to age, we selected toe clips of five small (90 mm $< \text{SVL} \leq 110$ mm) and five large (110 mm $< \text{SVL} \leq 130$ mm) females, as well as five small (130 mm $< \text{SVL} \leq 150$ mm), five medium (150 mm $< \text{SVL} \leq 170$ mm) and five large (SVL > 170 mm) males from each site except Bullfrog Pan, where only three large males were caught. Toe clips of the three largest males caught during the study (which included two males from Diepsloot and one from Glen Austin Pan with a SVL ≥ 190 mm) were added to the largest size category. Data for the size-age analysis were therefore obtained from 76 animals, including 30 females and 46 males (hereon referred to as the "size selected" animals). To compare the age of same-sex animals between the three locations we used the complete sample set again to randomly select toe clips of 10 females and 15 males from each site. Hence data for comparisons of animal age between the populations were obtained for 75 animals, including 30 females and 45 males (hereon referred to as the "randomly selected" animals).

Toe clips were decalcified in 8% formic acid for 48 h, and embedded in paraffin wax. A rotary microtome was used to cut 6 μm thick sections which were then stained with Ehrlich's haematoxylin. This method was adapted from the method used by Castanet & Smirina (1990), which involved nitric acid for decalcification, and a freezing microtome. We examined mounted sections under a standard light microscope at 4x, 10x, or 20x magnification, and selected for each sampled animal one or two sections with the smallest medullar cavity. We counted lines of arrested growth (LAG) from the endosteal bone outwards and treated the perimeter of the periosteal bone as a LAG if the animal had been sampled during the first spawning event of a season. False and double LAG were respectively, distinguished as incomplete or complete feint LAG within bands of summertime bone growth (Hemelaar & Van Gelder 1980; Sagor *et al.* 1998; Cvetković *et al.* 2005).

Data analysis

Statistical tests were performed in Statistica 7.0 (© StatSoft, Inc. 1984-2004 Tulsa). We used least squares regression analyses to examine relationships between the body mass, SVL, and LAG counts of size selected males or females from the three sites combined. We used ANOVA to compare LAG counts between small and large females, and between small, medium and large males. The SVL, body mass, body condition, or LAG counts between randomly selected males and females from the three sites treated separately, or combined, were compared using *t*-tests (when $n < 30$) or ANOVA (when $n > 30$). We used ANOVA to compare the SVL, body mass, body condition, or LAG counts of randomly selected same-sex animals between the three sites. In addition, we calculated the two-sample z- and Smirnov d-statistics to, respectively, compare the mean values and cumulative frequency distributions of the SVL or body mass of all males or females sampled during spawning events at Glen Austin Pan in 2004-06 (for this study) and in 1992-93 (for a separate study; Cook 1996). The data presented are mean ± standard deviation (SD) and the level of significance for all tests was $P = 0.05$. We applied sequential Bonferroni corrections to comparisons of SVL, body mass, body condition, or LAG counts between randomly selected males and females from the same site or same-sex animals from different sites, or all same-sex animals sampled at Glen Austin Pan for the two separate studies.

Results

Interpretation of sections

The most hematoxylinophilic LAG observed in the sections (Fig. 1) were arranged in a consistent geometric pattern among the samples (e.g. Fig. 1a and b in Bastien & Leclair 1992; Fig. 1c and d in Guarino *et al.* 2003; Fig. 1 in Tessa *et al.* 2007). We assumed that each of these LAG was deposited while an animal experienced torpor underground during a six- to eight-month winter period (Loveridge & Withers 1981). Animals experienced the most bone growth during their second summer. Growth declined slightly during the third summer and decreased dramatically thereafter. The number of complete and partially remaining LAG prior to this transition in bone growth revealed that complete or partial resorption of the first LAG and partial resorption of the second LAG was common among the samples. Within bands of summertime bone growth multiple false and double LAG were observed, which did not exhibit any consistent pattern among the samples.

Body size and age of size-selected animals

The females (*n* = 30) weighed 99-285 g, measured 92-136 mm in SVL and possessed 3-11 LAG. The males (*n* = 46) weighed 213-872 g, measured 130-198 mm in SVL and possessed 3-16 LAG. No animal possessed < 3 LAG. The oldest female (with 11 LAG) weighed 285 g, measured 136 mm in SVL and was sampled at Bullfrog Pan. The oldest male (with 16 LAG) weighed 704 g, measured 178 mm and was sampled at Glen Austin Pan. The three males with a SVL ≥ 190 mm possessed 7 or 8 LAG each.

Body mass increased exponentially with SVL in both males ($y = 42.10\ x^{2.465}$, $t = 11.7$, $P < 0.001$, $n = 46$) and females ($y = 26.05\ x^{2.317}$, $t = 8.0$, $P < 0.001$, $n = 30$; Fig. 2a). There was a weak linear increase in body mass with LAG counts in both males ($y = 29.92x + 289.63$, $r^2 = 0.2$, $P < 0.001$, $n = 46$) and females ($y = 18.52x + 17.63$, $r^2 = 0.6$, $P < 0.001$, $n = 30$). SVL increased logarithmically with LAG counts in males ($y = 31.96\mathrm{Ln}(x) + 103.27$, $r^2 = 0.3$, $P < 0.001$, $n = 46$) and linearly with LAG counts in females ($y = 4.09x + 90.36$, $r^2 = 0.5$, $P < 0.001$, $n = 30$; Fig. 2b). LAG counts differed significantly between small, medium and large males ($F_{2,\ 43} = 7.0$, $P = 0.002$, $n = 46$; Fig. 2c), and between small and large females ($F_{1,\ 28} = 6.9$, $P = 0.01$, $n = 30$; Fig. 2d). The lack of a strong sigmoid relationship between LAG counts and SVL precluded growth analysis to estimate maximal body size of males and females.

Males versus females

The SVL, body mass and body condition of all sampled *P. adspersus*, and LAG counts of the 45 males and 30 females whose toe clips were randomly selected for sectioning are shown in Table 1. At each of the three sites males were significantly longer and heavier than the females (Table 1). These differences, and the difference in LAG counts between males and females from the Diepsloot dams, or from all three sites combined, remained significant following Bonferroni corrections. The frequency distribution of LAG counts of the randomly selected males and females from the three sites combined is shown in Fig. 3. Eighty percent of the males possessed 4-7 LAG, and 83% of the females possessed 3-5 LAG. Only 13% of the males and 17% of the females possessed 8-11 LAG and 6-7 LAG, respectively.

Study site comparisons

There was no significant difference in the LAG counts of randomly selected same-sex animals between the three sites (Table 1). However, mean male SVL, body mass and condition was greatest at Glen Austin Pan and lowest at Bullfrog Pan. Mean female SVL was longest at Glen Austin Pan and shortest at Diepsloot.

Male (n = 510) and female (n = 204) *P. adspersus* sampled by Cook (1996) during 1992 or 1993 at Glen Austin Pan had a body mass of 561 ± 87 g (range: 320-970 g) and 173 ± 51 g (range: 60-400 g), and a SVL of 184 ± 13 mm (range: 137-227 mm) and 116 ± 10 mm (range: 95-141 mm), respectively. The mean SVL of males and females sampled at this site in 2004-06 for our study measured, respectively, 19 mm (z = 15.9, $P < 0.001$) and 3 mm (z = 2.1, P = 0.02) less than in 1992-93. These differences in mean SVL remained significant following sequential Bonferroni corrections. Male and female mean body mass measured 6 g (z = 0.5) and 4 g (z = 0.4) less, respectively, in 2004-06 than in 1992-93, but these differences in mean body mass were non-significant (P = 0.3 for both tests). Frequency distributions of the SVL or body mass of all male or female *P. adspersus* sampled in 1992-93 or 2004-06, are shown in Figures 4a-d. The cumulative frequency distributions of SVL and body mass differed significantly between the two studies for males (Smirnov d = 0.41 and 0.18, respectively, $P < 0.05$), but not for females (d = 0.20 and 0.24, respectively).

Discussion

Interpretation of sections

The reliability of skeletochronological age estimates can be affected by endosteal resorption of periosteal bone, deposition of false and double LAG, and rapprochement of LAG near the perimeter of the periosteal bone (Hemelaar & Van Gelder 1980; Castanet & Smirina 1990). Skeletochronological age estimates are also less reliable when phalanges (not long bones) are used or study species are long-lived (Wagner *et al.* 2011). Therefore the application of skeletochronology ideally requires reference to LAG in bones of known-age individuals. Known-age, wild *P. adspersus* were not available; therefore we attempted to sample captive specimens. However, most owners would not allow toe-clipping, accurate age records did not exist, and many specimens were fed and remained active throughout the year (CAY pers. obs.).

Nevertheless, the progressive increase in numbers of LAG with the body size of sampled *P. adspersus* (Fig. 2b-d) indicates that the use of LAG was effective. The geometric pattern of LAG deposition and the complete or partial resorption of the first and/or second LAG observed in the *P. adspersus* sections is also common among anurans (Smirina 1972; Hemelaar 1985; Patón *et al.* 1991). False and double LAG were however, unusually abundant among the sampled *P. adspersus* (Fig. 1). False LAG have been associated with periods of drought during the active season of other anurans (e.g. Rogers & Harvey 1994), and were probably deposited when sampled *P. adspersus* remained inactive for extended periods during summer between activity episodes following heavy rainfall. This was evident at the Diepsloot site where radio-tracked *P. adspersus* were buried for approximately 22-24 weeks cumulatively during the six-month (~ 26 week) summer period (Yetman & Ferguson 2011a, [Chapter 2]).

Body size and age

Pyxicephalus adspersus has a voracious appetite (Branch 1976; Paukstis & Reinbold 1984) and juveniles grow rapidly post-metamorphosis (Van Wyk *et al.* 1992; Douglas 1995). After nine months, which included winter torpor, newly metamorphosed *P. adspersus* kept under semi-natural conditions had grown 1 326% heavier and twice as long (Conradie *et al.* 2010). The consistently widest band of bone growth between the second and third LAG (Fig. 1) among the sections in this study suggests that sampled *P. adspersus* experienced the greatest somatic growth during their second active season. Subsequent reduction in bone growth appeared typical of a shift in resource allocation from growth to reproduction at sexual maturation (Caetano & Castanet 1993; Smirina 1994), suggesting that at our study sites, female and at least some male *P. adspersus* attained sexual maturity following their third active season. Other large anurans mature at an earlier or similar age, e.g. *Bufo marinus,* 1-2 years (Easteal 1982) and *R. catesbeiana,* 1-4 years (Howard 1981; Harding 1997).

We suspect, considering that the oldest aged male and female in this study possessed 16 and 11 LAG respectively, that at undisturbed sites male and female *P. adspersus* can reach ≥ 20 and ≥ 15 years of age respectively. Similar ages have been reached by other anurans in the wild, e.g. > 20 years for *Bombina variegata* (Płytycz & Bigaj 1993) and 15 years for *Bufo marinus* (Tyler 1975). In more arid areas such as the Kalahari, where *P. adspersus* can reportedly spend several successive years in torpor underground (Du Preez

& Carruthers 2009), individuals are likely to live much longer than 20 years. This is because, across different habitats, *P. adspersus* may have a similar mean physiological longevity calculated as, e.g. mean age in years multiplied by the mean number of summer rainfall days at a specific locality (modified from Bastien & Leclair 1992).

Males versus females

Male *P. adspersus* fight aggressively during spawning events, and since the largest males secure more matings and perform parental care of offspring (Cook 1996; Cook *et al.* 2001), there is probably strong selection for large male body size in *P. adspersus* (Shine 1979; Arak 1988). At our three study sites male *P. adspersus* were on average approximately three times (360 g) heavier, 1.5 times (54 mm) longer and possessed two more LAG than females suggesting shorter female longevity. There have been few similar studies on other anurans with reversed sexual size dimorphism (e.g. Briggs & Storm 1970; Sinsch *et al.* 2001). Although greater body size may be attained through greater longevity of the larger sex in anurans (Monnet & Cherry 2002), male *P. adspersus* were much larger than females of comparable LAG count (Fig. 2b). This was probably due to slower growth in females compared to males, which was observed in captive *P. edulis* (mistaken as *P. adspersus*, it seems) following the appearance of differentiated gonads in both sexes from approximately 60 days post-metamorphosis (Hayes & Licht 1992). Gonadectomy of a portion of these animals did not affect their subsequent growth, suggesting that sex hormones are not related to the divergence in the growth rates of male and female *Pyxicephalus*.

Study site comparisons

Differences in the size and condition of animals between the three study sites were not matched by differences in their age (Table 1). This was probably because the aged animals represented a small subset of all the sampled animals (*n* = 45 of 211 males; *n* = 30 of 68 females). When we compared the mass, SVL or condition of only the aged males or females (not shown here), there was no significant difference in their size or condition between the three sites. Therefore animals at Glen Austin Pan were larger because they were probably also older.

A plausible explanation for the smaller body size and poorer condition of males at Bullfrog Pan is the contamination of this site by toxic leachate from an adjacent disused landfill. Since 1993 the leachate was channelled by a plastic drain to a pump-house; but in 1996 a leak in the drain was discovered. At this time a study (Slater-Jones 1996) was performed when two newly metamorphosed *P. adspersus* with facial deformities were found in the pan. The study revealed an elevated concentration of lead (6.4 parts per million) in the tissue of newly metamorphosed *P. adspersus*, and a significant reduction in their body size with decreasing distance from the leak. Assuming in our study that males with 7-10 LAG were born between 1994 and 1999, those from Bullfrog Pan were possibly exposed to significant contamination during metamorphosis, which could have reduced their size and condition as froglets and later as adults. In 1997 the leak was fixed, which could explain why males with < 7 LAG (born after 1997) were larger than males with ≥ 7 LAG at this site. All but two females at this site possessed < 7 LAG. The one female with 7 LAG appeared to be small for her age (Fig. 2b). The other female with 11

LAG was large (Fig. 2b), possibly because she was born in 1993 when leachate was successfully diverted from the pan. Deformities and reduced growth, body size, condition and/or longevity of amphibians from chemically contaminated sites have been reported in various studies (e.g. Carey & Bryant 1995; Rowe *et al.* 2001; Spear *et al.* 2009; Brodeur *et al.* 2011).

The modest decrease in SVL of male and female *P. adspersus* between 1992-93 and 2004-06 at Glen Austin Pan was unlikely due to differences in sampling or measurement because these were performed in the same way (Cook 1996). Considering the increasing loss of habitat and mortality of *P. adspersus* with urban encroachment in Gauteng (CAY pers. obs.), the reduction in SVL of adults at Glen Austin Pan possibly indicates that very large males (SVL > 190 mm) and females (SVL > 130 mm) have become increasingly rare. This is suggested by all four graphs in Fig. 4. The significance of the size decrease of *P. adspersus* at Glen Austin Pan is emphasized by considering that the mean SVL and mass of adults at this site during 1992-93 was greater than at any of the three sites during our study. Mean SVL of males at Glen Austin Pan in 1992-93 was also greater than the maximum SVL of males at Bullfrog Pan in 2004-06. Although we cannot completely discount variation in ecological conditions (e.g., rainfall, food availability or predation; Reaser 2000) as a cause of the temporal or spatial differences in the body size or condition of *P. adspersus* in this study, indirect evidence suggests that large *P. adspersus* have become scarcer at several peri-urban breeding sites.

Conservation implications

Since some anurans take four or more years to mature (e.g. Metter 1967; Matthews & Miaud 2007), the generation time of *P. adspersus* is less than may have been predicted from the large adult size of this species (Peters 1983). Therefore *P. adspersus* populations could have greater resilience than has been assumed. However, population breeding success is likely to fluctuate dramatically because *P. adspersus* spawning and larval survival is strongly related to rainfall which varies greatly between years (Read 1990; Yetman & Ferguson 2001a, [Chapter 2]). In addition, juvenile *P. adspersus* probably experience high mortality prior to their sexual maturation when they spend ≥ 3 years moving large distances overland, as revealed by the philopatric behaviour of adults (Yetman & Ferguson 2011b, [Chapter 3]) and gene flow between populations ≥ 20 km apart (Yetman & Ferguson, unpubl. data, [Chapter 5]). Terrestrial habitat conservation is therefore critical for *P. adspersus* juvenile survival, dispersal and recruitment, which probably exerts a stronger influence than adult survival on the growth of populations (Conroy & Brook 2003; Grafe *et al.* 2004).

The oldest aged animal in this study was less than half the maximum age of 45 years reported for a captive *P. adspersus* (Channing 2001). Moreover, < 20% of males and females in this study had > 7 or > 5 LAG respectively (Fig. 3). This suggests that the life expectancy of *P. adspersus* at peri-urban sites is low, which would explain why very large males and females appear to have become increasingly rare, such as at Glen Austin and Bullfrog pans. This is of conservation concern because the largest male *P. adspersus* are reproductively most successful, partly because usually only they perform parental care of tadpoles, which are highly vulnerable to desiccation and predation (Cook 1996; Cook *et al.* 2001). As in other anurans, the largest female *P. adspersus* probably produce the greatest number of eggs and clutches in a season (Howard 1978; Reading & Clarke 1995; Reading 2007). Therefore efforts to reduce unnatural mortality of *P. adspersus* at threatened sites are strongly recommended. Habitat loss and road traffic generally represent the greatest threats to *P. adspersus* (Du Preez & Cook 2004). Therefore the destruction of undeveloped terrestrial habitat connected to breeding sites should be prevented (Yetman & Ferguson 2011b, [Chapter 3]), and safer movement of *P. adspersus* across roads near breeding sites should be ensured (Langton 1989; Puky 2005).

Differences in the size and condition of *P. adspersus* at different breeding sites, suggests that the species requires conservation management at site-specific, as well as broader spatial scales (Boyd *et al*. 2008). An important local concern is the poorer condition of *P. adspersus* at Bullfrog Pan, which is the largest known historical breeding site for this species in Gauteng, and perhaps South Africa. Research is warranted to determine if *P. adspersus* at Bullfrog Pan is being adversely affected by pollution or some other factor. Studies have shown that the body size and/or age of anurans can give useful indications of habitat quality. For example, SVL and age of *R. catesbeiana* was significantly lower at sites with high pesticide contamination (Spear *et al.* 2009), and age at maturity in males and longevity in females of *Bufo viridis* was negatively related to the intensity of human land use within a 1 km radius of breeding sites (Sinsch *et al.* 2007). Presuming that the spatio-temporal differences in body size and condition of the sampled *P. adspersus* reflect anthropogenic transformation of habitat, *P. adspersus* could serve as a valuable indicator of degradation of seasonal wetlands and grasslands, which are both highly threatened in South Africa (Low & Rebelo 1996; Le Roux 2002).

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Table 1. Mean ± standard deviation, and range, of the snout-vent length (SVL), body mass, body condition or age (estimated from LAG = lines of arrested growth in phalanges) of male or female *Pyxicephalus adspersus* from three peri-urban breeding sites. ANOVA F, t-, or z-test, and P values (*** $P < 0.001$) pertain to comparisons of variables for same-sex animals between the three sites (column 3), or between males and females from the three sites combined (column 4) or treated separately (columns 5, 6 and 7). Values of $P > 0.01$ were non-significant following sequential Bonferroni correction.

Column	2	3	4	5	6	7
	Se	F, P	All three sites	Diepsloot dams	Bullfrog Pan	Glen Austin Pan
SVL (mm)	♂	$F_{2, 208} = 8.5$ ***	163 ± 13 130-198 $n = 211$	160 ± 14 131-190 $n = 56$	155 ± 12 130-181 $n = 23$	165 ± 12 130-198 $n = 132$
	♀	$F_{2, 65} = 5.8$ $P = 0.004$	109 ± 9 92-136 $n = 68$	105 ± 8 93-129 $n = 30$	110 ± 12 92-136 $n = 16$	113 ± 6 100-123 $n = 22$
			$F_{1, 277} = 978.2$***	$F_{1, 84} = 376.0$***	$t = -11.6$***	$F_{1, 152} = 386.5$***
Body mass (g)	♂	$F_{2, 208} =$ 25.2 ***	512 ± 131 204-872 $n = 211$	454 ± 108 204-699 $n = 56$	405 ± 102 222-643 $n = 23$	555 ± 124 239-872 $n = 132$
	♀	$F_{2, 65} = 3.4$ $P = 0.04$	152 ± 43 90-294 $n = 68$	139 ± 33 95-233 $n = 30$	154 ± 53 90-285 $n = 16$	169 ± 42 117-294 $n = 22$
			$F_{1, 277} = 498.7$***	$F_{1, 84} = 242.1$***	$t = -9.0$***	$F_{1, 152} = 209.2$***
Body condition	♂	$F_{2, 208} =$ 30.2 ***	3.1 ± 0.6 1.5-4.4 $n = 211$	2.8 ± 0.5 1.5-3.7 $n = 56$	2.6 ± 0.5 1.7-3.7 $n = 23$	3.3 ± 0.6 1.8-4.4 $n = 132$
	♀	$F_{2, 65} = 2.5$ $P = 0.09$	1.4 ± 0.3 0.9-2.5 $n = 68$	1.3 ± 0.2 1.0-1.8 $n = 30$	1.4 ± 0.3 0.9-2.1 $n = 16$	1.5 ± 0.3 1.0-2.5 $n = 22$
			$F_{1, 277} = 504.4$***	$F_{1, 84} = 242.9$***	$t = -8.9$***	$F_{1, 152} = 218.6$***
LAG	♂	$F_{2, 42} = 1.3$ $P = 0.3$	6 ± 2 3-11 $n = 45$	6 ± 1 3-7 $n = 15$	6 ± 1 3-8 $n = 15$	7 ± 2 3-11 $n = 15$
	♀	$F_{2, 27} = 1.2$ $P = 0.3$	4 ± 1 3-7 $n = 30$	4 ± 1 3-5 $n = 10$	5 ± 2 3-7 $n = 10$	5 ± 1 3-6 $n = 10$
			$F_{1, 73} = 18.2$***	$t = -3.7$, $P = 0.001$	$t = -1.6$, $P = 0.1$	$t = -2.5$, $P = 0.02$

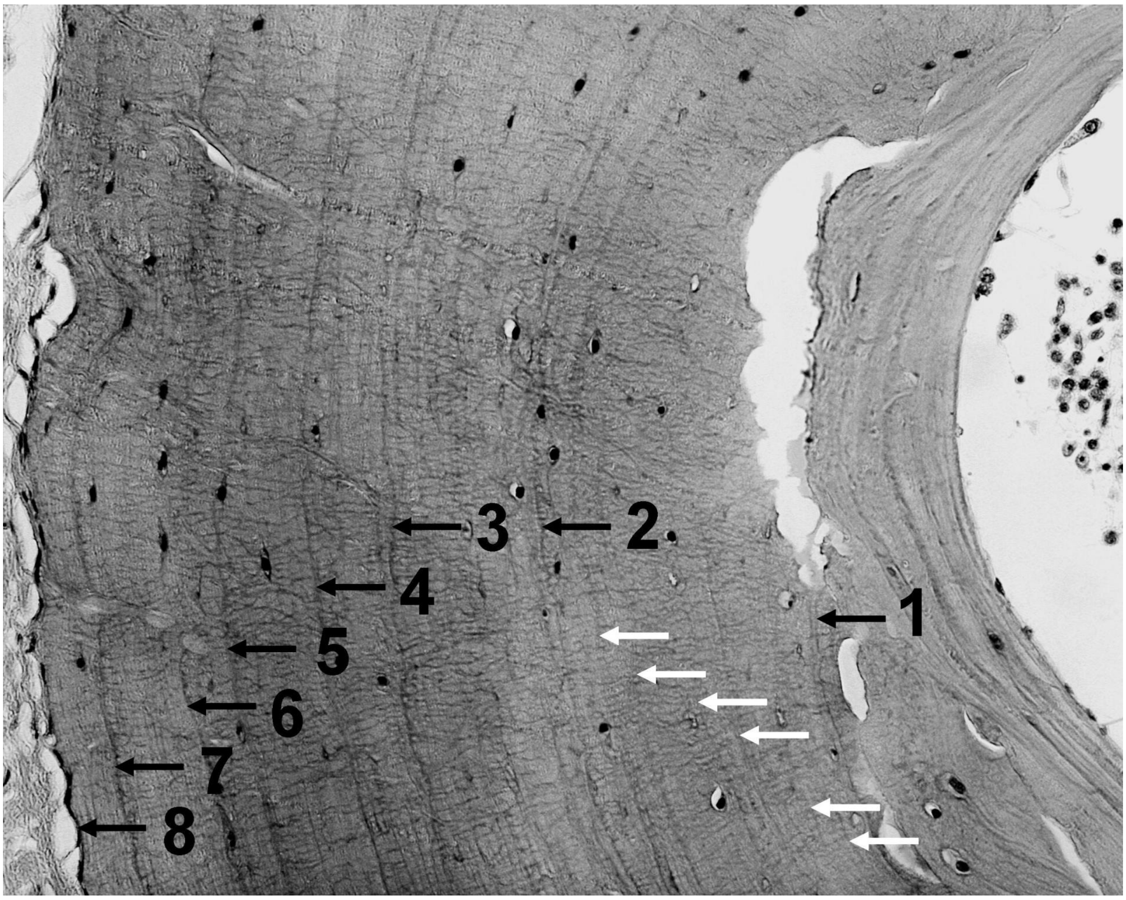

Figure 1. Black arrows indicate eight lines of arrested growth (LAG) visible in a phalangeal cross-section of an adult *Pyxicephalus adspersus*. These LAG, we assumed, were each deposited during 6-8 months of winter torpor and are numbered consecutively from the endosteal bone outwards. The first LAG has been partially resorbed. White arrows point to comparatively feint, incomplete LAG within bands of summer bone growth. These LAG were likely deposited during extended periods of rest in summer between bouts of animal activity following rainfall.

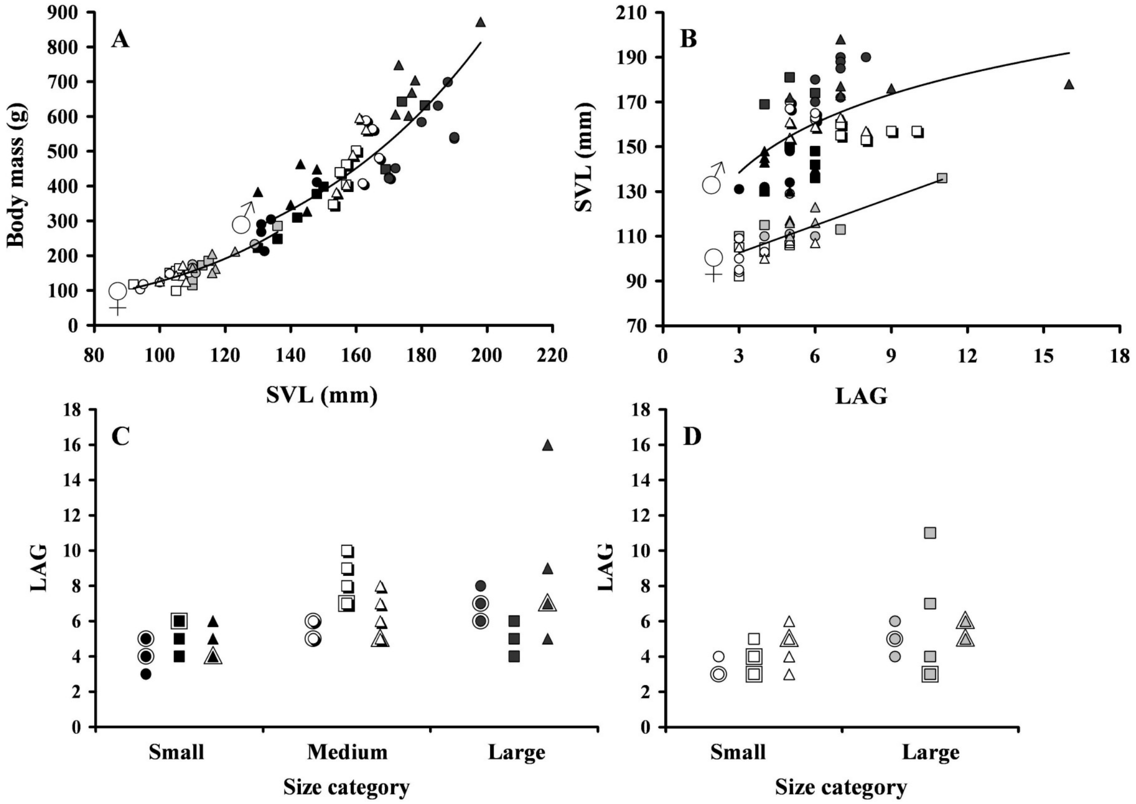

Figure 2. Scatterplots showing the relationship between A) snout-vent length (SVL) and body mass, B) the estimated age, and SVL; or the size category, and estimated age of C) male (n = 46), and D) female (n = 30) adult *Pyxicephalus adspersus* from three peri-urban breeding sites. Age was estimated as the number of lines of arrested growth (LAG) counted in cross-sections of animal phalanges. Circular, triangular, or square data points represent animals sampled at Diepsloot, Glen Austin or Bullfrog Pan, respectively. White or light grey data points represent small or large females, respectively. Black, white with a shadow, or dark grey data points represent small, medium, or large males, respectively. Data points with an extra outline represent more than one individual. Solid regression lines pertain to males (♂) or females (♀) from all three sites combined.

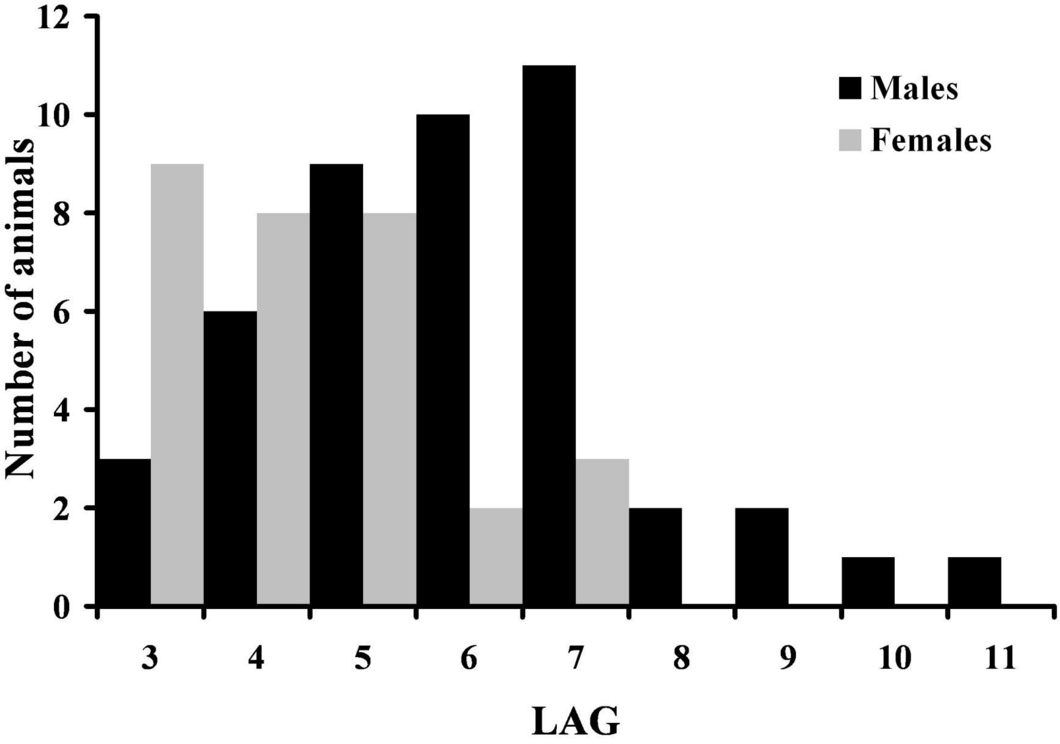

Figure 3. Frequency distribution of the estimated age of randomly selected adult male (*n* = 45) and female (*n* = 30) *Pyxicephalus adspersus* from three peri-urban breeding sites. Age was estimated as the number of annual lines of arrested growth (LAG) counted in cross-sections of animal phalanges.

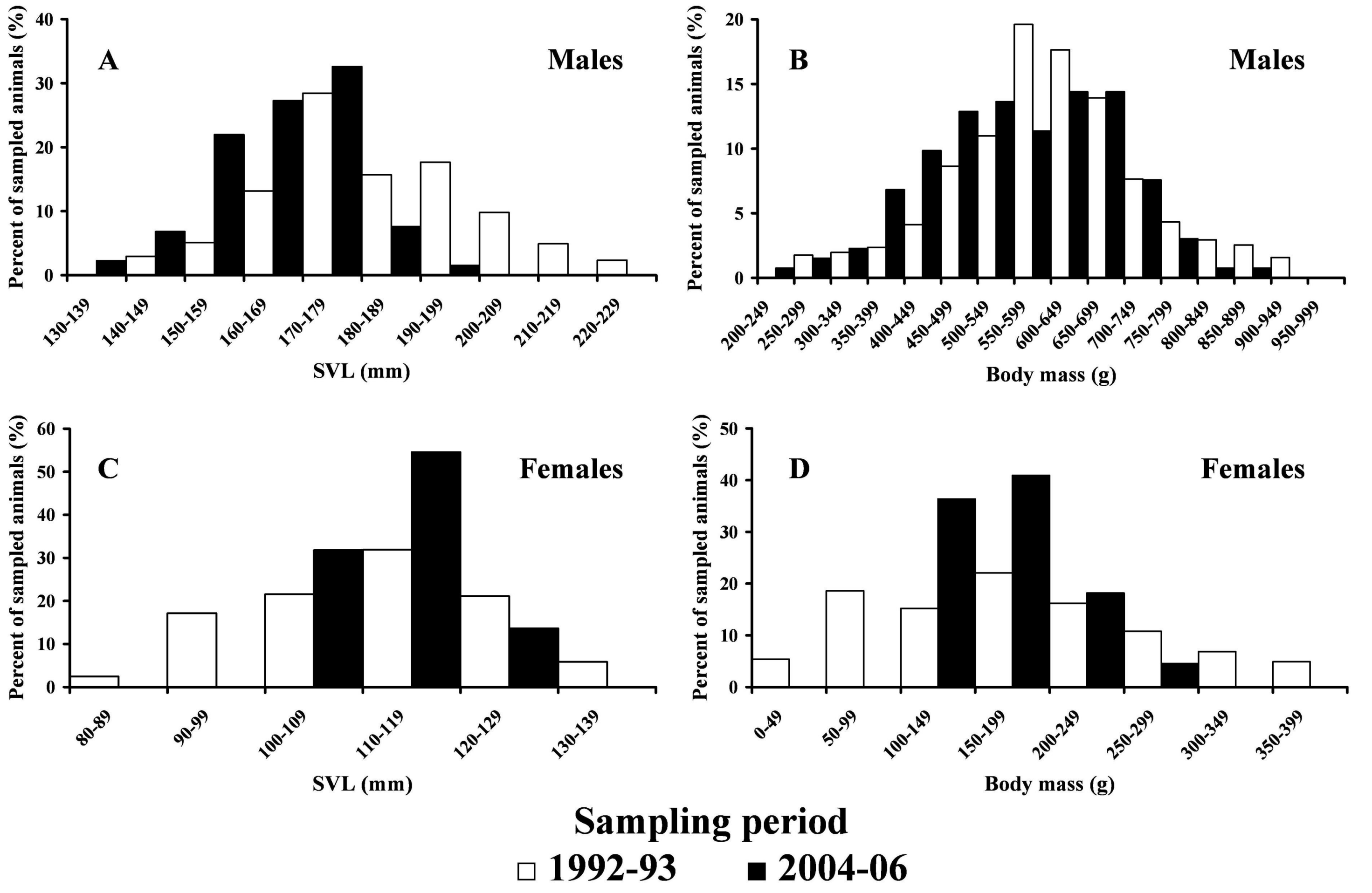

Figure 4. Frequency distribution of the snout-vent length (SVL) or body mass of male or female *Pyxicephalus adspersus* caught during spawning events at Glen Austin Pan in 1992-93 by Cook (1996) or in 2004-06 for this study.

Chapter 5

Conservation implications of giant bullfrog (*Pyxicephalus adspersus*) population genetic structure in Gauteng Province, South Africa

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Abstract.—The giant bullfrog (*Pyxicephalus adspersus*) is regarded as Near-Threatened in South Africa where many populations in Gauteng Province have been destroyed or remain increasingly threatened by habitat loss and other threats. As a first step towards identifying conservation management units for *P. adspersus*, we quantified genetic structure and gene flow for populations from 23 localities in Gauteng and seven additional localities in the north-eastern interior of South Africa, using 708 base pairs of the mitochondrial cytochrome *b* gene. Gene flow was limited between populations > 200 km apart in the north-eastern interior of South Africa, reflecting genetic differentiation at this scale (F_{ST} = 0.60). Populations in the Free State Province may represent an evolutionary significant unit of *P. adspersus*. In Gauteng, substantial gene flow between populations < 20 km apart was detected, and effective population size estimates were high. However, given recent male counts at remaining breeding sites in central Gauteng,

it appears that *P. adspersus* has declined by > 90 % in this area. The lack of correlation between genetic and geographic distance of samples suggested that the genetic differentiation in *P. adspersus* between the central, eastern and northern regions of Gauteng (F_{ST} = 0.26), and between Diepsloot, Glen Austin and Monavoni in central Gauteng (F_{ST} = 0.15) was due to genetic drift. The latter is possibly explained by reduced gene flow between *P. adspersus* populations with expansion of the Pretoria and Johannesburg metropolitan areas since the early 1900s. In northern Gauteng, where there has been considerably less human habitat transformation, no genetic differentiation between *P. adspersus* populations was found. Conservation of *P. adspersus* in South Africa's highly threatened Grassland biome is considered a priority and should involve separate protection of populations in Gauteng and the Free State Province, where *P. adspersus* is, respectively, highly threatened and genetically unique.

Introduction

Species populations lose potential to adapt to changing environmental conditions and may suffer from inbreeding depression when they become small and isolated (Reed & Frankham 2003; Willi *et al.* 2006). Inbreeding depression results because small, isolated populations lose genetic variation as the relative influence of genetic drift exceeds that of natural selection (Allendorf & Leary 1986; Rowe & Beebee 2003; Frankham 1995). The rate at which genetic variation is lost depends on a population's effective size, which is equal or less than the total number of breeding individuals. A population's effective size can be considerably smaller than its actual size if breeding individuals exhibit a skewed sex ratio or high variance in progeny production, or if population size varies greatly between generations (Lande 1993; Charlesworth 2009). It is therefore desirable to maintain high genetic variation in species by maintaining a number of large, well-connected breeding populations (Lande & Barrowclough 1987; Lande 1988).

Of the five Vertebrate classes the Amphibia has the greatest proportion (32.5%) of species that are globally threatened (Stuart *et al.* 2004; Beebee & Griffiths 2005). Relative to other vertebrates that are less moisture dependent and more vagile, amphibians are particularly vulnerable to habitat loss and climate change (Cushman 2006; Wake & Vredenburg 2008). Due to their permeable bodies and aquatic larval life stages amphibians may also be especially sensitive to environmental contamination and UV-B radiation (e.g. Blaustein *et al.* 1994; Relyea 2005; but see Crump *et al.* 1999 and Kerby *et al.* 2010). Amphibian survival and breeding are known to fluctuate greatly in response to e.g. weather variation (Seppä & Laurila 1999; Marsh 2001). Amphibian populations are susceptible to population bottlenecks and genetic drift (Lande 1993), caused by skewed sex ratios (Alho *et al*. 2008), short life expectancies (Monnet & Cherry 2002) and small effective population sizes (Funk *et al.* 1999). As a result of low genetic variation, reduced fitness has been detected in various amphibian populations (Rowe & Beebee 2003; Andersen *et al*. 2004; Pearman & Garner 2005).

The giant bullfrog (*Pyxicephalus adspersus*) is a large, aggressive anuran that breeds explosively in shallow, seasonal wetlands following heavy rainfall in summer (Balinsky & Balinsky 1954; Cook 1996). The species is widespread in southern Africa (Channing 2001; Du Preez & Carruthers 2010) but is considered to be Near-Threatened in South Africa (Minter *et al.* 2004), where estimated population declines of between 50 and 80% have been reported (Harrison *et al.* 2001). During the past two decades many *P. adspersus* populations have been destroyed or remain increasingly threatened by habitat loss and other factors in Gauteng Province (Carruthers 2007), South Africa's economic centre. Although Gauteng covers only 1.4% of the land area in South Africa, it has the highest provincial human population growth rate and is experiencing rapid urban development even outside the formal urban edge (GDACE 2004).

Unfortunately, *in situ* conservation of *P. adspersus* in Gauteng has been limited to physical protection of the populations concentrated around Glen Austin and Bullfrog pans near Johannesburg. Bullfrog Pan has a history of chemical contamination from a nearby landfill, which may explain why juvenile and adult *P. adspersus* at this site have, respectively, exhibited deformities and poor body condition (Slater-Jones 1996; Yetman *et al.*, in press, [Chapter 4]). At Glen Austin Pan there has been a significant decline in the body size of adult *P. adspersus* since 1992-93 (Yetman *et al.*, in press, [Chapter 4]). No doubt, protection of *P. adspersus* at these two breeding sites alone is unlikely to ensure the long term persistence of this species in Gauteng (Hitchings & Beebee 1998; Hamer & McDonnell 2008). With increasing isolation of these populations and loss of remaining natural habitat in Gauteng, there is an urgent need to investigate the genetic structure of remaining *P. adspersus* to evaluate the past connectedness, present viability and future conservation management of populations in this province (Nunney & Campbell 1993; Crandall *et al*. 2000; Sutherland *et al*. 2004). The objectives of this study were, therefore, to:

- quantify *P. adspersus* population genetic structure and gene flow in Gauteng Province.
- quantify *P. adspersus* population genetic structure and gene flow in other parts of South Africa, where possible.
- identify important, genetically unique *P. adspersus* populations in the study area.
- infer consequences of the results on the conservation management of *P. adspersus.*

Materials and Methods

Sampling

Sampling was performed during the 2003/2004-2005/2006 summer seasons but opportunities to encounter specimens were limited due to the unpredictable and sporadic activity of *P. adspersus* (Yetman & Ferguson 2011a, [Chapter 2]). A total of 129 samples were collected (Fig. 1), of which 107 were obtained from 23 localities in Gauteng Province. Of the remaining 22 samples we obtained nine from three localities in the Free State Province, five from three localities in Mpumalanga Province, and eight from one locality in Limpopo Province (Table 1). The mean minimum distance between all 30 localities was 32 km (range: 2-186 km). Samples constituted tail clips from tadpole (n = 7) or toe clips from froglet (n = 12) or adult (n = 110) specimens found live or dead. Adult specimens were targeted as far as possible to minimize the likelihood of sampling siblings at a site. Live specimens were handled in a manner complying with the "Guidelines for Use of Live Amphibians and Reptiles in Field Research" (Society for the Study of Amphibians and Reptiles, the American Society of Ichthyologists and Herpetologists, and The Herpetologists' League). Samples were preserved in 70-99% ethanol or frozen dry at -70º C prior to DNA extraction.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from each sample using the High Pure PCR Template Preparation Kit (Roche Diagnostics). To increase the concentration of eluted DNA, half (100 µl) of the standard volume of elution buffer was used. A 708 base pair segment of the mitochondrial gene cytochrome *b* was amplified by polymerase chain reaction (PCR) using the protocol and primers L14841 and CB3-H developed by Mausfeld *et al*. (2000) to analyze Scincid lizard populations. For amplification, 100 ng extracted DNA was added to a 50 µl reaction containing 20 pmol of each primer (Integrated DNA Technologies), 10 mM dNTPs, and 10X buffer in the presence of 1 unit of BIOTOOLS

DNA polymerase (BIOTOOLS B&M Labs). The thermo-cycling profile entered into a Perkin Elmer Gene Amp 2 400 thermocycler (Applied Biosystems) included an initial denaturing step at 96°C for 30 s, followed by 34 cycles of denaturing at 95°C for 90 s, annealing at 50°C for 60 s, and extension at 72°C for 90 s, with a final elongation step at 72°C for 50 s.

PCR product (5 µl) was combined with 2 µl loading dye, electrophoresed on a 1.5% agarose gel containing ethidium bromide or GoldView Nucleic Acid Stain (SBS Genetech), and the quality of the product visually assessed under a UV light. PCR product was purified using the High Pure PCR Product Purification Kit (Roche Diagnostics). For cycle sequencing (in the forward and reverse directions), depending on the quality of purified PCR product, 2-6 µl was added to a 10 µl reaction containing 1 µl 5X buffer, 3.2 pmol primer, 2 µl BigDye v.3.1.1 (Applied Biosystems), and 0-4 µl water. The cycle sequencing thermo-cycling profile included initial denaturation at 96°C followed by 25 cycles of denaturing at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min. Cycle sequencing product was precipitated using a 70% ethanol sodium acetate method, and sequenced on an Applied Biosystems 3 130*xl* Genetic Analyzer.

Sequence chromatograms were edited, aligned, and the forward and reverse sequences for each sample converted into one contiguous sequence (contig) using MEGA 4.0.1 (Tamura *et al.* 2007). The contigs were deposited in the GenBank database under accession numbers FJ613 658-786. Sequences for two outgroup species were obtained from GenBank for *Rana perezi* and *Rana nigromaculata* (accession numbers DQ902146 and DQ006267). (Comparative sequence data for suitable African anuran species were not available at the time).

Spatial scales of analyses

Population genetic structure and gene flow were estimated at various spatial scales as encouraged by Boyd *et al.* (2008). These scales were: i) the north-eastern interior of South Africa; ii) Gauteng Province; and iii) two local spatial scales in Gauteng (Fig. 1; Table 1). For the north-eastern interior of South Africa localities from the Free State, Gauteng plus Mpumalanga, and Limpopo provinces were separately grouped for analysis given the large (200-400 km) distances separating these groups. In Gauteng Province localities from the central, eastern and northern regions of this province (20-100 km apart) were separately grouped for analysis based on differences in climate and habitat between these regions (Table 1). While northern Gauteng is characterized by warmer savanna, the rest is characterized by cooler grassland (Mucina *et al.* 2005), and in eastern Gauteng there is an unusually high density of pans and other wetlands where *P. adspersus* may breed (GDACE 2004). Samples from Diepsloot, Glen Austin and Monavoni (10-15 km apart; Table 1; Fig. 1) were used for the local spatial scale analysis in central Gauteng, and samples from Buffelsdrif, Wallmannsthal and Hammanskraal (7-19 km apart; Table 1; Fig. 1) were used for the local spatial scale analysis in northern Gauteng (Table 1). Samples from other localities in these two sub-regions of Gauteng were too few for inclusion in these local spatial scale analyses.

Analysis methods

An unrooted haplotype network of the 129 *P. adspersus* contigs (hereon referred to as the dataset) was generated using TCS 1.21 (Clement *et al.* 2000). A neighbour-joining dendrogram based on *p*-distances among all haplotypes in the dataset plus the two outgroup species was constructed from 1 000 bootstrap replicates in MEGA 4.0.1 (Tamura *et al*. 2007).

At each spatial scale, comprising a separate analysis, haplotype frequency variation and differentiation within and among populations was quantified in Arlequin 3.11 (Excoffier *et al*. 2005) from 1 000 permutations. Since the indirect estimates of migration rate from F_{ST} involve often-unrealistic assumptions (e.g. equal-sized populations, equal rates of inter-population gene flow, etc.; Whitlock & McCauley 1999), migration rates and effective population sizes here were estimated directly using the maximum likelihood estimate (MLE) approach of Beerli & Felsenstein (1999, 2001), implemented in Migrate 3.0.3.

To increase the probability of estimating θ and M more accurately, for each spatial scale five analyses with different settings were tested. The first analysis was based strictly on the default MLE “search” settings in Migrate 3.0.3. The second analysis matched the first except that the number of short and long sampling chains was doubled to 20 and 6, respectively. The third and fourth analyses matched the second except that an adaptive heating scheme was implemented, which involved four chains with starting temperatures ranging logarithmically from 1 to 10, and 1 to 10 000, respectively. The fifth analysis matched the fourth except that the heating scheme used 10 (not four) chains. Each analysis was repeated five times using different automatically-generated seed numbers. From the 25 analyses for each spatial scale the mean value of the five most consistent estimates of each parameter was used. Effective population size was calculated from θ using a mitochondrial nucleotide mutation rate of $\mu = 15.43 \times 10^{-9}$ base substitutions per site per year as reported for amphibians and reptiles by Lynch *et al*. (2006).

A Mantel test (of 1 000 permutations) was performed in Arlequin 3.11 to establish whether sampled *P. adspersus* populations showed isolation by distance. The 30 sampled localities were split into five geographical groups including Limpopo Province, Free State Province, central Gauteng, northern Gauteng, and eastern Gauteng plus Mpumalanga Province. Geographic distance between group centroids was calculated using Hawth's Analysis Tools 3.26 (© 2002-06) in ArcMap 9.2 (© ESRI, Inc. 1999-2006). Genetic distance was calculated in Arlequin as $F_{ST}/(1 - F_{ST})$.

Results

Eighteen single-base pair (bp) polymorphisms were found in the 708 bp region of cytochrome *b* sequenced for all 129 samples. The mean base composition of the 129 *P. adspersus* contigs was A = 23.8%, C = 34.0%, G = 16.1% and T = 26.1%, with a transition/transversion ratio of 8.5.

Of the 15 haplotypes in the unrooted network (Fig. 2), 10 (67%) were defined by only one bp polymorphism. The most common haplotype among samples in the study is labelled "Haplotype 1," which was found in Gauteng, Mpumalanga and Limpopo provinces, and in all three sub-regions in Gauteng. It represents 83 (64%) of the 129 samples in the dataset. Except for haplotypes 1 and 13 the remaining 13 (87% of) haplotypes were each unique to a particular province (Fig. 2). Haplotypes 2-9, 10, 11-12 and 14-15 were respectively found exclusively in Gauteng, Mpumalanga, Limpopo and Free State Province.

The neighbour-joining dendrogram comprises two main *P. adspersus* groups hereon referred to as southern and northern (with respectively 91 and 82% bootstrap support). The southern group contains haplotype 15 (found in the Free State only) and a nested group with 55% bootstrap support containing haplotypes 14 (Free State) and 13 (Free State and central Gauteng). The northern *P. adspersus* group contains the remaining 12 haplotypes, represented by samples collected in Gauteng, Mpumalanga and Limpopo provinces. Haplotypes 11 and 12 (both found in Limpopo only) formed a nested group with 84% bootstrap support. No other haplotypes in the dataset grouped with more than 50% bootstrap consensus.

Haplotype frequency distributions, and results obtained from the AMOVA and Migrate analyses performed for each spatial scale are shown in Figures 4a-d. In the north-eastern interior of South Africa (Fig. 4a) between-province haplotype frequency variation contributed 45.2% of the total haplotype variance. Between-province genetic differentiation was significant (AMOVA $F_{ST} = 0.60$, $P < 0.001$), and estimates of effective population size (which ranged between 177 000 and 1 400 000 animals) were indicative of large populations. Estimates of gene flow were below one migrant per generation between the three provincial groups except from Gauteng-Mpumalanga to Limpopo ($N_{E}m$ ~ 5 migrants/generation).

In Gauteng Province, 73.5% of the total amount of haplotype frequency variation measured was estimated within sampled localities and 28.0% among localities (Fig. 4b). Genetic differentiation was significant at this scale (AMOVA $F_{ST} = 0.26$, $P < 0.001$), and estimates of effective population size (which ranged between 164,000 and 584,000 animals) indicated large populations for the central, northern and eastern regions of Gauteng. Between these regions moderate rates of gene flow were estimated ($1 < N_{E}m < 150$ migrants/generation mostly).

At the two local spatial scales in Gauteng (Figures 4c and d) 85-100% of the total amount of haplotype frequency variation measured was attributable to variation within the three selected localities. Genetic differentiation was significant between Diepsloot, Glen Austin and Monavoni (AMOVA $F_{ST} = 0.15$, $P < 0.001$) but non-existent between Buffelsdrif, Hammanskraal and Wallmannsthal ($F_{ST} = -0.03$, $P > 0.05$). For the three localities in the central region of Gauteng large effective population size estimates (between 61 000 and 455 000 animals) and moderate rates of gene flow ($8 < N_{E}m < 565$ migrants/generation) were found. For the three localities in the northern region of Gauteng large effective population size estimates (around roughly 250 000 animals) and high rates of gene flow ($585 < N_{E}m < 62\ 159$ migrants/generation) were estimated.

The Mantel test revealed a non-significant relationship ($r = 0.002$, $P = 0.13$) between genetic and geographic distance calculated for the five geographical groups of samples *viz.* Limpopo Province, Free State Province, central Gauteng, northern Gauteng, and eastern Gauteng plus Mpumalanga Province.

Discussion

Population genetic structure in South Africa's Gauteng Province

Effective population size and gene flow estimates (Fig. 4b) indicated that in South Africa's Gauteng Province, *P. adspersus* has been abundant, and populations 20-100 km apart were connected by significant gene flow. In central and northern Gauteng there was substantial movement of *P. adspersus* among localities < 20 km apart (Fig. 4c and d). In contrast, studies on other anurans have generally reported effective population sizes of < 500 individuals (Merrell 1968; Easteal 1985; Berven & Grudzien 1990; Rowe & Beebee 2004), and gene flow of < 10 migrants per generation (Morjan & Rieseberg 2004; Funk *et al.* 2005). Populations are effectively panmictic when gene flow is > 4 migrants/generation (Hartl & Clark 1997 cited in Morjan & Rieseberg 2004). Therefore, the effective size estimates in Figures 4c and d must pertain to *P. adspersus* from larger, overlapping geographical areas encompassing the sampled localities. Among these localities *P. adspersus* would be expected to exhibit little genetic differentiation (Caughley 1994; Frankham 1996; Kirkpatrick & Barton 1997).

Indeed, within the three localities in northern Gauteng, between-population differences did not contribute in a measurable way (-3.1%) to the overall genetic variation (Fig. 4d). However, between the three localities in central Gauteng there was significant genetic differentiation (F_{ST} = 0.15; Fig. 4c). We suggest that since no relationship between the genetic and geographic distance of samples was found in this study (Wright 1943; Slatkin 1993; Hutchinson & Templeton 1999), the genetic differentiation between *P. adspersus* populations in central Gauteng may be due to genetic drift caused by a reduction in gene flow with expansion of the Pretoria and Johannesburg metropolitan cities since the early 1900s. This may also explain the genetic dissimilarity between samples from northern versus central and east Gauteng (F_{ST} = 0.26; Fig. 2, 4b). In northern Gauteng, where there has been considerably less human transformation of habitat (GDACE 2004; [Chapter 6]), gene flow estimates were especially high, and genetic differentiation was insignificant between populations (Fig. 4d) compared to central and east Gauteng (Fig. 4b and c). Alternatively, the lack of genetic structure among populations in northern Gauteng may reflect a range expansion of *P. adspersus* into this area following a population bottleneck, range contraction or colonization event (Wade & McCauley 1988; Kirkpatrick & Barton 1997; Luikart *et al.* 1998; Leberg 2002).

Genetic structure in the north-eastern interior of South Africa

The total number of haplotypes found in this study for *P. adspersus* was similar to that reported for other anurans sampled at large spatial scales (Monsen & Blouin 2003; Wilson *et al.* 2008). Although few samples were collected outside Gauteng, the confidence intervals around the large effective size estimates in the Free State and Limpopo provinces (Fig. 4a) indicate that it would be reliable to conclude that *P. adspersus* has been common across the north-eastern interior of South Africa.

Unlike at the smaller spatial scales studied, inter-provincial *P. adspersus* gene flow estimates were almost negligible except from Gauteng-Mpumalanga to Limpopo (Fig. 4a). This probably explains why 87% of haplotypes in this study were unique to a particular province (Fig. 2), and genetic differentiation between Free State, Limpopo and Gauteng-Mpumalanga was significant (F_{ST} = 0.60). For a large anuran with good dispersal ability, high gene flow between local populations, and non-specific non-breeding habitat requirements, the level of genetic differentiation found among *P. adspersus* populations in the north-eastern interior of South Africa is exceptional (compared with e.g. Rowe *et al.* 1999; Squire & Newman 2002; Garner *et al.* 2004; Telles *et al.* 2006; Wilson *et al.* 2008).

Evolutionary significant units

Pyxicephalus adspersus in the Free State may represent an evolutionary significant unit (ESU) of this species in South Africa as limited data support the three main criteria to distinguish ESUs, *viz.*: long term reproductive isolation (Waples 1991); ecological distinctness or adaptive uniqueness (Waples 1991, Dizon *et al.* 1992); and reciprocal monophyly (Moritz 1994; Kizirian & Donnelly 2004). First, the near-zero gene flow estimates suggest reproductive isolation of *P. adspersus* between the Free State and Gauteng-Mpumalanga (Fig. 4a). Second, in this study *P. adspersus* was genetically most different in the Free State (Fig. 2, 3), where adult specimens are significantly smaller (SVL: 70-133 mm, Channing *et al.* 1994) and apparently produce a slightly different call (Louis Du Preez, pers. comm.) compared to those in Gauteng (SVL: 95-184 mm; Cook 1996). Third, haplotypes 14 and 15 (found in the Free State only) are reciprocally monophyletic to haplotypes 2-10 (from Gauteng-Mpumalanga; Fig. 2). ESUs have been similarly identified for other anurans (Shaffer *et al.* 2000; Monsen & Blouin 2003; Allentoft *et al*. 2008).

Conservation and research recommendations

Pyxicephalus adspersus has declined sharply at least in central Gauteng. During the 2003/2004-2005/2006 summer seasons, maxima of ~ 1 000 and ~ 500 males, respectively, were counted at Glen Austin Pan (Yetman, unpubl. data) and Diepsloot (Yetman & Ferguson 2011a), which appeared to be the two largest *P. adspersus* breeding populations remaining in central Gauteng. The effective sizes of these populations would have been smaller, however, since the sex ratio of spawning *P. adspersus* is always skewed towards males (Channing *et al.* 1994; Cook 1996). There is apparent contradiction between, on the one hand, the substantial population sizes suggested by genetic variation within Gauteng (Fig. 4b) and, on the other hand, the modest number of bullfrogs encountered at major known breeding sites in this area. However, current mitochondrial genetic variation reflects population events over the last number of decades and does not necessarily reflect population sizes at this moment (Swart *et al.* 1994). The genetics-based population size estimates in conjunction with the relatively modest contemporary counts of frogs are therefore consistent with the claim of Harrison *et al.* (2001) and Cook (2002) that in Gauteng, especially, some *P. adspersus* populations had declined by 50-80% near the end of the 1990s.

Since *Pyxicephalus adspersus* populations are highly threatened in Gauteng and genetically more unique in the Free State, their conservation in both of these South African provinces is considered a priority. Given the lack of gene flow found between the Free State, Gauteng-Mpumalanga and Limpopo, and to prevent disrupting any adaptive evolution of *P. adspersus* living in distinctly different habitats (Crandall *et al.* 2000; Allendorf & Luikart 2007 cited by Wilson *et al.* 2008), we recommend that there should be no artificial movement of live specimens over distances > 100 km or between different biomes.

Within a biome ideally ≥ 2 breeding populations of *P. adspersus* should be protected, and where possible, connected by natural gene flow to avoid reduction in population fitness, persistence and adaptive potential (Cushman 2006; Willi *et al.* 2006). Conservation of a *P. adspersus* meta-population in South Africa's highly threatened Grassland biome (Low & Rebelo 1996) is considered a priority (Cook 2002). Since natural gene flow between some populations may not be possible, translocation of *P. adspersus* could be necessary (Wilson *et al.* 2008) and therefore warrants research (Germano & Bishop 2009). However, without sufficient suitable habitat, translocation of *P. adspersus* will be of limited benefit (Denton *et al*. 1997; Fischer & Lindenmayer 2000; Bouzat *et al*. 2009). Habitat conservation should, therefore, be a conservation priority for *P. adspersus*.

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Table 1 Number of sampled localities and specimens (# localities: # specimens) included in analyses of genetic structure and gene flow at different spatial scales (corresponding with the table rows) for populations of *Pyxicephalus adspersus* in the north-eastern interior of South Africa (NE SA). South African province or Gauteng sub-region or suburb names, respectively, are shown in bold or italic text, or as single capital letters where D = Diepsloot, G = Glen Austin, M = Monavoni, B = Buffelsdrif, H = Hammanskraal and W = Wallmannsthal.

NE SA	**Limpopo**	**Free State**	**Mpumalanga**	**Gauteng**								
	1:8	3:9	3:5	23:107								
Gauteng				*East*	*Central*				*North*			
				5:7	9:69				9:31			
Local					D	G	M	Other	B	H	W	Other
					1:20	1:20	1:15	6:14	1:8	1:8	1:5	6:10

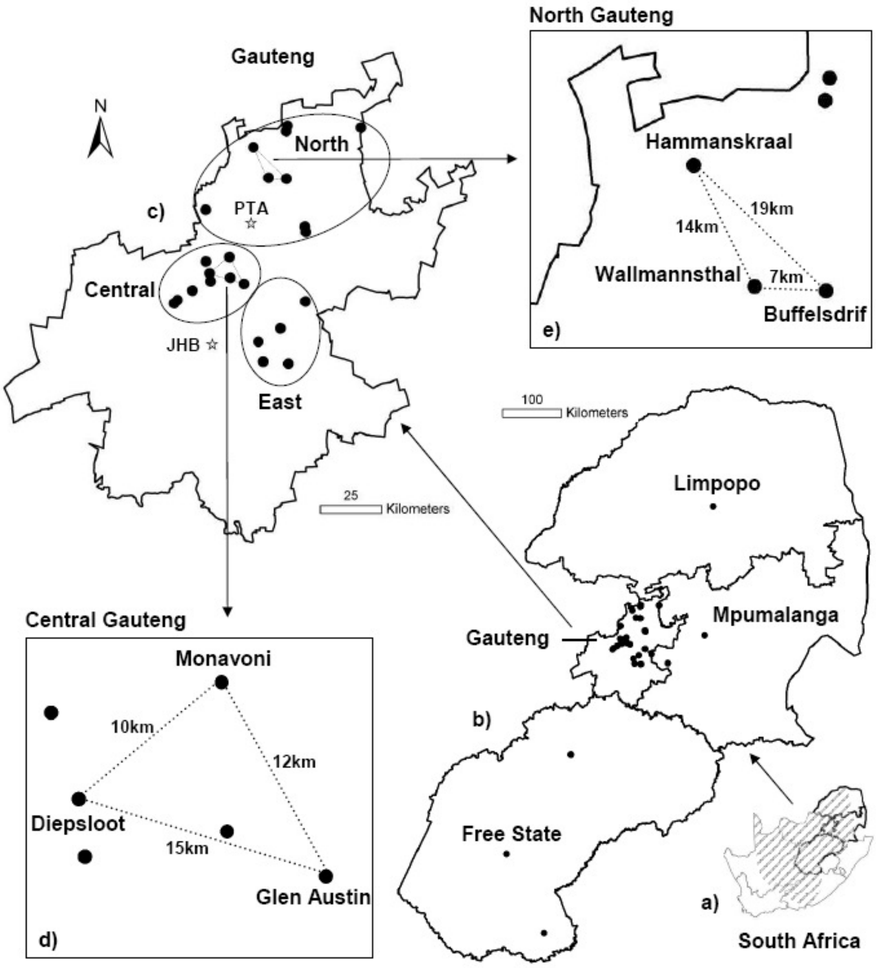


Figure 1. 30 localities (●) where 129 *Pyxicephalus adspersus* samples were obtained from four provinces shown in a) with bold outlining, in the north-eastern interior of South Africa, and used to investigate genetic structure and gene flow at different spatial scales, i.e. between: b) Free State, Gauteng-Mpumalanga and Limpopo provinces; c) the central, east and northern regions of Gauteng Province; and between three nearby localities in the d) central and e) northern region of Gauteng. In a) the hatching represents the geographic distribution of *P. adspersus* in South Africa. In d) and e) approximate straight-line distances between the three selected localities are shown in km. PTA = Pretoria and JHB = Johannesburg metropolitan city centres.

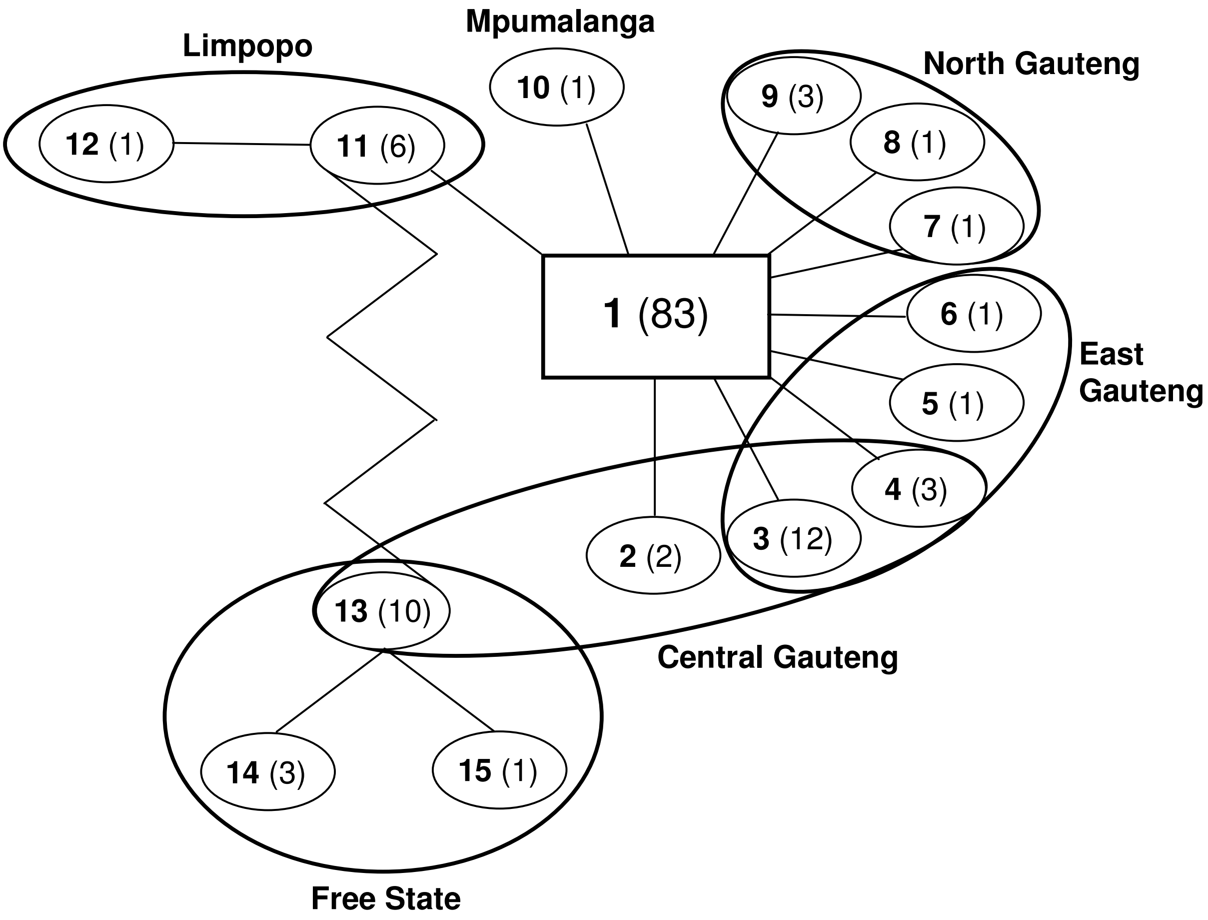


Figure 2. Unrooted haplotype network for 15 haplotypes (numbered 1-15 in bold) found in a 708 base pair segment of cytochrome *b* sequenced for 107 *Pyxicephalus adspersus* sampled from 23 localities in Gauteng Province, and 22 *P. adspersus* sampled from seven additional localities in the north-eastern interior of South Africa. The sample size for each haplotype is shown in parentheses. Haplotype 1 was present in Limpopo, Mpumalanga, (north, east and central) Gauteng, but not Free State Province. Each line segment represents one base pair difference. The bold ellipses and text indicate the geographic source of samples that represent the different haplotypes.

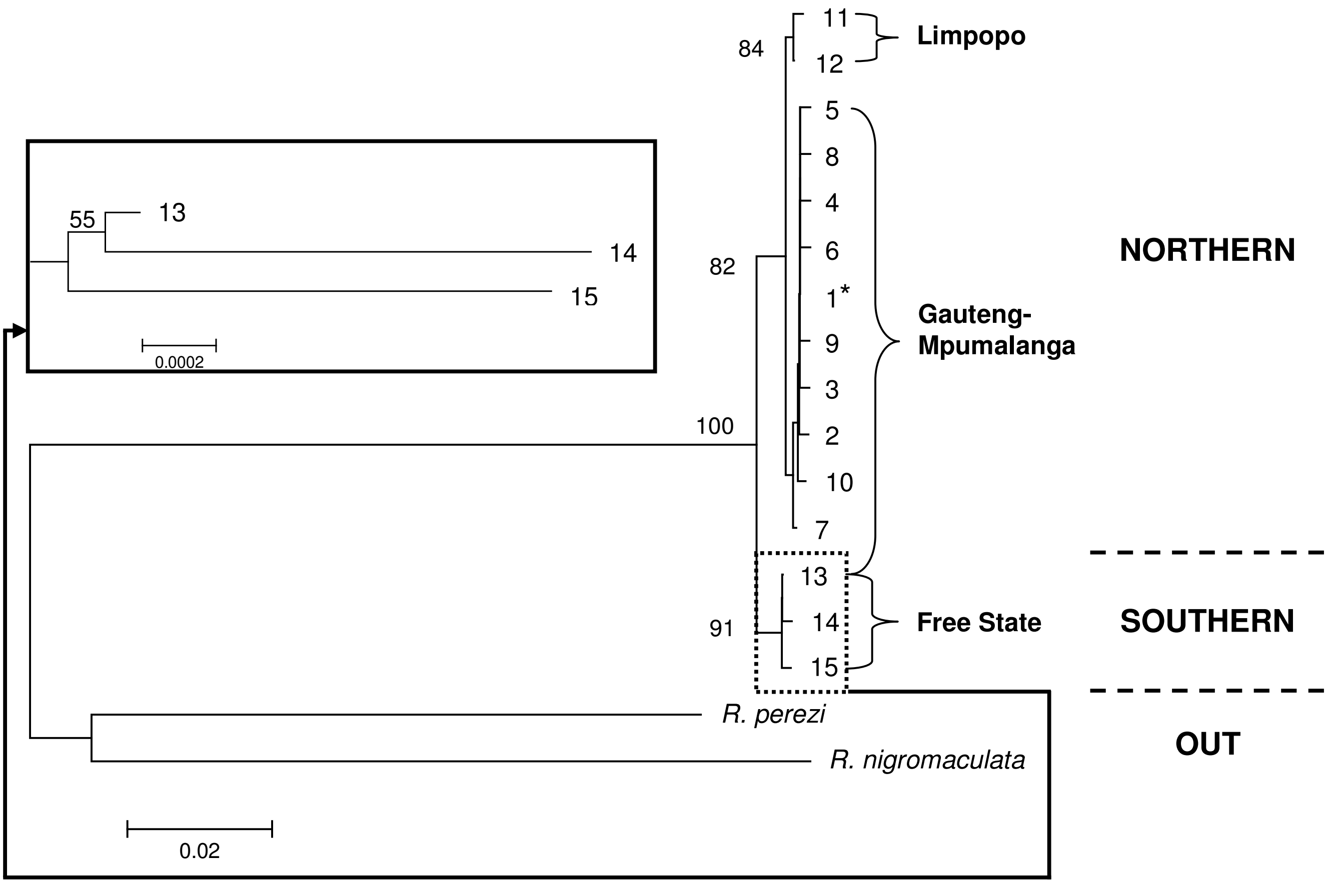
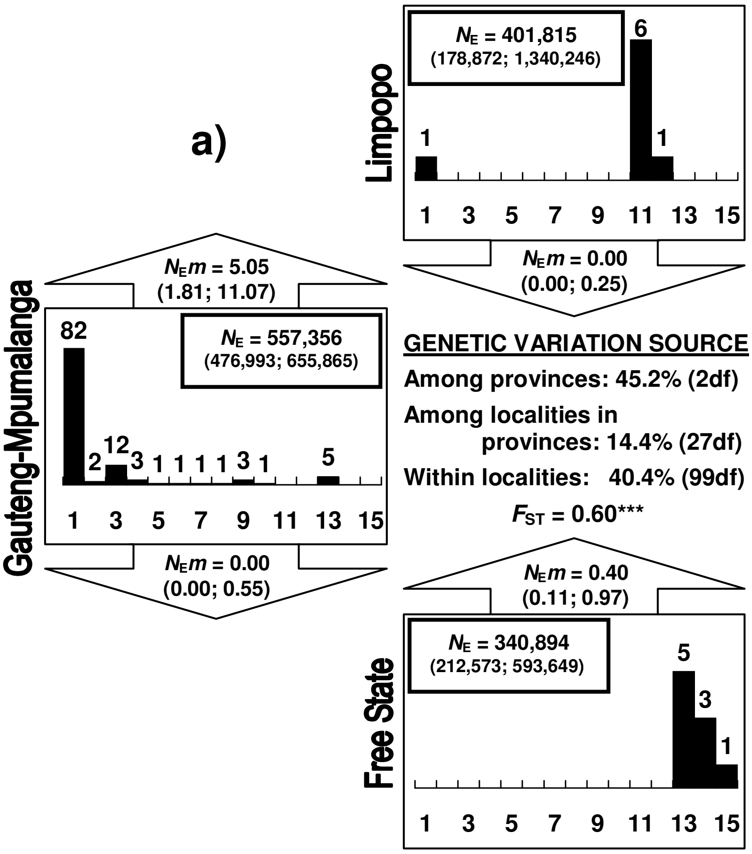


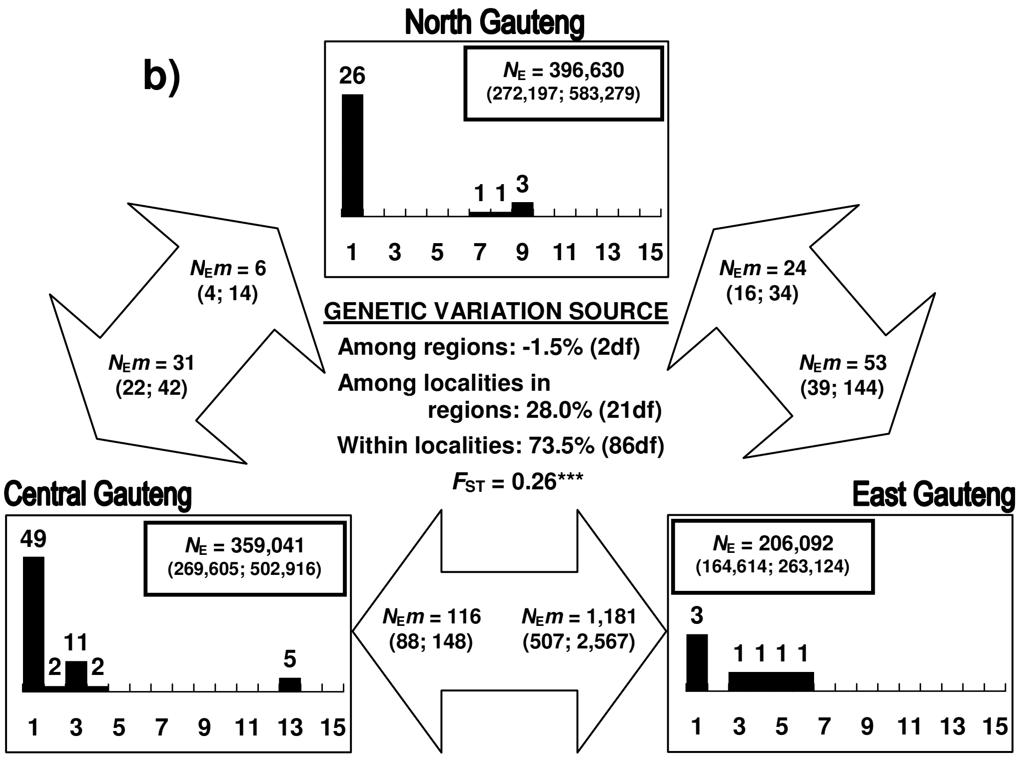
Figure 3. Neighbor-joining dendrogram for 15 haplotypes (numbered 1-15 at branch tips) found in a 708 base pair region of cytochrome *b* sequenced for 107 *Pyxicephalus adspersus* sampled from 23 localities in Gauteng Province, and 22 *P. adspersus* sampled from seven additional localities in the north-eastern interior of South Africa. Branch lengths correspond with the *p*-distance (see scale bar) among haplotypes. Bootstrap values (greater than 50%) from 1 000 iterations are shown to the left of branch nodes. * denotes that haplotype 1 was represented by samples collected in Limpopo, Mpumalanga, Gauteng but not the Free State Province. *Rana perezi* and *R. nigromaculata* were used as an outgroup. Broken lines distinguish the outgroup and two main haplotype groups labelled southern and northern (reflecting the relative latitude of sample localities).

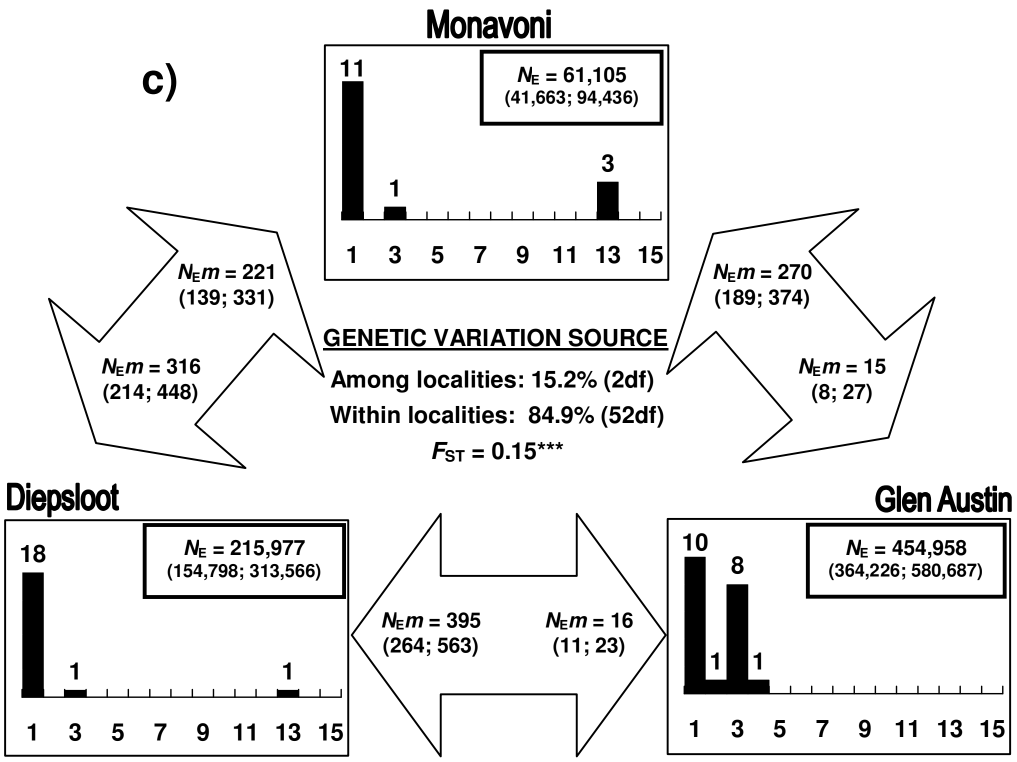
Figures 4a-d Estimates of genetic structure and gene flow for populations of *Pyxicephalus adspersus* sampled a) in the north-eastern interior of South Africa, b) in Gauteng Province only; and at a local spatial scale in the c) central or d) northern region of Gauteng. At each spatial scale a-d) each of three populations is represented by its name and a graph. The 15 haplotypes found among the study's 129 samples are numbered 1-15 on the x-axis of each histogram. The number of samples of a specific haplotype from a population appears as a numbered graph bar. Results of an AMOVA performed for each spatial scale are centred in a-d). Here, contributing sources to the total amount of haplotype frequency variation measured are listed together with their percentage contribution (with degrees of freedom in parentheses). This is followed by the degree of genetic differentiation (F_{ST}) measured at that scale (*** $P < 0.001$). The inset in each population graph contains mean values of respectively the estimated effective size (N_E), and the 0.05 and 0.95 percentiles thereof (in parentheses), for the population. Arrows in a-d) reflect the direction of gene flow between populations. Arrow heads show mean values of the estimated number of migrants per generation ($N_E m$) and the 0.05 and 0.95 percentiles thereof (in parentheses).

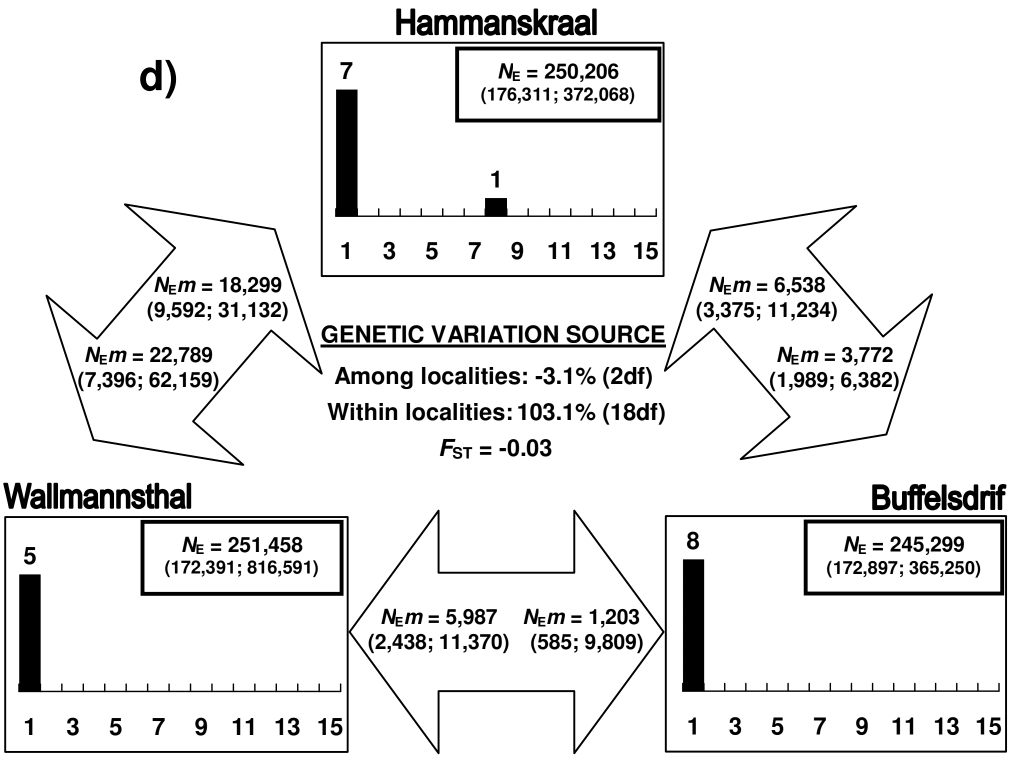
a)


Limpopo
N_E = 401,815
(178,872; 1,340,246)
6
1
1
1 3 5 7 9 11 13 15
N_Em = 0.00
(0.00; 0.25)
N_Em = 5.05
(1.81; 11.07)
Gauteng-Mpumalanga
N_E = 557,356
(476,993; 655,865)
82
2 12 3 1 1 1 1 3 1
5
1 3 5 7 9 11 13 15
N_Em = 0.00
(0.00; 0.55)
GENETIC VARIATION SOURCE
Among provinces: 45.2% (2df)
Among localities in provinces: 14.4% (27df)
Within localities: 40.4% (99df)
F_{ST} = 0.60***
N_Em = 0.40
(0.11; 0.97)
Free State
N_E = 340,894
(212,573; 593,649)
5
3
1
1 3 5 7 9 11 13 15

b)


North Gauteng
26
N_E = 396,630
(272,197; 583,279)
1 1 3
1 3 5 7 9 11 13 15
N_Em = 6
(4; 14)
N_Em = 31
(22; 42)
N_Em = 24
(16; 34)
N_Em = 53
(39; 144)
GENETIC VARIATION SOURCE
Among regions: -1.5% (2df)
Among localities in regions: 28.0% (21df)
Within localities: 73.5% (86df)
F_{ST} = 0.26***
Central Gauteng
49
N_E = 359,041
(269,605; 502,916)
11
2 2
5
1 3 5 7 9 11 13 15
N_Em = 116
(88; 148)
N_Em = 1,181
(507; 2,567)
East Gauteng
N_E = 206,092
(164,614; 263,124)
3
1 1 1 1
1 3 5 7 9 11 13 15


c)
Monavoni
NE = 61,105
(41,663; 94,436)
11
1
3
1 3 5 7 9 11 13 15
NEm = 221
(139; 331)
NEm = 316
(214; 448)
NEm = 270
(189; 374)
NEm = 15
(8; 27)
GENETIC VARIATION SOURCE
Among localities: 15.2% (2df)
Within localities: 84.9% (52df)
FST = 0.15***
Diepsloot
NE = 215,977
(154,798; 313,566)
18
1
1
1 3 5 7 9 11 13 15
NEm = 395
(264; 563)
NEm = 16
(11; 23)
Glen Austin
NE = 454,958
(364,226; 580,687)
10
1
8
1
1 3 5 7 9 11 13 15


d)
Hammanskraal
NE = 250,206
(176,311; 372,068)
7
1
1 3 5 7 9 11 13 15
NEm = 18,299
(9,592; 31,132)
NEm = 22,789
(7,396; 62,159)
NEm = 6,538
(3,375; 11,234)
NEm = 3,772
(1,989; 6,382)
GENETIC VARIATION SOURCE
Among localities: -3.1% (2df)
Within localities: 103.1% (18df)
FST = -0.03
Wallmannsthal
NE = 251,458
(172,391; 816,591)
5
1 3 5 7 9 11 13 15
NEm = 5,987
(2,438; 11,370)
NEm = 1,203
(585; 9,809)
Buffelsdrif
NE = 245,299
(172,897; 365,250)
8
1 3 5 7 9 11 13 15

Chapter 6

Conservation implications of habitat preference and geographic range of the giant bullfrog (*Pyxicephalus adspersus*) at two spatial scales

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Abstract.—The geographic range of the giant bullfrog (*Pyxicephalus adspersus*) has been difficult to accurately assess due to the sporadic activity and superficial morphological similarity of species in this genus. *P. adspersus* appears to be widespread in southern Africa but is Near-Threatened in South Africa where populations in Gauteng Province are highly threatened by habitat loss. In this study we created a model in MaxEnt to predict the potential geographic range of *P. adspersus* in southern Africa, and used recent land cover data to determine the remaining availability of suitable habitat for this species in South Africa's Gauteng Province. The model was trained using records of *P. adspersus* that were accompanied by DNA sequence data, photographed or preserved specimens, or a recording of male advertisement calling. Three interpolated bioclimatic variables were used as environmental predictors, and predicted suitable conditions were projected over Swaziland, Lesotho, Botswana and Mozambique, where no reliable

records of *P. adspersus* were available. Predicted suitable conditions for *P. adspersus* included the temperate to semi-arid interior, but excluded the low-lying eastern subtropical and arid western sides of southern Africa. We encourage field surveys and phylogeographic research to validate our model prediction, which suggests that *P. adspersus* has a slightly smaller geographic range in southern Africa than has generally been reported. In Mozambique, *P. edulis* has likely been misidentified as "*P. adspersus*." In South Africa's Gauteng Province, the largest remaining patches of suitable habitat for *P. adspersus*, which included natural grassland, savanna and wetlands, were found in the north, north-east, west, south-west and south-eastern provincial regions. In the Savanna biome in northern Gauteng, conservation of a *P. adspersus* meta-population could possibly be achieved. Elsewhere in Gauteng successful dispersal of *P. adspersus* between grassland populations of this species seems unlikely. Long term persistence of these populations may therefore require translocation.

Introduction

The giant bullfrog (*Pyxicephalus adspersus*) is a large (up to 1 kg), aggressive and elusive anuran. Individuals spend most of the year in a state of torpor underground, but emerge during summer after heavy rain to breed and forage. Breeding occurs explosively in shallow, seasonal wetlands, and males fight fiercely over favoured oviposition sites and females (Cook 1996). During a six-month summer period most adults cumulatively spend a few days at breeding sites, and 2-3 weeks foraging near their burrows at night (Yetman & Ferguson 2011a, [Chapter 2]). Adults excavate burrows up to 1 km away from a breeding site, and are highly faithful to both their burrows and breeding site (Yetman & Ferguson 2011b, [Chapter 3]). Substantial gene flow, however, has been detected between localities up to 20 km apart (Yetman & Ferguson, unpubl. data, [Chapter 5]), suggesting that juveniles facilitate gene flow by dispersing large distances from their natal site during the ≥ 3 year period prior to their maturation (Yetman *et al.*, in press, [Chapter 4]).

Pyxicephalus adspersus is not considered threatened on a global scale (Measey 2011) because it reportedly occurs across most of southern and in parts of east Africa (Channing 2001). However, there are few reliable records of *P. adspersus* from countries other than South Africa, and discrepancies exist between distribution maps compiled by different authors for various geographic regions (e.g., Lambiris 1988; Channing 2001; Minter *et al.* 2004; Du Preez & Carruthers 2009; Clauss & Clauss 2002). This is possibly explained by the sporadic activity (Yetman & Ferguson 2011a, [Chapter 2]) and infrequent detection of *P. adspersus* in remote areas, and the superficial morphological (Poynton & Broadley

1985; Haagner 1990) and behavioural (Cook & Minter 2004; Braack & Maguire 2005) similarity of metamorphosed *P. adspersus* and *P. edulis*. Although these two species can be readily distinguished using the advertisement call of males, tadpole morphology (Du Preez & Carruthers 2009) or certain genetic markers (Yetman, unpubl. data), voucher material accompanying most records of *Pyxicephalus* is represented by preserved or photographed adult or juvenile specimens (as in e.g., Minter *et al*. 2004, and this study). *P. adspersus* and *P. edulis* juveniles are difficult to identify because they do not exhibit a distinct morphological difference, and adults of the two species exhibit overlapping body size and skin colour variation. Nevertheless, using the additional morphological differences described in Table 1, most adult *P. adspersus* and *P. edulis* can be successfully distinguished. Using these characters to re-examine *Pyxicephalus* voucher material, a significant number of mis-identified specimens were uncovered in museums and in the literature (e.g. Haagner 1990; Channing *et al*. 1994; Minter *et al*. 2004). Hence the geographic range of *P. adspersus* should be reassessed.

Species distribution models (SDMs) exploit relationships between species' occurrence records and environmental conditions at record localities (Franklin 2009), and have been applied to a large diversity of taxa to investigate, among other things, their potential geographic ranges (for a review see Elith & Leathwick 2009). The reliability of a SDM is influenced by the choice of model, environmental predictors, data resolution, and the extent of extrapolation (Elith & Leathwick 2009). Studies on amphibians have involved SDMs to investigate e.g., geographical variation in morphology (Schäuble 2000), environmental limits (Penman *et al*. 2005), and the potential geographic range of native or invading taxa under different climate or habitat conditions (Negga 2007; Elith *et al.* 2010). MaxEnt (Phillips *et al.* 2006; Phillips & Dudik 2008) is a popular SDM for predicting the geographic range of species when only presence data are available (Elith *et al.* 2011). Statistically speaking this method minimizes the relative entropy between the estimated probability densities of the species presence and background environmental data in co-variate space (Phillips *et al.* 2006; Elith *et al.* 2011). MaxEnt compares favourably to alternative approaches (Elith *et al.* 2006; Wisz *et al.* 2008) and is useful even when very few species records are available (Jackson & Robertson 2011), or extrapolation in space and/or time is necessary (Elith *et al.* 2010). Therefore, as the main objective of this study, we wanted to predict the potential geographic range of *P. adspersus* in southern Africa using MaxEnt.

Since 2001 *P. adspersus* has been considered to be extinct in Swaziland (Boycott 2001) and Near-Threatened in South Africa, where estimated population declines of between 50 and 80% have been reported due to habitat loss, road traffic and harvesting for human consumption (Harrison *et al.* 2001; Minter *et al.* 2004). *Pyxicephalus adspersus* seems to be especially threatened in Gauteng Province, the economic centre of South Africa. Gauteng covers only 1.4% of the land in South Africa (Fig. 1), but has the highest provincial human population growth rate, and is experiencing rapid "urban sprawl" (i.e. urban development outside the delineated urban edge; GDACE 2004). More than 17% of the land is urban, > 96% of wetlands are threatened, < 4% of vegetation types are conserved (GDACE 2004), and many *P. adspersus* breeding populations have been destroyed or are highly threatened, in Gauteng (Carruthers 2007). Effective population size estimates from a genetic study (Yetman & Ferguson, unpubl. data, [Chapter 5]) indicate that *P. adspersus* was widespread and abundant in Gauteng, but counts of breeding adults indicate that populations may have declined by > 90% in the central region of this province. Of > 40 formally protected areas in Gauteng (including six provincial and 24 municipal reserves), only three (i.e. Abe Bailey, Diepsloot, and Leeuwfontein Collaborative nature reserves) are known to support *P. adspersus* breeding (Yetman, unpubl. data). Bullfrog and Glen Austin pans near Johannesburg represent two of the last large, natural breeding sites for *P. adspersus* in Gauteng, and both sites are highly threatened by encroaching urbanization (Yetman *et al.*, in press, [Chapter 4]). Therefore, as a second objective of this study, using recent high-resolution land cover data we wanted to determine the remaining availability of suitable habitat for *P. adspersus* in Gauteng. Following from this, as a third objective, we wanted to examine whether recent occurrence records for *P. adspersus* in Gauteng coincided with the remaining availability of habitat that we classified as suitable for this species in this province. Finally, we inferred consequences of the results on the conservation management of *P. adspersus.*

Materials and Methods

Occurrence data

We obtained occurrence records for *P. adspersus* from museum specimens, the South African Frog Atlas Project (SAFAP; Minter *et al.* 2004), the Gauteng Department of Agriculture and Rural Development (GDARD), herpetologists, peer-reviewed journal articles, grey literature, and our own field surveys. We also obtained data through a citizen science programme in which we encouraged members of the public to submit information with photographs of *Pyxicephalus* sightings via a website (www.giantbullfrog.org), which was advertised on South African television and radio during the 2004/2005 and 2005/2006 summer seasons. Records from the different sources were combined into a single dataset, and where necessary, records were geo-referenced using Google Earth (http://earth.google.com) or a gazetteer (e.g., http://earth-info.nga.mil).

Records that were accompanied by DNA sequence data, filmed, photographed or preserved specimens, or a recording of male advertisement calling, are hereafter referred to as the "voucher records." Specimens for which a 708 base pair segment of the mitochondrial gene cytochrome b had been sequenced were distinguished as *P. adspersus* or *P. edulis* based on several consistent differences in this gene between the two species (Yetman, unpubl. data). We used as many of the morphological characters as possible in Table 1, obtained from the literature (Parry 1982; Poynton & Broadley 1985; Lambiris 1988; Carruthers 2001; Channing 2001), to identify adult *Pyxicephalus* specimens that were filmed or photographed, or preserved at the Ditsong, Iziko, or Port Elizabeth museums in South Africa, or the National Museum in Zimbabwe. Preserved adult *Pyxicephalus* at other international museums (listed in the Acknowledgements) were only recognized as *P. adspersus* when measurements taken by a curator revealed that the distance between the eye and tympanum exceeded the diameter of the tympanum (Table 1; Carruthers 2001). Filmed, photographed or preserved larval or juvenile *Pyxicephalus* were only regarded as *P. adspersus* when records originated from a part of southern Africa (including Namibia except the Caprivi strip, and the Northern, Western and Eastern Cape, the Free State, and the southern half of North-West and Mpumalanga provinces in South Africa) where *P. edulis* was safely considered to be absent (Channing 2001; Clauss & Clauss 2002; Minter *et al*. 2004; Du Preez & Carruthers 2009). *P. edulis* was also considered to be absent from Gauteng Province based on extensive field surveys and DNA sampling of *Pyxicephalus* in this province (Yetman & Ferguson, unpubl. data, [Chapter 5]).

To reduce the bias of voucher records from South Africa, and particularly Gauteng, we used Hawth's Analysis Tools 3.26 (© 2002-2006) in ArcMap 9.2 (© ESRI, Inc. 1999-2006) to randomly select one voucher record from every degree grid cell in southern Africa that contained > 1 voucher record (similar to Trethowan *et al.* 2011). Only the remaining voucher records were then used for modelling, and are hereafter referred to as the "model records." Records from peer-reviewed or grey literature that were not associated with voucher material, and reports of *P. adspersus* that were seen or heard from a part of southern Africa where *P. edulis* is absent (as previously described), were used as an additional, independent data set to evaluate the model prediction, and are hereafter referred to as the "no-voucher records."

Species distribution modelling

We used MaxEnt (Phillips *et al*. 2006; Phillips & Dudik 2008) to predict the geographic range of *P. adspersus* in southern Africa, which included South Africa, Lesotho, Swaziland, Mozambique, Zimbabwe, Botswana and Namibia (Fig. 1). Data from e.g., the South African Frog Atlas Project, limited the model to a quarter degree (15 arc minute) spatial resolution. The predicted distribution was assumed to represent the geographic extent of suitable habitat for *P. adspersus* prior to European colonization and subsequent habitat transformation in southern Africa. The model background was defined by South Africa, Zimbabwe and Namibia, since these were the only countries with voucher records.

Initially we tested 19 bioclimatic variables as environmental predictors, at a 10 arc minute resolution from the WorldClim project (http://www.worldclim.org), and re-sampled at a 15 arc minute resolution using ArcMap 9.2. The quality of the interpolated bioclimatic surfaces was least reliable where the density of weather stations was low e.g., in Botswana (Hijmans *et al*. 2005). Using the default jackknife tests of predictor variable importance in MaxEnt 3.3.2, we selected for the final model, the three environmental predictors with the highest training gain when used in isolation, and/or which decreased the gain the most when omitted from the model training. These variables included minimum temperature of the coldest month, mean temperature of the driest quarter, and annual precipitation, which were considered to be biologically significant because cold, dry conditions cause inactivity or torpor (Loveridge & Crayé 1979; Loveridge & Withers 1981), and precipitation exerts a strong influence on the breeding and non-breeding activity of *P. adspersus*. Annual precipitation, specifically, has been found to significantly affect the annual frequency of *P. adspersus* spawning (Yetman & Ferguson 2011a, [Chapter 2]).

The predicted suitability of environmental conditions was projected over Lesotho, Swaziland, Botswana and Mozambique, and the model was cross-validated 10 times. The model was thus tested with a different tenth of the 53 model records for each of 10 model repetitions. To further evaluate the model prediction, the minimum training presence threshold (Pearson *et al.* 2007) was used to determine the omission error rate for all voucher and no-voucher records. Using the latter records thus allowed the model prediction to be tested with an independent dataset.

Gauteng land cover analysis

We used a raster of land cover data with 10 m resolution to determine the availability of suitable habitat remaining for *P. adspersus* in Gauteng, South Africa. The data were derived from a combination of single date 2009-08 10 m resolution SPOT5 satellite imagery (mostly from late summer, i.e. January-March), and 2009 50 cm resolution aerial photography (GeoTerraImage Pty Ltd, http://www.geoterraimage.com). The spatial extent of the data included the current Gauteng boundary, parts of the Cradle of Mankind, and Dinokeng conservation areas (shared with North-West and Limpopo provinces, respectively), the proposed Merafong City Municipality (situated on the south-west boundary of Gauteng), and a 2 km wide buffer around all this land (hereafter referred to as the Gauteng study area). The raster contained 41 different land cover types, which were mapped using "conventional pixel based image classifiers, object-based modelling and direct image photo-interpretation" (GeoTerraImage Pty Ltd).

We re-classified the 41 land cover types as either “suitable,” “unsuitable” or “intermediate” for *P. adspersus* (Table 2). Seasonal wetlands, and grassland and open savanna, which comprise the natural habitat of *P. adspersus* (Du Preez & Carruthers 2009), were considered to be suitable. Agricultural, urban and industrial areas were considered to be unsuitable. Land of intermediate suitability included dense bush/trees (i.e. with 70-100% canopy closure), rocky terrain with grass (found mainly on elevated ridges), grassland or savanna on agricultural small-holdings, and regenerated grassland or savanna on old (cultivated) lands, where *P. adspersus* may occur but usually at low densities (CAY pers. obs.).

Based on the reasoning that a *P. adspersus* population utilizing a small (< 1 ha) breeding site requires 1-4 km^2 of suitable terrestrial habitat for foraging and overwintering (see Yetman & Ferguson 2011b, [Chapter 3]), we re-sampled the original land cover raster in ArcMap 9.2 to create a raster with 1 x 1 arc minute (or ~ 3.5 km^2) grid cells. Each new grid cell represented the land cover type with the greatest surface area contained within it. Thus patches of suitable habitat smaller than ~ 1.7 km^2, which were considered insufficient to sustain a *P. adspersus* population over the long term, were incorporated in grid cells classified as intermediate or unsuitable. The total amount of remaining suitable or intermediate habitat for *P. adspersus* within the Gauteng study area was estimated as the combined area of all the 3.5 km^2 grid cells that were dominated by suitable or intermediate habitat, respectively.

Finally, using ArcMap we visually examined the spatial coincidence in the Gauteng study area between *P. adspersus* voucher records dating between 1995 and 2010 and: i) the provincial urban edge in 2002 i.e., mid-way between 1995 and 2010 (defined by land use patterns and property boundaries for legislative purposes, and supplied as a shapefile by GDARD); ii) the 1 arc minute raster of the 2008-09 land cover data (derived from satellite images and aerial photographs, and supplied by GeoTerraImage Pty Ltd); and iii) the existing network of provincial and municipal protected areas in Gauteng (supplied as a shapefile by GDARD).

Results

Predicted range of P. adspersus *in southern Africa*

The total of 759 unique *P. adspersus* occurrence records comprised 348 voucher (Fig. 1) and 411 no-voucher (Fig. 2) records. When the voucher records were filtered to reduce the bias of these records from South Africa, and in particular Gauteng, 53 records remained for modelling (Fig. 1). The numbers of different voucher, no-voucher, and model record types are shown in Table 3.

Jackknife tests in MaxEnt revealed that minimum temperature of the coldest month, mean temperature of the driest quarter, and annual precipitation made similar percentage contributions to the model (Table 4). Minimum temperature of the coldest month and mean temperature of the driest quarter contributed approximately equally to the model’s training and testing gain, while annual precipitation contributed additional useful information that was not provided by the other two variables (Table 4). The mean training and testing area under the (Receiver Operator Characteristic) curve (AUC; Swets 1988; Thuiller *et al*. 2006) was, respectively, 0.77 and 0.75 for the 10 model repetitions. The predicted geographic range of *P. adspersus* is shown as a logistic probability map upon which the 411 no-voucher records have been superimposed (Fig. 2). The mean minimum training presence logistic threshold for the 10 model repetitions was 0.12. All no-voucher and 98.9% of voucher records occupied quarter degree grid cells where the predicted probability of suitable conditions for *P. adspersus* was ≥ 0.12 (Fig. 2).

Probabilities of predicted suitable conditions for *P. adspersus* in southern Africa were categorized as very high (0.58-0.77), high (0.38-0.58), intermediate (0.38-0.19) or low (0-0.19; Fig. 2). High to very high probabilities were found in the eastern interior of South Africa, including Gauteng, most of the Free State and North-West Province, the south-western region of Limpopo, the western half of Mpumalanga, the western side of KwaZulu-Natal, and the north-central region of the Eastern Cape. Probabilities were intermediate to high in the central and eastern regions of Namibia, across most of Botswana (excluding the Makgadikgadi Pans), and the central region of Zimbabwe. Probabilities were low along the eastern side of Mpumalanga and Limpopo provinces, and on the eastern coastline and western side of South Africa. In Namibia, probabilities were low in the southern region and along the western side of this country. Probabilities were low in the north-west and south-east parts of Zimbabwe, and across most of Swaziland (maximum = 0.34) and Mozambique (maximum = 0.20).

Habitat and P. adspersus *in Gauteng Province, South Africa*

The raster of land cover types in the Gauteng study area (1 arc minute), which included the entire province, some additional conservation and proposed municipal land, and an all-inclusive 2 km wide buffer, had a total area of 23 726 km^2. Of this, approximately 12 634 km^2 (53%) was considered suitable, 1 059 km^2 (4%) was considered intermediate, and 10 033 km^2 (42%) was considered unsuitable for *P. adspersus*. Habitat that was considered suitable for *P. adspersus* was found mainly beyond the Gauteng 2002 urban edge, in the distal reaches of this province. The largest patches of suitable habitat were found in the north, north-east, west, south-west and south-eastern regions of Gauteng, where the Wallmansthal, Leeuwfontein and Ezemvelo, Cradle of Mankind, Abe Bailey, and Suikerbosrand protected areas are situated, respectively (Fig. 3).

Of 128 *P. adspersus* records in the Gauteng study area, which dated between 1995 and 2010, 42 (33%) were located inside and 86 (67%) were located outside the 2002 provincial urban edge (Fig. 3). Fifty-two (41%), 13 (10%) and 63 (49%) of the 128 records were located in raster grid cells that were dominated in 2008-09 by habitat that we classified as suitable, intermediate or unsuitable for *P. adspersus* (Fig. 3). More specifically, 43 (34%), 41 (32%) and 11 (9%) of the 128 records were, respectively, located in urban areas, grassland, and grassland on agricultural small-holdings (Fig. 4). Eight (6%) of the 128 records were located in Gauteng provincial or municipal protected areas (Fig. 3).

Discussion

Model

The MaxEnt model prediction that was obtained in this study probably provides the most accurate depiction of the potential geographic range of *P. adspersus* in southern Africa to date. Only occurrence records with unambiguous voucher material and locality data were used for the model, and the geographic sampling bias of records was minimized. The model was tested with an independent dataset, and there was good overlap between voucher or no-voucher records and areas with high probabilities of predicted suitable conditions for *P. adspersus*. Only four (1%) of the 348 voucher records were not included by the mean minimum training presence logistic threshold of 0.12. These four voucher records occupied the same quarter degree grid cell over Port Elizabeth on the southern South African coast, and were no doubt excluded because half of this grid cell included sea, which would have strongly influenced local bioclimatic conditions.

Species distribution models tend to involve fewer explanatory variables when conditions are projected over larger areas (VanDerWal *et al.* 2009), therefore, it is not surprising that of the 19 bioclimatic variables that were tested, three (i.e. minimum temperature of the coldest month, mean temperature of the driest quarter, and annual precipitation) contributed the most to our model. These variables have biological significance because due to the ectothermic, permeable body and aquatic breeding habits of *P. adspersus*, the activity, survival and reproduction of this species is directly affected by temperature and precipitation. Therefore, the selected predictors (in contrast to indirect predictors like vegetation; Austin 2002) were considered to be suitable and useful for the required model projection over Lesotho, Swaziland, Botswana and Mozambique, where no reliable occurrence data for *P. adspersus* were available.

Predicted range of P. adspersus *in southern Africa*

Our model prediction indicated that temperate to semi-arid habitats situated mainly in the interior of southern Africa were most suitable for *P. adspersus*. The eastern, low-lying subtropical and western, arid sides of southern Africa and cool, high-altitude habitat in and around Lesotho, were unsuitable for *P. adspersus*. In contrast to the geographic range of *P. adspersus* reported by other authors (e.g. Channing 2001; Du Preez & Carruthers 2009), our model did not predict suitable conditions for this species in Mozambique. It is unlikely that this was due to the lack of *P. adspersus* occurrence records from this country. Records were also not available from Botswana, where probabilities of predicted suitable conditions for *P. adspersus* were mostly intermediate to high. We argue that in Mozambique, *P. edulis* has been misidentified as "*P. adspersus*," while *P. angusticeps* (Parry 1982) has been misidentified as "*P. edulis*."

Our model prediction also differs from maps for *P. adspersus* compiled by other authors (e.g., Channing 2001; Minter *et al.* 2004; Du Preez & Carruthers 2009), in that a small area in the western half of the Western Cape Province in South Africa was predicted to be suitable for this species. There are several possible explanations for this. The model may be inaccurate as a result of unique environmental conditions in this area, which is a hotspot of floral and amphibian species richness (Van Wyk & Van Wyk 1997; Minter *et al.* 2004). Alternatively, if conditions were indeed at least historically suitable for *P.*

adspersus in this area, it may be that this species was unable to disperse to this area due to geographic or climatic barriers, or was present in the area but has since gone extinct (Soberón & Peterson 2005).

Parry (1982) reported that *P. adspersus* and *P. edulis*, respectively, occupy temperate and subtropical habitats in southern Africa. Our model prediction lends support for Parry's assertion about *P. adspersus.* If *P. adspersus* and *P. edulis* occupy different habitats and, therefore, largely different ecological niches, it is reasonable to expect that their geographic ranges should be largely allopatric. There are, however, indications that the two species occur sympatrically in some places in e.g., Limpopo Province in South Africa (Minter *et al.* 2004), and various parts of Botswana (Clauss & Clauss 2002). Where this may occur it would be interesting to investigate the factors that facilitate niche separation between these two species, and whether *P. adspersus* preys on the smaller *P. edulis* in addition to other anurans and animals (Channing 2001; Du Preez & Cook 2004).

Habitat and P. adspersus *in Gauteng Province, South Africa*

Although > 50% of habitat in the Gauteng study area included natural grassland, open savanna, and vegetated water, and was, therefore, classified as suitable for *P. adspersus*, this habitat is clearly fragmented and remaining patches are becoming increasingly isolated and threatened by human activities. The largest patches of suitable habitat for *P. adspersus* in Gauteng remain in and around protected areas in the distal reaches of this province.

Ironically, in Gauteng, most known *P. adspersus* breeding populations are situated in peri-urban areas (Yetman, unpubl. data), and 33% and 34% of *P. adspersus* records from 1995-2010 were, respectively, located inside the 2002 urban edge, and within habitat identified as urban in 2008-09. There are several explanations for this. Firstly, areas have become urbanized since some records were collected. Secondly, *P. adspersus* is more likely to be detected in more densely human populated areas. Thirdly, individual *P. adspersus* may survive for several years in urban gardens due to the longevity of this species (Channing 2001; Yetman *et al.*, in press, [Chapter 4]). In the more remote, less transformed reaches of Gauteng *P. adspersus* is probably present, but remains undetected since genetic data indicated that this species was common in this province (Yetman & Ferguson, unpubl. data, [Chapter 5]). However, large *P. adspersus* breeding populations are probably rare in Gauteng given the large terrestrial spatial habitat requirements of this species (Yetman & Ferguson 2011b, [Chapter 3]).

Conservation implications

Our model prediction suggests that the geographic range of *P. adspersus* in southern Africa is slightly smaller than that reported by e.g., Channing (2001) and Du Preez & Carruthers (2009). This is mainly because conditions in Mozambique were predicted to be unsuitable for *P. adspersus*. In Swaziland it appears probable that *P. adspersus* has gone extinct (Boycott 2001), considering that suitable conditions for this species were predicted to occur only marginally on the western boundary of this country. In Lesotho, where predicted suitable conditions for *P. adspersus* were also limited, the presence of this species is unknown. In Zimbabwe, where predicted suitable conditions coincide with the more densely populated metropolitan areas around Bulawayo in the south-west, and Harare in the north-east of this country, *P. adspersus* populations are likely to be threatened. Thus it would appear that in southern Africa, the stronghold of *P. adspersus* remains in Botswana, Namibia and the more remote regions of South Africa. We encourage phylogeographic research to validate our model prediction, and strongly recommend field surveys in sub-Saharan African countries with no or few reliable records of *P. adspersus.*

In South Africa's densely populated Gauteng Province, suitable natural habitat for *P. adspersus* is fragmented, and remaining *P. adspersus* breeding populations will become increasingly isolated in the distal reaches of this province. In the warmer Savanna biome in northern Gauteng (Mucina *et al.* 2005), juvenile dispersal and gene flow between *P. adspersus* populations in e.g., the Leeuwfontein and Wallmansthal protected areas might still occur (Yetman & Ferguson, unpubl. data, [Chapter 5]). However, in the cooler Grassland biome elsewhere in Gauteng (Mucina *et al.* 2005), successful dispersal of *P. adspersus* between e.g., the Cradle of Mankind, Abe Bailey, Suikerbosrand, Rietvlei Dam and Ezemvelo protected areas is unlikely. In fact, limited genetic data revealed that populations in central Gauteng < 20 km apart exhibited significant genetic differentiation, which was likely due to reduced gene flow and genetic drift (Yetman & Ferguson, unpubl. data, [Chapter 5]). Although conservation of a *P. adspersus* meta-population may still be possible in northern Gauteng, translocation of specimens between grassland populations may become necessary and, therefore, warrants research.

The Harts River system (some 400 km south-west of Johannesburg) yielded the highest density of voucher records outside of Gauteng Province (Fig. 1), indicating that this area warrants attention for the conservation of *P. adspersus*. However, the upper Harts River is impacted by intensive commercial agriculture (mainly maize cultivation) and, therefore, conservation of suitable natural habitat for *P. adspersus* would be an important issue in this area, as in Gauteng Province.

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Table 1. Characters used to identify filmed, photographed or preserved adult *Pyxicephalus*.

Character	*P. adspersus*	*P. edulis*
Distance between eye and tympanum	> tympanum diameter	< tympanum diameter
Mandible skin barring	Absent or indistinct	Pronounced
White tympanic spot	Absent or indistinct	Pronounced
Vertebral stripe	Absent or indistinct	Pronounced
Dorsal skin blotching	Absent or indistinct	Pronounced
Dorsal skin colour	Light to dark olive-green	Brown, or yellow- to olive-green
Snout-vent length	< 230 mm	< 150 mm

Table 2. Classification of land cover types in Gauteng Province, South Africa, for *Pyxicephalus adspersus.*

Habitat quality	Land cover types
Suitable	Natural, vegetated water (including pans).
	Grassland or open savanna.
Intermediate	Natural open water (including open water in wetlands, streams and rivers).
	Dense bush / trees or rocky terrain with grass.
	Grassland or open savanna on agricultural small-holdings.
	Grassland or open savanna representing regenerated vegetation on old (cultivated) lands.
Unsuitable	Man-made water (e.g. irrigation dams, quarry pits, sewerage farms, etc.).
	Degraded and non-vegetated natural habitat, including bare rock.
	Plantations and woodlots.
	Buildings, roads and other urban infrastructure and vegetation, including sports and other recreation fields.
	Cultivated land and intensive animal camps.
	Mines.

Table 3. Number of different *Pyxicephalus adspersus* occurrence records that were obtained for southern Africa. Records representing more than one type were assigned to the highest tabulated type. Voucher records were accompanied by voucher material, unlike no-voucher records. Due to sampling bias only a small selection of the voucher records was used to model the potential geographic range of *P. adspersus*.

		No. of records	
	Type	Obtained	Used for modelling
Voucher records	DNA sequence data	28	3
	Video footage	4	0
	Call recording	5	2
	Preserved specimen(s)	170	30
	Photograph(s)	138	15
	Peer-reviewed article	3	3
Non-voucher records	Peer-reviewed article	29	-
	Grey literature	10	-
	Specimen(s) seen	367	-
	Calling only heard	5	-
Total		759	53

Table 4. Jackknife test results for three interpolated bioclimatic variables (each represented by *x*) that were used to predict the geographic range of *Pyxicephalus adspersus* in southern Africa using MaxEnt (Phillips *et al.* 2006). Tabulated values represent the mean of values obtained from 10 model repetitions.

	Variable (*x*)		
	Min. temp. of coldest month	Mean temp. of driest quarter	Annual precipitation
Percent contribution	30.5%	32.0%	37.5%
Training gain without *x*	0.41	0.41	0.28
Training gain with only *x*	0.23	0.26	0.17
Test gain without *x*	0.40	0.40	0.24
Test gain with only *x*	0.21	0.24	0.18
AUC without *x*	0.75	0.74	0.71
AUC with only *x*	0.70	0.71	0.66

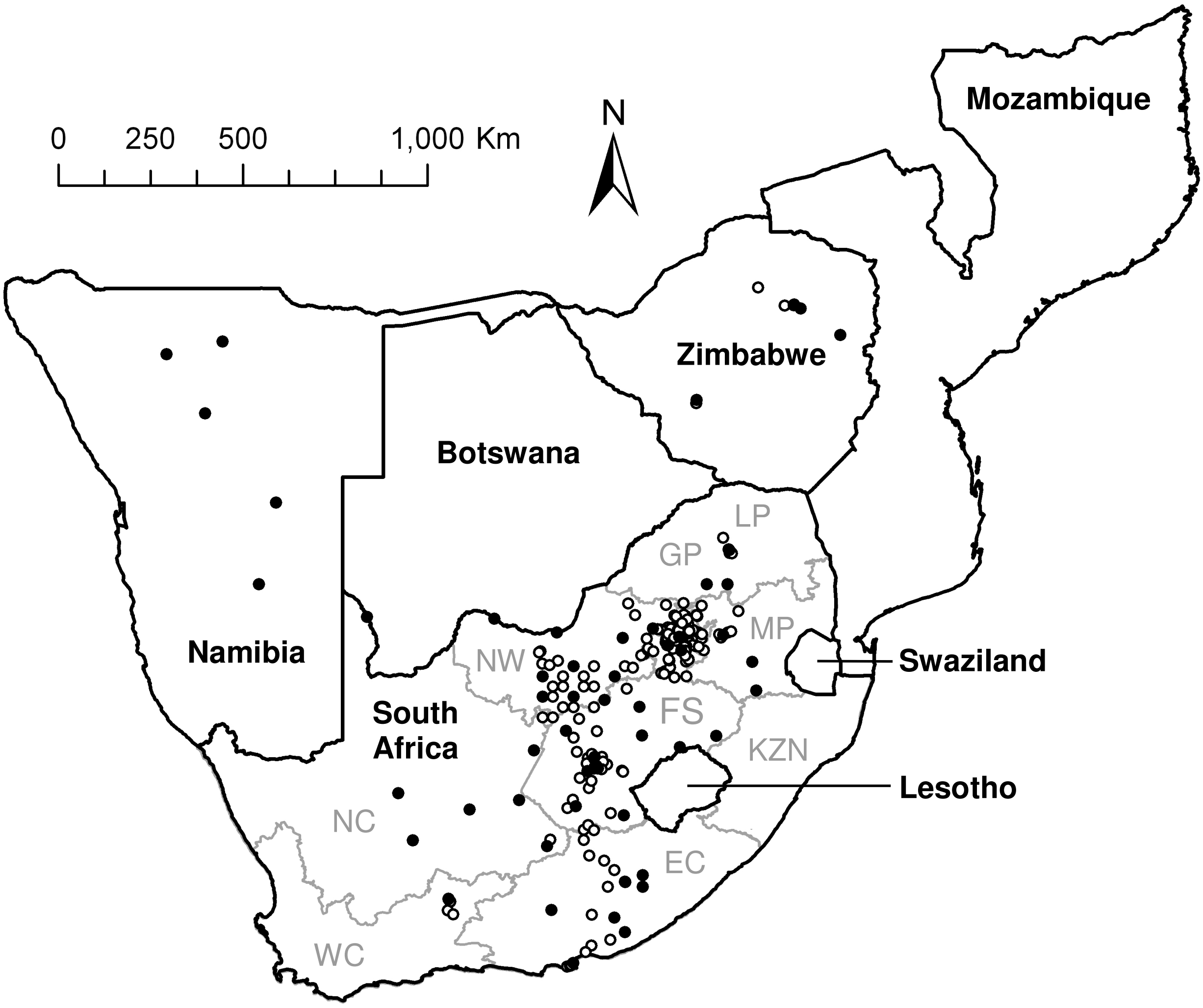

Figure 1. The southern African study area where LP = Limpopo, NW = North-West, NC = Northern Cape, WC = Western Cape, EC = Eastern Cape, FS = Free State, KZN = KwaZulu-Natal, MP = Mpumalanga and GP = Gauteng provinces in South Africa. Dots represent *Pyxicephalus adspersus* occurrence records with voucher material, which were filtered to reduce their biased distribution in South Africa, and particularly Gauteng. Black dots represent the 53 records that were used to model this species' geographical range. White dots represent records that remained after filtering and were not used for the modelling.

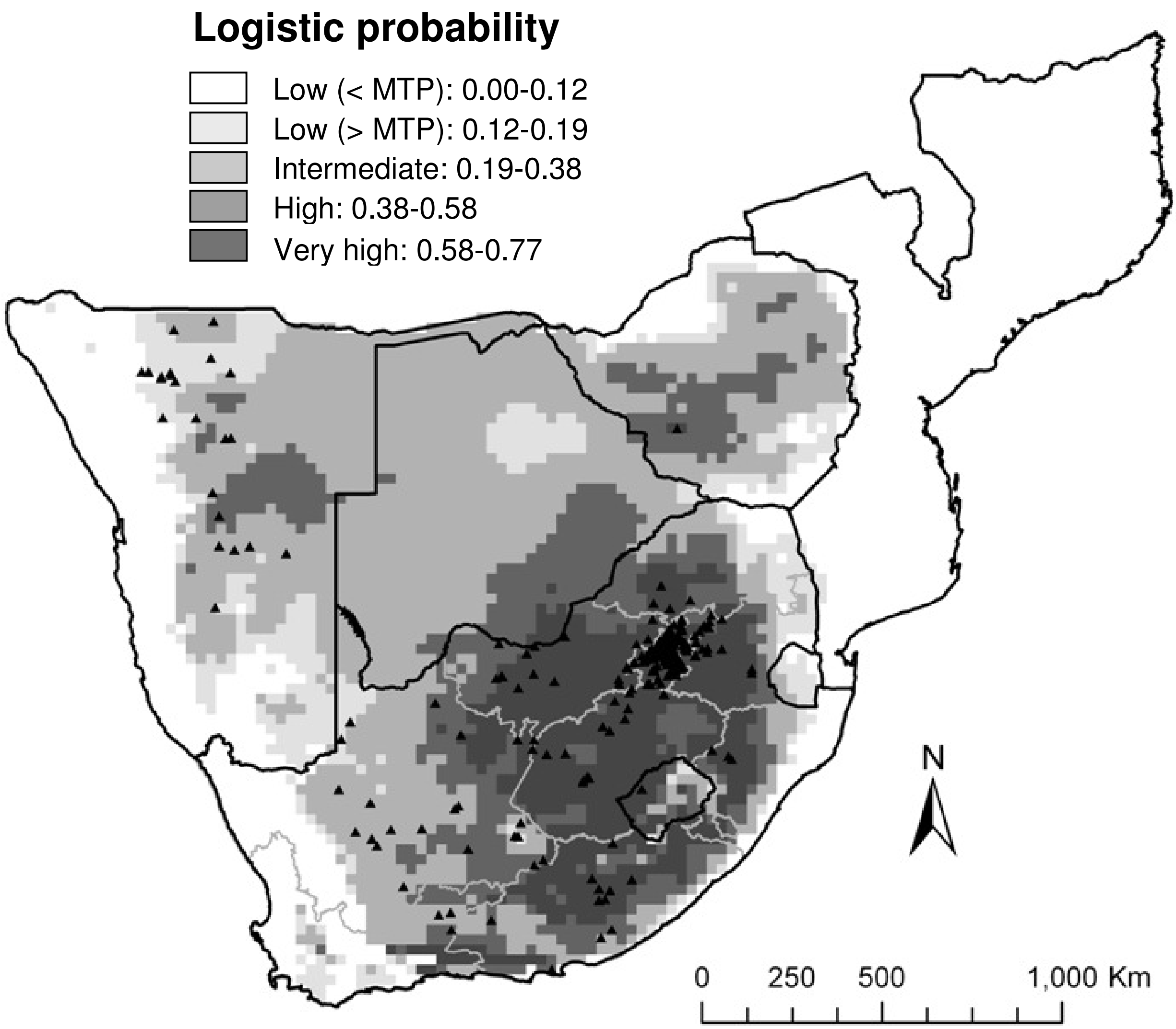

Figure 2. Black triangles represent *Pyxicephalus adspersus* occurrence records without voucher material. These have been superimposed on a 15 arc minute raster showing the predicted suitability of environmental conditions for this species, as determined from ten repetitions of a model created in MaxEnt. The minimum training presence (MTP) threshold was 0.12, and darker grid cells represent more suitable predicted conditions for *P. adspersus*. The model was trained using *P. adspersus* occurrence records with voucher material, which are shown as white and black dots in Fig. 1.

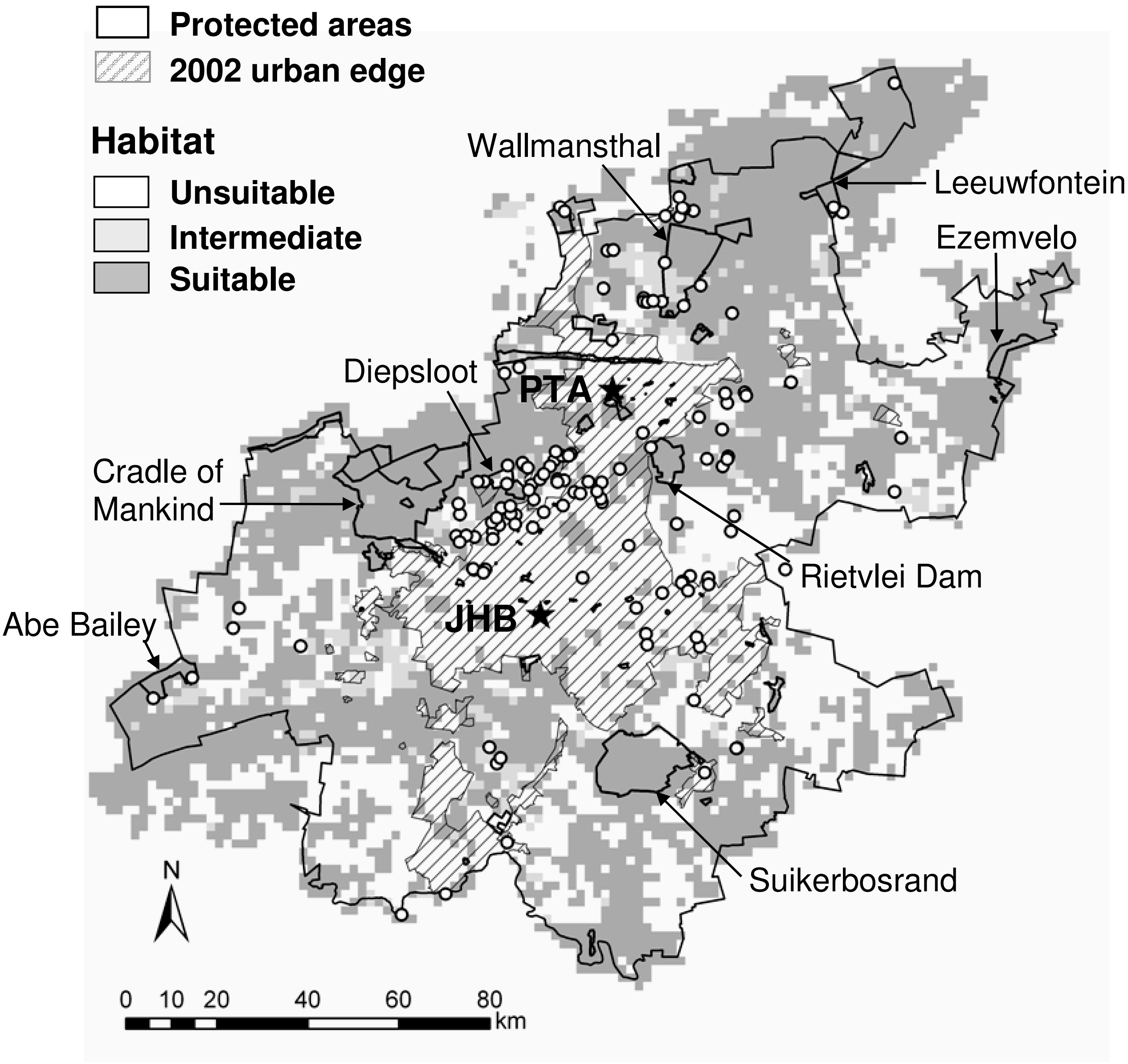

Figure 3. A 1 arc minute raster showing where habitat in Gauteng Province, South Africa, was classified as suitable, unsuitable or intermediate for *Pyxicephalus adspersus*. White dots represent *P. adspersus* occurrence records with voucher material, dating from 1995 to 2010. The largest protected areas in Gauteng are indicated with labelled arrows. PTA = Pretoria and JHB = Johannesburg metropolitan cities.

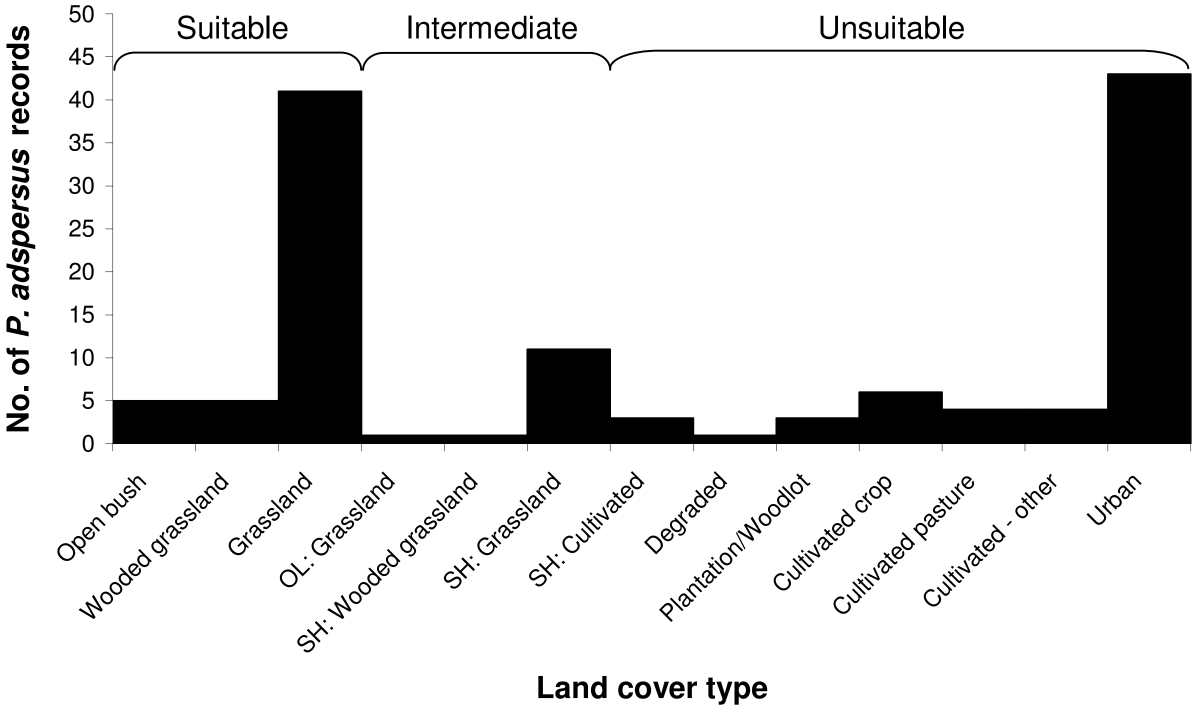

Figure 4. The number of *Pyxicephalus adspersus* occurrence records with voucher material, dating between 1995 and 2010, which were located in raster grid cells (1 arc minute) dominated by different land cover types identified in 2008-09, in Gauteng Province, South Africa. Land cover types were broadly categorized as suitable, intermediate or unsuitable for *P. adspersus.* SH = small-holding and OL = old (re-vegetated) land. "Cultivated – other" refers to small cultivated areas around rural farm building clusters.

Chapter 7

Conclusion

Summary of results

This thesis was designed to investigate several poorly understood aspects of the ecology of *Pyxicephalus adspersus*, which would contribute towards improved conservation management of this species in South Africa, and in particular, Gauteng Province. The main findings from the different research chapters comprising this thesis are described below.

Chapter 2

To better understand the sporadic activity of *P. adspersus* for improved monitoring and conservation management of this species, a population's spawning and non-breeding activity was monitored during five summers at a site in Diepsloot, Gauteng Province, South Africa. This research revealed that:

- Greater proportions of study animals moved overland and/or foraged at night around full moon, after heavy rainfall, when cooler and less windy conditions prevailed.
- Most study animals cumulatively spent a few days at breeding sites and 2-3 weeks foraging near their burrows at night during the six-month summer period.

- Males spent significantly more days at the breeding site, and more nights active (moving overland and foraging) during summer, compared to females. Therefore, male *P. adspersus* are more vulnerable to human harvesting at breeding sites than females, which spend virtually their entire lives in terrestrial habitat.
- Population spawning occurred most frequently in December and six times on average, annually. Both numbers of annual spawning events and numbers of spawning males were positively correlated with rainfall.
- Numbers of spawning males varied greatly, therefore, single counts of spawning adults will often result in gross underestimates of population size. However, given the sporadic activity of adult *P. adspersus* it may be more practical to monitor larvae and/or numbers of successful breeding sites in certain areas.

Chapter 3

To investigate the unknown spatial habitat requirements of *P. adspersus* for more effective physical protection of populations, the movements of 70 adult frogs were radio- or spool-tracked during five summers at a site in Diepsloot, Gauteng. This research revealed that:

- Study animals moved directly between their breeding site and burrows, covering a maximum overnight distance of 350 m. Individuals often followed human-created footpaths and/or dirt roads, and were not deterred by electric fencing or vehicle traffic.
- Limited data suggested that adult *P. adspersus* forage mostly within 20 m of their burrows.

- Study animals used one "home" burrow, on average, within and between summers. The home burrows of females were situated almost four times further from the breeding site than those of males, and female body condition was significantly positively correlated with distance of their burrows from the breeding site.
- A 950-1 000 m wide buffer would be necessary around the Diepsloot breeding site to protect the home burrows of all the radio-tracked study animals.
- Since adult *P. adspersus* appear to be philopatric, juvenile dispersal is predicted to be largely responsible for gene flow between populations.

Chapter 4

To investigate the unknown age range of wild *P. adspersus*, skeletochronology was used to compare the age distribution of specimens from three peri-urban breeding sites including that at Diepsloot and at Glen Austin and Bullfrog pans near Johannesburg, Gauteng. This research revealed that:

- Males and females from the three breeding sites, respectively, possessed 6 ± 2 (max. 16) and 4 ± 1 (max. 11) annual phalangeal lines of arrested growth (LAG), suggesting that juveniles take ≥ 3 years to mature, and females experience shorter longevity than males.
- LAG counts of same-sex animals did not differ between the three sites, but mean male snout-vent length, body mass and condition was greatest at Glen Austin Pan and lowest at Bullfrog Pan.

- At Glen Austin Pan *P. adspersus* sampled in 2004-06 for this study were significantly shorter than specimens sampled in 1992-93 for a different study, suggesting that at some peri-urban breeding sites, adult *P. adspersus* life expectancy is declining.
- Juvenile *P. adspersus* are most threatened by terrestrial habitat transformation because they take ≥ 3 years to mature, during which time they may disperse far from their natal site.
- Between-site differences in the size and condition of *P. adspersus* indicates that this species requires conservation management at site-specific and larger spatial scales.

Chapter 5

As a step towards identifying *P. adspersus* conservation management units, genetic structure and gene flow for populations from 23 localities in Gauteng and seven additional localities in the north-eastern interior of South Africa was quantified using 708 base pairs of the mitochondrial gene cytochrome *b*. This research revealed that:

- Gene flow was limited between populations > 200 km apart in the north-eastern interior of South Africa, which reflected genetic differentiation at this scale (F_{ST} = 0.60). Populations of *P. adspersus* in the Free State Province fit the three main criteria used to distinguish evolutionary significant units in species.
- There was a high abundance of *P. adspersus* and significant gene flow between populations < 20 km apart in Gauteng, but recent counts of spawning adults suggest that populations in central Gauteng have declined by > 90 %.

- The lack of correlation between genetic and geographic distance of samples suggested that the genetic differentiation in *P. adspersus* between the central, eastern and northern regions of Gauteng (F_{ST} = 0.26), and between Diepsloot, Glen Austin and Monavoni in central Gauteng (F_{ST} = 0.15), was likely due to genetic drift.
- Results of this research suggest that there should be no artificial movement of live specimens over distances > 100 km or between different biomes. Within a biome ideally ≥ 2 *P. adspersus* breeding populations should be protected, and where possible, connected by natural gene flow for long term persistence.
- Conservation of *P. adspersus* in South Africa's highly threatened Grassland biome is considered a priority, and should involve separate protection of populations in Gauteng and the Free State Province where *P. adspersus* is, respectively, highly threatened and genetically unique.

Chapter 6

A MaxEnt model was created to predict the potential geographic range of *P. adspersus* in southern Africa, and recent land cover data were used to determine the amount of suitable habitat remaining for this species in Gauteng Province, South Africa. This research revealed that:

- Predicted suitable conditions for *P. adspersus* included the temperate to semi-arid interior, but excluded the low-lying eastern subtropical and arid western sides of southern Africa.
- In Mozambique, *P. edulis* has likely been misidentified as "*P. adspersus.*"

- In South Africa's Gauteng Province the largest remaining patches of suitable habitat for *P. adspersus*, which included natural grassland, savanna and wetlands, were found in the north-east, west, south-west and south-eastern provincial regions.
- In the Savanna biome in northern Gauteng, where there has been less destruction and fragmentation of habitat, the conservation of a *P. adspersus* meta-population is possible.
- Elsewhere in Gauteng, the successful dispersal of *P. adspersus* between fragmented grassland populations of this species is unlikely. Long term persistence of these populations may, therefore, require translocation.

Concluding remarks

How does this research contribute to the conservation of P. adspersus?

Valuable information on the ecology of *P. adspersus* was obtained during multiple seasons at spatial scales ranging from discrete breeding sites to the whole of southern Africa. Findings described in Chapter 2 should improve the detection and monitoring of *P. adspersus*. Results in Chapter 3 should guide the physical protection of habitat for, and Chapter 4 contains benchmark data on the age structure of *P. adspersus* populations. Findings described in Chapter 5 should contribute towards the conservation management of *P. adspersus* meta-populations, and results in Chapter 6 indicate where this may be feasible given the remaining extent of suitable habitat for *P. adspersus* in South Africa's rapidly urbanizing Gauteng Province.

Is the conservation status of P. adspersus *at different spatial scales, appropriate?*

The global Least Concern conservation status of *P. adspersus* (IUCN 2008; Measey 2011; IUCN 2011) is considered to be appropriate, as *P. adspersus* is likely to occur across much of Namibia and Botswana [Chapter 6] where human population densities are relatively low and habitat transformation is limited. However, in South Africa, Lesotho and Swaziland where habitat transformation has been more extensive and few large *P. adspersus* breeding populations are known (Yetman, unpubl. data), it would be conservative to retain the current Near-Threatened conservation status of *P. adspersus* (Minter *et al.* 2011). In South Africa's Gauteng Province where natural habitat is severely fragmented [Chapter 6] and known *P. adspersus* breeding populations are becoming increasingly isolated [Chapter 5], the provincial Near-Threatened status of this species is warranted (GDACE 2004).

What research on P. adspersus *is recommended?*

Research to determine the feasibility of translocation as a conservation management tool for *P. adspersus* is an urgent priority, and a study on dispersal and survival of juvenile *P. adspersus* is strongly recommended. Additional ecological data of significant conservation value would be obtained by research on geographic variation in the behaviour, habitat requirements and genetic structure of *P. adspersus* across its global sub-Saharan geographic range. Research to elucidate factors that may facilitate niche separation between *P. adspersus* and *P. edulis* would give greater insight into the biology of these frogs, and a study of the age structure of a *P. adspersus* population at an undisturbed site would provide useful comparative data.

What are the conservation priorities for P. adspersus?

The remaining extent of suitable habitat for *P. adspersus* in the other eight South African provinces deserves to be quantified for improved evaluation of this species' conservation status in each province. Citizens should be encouraged to provide information about *P. adspersus* breeding populations, as very few of these are known (Yetman, unpubl. data). Populations of *P. adspersus* in South Africa's extensively disturbed Grassland biome require the most urgent protection. South Africa's Free State Province *P. adspersus* populations require improved protection given their reported genetic uniqueness. In Gauteng Province, where remaining grassland *P. adspersus* populations are increasingly threatened by encroaching urbanization, protection of remaining terrestrial habitat adjacent to breeding sites is critical. In isolated, protected, grassland areas where *P. adspersus* is known to occur but evidence of breeding by this species has not been found in the past decade (e.g. in Suikerbosrand and Rietvlei Dam Game Reserves in Gauteng; Gavin Masterson and Riaan Marais, pers. comm.), the creation of artificial breeding habitat for *P. adspersus* is recommended. The importance of the Hart River system as an area with a relatively high abundance of *P. adspersus* needs to be investigated. Programmes should be implemented to monitor the breeding success (e.g. metamorphosis of tadpole schools) of important threatened *P. adspersus* populations (e.g. at Bullfrog and Glen Austin pans near Johannesburg, Gauteng). Finally, since Bullfrog Pan is one of the largest historical breeding sites for *P. adspersus* in South Africa, the poor condition of adults at this site deserves investigation.

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