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Microhabitat preference of the critically endangered golden mantella frog in Madagascar

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The golden mantella (*Mantella aurantiaca*) is a critically endangered (CR) frog, endemic to the eastern rainforests of Madagascar. Although the species is very popular in the pet trade and widely bred in captivity, its specific habitat requirements in the wild are poorly understood. Ten forested sites in the Moramanga district of Madagascar were surveyed for microhabitat and environmental variables, and the presence or absence of golden mantellas in quadrats positioned along transects in the vicinity of breeding sites. Mixed models were used to determine which variables best explained microhabitat use by golden mantellas. Sites where golden mantellas were found tended to have surface temperatures of 20–23 °C, UVI units at about 2.9, about 30 % canopy cover, and around 30 % herbaceous cover. Within sites, golden mantellas preferred microhabitats that had 70 % leaf litter coverage and relatively low numbers of tree roots. This information can be used to improve the identification and management of habitats in the wild, as well as to refine captive husbandry needs.

Keywords: mantella, Madagascar, amphibian, montane, rainforest, protected area

INTRODUCTION

Conservation of critically endangered species requires information at different spatial scales. Species distribution models (SDMs) can combine climatic and landscape variables from regional or national sources to provide large-scale pictures of habitat preferences and predicted distribution ranges (Guisan & Thuiller, 2005). However, within the predicted range a species is likely to be patchily and unevenly distributed, with occurrence within a habitat patch dependent on microhabitat and its associated microclimate. Microhabitat variables cannot usually be extracted from remote sensing or landcover maps and need to be measured directly on the ground (Stanton et al., 2012). This can be problematic for small, microhabitat specialist species that are difficult to observe. However, understanding microhabitat preferences is crucial to both providing appropriate habitat management in the field, and for informing captive management conditions in ex situ programmes (Semlitsch et al., 2009; Piludu et al., 2015; Tapley et al., 2015).

The golden mantella (*Mantella aurantiaca*) is a small, montane, diurnal, frog endemic to the eastern rainforests

of Madagascar (Glaw & Vences, 2007). Its extent of occurrence is 699 km² centred in the Moramanga district (Piludu et al., 2015). The known area of occupancy for this species is low at less than 10 km² (Vences & Raxworthy, 2008) with two main population clusters, one to the north of Moramanga at Ambatovy, Torotorofotsy forest and Analabe forest (Piludu et al., 2015). South of Moramanga clusters of breeding ponds are also found within fragments of Mangabe forest (Piludu et al., 2015). Due to a low area of occupancy, fragmented distribution and a decline in both numbers and suitable forest habitat, this species is categorised as critically endangered (CR) B2ab (iii, v) and listed on CITES Appendix II (Vences & Raxworthy, 2008). Current threats to the golden mantella and their rainforest habitat include logging, illegal collection for the pet trade, the destruction of breeding ponds due to mining activity, forest clearance to make way for subsistence agriculture and climate change (Andreone et al., 2008; Vences & Raxworthy, 2008; Piludu et al., 2015). The golden mantella therefore continues to be a prime candidate for in situ and ex situ conservation initiatives, but further research on habitat needs could help fill some knowledge gaps (Randrianelona et al., 2010).

Most of the forest fragments inhabited by golden

mantellas are deemed to have protected status (Piludu et al., 2015). In reality, the actual practical protection afforded to these areas is low, and forest clearance, mining and the illegal collection of golden mantellas continues regardless. According to Piludu et al. (2015) there are now more threatened golden mantella populations in forests with protected status than there are in forests without protected status. There is clearly a need to identify and prioritise new sites for future conservation actions such as assisted colonisation (Piludu et al., 2015; Andreone et al., 2016). However, without an in-depth knowledge of specific environmental/habitat requirements for the species, finding, creating, restoring or protecting optimum habitat is difficult. This study was therefore designed to determine the environmental and microhabitat variables that influence the presence of golden mantellas in the wild. The results will help to identify areas where this species is most likely to persist and thrive.

METHODS

Data Collection

Ten sites within the protected area of Mangabe-Ranomena - Sahasarotra, Moramanga District, eastern Madagascar, each containing or bordering known golden mantella breeding ponds, were targeted for surveys. Nine of these sites were surveyed between 28 November 2014 – 12 December 2014, and the tenth earlier on in the year in March 2014. These periods correspond to the main breeding activity periods for this species.

All surveys took place between 0700-1400 hrs each day, one visit per site. The surveys were centred on breeding pools located in shallow valleys. A series of transects were established on the slope running down to each pool. The first transect was positioned at the valley bottom and ran parallel to the pool. Subsequent transects were positioned at 30 m intervals up the slope, each following the contour at that position, with the last transect positioned along the crest of the slope (Fig. 1). The number of transects and the number of associated quadrats surveyed depended on the length, width and topography of the slope accessible to the survey team, i.e. two sites contained five transects, seven sites had three transects and one site had two transects. Where the top of a slope was bordered by a pathway the crest transect was placed 3 m down slope from the pathway, two further transects were then surveyed, one either side of the path. This meant that the two sites with crest paths had five transects in total. Along each transect 1 m x 1 m quadrats were established at 4 m intervals and transects contained between 10–20 quadrats, sites with more transects therefore having more associated quadrats. A two-person research team moved along the transect line stopping, surveying and recording environmental variables (Table 1) and the number of golden mantellas counted in each quadrat. Transect lines at the valley bottom were surveyed first, followed by next nearest transect as the slope was ascended. Golden mantellas observed outside the transects were also recorded and microhabitat variables measured within 1 m² of these locations.

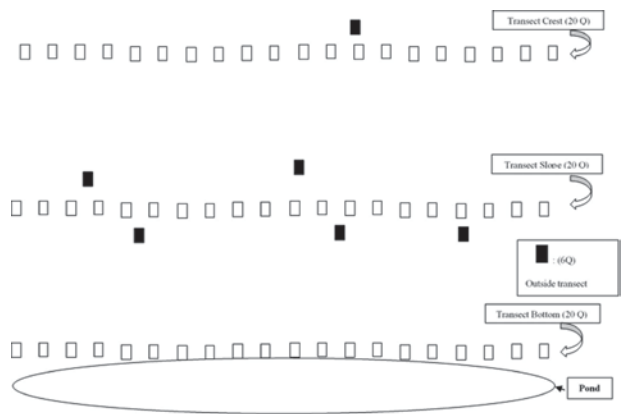


Figure 1. Diagrammatic representation of transect lines of twenty 1 x 1 m quadrats (white boxes) spaced at 30 m intervals running parallel to the breeding pond (white oval). Black boxes indicate where a golden mantella was seen outside of the transect/quadrat line and all environmental and microhabitat data within 1 m² of the individual were recorded. (Courtesy of Rakotondrasoa et al., 2015; unpublished report).

Statistical analysis

Statistical analyses were carried out using the statistical software R (R Core Team, 2017). The quadrats from the ten forests were classified into presence or absence of golden mantella categories and then initially tested for significant differences in microhabitat variables using the Wilcoxon Rank Sum Test. A generalised linear mixed model (GLMM) was then developed using the number of quadrats occupied and unoccupied to determine which independent variables (Table 1) were most likely to influence the microhabitat preference of golden mantellas (Table 2). We then followed Zuur et al. (2009) by removing the independent variable with the highest p value and re-running the GLMM. This procedure was repeated until only significant ($p \leq 0.05$) independent variables were left. Site was used as a random factor in the models, and we assumed a binomial error distribution with a logit link function.

RESULTS

Our analyses showed that for all ten sites combined, two microhabitat variables differed between quadrats with and without mantellas: litter cover and number of tree roots (Wilcoxon tests all $P < 0.001$). The GLMM also identified litter cover, number of tree roots and surface temperature as important predictors of golden mantellas (Table 2). Although not important at the microhabitat selection level, at the time of the surveys the sites where golden mantellas were found tended to have surface temperatures of 20-23 °C, UVI units at about 2.9, and about 30 % canopy cover and 30 % herbaceous cover (Table 3; Figs 2-3).

Within the sites, golden mantellas tended to occupy quadrats with at least 70 % leaf litter coverage and low (mean = 1.73) numbers of tree roots rather than quadrats with no or very low numbers of tree roots (Table 2).

Table 1. Variables, type and method of measurement used to collect data

Variable	Method of collection
Surface temperature (°C)	Rolson™ Infrared thermometer
Ultra-Violet B (UVI units)	Solarmeter 6.5TM Ultra-Violet Index (UVI) meter
Canopy cover (%)	Estimate
Herbaceous cover (%)	Estimate
Moss cover (%)	Estimate
Litter Cover (%)	Estimate
Litter depth (cm)	Tape measure
N° dead trees	Count
N° large trees (diameter < 1 m)	Count
N° small trees (~ 1.5 m height)	Count
N° trees cut	Count
N° trees damaged by cyclone	Count
Canopy height (m)	Estimate
Number of tree roots	Count

Table 2. Generalised Linear Mixed Model results showing potentially important predictor variables associated with golden mantellas (As canopy cover is alphabetically first in the list of variables it is labelled by R software as the Intercept and then used as a reference point). We provide the z-value ($z = x - \bar{x} / s$) and corresponding p-value for testing the null hypothesis that the slope and intercept is equal to 0 (Zuur et al., 2009).

Variable	Estimate	Std Error	z value	p (> z)
Intercept	-0.682018	0.858396	-0.795	0.42689
Surface temperature	-0.085088	0.037610	-2.262	0.02368
Litter cover	0.011037	0.003636	3.035	0.00240
Litter depth	0.038146	0.020607	1.851	0.06415
Tree roots	0.173847	0.050318	3.455	0.00055

However, across the sites, the number of golden mantellas declined in areas with very dense tree roots (Fig 2).

DISCUSSION

Although the relative number of occupied quadrats varied between sites, this may have been a result of environmental conditions on those survey days being particularly propitious for mantella activity, rather than reflecting real differences in abundance between sites. Nevertheless, our results show that at quadrat or transect level, the number of frogs encountered increases as percentage litter cover increased. Golden mantellas are a tropical forest floor species and are dependent on leaf litter to provide cover, create territories, forage, breed, and more easily regulate hydration state and body temperature. Like all frogs, golden mantellas can mitigate for the effects of evaporative water loss via the skin in dryer or warmer conditions by morphological and/or behavioural means (Duellman & Trueb, 1994). Adult frogs take up water via absorption across the skin surfaces when in close contact with moist soils and substrates (Duellman & Trueb, 1994). Granular skin on the ventral surface then facilitates increased capillary action drawing water up from moist soils and provides increased skin surface areas for absorption. However, morphological adaptations such as cutaneous sculpturing or increased permeability and vacuolisation will only

be advantageous in moist microhabitat (Hillyard et al., 1998). Therefore, the frogs must move between, or remain in, microhabitats where they are able to reduce the evaporation gradient of water from the body to the surrounding environment and rehydrate at a rate that offsets the amount of water lost. Blomquist and Hunter (2010) obtained similar results for wood frogs (*Rana sylvatica*), which were more likely to inhabit areas with greater humidity, substrate moisture, canopy cover, leaf litter depth and coverage. Seymour (1972) and Walvoord (2003) found that green toads (*Bufo debilis*) and cricket frogs (*Acris crepitans*) were more likely to select moist habitat when exposed to higher temperatures. Several other amphibian studies have obtained similar results and demonstrated that core temperatures, evaporative water loss and subsequent habitat selection were all highly influenced by ambient temperature and humidity (Tracy, 1975; Tracy, 1976; Pough et al., 1983; Semlitsch et al., 2009; Kohler et al., 2011; Tracy et al., 2013). It is now widely regarded that anuran activity is more limited by the effects of dehydration than by temperature, and as such hydoregulation is more important than thermoregulation (Seymour, 1972; Prest & Pough 1987; Tracy et al., 1993; Prest & Pough, 2003; Tracy et al., 2013).

Our results suggest that golden mantellas prefer sites with about 30 % canopy cover, and there is a tendency for fewer frogs to be observed in areas with

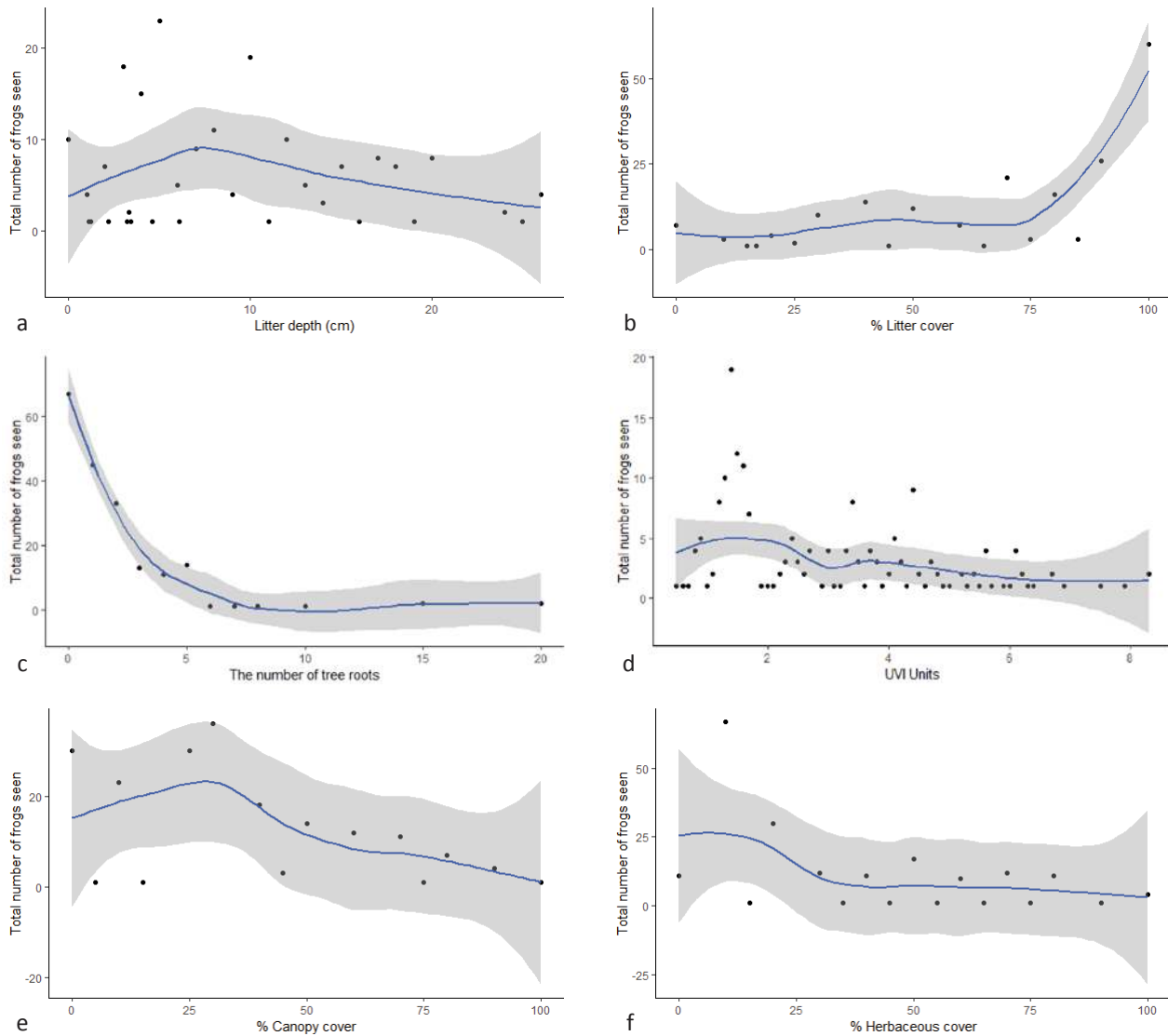


Figure 2. The total number of frogs observed combined for all ten sites versus **(a)** percentage litter cover, **(b)** litter depth in cm, **(c)** the number of tree roots, **(d)** UVB intensity (UVI units), **(e)** percentage canopy cover and **(f)** herbaceous cover. Each of the data points (black dots) represent the specific number of frogs recorded at each associated level of independent variable and are fitted with a LOESS smoother (blue line) to most closely model the relationship between independent variables and the total number of frogs seen. The shaded area represents a 95 % confidence interval.

dense canopy cover and tree roots. Golden mantellas are known to frequent sun-exposed areas within forest (Glaw & Vences, 2007) and the time of day or weather patterns may have an influence on mantella activity in these areas. Sunlight interception and irradiance at ground level depends to a certain extent on the height and positioning of the canopy (Dodd, 2010). The amount of cloud cover and orientation of the sun to the canopy gap can also be important in determining UVB and temperature levels at the forest floor (Pringle et al., 2003). Higher levels of UVB and herbaceous cover may be indicative of higher levels of disturbance or more extensive gaps in the canopy. Larger gaps in the canopy allow more solar radiation to penetrate further towards the forest floor which in turn increases soil and surface temperatures, lowers humidity, reduces leaf litter and food sources, these effects are amplified as canopy gap size increases (Carlson & Groot, 1997, Semlitsch et al., 2009).

It is plausible that as litter depth and the number of tree roots in a given quadrat increase, frog detectability becomes compromised. Greater coverage of herbaceous plants may also impede the ability of researchers to observe the frogs. According to an unpublished report by Rakotondrasoa et al. (2015), direct counts of golden mantella can be biased and challenging. An example is given where a count was carried out and around 400 mantellas were observed, yet further surveys were carried out and 2000 individuals were later captured in the same area. Indeed, it is generally acknowledged that at the population level count data for amphibians may be unreliable given imperfect detection, and where possible should be underpinned by capture mark recapture techniques, good quality habitat data and expert opinion (Schmidt, 2003; Sewell et al., 2010; Griffiths et al., 2015; Barata et al., 2017).

The rainy season begins in November in Madagascar, and this corresponds to the start of the breeding season

Table 3. Percentage of quadrats surveyed with or without golden mantellas at each of the ten forested sites. The range and mean of the predictor variables associated with mantella presence are also shown (% Litter cover, Litter depth, Number of tree roots, Surface temperature, UVI units, % Canopy cover and % Herbaceous cover). The percentage of quadrats not containing mantellas with associated ranges and means for predictor variables are also shown for each site. The bottom two rows show the differences between predictor variable means for quadrats with or without golden mantellas at all ten sites combined.

Site	Golden mantella	% of Quadrats	% Litter cover		Litter depth (cm)		Number of tree roots		Surface temp (°C)		UVB (UVI units)		% Canopy cover		% Herb cover	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Sassarotra 25	with	51	0-100	65	0-24	10.4	0-8	1.6	18.1-27.7	22.3	3.0-8.3	5.0	0-100	30	0-100	32
	without	49	0-90	39	0-17	4.4	0-3	0.5	19.2-29.8	23.2	3.6-8.8	6.0	0-70	22	0-100	50
Sassarotra 17	with	47	30-100	74	2-26	13.0	0-20	4.7	19.5-27.8	22.4	1.2-3.6	1.6	0-60	29	0-80	42
	without	53	30-100	67	2-16	8.5	0-10	2.2	18.7-27.9	22.1	1.2-4.9	2.3	0-80	25	10-90	42
Antanimbarit-sara	with	36	0-100	75	0-18	6.0	0-3	1.1	16.9-22.9	20.0	0.9-1.7	1.4	0-90	36	0-100	25
	without	64	0-100	72	0-12	5.0	0-4	1.2	15.6-24.3	19.2	0.3-1.6	1.2	0-90	33	0-100	29
Andriamaro-hangotra	with	31	0-100	39	0-5	2.2	0-2	0.5	18.6-20.3	19.3	1.5-6.1	4.3	0-60	15	0-80	32
	without	69	0-90	51	0-10	3.2	0-4	0.4	19.2-20.4	19.7	1.6-6.4	3.7	0-70	21	0-100	37
Andravinala	with	27	40-100	79	10-25	16.5	0-5	1.4	18.6-21.1	20.1	2.7-5.6	3.9	0-60	27	0-50	14
	without	73	10-100	76	5-30	14.0	0-4	0.8	17.7-21.6	19.8	2.1-5.7	3.1	0-80	25	0-80	18
Andavaioaka 4	with	19	20-100	73	2-20	7.4	0-8	2.5	18.1-22.1	20.1	2.0-3.0	2.4	0-90	49	0-80	29
	without	81	10-100	65	1-30	9.1	0-7	1.9	15.4-25.8	20.2	1.6-3.2	2.3	0-100	27	0-100	30
Ambinanin'i	with	18	40-100	92	3-18	6.7	0-5	1.7	16.5-26.9	19.0	0.5-4.5	1.4	0-80	24	10-80	33
	without	82	0-100	61	0-18	6.9	0-5	1.1	15.1-43.2	24.3	0.3-7.8	1.9	0-80	18	0-90	40
Lemafy	with	14	0-85	58	1-7	3.2	0-1	0.1	16.8-22.9	19.1	3.3-7.5	4.6	0-80	38	10-75	39
	without	86	0-95	52	0-12	3.9	0-4	0.2	14.3-27.7	19.1	0.8-9.6	3.9	0-90	39	0-90	32
Bekomy	with	11	40-80	81	2-5	3.9	0-6	1.7	17.3-22.2	19.2	0.8-1.3	1.1	40-80	57	10-10	10
	without	89	10-100	73	0-22	6.1	0-4	1.2	17.0-36.7	21.4	0.9-5.8	1.7	0-80	40	0-90	16
Antoko	with	9	90-100	98	5-12	9.0	0-5	2.0	19.8-22.6	21.2	2.4-3.7	3.2	0-90	17	10-80	38
	without	91	10-100	84	1-18	5.5	0-15	1.6	18.2-40.4	22.0	1.3-6.3	2.8	0-100	40	10-90	33
Mean of Sites	with	26		73.4		7.8		1.73		20.2		2.9		32.2		29.4
	without	74		64.0		6.7		0.9		21.1		2.9		29.0		32.7

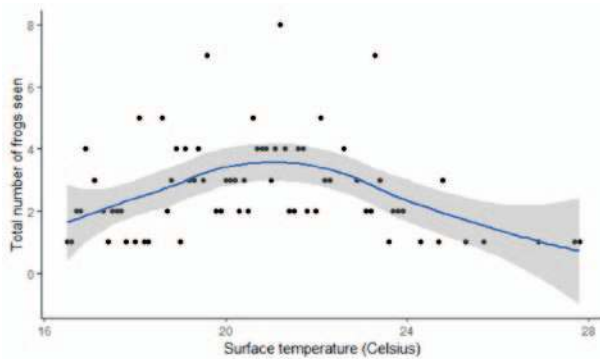


Figure 3. The total number of golden mantellas encountered combined for all ten sites and associated surface temperatures. Each of the data points (black dots) represent the specific number of frogs recorded at each temperature and are fitted with a LOESS smoother (blue line) to most closely model the relationship between surface temperature and the total number of frogs seen. The shaded area represents a 95 % confidence interval.

for golden mantellas. The Bejofo site was surveyed in March, towards the end of the breeding season when frogs may have migrated back up the hill away from ephemeral breeding ponds. Indeed, all golden mantellas encountered in Bejofo were recorded in the

hill-top quadrats. The other nine sites were surveyed in November and as such we would expect to observe more frogs in the valley bottom transects near to the breeding ponds. However, this was not the case, as more frogs were observed in the higher transects on the slope or crest of the hill. It may be that the frogs are migrating down to the breeding ponds and laying eggs in leaf litter, then migrating back up to warmer surface temperatures on the slope and crest. Lower average temperatures recorded in valley bottom transects may also mean fewer frogs are active outside of leaves and observed. The timing of the surveys was dictated by logistics and weather, but either way, there was no evidence that the difference in the timing of surveys between sites made any difference to observations of microhabitat use.

CONCLUSIONS

We recommend maintaining the integrity of current golden mantella forest habitat, increasing connectivity between breeding ponds and keeping disturbance of these areas to a minimum by increasing the levels of protection. Piludu et al. (2015) recommend an increase in effort or a new approach to safeguard breeding ponds, involving sampling and surveillance for detection of emerging pathogens, such as the chytrid

fungus *Batrachochytridium dendrobatidis* (e.g. Bletz et al., 2015). The monitoring of local climate and the study of predicted climate change effects and further development of species distribution and population viability models to determine future relevant sites should continue (Piludu et al., 2015). Like Rakotondrasoa et al. (2015), we recommend continuing the search for new ponds and the continued monitoring of existing ponds, as well as continuation of research and estimations of population sizes using capture-mark-recapture techniques. Understanding the relationship between rare species and subsequent avoidance by animals of certain microhabitats within their range is vital if we are to plan future management strategies in important forest habitat (Semlitsch et al., 2009; Irwin et al., 2010; Pike et al., 2010). Information on such factors as day-time surface temperatures, canopy cover and litter cover can be used to inform the identification, creation and restoration of suitable habitats in the wild, as well as the requirements of the species in captivity.

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