

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

1

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

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Amphibians are more threatened than any other vertebrate class, yet evidence for many threats is missing. The Cape lowland fynbos 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.

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Abstract

Amphibians are more threatened than any other vertebrate class, yet evidence for many threats is missing. The Cape lowland fynbos 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:

Introduction

Following recognition of global amphibian decline in the early 1990s, amphibian conservation has centred around three major themes: habitat loss, disease and invasive species (Grant et al., 2019). Although disease is recognised to have caused the most severe biodiversity loss for any vertebrate class (Scheele et al., 2019), habitat loss 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, the evidence for impacts and their commensurate conservation measures for 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 by all authors as causing severe impacts on many amphibian communities (Hecnar & M'Closkey, 1997; Ficetola & De Bernardi, 2004; Hartel et al., 2007; Holbrook & Dorn, 2016). 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). 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, especially with respect to amphibian communities.

The low-lying fynbos of South Africa's Cape region carries an important community of amphibians that have high conservation concern (Measey 2011; Schreiner, Rodder & 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 or habitat characteristics (especially temporary vs. permanent water) are the proximate drivers of 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. ~~Therefore, we designed our sampling strategy to include different types of water bodies in the area, including natural temporary pools, rivers, ponds and impoundments (large and small). The sampling took place at 50 sites of different freshwater aquatic types including permanent impoundments and seasonal water bodies over two catchments on the Agulhas Plain.~~

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 m² including ponds), and large dams (artificial impoundments >2000 m²). 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 encompassed two separate catchments, the spatial proximity and different water body types (see Fig. 1; Supp Info; Table S1).

>>Figure 1

Anuran data collection

We set audio recorders at each site for two nights to collect calling data between May and August 2016-2017. We conducted three nights of searches around each site to look for adults. 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). Taxonomy for all species was corrected to Frost (2022), 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 approached local recreational fishermen for images of species caught within our sampling period.

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 and to correspond with our sampling times. We also noted the latitude and longitude of the centre point of each site. During our visits to sites, we used a Hannah instrument to measure water temperature, pH and conductivity.

Data Analyses

We used the function `ggpairs` in the package `GGally` (Schloerke, Crowley & Cook, 2018) in R (v4.2.1; R Core Team, 2021) to determine whether measured environmental variables were correlated using a cut off at $R^2 > 0.3$. When deciding between correlated variables, we chose those that have been considered biologically meaningful in the context of anuran biology in the southwestern Cape. For example, we chose to use pH over conductivity ($R^2 = 0.508$) as low pH has been considered important to species inhabiting naturally acidic fynbos pools (e.g. Picker, McKenzie & Fielding, 1993). We chose the perimeter of water bodies to be more important to anurans than their area, and the presence or absence of all invasive fish over particular species.

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. Using this matrix of presence/absence data for anuran species and sites, we ran a non-metric multidimensional scaling (NMDS) analysis using `metaMDS` with the Jaccard similarity index (suitable for presence/absence data) in package `vegan` (v.2.6-2; Oksanen et al., 2022) in R 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 `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. Similarly, we also 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 tested separately for their impact on amphibian communities in a reduced dataset (36 permanent water sites) using a multivariate analysis of variances on distance matrices (`adonis2`) in `vegan`. We used the coefficients of the output from `adonis2` to determine amphibian species sensitivity to fish.

We then used `ggvegan` (Simpson 2019), `ggpubr` (Kassambara 2020) and `ggplot2` (Wickham 2016) to visualise our data.

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. In addition, we had evidence of 3 invasive fish species: Large-Mouth Bass *Micropterus salmoides*, Small-Mouth Bass *M. dolomieu* and Mozambique Tilapia *Oreochromis mossambicus* from 14 sites.

>>Table 1

The NMDS stress level for the anuran community data of 11 species from 50 sites was 0.12 with 1 convergent solution after 20 runs with 3 dimensions (Figure 2).

The best measured environmental determinant of the amphibian community was the type of wetland habitat ($R^2 = 0.2822$; $P < 0.001$; Figure 2a,b), followed by whether sites were temporary or permanent ($R^2 = 0.0896$; $P = 0.012$; Figure 2c,d), and whether or not invasive fish were present ($R^2 = 0.0719$; $P = 0.016$). The catchment sampled, and the position of the wetland (latitude and longitude) were not significant. Neither were other measures of perimeter and pH (for full results of envfit analysis, see Table S2).

The species most responsible for determining amphibian community structure was *Xenopus laevis* ($R^2 = 0.2808$; $P < 0.001$); together with *Amietia fuscigula* ($R^2 = 0.1498$; $P = 0.014$) and *Tomopterna delalandii* ($R^2 = 0.1449$; $P = 0.023$), these species significantly indicate permanent water communities. At the other extreme, *Strongylopus bonespei* ($R^2 = 0.1793$; $P = 0.005$) and *Hyperolius horstockii* ($R^2 = 0.2426$; $P = 0.001$) significantly indicate communities with temporary water (see Table 1).

>> Figure 2

Our test to determine whether 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 ($R^2 = 0.08916$; $P = 0.025$; Fig 2d). 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 et al., 2017, 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: *Xenopus laevis*, *Amietia fuscigula* and *Tomopterna delalandii*. These species are not endemic to the area, while those associated with temporary water have much smaller distributions (~20 000 km²). 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).

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 et al., 2017, 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 (see Bond et al., 2019). While our research is pertinent to low-lying areas of the fynbos, hydroperiod and invasive fish have been found to significantly impact amphibian communities elsewhere (e.g. Holbrook & Dorn, 2016). Therefore, it may be that upland fynbos areas and 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.

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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). Tertiary catchments are shown in different colours. Candidate sites, from which sample sites were selected, are shown as triangles. For details of the candidate (Table S1) and selected sites see Suppl Mat.

Figure 2 The relationship between 50 sites sampled and their amphibian communities in the Overberg region of South Africa. (a) for NMDS1 and NMDS2, and (b) for NMDS2 and NMDS3. Points and ellipses are coloured by wetland type: Temporary vlei purple, River edge green, Large dam brown, Small dam blue and Fynbos pool red (but with too few points to draw an ellipse). Ellipses demonstrate how site types are differentiated. The position and influence of species are shown with arrow lengths. (c) Points and ellipses are coloured by temporary (blue) or permanent (red) wetland types for NMDS1 and NMDS2, and (d) for NMDS2 and NMDS3. Species names are abbreviated to the first letters of genus and specific name (see Table 1).

Figure 3 The relationship between 36 permanent water sites sampled and their amphibian communities in the Overberg region of South Africa. (a) NMDS1 and NMDS2. 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 1).

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). Tertiary catchments are shown in different colours. Candidate sites, from which sample sites were selected, are shown as triangles. For details of the candidate (Table S1) and selected sites see Suppl Mat.

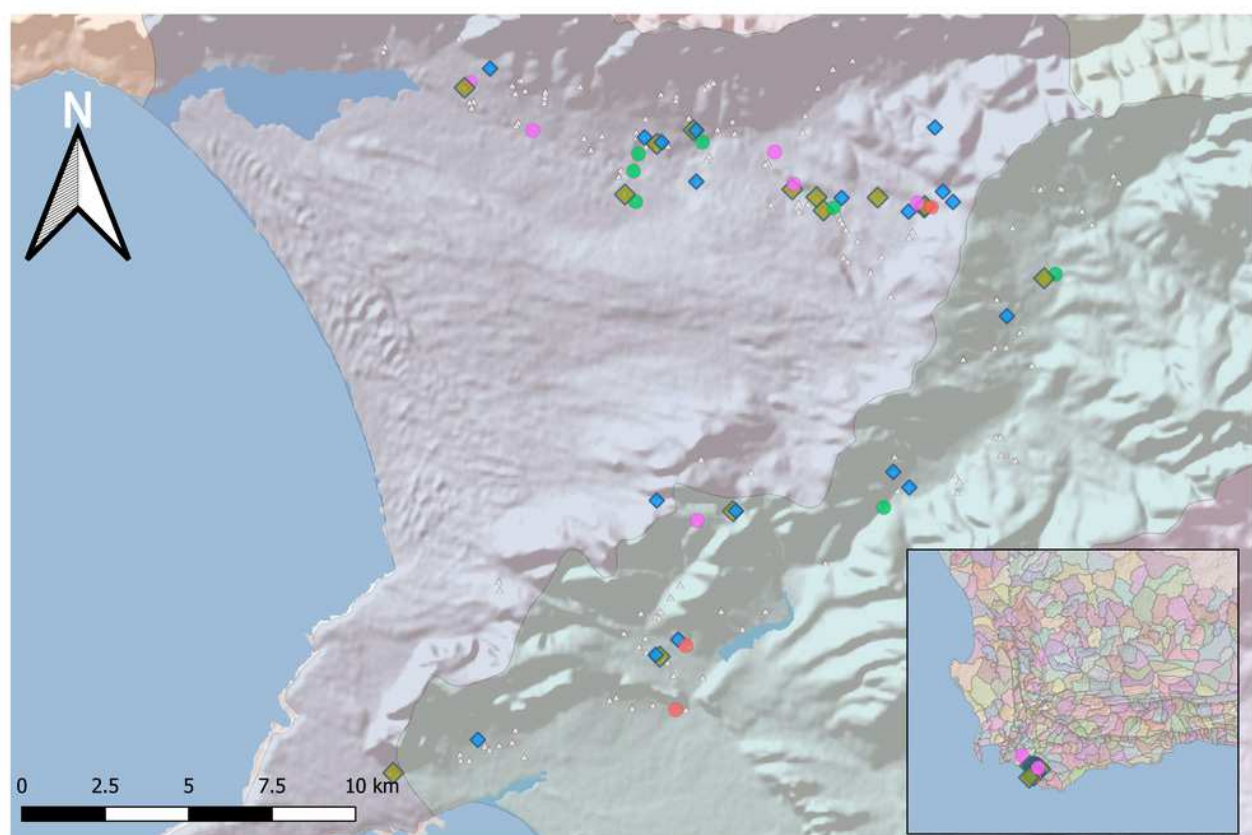


Figure 2

The relationship between 50 sites sampled and their amphibian communities in the Overberg region of South Africa.

(a) for NMDS1 and NMDS2, and (b) for NMDS2 and NMDS3. Points and ellipses are coloured by wetland type: Temporary vlei purple, River edge green, Large dam brown, Small dam blue and Fynbos pool red (but with too few points to draw an ellipse). Ellipses demonstrate how site types are differentiated. The position and influence of species are shown with arrow lengths. (c) Points and ellipses are coloured by temporary (blue) or permanent (red) wetland types for NMDS1 and NMDS2, and (d) for NMDS2 and NMDS3. Species names are abbreviated to the first letters of genus and specific name (see Table 1).

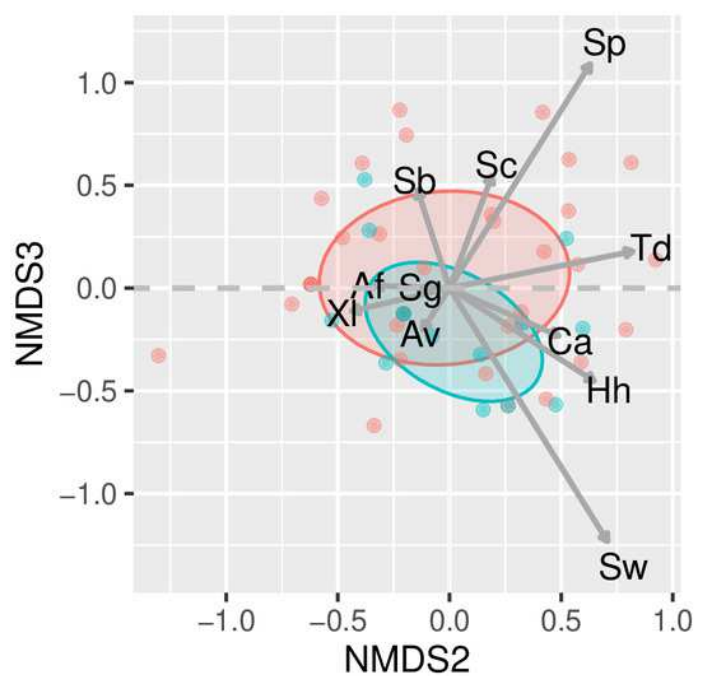
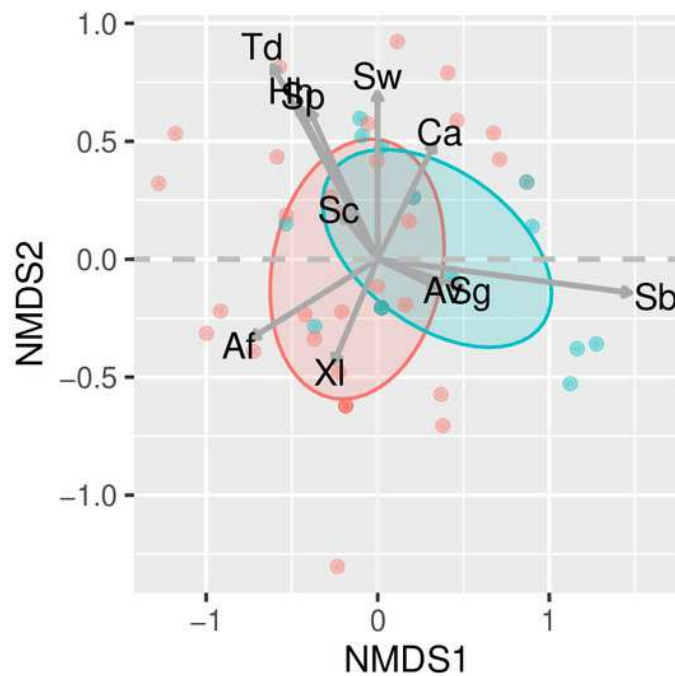
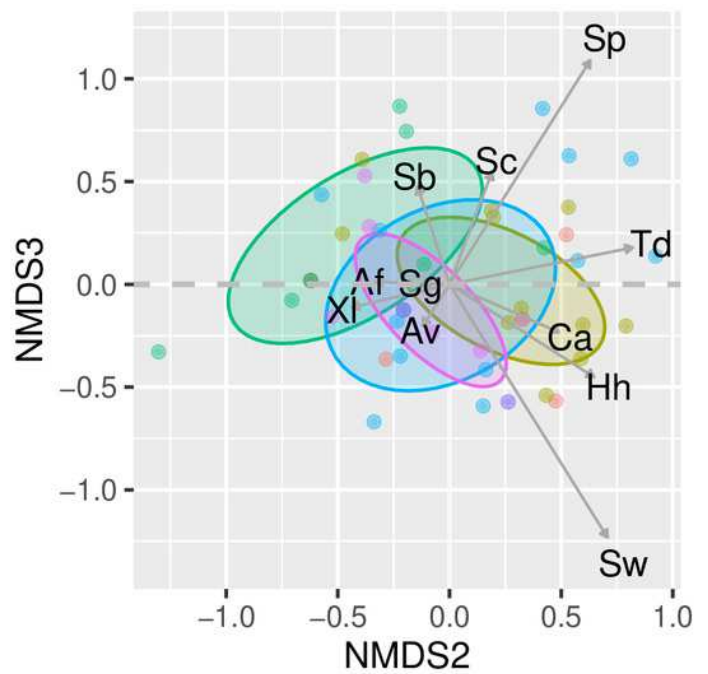
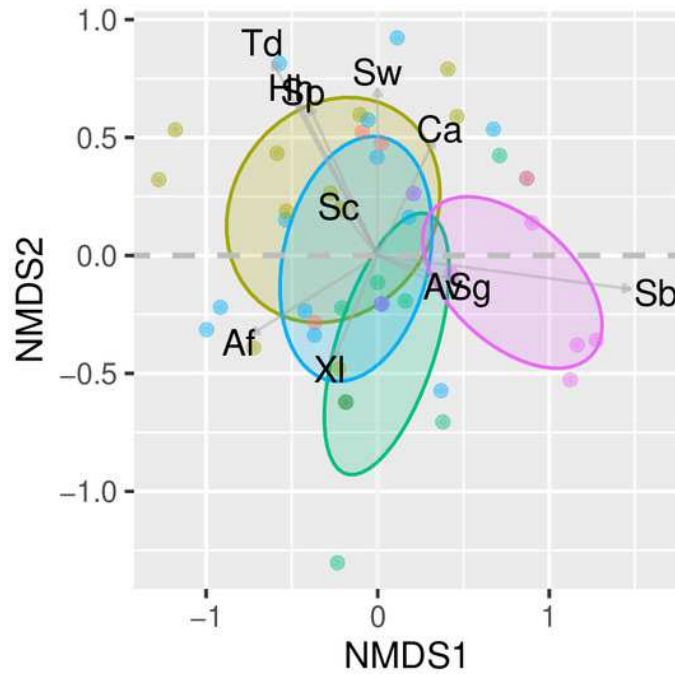


Figure 3

The relationship between 36 permanent water sites sampled and their amphibian communities in the Overberg region of South Africa.

(a) NMDS1 and NMDS2. 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 1).

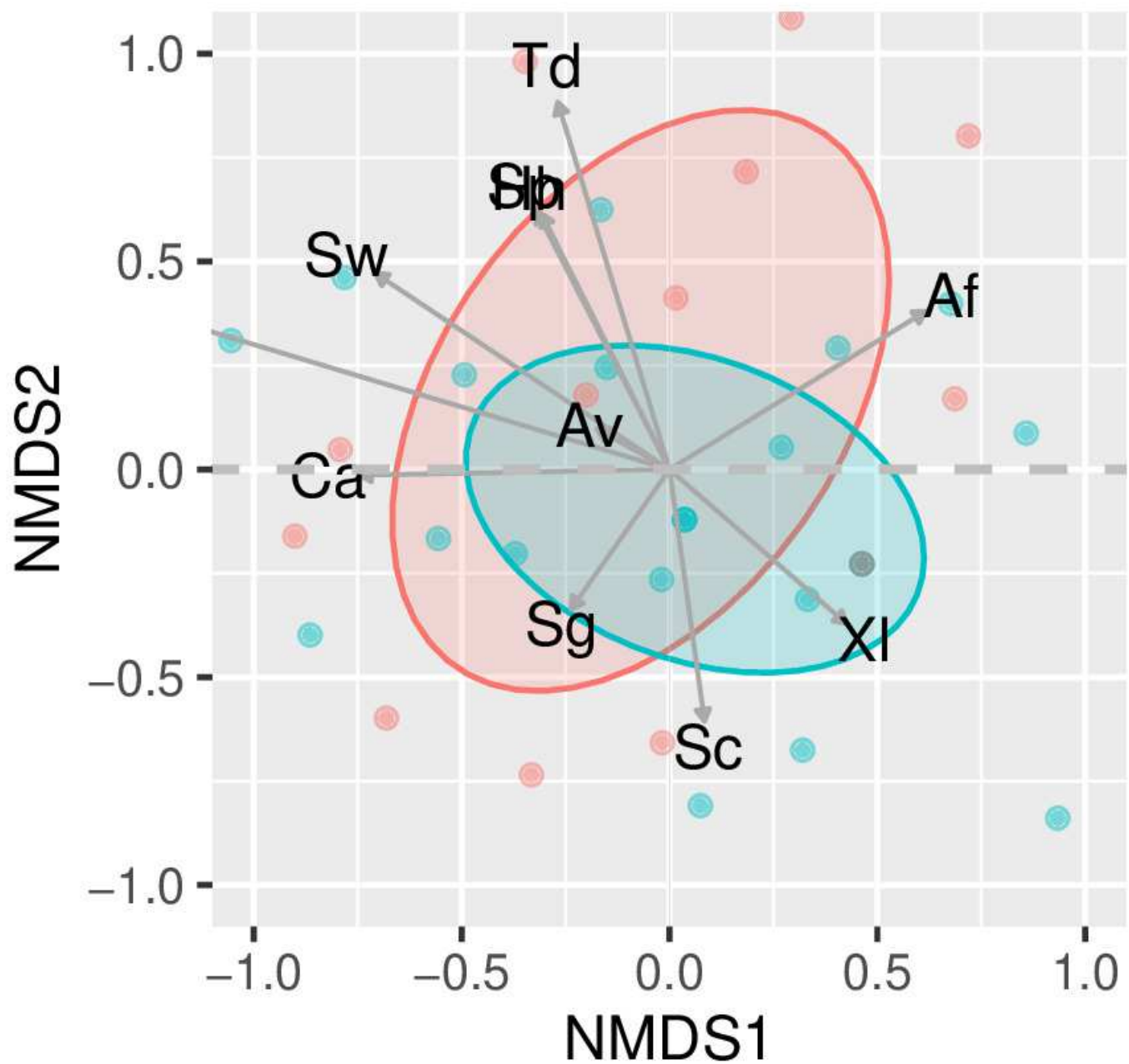


Table 1(on next page)

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

Their position in ordinal space and from NMDS calculations demonstrate affinity. 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).

Table 1. The 11 species of amphibians found at 50 lowland sites in the Overberg. Their position in ordinal space and from NMDS calculations demonstrate affinity. 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	Number of sites	NMDS1	NMDS2	R ²	Pr(>r)	IUCN range (km ²)
<i>Amietia fuscigula</i>	27	-0.99664	0.08195	0.1498	0.0144	598013
<i>Arthroleptella villiersi</i>	8	0.80275	-0.59631	0.0231	0.5880	6382
<i>Cacosternum australis</i>	29	0.99250	0.12226	0.0176	0.6654	17037
<i>Hyperolius horstocki</i>	19	-0.31054	0.95056	0.2426	0.0014	18110
<i>Strongylopus bonaespei</i>	4	0.94845	-0.31694	0.1793	0.0051	28077
<i>Scelerophys capensis</i>	12	-0.84920	0.52806	0.0723	0.1814	732181
<i>Scelerophys pantherina</i>	12	0.92560	0.37851	0.0502	0.3077	3824
<i>Strongylopus grayii</i>	39	0.45857	0.88866	0.0699	0.1901	580275
<i>Semnodactylus wealii</i>	4	-0.08281	0.99657	0.0373	0.4258	376520
<i>Tomopterna delalandii</i>	10	-0.39310	0.91950	0.1449	0.0230	215909
<i>Xenopus laevis</i>	34	-0.35219	-0.93593	0.2808	0.0007	3761124