A critical evaluation of field survey methods for establishing the range of a small, cryptic tortoise (*Pyxis arachnoides*)

Ryan C.J. Walker[1,2]

[1]Nautilus Ecology, 1 Pond Lane, Greetham, Rutland, LE15 7NW, United Kingdom
[2]Department of Life Sciences, The Open University, Milton Keynes, MK7 6AA, United Kingdom

Understanding the range of threatened species is important for developing sound conservation initiatives. However, different survey methods can yield varying results when applied to cryptic vertebrates. Here, I established detection probabilities and compare detection rates of time-dependent searches against line-transect sampling for a rare, small and cryptic spider tortoise (*Pyxis arachnoides*) in the coastal dry forests of southwest Madagascar. The detection probability was 1.00 for field surveys undertaken during periods of highest tortoise activity. Significant differences in mean detection rates of 4.15 and 2.29 tortoises per man hour were recorded for time-constrained searching and line-transect sampling, respectively. Only time-constrained searches detected tortoises at all survey sites. There was no size-dependent variation in tortoise detection for either method. A GIS-based spatial model revealed that 12.54% of the range detected through timed searching would have been missed if transect sampling alone was applied. Higher detection rates for the timed search method are probably a result of surveyors applying greater effort to the species preferred microhabitat. Dependent on the desired output of the study, time-dependent searches or a combination of time-dependent searching and linear transect sampling is suggested.

Key words: arid forest environments, cryptic species, distribution mapping, line-transect sampling, *Pyxis arachnoides*, time-constrained search

INTRODUCTION

A concern for conservation biologists addressing the management of any species is to allocate limited resources in the most effective manner, for example when undertaking baseline field surveys of species for which few distribution data are available (Franco et al., 2007). Baseline distribution mapping of a species suffering range contraction and subsequent population decline is often the first step in the development of sound conservation initiatives (Scott et al., 1993; Smith et al., 1999; Quinn & Keough, 2002). Early vertebrate biologists often used locations of museum records and the boundaries of major biomes in which specimens were discovered to produce range maps (Baker, 1956; Armstrong, 1972). As a result, these early range maps rarely excluded areas of unsuitable habitat within these broader biomes (Scott et al., 1993).

Ecological thinking now accepts that many species only exploit certain zones within broad-scale habitat types (Quinn & Keough, 2002), coupled with the fact that ranges are often fragmented due to anthropogenic pressures such as hunting, illegal poaching and habitat degradation (Harper et al., 2007; Walker, 2010). The accurate depiction of a species range requires extensive ground truthing through biological field surveying (Quinn & Keough, 2002; Pullin et al., 2004), with Geographical Information Systems (GIS; McCoy et al., 2002; Walker, 2010) allowing for a quantitative, spatial assessment. Amassing rigorous and comprehensive field data for the production of distribution maps for a particular species becomes challenging in the case of small, cryptic or rare species. As a result, the selection of inappropriate surveying techniques can sometimes compromise the rigour of the data collected, due to the failed detection of some individuals or populations (Silveira et al., 2003; Somers & Mansfield-Jones, 2008).

The critically endangered Madagascar spider tortoise *Pyxis arachnoides* is one of the world's smallest tortoises (Pritchard, 1979; Leuteritz & Walker, 2008), and displays cryptic, crepuscular and habitat-dependent behaviour (Walker et al., 2007). This species also remains in a state of aestivation for up to eight months per year (Walker et al., 2007; Pedrono, 2008). Spider tortoises inhabit the dry coastal forests of southwestern Madagascar, which are threatened by slash and burn agriculture, charcoal production and subsistence grazing (Seddon et al., 2000; Fenn, 2003; Harper et al., 2007; Gardner, 2009). The spider tortoise is also facing significant threats as a result of collection to support the pet trade, and collection for local consumption, particularly within the north of its range (Walker et al., 2004; Walker, 2010). Its ecology, life-history and contraction in available habitats have attributed to a lack of reliable data on the current range (Bour, 1981; Pedrono, 2008; Walker, 2009), as is the case with many species of tortoise (Baillie et al., 2004).

Recent field survey techniques for tortoises in arid environments have generally fallen into two categories: Time-constrained visual searches of either a quantified or unquantified unit area of habitat (Smith et al., 1999; Loehr, 2002; Nomani et al., 2008; Attum et al., 2008), or transect sampling undertaken over a predetermined distance (O'Brien et al., 2003; Leuteritz et al., 2005; Walker, 2010). Both techniques require a large investment in

Correspondence: Ryan C.J. Walker, 1 Pond Lane, Greetham, Rutland, LE15 7NW, United Kingdom;
E-mail: ryan@nautilusecology.org

manpower, and need to consider individual detectability for estimating abundance (Seber, 1982; Buckland et al., 2001; Thomas et al., 2010). Here I establish the detection probability of the spider tortoise for surveys carried out when the tortoises are most active (Walker et al., 2007; Pedrono, 2008). I present a critical analysis of the effectiveness of single-visit, time-constrained searches versus line-transect sampling in detecting the occurrence of a small and cryptic tortoise, and compare the results when both methods are applied to distribution mapping.

MATERIALS AND METHODS

Pyxis arachnoides is assumed to inhabit an approximately 10 km wide coastal strip of dry forest unique to south-western Madagascar (Bour, 1981; Seddon et al., 2000; Pedrono 2008; Fig. 1), experiencing deforestation rates of up to 1.2% per year (Harper et al., 2007). I selected a portion of coastline within the centre of the species suspected area of occurrence (Pedrono, 2008) between the Onilahy and Linta rivers (approximately 150 km in length) (Fig. 1). The forests within this region support low lying, xerophytic vegetation, with a canopy typically 2–3 m high (Seddon et al. 2000) and a canopy coverage of up to 80% in areas unaffected by anthropogenic impact (Harper et al., 2007). Tortoises typically favour areas of >40% canopy cover (Walker et al., 2007).

Walker et al. (2007) have established that field surveys undertaken during the austral winter result in 55.1% lower detection levels for *P. arachnoides* than surveys undertaken during the warmer and damper summer months (November to April). However, the detection function (i.e. the amount of time the tortoise remains visible) is unknown, and critical to establish the effectiveness of any survey (Buckland et al., 2001). To measure detection probability, nine tortoises were selected on separate days during February 2003 for continuous focal observations (Altmann, 1974; Martin & Bateson, 1993). Each tortoise was located at approximately 0600 hours on clear, cloudless days. Observers used 10× binoculars at a observation distance of ~10 m to watch each tortoise for the whole day (0630–1830, Hailey & Coulson, 1999; Lagarde et al., 2003), undertaking 3 hour shifts. Observers recorded in minute intervals if the tortoise was visible or fully impaired from view. The total number of minutes that each of the nine tortoises were out of view were divided into the number of minutes for the nine days within the following pre-determined times; 0630–1030, 1030–1530 and 1530–1830, and compared using a one-way ANOVA (α=0.05) with Tukey's post hoc test. Prior to analysis, data were tested for normal distribution using a Ryan-Joiner test and where necessary transformed using a Box-Cox transformation. All statistical analyses were performed using Minitab 12.

Twenty survey sites were selected using high-resolution remotely sensed imagery (IKONOS and QuickBird) derived from Google Earth™, based upon apparently intact habitat across the species range as described by Bour (1981) and Pedrono (2008). Within each of these varying sized survey areas, a 1 km line transect was surveyed concurrently with a time-constrained search. Field work took place in February 2010, coinciding with the annual period of heightened tortoise activity. Surveying was limited to 0630–1030 and 1530–1830 (mean temperature at ground level: 32.4 °C ±4.6), when the tortoises are most active (Walker et al., 2007; Pedrono, 2008).

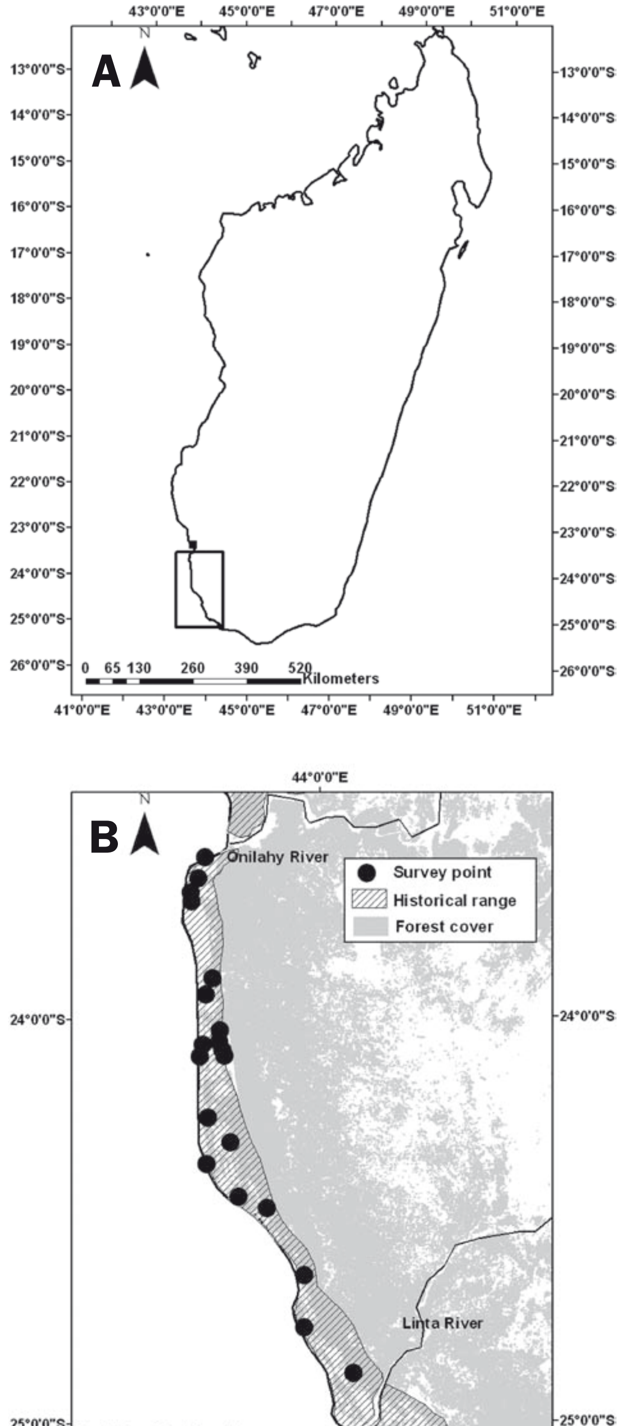

Fig. 1 (A) Location of the study area. (B) Suspected historical area of occurrence of the spider tortoise *Pyxis arachnoides* within the portion of its range between the Onilahy and Linta Rivers as described by Bour (1981) & Pedrono (2008). Grey areas denote vegetation cover derived from Landsat TM7 images.

The same two surveyors were used for the entirety of the study. The surveyors adopted the line-transect distance-sampling method described by Buckland et al. (2001) and Thomas et al. (2010), using a GPS tracking function to determine the distance covered. Upon detection of each tortoise, the perpendicular distance between the point of first detection to the centre of the transect line was measured (Buckland et al., 2001). The curved carapace length (CCL) of each tortoise was measured in mm using a flexible tape measure.

Concurrently, an additional two surveyors undertook a time-constrained search (Smith et al., 1999; Attum et al., 2008) within the same area. Each timed search lasted the length of the time taken for the transect surveyors to the traverse the 1 km transect. Timed searching was undertaken at least 10 m from the transect team to eliminate the possibility of duplicate detection by the two teams. Timed searchers focused their search on the base of low-lying vegetation, a microhabitat favoured by the species (Walker et al., 2007; Pedrono, 2008), and also used tracks in sandy substrate to locate animals. The CCL of each tortoise was also recorded. When either member of a team stopped searching as a result of tortoise detection, all four surveyors stopped surveying to ensure consistent searching at the same time. Transects and timed searches lasted on average 34.7 (±5.1) minutes. All detected tortoises were marked using a small dot of nail polish on the top of the carapace to avoid duplicate counting. The CCLs of each tortoise detected were grouped into 'large' (>150 mm CCL) and 'small' (<150 mm CCL).

Using the program ArcMap (ArcGIS 9.0), waypoints marking the start of each transect/timed search were plotted in a GIS database, adding habitat cover derived from Landsat TM 7 imagery (Fig. 1B). Each survey point was coded with either presence or absence of tortoises recorded by each survey method. By using Google Earth it was possible to identify areas of degraded and suitable habitat. The perimeter of occupied areas of habitat were digitized to form polygons for the tortoises detected through timed searching and line-transect sampling, respectively, added as a layer to the GIS. A further layer representing the suspected historical area of occurrence of the tortoise between the two rivers as described by Pedrono (2008) was added. By applying the area calculation function in ArcGIS 9.1 to each polygon, it was possible to establish the current range using both survey methods in comparison to the historically assumed area of occurrence. All spatial data were georeferenced and projected to WGS84.

RESULTS

The warmer part of the day (1030–1530) resulted in a significant drop in detection (one way ANOVA P=0.019, Tukey's post hoc test), with tortoises spending on average 5.4 minutes (n=49) hidden from view during the nine survey days. During mornings (0630–1030) and late afternoons (1530–1830), tortoises were on average only hidden for 1.0 (n=9) and 1.6 minutes (n=13, Fig. 2). The tortoises that were continually watched had a detection function of 1, therefore negating the need for a multiplier to be added to the occurrence data.

A total of 97 and 54 tortoises were detected during a total of 11 hours and 59 minutes of time-constrained searching and line-transect sampling, respectively. The mean detection rate of tortoises per man hour for time-constrained searching (4.15±2.77) was significantly different from line-transect sampling (2.29±1.72, paired t test; P=0.014, Fig. 3). There was no difference between the methods in the size of tortoises detected (x^2=0.973, P=0.324). Mean tortoises detection distance from the middle of the transect line was 264±184 cm. At least one tortoise was detected at each site using both methods. However, line-transect sampling failed in tortoise detection at three sites where time-constrained searching recorded at least one animal. Applying line-sampling data to the GIS results in a range that is 12.54% smaller compared to the range determined through time-constrained searches (Fig. 4). The combined results from both sampling techniques shows a smaller range (1,105.4 km^2) compared with the previously published suspected area of occurrence (1,514.3 km^2; Bour, 1981; Pedrono, 2008) between the Onilahy and Linta rivers.

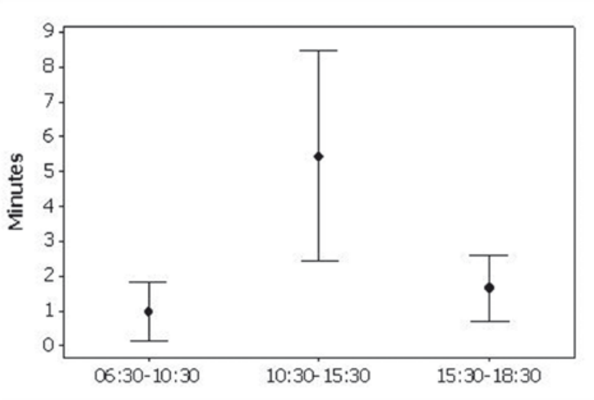

Fig. 2. Mean number of minutes during three time intervals (0630–1030, 1030–1530 and 1530–1830) when nine individual tortoises were completely invisible to the observer.

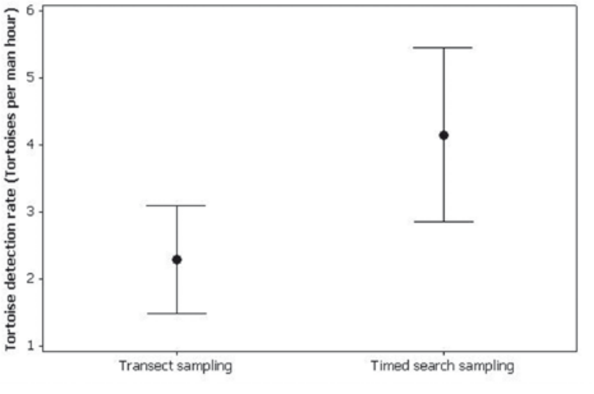

Fig. 3. Detection rate of *P. arachnoides* represented as tortoises per man hour of survey effort for line transect sampling and timed searches respectively.

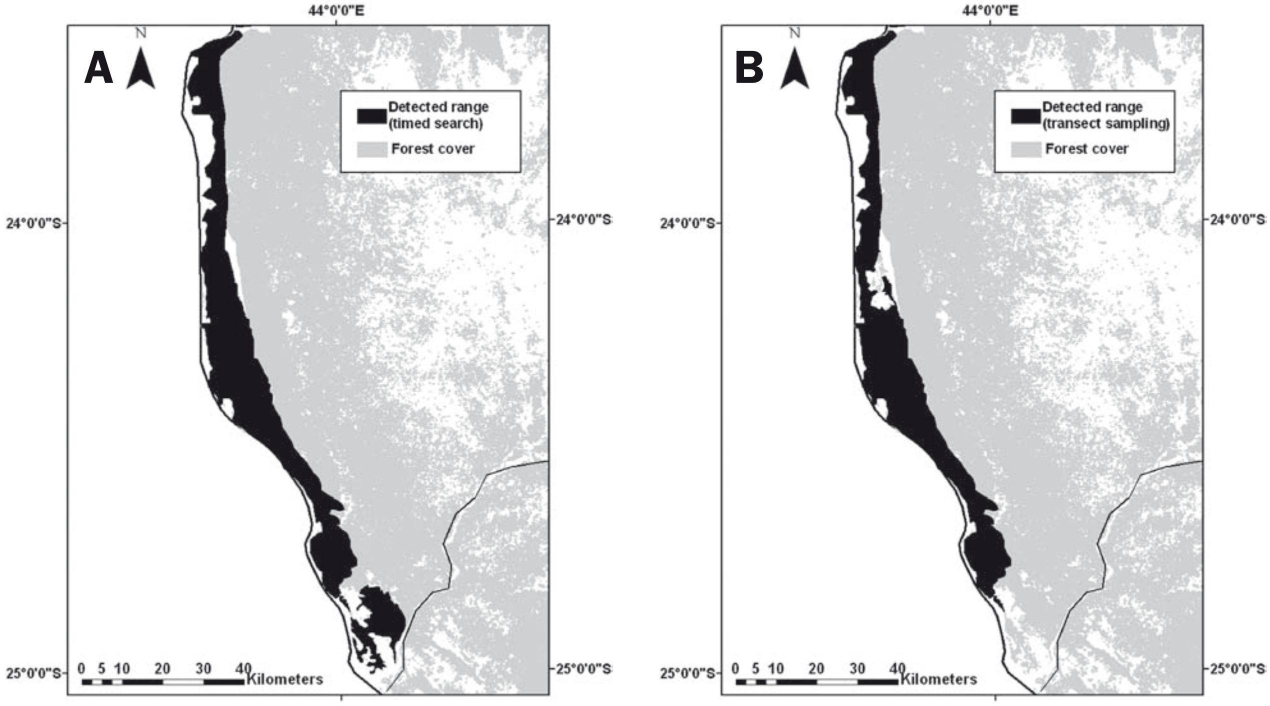

Fig. 4. Detection interpreted as spatial distribution of *P. arachnoides* between the Onilahy and Linta Rivers using (A) a timed search sampling as a survey technique, (B) a line-transect survey technique.

DISCUSSION

The results demonstrate the importance of selecting appropriate survey methods when working with small, cryptic, crepuscular, seasonally active chelonians. If surveys are undertaken during the austral winter, Walker et al. (2007) report a 55% reduction in tortoise detection on account of seasonal aestivation. If surveys are undertaken during the warmer period of the day (1030–1530), the detection function is below 1. Both line-transect sampling and time-dependant searches proved equally effective at detecting juvenile tortoises. Gardner et al. (1999) and Anderson et al. (2001) however showed that, in the case of *Psammobates geometricus* and *Gopherus agassizii,* detection probability was lowest for juveniles and overall detection function was as low as 0.5 for the small, cryptic *P. geometricus*. Young et al. (2008) report a detection function of 0.94 for *P. planicauda*, and tortoise species from arid environments can support detection functions as low as 0.85 (the burrowing *G. agassizii*, Swann et al., 2002).

The lower the detection function for a species, the more relevant adaptive surveying methods become. MacKenzie et al. (2002) describe a method for assessing site occupancy of rare, cryptic species with a detection function below 1. This method involves undertaking multiple surveys at the same site to establish an occupancy model allowing for missing observations. The drawback to this method is the labour-intensive nature of repeat surveys over a wide geographical range. In the present study, this method would likely enable more reliable and robust results, as tortoises were missed at 3 of the 20 sites using the line-transect method. Alternatively, a mark-recapture approach (e.g. Otis et al., 1978) could yield robust results for establishing species occupancy at a site, requiring at least iwo visits to each site. Gu & Swihart (2004) propose logistic regression models to infer a species presence when the population size is small, individuals are difficult to sample or sampling effort is limited. Their model predicts the presence of a species based on habitat quality. However, this method has limited application for the present study, as some suitable areas do not support tortoises due to poaching pressure (Walker et al., 2004; Walker, 2010).

If line transect sampling alone was used in this study, the ground truthing for the GIS analysis would have resulted in an underestimation of 12.5% of the range detected through time-dependent searching. An underestimation of range can have important implications for endangered species such as *P. arachnoides,* as undetected populations would be omitted from conservation management strategies (Rabesahala Horning, 2003; Rabearivony et al., 2010; WWF, 2010). Time-dependent search surveys were more effective at establishing the presence of spider tortoises than line transects, as the surveyors in addition to using tortoise tracks focused their attention on microhabitats favoured by the species, as opposed to line transects which often cover large areas of open ground not favoured by the tortoises. Indeed, time-dependent searches detected tortoises at all 20 survey sites, establishing a 27% loss of range compared to what was previously considered the tortoise's range (Walker, 2010).

Methods for sampling the presence or absence of tortoises within a particular area are less developed than sampling protocols for other taxa such as birds (Bibby et al., 2000). Field-surveyor sampling is the only effective way of detecting the presence of tortoises within a natural habitat. Methods such as pitfall trapping can only be applied for other reptiles such as lizards (Moseby & Read, 2001; Doan, 2003), and cage traps are usually only applicable to aquatic turtles (Somers & Mansfield-Jones, 2008). One addition to active, observation-based sampling for tortoises is the use of trained detection dogs, which have been successfully used in the surveying of *G. agassizii* (Cablk & Heaton, 2006; Nussear et al., 2008). Feasibility studies using dogs are also underway in Vietnam for a variety of tropical forest chelonian species (McCormack, 2010). However, the use of dogs can place added logistical pressures to a surveying project, as trained detection dogs are generally limited in availability (Cablk & Heaton, 2006). There is a lack of published critical evaluations of survey methods applied to cryptic, dry forest tortoise species. Most of the literature focuses on studies investigating observer bias (field biologists versus untrained volunteers) using excepted surveying methods such as distance sampling (Anderson et al., 2001). Only Somers & Mansfield-Jones (2008) described the trapping effort required in the detection of small bog turtles, and Normani et al. (2008) compared the effectiveness of different methods in detecting gopher tortoise burrows.

Line-transect sampling misses the base of low lying scrub, the microhabitat favoured by *P. arachnoides*, and the species small size limits its detectability to about 3.5 m from the transect line (Walker & Rafeliarisoa, in press). This suggests that line-transect sampling is more suited for larger species. This method has been applied extensively to the larger species *Astrochelys radiata*, which in much of its range is sympatric with *P. arachnoides* (O'Brien et al., 2003; Leuteritz, et al., 2005). Despite time-constrained searching being the more suitable surveying method, the data generated can be somewhat limited in application. Time-constrained search methods establish the presence/absence of a species within an area, which can be applied to distribution mapping (Smith et al., 1999). For management purposes, quantitative data on the abundance and density of a population in a particular area often become important after the range of a particular species has been established (Anderson et al., 2001; Young et al., 2008). Line-transect sampling is widely regarded as the most effective method to establish population estimates for tortoises (Anderson et al., 2001; Swann,et al. 2002; Young et al., 2008).

The present study suggests that timed searches are the preferred method to establish a small, cryptic tortoise species range. Further improvements could be achieved using a multiple-visit site-occupancy model, or a mark-recapture model. If a survey requires quantitative data on population size or density in addition to the presence/absence of a species within a particular area, then it is suggested that timed searches are undertaken concurrently with line-transect distance sampling and occupancy modeling.

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