## Microhabitat use by the Critically Endangered Madagascar endemic tortoise, Pyxis arachnoides

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We studied microhabitat use by the threatened Malagasy endemic spider tortoise (*Pyxis arachnoides*) during the wet and the dry season of Madagascar's Southern Dry Forest. In total, 282 tortoises were captured and marked. Microhabitat selection was dependent on the interaction term 'age  $\times$  sex' (with curved carapace length as a covariate), but neither dependent on season, sex and age taken independently. Juveniles favoured areas of denser vegetation cover than more mature animals, which typically selected the microhabitat types in relation to their relative availability. Controlling the subsistence harvest of fuel wood within the region is an important conservation initiative to increase the recruitment of reproductively mature tortoises into the population.

Key words: ecology, Madagascar, microhabitat, spider tortoise

helonians are among the most threatened vertebrates, with a present global turtle survival crisis currently being recognised by biologists and conservationists worldwide, and especially in tropical regions (Turtle Conservation Fund, 2002; Leuteritz et al., 2005; Luiselli, 2009). Thus, collection of ecological data on threatened chelonian species is crucial for their conservation (Forero- Medina et al., 2007; Bombi et al., 2011). The threatened Madagascar spider tortoise Pyxis arachnoides, listed as Critically Endangered by the IUCN (2008), inhabits the narrow coastal strip (up to 10 km inland) of southwest Madagascar. The former continuous coastal range from Morombe to Fort Dauphin (Pedrono, 2008) is currently thought to be fragmented into eight populations (Walker, 2010; Walker & Rafeliarisoa, in press). The northern fragmented populations inhabit the Mikea forest habitat, which spreads south of the Onilahy River through the Southern Dry Forests, a very distinctive yet least protected ecosystem which supports the highest percentage of plant endemism in Madagascar (Jolly et al., 1984; Davis et al., 1994; Seddon et al., 2000). The spider tortoise is the least studied of Madagascar's endemic tortoises (Pedrono, 2008), and faces threats from habitat destruction and over-collection for the exotic pet and food trade despite protection under CITES Appendix 1 (Behler, 2000; Walker et al., 2004). In addition to poaching, P. arachnoides is believed to be suffering from serious loss of habitat (Seddon et al., 2000; Harper et al., 2007; Walker, 2012) and is thought to now inhabit only ~30% of its former range (Walker & Rafeliarisoa, in press). Because of its threatened status and concurrently poor knowledge of its ecology, collection of ecological data for this species in the wild is a priority. Pyxis arachnoides is sympatric in its range with the larger radiated tortoise Astrochelys radiata (Pedrono, 2008). Unlike A. radiata, which appears to be a habitat generalist (Pedrono, 2008), P. arachnoides is linked to xerophilic spiny forest, with a requirement for vegetation cover (Walker et al., 2007). Data on the population size, density, current range and age structure of P. arachnoides have been published by Jesu & Schimmenti (1995); Walker et al., (2007); Walker & Rafeliarisoa (in press) and Walker (2010). Also, Walker (2012) describes the effectiveness of various field surveying techniques and the detectability of spider tortoises in their natural habitat. This paper presents detailed data of the effects of season variation, sex, and age in relation to microhabitat preferences of a population of P. arachnoides.

An area of Southern Dry Forest, approximately 3 km east of the coastal fishing community of Anakao, south of the Onilahy River, was selected (23°37.850' S, 43°39.763' E) during 2002 and 2003. The area was considered a favourable habitat for P. arachnoides (e.g. Pedrono, 2008; Walker et al., 2007), with dominant vegetation including Didiereaceae and species of Euphorbia. However, the Southern Dry Forests are one of the most threatened habitats within Madagascar, with an estimated forest loss of 1.2% per year (Harper et al., 2007). The structure of the forest within the study area typically ranges from areas of habitat degraded though charcoal production and slash and burn agriculture (Seddon et al., 2000; Fenn, 2003) to areas of up to 80% vegetation cover. Vegetation is generally uniform in structure throughout un-impacted areas, with low lying forest vegetation cover typically in the range of 2-3 m in height (Fenn, 2003).

A survey area of 13.7 km<sup>2</sup> (1369 ha) of forest was selected for field surveys, and a grid of 400 quadrants (100 m x 100 m) was devised using GPS. Forty quadrats were selected at random for survey during the dry season (July to November) over a period of 11 days, and a further 43 during the warmer wet season (December to June) over a period of 17 days, using a GPS to ensure accurate quadrat location. The field research was conducted by ten surveyors, spread out on a compass bearing of 180° from the GPS point that marked the corner of each quadrat. A 10 m rope was stretched out between each surveyor to ensure accurate spacing. The rope was then transferred and laid out for 10 m in a straight line along a compass bearing of

Correspondence: Ryan C.J. Walker, 1 Pond Lane, Greetham, Rutland, LE15 7NW, UK; E-mail: ryan@nautilusecology.org 90° in front of each surveyor. Each surveyor walked up the line and searched for 5 m on either side of the rope in a 10 m wide transect, moving as quietly as possible so as not to disturb the behaviour of any tortoises encountered. Both exposed areas and ground covered by low-lying vegetation were searched for the presence of *P. arachnoides*. Each surveyors rope was then laid out another 10 m on a 90° bearing and the process was repeated until an area of 1 ha was surveyed.

Each tortoise encountered was weighed using an electronic balance (precision  $\pm 0.5$  g), and an approximation of age was recorded by counting growth annuli rings (Jesu & Schimmenti, 1995; Germano, 1998; Walker et al., 2007). Curved carapace length (mm) was also measured. Since curved carapace length and (log) weight are strongly positively correlated (*r*=0.485, *P*<0.01), we used curved carapace length as a measure of size. To avoid duplicating counts (Hurlbert, 1984), tortoises were marked with an identifying number using nail varnish on the rear of the carapace before release.

Given that the study area was homogenous in terms of vegetation composition (Walker et al., 2007), microhabitat type was defined as percent vegetation cover. Microhabitat selection by tortoises was recorded using a visual estimate of percentage area cover of shade provided by vegetation for each 10 m x 10 m subquadrat within the larger 1 ha quadrat. The precision of our visual estimates of vegetation cover was 10%. All surveys were carried out between 0700 and 1300 hours (Antananarivo local time) during the cooler period of the day, when P. arachnoides was most active (Walker et al., 2007; Pedrono, 2008). Consistency in weather conditions were maintained by undertaking surveys on clear, cloudless days. Time data were divided into onehour blocks for ease of analysis. Statistical analyses were performed with Minitab and SPSS software. Since data satisfied the assumptions of homogeneity and homoscedasticity of variances, parametric tests were used (Pearson's correlation coefficients, Analysis of Variance, and General Linear models). Microhabitat analyses were done with a General Linear Model (GLM, full factorial design) procedure. In microhabitat analyses, the dependent variable was the vegetation cover for each quadrat where tortoises were observed. The factors were sex (males, females and juveniles) and age, and the curved carapace length was the covariate. The interaction terms between these factors were also taken into consideration in the GLM models.

The frequency distribution of the various microhabitat categories at the study area is given in Figure 1. Vegetation cover categories of 30–40% were most abundant. In total, 282 tortoises (44 juveniles, 158 females and 79 males) were recorded across 83 quadrats. Of these, 82 animals were captured in the dry season and 200 were captured in the wet season, with no animal recaptured more than once. Data on microhabitat use by tortoises in relation to sex, age and size are summarized in Table 1. A GLM full factorial model (with age and sex as factors, carapace length as covariate and microhabitat (=vegetation) selection was strongly dependent on the interaction term 'sex  $\times$  age', with curved carapace length as a covariate



Fig. 1. Vegetation cover (%) across 83 survey quadrants.

positively correlated with age (r=0.412, P<0.01, Table 2). Nonetheless, microhabitat selection was neither dependent on age nor sex when considered independently (Table 2). Tukey HSD post-hoc tests, however, revealed that males were significantly more frequently observed than females (P<0.01) associated with reduced vegetation cover (<40%) areas as well in greater vegetation cover (>50%). Season was not important for determining microhabitat of tortoises (one-way ANOVA:  $F_{1,279}=1.117$ , P=0.298).

As a result of anthropogenic pressure from timber harvest, suitable tortoise habitat is limited. Our results revealed that, despite living in a strongly seasonal environment, P. arachnoides shows little variation between seasons in terms of its microhabitat selection. We suggest that these tortoises continue feeding during the dry months in-between extended dormant periods (Walker et al., 2007, Pedrono, 2008), and thus are forced to move around when foraging for their food that is concentrated in the same microhabitat spots all year round (Walker et al., unpubl.). Size and the interaction term 'age  $\times$  sex' played a significant role in determining the microhabitat selection of tortoises, with adults selecting more exposed sites. This may be due to predation effects, as predation rates are size-dependent in chelonians (juveniles being more intensely preved than adults, e.g. Frazer & Gibbons, 1991a, 1991b; Janzen, 1993; Tucker et al., 1999). In P. arachnoides, males are larger than females (on average 455 g versus 398 g; Walker et al., 2007), and both sexes are rather large for predators occurring in the study area, whereas juveniles need well vegetated spots to remain hidden (Butler & Graham, 1995; McMaster & Herman, 2000). Potential predators within this study area were snakes (i.e. Boa dumerili) and birds of prey (i.e. Milvus aegyptius), which would typically focus their foraging upon smaller size classes (individuals below three years of age, Walker et al., unpubl.).

Mean vegetation cover for surveyed quadrats was 45.4%, and it appears that *P. arachnoides* is selecting habitat representative for the area. Further surveys over a greater geographical range should be undertaken to establish if this is the case across the species range, or if the species is only locally utilizing the main available habitat (Harper et al., 2007; Walker et al., 2007). Male tortoises have been encountered both in reduced (<40%) as well as greater vegetatation cover (>50%) areas. However, a more detailed study is required to understand the precise reasons for this pattern. Recent studies on a European tor-

Vegetation cover	Females	Males	Juveniles	Age (years)	CCL (mm)
(76)	0	2.5	0	100/0/0	0/100/0
20	7.4	6.3	7.0	28.6/57.1/14.3	14.3/42.8/42.9
30	9.6	13.9	11.6	33.3/36.1/33.9	10.8/35.1/54.1
40	34.5	36.7	37.2	38.3/40/21.7	11.9/15.3/72.8
50	26.1	17.7	32.6	36.6/35.0/28.4	10.2/44.1/45.7
60	9	12.7	4.7	28.6/50.0/21.4	42.9/7.1/50.0
70	6.3	7.3	7.0	38.9/38.8/22.1	38.9/33.3/27.8
80	5.1	2.9	0.3	33.3/33.3/33.3	0/33.3/66.6
90	0	0	0.3	0/0/100	100/0/0

**Table 1.** Summary table of relative numbers (%) of *Pyxis arachnoides* found in each microhabitat category. Age and carapace curved lengths (CCL) were split into three groups of equal size (age: >8 years old, 9–16 years old, and >16 years old; CCL: <68 mm, 68–136 mm, >136 mm).

toise (*Testudo hermanni*) demonstrated that microhabitat utilisation may be linked to very specific floral aspects (Del Vecchio et al., 2011a), and that this narrow choice is not linked to foraging needs alone (Del Vecchio et al., 2011b).

local communities into overexploitation of forest resources. Our data suggest that forest loss might result in high juvenile mortality, potentially with profound impacts on the recruitment of tortoises into the population. Specific monitoring and actions should be taken in order to control this potential threat to the local spider tortoise population.

For efficient conservation measures it is important to locally address the poverty-induced drivers which force

**Table 2.** General Liner Model (full factorial design) for determining the effects of sex and age (as factors) with curved carapace length as covariate, on microhabitat selection (vegetation cover) of *Pyxis arachnoides*.

Source	Type III Sum of Squares	df	Mean Square	F	Р
Corrected model	8125.339	42	193.46	1.196	0.219
Intercept	48790.628	1	48790.628	301.559	0.0001
Curved carapace length	943.133	1	943.133	5.829	0.017
Sex	6.726	1	6.726	0.042	0.839
Age	3552.183	24	148.008	0.915	0.582
Sex x Age	4763.749	15	317.583	1.963	0.022
Error	23298.458	144	161.795		

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## REFERENCES

- Behler, J. (2000). Letter from the IUCN tortoise and freshwater turtle specialist group. Turtle and Tortoise Newsletter 1, 4–5.
- Bombi, P., Luiselli, L., D'Amen, M. (2011). When the method for mapping species matters: defining priority areas for conservation of African freshwater turtles. *Diversity and Distributions* 17, 581–592.
- Butler, B.O. & Graham, T. (1995). Early post-emergent behavior and habitat selection in hatchling Blanding's turtles, *Emydoidea blandingii*, in Massachusetts. *Chelonian Con*servation Biology 1, 183–196.
- Davis, S.D., Heywood, V.H. & Hamilton, A.C. (1994). Centres of plant diversity, a guide and strategy for their conservation. WWF and IUCN, Oxford, UK.

Del Vecchio, S., Burke, R.L., Rugiero, L., Capula, M. & Lu-

iselli, L. (2011a). The turtle is in the details: Microhabitat choice by *Testudo hermanni* is based on microscale plant distribution. *Animal Biology* 61, 249–261.

- Del Vecchio, S., Burke, R.L., Rugiero, L., Capula, M. & Luiselli, L. (2011b). Seasonal changes in the diet of *Testudo hermanni hermanni* in central Italy. *Herpetologica* 67, 236– 249.
- Fenn, M. (2003). The spiny forest ecoregion. In *The Natural History of Madagascar*: 1525–1530. Goodman, S.M. & Benstead, J.P (Ed.). Chicago, Illinois: University of Chicago Press.
- Forero-Medina, G., Castano-Mora, O.V. & Montenegro, O. (2007). Abundance, population structure, and conservation of *Kinosternon scorpioides albogulare* on the Caribbean island of San Andrés, Colombia. *Chelonian Conservation Biology* 6, 163–169.
- Frazer, N. B. & Gibbons, J.W. (1991a). Life history and demography of the common mud turtle, *Kinosternon subrubrum* in South Carolina, USA. *Ecology* 72, 2218–2231.
- Frazer, N. B. & Gibbons, J.W. (1991b). Growth, survivorship and longevity of painted turtles *Chrysemys picta* in a southwestern Michigan marsh. *American Midland Naturalist* 125, 245–258.

- Germano, D.J. (1998). Scute and age determination of desert tortoises revisited. *Copeia* 1998, 482–484.
- Harper, G., Steininger, M., Tucker, C., Juhn, D. & Hawkins, F. (2007). Fifty years of deforestation and forest fragmentation in Madagascar. *Environmental Conservation* 34, 325–333.
- Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. <u>Ecological Monographs 54</u>, 187–211.
- IUCN. (2008). IUCN Red List of Threatened Species. IUCN, Gland, Switzerland.
- Janzen, F.J. (1993). An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* 74, 332–341.
- Jesu, R. & Schimmenti, G. (1995). A preliminary study on the status of a population of Malagasy Spider tortoise (*Pyxis* arachnoides Bell, 1927) from SW Madagascar. International Congress of Chelonian Conservation, Gonfaron, France. 6th –10th July 1995.
- Jolly, A., Oberlé, P. & Albignac, R. (1984). *Key Environments: Madagascar*. Pergamon Press, Oxford.
- Leuteritz, T.E.J., Lamb, T. & Limberaza, J.C. (2005). Distribution, status, and conservation of radiated tortoises (*Geochelone radiata*) in Madagascar. *Biological Conservation* 124, 451–461.
- Luiselli, L. (2009). A model assessing the conservation threats to freshwater turtles of Sub-Saharan Africa predicts urgent need for continental conservation planning. *Biodiversity* and Conservation 16, 1349–1360.
- McMaster, L. & Herman, T.B. (2000). Occurrence, habitat selection, and movement patterns of juvenile Blanding's turtles (*Emydoidea blandingii*) in Kejimkujik National Park, Nova Scotia. *Chelonian Conservation Biology* 3, 602–610.
- Pedrono, M. (2008). The Tortoises and Turtles of Madagascar. Natural History Publications (Borneo), Kota Kinabalu, Malaysia.

- Seddon, N., Tobias, J., Yount, J.W., Ramanampamonjy, J.R., Butchart, S. & Randrianizahana, H. (2000). Conservation issues and priorities in the Mikea Forest of South-west Madagascar. *Oryx* 34, 287–304.
- Tucker, J.K., Filoramo, N.J. & Janzen, F.J. (1999). Size-biased mortality due to predation in a nesting freshwater turtle, *Trachemys scripta*. <u>American Midland Naturalist 141</u>, 198–203.
- Turtle Conservation Fund. (2002). A global action plan for conservation of tortoises and freshwater turtles. Conservation International, Washington D.C.
- Walker, R.C.J. (2010) The decline of the northern Madagascar spider tortoise (*Pyxis arachnoides brygooi*). *Herpetologica* 6, 411–417.
- Walker, R.C.J. (2012) A critical evaluation of field survey methods for establishing the range of a small, cryptic tortoise: (*Pyxis arachnoides*). *The Herpetological Journal* 22, 7–12.
- Walker, R.C.J & Rafeliarisoa, T.H. (in press). Status of the relict population of the Critically Endangered Madagascar spider tortoise *Pyxis arachnoides*. *Oryx*.
- Walker, R.C.J., Rix, C.E. & Woods-Ballard, A.J. (2004). The legal and illegal trade in the Madagascar spider tortoise *Pyxis* arachnoides, for the exotic pet trade. *Herpetological Bulletin* 90, 2–9.
- Walker, R.C.J., Woods-Ballard, A.J. & Rix, C.E. (2007). Population density and seasonal activity of the threatened Madagascar spider tortoise (*Pyxis arachnoides arachnoides*) of the southern dry forests, South West Madagascar. *African Journal of Ecology* 46, 67–73.

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