The conservation value of freshwater habitats for frog communities of lowland fynbos (#79676)

First revision

Guidance from your Editor

Please submit by **9 Mar 2023** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
3 Figure file(s)
2 Table file(s)
1 Other file(s)

Custom checks

Vertebrate animal usage checks

- Have you checked the authors ethical approval statement?
- Were the experiments necessary and ethical?
- Have you checked our animal research policies?

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

The conservation value of freshwater habitats for frog communities of lowland fynbos

Ignatius L Terblanche [1] , **John Measey** [Corresp. 2]

[1] Riverglade Retirement Village, Parklands 7441, unaffiliated, Cape Town, South Africa
[2] Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Stellenbosch, South Africa

Corresponding Author: John Measey
Email address: johnmeasey@gmail.com

Amphibians are more threatened than any other vertebrate class, yet evidence for many threats is missing. The Cape lowland fynbos (endemic scrub biome) is threatened by habitat loss, and natural temporary freshwater habitats are removed in favour of permanent impoundments. In this study, we determine amphibian assemblages across different freshwater habitat types with special attention to the presence of invasive fish. We find that anuran communities differ primarily by habitat type, with permanent water habitats having more widespread taxa, while temporary water bodies have more range restricted taxa. Invasive fish are found to have a significant impact on frogs with toads most tolerant of their presence. Temporary freshwater habitats are a conservation priority in the area, and their amphibian assemblages represent endemic taxa that are intolerant of invasive fish. Conservation of a biodiverse amphibian assemblage in lowland fynbos areas will rely on the creation of temporary freshwater habitats, rather than a northern hemisphere pond based solution.

The conservation value of freshwater habitats for frog communities of lowland fynbos

I.L. Terblanche[1] & J. Measey[2]*

1. Riverglade Retirement Village, Parklands 7441, Cape Town, South Africa
2. Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa

*Corresponding Author:
John Measey
Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa
Email address: john@measey.com
John Measey: http://orcid.org/0000-0001-9939-7615

Abstract

Amphibians are more threatened than any other vertebrate class, yet evidence for many threats is missing. The Cape lowland fynbos (endemic scrub biome) is threatened by habitat loss, and natural temporary freshwater habitats are removed in favour of permanent impoundments. In this study, we determine amphibian assemblages across different freshwater habitat types with special attention to the presence of invasive fish. We find that anuran communities differ primarily by habitat type, with permanent water habitats having more widespread taxa, while temporary water bodies have more range restricted taxa. Invasive fish are found to have a significant impact on frogs with toads most tolerant of their presence. Temporary freshwater habitats are a conservation priority in the area, and their amphibian assemblages represent endemic taxa that are intolerant of invasive fish. Conservation of a biodiverse amphibian assemblage in lowland fynbos areas will rely on the creation of temporary freshwater habitats, rather than a northern hemisphere pond based solution.

Key Words: Anura; freshwater habitats; frogs; invasive fishes; ponds; toads; seepage

Introduction

Amphibian conservation has centred around three major themes: habitat change, disease and invasive species (Grant et al., 2019). Freshwater habitats are in particular peril, with declines that are far greater than terrestrial ecosystems (Dudgeon et al., 2006). However, the construction of artificial impoundments is increasing rapidly (Downing et al., 2006), and changing the nature of landscapes especially in arid ecosystems, where these permanent impoundments facilitate invasions of freshwater species (Johnson, Olden & Vander Zanden, 2008). Habitat change and invasive species impact more species globally and are the proximate causes of conservation concern for the majority of amphibian species (IUCN 2022). Despite a general acknowledgement of these mechanisms, conservation evidence for impacts and their commensurate measures for the conservation of amphibians continues to be low (Meredith, Van Buren & Antwis, 2016).

The impacts of invasive species on amphibians have been assessed qualitatively (Bucciarelli et al., 2014; Falaschi et al., 2020), and quantitatively (Nunes et al., 2019). Invasive freshwater fish are ranked highly in reviews of invasive species as causing severe impacts on many amphibian communities (Hecnar & M'Closkey, 1997; Ficetola & De Bernardi, 2004; Hartel et al., 2007; Holbrook & Dorn, 2016). Excluding invasive fish from sites with threatened frog species has resulted in recovery of anuran populations in Spain and Portugal (*Rana iberica* Bosch et al., 2019) and California (*Rana mucosa* Knapp, Boiano & Vredenburg, 2007), leading those workers to identify the proximate role of invasive fish as a threat to amphibian populations. But the impacts of invasive fish are poorly described in the southern hemisphere (except Australia), especially with respect to amphibian communities. However, many amphibian communities are driven by natural environmental factors as well as anthropogenically driven creation and modifications of freshwater habitats (Ficetola & De Bernardi, 2004; Hartel et al., 2007; Kruger, Hamer & Du Preez, 2015).

The low-lying fynbos of South Africa's Cape region is a biodiversity hotspot (Myers et al., 2000), composed of evergreen, Mediterranean scrub vegetation (Mucina & Rutherford, 2006). The fynbos also holds an important community of amphibians that have high conservation concern (Measey, 2011; Schreiner, Rödder & John Measey, 2013; Mokhatla, Rödder & Measey, 2015).

Much of the habitat where amphibians and other flora and fauna were once abundant has been transformed for agriculture and more recently for housing (Measey & Tolley, 2011; Rebelo et al., 2011; Measey et al., 2014). Where land has been transformed, temporary wetlands have been infilled and permanent impoundments (dams) or ponds added to the landscape. The addition of permanent water and the introduction of alien fish has been ongoing for ~200 years (Ellender & Weyl, 2014). Angling is a popular pastime in the region, and anglers introduce fish to new impoundments and natural waterbodies (Ellender et al., 2014).

Southern Africa has no salamanders or caecilians, but several major radiations of anurans, many of which specialise in lowland temporary aquatic habitats (Poynton, 1964). The extreme southwestern corner of the continent has a mediterranean climate with winter rains and dry hot summers (Wilson et al., 2020). Several species that rely on temporary water have become threatened, while those that thrive in permanent water have become abundant and ubiquitous even in arid areas. ~~Examples of IUCN threatened species include the Western Leopard Toad *Sclerophrys pantherina* (EN), the Cape Platanna *Xenopus gilli* (EN), the Microfrog *Microbatracella capensis* (CR), and the Flat Caco *Cacosternum platys* (NT).~~

In this paper, we ~~aim to determine~~ whether invasive fish and habitat characteristics (especially temporary vs. permanent water) ~~correlate with Cape lowland amphibian communities~~. In particular, we were interested ~~to find out~~ whether anthropogenically constructed impoundments are useful sites for threatened amphibian communities, in the presence or absence of invasive fish.

Methods & Materials

Site selection

Using Google Earth imagery from 2017, we classified every waterbody visible within our study area (2 catchments) into our nominate freshwater body types being natural: vleis (natural temporary shallow water bodies), natural pools and river edges in the fynbos, and anthropogenically created: small dams (artificial impoundments <2000 m^2 including ponds), and large dams (artificial impoundments >2000 m^2). In lowland fynbos, slow moving rivers are effectively temporary water bodies and have the same amphibian species assemblages (Channing, 2019). This gave us a candidate list of 196 sites (see Table S1) all chosen from within the fynbos biome (see Mucina & Rutherford, 2006).

We made our initial stratified sampling selection from within these 196 sites to represent balanced numbers of freshwater body types, equally represented across space, and these were further refined once we requested permission to access sites from landowners. The final 50 sites selected fell within two separate catchments, varied in their spatial proximity and different water body types (see Fig. 1; Supp Info; Table S1).

>>Figure 1

Anuran data collection

Each site was visited three times during the day and into the first half of the night in the active winter period (between May and August 2016-2017), with a pseudorandom order of sites as permission to visit was granted. At each site we performed a standardised method to survey anurans. Firstly, we walked around the waterbody during the day and again at night with a headlight looking for adult anurans. We set audio recorders at each site for two nights on each site visit to collect calling data. Lastly, we set funnel traps over two nights for tadpoles and adult aquatic frogs (Xenopus species). After identification, all individuals were immediately released on site. All fieldwork was authorised by CapeNature (permit number: AAA043-00449). The research protocol was approved by Stellenbosch University Research Ethics Committee: Animal Care and Use (ethics number: SU-ACUD15-00101).

We identified calls using spectrograms in Audacity (http://audacityteam.org/) against a set of calls for species in South Africa (Du Preez & Carruthers, 2017). Adults were identified against descriptions and keys in two field guides (Du Preez & Carruthers, 2017; Channing, 2019). Tadpoles were identified from their mouthparts according to du Preez & Carruthers (2017) using a stereomicroscope for exemplars from each site. Taxonomy for all species was corrected to Frost (2020), and we consulted relevant new literature with respect to newly described cryptic species. For example, the genus of Dainty Frogs, *Cacosternum*, was found to have multiple cryptic species by Channing et al. (2013), but only one of these, *C. australis*, has been identified within this area (see Vogt et al., 2017).

Fish data collection

To determine whether fish were present at each locality, we consulted landowners and local recreational fishermen for images of species caught within our sampling period. This evidence was added to our own sampling when reports were ambiguous. Sites with temporary water were assumed to be fishless.

Site data collection

For each site, we measured the area and perimeter of the waterbody using tools in Google Earth with images from mid-Winter (June and July) when they were at their maximum size, to correspond with our sampling date. We also noted the latitude and longitude of the centre point of each site, and its catchment. Previous studies have been suggested area and perimeter to be of importance in structuring amphibian communities (Hamer & Parris, 2011; Kruger, Hamer & Du Preez, 2015). We measured conductivity as some sites were in close proximity to the sea, and pH (Hannah Instruments) as low pH has been considered important to species inhabiting naturally acidic fynbos pools (e.g., Picker, McKenzie & Fielding, 1993). As vegetation in the fynbos is typically low, we did not record vegetation surrounding aquatic habitats. Hydroperiod of sites is known to be of great importance for amphibian community structure (Pechmann et al., 1989; Wellborn, Skelly & Werner, ~~2003).~~ Together with our waterbody class (see site selection above), these measures were used as environmental covariates in the data analysis.

Data Analyses

We first investigated our site covariates for multicollinearity using Variance Inflation Factors (VIF) in the car package (Fox & Weisberg, 2019). We considered a VIF threshold value of <5 as causing negligible collinearity effects. Subsequently, we divided the site environmental variables into two groups: those environmental variables with the potential to structure amphibian communities (pH, conductivity, hydroperiod, area, perimeter and invasive fish), and covariates which might confound the analysis through spatial autocorrelation, catchment assignment or the order of visit (date visited, latitude, longitude and catchment).

To test which of our environmental variables explained the frog community, we ran a partial Redundancy Analysis (partial RDA) in package vegan (Oksanen et al., 2022). RDA is a constrained ordination analysis that models the effects of a matrix of explanatory variables (here environmental variables) on a complementary community matrix (here species of amphibians) using multiple regression (Legendre & Legendre, 2012). The partial RDA used in this study allows for a third matrix of covariates (here spatial and date data) to be controlled for when conducting the RDA by calculating the residuals of the two covariate matrices before conducting the RDA (Legendre & Legendre, 2012). In effect, this removes the effect of these background variables before the RDA proper. For our frog community data, we used presence/absence data for each of the identified anuran species at each of the 50 sites. Presence was determined through either adults captured, calls recorded or tadpoles in traps.

In our partial RDA model we used frog species as our community matrix (X), the environmental variables as our explanatory matrix (Y), and the spatial and date covariates as the condition matrix (Z) in the formula: rda(X ~ Y + Condition(Z). We calculated the adjusted R^2 of the model to determine the proportion of the percentage of the variance in the amphibian community explained by the variation in frog community composition across sites, and we performed a permutation test with 10,000 steps (using anova.cca in package vegan) to test whether the model is significant. We used further permutation tests (with 10,000 steps each using anova.cca) to test for the significance of each variable and each canonical axis (using "term" and "axis", respectively). Lastly, we used ggplot2 (Simpson, 2015) to plot the partial RDA model results.

To further explore the influence of invasive fish and other environmental variables on the anuran community, we used an unconstrained ordination analysis on a reduced dataset with a non-metric multidimensional scaling (NMDS) analysis using metaMDS with the Jaccard similarity index (suitable for presence/absence data) in package vegan with a maximum of 1000 tries, and no autotransformation. We increased the number of dimensions (k) until increases failed to reduce the stress value by more than 0.05. We then used envfit in package in vegan with our reduced set of continuous and discrete environmental variables (see above for removal of correlated environmental variables) to determine whether they were significant determinants of our amphibian species assemblages. We used envfit to discover which of the amphibian species were contributing most to the community distributions. Because invasive fish can only occur in permanent water, we reduced the dataset to the 36 permanent water sites. We then used a permanova on distance matrices (adonis2) in vegan. We used the coefficients of the output from the permanova to determine amphibian species sensitivity to fish.

Results

We identified 11 different anuran species (Table 1) across the 50 sites sampled. All sites sampled were found to have at least one species of anuran, with a maximum of nine and a minimum of one. No species were found exclusively at permanent or temporary water sites (Table S2). In addition, we had evidence of three invasive fish species: Large-Mouth Bass

Micropterus salmoides, Small-Mouth Bass *M. dolomieu* and Mozambique Tilapia *Oreochromis mossambicus* from 14 sites.

>>Table 1

The partial RDA model explains 10.0% of the variation in frog community composition across all sites, and is statistically significant (F = 1.507; P = 0.005). From the partial RDA, we found that the best measured environmental determinants of the amphibian community were the presence or absence of invasive fish (F = 2.412; P = 0.009; Figure 2; Table 2), and the type of wetland habitat sampled (F = 1.548; P = 0.033). In figure 2, only the first axis was found to be significant (F = 6.472; P < 0.001), but the opposing direction of the arrows on wetland type show that anthropogenic impoundments (small and large dams) have an important and opposite impact on the amphibian community to natural sites (temporary vleis, pools and river edges).

>> Table 2
>> Figure 2

Our exploratory analysis on how invasive fish impact amphibian assemblages in permanent water (using 36 of the 50 sites) produced a 2-dimensional NMDS fit with stress of 0.192, which showed significant differences using adonis2 (R^2 = 0.089; P = 0.025; Figure 3). The two species most tolerant of the presence of fish were the toads: *Sclerophrys capensis* and *S. pantherina*, while the species most intolerant of fish was *X. laevis* (Figure 3; Table S3).

>> Figure 3

Discussion

Our study stresses the importance of freshwater types which determine the type of amphibian community in the southwestern Cape, with an important division between anthropogenically created water bodies (irrespective of size), and those that occur naturally in the fynbos. Permanent water bodies generally hold widespread species, while temporary sites typically hold fynbos endemic species. In addition, we show that the presence of invasive fish in permanent water bodies also impacts amphibian assemblages. Our results indicate that building permanent water bodies, whether they be large impoundments for agricultural water supply or small garden ponds, will favour different amphibian communities from those present in sites with temporary water. Many urban homeowners create permanent small ponds in their gardens with conservation goals. However, our results indicate that trends for increasing biodiversity in urban areas by creating ponds championed in the northern hemisphere (Hassall, 2014; Hill, Lawson & Tuckett, 2017; Hill et al., 2018) are inappropriate in the fynbos where large impoundments already provide for assemblages that require permanent water. Permanent impoundments also promote invasions of both fish and amphibians (Davies et al., 2013; Ellender et al., 2014). Currently, amphibians that rely on temporary water in lowland fynbos are poorly served by anthropogenically created wetlands, but could be better conserved by the promoting construction of temporary water bodies instead of ponds.

While no anuran species was exclusive to permanent water, these types of water bodies were commonly associated with more widespread species: *Amietia fuscigula, Hyperolius horstocki, Xenopus laevis* and *Tomopterna delalandii*. These species are not endemic to the area, while those associated with temporary water have much smaller distributions (~20 000 km^2). Toads (*Sclerophrys capensis* and *S. pantherina*) were most tolerant of the presence of invasive fish, presumably because their eggs and larvae are toxic and adults have prominent parotid glands (Hecnar & M’Closkey, 1997; Crossland & Alford, 1998; Caller & Brown, 2013). The species most intolerant to the presence of fish was *X. laevis*, which may be because they are principally aquatic and encounter fish more often than other frogs. The area we sampled did not include some threatened species present in the lowland fynbos, for example *X. gilli* (EN) and *Microbatrachella capensis* (CR), but these are most commonly associated with temporary water (JM pers. obs.).

Historically, these areas of lowland fynbos would have had very few permanent water bodies. The sediment is typically sand or silty soils over young Quaternary sediments, largely derived from weathering Table Mountain sandstones and Cape Supergroup shales (Cawthra et al., 2020). Rivers that flow year round may well have been augmented by the movements of large mammals to increase the permanent water features associated with them (Venter et al., 2020). Away from rivers, most water bodies would have formed through rainfall, or be fed by underground seepages, during the wet winter period, and completely dry out during summer. Much of the lowland fynbos areas have been developed and habitat loss continues to the present day (Skowno, Jewitt & Slingsby, 2021). The Cape Lowland Freshwater Wetlands are considered to be Critically Endangered in the National Ecosystem Status for South Africa (Dayaram et al., 2021).

Because we were not able to sample each site systematically for fishes, there is a chance that we have false negative data among some of the permanent water sites. For example, we found no mosquitofish, *Gambusia affinis*, in the water bodies we sampled, but they are known to be invasive in many drainages of South Africa (Weyl et al., 2020). Additionally, sites along river edges are likely to have more transient impacts from fish, unlike those of ponds of similar sizes. These false negatives may impact the reported position of anuran species in relation to invasive fish, but they are unlikely to change the overall result. Similarly, we cannot discount the possibility that some anuran species went undetected during our surveys.

We did not include native fish in our scoring. To our knowledge, none of the impoundments that we surveyed contained any native fishes. Sites along the river are reported to have Cape Kurper *Sandelia capensis* and *Galaxias* sp. 'Klein' (see Chakona, Swartz & Gouws, 2013). Of these, the Cape Kurper may have exerted some predation impact on amphibians. There are other native predatory species that may exert an impact on amphibian communities, such as the Cape Clawless Otter *Aonyx capensis*, Cape Terrapin *Pelomedusa galeata* and the Western Cape River Crab *Potamonautes perlatus*. All of these species are present in the area sampled and further study would be required to interpret their impact on amphibian communities.

Conclusions

Anthropogenically created permanent water bodies (regardless of size) and the presence of invasive fish significant alter amphibian communities in lowland fynbos by favouring widespread species. Our results question the dogma of creating urban ponds to increase biodiversity (Hassall, 2014; Hill, Lawson & Tuckett, 2017; Hill et al., 2018), at least for amphibian communities but possibly for other species. Recent success in restoring European amphibian populations with pond construction (Moor et al., 2022) needs to be taken in context, and not as a freshwater biodiversity panacea. Rather like the popular fixation on planting trees, the evolutionary and climatic context must take precedence when considering future conservation actions (Bond et al., 2019). While our research is pertinent to low-lying areas of the fynbos, the importance of hydroperiod in structuring aquatic communities, including amphibian communities, has been stressed before (e.g., Pechmann et al., 1989; Welborn, Skelly & Werner, 1996; Werner et al., 2007; Holbrook & Dorn, 2016). This is even more important in Mediterranean biomes where permanent water is an unusual natural feature, but anthropogenic need for access to water particularly for agriculture have made it the most abundant freshwater aquatic features. It may be that other southern African biomes may also have their amphibian communities strongly impacted by hydroperiod, but this remains untested (Kruger, Hamer & Du Preez, 2015). When opportunities arise for mitigation effects that call for creation of wetland habitats in the fynbos, we strongly encourage creation of temporary water features that are allowed to dry out during the summer months. This effectively excludes populations of invasive fish and increases the diversity of amphibian fauna endemic to the southwestern Cape lowlands.

Acknowledgements

We would like to thank all landowners for permission to survey fish and frogs on their property. We would like to thank Andrew Turner and Martine Jordaan from CapeNature for their help and assistance during this study.

Literature

Bond WJ, Stevens N, Midgley GF, Lehmann CER. 2019. The Trouble with Trees: Afforestation Plans for Africa. *Trends in Ecology & Evolution* 34:963–965. DOI: 10.1016/j.tree.2019.08.003.

Bosch J, Bielby J, Martin-Beyer B, Rincón P, Correa-Araneda F, Boyero L. 2019. Eradication of introduced fish allows successful recovery of a stream-dwelling amphibian. *PLOS ONE* 14:e0216204. DOI: 10.1371/journal.pone.0216204.

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB. 2014. Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. *Copeia* 2014:611–632. DOI: 10.1643/OT-14-014.

Caller G, Brown C. 2013. Evolutionary Responses to Invasion: Cane Toad Sympatric Fish Show Enhanced Avoidance Learning. *PLOS ONE* 8:e54909. DOI: 10.1371/journal.pone.0054909.

Cawthra HC, Cowling RM, Andò S, Marean CW. 2020. Geological and soil maps of the Palaeo-Agulhas Plain for the Last Glacial Maximum. *Quaternary Science Reviews* 235:105858. DOI: 10.1016/j.quascirev.2019.07.040.

Chakona A, Swartz ER, Gouws G. 2013. Evolutionary Drivers of Diversification and Distribution of a Southern Temperate Stream Fish Assemblage: Testing the Role of Historical Isolation and Spatial Range Expansion. *PLOS ONE* 8:e70953. DOI: 10.1371/journal.pone.0070953.

Channing A. 2019. *Amphibians of Central and Southern Africa*. Cornell University Press.

Channing A, Schmitz A, Burger M, Kielgast J. 2013. A molecular phylogeny of African Dainty Frogs, with the description of four new species (Anura: Pyxicephalidae: Cacosternum). *Zootaxa* published. DOI: 10.11646/zootaxa.3701.5.2.

Crossland MR, Alford RA. 1998. Evaluation of the toxicity of eggs, hatchlings and tadpoles of the introduced toad Bufo marinus (Anura: Bufonidae) to native Australian aquatic predators. *Australian Journal of Ecology* 23:129–137. DOI: 10.1111/j.1442-9993.1998.tb00711.x.

Davies SJ, Clusella-Trullas S, Hui C, McGeoch MA. 2013. Farm dams facilitate amphibian invasion: Extra-limital range expansion of the painted reed frog in South Africa. *Austral Ecology* 38:851–863. DOI: 10.1111/aec.12022.

Dayaram A, Skowno AL, Driver A, Sink K, Van Deventer H, Smith-Adao L, van Niekerk L, Harris L, Job N, Nel JL. 2021. *South African National Ecosystem Classification System Handbook*. SANBI.

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegl RG, McDowell WH, Kortelainen P, Caraco NF, Melack JM, Middelburg JJ. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. *Limnology and Oceanography* 51:2388–2397. DOI: 10.4319/lo.2006.51.5.2388.

Du Preez L, Carruthers V. 2017. *Frogs of Southern Africa – A Complete Guide*. Penguin Random House South Africa.

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society* 81:163–182. DOI: 10.1017/S1464793105006950.

Ellender B, Weyl O. 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* 9:117–132. DOI: 10.3391/ai.2014.9.2.01.

Ellender BR, Woodford DJ, Weyl OLF, Cowx IG. 2014. Managing conflicts arising from fisheries enhancements based on non-native fishes in southern Africa. *Journal of Fish Biology* 85:1890–1906. DOI: 10.1111/jfb.12512.

Falaschi M, Melotto A, Manenti R, Ficetola GF. 2020. Invasive Species and Amphibian Conservation. *Herpetologica* 76:216–227. DOI: 10.1655/0018-0831-76.2.216.

Ficetola GF, De Bernardi F. 2004. Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. *Biological Conservation* 119:219–230. DOI: 10.1016/j.biocon.2003.11.004.

Fox J, Weisberg S. 2019. *An R companion to applied regression*. Thousand Oaks CA: Sage.

Frost DR. 2020. Amphibian species of the world: an online reference, version 5.4.

Grant EHC, Muths E, Schmidt BR, Petrovan SO. 2019. Amphibian conservation in the Anthropocene. *Biological Conservation* 236:543–547. DOI: 10.1016/j.biocon.2019.03.003.

Hamer AJ, Parris KM. 2011. Local and landscape determinants of amphibian communities in urban ponds. *Ecological Applications* 21:378–390. DOI: 10.1890/10-0390.1.

Hartel T, Nemes S, Cogălniceanu D, Öllerer K, Schweiger O, Moga C-I, Demeter L. 2007. The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia* 583:173–182. DOI: 10.1007/s10750-006-0490-8.

Hassall C. 2014. The ecology and biodiversity of urban ponds. *Wiley Interdisciplinary Reviews: Water* 1:187–206.

Hecnar SJ, M'Closkey RT. 1997. The effects of predatory fish on amphibian species richness and distribution. *Biological Conservation* 79:123–131. DOI: 10.1016/S0006-3207(96)00113-9.

Hill MJ, Hassall C, Oertli B, Fahrig L, Robson BJ, Biggs J, Samways MJ, Usio N, Takamura N, Krishnaswamy J. 2018. New policy directions for global pond conservation. *Conservation Letters* 11:e12447.

Hill J, Lawson K, Tuckett Q. 2017. First record of a reproducing population of the African clawed frog *Xenopus laevis* Daudin, 1802 in Florida (USA). *BioInvasions Records* 6:87–94. DOI: 10.3391/bir.2017.6.1.14.

Holbrook JD, Dorn NJ. 2016. Fish reduce anuran abundance and decrease herpetofaunal species richness in wetlands. *Freshwater Biology* 61:100–109. DOI: 10.1111/fwb.12683.

Johnson PT, Olden JD, Vander Zanden MJ. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment* 6:357–363. DOI: 10.1890/070156.

Knapp RA, Boiano DM, Vredenburg VT. 2007. Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, Rana muscosa). *Biological Conservation* 135:11–20. DOI: 10.1016/j.biocon.2006.09.013.

Kruger DJD, Hamer AJ, Du Preez LH. 2015. Urbanization affects frog communities at multiple scales in a rapidly developing African city. *Urban Ecosystems* 18:1333–1352. DOI: 10.1007/s11252-015-0443-y.

Legendre P, Legendre L. 2012. Chapter 11 - Canonical analysis. In: Legendre P, Legendre L eds. *Developments in Environmental Modelling*. Numerical Ecology. Elsevier, 625–710. DOI: 10.1016/B978-0-444-53868-0.50011-3.

Measey GJ. 2011. Ensuring a future for south africa's frogs: a strategy for conservation research. In: Measey GJ ed. *SANBI biodiversity series 19*. Pretoria: South African National Biodiversity Institute, 2–6. DOI: 10.1080/0035919x.2011.586444.

Measey J, Annecke W, Davies SJ, Dorse C, Stafford L, Tolley K, Turner A. 2014. Cape collaborations for amphibian solutions. *FrogLog* 109:46–47.

Measey GJ, Tolley KA. 2011. Sequential fragmentation of Pleistocene forests in an East Africa biodiversity hotspot: Chameleons as a model to track forest history. *PLoS ONE* 6. DOI: 10.1371/journal.pone.0026606.

Meredith H, Van Buren C, Antwis RE. 2016. Making amphibian conservation more effective. *Conservation Evidence* 13:1–6.

Mokhatla MM, Rödder D, Measey GJ. 2015. Assessing the effects of climate change on distributions of Cape Floristic Region amphibians. *South African Journal of Science* 111:1–7. DOI: 10.17159/sajs.2015/20140389.

Moor H, Bergamini A, Vorburger C, Holderegger R, Bühler C, Egger S, Schmidt BR. 2022. Bending the curve: Simple but massive conservation action leads to landscape-scale recovery of amphibians. *Proceedings of the National Academy of Sciences* 119:e2123070119. DOI: 10.1073/pnas.2123070119.

Mucina L, Rutherford MC (eds.). 2006. *The vegetation of South Africa, Lesotho and Swaziland*. Pretoria: South African National Biodiversity Institute.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858. DOI: 10.1038/35002501.

Nunes AL, Fill JM, Davies SJ, Louw M, Rebelo AD, Thorp CJ, Vimercati G, Measey J. 2019. A global meta-analysis of the ecological impacts of alien species on native amphibians.

Proceedings of the Royal Society B: Biological Sciences 286. DOI: 10.1098/rspb.2018.2528.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P. 2022. vegan: Community Ecology Package. R package version 2.5–7. 2020.

Pechmann JHK, Scott DE, Whitfield Gibbons J, Semlitsch RD. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. *Wetlands Ecology and Management* 1:3–11. DOI: 10.1007/BF00177885.

Picker MD, McKenzie CJ, Fielding P. 1993. Embryonic Tolerance of Xenopus (Anura) to Acidic Blackwater. *Copeia* 1993:1072–1081. DOI: 10.2307/1447086.

Poynton JC. 1964. Amphibia of southern Africa; a faunal study. *Annals of the Natal Museum* 17:1–334.

Rebelo AG, Holmes PM, Dorse C, Wood J. 2011. Impacts of urbanization in a biodiversity hotspot: Conservation challenges in Metropolitan Cape Town. *South African Journal of Botany* 77:20–35. DOI: 10.1016/j.sajb.2010.04.006.

Schreiner C, Rödder D, John Measey G. 2013. Using modern models to test Poynton's predictions. *African Journal of Herpetology* 62:49–62. DOI: 10.1080/21564574.2013.794865.

Simpson GL. 2015. ggvegan:'ggplot2'Plots for the 'vegan'Package. *R package version 00–3*.

Skowno AL, Jewitt D, Slingsby JA. 2021. Rates and patterns of habitat loss across South Africa's vegetation biomes. *South African Journal of Science* 117:1–5. DOI: 10.17159/sajs.2021/8182.

Venter JA, Brooke CF, Marean CW, Fritz H, Helm CW. 2020. Large mammals of the Palaeo-Agulhas Plain showed resilience to extreme climate change but vulnerability to modern human impacts. *Quaternary Science Reviews* 235:106050. DOI: 10.1016/j.quascirev.2019.106050.

Vogt S, Villiers FA de, Ihlow F, Rödder D, Measey J. 2017. Competition and feeding ecology in two sympatric *Xenopus* species (Anura: Pipidae). *PeerJ* 5:e3130. DOI: 10.7717/peerj.3130.

Welborn GA, Skelly DK, Werner EE. 1996. Mechanisms Creating Community Structure Across a Freshwater Habitat Gradient. *Annual Review of Ecology and Systematics* 27:337–363.

~~Wellborn GA, Skelly DK, Werner EE. 2003. Mechanisms creating community structure across a freshwater habitat gradient. *https://doi.org/10.1146/annurev.ecolsys.27.1.337* 27:337–363. DOI: 10.1146/annurev.ecolsys.27.1.337~~.

Werner EE, Skelly DK, Relyea RA, Yurewicz KL. 2007. Amphibian species richness across environmental gradients. *Oikos* 116:1697–1712. DOI: 10.1111/j.0030-1299.2007.15935.x.

Weyl OLF, Ellender BR, Wassermann RJ, Truter M, Dalu T, Zengeya TA, Smit NJ. 2020. Alien Freshwater Fauna in South Africa. In: van Wilgen BW, Measey J, Richardson DM, Wilson JR, Zengeya TA eds. *Biological Invasions in South Africa*. Invading Nature - Springer Series in Invasion Ecology. Cham: Springer International Publishing, 153–183. DOI: 10.1007/978-3-030-32394-3_6.

Wilson JR, Foxcroft LC, Geerts S, Hoffman MT, MacFadyen S, Measey J, Mills A, Richardson DM, Robertson MP, van Wilgen BW. 2020. The Role of Environmental Factors in Promoting and Limiting Biological Invasions in South Africa. In: van Wilgen BW, Measey J, Richardson DM, Wilson JR, Zengeya TA eds. *Biological Invasions in South Africa*. Invading Nature - Springer Series in Invasion Ecology. Cham: Springer International Publishing, 355–385. DOI: 10.1007/978-3-030-32394-3_13.

Figure Legends

Figure 1. Fifty sampling sites (coloured by wetland type: Temporary vlei purple, River edge green, Large dam brown, Small dam blue and Fynbos pool red) are constructed (diamonds) or natural (circles) in the Overberg region of South Africa (inset shows extreme southwest of southern Africa). For details of the selected sites see Suppl Mat (Table S1).

Figure 2 The relationship between 50 sites sampled and their amphibian communities in the Overberg region of South Africa from a reduced redundancy analysis (reduced RDA). The position and influence of environmental variables are shown with arrow lengths. Species names are abbreviated to the first letters of genus and specific name (see Table 2).

Figure 3 The relationship between 36 permanent water sites sampled and their amphibian communities in the Overberg region of South Africa using a non-metric multidimensional scaling (NMDS) analysis. Points and ellipses are coloured by whether fish are present (red) or absent (blue). The position and influence of species are shown with arrow lengths. Species names are abbreviated to the first letters of genus and specific name (see Table 2).

Figure 1

Fifty sampling sites in the Overberg region of South Africa.

Freshwater bodies (coloured by wetland type: Temporary vlei purple, River edge green, Large dam brown, Small dam blue and Fynbos pool red) are constructed (diamonds) or natural (circles) (inset shows extreme southwest of southern Africa). For details of the selected sites see Suppl Mat. Table S1.

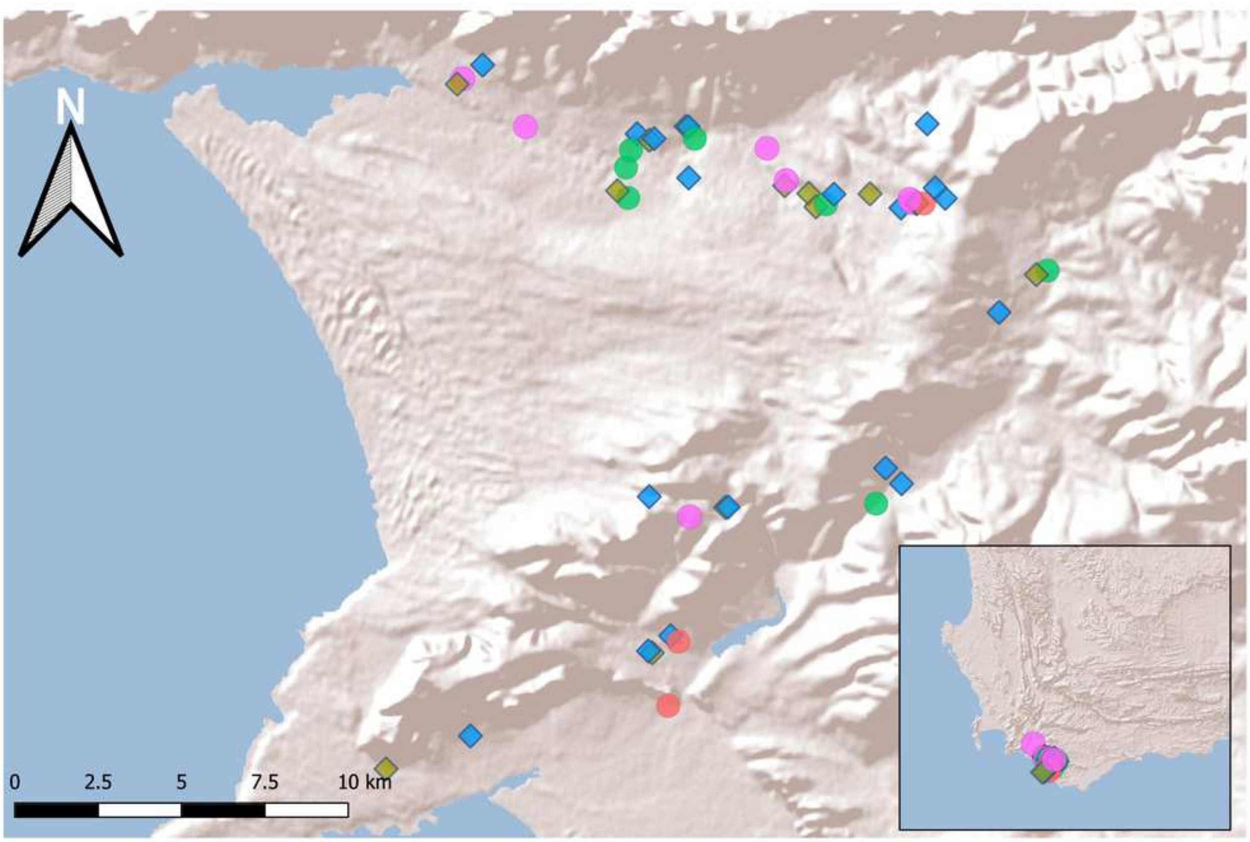

Figure 2

The relationship between 50 sites sampled and their amphibian communities in the Overberg region of South Africa from a reduced redundancy analysis (reduced RDA).

The position and influence of environmental variables are shown with arrow lengths. Species names are abbreviated to the first letters of genus and specific name (see Table 2).

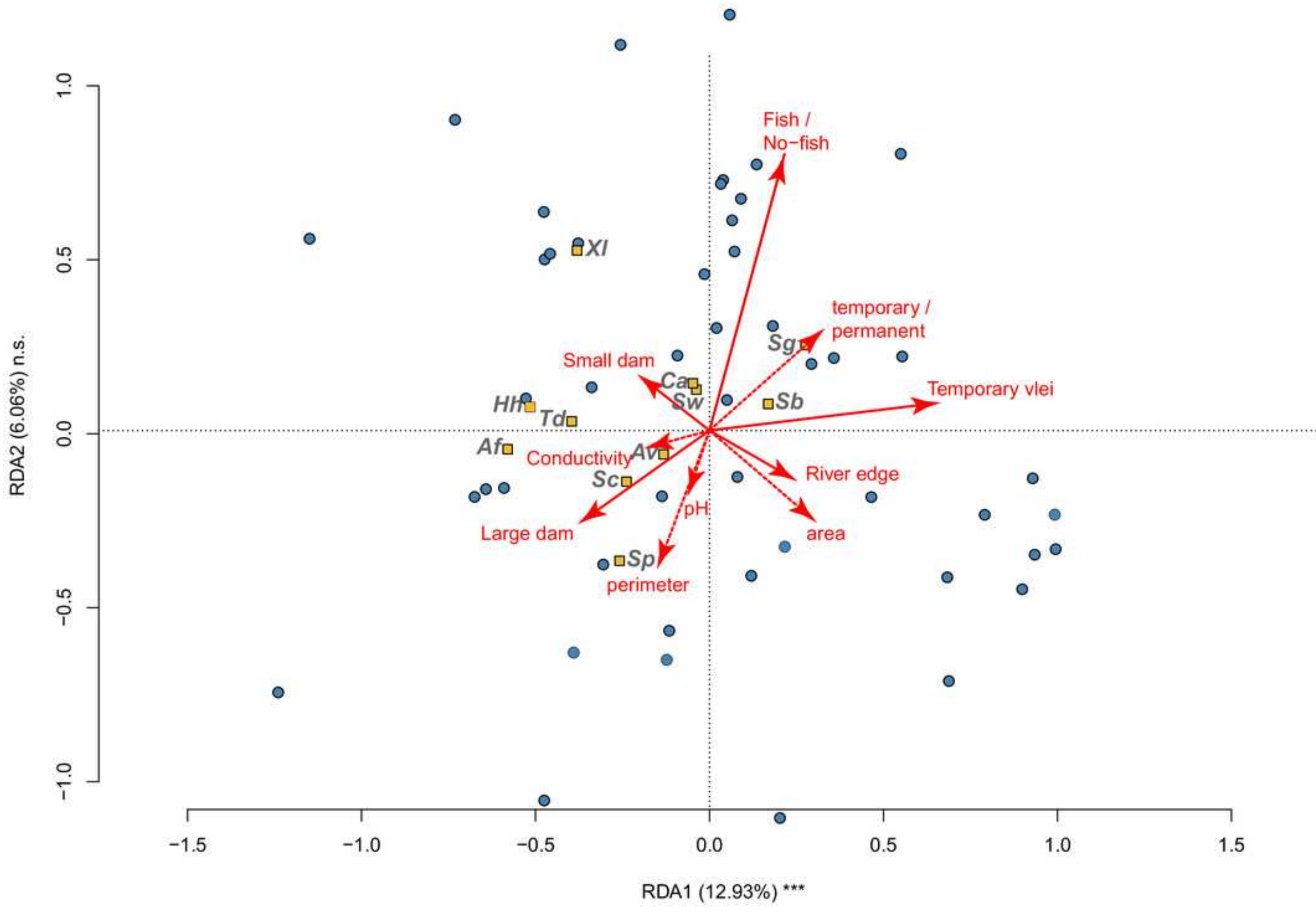

Figure 3

The relationship between 36 permanent water sites sampled and their amphibian communities in the Overberg region of South Africa using a non-metric multidimensional scaling (NMDS) analysis.

Points and ellipses are coloured by whether fish are present (red) or absent (blue). The position and influence of species are shown with arrow lengths. Species names are abbreviated to the first letters of genus and specific name (see Table 2).

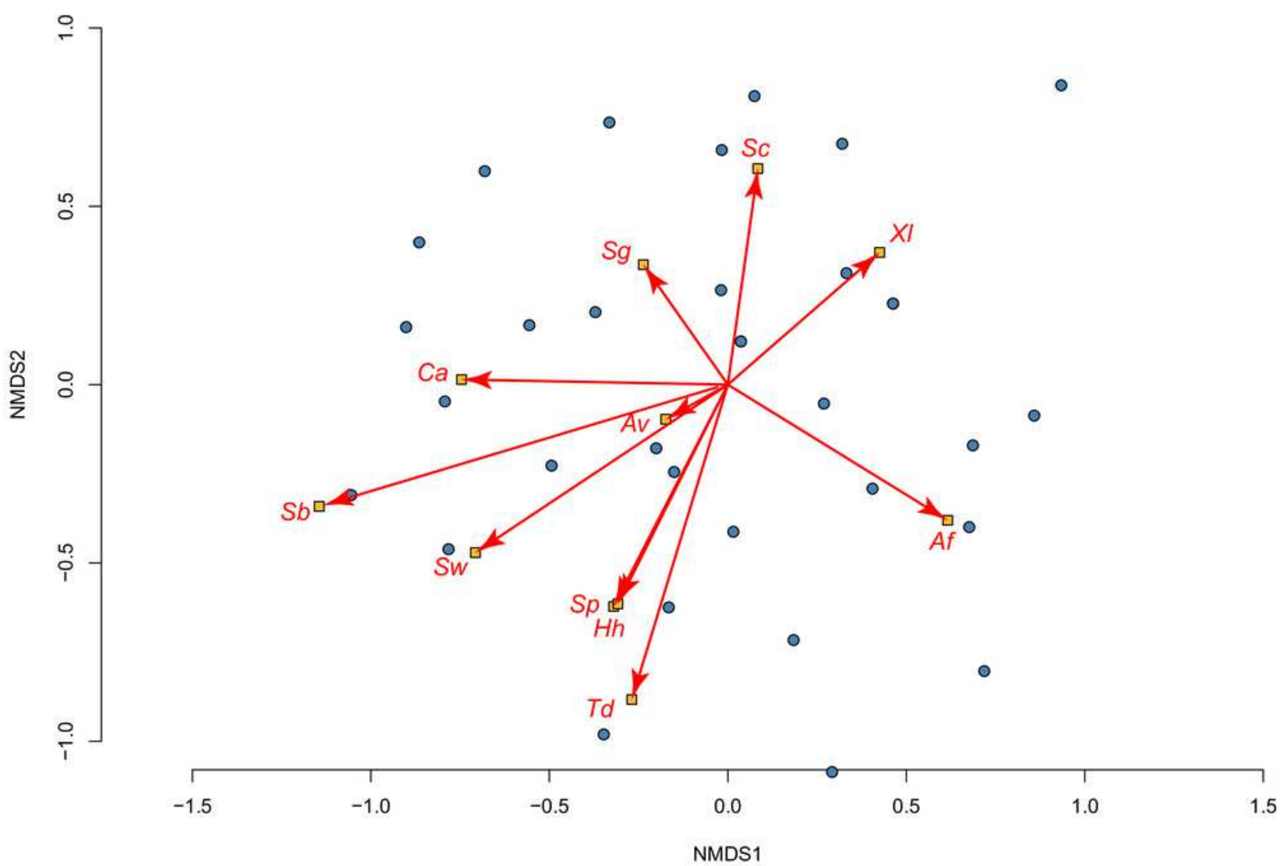

Table 1(on next page)

The 11 species of amphibians found at 50 lowland sites in the Overberg, South Africa.

The number of sites is provided with the number of temporary sites in brackets. Their position in ordinal space and from a partial RDA analysis demonstrate affinity. Species are sorted according to their position along RDA1 (see Figure 1). Mean snout-vent length (SVL) is taken from AmphiBIO (Oliveira et al., 2017). Range sizes are calculated from Extent of Occurrence from the IUCN RedList (www.iucnredlist.org).

Table 1. The 11 species of amphibians found at 50 lowland sites - in the Overberg. The number of sites is provided with the number of temporary sites in brackets. Their position in ordinal space and from NMDS calculationsa partial RDA analysis demonstrate affinity. Species are sorted according to their position along RDA1 (see Figure 1). Mean snout-vent length (SVL) is taken from AmphiBIO (Oliveira et al., 2017). Species highlighted in bold contribute significantly to community structure. Figures are taken from output of envfit using species on the chosen NMDS model (see Figure 1). Range sizes are calculated from Extent of Occurrence from the IUCN RedList (www.iucnredlist.org).

Species	**Family**	**Number of sites**	**RDA1**	**SVL (mm)**	**IUCN range (km^2)**
Amietia fuscigula	Pyxiecephalidae	27 (5)	-0.5800	125	598013
Hyperolius horstocki	Hyperoliidae	19 (6)	-0.5154	43	18110
Tomopterna delalandii	Pyxiecephalidae	10 (2)	-0.3960	41	215909
Xenopus laevis	Pipidae	34 (8)	-0.3807	147	3761124
Scelerophys pantherina	Bufonidae	12 (1)	-0.2584	140	3824
Scelerophys capensis	Bufonidae	12 (2)	-0.2387	115	732181
Arthroleptella villiersi	Pyxiecephalidae	8 (3)	-0.1318	22	6382
Semnodactylus wealii	Hyperoliidae	4 (2)	-0.0478	44	376520
Cacosternum australis	Pyxiecephalidae	29 (10)	-0.0378	26	17037
Strongylopus bonaespei	Pyxiecephalidae	4 (3)	0.1689	42	28077
Strongylopus grayii	Pyxiecephalidae	39 (13)	0.2761	64	580275

Species	**Number of sites**	**IUCN range (km^2)**
Amietia fuscigula	**27**	**598013**
Arthroleptella villiersi	8	6382
Cacosternum australis	29	17037
Hyperolius horstocki	**19**	**18110**

~~***Strongylopus bonaespei***~~	**4**	~~**28077**~~
~~*Scelerophys capensis*~~	~~12~~	~~732181~~
~~*Scelerophys pantherina*~~	~~12~~	~~3824~~
~~*Strongylopus grayii*~~	~~39~~	~~580275~~
~~*Semnodactylus wealii*~~	4	~~376520~~
~~*Tomopterna delalandii*~~	~~10~~	~~215909~~
~~***Xenopus laevis***~~	~~**34**~~	~~**3761124**~~

* Note that RDA 2 is not shown here as, although it is shown in Figure 2, it was not significant.

Table 2 (on next page)

The significance of environmental variables measured in structuring the community of 11 species of amphibians found at 50 lowland sites in the Overberg, South Africa.

Outputs are from a partial Redundancy Analysis (partial RDA) controlling for spatial position of the sites and their day of sampling.

Table 2. The significance of environmental variables measured in structuring the community of 11 species of amphibians found at 50 lowland sites in the Overberg, South Africa. Outputs are from a partial Redundancy Analysis (partial RDA) controlling for spatial position of the sites and their day of sampling.

Environmental Variable	**df**	**Variance**	**F**	**P**
Fish / No Fish	1	0.0860	2.4115	0.009 **
Temporary / Permanent	1	0.0570	1.5985	0.096
Area	1	0.0500	1.4029	0.179
Perimeter	1	0.0576	1.6141	0.107
pH	1	0.0156	0.4364	0.927
Conductivity	1	0.0504	1.4144	0.171
Wetland Type	4	0.2208	1.5476	0.033 *
Residual	35	1.2483		