Notes on the identification of *Uperoleia* Gray, 1841 toadlets from the Darwin region of the Northern Territory, with comments on the ecology, detection, and conservation management of the Vulnerable Howard River Toadlet (*U. daviesae*)

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ABSTRACT – Three species of *Uperoleia* toadlets occur in the Darwin region, and are difficult to tell apart due to similar size and colouration. Identification has generally relied on differences in male advertisement calls. *Uperoleia daviesae* is the Northern Territory's only threatened frog and is impacted by urban development and sand mining. Given their threatened status and significance in development impact assessments, having a method of species identification that does not rely on calling males is particularly important. Here we outline a reliable and simple method for the morphological identification of each of the three species based on the shape, size and placement of the parotoid and inguinal glands. We also provide comments on the ecology and habitat of *U. daviesae*, and key information on detectability to improve survey work on this threatened species. We broadly characterise *U. daviesae* sites as 'persistent flowing' or 'intermittent flowing' based on our observations of surface water flow and calling patterns. Persistent sites have surface flow and support *U. daviesae* calling for weeks or months after the first significant rainfall, whereas intermittent sites may require 10-day cumulative rainfall totals of >100 mm to trigger calling which may persist for a few days only. Detectability of *U. daviesae* from calling is therefore site specific. Effective conservation planning and species recovery would be aided by research into *U. daviesae* population dynamics, hydrology of sandsheet heath habitats and the potential for sand mining rehabilitation.

KEYWORDS: Parotoid gland, inguinal gland, species identification, detectability

INTRODUCTION

The Darwin region of the Northern Territory is home to three species in the genus *Uperoleia*: the Darwin region endemic *U. daviesae* Young, Tyler, and Kent, 2005; the widespread *U. crassa* Tyler, Davies, and Martin 1981 that is distributed across the Kimberley and Top End (Jaya et al. 2022); and *U. lithomoda* Tyler, Davies, and Martin, 1981 that is also widespread, distributed across the east Kimberley, Top End and far north Queensland (Catullo et al. 2014; Catullo and Keogh 2014). Males from each species can be easily distinguished by their distinctive advertisement calls (Catullo et al. 2014; Jaya et al. 2022; Tyler et al. 1980; Young et al. 2005), which differ in call duration, number of pulses and dominant frequency. These species are also highly genetically distinct, belonging to three separate clades; indeed *U. daviesae* is the most phylogenetically distinct species within the genus, estimated to have diverged approximately 4 MYA (Catullo and Keogh 2014).

Despite the acoustic and genetic differences, there has been substantial difficulty in the morphological identification of each species. The description of *U. daviesae* (Young et al. 2005) distinguishes it from the two sympatric species by being dentate (a characteristic that is not easily used in the field), having 'indistinct' dermal glands and orange-red inguinal pigmentation. *Uperoleia crassa* is described as larger, with well-defined dermal glands, a smooth dorsum, and light orange inguinal pigmentation. *Uperoleia lithomoda* is described as similar in size, but with well-defined dermal glands and dark, slightly raised markings. More recent field guides refer to *U. daviesae* having 'indistinct or moderately developed' parotoid glands (Clulow and Swan 2018), or no clear distinguishing characteristics (Eipper and Rowland 2023). However, observations by the authors are that *U. daviesae* cannot be distinguished by these characters; that *U. daviesae* generally has well-defined, if small, dermal glands and both it and *U. crassa* have variable dorsal texture. The colour of inguinal pigmentation is variable within many *Uperoleia* species, particularly across the orange/red colour spectrum (RC; pers. obs.).

The lack of ability to identify the species morphologically is problematic because *U. daviesae* is listed as Vulnerable in the Northern Territory under the Territory Parks and Wildlife Conservation Act (Department of Environment, Parks and Water Security 2021) and nationally under the Environmental Protection and Biodiversity Conservation Act (Threatened Species Scientific Committee 2021). The species is restricted to sandsheet heath habitats characterised by sand substrates, low and sparse vegetation and seasonal inundation (Department of Environment, Parks and Water Security 2021). With most of its distribution within the peri-urban growth areas of Darwin, *U. daviesae* is at threat from habitat loss, hydrological alteration from the expansion of Darwin and sand mining (Department of Environment, Parks and Water Security 2021). If possible, reliable morphological identification of *U. daviesae* versus sympatric congeners will enable higher confidence in ecological survey work, open the opportunity for survey methods that assess female frogs, and provide the ability to undertake methods of survey such as pitfall trapping outside the breeding season.

Given the frequent reliance on advertisement calls for *U. daviesae* detection, information on the timing of calling activity must underpin the design and planning of targeted acoustic surveys. Reynolds and Grattidge (2012) and (Young et al. 2005) identified that *U. daviesae* calling activity was associated with shallow surface flows and that substantial wet season rainfalls were required to create suitable breeding conditions. They also identified that spatial variation in *U. daviesae* calling may relate to site-specific characteristics (Reynolds and Grattidge 2012). However, Dostine et al. (2013) found continuous calling at a single site when surveying over longer periods. These observations point to the need for further investigation into the relationships between antecedent rainfall and calling activity, and how this may vary between sites.

We here present a method to identify all *Uperoleia* species in the Darwin region based on morphology alone. We also outline biological observations on the detectability of the species based on site characteristics that should be considered in future surveys. Finally, we outline areas of research needed to underpin conservation planning and management for this distinct species.

MORPHOLOGICAL IDENTIFICATION

Differences in gland shape and placement as diagnostic characters in *Uperoleia* were outlined in previous work (Catullo and Keogh 2021), which had identified clear differences in inguinal gland shape and placement between *U. crassa* and *U. lithomoda*. The original description of *U. daviesae* identified 'indistinct dermal glands' and small body size as methods to distinguish from sympatric congeners (Young et al. 2005). Reynolds and Grattidge (2012) has also noted 'indistinct dermal glands' of *U. daviesae,* but consistent with our approach outlined below, that they were less prominent than *U. crassa*.

The identification method described here is based on surveys carried out by the authors in January/February 2023, where we reviewed the gland patterns by species. During these surveys, which were focused on the collection of genetic samples for conservation planning, we captured *U. daviesae* (n = 130). Each individual was morphologically identified, photographed, most were measured for snout-urostyle length, and all were finger clipped for ongoing genetic assessment (Charles Darwin University AEC approved project no. A21021, NT Parks and Wildlife Commission permit no. 71982). All acoustic/morphological identifications of *U. daviesae* as well as 6 *U. crassa* from 2023 were genetically confirmed as part of ongoing research (not shown). Identifications of *U. lithomoda* from outside the Darwin areas were genetically confirmed as part of previous research projects (Catullo et al. 2014; Catullo and Keogh 2014), as we did not observe them in the sandsheet heath habitat.

A review of captured *U. daviesae* individuals identified consistent differences in the shape and size of the parotoid and inguinal glands relative to *U. crassa* and *U. lithomoda* (Figure 1; Reynolds and Grattidge 2012; Catullo and Keogh 2021). In particular, our approach differs from previous descriptions in that we do not identify the parotoid gland of *U. daviesae* as 'indistinct'. Indeed, the parotoid gland is generally well-defined but differs in size and placement. The parotoid gland of *U. daviesae* is small, located entirely on the lateral surface above the arm, and rarely extends above the level of the eye. It does not extend ventrally to the arm, nor anteriorly to the eye. The inguinal gland of *U. daviesae* is small, round, and restricted to the rear half of the dorsal surface, often covered when the leg is in resting position.

The parotoid gland of *U. crassa* is large and triangularish. It often extends towards the dorsal midline (often on the dorsal surface), ventrally to the arm, forward to the posterior edge of the eye, and often extends dorsally to above the level of the eye. It is substantially larger and more obvious than that of *U. daviesae*. The inguinal gland of *U. crassa* is similar to

U. daviesae, in that it is small, round, and restricted to the rear half of the dorsal surface, often covered when the leg is in resting position. The inguinal gland of *U. lithomoda* distinguishes it from both *U. daviesae* and *U. crassa*. Inguinal glands of *U. lithomoda* are long and thin, and located dorsolaterally. The parotoid glands of *U. lithomoda* are large and triangular, but not as large as *U. crassa*. It is important to note that this diagnosis applies to the extent of the gland itself. Colour patterning on the parotoid glands can be variable and sometimes misleading; note in Figure 2 that the lateral and dorsal areas of the large parotoid glands often have differently coloured surfaces in *U. crassa* and *U. lithomoda*.

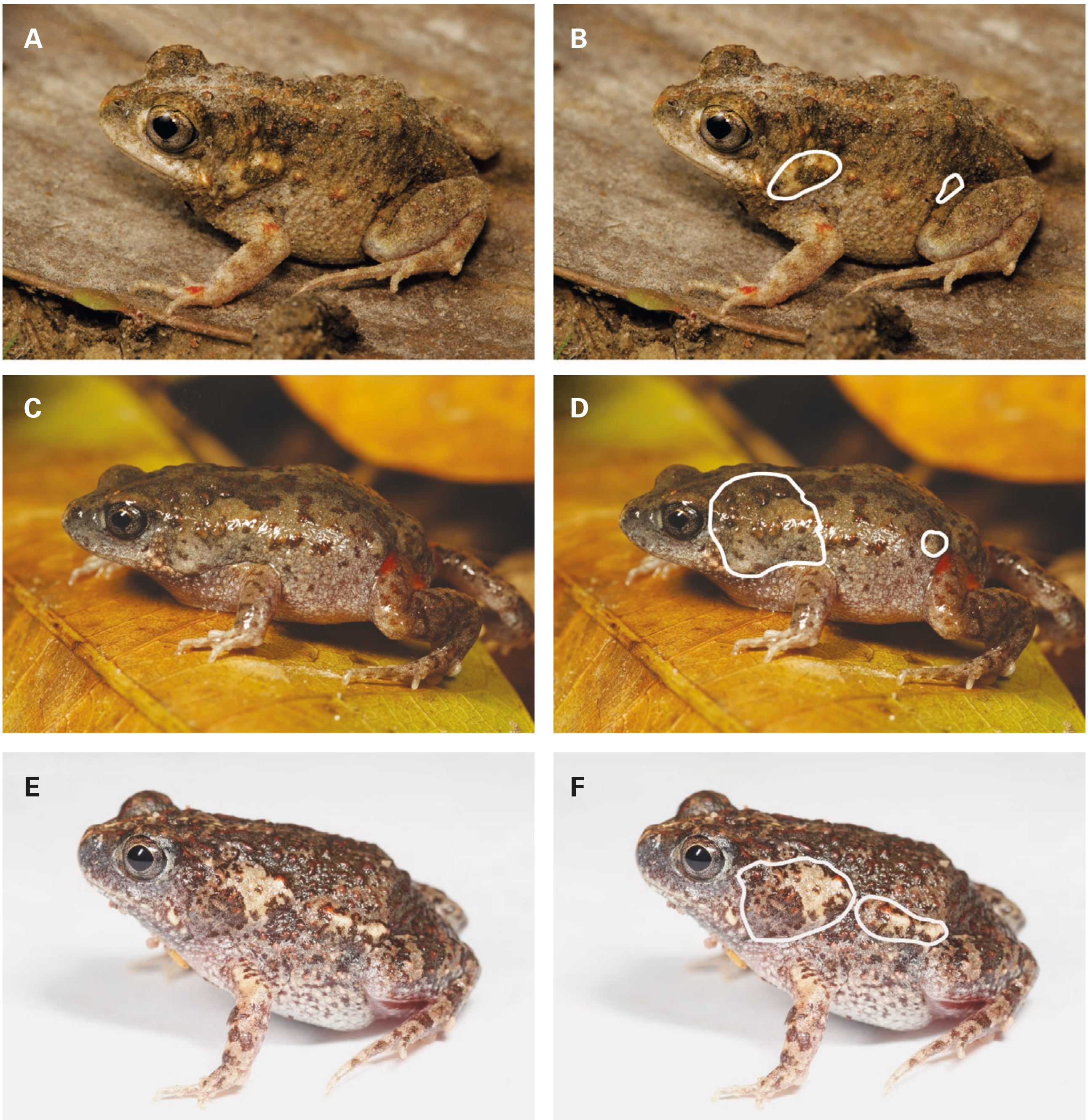

FIGURE 1 Diagnostic differences in gland pattern of the three species of *Uperoleia* in the Darwin region (B, D, F outline the glands shown in A, C and E): A–B) *U. daviesae*, the parotoid glands are small and oval and located laterally above the arm, rarely extending dorsally above the level of the eye. The inguinal glands are small, round and restricted to the posterior dorsal surface; C–D) *U. crassa*, the parotoid glands are large, often extending above the level of the eye, and cover an extensive dorsolateral area of the frog. The inguinal glands are similar to those of *U. daviesae*; E–F) *U. lithomoda*, the inguinal glands are long, thin and distributed dorsolaterally. The parotoid glands of *U. lithomoda* are larger than those of *U. daviesae* and smaller than those of *U. crassa*. (Photos: *U. daviesae* by M. Clancy; *U. crassa* and *U. lithomoda* by D. Esquerre.)

FIGURE 2 Variation in the parotoid gland in males from the three species of *Uperoleia* in the Darwin region: A–F) *U. daviesae*; G–L) *U. crassa*; M–R) *U. lithomoda*. All *U. daviesae* and *U. crassa* were photographed in the Darwin region during January/February 2023, from sites where the species are sympatric. All individuals were accurately identified morphologically by the authors using parotoid gland shape and size, with confirmation through subsequent genotyping. *Uperoleia lithomoda* are shown from previous morphological and genetic work by Renee Catullo. (Photos: *U. daviesae* and *U. crassa* by A. Stewart and R. Catullo; *Uperoleia lithomoda*: M, Umbrawarra Rd by D. Esquerre; N, Roper Hwy by M. Whitehead; O, Point Stuart Rd by D. Esquerre; P, Katherine Gorge Rd by M. Whitehead; Q, Gregory National Park by M. Whitehead; R, Keep River National Park by M. Whitehead.)

All three species are overlapping in size, with a trend towards *U. crassa* as the largest. The mean size of calling adult male *U. daviesae* (n = 97) was 22.9 mm [19–26]. In broader the Northern Territory, the mean size of *U. crassa* males (n = 11) was 24.5 mm [21.8–26.5] (Catullo and Keogh 2021), and the mean size of *U. lithomoda* males (n = 17) was 22.2 mm [20.2–24.9] (Catullo et al. 2014).

SITE CHARACTERISTICS AND DETECTABILITY

During the field season, we visited most confirmed *U. daviesae* sites at least twice, and up to six times, and observed striking differences in the consistency of call activity (Table 1, Appendix). We found that sites can be broadly characterised as 'persistent flowing' or 'intermittent flowing' according to patterns in surface water flow persistence. Persistent flowing sites have persistent surface water flow and support *U. daviesae* calling activity for weeks or even months after the last significant rainfall, as demonstrated by repeated visits to such sites (Table 2). The surface flow in these sites is fed by adjacent laterite which, after becoming saturated, slowly releases water across the sandsheet (D. Cobban, pers. comm.). In contrast, intermittent sites have surface water flow only in the days following significant rainfall and calling activity ceases once the flows have stopped. Presumably, the intermittent sites have smaller or disrupted areas of adjacent laterite than flowing sites or surface flow is derived from other substrates. These observations are supported by previous work that found *U. daviesae* active during weekly surveys of a single site over a 10-week period

TABLE 1 Observations of variation in *Uperoleia daviesae* calling activity between sites with variable antecedent rainfall during the 2023 summer breeding season. '10-day rainfall' refers to 10 consecutive 24 hour periods up until 9 am on the sampling day.

Site	Date	10-day rainfall (mm)	Calling Y/N	Classification
UD01	23 January	17.8	Y	Persistent
UD21	24 January	33.6	N	Intermittent
UD21	27 January	59.8	Y	Intermittent
UD29	24 January	33.6	Y	Persistent
UD10	24 January	88.4	Y	Intermittent
UD10	1 February	31.8	N	Intermittent
UD18	26 January	99.4	N	Intermittent
UD18	2 February	182.9	Y	Intermittent
UD28	1 February	33.3	N (1 individual only)	Intermittent

TABLE 2 Soil temperature, water temperature and 24-hour rainfall for repeated visits to site UD1, a persistent flow site, during the summer breeding season in 2023. Male calling activity was generally continuous regardless of rainfall, with a trend for less calling at lower temperatures.

Date	Soil (°C)	Water (°C)	24-hour rainfall (mm)	*U. daviesae* activity
26 January	26.3	26.9	1.2	Present but low calling activity
27 January	26.4	27.0	1	One individual calling
30 January	28.4	28.7	1	Many individuals calling
31 January	28.6	27.6	1.2	Many individuals calling
1 February	28.6	30.2	1	Many individuals calling
2 February	28.0	29.6	1.2	Many individuals calling, actively raining

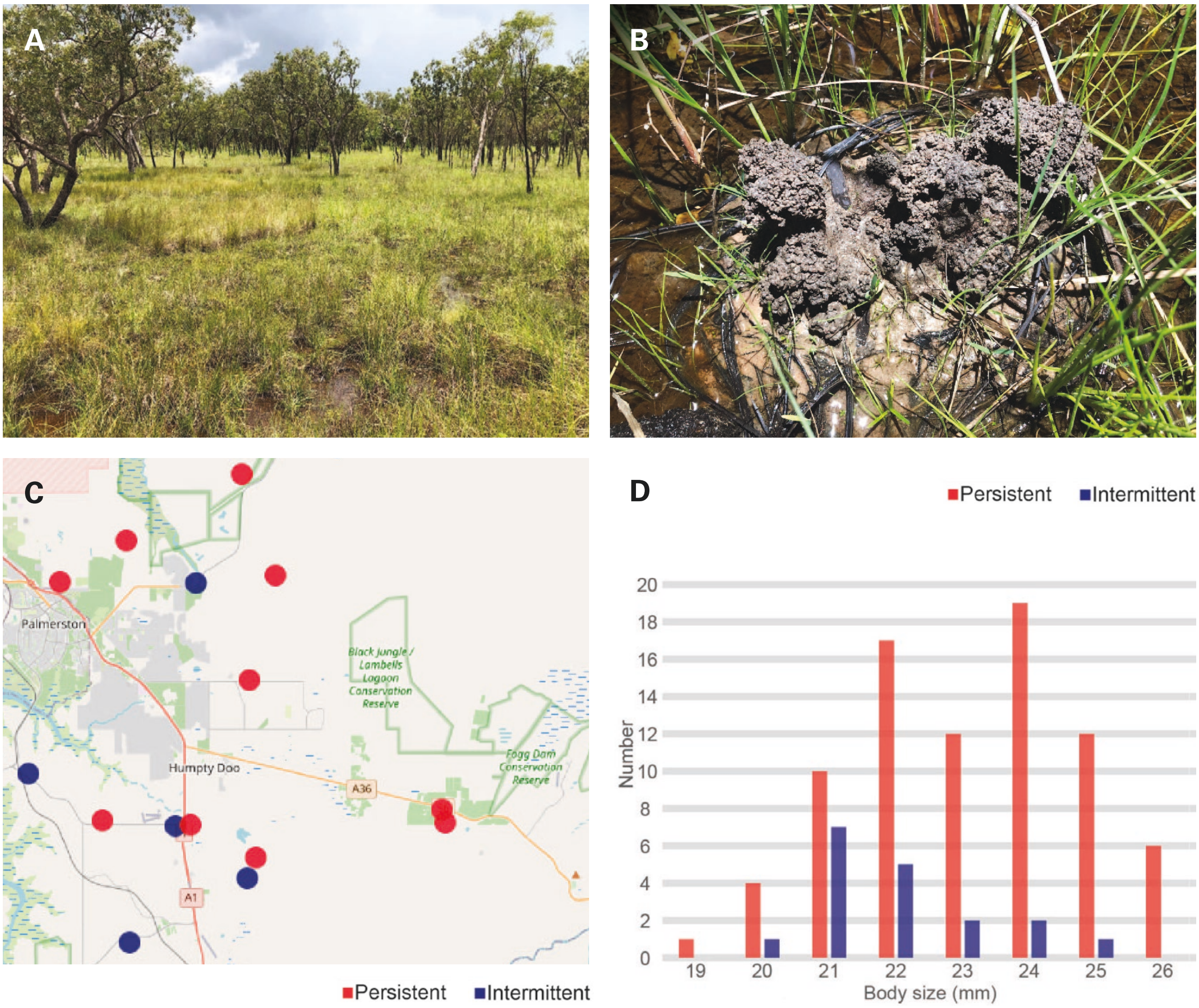

FIGURE 3 A) Sandsheet heath habitat at Lambell's lagoon (UD1); B) Closeup of a debil-debil mound built from annelid worm tailings; C) Visited sites were classified as persistent or intermittent based on our observations; D) Male size differences between persistent and intermittent sites (photos Renee Catullo).

between mid-December 2008 to late February 2009 (Dostine et al. 2013), despite fluctuations in calling activity in other co-distributed species.

Given our observation of high levels of variation in surface water flow across sites (Table 1), together with the fact that monsoonal rainstorms can be highly localised, reference sites for calling activity are unlikely to be useful in predicting calling activity at other sites; reference sites will be active. This was highlighted in our observations of sites UD21 and UD29, situated 1 km apart either side of the Stuart Hwy site. Site UD29 is a well-known *U. daviesae* location with persistent surface flow from a large area of adjacent laterite, and we observed surface flow and calling activity during a relatively dry period (Table 1; Appendix). In contrast, site UD21 is relatively flat with no obvious adjacent laterite water source. Despite the close proximity to site UD29, *U. daviesae* were only recorded here after a period of higher rainfall resulted in surface flow (Table 1; Appendix). For *U. daviesae* call detection at intermittent sites, our observations show that 10-day cumulative rainfall totals of >50 to >100 mm may be required (Table 1; Appendix). Using nearby persistent sites as references sites may result in incorrect inferences of absence; we note that previous survey work has used UD028, one of our persistent sites, as a reference site (EcOz Environmental Consultants 2014). We stopped to listen as we drove past this site repeatedly and frogs were actively calling regardless of prior rainfall, even when other nearby populations were inactive.

While surveying, we noticed a trend that males from intermittent sites ($n = 18$) appeared smaller than males from flowing sites ($n = 81$). This was confirmed by a two-sample t-test that identified a significantly smaller body size in intermittent sites ($p = 0.005$; mean intermittent 22.0 mm [20–25] *versus* mean flowing 23.1 mm [19–26], equating to a 5% average difference in body size in intermittent sites (Figure 3D).

CONSERVATION

Our observations made here that 1) sites differ in hydrology, and 2) there is a size difference between persistent and intermittent sites, suggests substantial site-level differences in habitat quality. Given these patterns, we propose that there are two possible landscape-level population processes for the species: source-sink dynamics or a metapopulation (Heard et al. 2012). The distinction between these is important for management: a source-sink population has key stable populations that are critical to the persistence of the species in an area and to the maintenance of peripheral populations. Under this population dynamic, identification and protection of source populations would be vital. A metapopulation consists of a network of demographically independent populations connected by infrequent, distance-limited dispersal (Levins 1969), characterised by extinction and colonisation of individual populations (Heard et al. 2012). Under a metapopulation dynamic, connectivity between habitats must be maintained to enable colonisation and reestablishment as habitat quality shifts. Appropriate management of *U. daviesae* would be aided by research into the population dynamics of the species. Until such information is known, it will be difficult to accurately assess the impact of habitat changes on the species. The population genetics study underway may also provide some resolution of *U. daviesae* population processes.

The role of the debil-debil mounds in the system needs further investigation (Figure 2B). These mounds appear characteristic of healthy heath systems and are the main calling site for *U. daviesae*, and likely the primary daytime retreat sites for males. The mounds resemble earthworm tailings, and are quickly built up over existing vegetation. We dug up several mounds and sorted through the sand, finding large numbers of earthworms (RC and AS). These earthworms were two distinct morphologies (large and proportionally thicker *versus* small and proportionally thinner). We speculate that worms construct the mounds and it is possible this repeated turnover of soil contributes to the low level of vegetation characteristic of the heath. Confirmation of the mounds being built by annelid worms and an assessment of the taxonomy and distribution of the worms could provide insight on the factors underpinning *U. daviesae* habitat suitability.

Effective conservation planning and species recovery for *U. daviesae* would benefit from research into their population ecology and landscape-scale population dynamics, the hydrology of sandsheet heath habitats and neighbouring laterite horizons, and the potential for sand mining rehabilitation, as well as targeted grassy weed management (Northern Territory Government 2017; Threatened Species Scientific Committee 2021).

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APPENDIX The following observations of variation in *Uperoleia daviesae* calling activity across sites with variable antecedent rainfall were recorded during targeted genetic sampling fieldwork in the Darwin rural area between 23 January and 2 December 2023:

OBSERVATION 1

On 23/01/2023 we surveyed a site for *U. daviesae* in the Lambell's Lagoon area just south of the Arnhem Highway (UD01; -12.60396°, 131.23567°). Many (>30) male *U. daviesae* were heard calling at this site and we observed surface water flow. The nearest active BOM weather station (Middle Point, station no. 14041, ~10 km north-east of the survey site) recorded 1.8 mm of rainfall in 24 hours to 9 am the following day (24/01/2023) and a 10-day cumulative total of 17.8 mm. The site was revisited multiple times (Table 1) to assess male call activity relative to temperature.

OBSERVATION 2

On 24/01/2023 we surveyed two known *U. daviesae* sites 1 km apart either side of the Stuart Hwy in the Noonamah area – Goode Road (UD29; -12.61345°, 131.07710°) and Jenkins Road (UD21B; -12.613902°, 131.067731°). The nearest active BOM weather station (Humpty Doo Collard Road, station no. 14226, ~4.5 km north of the surveys sites) recorded 6.8 mm of rainfall in 24 hours to 9 am on 25/01/2023 and a 10-day cumulative total of 33.6 mm. Numerous male *U. daviesae* were calling at the Goode Road site (with persistent flowing water). No individuals were calling at the Jenkins Road site nor in the intervening potentially suitable habitat between this site and the Stuart Highway, where there was water but only in pools. We returned to the Jenkins Road site on 27/01/2023 after late afternoon storm activity (11.8 mm of rain in 24 hours to 28/01/2023 and a 10-day cumulative total of 59.8 mm) and recorded numerous (>10) *U. daviesae* calling along a sandy vehicle track with a sheet of flowing water, and in adjacent sandsheet heath to the west.

OBSERVATION 3

On 24/01/2023 we surveyed a known site in the southern Weddell area (UD10; -12.68561°, 131.03849°). The nearest active BOM weather station (Territory Wildlife Park, station no. 14264, ~6 km south-west of the site) recorded 13.8 mm of rainfall to 9am on 25/01/2023 and a 10-day cumulative total of 88.4 mm. Approximately 15 male *U. daviesae* were recorded in a small area of sandsheet heath with surface flow during this survey. We returned to this site on 01/02/2023 and detected no calling *U. daviesae*. There was little or no surface flow at this time and the nearest weather station recorded 2 mm of rainfall to 9 am on 02/03/2023 and a 10-day cumulative total of 31.8 mm.

OBSERVATION 4

On 26/01/2023 we surveyed a known *U. daviesae* site in the Howard Springs area near the Gunn Point Road crossing of the Howard River (UD18; -12.46507°, 131.08033°). No *U. daviesae* were detected at this site, despite an abundance of surface water in the general area. The nearest active BOM weather station (Howard Springs Nature Park, station no. 14149, ~3 km west of the site) recorded 47.0? mm of rainfall to 9 am on 27/01/2023 and a 10-day cumulative total of 99.4 mm. We returned to this site during and after widespread storm activity on 02/02/2023 and found approximately 10 *U. daviesae* calling. Surface flow was observed at this time and the nearest weather station recorded 30.6 mm of rainfall to 9 am on 27/01/2023 and a cumulative total of 182.9 mm across the previous 10 days.

OBSERVATION 5

On 1/02/2023 we surveyed a site on Hopewell Road in the Berry Springs area (UD28; -12.71104°, 131.01004°) where *U. daviesae* was previously recorded in January 2019 (Atlas of Living Australia). The nearest active BOM weather station (Territory Wildlife Park, station no. 14264, ~2 km west of the site) recorded 2 mm of rainfall to 9 am on 02/03/2023 and a 10-day cumulative total of 33.3 mm. A single male was detected calling intermittently at this site and no other *U. daviesae* were detected in a wider search of this Crown Land block. Most of the potentially suitable habitat had no surface water and there was no observed surface water flow.

OBSERVATION 6

Prior surveys identified a site on the southern end of Redcliffe Road in the Noonamah area as having *U. daviesae*. This area was revisited on multiple occasions following a variety of rainfall. On all occasions *U. daviease* males were active at a nearby persistent flow site (UD29, ~2.5 km away), regardless of rainfall. On 02/02/2023 following extensive rain, two intermittent sites on Horsnell Road (~1.5 km away) had active choruses. During an exploration of this location on the date of positive Horsnell Road activity, we observed water flowing as a creek, and tall (~2 m) and thick grass across the site. No *U. daviesae* were detected.