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A Composite Method to Quantitatively Survey Fossorial Herpetofaunal Communities in Sandy Substrate

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Abstract.—**The monitoring of fossorial herpetofauna has been constrained by a lack of reliable baseline data for even commonly occurring species. Here we report on a quantitative survey method composed of selected elements from established techniques and adapted to promote the optimal exposure of subterranean amphibians and reptiles in aeolian sands as conducted in wooded grassland vegetation. To test the effectiveness of this new composite method to detect variations in the diversity, demography, and structure of a fossorial herpetofaunal community, we compared the results of localized austral winter and summer surveys from a site in South Africa. We then tested the performance of this new composite technique against an established method that has been implemented within the same general region. We identified notable differences in the community structure and the demography of the two most abundant species between the winter and summer. We documented significantly higher fossorial herpetofaunal densities using the new composite technique compared with the established survey method. We discuss these results along with the constraints and the practical implementation of the new composite technique as a survey methodology and as a monitoring tool to assess changes in detectable fossorial herpetofaunal community structure.**

Key Words.—*Breviceps*; burrowing herpetofauna; fossorial vertebrate monitoring; Sileza Nature Reserve; surveying; *Zygaspis*.

Introduction

The quantitative surveying of fossorial herpetofauna requires specialized techniques (Measey et al. 2003; Maritz and Alexander 2008). Such methods generally entail the excavation of substrate from standardized quadrats to expose resident subterranean amphibians and reptiles, providing density and diversity data that may be implemented over time as a monitoring tool (Measey 2006). The strain and physical labor associated with these techniques limit the appeal of such studies (Maritz and Alexander 2008), evidenced by the rarity of published accounts from only a small group of authors. This general lack of historical baselines greatly handicaps efforts aimed at monitoring fossorial reptile and amphibian responses to environmental change (Measey et al. 2009), which may lead to undocumented or uncalibrated declines (Martin et al. 2015; Henderson et al. 2016).

Where survey objectives relate to the optimal quantification of fossorial herpetofauna, methodologies should effectively conform to three underlying assumptions to promote confidence in a technique, as put forward by Heatwole (2012): (1) adequate habitat representation of the total sample; (2) the inability of specimens to vacate the quadrat during the survey; and (3) the ability of the method to reliably detect all specimens present within the parameters of the surveyed soil quadrat. Moreover, as most quantitative surveying techniques aimed at assessing fossorial herpetofauna rely on the disturbance of substrate in one way or another, such techniques are considered inherently destructive (Maritz and Alexander 2008; Henderson et al. 2016).

Where prospective sites are earmarked for development (e.g., for mining, intensive agriculture, or property development), such survey-associated disturbances are effectively irrelevant (e.g., Maritz and Alexander 2008); however, the mitigation of such impacts may play a significant role in the feasibility of conducting surveys in sensitive, rehabilitated, or protected areas. To limit the degree of environmental disturbance incurred during such surveys, the size and distribution of quadrats need to be mitigated while maintaining the reliability and functionality of the methods employed. Thus, in addition to the underlying assumptions (Heatwole 2012, see above), the general integrity of the surveyed habitat needs to be preserved by limiting the standardized size and number of sampling units in accordance with the study objectives, as well as by conducting rehabilitation measures such as replacing excavated substrate and replanting extracted plants following field assessments (e.g., Henderson et al. 2016).

Following the general distinction promoted by Measey (2006), Maritz and Alexander (2008) divided soil-living amphibians and reptiles into what they called strictly fossorial, exhibiting specialized morphological adaptations for obligatory subsurface existence, as well as the more common fossorial species, which largely use detritus layers and soils as temporary shelter. Fossorial species have varying affinities to subsurface environments and niches driven by daily or seasonal requirements (Measey and Barot 2006; Martin et al. 2011). Weather conditions, most notably precipitation events, may also affect the presence and depth of certain species within substrate, ultimately impacting the effectiveness of survey methods, which rely on the presence of soil-living animals within specific soil depths to make them available for detection using these methods. Additionally, fossorial herpetofaunal occupancy may be influenced by the disturbance of soil or vegetation structure (Kuhnz et al. 2005; Wong et al. 2021), limiting the usefulness of direct comparisons of resurveyed quadrats over time. Similarly, surface conditions and activities have been shown to impact facets of subterranean herpetofaunal ecology and biology, including the size of populations, the composition of communities, body condition, and movement patterns (e.g., Measey et al. 2009; Driscoll et al. 2012; Martin et al. 2015).

As most fossorial herpetofauna reside within the top 0.25 m to 0.3 m of the soil column (Measey 2006; Henderson et al. 2016), quantitative methodologies generally focus efforts on surveying these relatively shallow depths. Subsequently, fossorial herpetofaunal density is expressed per excavated area (e.g., m^2) rather than by volume of excavated substrate. Notable variations in survey parameters occur between published methodologies with authors selecting appropriate techniques to fulfil study objectives or to fit practical considerations such as the effective soil depth, the habitat or depth selection of targeted species, and vegetation characteristics, among others. Such variation between techniques may complicate direct comparisons between assessments in terms of the post excavation search measures and the confidence in the effective survey parameters (see Measey 2006). It should, however, be kept in mind that some techniques possibly underestimate population densities (Kuhnz et al. 2005), further complicating comparisons between survey estimates derived from different methodologies (Jordaan et al. 2023).

The objective of our study was to develop and validate a composite quantitative approach to survey subterranean amphibians and reptiles within aeolian substrates to optimally comply with the underlying assumptions of Heatwole (2012) while assuring that the method can be conducted in an environmentally responsible way for application in sensitive or protected areas. Here we report on such a technique, which combines and modifies facets from previously published survey methodologies into an Adapted Composite Method (ACM). This included using nested random quadrats that ensure representative sampling of the selected habitat type with adapted excavation, sorting, and sieving processes employed to expose specimens from extracted substrate (Measey et al. 2003, 2009; Measey and Barot 2006; Maritz and Alexander 2008). We first compared the ACM between winter and summer conditions, assessing the potential to quantify seasonal shifts in population demography and density, followed by a trial to compare the effectiveness of our ACM with the methodology of Measey et al. (2009), a method that has been implemented in the same general region as our study site to assess fossorial herpetofaunal communities. We also discuss possible inherent biases, interpretations of the derived data, and practical aspects surrounding this ACM.

Materials and Methods

Study site.—This study took place in Sileza Nature Reserve (SNR), KwaZulu-Natal province, South Africa (-27.10750° 32.62407°), a protected area managed by Ezemvelo KwaZulu-Natal Wildlife (EKZNW), the provincial conservation authority (EKZNW 2013). The protected area encompassed 2,300 ha of Indian Ocean Coastal Belt vegetation along the Mozambique coastal plain (Mucina and Rutherford 2006), which is known for harboring a high diversity and density of fossorial herpetofauna (Maritz and Alexander 2008). Undulating vegetated dunes composed of aeolian sand and underlain with district regisols dominate the landscape (Matthews et al. 1999). We conducted surveys in Maputaland wooded grassland (Matthews et al. 1999) along the eastern section of the protected area. This vegetation type inherently harbors high densities of geoxylic suffructices (trees or plants with extensive woody structures belowground; Matthews et al. 1999), with considerable levels of Sicklebush (*Dichrostachys cinerea*) and Straw Everlasting (*Helichrysum kraussii*) shrub encroaching throughout the area.

Surveys and data collection.—We employed a randomized quadrat selection process within constrained and localized sector blocks with comparable habitat features to position survey quadrats, assuring adequate habitat representation between paired surveys and unbiased site selection. We accomplished this by applying different levels of randomization to the general study structure using R version 3.0.2 (R Development Core Team, 2013) to generate random numbers corresponding with quadrat locations in selected sub-sectors (see below). To facilitate non-biased site selection, we superimposed a digitized 50 × 50 m numbered grid over a georeferenced map of Maputaland wooded grassland vegetation within the eastern section of SNR after delineating vegetation types presented in the SNR Management Plan (EKZNW 2013), totaling 1,083 complete sectors (equivalent to 270.75 ha). We selected a single random number generated between one and 1,083 and used the corresponding numbered sector as the reference point for Survey 1. This sector block fell on the eastern slope of a small dune orientated in a north-south direction, with the sector stretching from the dune crest to the bottom of the interdune depression. To standardize the physical terrain features assessed between winter (Survey 1) and summer (Survey 2), we chose the sector block directly north of the Survey 1 sector in the overlain 50 × 50 m grid as the site for Survey 2. We then divided these two sectors into 25 sub-sectors of 10 × 10 m each, which we then further divided into 25 potential quadrats of 2 × 2 m. We selected 21 sub-sectors per survey using random numbers. Using a second round of randomization, we selected a single 2 × 2 m quadrat within each selected sub-sector. This resulted in an excavated area of 84 m^2 (21 quadrats of 4 m^2 each) assessed with the ACM across each of the Survey 1 and Survey 2 sectors. We conducted Survey 1 during the Austral winter (August 2018) and Survey 2 during the following summer (November and December 2018) to compare fossorial herpetofaunal density and community demography between seasons as detected by the ACM.

To test the effectiveness of the ACM (Survey 3) against the technique used in Measey et al. (2009; our Survey 4), we selected terrain features harboring high fossorial herpetofaunal density based on the results of the prior two surveys. The stratified results from Surveys 1 and Survey 2 across subsectors identified proportionately higher fossorial herpetofaunal densities on the dune crest, leading to the subjective placement of the Survey 3 and Survey 4 sectors immediately south of the initial Survey 1 sector across the top of the dune. Of the 25 sub-sectors, we selected 15 sub-sectors and quadrats by using random numbers, translating to 60 m^2 in total area that we assessed during both Survey 3 and Survey 4, the same effort applied by Measey et al. (2009) per survey.

We programmed the central coordinates of each predetermined quadrat into a handheld GPS to locate sampling locations, then used nylon rope and four metal pegs to demarcate quadrat parameters (Fig. 1) prior to

Figure 1. The adapted composite method for fossorial herpetofuanal surveys begins with (A) the demarcation of the 2 × 2 m quadrat, (B) the excavation of the peripheral border trench, (C) then the excavation of the remainder of the central block until (D) the entire quadrat has been dug out and the extracted substrate with its associated vegetation is temporarily stored in a series of buckets. (E) The excavated substrate is then processed by pouring substrate from a bucket onto iron mesh netting. (F) Vegetation is removed from the sample as part of the sorting process before the sample is sifted. (Photographed by Philip R. Jordaan).

sampling. We took care not to disturb quadrats before initiating surveys to prevent specimens from moving out of the demarcated area (e.g., see Heatwole and Stuart 2008), and, therefore, the herbaceous layer was left intact and only removed with the substrate during the excavation process both during Surveys 1, 2, and 3, using the ACM, as well as during Survey 4 using the Measey et al. (2009) technique.

When survey quadrats fell next to each other, we instructed field assistants to traverse the unsampled quadrat area as little as possible. To decrease disturbance bias from sampling neighboring quadrats, we postponed the sampling of these quadrats until the end of the survey period to allow any fossorial herpetofauna that may have been disturbed time to return before sampling (only two such cases occurred throughout the entire study). We assessed habitat features for each quadrat between paired surveys to determine if results could be adequately compared to each other, using the overall vegetation structure and the relative position of the quadrat with respect to terrain features for this comparison.

The ACM entails two field workers, positioned at opposite corners of a quadrat, to initiate surveying with the simultaneous and synchronized excavation of the quadrat border line, using spades, to create a trench around the periphery of the demarcated area, 0.2 m wide with a minimum depth of 0.25 m (Fig. 1). The preferred method of excavation entailed the removal of sand in intact structured blocks (approximately 0.2 × 0.2 × 0.25 m), including the vegetation growing in it from the quadrat (Fig. 1). We removed loose unconsolidated sand at the bottom of the excavated quadrat as well. We temporarily stored all excavated substrates and associated vegetation in a series of 20-L buckets to prevent herpetofauna from escaping. After we excavated each quadrat (Fig. 1), we calculated its mean survey depth by measuring the difference between the soil surface and the bottom of the quadrat at five random points and averaging the value.

To process excavated substrate, we initially poured the contents of a bucket onto a 1.2 × 1.2 m sheet of enameled iron wire mesh netting with an aperture of 2 × 2 mm to facilitate the initial sorting process (Fig. 1). To carry out sorting, we broke apart the amalgamated rooted sand blocks by hand and removed as much plant material from the sample as possible, taking care to inspect root clumps for sheltering reptile or amphibian specimens (Fig. 1). The removal of woody roots and other woody structures also prevented damage to the mesh netting. Additionally, the sorting process included an active search for specimens by hand in the loosened substrate before we sifted the sand through the mesh. Sifting removed all the sand through the mesh sheet, exposing any reptiles or amphibians that may have avoided detection when excavating the quadrat or during the sorting process.

Table 1. The sample percentages (and number) of fossorial herpetofaunal specimens extracted from the three survey processes (excavation, sorting, and sifting) employed during the adapted composite method for Surveys 1, 2, and 3 in Sileza Nature Reserve, KwaZulu-Natal province, South Africa.

	Excavation	Sorting	Sifting
Survey 1	10.0% (5)	24.0% (12)	66.0% (33)
Survey 2	13.2% (7)	34.0% (18)	52.8% (28)
Survey 3	4.84% (3)	35.5% (22)	59.7% (37)

We also recorded the survey element (i.e., excavation, sorting, or sifting) responsible for the exposure of each encountered individual (Table 1). The Measey et al. (2009) method employed during Survey 4 used a similar system of synchronized excavation, in which we started by excavating the peripheral borders using hoes and the extracted substrate was not sifted. We identified to species, weighed (g), and measured to 1 mm snout-vent length (SVL), tail length, and total length (TL) all herpetofauna exposed during surveys. We calculated total herpetofaunal density as well as species specific densities (individuals per m^2) both for quadrats and across each survey.

We used Mean Fossorial Herpetofaunal Density (MFHD), defined as the total number of encountered herpetofauna, subgroup, or specific species, divided by the area surveyed, to compare survey results. To assess if seasonal differences in fossorial herpetofaunal community structure, density, and demography could be identified between winter and summer surveys, we compared recorded frequencies between Surveys 1 and 2. To ensure that the comparisons between the effectiveness of the ACM and the Measey et al. (2009) method could be adequately assessed, we conducted Surveys 3 and 4 along the crest of the same dune, which appeared to harbor the highest densities of fossorial herpetofauna when we compared results from the preceding two surveys across delineated sub-sectors and their associated terrain features (see Results below). As with the ACM, we did not remove the vegetation layer prior to surveying quadrats with the Measey et al. (2009) technique during Survey 4 to limit the disturbance of resident herpetofauna and prevent animals from leaving the quadrat before the survey excavation was initiated.

We used the R version 3.0.2 (R Core Team 2013) for statistical analyses. We used a Mann-Whitney U Test (also called a Wilcoxon Rank-sum Test) to assess observed differences in the medians between paired samples for the following comparisons. We compared Surveys 1 and 2, to test for the ability to detect changes in fossorial diversity and abundance. We also compared the results of Surveys 3 and 4, testing the efficacy of the new ACM to that of Measey et al. (2009).

Table 2. Observed mean densities of fossorial herpetofauna individuals per m^2 (counts per survey), the size of each survey, habitat representation, and mean sampling depth for each of the four fossorial surveys conducted on Sileza Nature Reserve, South Arifica. An asterisk (*) indicates this species was not included due to the ability of *Leptotyphlops* to move through the sieve aperture.

	Density per Survey			
Species	Survey 1	Survey 2	Survey 3	Survey 4
Mozambique Rain Frog (*Breviceps mossambicus*)	0.31 (26)	0.50 (42)	0.72 (43)	0.02 (1)
Peter's Thread Snake (*Leptotyphlops scutifrons*)*	(1)	(0)	(0)	(0)
Slender Worm Lizard (*Monopeltis sphenorhynchus*)	0.01 (1)	0 (0)	0.02 (1)	0 (0)
Wahlberg's Snake-eyed Skink (*Panaspis wahlbergii*)	0.05 (4)	0.01 (1)	0 (0)	0 (0)
Zululand Dwarf Burrowing Skink (*Scelotes arenicolus*)	0.02 (2)	0.04 (3)	0.08 (5)	0 (0)
Sand-dwelling Dwarf Worm Lizard (*Zygaspis arenicola*)	0.20 (17)	0.11 (9)	0.22 (13)	0.02 (1)
Total fossorial herpetofaunal density	0.60 (50)	0.65 (55)	1.03 (62)	0.03 (2)
Total number of quadrats	21	21	15	15
Total surveyed area in m^2	84	84	60	60
Mean measured sampling depth in m	0.28	0.27	0.30	0.27

Results

We recorded six herpetofaunal species consisting of one amphibian, four lizards, and one typhlopid snake species (Table 2). Apart from Wahlberg's Snake-eyed Skink (*Panaspis wahlbergii*), which is considered fossorial (Maritz and Alexander 2008) or semi-burrowing (Branch 1998) in nature, the remaining herpetofauna exhibit obligate fossorial lifestyles (Maritz and Alexander 2008). Peter's Thread Snake (*Leptotyphlops scutifrons*) was deemed slender enough to escape through the iron mesh netting during the sorting and sifting processes, which meant that the number recorded using the ACM may not be a true reflection of the number in the sample quadrats. As such, we excluded this species from our analytical comparisons.

Habitat representation between the paired surveys proved near identical. Surveys 1 and 2 were similarly delineated along the dune and sampled very similar vegetation profiles. Due to the subjective placement of the Survey 3 and 4 sectors on the crest of the dune, the two surveys sampled identical landscape features (10 quadrats on the crest and five quadrats on the upper slope each) and very similar vegetation features (Table 3).

Survey 1 produced a MFHD (mean ± standard error) of 0.6 ± 0.19 individuals/m^2 and Survey 2 a MFHD of 0.65 ± 0.18 individuals/m^2. Survey 3 produced a MFHD of 1.03 ± 0.15 individuals/m^2 and Survey 4, 0.03 ± 0.02 individuals/m^2. The most abundant species was the Mozambique Rain Frog (*Breviceps mossambicus*), which was the only amphibian detected during these surveys, followed by the Sand-dwelling Dwarf Worm Lizard (*Zygaspis arenicola*; Table 2). Although we did not test for significance, there were notable differences between the frequencies of specimen extraction arising from digging, sorting, and sifting, which we conducted using the novel ACM (Table 1). Despite the manual sorting of substrate samples by hand, the extraction element that appears most effective in all three surveys conducted with the ACM appears to be sifting.

The median relative abundances did not differ significantly between Surveys 1 and 2 (U = 189, P =

Table 3. The vegetation characteristics and location relative to the dune slope of quadrat surveys for fossorial herpetofauna on Sileza Nature Reserve, South Africa.

Habitat characteristic	Survey 1	Survey 2	Survey 3	Survey 4
Vegetation				
Grass dominated quadrats (%)	13 (61.9)	13 (61.9)	11 (73.3)	10 (66.7)
Woody dominated quadrats (%)	5 (23.8)	6 (28.6)	2 (13.3)	3 (20.0)
Grass-woody dominated quadrats (%)	3 (14.3)	2 (9.52)	2 (13.3)	2 (13.3)
Positions on slope				
Quadrats on dune crest (%)	5 (23.8)	6 (28.6)	10 (66.7)	10 (66.7)
Quadrats on upper slope of dune (%)	5 (23.8)	6 (28.6)	5 (33.3)	5 (33.3)
Quadrats on mid slope of dune (%)	6 (28.6)	5 (23.8)	0 (0.0)	0 (0.0)
Quadrats at base of dune (%)	5 (23.8)	4 (19.1)	0 (0.0)	0 (0.0)

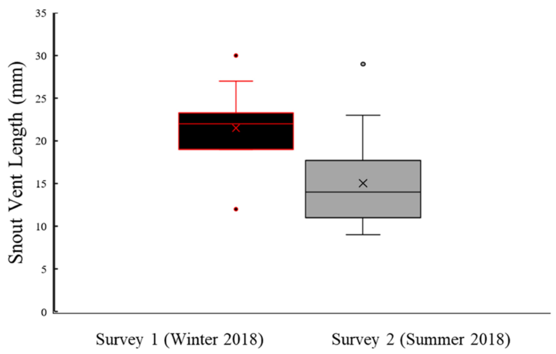

Figure 2. Box plots showing the body length measurements (mm) of the Mozambique Rain Frog (*Breviceps mossambicus*) captured during Survey 1 in the austral winter (n = 26) and during Survey 2 in the austral summer (n = 40) on the Sileza Nature Reserve, South Africa, using the adapted composite method. The box indicates the interquartile range, the horizontal line running through each box indicates the median, the x in each box indicates the mean, and the dots indicate the outliers in body length measurements.

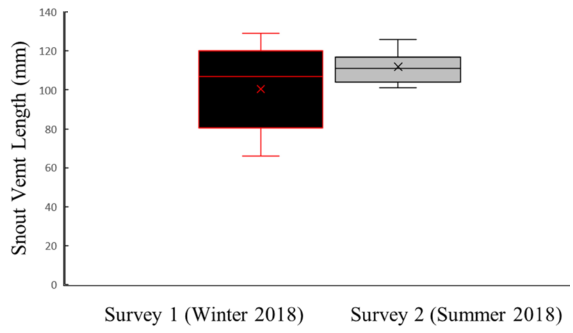

Figure 3. Box plots showing the snout-vent length measurements (mm) of the Sand-dwelling Dwarf Worm Lizard (*Zygaspis arenicola*) as derived from Survey 1 during the austral winter (n = 14) and Survey 2 during the summer (n = 7) assessments on the Sileza Nature Reserve, South Africa, using the adapted composite method. The box indicates the interquartile range, the horizontal line running through each box indicates the median, the x in each box indicates the mean, and the dots indicate the outliers in in the body length measurements.

0.218). Mozambique Rain Frog density increased from the winter to the summer survey, but the mean SVL for the measured sample decreased from 22.3 ± 5.49 mm (n = 26) from Survey 1 (winter) to 15.5 ± 4.79 mm (n = 40) in Survey 2 (summer), with 40.0% (n = 16) of the measured Survey 2 sample made up of individuals ≤ 12 mm in comparison to 5.6% (n = 1) of the measured Survey 1 sample (Fig. 2). The reverse proved true for the Sand-dwelling Dwarf Worm Lizard samples. Survey 2 exposed lower densities for the Sand-dwelling Dwarf Worm Lizard (n = 7) with a slightly increased mean SVL (111.9 ± 8.70 mm) when compared to Survey 1 (n = 14) and the complete lack of individuals < 100 mm, which made up 48.5% of the winter sample (Fig. 3). We found three gravid Sand-dwelling Dwarf Worm Lizard females, as well as a single egg, during the summer survey. The medians for exposed fossorial herpetofauna differed significantly between Survey 3 and Survey 4 ($U = 9.5$, $P < 0.001$).

Discussion

The development of the ACM (described herein) aimed to amalgamate and adapt elements from existing survey techniques to better adhere to the three underlying conditions set out by Heatwole (2012) while simultaneously making the technique eligible for implementation in protected areas. The ACM incorporates randomized quadrats of standard parameters within localized sector and sub-sector block, the temporary storage of sand in buckets to prevent the escape of herpetofauna prior to its processing, and the active sorting and sifting through sand to expose fossorial reptiles and amphibians. This method also employed the smallest sift aperture (2 × 2 mm) to date, allowing only the smallest of species to potentially evade capture. Although none of the basic elements incorporated into the ACM are unique to the survey technique, the methodology is novel due to the combination of the selected physical survey and quadrant parameters with practical aspects such as the temporary storage of substrate, decrease in sieve aperture, use of spades instead of hoes, and others. These modifications optimize the reliability of generalized subterranean herpetofaunal surveying through increased adherence to the three underlying conditions of Heatwole (2012) while limiting the scope of environmental impact.

Mitigating survey induced environmental disturbance.—Despite the ease with which earth moving machinery can conduct large scale excavations that might facilitate quantitative fossorial herpetofaunal surveys, the use of such equipment in sensitive locations or in conservation areas proved unacceptable to conservation authorities due to the potential environmental impact. We determined that manual labor was the more acceptable and financially viable excavation option for the ACM. We also believe that the relatively small size and spacing of the quadrat locations will likely result in relatively rapid rates of vegetation succession. Limiting the number of replicates across a defined area also prevented large sections of protected habitat from being impacted. In total, the extent of the four surveys we conducted impacted 288 m^2, which was equivalent to 0.001% of the surface area of the SNR, limited to 1 ha (four sectors of 50 × 50 m) of the protected area.

Table 4. A comparison of several facets of quantitative methodologies of fossorial herpetofaunal surveys illustrating how techniques compare. References in parentheses are 1 = Pooley et al. 1973, woodland quadrats; 2 = Pooley et al. 1973, sand forest quadrats; 3 = Measey et al. 2003/Measey et al. 2006; 4 = Heatwole and Stuart 2008; 5 = Maritz and Alexander 2008, manual excavation method; 6 = Maritz and Alexander 2008, mechanical excavation method; 7 = Measey et al. 2009/Jordaan and Hanekom 2019/Survey 4 current study; and 8 = Adapted composite method Survey1, 2 and 3 in current study. The abbreviation NA = not applicable.

Quadrat Selection Process	Subjective placement (1)	Subjective placement (2)	Randomized placement (3)	Subjective placement (4)
Size of selected area per survey	NA	NA	10 × 10 m (100 m² × 3)	NA
Number of quadrats	Nonstandard	5	15	8
Quadrat size and dimensions	Nonstandard	1,67 m²	1 m² (1 × 1 m)	100 m² (10 × 10 m)
Depth	Nonstandard	0.76 m	0.25 m	Detrital material until mineral soil
Quadrat border barriers	No	No	Yes (Trench)	Yes (Trench and mosquito netting)
Excavation method	Manual excavation	Manual excavation	Manual excavation with hoes	Manual detrital removal by hand
Sorting through substrate by hand	Yes	Yes	Yes	Yes
Sifting component (sieve aperture) [sieve dimensions]	No	No	No	No
	Randomized placement (5)	Subjective placement (5)	Subjective placement for historical assessment (7)	Randomized placement (8)
Size of selected area per survey	10 × 10 m (100 m²)	NA	NA	50 × 50 m (2,500 m²)
Number of quadrats	28	19	15	Survey 1, 2: 21 Survey 3: 15
Quadrat size and dimensions	1 m² (1 × 1 m)	9 m² (3 × 3 m)	4 m² (2 × 2 m)	4 m² (2 × 2 m)
Depth	0.3 m	1 m	0.25 m	0.25 m (minimum)
Quadrat border barriers	No	Yes (Trench)	Yes (Trench)	Yes (Trench)
Excavation method	Manual excavation with spades	Large earth moving machinery	Manual excavation with hoes	Manual excavation with spades
Sorting through substrate by hand	No	Yes	Yes	Yes
Sifting component (sieve aperture) [sieve dimensions]	Yes (25 × 15 mm) [1 × 0.75 m]	No	No	Yes (2 × 2 mm) [1.2 × 1.2 m]

A series of other less disruptive semi-quantitative and quantitative techniques (Measey 2006) have also been employed to either locate or assess the density of fossorial herpetofauna (Henderson et al. 2016). These include pitfall trapping (Goodyear and Pianka 2008), structured cover board assessments (Henderson et al. 2016), and active searches involving both the inspection of naturally occurring cover objects, such as rocks and logs (Martin et al. 2015; Wong et al. 2021) or actively searching through loose sand either by hand (Huey et al 1987) or with the use of rakes (Mashinini et al. 2011), which may or may not be timed or otherwise constrained. The implementation of such low-impact survey methods has been shown to underestimate population numbers, diminishing the value of such estimates (Kuhnz et al. 2005).

With respect to the survey sites of this study on SNR, the high load of subsurface woody roots and stems made the use of rakes during surveys unfeasible. Environmental and seasonal factors, as well as species-specific movement and habitat selection, may inherently affect pitfall trapping results (Driscoll et al. 2012), which require the surface or shallow subsurface lateral movement of animals to make themselves available for capture (Henderson et al. 2016; Willson 2016). Considering that fossorial reptiles and amphibians may only infrequently move over the surface of the soil and that some obligate fossorial herpetofauna seldom move large distances (see Martin et al. 2021), these survey techniques may have limited application depending on the targeted species or a specific group of fossorial herpetofauna. It should be stated, however, that terrain and habitat features (e.g., excessive rocks, stony or structured soils, and forests with many trees and roots) may limit the feasibility of conducting quantitative quadrat surveys, including the ACM, leaving only semi-quantitative methods to assess the composition of fossorial herpetofaunal communities.

Adequate habitat representation.—The constraint of the quadrat selection to within sectors and sub-sectors during this study largely resulted in quadrats being spread relatively evenly, allowing the assessment to survey homogenous vegetation and comparable terrain features. The randomized selection process, which dispersed the sample quadrats over the sub-sectors, produced similar microhabitat profiles across Surveys 1 and 2, which were situated along the dune slope. The vegetation characteristics surveyed by Surveys 3 and 4, which were positioned on the dune crest and upper slope, were also nearly identical to each other. Some quantitative fossorial herpetofaunal surveying techniques subjectively select areas suspected of harboring high densities of fossorial herpetofauna (sand forest surveys in Pooley et al. 1973; Verburgt et al. 2018) or only follow qualitative methods indicating detectable densities (Measey 2006). The different layers of randomized selection informing the ACM quadrat locations largely eliminated subjective selections, potentially providing more generalized densities across the sector blocks.

Preventing the escape of herpetofauna.—The selected standard size of quadrats for the ACM allowed for the implementation of better measures to prevent specimens from escaping compared to methods that employed 1 m^2 quadrats (e.g., Measey et al. 2003; Measey 2006). The removal of substrate from inside the quadrat borderline during the ACM effectively functioned as a barrier to prevent undetected lateral movement of animals from the central quadrat block or caused them to be sighted when trying to cross the bottom of the trench. During Surveys 1, 2, and 3, we first observed several specimens in the peripheral trench trying to vacate the central quadrat block during the excavation process, but individuals were unable to scale the sides of the trench and did not move so quickly that we could not detect and catch them.Additionally, the complete removal of substrate from the quadrat and its temporary storage in a series of 20-L buckets allowed the excavation and processing of the rest of the sample to continue without fear of herpetofauna escaping from unattended extracted sand. We note that even with our method, herpetofauna could move vertically down into the substrate while still in the quadrat during excavation, a relevant flaw in most quantitative fossorial quadrat surveys, as some obligate fossorial herpetofauna (such as amphisbaenians) may have vertical galleries or tunnel networks into which they can quickly escape. We used spades (used in the manual excavation method of Maritz and Alexander 2008), with which survey staff had more experience rather than hoes (Measey et al. 2003, 2009; Measey and Barot 2006) or rakes (e.g., Mashinini et al. 2011; Verburgt et al. 2018) as excavation tools. This effectively maintained the standard quadrat parameters and assisted with the removal of loose unconsolidated sand as well as the consolidated blocks of vegetated sand.

Exposing all specimens within the surveyed substrate.—While the sorting of extracted substrate by hand produced the second highest number of herpetofauna during the survey process using the ACM, we took additional steps to sift and remove all the sand from a sample to ensure no specimens avoided detection by sheltering under or in substrate. We put all excavated substrate through a sieve following sorting in accordance with the heavy machinery method of Maritz and Alexander (2008) to facilitate the extraction of specimens, although we selected a much finer sift aperture (2 × 2 mm) to decrease the physical possibility of specimens escaping detection. The removal of plant materials from the substrate during the sorting process allowed for the additional inspection of embedded vegetation to ensure no specimens avoided detection by hiding between root or grass clumps, with the added benefit of also protecting the sieve during sifting, which can be damaged by stiff or sharp roots and stems pushing holes through the enameled iron mesh.

Despite the surveyed sectors of Survey 3 and Survey 4 neighboring each other and falling over the same terrain and habitat features, which presumably harbor similar densities of soil living reptiles and amphibians, the results indicate a significantly higher detection ability for fossorial herpetofauna in favor of the ACM compared to the Measey et al. (2009) methodology. The higher success rate of the ACM may be due to the complete removal of substrates in structured blocks from the quadrat, which were then temporarily stored in buckets, sorted through by hand and then sifted through a fine apertured sift. This allowed for the extraction of specimens from the substrate instead of relying only on visual observations of exposed or moving specimens in or through the substrate as the primary method of detection, which is employed by some other quantitative methods of fossorial herpetofauna surveys. Few studies have tested or compared the effectiveness of fossorial herpetofaunal survey techniques against each other (e.g., Kuhnz et al. 2005; Maritz and Alexander 2008; Jordaan et al. 2023).

Parameters, application, biases, and interpretation.—As most fossorial herpetofauna occurs in shallow soil layers, we set a minimum depth of 0.25 m at which quadrats are excavated during the ACM. A standard quadrat size of 4 m^2 (2 × 2 m) was deemed more likely to produce results as found in Measey et al. (2009) than the 1 m^2 quadrats used by Measey et al. (2003), Measey and Barot (2006), and in the manual digging surveys of Maritz and Alexander (2008). We selected these parameters to ensure that measures of confinement could be implemented to better prevent the escape of specimens (see above), while covering a greater area using fewer quadrats but still limiting the size of their disturbance.

Despite non-significant differences in total herpetofauna encountered between the winter and summer surveys, notable differences in species density and the physical size for the *B. mossambicus* and *Z. arenicola* populations were evident. This would suggest that the ACM can detect general changes in community diversity and population structure for fossorial herpetofauna which occur within the top 0.25 m of the soil column at high densities between seasons and could likely detect similar changes when conducting assessments over time. This would make the ACM a viable option as a monitoring tool or potentially assist with the calibration of less destructive techniques, taking its limitations and biases into consideration. Several practical caveats should be considered when conducting surveys using the ACM. For instance, the time required to conduct sampling using the ACM is significantly longer than when using the Measey et al. (2009) method. This is evident when comparing the average time per quadrat between Surveys 3 and 4 (111.0 and 29.6 min, respectively). Additionally, due to the fine aperture of the sieve, this method is likely best applied to fine sandy substrates. Increased soil moisture following rain caused sand to clump together, making it more difficult to pass through the mesh netting. The onset of rainfall may also increase the surface movement of fossorial herpetofauna (Branch 1998; Du Preez and Carruthers 2017). With increased surface activity, observable fossorial herpetofaunal density will likely decrease as the sampling strategy depends on the presence of specimens within the first 0.25 m of the soil column.

An evident seasonal shift in the demography and density of *B. mossambicus* occurred between Surveys 1 and 2, presumably related to the increased breeding activities of the species at the onset of summer with an influx of juvenile animals to the population. The detectable density of *Z. arenicola* decreased between winter and summer but with a corresponding increase in the median and mean SVL of the population. This may either imply that juvenile animals grow rapidly, or an ontogenetically associated seasonal shift in fossoriality may exclude young animals from the population available for detection by the survey technique.

Concluding remarks.—The general lack of information on the ecology and conservation status of fossorial herpetofauna when compared to other terrestrial faunal groups (Measey 2006; Böhm et al. 2013; Bates et al. 2014) has prompted several authors to advocate for the standardized inclusion of fossorial assessments during herpetofaunal surveys to aid biodiversity assessments (Measey 2006, Maritz and Alexander 2008). Such inclusions in biological diversity assessments have led to recent discoveries of new taxa (Verburgt et al. 2018) and may contribute significantly to either monitoring soil-based ecosystem responses to environmental change directly (Measey et al. 2009) or to calibrating less destructive semi-quantitative survey methods. The responses of soil vertebrates to large-scale agriculture, post-mining rehabilitation, the invasion of alien or encroaching vegetation, exotic plantations, fire regimes, increased herbivore stocking rates, and urban sprawl have not received adequate attention from either conservation or academic communities (e.g., Measey et al. 2009; Measey 2014; Jordaan et al. 2023).

The ACM described and tested here, appears to produce significantly higher density estimates for abundant medium-sized fossorial herpetofauna than the established method it was tested against while still limiting environmental disturbance. The ability of the method to detect alterations to the fossorial herpetofaunal community structure between seasons, suggests that similar differences may be detected by incorporating the method into monitoring strategies to track changes in density and diversity associated with environmental change in areas with unconsolidated aeolian substrate. Additional testing of the efficacy of this method to explore its functionality and application in other vegetation types and soils will be required to expose the inherent biases of this technique. With the increased publication of accurate data from quantified subterranean herpetofaunal assessments, the impact of environmental changes on fossorial ecosystems and the species-specific conservation status of fossorial herpetofauna can be better assessed.

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