A biogeographical assessment of anthropogenic threats to areas where different frog breeding groups occur in South Africa: implications for anuran conservation

Mohlamatsane M. Mokhatla^{1*}, G. John Measey^{2,3}, Christian T. Chimimba^{1,4} & Berndt J. van Rensburg¹

¹Department of Zoology and Entomology, Centre of Excellence for Invasion Biology (C·I·B), University of Pretoria, Pretoria 0002, South Africa, ²Applied Biodiversity Research, South African National Biodiversity Institute, P/Bag X7, Claremont 7735, South Africa, ³Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa, ⁴Department of Zoology & Entomology, Mammal Research Institute (MRI), University of Pretoria, Pretoria 0002, South Africa

**Correspondence:* Mohlamatsane M. Mokhatla, Department of Zoology & Entomology, Centre of Excellence for Invasion Biology (C·I·B), University of Pretoria, Pretoria, 0002 South Africa.

Tel: +27 (12) 4204627; Fax: +27 (12) 3625242; E-mail: mmmokhatla@zoology.up.ac.za

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ABSTRACT

Aim To determine the spatial relationship between areas where different frog breeding groups occur and elevated anthropogenic activities, and the conservation implications thereof.

Location South Africa.

Methods Data on frog distribution ranges for the southern African sub-region were used to identify biogeographic areas within South Africa. A random draw technique was used to

determine whether areas where different frog breeding groups occur were characterised by higher levels of anthropogenic threats than expected by chance. Four measures (human population density, percentage land transformation, percentage protected area, and invasive alien plants richness) expected to reflect threats were analysed.

Results Terrestrial-breeders were more often spatially associated with areas of threat than expected by chance in three of the seven biogeographical regions examined with land transformation and invasive alien plant richness being most significant. The south central was the only region where terrestrial-breeders were spatially congruent with protected areas. Areas where stream-breeders occur were spatially congruent with anthropogenic threats (with alien plants being most consistent) in five of the seven regions examined while protected areas were well represented in four of the seven regions. Non-significant results were found for permanent and temporary aquatic-breeders at both the national and the biogeographic scale.

Main conclusions By analyzing data at the sub-continental scale we were able to identify regional threats to amphibians traditionally classified at species specific scales. Our study recognised land transformation and alien invasive plants as significant threats to areas important for the long-term breeding success of stream and terrestrial amphibians in South Africa. Areas where different breeding groups occur in the southwestern Cape showed the greatest spatial congruence with the threats examined. Areas where terrestrial breeding frogs occur are not well represented in the current conservation network. This has important implications in addressing the current status of threats on amphibians in a biogeographical context.

Keywords: anthropogenic threats, biogeographical scale, areas where different frog breeding groups occur, national scale, protected areas network, South Africa.

INTRODUCTION

Based on recent assessments of population declines, amphibians represent the most threatened animal class globally (Stuart *et al.*, 2004; Mendelson *et al.*, 2005). It has been suggested that the size, growth and resource demands of the human population, ultimately leading to climate change, is the single most important reason for the recently observed changes in amphibian phenology, species range shifts, and an increase in rates of spread of infectious diseases (Vitousek *et al.*, 1997; Sala *et al.*, 2000; also see Hughes, 2000; Carey & Alexander, 2003; Pounds *et al.*, 2006). Other well-known threats also include land-use change, commercial over-exploitation, and the introduction of exotic species (Drost & Fellers, 1996; Blaustein & Kiesecker, 2002; Collins & Storfer, 2003; Beebee & Griffiths, 2005). An additional alarming aspect concerning amphibian conservation is that compared to other animal groups such as birds and mammals, it is only during the last two decades that scientists have become aware of the global extent and rate at which amphibians are declining (Halliday, 1998; Houlahan *et al.*, 2000, Pounds *et al.*, 2006). Although historical data from the 1970s indicated amphibian declines in several countries globally, scientists only acknowledged the magnitude of the amphibian problem at the First World Congress of Herpetology in 1989 (see Stuart *et al.*, 2004 and references therein).

In South Africa, the majority of conservation planning efforts conducted at the national scale have focused mainly on vegetation types (e.g., Reyers *et al.*, 2001; Rouget *et al.*, 2003; Driver *et al.*, 2005; Reyers *et al.*, 2007), birds (e.g., Bonn *et al.*, 2004; van Rensburg *et al.*, 2004a; Storch *et al.*, 2005), mammals (e.g., Andrews & O'Brien, 2000; Keith *et al.*, 2007), and to some extent, tortoises and terrapins (Branch *et al.*, 1995). However, limited studies have focused their attention on the anurans of the southern African sub-region and their conservation. Previous studies by Poynton (1999), Seymour *et al.* (2001), and

Alexander *et al.* (2004) have contributed significantly towards our understanding of anuran biogeographical patterns in southern Africa. In addition, Drinkrow & Cherry (1995) and Alexander *et al.* (2004) identified areas harbouring exceptional amphibian diversity as well as biologically important hotspots for South Africa, Lesotho and Swaziland. Furthermore, using complementarity techniques, Seymour *et al.* (2001) identified areas important for frog conservation in the sub-region. However, anuran diversity in southern Africa is relatively high, particularly at the family level, and with it the diversity in how each species utilises its environment. The incorporation of life history traits (e.g., areas where different frog breeding groups occur) in stream-lining conservation efforts is currently gaining momentum (Becker & Loyola, 2008; Loyola *et al.*, 2008; Becker *et al.*, 2010). Conservation efforts neglecting to consider amphibian breeding habitat fall short of acknowledging that different life histories are affected differently by anthropogenic threats (Becker *et al.*, 2007).

South Africa holds a markedly rich anuran diversity with of 12 out of the 15 anuran families in sub-Saharan Africa represented in South Africa. For example, one small family of six ghost frog species (Heleophrynidae) is a near-endemic, forming a clade which has been placed basal to other Neobatrachia (Frost *et al.*, 2006). South Africa also has the largest radiation of pyxicephalid frogs (40 of 69 species) which display not only a wide range of body sizes from the tiny micro-frog (*Microbatrachella capensis*, ca. 15 mm in body length) to the giant bullfrog (*Pyxicephalus adspersus*, ca. 245 mm), but also a range of reproductive modes including direct development, thought to have evolved at least twice within this group (van der Meijden *et al.*, 2011). Of the 118 frog species currently reported to occur in South Africa, 51 (43%) are endemics (Angulo *et al.*, 2011), and this figure is likely to increase (Channing *et al.*, 2011). South Africa also has remarkably good distribution records compared to other African countries. A frog atlas produced in 2004 (Minter *et al.*, 2004)

provides distribution records for 117 species across southern Africa and these species have been recently up-dated for IUCN Red Listing (Measey, 2011).

In the present study, we assess the degree of spatial congruence between frog species representing different biological traits, focusing on areas where different breeding groups occur and elevated anthropogenic activities assuming that most of these activities potentially have negative impacts, either directly or indirectly, on the survival rates of frog populations. More specifically, we wanted to determine the spatial congruence of different frog breeding groups with anthropogenic threats.

METHODS

Data

Given the high degree of variation in anthropogenic threats in geographic space, the effect of variation at the spatial scale was incorporated in our analyses. Consequently, spatial congruence between anthropogenic activities and areas where frog breeding groups occur were assessed at two spatial scales as follows: 1) the whole of South Africa (hereafter referred to as the ‘national scale’); and 2) at smaller biogeographical areas representing different frog assemblages within South Africa.

Although our analyses were restricted to South Africa, mainly due to the restricted availability of data on anthropogenic activities in other southern African countries, the actual identification of biogeographical areas was based on anuran presence/absence data encompassing South Africa, Lesotho, Swaziland, Namibia, Botswana, Zimbabwe, Mozambique, Zambia, Malawi and Angola (hereafter referred to as the southern African sub-region). The identification of biogeographic areas based on data from the entire southern African sub-region, thus excluding political boundaries, may allow for more biologically

meaningful insights into anuran assemblages in South Africa. Data on frog distributions representing the southern African sub-region were obtained from the South Africa frog atlas project (Minter *et al.*, 2004), Global Amphibian Assessment (IUCN, GAA 2008; www.iucnredlist.org/initiatives/amphibians/) and the Global Biodiversity Information Facility (GBIF; www.gbif.org).

Species occurrence records on the GBIF dataset are continuously up-dated by specialists and the general public as new occurrence records are reported. The accuracy of this dataset depends highly on correct species identification, and hence, the following data were excluded from our analysis: 1) all unconfirmed or doubtful species occurrence records (i.e., occurrence of species outside their known range – using GAA polygons as ‘known range’); 2) species records based on fossil data because of the inconclusiveness of such data and also given that many of these species have become extinct; and 3) records that were not identified to the species level.

The identification of biogeographic areas was based on the latest authoritative amphibian taxonomic treatment by Frost *et al.*, (2006), based on occurrence records of 245 species at a quarter degree grid resolution. A hierarchical clustering technique was used to group these species based on similarities in their distributions (Legendre & Legendre, 1998; Seymour *et al.*, 2001; Alexander *et al.*, 2004; Chen & Bi, 2007). The aim was not to impose any clustering pattern on the distributional data by defining the number of clusters *a priori* (see Heikenheimo *et al.*, 2007), but rather to observe the naturally occurring structure of the data. We used Ward’s minimum variance (see Everitt, 1993) and Euclidian distance (Heikenheimo *et al.*, 2007) as the clustering and linkage methods, respectively (see also Gagné & Proulx, 2009; Hiekenheimo *et al.*, 2009). Although hierarchical clustering is a well-established multivariate technique, its algorithm does not include testing for statistical

significance (Everitt, 1993) such that decisions on the extent to which clusters differ from each other are subjective and/or based on prior knowledge. Consequently, the present study was based on optimisation methods (see Everitt, 1993) in an attempt to objectively identify biologically meaningful clusters within the generated southern African amphibian dataset.

It is inevitable for biogeographic areas to depict some degree of spatial overlap with each other, translating into pseudo-replication in subsequent analyses. Consequently, we used a systematic approach to reduce such overlaps, where two or more biogeographic areas overlap. Beta diversity measures highlight species compositional differences between focal and neighbouring cells (Williams, 1996; Williams *et al.*, 1999) and are thus commonly used to identify transitional areas between biogeographical regions (see van Rensburg *et al.*, 2004b and references therein). We used Simpson's measure of beta diversity (hereafter referred to as β_{sim} -diversity) for this procedure (see Lennon *et al.*, 2001 and Koleff *et al.*, 2003 for more information on this diversity measure).

Areas with high β_{sim} -diversity values would, therefore, highlight areas with significant levels of species compositional differences when compared with neighbouring areas (van Rensburg *et al.*, 2004b). Thus, for each anuran biogeographical area that was identified for the greater southern African sub-region, we identified grid cells showing spatial overlap with neighbouring biogeographical areas. Within each set of overlapping grid cells, a β_{sim} -diversity value was calculated for each grid cell (averaged across all pair-wise comparisons with adjacent cells) based on the frog species occurrence data. Overlapping cells with low (< 50%) β_{sim} -diversity values can, therefore, be treated as areas sharing a high level of species compositional similarities when compared between the given biogeographical areas. Hence, such areas would be less likely to represent the anuran assemblage of a specific biogeographical area and can thus be shared between biogeographical areas. In contrast, grid cells with high (> 50%) β_{sim} -diversity values can be regarded as areas sharing a low level of

species compositional similarities and were, therefore, excluded from subsequent analyses in order to reduce pseudo-replication.

Eleven biogeographical areas were identified based on the anuran distribution data for the southern African sub-region, with seven of these being represented in South Africa. Of significance is that these 11 biogeographical areas are similar to those previously identified by Poynton (1999), Seymour *et al.* (2001), and Alexander *et al.* (2004) despite being based on different data quality (the present study being based on the most recent available data), spatial scales and resolutions, and clustering approaches. Consequently, the naming of the biogeographical areas in the present study largely follows that of the studies cited above, and also with reference to the phytogeographic regions followed by Burgess *et al.* (2004) (see also Alexander *et al.*, 2004). Subsequent analyses at the biogeographical scale focused on the seven biogeographic areas represented in South Africa that included: 1) South-western Cape; 2) Southwest arid; 3) South central, 4) Maputaland; 5) East African lowlands; 6) South east lowland; and 7) Zambesian/Bushveld woodland assemblages (see Appendix S1 in “Supporting Information” for a map of biogeographic areas occurring in South Africa relative to the southern African sub-region).

Analyses

To assess the degree of spatial congruence between areas where breeding groups occur and anthropogenic threats, we grouped all 117 frog species occurring in South Africa (du Preez & Carruthers, 2009; IUCN 2010; we did not include the monotypic *Cacosternum poyntoni* as we considered it to be a synonym of *C. nanum*) according to their developmental modes. These included 92 species of aquatic-breeders and 25 species of terrestrial-breeders (see Becker & Loyola, 2008; Loyola *et al.*, 2008). Aquatic-breeders were defined as species that require an aquatic stage to complete their life cycle and terrestrial-breeders as species that do

not consist of an aquatic life stage to complete their life cycle. Depending on where the aquatic life stage occurs (see Becker *et al.*, 2010), eight species of the aquatic-breeders were further grouped into stream-breeders, 43 species into permanent aquatic-breeders, and 41 species into temporary aquatic-breeders. Using Arc View GIS 3.3 (ESRI, 1998), we mapped the spatial extent of areas where each of the four different breeding groups occur (i.e., terrestrial-, stream-, permanent aquatic-, and temporary aquatic-breeders) across South Africa based on the spatial distribution of each frog species representing a given category at the quarter-degree resolution (see Appendix S2 in “Supporting Information” for a map showing the spatial extent of areas where breeding groups occur).

To assess the degree of spatial congruence between anthropogenic threats and each of the areas where breeding groups occur, data on four potential anthropogenic threat variables were obtained for South Africa at the quarter-degree grid cell resolution (see Appendix S3 in “Supporting Information” for the distribution of potential anthropogenic threats considered). These anthropogenic threats included: 1) human population density based on a 2001 census data for South Africa (Anonymous, 2001); 2) percentage land transformation based on 1994–1995 data from Thompson (1996) and Fairbanks *et al.* (2000); 3) percentage protected area based on 637 national protected areas mapped in the World Database on Protected Areas (WDPA, 2004); and 4) alien plant species richness data. The alien plant species richness data were obtained from three sources, namely: 1) the *Southern African Plant Invaders Atlas* (“SAPIA”) with records for over 500 species (Henderson, 1998; 1999; 2001); 2) the National Herbarium Pretoria Computerized Information Service (“PRECIS”) comprising over 800 000 herbarium specimens with records for over 24 000 taxa collated from all major South African herbaria (Germishuizen & Meyer, 2003); and 3) the “*Catalogue of Problem Plants in Southern Africa*” (Wells *et al.*, 1986) using a filtered list of taxa including 711 species alien to South Africa (for details, see Richardson *et al.*, 2003, pp. 295). It should be noted,

however, that the available data only included woody invasive plant species, especially in natural and semi-natural ecosystems (Richardson *et al.*, 2005). We acknowledge that other factors such as infectious diseases, pollution and chemical contaminations are important threats to anurans (Branch & Harrison, 2004). These were, however, not included in our analyses because of the lack of appropriate data at the resolution and scale at which this study was conducted.

Co-linearity levels between the different potential anthropogenic threats were first determined prior to any statistical analysis (see Wilson *et al.*, 2008). To determine the co-linearity levels, the tolerance value for each predictor (i.e., anthropogenic threat) variable was determined. Tolerance is defined as 1 minus the squared multiple of a predictor variable with all other independent variables in the regression equation (StatSoft Inc., 2005). The lower the tolerance level of a given variable, the stronger the correlation between the variable in question and one or more of the other predictor variables. Following Quinn & Keough (2002), variables with tolerance values < 0.1 should be eliminated from subsequent analyses due to redundancy. None of the predictor variables were found to be redundant due to co-linearity (tolerance values ranged between 0.2 and 0.96). Human population density was found to be the strongest predictor of the other potential anthropogenic variables examined, except for percentage protected area which, in contrast, showed its strongest co-linearity with alien plants species richness.

To assess whether the grid cells representing areas where breeding groups occurs are likely to be characterized by higher human population density values than expected by chance, firstly, we calculated the mean human population density of the 1 147 grid cells representing terrestrial-breeders. The observed mean value was then compared with the mean human population density values found for 10 000 sets of randomly selected grid cells (selected from a pool of all possible grid cells, namely, 1 954 grid cells). The number of

randomly selected grid cells was equivalent to the number of grid cells within which terrestrial-breeders are found (i.e., 1 147 grid cells; see van Rensburg *et al.*, 2004a where a similar approach was followed). Secondly, following van Rensburg *et al.* (2004a), mean human population density values were calculated for the remaining three groups of areas where frog breeding groups occur (i.e., stream-, permanent aquatic-, and temporary aquatic-breeders). Thirdly, data for the remaining three potential anthropogenic threat (i.e., percentage land transformation, percentage protected area, and alien plant species richness) with reference to each of the areas where different breeding groups occur were similarly analysed as outlined above. Finally, mean values for each threat variable were compared between the different breeding habitat categories at the national scale, and across the different biogeographical areas using non-parametric Kruskal-Wallis analysis of variance (ANOVA; Zar, 1996). Where statistically significant differences were detected, maximally non-significant subsets ($P > 0.05$) were derived by the *a posteriori* Duncan's *post-hoc* test procedure using ranked means (Sokal & Rohlf, 1981).

RESULTS

Although areas representing terrestrial- and stream-breeders show a strong association with protected areas at the national scale (Tables 1 & 2), these areas had significantly larger mean human population density, land transformation and alien plant richness values ($P < 0.001$; 10,000 permutations) than expected by chance (Table 1). In contrast, all these anthropogenic variables scored their lowest mean values in areas representing both permanent aquatic- and temporary aquatic-breeders (Table 2), and spatially, these areas (representing aquatic-breeders) showed no significant congruence with any of the threat variables (Table 1).

Considering the biogeographical scale, terrestrial-breeders were spatially congruent with anthropogenic threats than expected by chance in three of the seven biogeographical regions examined, namely the south central, the southwest arid, and the southwestern Cape regions (Table 1). Land transformation and alien plant richness were most significant in these regions. Moreover, the south central was the only region where terrestrial-breeders showed significant spatial congruence with protected areas (Table 1). In contrast, stream-breeders showed significant anthropogenic threats in five of the seven biogeographical regions examined, the most notable results being: 1) the consistent significant threat posed by alien plant species richness (in four of the five regions), and 2) the overall strong association with protected areas, although a significant negative association was true for the Maputaland region (Table 1). No statistically significant results were found for permanent aquatic- and temporary aquatic-breeders (Table 1). Compared to all the biogeographical regions examined, the east African lowlands was the only region where none of the threats were spatially congruent with any of the areas where frog breeding groups occur (Table 1).

Comparing mean values for each threat variable across all the biogeographical areas representing different frog assemblages, the southwestern Cape together with Maputaland region showed significantly higher mean human population density, land transformation and alien plant species richness compared to the other regions examined (Table 3). The south central followed by the south east lowlands and the Maputaland are the least protected regions; while the Zambesian/Bushveld woodlands followed by east African lowlands were highly congruent with the percentage of protected area (Table 3).

DISCUSSION

The distribution ranges of stream- and terrestrial-breeding frog species in South Africa, at both the national and biogeographical scales, were spatially congruent with anthropogenic activities that are likely to threaten their existence. Of concern is that most of the threatened stream-breeders in South Africa are from a single near-endemic family, the Heleophrynidae (which also occurs in Lesotho and Swaziland). Heleophrynidae tadpoles are especially vulnerable to factors affecting water flow and quality such as alien invasive plants (see further discussion below) because of their long period ($>$ one year) to complete metamorphosis and thus requires permanent streams (du Preez & Carruthers, 2009).

At the national scale, the present study suggests that terrestrial- and stream-breeders are well-represented by the current protected area network in South Africa. These results support Drinkrow & Cherry (1995) who found that *ca.* 90% of anuran species in South Africa were also congruent with protected areas. However, careful consideration of factors that may negate the significance of these positive results is warranted. Firstly, although a given species is present in one or more protected areas, the extent to which its full distributional range is being represented (or not) by a matrix of protected areas is often unknown. This is because the presence of a given species within a protected area does not guarantee long-term protection of viable populations of the species (Armstrong, 2001). Secondly, should the sampling of frog atlas data be biased towards protected areas, spurious results may be obtained when examining spatial patterns between frog diversity and protected areas (Botts *et al.*, 2011). Thirdly, at the biogeographic scale, both stream- and especially terrestrial-breeders were poorly represented in the reserve network within most of the biogeographic regions (see further discussion below). Fourthly, the current analysis was undertaken at a QDS resolution, which covers a much larger area than frog breeding habitats. Several studies have highlighted

positive relationship between human induced threats and biodiversity at such coarse spatial scales (Chown *et al.*, 2003; Pautasso, 2007). We do acknowledge that negative relationship might be true at more local level investigations. Finally, the potential influence of a combination of the above factors also needs to be taken into consideration.

Our non-significant results for both permanent and temporary aquatic-breeders compliment Darwall *et al.* (2009) who also highlighted the paucity of protected areas within freshwater systems (see also Nel *et al.*, 2007). Based on a global dataset, Becker & Loyola (2008) found that aquatic-breeders have an exceptionally high extinction risk due to their generally low congruence with protected areas. It is possible that the general lack of spatial congruency with protected areas globally, especially for amphibians (Rodrigues *et al.*, 2004; Sohdi *et al.*, 2008) may contribute towards high extinction risk. This may be due to limited options to build more comprehensive conservation networks, especially in areas important for conservation because of the positive relationship between species richness and human densities (or impacts) as has been shown for many countries globally (Chown *et al.*, 2003; Gaston, 2005; Hugo & van Rensburg 2008; Luck *et al.*, 2010). An additional explanation for poor reserve representation that may especially be true for stream-breeders where individuals generally have small distribution ranges, is that conservation decisions may often be implemented at a scale simply too coarse to capture fine-scale heterogeneity in species distributions (Rebelo, 1997; Reyers *et al.*, 2001; Cowling & Pressey, 2003; Reyers, 2004).

Given that none of the threats examined in this study showed significant spatial congruence with both permanent and temporary aquatic-breeders at both the national and biogeographic scales, it may be possible that the threats used in our analysis were not sensitive enough to be applied in the analysis of aquatic systems despite some significant relationships between stream-breeders and some threats used herein. It may be possible that

other threats such as water pollution, chemical contamination as well as the chytrid fungus (*Batrachochytrium dendrobatidis*) may have more influence on the survival of aquatic-breeding populations locally (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003; see also Kerby *et al.* (2010) on why amphibians may be poor environmental indicators). Although there is a need for further research, the presence of the chytrid fungus has been confirmed in some aquatic-breeders in South Africa (Hopkins & Channing, 2003; Weldon *et al.*, 2004), despite having not resulted in any known mass frog die-offs.

Other potential explanations for the low spatial congruence between aquatic species distributions and anthropogenic threats (including protected area distribution) may include factors such as: 1) spatially, the aquatic-breeders are found in almost every grid cell spanning the study area (see Measey, 1998; Lobos & Measey, 2002; Lobos & Jaksic, 2005; Tolley *et al.*, 2010 for more information on the distribution, invasion patterns and adaptability of these species), thus there is a high likelihood for these species to maintain viable populations in areas that are not yet highly threatened and/or under any formal protection (see Appendix S2, B & C); and 2) given the large spatial range of the species within these breeding groups, there is a low likelihood that a given mean threat value calculated across the grid cells representing the observed spatial range (for a given breeding group) will show a significantly higher (or lower) value compared to the randomly selected means due to the large spatial overlap between the grid cells representing the observed *versus* those representing a randomly selected mean. More detailed studies of anthropogenic threats to aquatic breeders at the regional scale are therefore needed.

There is poor spatial overlap between protected areas and areas where different frog breeding groups occur at the biogeographical scale. Compared to the other breeding groups, stream-breeders were the best represented group within the current reserve network although

significance was only reached in the south east lowlands, southwestern arid and southwestern Cape biogeographic areas. These results may be explained by the way protected areas were historically designated in South Africa such as the bias towards the savanna and the fynbos biomes (Reyers *et al.*, 2001) with an emphasis for conserving riparian zones, high altitude sites and forested habitat types (Rebelo, 1997). A conservation bias towards riparian zones may contribute positively to the conservation of frog species in general. However, riparian zones in South Africa (le Maitre *et al.*, 2000; van Wilgen *et al.*, 2001; Meek *et al.*, 2010) and elsewhere (Greenwood *et al.*, 2004) are among the most heavily invaded habitat types (mainly by plants) and this is also true in protected areas (Foxcroft & Richardson, 2003; Foxcroft *et al.*, 2007). Our analyses support the findings that alien plant species richness was the most prominent threat to stream-breeders across the different biogeographic areas.

Biological invasions in general are known to have dire consequences for anurans. In South Africa, compared to global trends, biological invasions (mainly due to invasive alien plants species) affect a disproportionate number of threatened anuran species (37% compared to 16% globally; Angulo *et al.*, 2011). For example, Branch & Harrison (2004) reported that exotic pine stands have negative outcomes on the recruitment rates of Hewitt's ghost frog (*Heleophryne hewitti*) due to a reduction in stream flow. The impacts of spreading invasive alien vegetation and afforestation is also well-known for affecting amphibians in fire-driven biomes such as the fynbos due to increased fuel and therefore fire intensity from which many threatened amphibians struggle to recover (Minter *et al.*, 2004; Angulo *et al.*, 2011). This was supported by our analyses where alien plant species richness was prominent in the southwestern Cape for both terrestrial- and stream-breeders. Alien plant species may also lead to a reduction in both the number and abundance of native insect species which support indigenous amphibian biota (Maerz *et al.*, 2005). Data on invading amphibians in South Africa is however lacking (van Wilgen *et al.*, 2008). Nonetheless, there have been reports of

human-mediated range expansion of the painted reed frog (*Hyperolius marmoratus*) in the western Cape province of South Africa (Tolley *et al.*, 2008), and the introduction of the Guttural Toad (*Amietophrynus gutturalis*) in sub-urban Cape Town (de Villiers, 2006), which has begun to rapidly expand in recent years (Measey & Davies, in press).

Land transformation and alien plant richness seem to be the most dominant threats to terrestrial-breeders with significant results found in the south central, the southwest arid, and the southwestern Cape regions (Angulo *et al.*, 2011; Table 1). The grassland biome represents one of the most highly populated and highly transformed parts of South Africa. This biome has a number of densely populated centres mainly due to employment opportunities in the mining and agricultural industries (O'Connor & Bredenkamp, 1997; Bredenkamp *et al.*, 2006) which may elevate the vulnerability of terrestrial-breeders. However, significant spatial overlap between areas where terrestrial breeders occur and the proportion of protected areas was only found in the south central region, despite only 2% of this region being under formal protection. O'Connor & Bredenkamp (1997) found that a high proportion of plant species (*ca.* 78%) were congruent with protected areas within the grassland biome (which spans the bulk of the south central biogeographic area), despite low levels of protection in this biome. Of significance for amphibians in these areas is that most of the terrestrial-breeders are brevipeptides. Some of these species occur only in the low-lying and highly transformed areas of the Western Cape Province (Minter, 2004). Conversely, members of the other terrestrial-breeding genus *Arthroleptella* (Pyxicephalidae) occur mostly in high altitude protected sites of the Western Cape Province (Channing, 2004).

In conclusion, both globally and in South Africa, Red List assessments have highlighted the consistency between patterns of major threats affecting amphibian conservation; most notably agricultural and aqua-cultural activities, and in South Africa,

these are coupled with biological invasions (Angulo *et al.*, 2011). In a recent global assessment, compared to birds and mammals, the extinction risk of amphibians showed the greatest increase over time because of biological invasions (McGeoch *et al.*, 2010). Using all species at a sub-continental scale (at a QDS resolution), rather than a single species approach used by Red List Assessors, our study identified land transformation and alien invasive plants as significant threats to areas important for the long-term breeding success of both stream- and terrestrial-breeders in South Africa. At the biogeographical scale, the areas where different frog breeding groups occur in the southwestern Cape showed the greatest spatial congruence with the threats examined, especially alien invasive plants. Finally, our study suggests that areas where different frog breeding groups occur are, in general, not well-represented in the current conservation network when examined at the biogeographical scale and this was true especially for terrestrial-breeders. In areas where a breeding group was reasonably well-represented in the protected area network (mainly stream-breeders), there is a need for further research to investigate the extent to which frog species distributional ranges are being captured by the reserve network in order to assess long-term population viability. This is an important, but currently outstanding quantification that needs attention to further our efforts in amphibian conservation. The results presented here have important implications in addressing the current status of threats on amphibians in a biogeographical context, which to date has largely been anecdotal (see Minter *et al.*, 2004).

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Table 1. Results indicating whether grid cells in South Africa representing different groups of frogs, based on where each group occur, have significantly greater values of anthropogenic threats than expected by chance based on 10 000 permutations. The assessment was based on two spatial scales, namely, a national scale and a smaller biogeographical scale (consisting of seven biogeographical areas (see the Methods section on how these areas were identified)). 1 = Human population density; 2 = Percentage land transformation; 3 = Alien plant species richness; and 4 = Percentage protected area.

Region	Areas where different breeding habitats occur			
	Terrestrial	Stream	Permanent aquatic	Temporary aquatic
National	(1-4) ⁺⁺⁺	(1-4) ⁺⁺⁺	NS	NS
South central	(1,2) ⁺⁺⁺	NS	NS	NS
	(3,4) ⁺⁺			
Zambesian/ Bushveld woodlands	NS	3 ⁺⁺ (2,4) ⁺	NS	NS
East African lowlands	NS	NS	NS	NS
Southeast lowlands	NS	3 ⁺⁺⁺ 4 ⁺⁺	NS	NS

Region	Areas where different breeding habitats occur			
	Terrestrial	Stream	Permanent aquatic	Temporary aquatic
Maputaland	NS	4 ⁻⁻⁻	NS	NS
Southwest arid	2 ⁺⁺	4 ⁺⁺⁺	NS	NS
	3 ⁺	3 ⁺⁺		
Southwestern	3 ⁺	4 ⁺⁺⁺	NS	NS
Cape		3 ⁺⁺		
		2 ⁻⁻⁻		

Statistical significance higher than expected by chance: ⁺⁺⁺ = $P < 0.001$; ⁺⁺ = $P < 0.01$; ⁺ = $P < 0.025$; statistical significance lower than expected by chance: ⁻⁻⁻ = $P > 0.001$; NS = not statistically significant.

Table 2. Kruskal-Wallis analysis of variance (ANOVA) test of anthropogenic threat variables in areas where each frog breeding group occur identified in South Africa at the national scale.

Areas where different frog breeding groups occur	Anthropogenic threats			
	Human population density (2001)	Percentage land transformation	Alien plants species richness	Percentage protected area
	Mean $\pm$ S.E			
	$H_{(1,3)} = 278.2;$	$H_{(1,3)} = 187.4;$	$H_{(1,3)} = 264.5;$	$H_{(1,3)} = 141.7;$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Permanent aquatic	$22,932 \pm 2,028.2^a$	21 ± 0.6^a	17 ± 0.6^a	6 ± 0.4^a
Temporary aquatic	$22,982 \pm 2,033.3^a$	21 ± 0.6^a	17 ± 0.6^a	6 ± 0.4^a
Terrestrial	$35,439 \pm 3391.9^b$	28 ± 0.8^b	22 ± 0.9^b	9 ± 0.6^b
Stream	$46,117 \pm 6,371.5^c$	35 ± 1.5^c	36 ± 2.0^c	11 ± 1.1^c

S.E. = Standard Error; superscripts denote the significantly different subsets based on Duncan's *post hoc* test procedure

Table 3. Kruskal-Wallis analysis of variance (ANOVA) test of anthropogenic threat variables and biogeographical regions representing different anuran assemblages in South Africa.

Biogeographic region		Anthropogenic threats			
		Human population density (2001)	Percentage land transformation	Alien plants species richness	Percentage protected area
		Mean $\pm$ S.E			
		$H_{(1,6)} = 416.0;$ $P < 0.001$	$H_{(1,6)} = 536.3;$ $P < 0.001$	$H_{(1,6)} = 468.0;$ $P < 0.001$	$H_{(1,6)} = 425.1;$ $P < 0.001$
South central		19,929 $\pm$ 3,609.6 ^a	15 $\pm$ 0.8 ^a	12 $\pm$ 0.7 ^a	2 $\pm$ 0.3 ^a
Zambesian/ Bushveld woodlands		35,579 $\pm$ 4,671.9 ^b	24 $\pm$ 1.4 ^b	22 $\pm$ 2.0 ^b	18 $\pm$ 1.8 ^b
East African lowlands		23,895 $\pm$ 4,139.9 ^b	36 $\pm$ 5.0 ^{b, c}	26 $\pm$ 3.4 ^{b, c}	16 $\pm$ 3.0 ^b
South lowlands	east	22,674 $\pm$ 3,125.3 ^b	26 $\pm$ 1.3 ^c	23 $\pm$ 1.3 ^c	4 $\pm$ 0.5 ^c
Maputaland		86,090 $\pm$ 20,887.0 ^c	45 $\pm$ 2.8 ^c	42 $\pm$ 4.2 ^c	7 $\pm$ 1.3 ^{c, d}

Biogeographic region	Anthropogenic threats			
	Human population density (2001)	Percentage land transformation	Alien plants species richness	Percentage protected area
	Mean $\pm$ S.E			
Southwest arid	7,514 $\pm$ 3,971.5 ^d	6 $\pm$ 0.8 ^a	12 $\pm$ 1.1 ^a	11 $\pm$ 1.3 ^d
Southwestern Cape	55,812 $\pm$ 14,035.8 ^{b, c}	59 $\pm$ 2.0 ^c	51 $\pm$ 3.2 ^c	14 $\pm$ 1.3 ^b

S.E. = Standard Error; superscripts denote the significantly different subsets based on Duncan's *post hoc* test procedure.

SUPPLEMENTARY MATERIAL

Appendix S1. A quarter-degree grid cell map of seven anuran biogeographic regions occurring within the political boundaries of South Africa.

Appendix S2. Maps indicating the spatial distribution of anuran species richness based on four (A to D) areas where different frog breeding groups occur in South Africa. A = stream breeding species, B = permanent aquatic breeding species, C = temporary aquatic breeding species, D = terrestrial breeding species.

Appendix S3. Maps showing the extent of the distribution of factors affecting threats to anurans (A to D) in South Africa. A = human population density, B = land use transformation, C = alien plants species richness, D = percentage protected area.