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Movement and Habitat Use of the Endangered Australian Frog *Nyctimystes dayi*

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The Australian Lacelid, *Nyctimystes dayi*, is an endangered, stream-breeding hylid frog endemic to rainforests in the wet tropics of northeastern Queensland, Australia. During the 1990s, populations of *N. dayi* declined dramatically, with the species disappearing from all upland (>300 m) areas, where they were once common (Richards et al. 1993; Northern Queensland Threatened Frogs Recovery Team 2001; Trenerry et al. 1994). The proximate cause of these population declines and disappearances was the amphibian disease chytridiomycosis (Berger et al. 1998). This disease had similar effects on several other species sympatric with *N. dayi* (Berger et al. 1998). Habitat modification and fragmentation are also potential stressors for *N. dayi* populations, since approximately 20% of the wet tropics region has been clear cut since 1880 (Winter et al. 1987), and smaller-scale clearing still occurs in non-protected areas (e.g., for pastures, human settlement and associated infrastructure; Department of Natural Resources and Water 2007).

In order to make informed management decisions aimed at species conservation, it is necessary to have species-specific information on movement and habitat use, information that is lacking for the vast majority of amphibians (Johnson et al. 2007; Semlitsch and Bodie 2003; Trenham and Shaffer 2005). This is especially true for stream-breeding species such as *N. dayi*.

The study of *N. dayi* in the field has been hampered by several factors. First, the species is cryptic in both appearance and behaviour, and inhabits complex, densely vegetated habitats. Only males calling along streams in the breeding season are readily detectable. Second, males weigh less than 4 g, making them too small to track remotely using either radio-telemetry or harmonic direction finding (see Rowley and Alford 2007a), and females are rarely encountered (Czechura 1987; Hodgkison and Hero 2002), with male *N. dayi* 24 times more common than females along stream transects (Czechura 1987). As a result, the only information available on the ecology and habitat use of *N. dayi* is on males, and is derived from nocturnal surveys along streams or in riparian vegetation within 10 m of the stream (Czechura 1987; Hodgkison and Hero 2002).

Our objective was to track to track *N. dayi* females in the field, obtaining information on their movement and habitat use. The study was conducted at Tully Falls Forest Reserve (145.68°E, 17.80°S; 70 m elev.), in northern Queensland, Australia. Individuals were initially captured along a low-gradient stream containing pools, riffles, and a number of waterfalls. The stream was surrounded by relatively undisturbed rainforest vegetation, except for a sealed, two-lane road, which crosses the stream via a bridge approximately 20 m above the stream bed.

The study was conducted during the warm/wet season from 22 February to 8 March 2005 and the cool/dry season from 25 August to 8 September 2005. Tracking methodology followed that of Rowley and Alford (2007b). Each frog was captured by hand, weighed, fitted with radio transmitters (model BD-2NT; Holohil Systems Ltd., Ontario, Canada; weighing ca. 0.67 g including silicone tubing harness) *in situ* and released at point of capture within five minutes. Frogs did not carry more than 6% of their total body weight (the recommended maximum relative weight for an attached tag is 10% of the body weight; Richards et al. 1994). We tracked each frog using a HABIT Research HR2500 Osprey VHF Receiver (HABIT Research, Victoria, B.C., Canada), fitted with a three-element folding Yagi antenna (A.F. Antronics, Urbana, Illinois, USA).

The location of each frog was determined during two surveys per 24-h period, once during the day (0700–1900 h) and once at night (1900–0700 h) over the duration of the study. We established a marked transect along the stream edge to serve as a reference for frog locations, and when we located a frog, we recorded its three-dimensional position in meters as its location along the transect, its horizontal distance from the stream, and approximate elevation above stream. Distances were measured using tape measures, range finders and transect markers. If the frog was more than 20 m from the stream, distance from the stream was recorded to the nearest 5 m. We also recorded the environmental substrate frogs were using: bare ground, dry rock, leaf litter, vegetation, wood, on wet rock in stream, and under rock in stream.

TABLE 1. Movement patterns and habitat use of female *Nyctimystes dayi*. Values represent medians (and ranges) of data obtained using individual frogs as replicates.

Individual ID	Season	Total number of relocations	Percent of observations individuals moved between surveys	Distance moved between surveys (m)	Elevation above stream (m)		Horizontal distance from stream (m)	
					Diurnal	Nocturnal	Diurnal	Nocturnal
Frog 1	Cool/dry	24	60.9	8 (0–91.3)	3 (0–12)	3 (0–10)	8 (0–50)	3 (0–50)
Frog 2	Cool/dry	28	33.3	0 (0–27.1)	13 (0–20)	13 (0–20)	0 (0–3)	0 (0–3)
Frog 3	Cool/dry	15	64.3	4.2 (0–91.3)	0 (0–3)	0.75 (0–3)	0 (0–4)	0 (0–4)
Frog 4	Warm/wet	28	55.6	0 (0–20.6)	5 (0–10)	6 (0–10)	5 (0–20)	2.5 (0–10)
Frog 5	Warm/wet	19	66.7	4.7 (0–17.1)	5 (0–12)	5 (0–12)	4 (0–5)	5 (0–5)

We used individuals as replicates and compared summary statistics calculated for each animal, in order to avoid pseudoreplication and biasing our results by including more data on frogs that were located more often (Rowley and Alford 2007b). Due to obvious differences between the diurnal and nocturnal behavior of *Nyctimystes dayi*, we examined diurnal and nocturnal data on habitat use separately.

Five female *N. dayi* were tracked during the study period, three in the cool/dry season and two in the warm/wet season (Table 1). Our sample size was limited by the low availability of females for initial capture. We successfully relocated all frogs on every attempt, resulting in between 15–28 relocations per frog, and a total of 117 relocations for the species.

Tracked frogs moved to a new position between 33.3–66.7% of surveys (Table 1). The median distance moved between surveys (over ca. 12 h) ranged from 0–8 m, however two individuals moved more than 90 m between surveys (Table 1). The elevation of frogs above the stream during surveys varied from 0–20 m and their horizontal distance from the stream ranged from 0–50 m (Table 1). Individual movement patterns were variable; one frog remained within 5 m of the stream in both elevation and horizontal distance, while others moved extended distances vertically and horizontally from the stream for several days at a time (Fig. 1). No frogs were observed to have moved during the day. Tracked *N. dayi* were always relocated in rainforest. A single individual, "Frog 1," traversed the sealed, two-laned road twice during the study period.

During both diurnal and nocturnal observations, tracked frogs were most often located in vegetation (Table 2), typically in the canopy of large trees (>10 m height). When we were able to visually locate tracked frogs in the canopy, all were on the leaves of trees. During a single instance in which we opportunistically sighted a male *N. dayi* during the day, it was also located in vegetation, on leaves overhanging the stream. Frogs also spent up to several days at a time under rocks in the stream, and were occasionally observed during the night on wet rocks in the stream (Table 2). A single frog was observed on leaf litter during the study (Table 2).

We observed three of our tracked frogs in amplexus; two in the cool/dry season and one in the warm/wet season. "Frog 2" spent between 24–36 h in amplexus, on vegetation 1–2 m above the stream and 2 m in horizontal distance from the stream. "Frog 3" spent <12 h in amplexus on 3 m high vegetation, 4 m from the stream, and "Frog 5" was found in amplexus under a rock in the middle of the stream when we removed its transmitter at the end of the study.

Nyctimystes dayi females made relatively large (up to 50 m), extended journeys away from streams, spent a high proportion of their time in canopy vegetation, and were always observed in rainforest. Similar behavior has been recorded in females of the sympatric hylid *Litoria genimaculata* (Rowley and Alford 2007b). Although we did not track male *N. dayi*, due to their small size, we can make predictions about their behavior, based on the behavior of females. First, it is likely that the frequency and distance of movements in male *N. dayi* is lower than for females, as it is in many species, especially when males are smaller in size than females (Bartelt et al. 2004; Bellis 1965; Johnson et al.

TABLE 2. Substrate use of female *Nyctimystes dayi*. Values represent proportions of observations each frog was on each substrate type during diurnal and nocturnal surveys.

Individual ID	Season	Nocturnal				Diurnal			
		Vegetation	Under rock in stream	Leaf litter	On wet rock in stream	Vegetation	Under rock in stream	Leaf litter	On wet rock in stream
Frog 1	Cool/dry	0.73	0.18	0.09	0	0.69	0.23	0	0.08
Frog 2	Cool/dry	0.77	0.23	0	0	0.8	0.13	0	0.07
Frog 3	Cool/dry	0.29	0.71	0	0	0.5	0.5	0	0
Frog 4	Warm/wet	0.85	0.15	0	0	0.87	0	0	0.13
Frog 5	Warm/wet	0.78	0.22	0	0	0.8	0.1	0	0.1

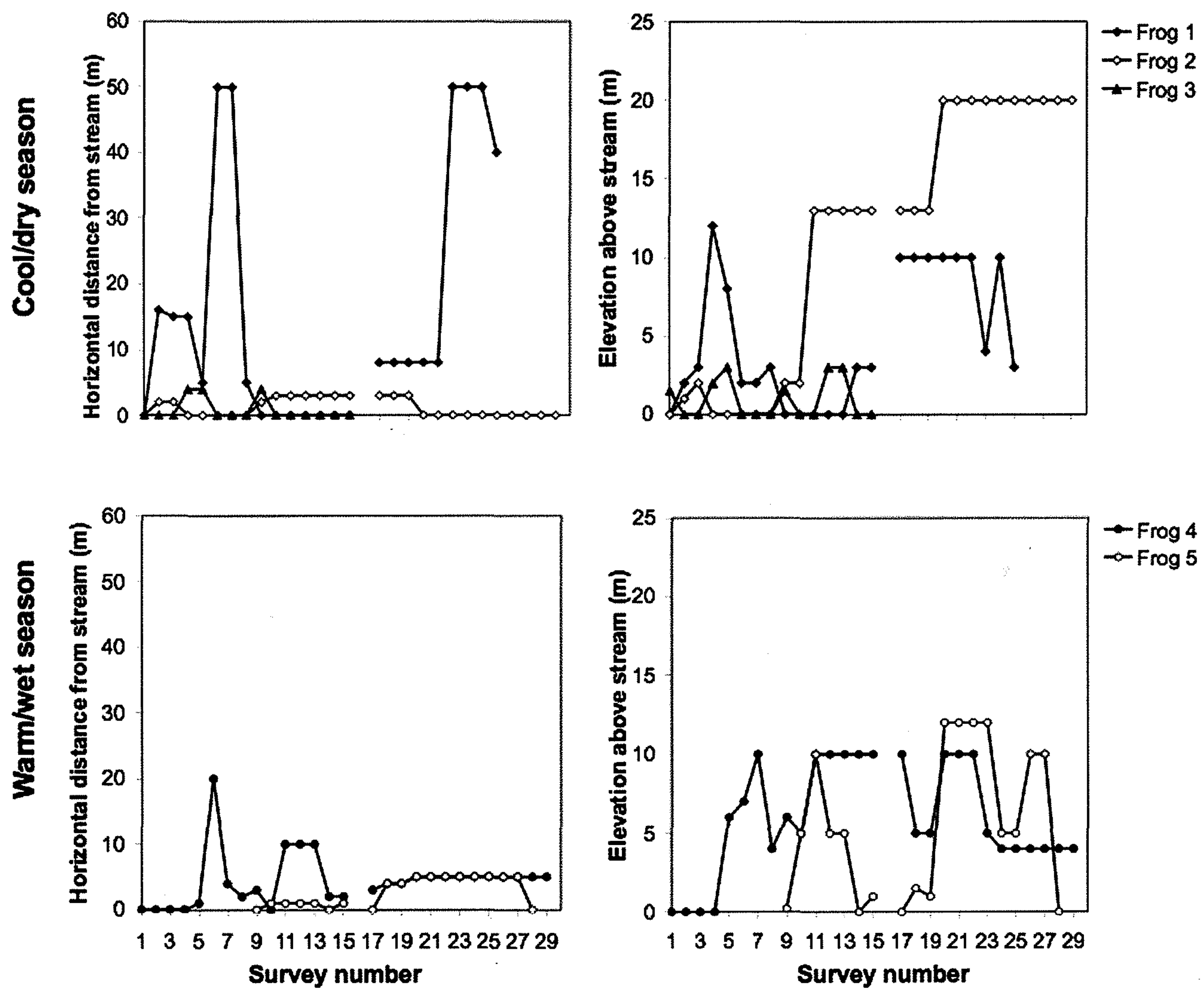

FIG. 1. Individual movement patterns of female *Nyctimystes dayi* during the cool/dry and warm/wet seasons. Values represent position of single frogs in terms of horizontal distance from stream (m) and elevation above stream (m) during successive surveys. Even numbered surveys represent diurnal surveys and odd numbered surveys represent nocturnal surveys.

2007; Miaud et al. 2000; Muths 2003; Ovaska 1992; Rowley and Alford 2007b). It is also likely that male *N. dayi* are more stream-restricted, especially considering their high relative abundance along streams, fidelity to calling-sites along streams (Hodgkison and Hero 2002; Rowley and Alford, unpubl. data), and inter-male aggression (Rowley 2007) during the breeding season.

Results of this study have important consequences for population censuses of *N. dayi* using techniques such as mark-recapture. The large movements of *N. dayi* both along and away from streams are likely to make it difficult to distinguish between mortality and emigration from fixed sites, an issue previously recognized for several other amphibian species (Rowley and Alford 2007b; Schmidt et al. 2007). In addition, it is likely that the high relative abundance of male *N. dayi* encountered during population surveys is simply a reflection of greater detectibility, with females spending large proportions of time farther away from the stream in terms of both horizontal distance and in elevation. This is probably exacerbated by the cryptic behavior of females. When they were adjacent to streams at night, they were often concealed from sight from the stream itself, and would have remained undetected had they not been fitted with tracking devices.

As *N. dayi* were always observed in rainforest, and used habitat at relatively large distances from streams, management actions aimed at conserving the species should maintain terrestrial vegetation buffer zones along streams and habitat corridors between streams. Although a single *N. dayi* successfully traversed a sealed road during the study, we do not know how common these movements are, whether male *N. dayi* are capable of traversing such potential barriers, and how great the risk presented by such traversals is. Until further research is carried out, it should be assumed that roads and other habitat disturbances are barriers to movement for *N. dayi*.

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TECHNIQUES

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Cross-Species Amplification of Emydid Microsatellite Loci in Three *Graptemys* Species and Their Utility as a Conservation Tool

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Microsatellites are a powerful set of highly variable molecular markers gaining widespread use in population genetics (Avise 2004) and increasingly in studies with a conservation focus (e.g., Beaumont and Bruford 1999; Jehle and Arntzen 2002). However, one drawback of microsatellites is that the development of these markers for use *de novo* typically involves the costly and time-consuming process of creating a genomic library, isolating microsatellite-containing clones, designing primers for the loci, and then optimizing the primers (Selkoe and Toonen 2006). Fortunately, the priming sites within the flanking sequences of microsatellite loci are sometimes conserved across species (e.g., Engel et al. 1996; Holman et al. 2005; Primmer et al. 1996; Primmer et al. 2005; Rico et al. 1996), including turtles (FitzSimmons et al. 1995). This allows the primers designed for one species (the focal species; Hutter et al. 1998) to successfully amplify loci in other species. In fact, many authors routinely test novel microsatellite loci on closely related species, usually within the same genus.

Turtles are considered one of the most endangered animal taxa, with 129 of the 205 species assessed by the World Conservation Union (IUCN) listed as critically endangered, endangered or vulnerable (http://www.iucnredlist.org/info/tables/table4a; accessed 27 May 2007). Genetic studies are playing an increasing role in the conservation of chelonians including the use of microsatellites, either alone (Pearse et al. 2006; Roberts et al. 2005; Schwartz and Karl 2005) or in combination with other genetic markers (Bowen et al. 2005; Sites et al. 1999). Interest in genetic studies of several species within the family Emydidae has been indicated by the recent publication of papers describing microsatellite loci for the Diamondback Terrapin (*Malaclemys*