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METHODS FOR ASSESSING POPULATION SIZE IN SAND DUNE LIZARDS (*LIOLAEMUS MULTIMACULATUS*)

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Abstract: Basic information, such as population size and density, is needed for conservation and management of many species, especially threatened species. Thus, well-designed population monitoring programs that use appropriate methods for estimating parameters of interest, including density and survival, are needed as well. Mark-recapture and distance-sampling are established methods for estimating density in wildlife surveys. The sand dune lizard (*Liolaemus multimaculatus*) is an endemic and vulnerable species that inhabits dune habitats in Argentina. At present, however, there are no accurate estimates of density of this species and no established monitoring programs. The objectives of this study were (1) to test the use of mark-recapture and distance-sampling methods and (2) to estimate density of this species in Mar Chiquita Reserve (37° 37′ S–57° 16′ W), an important area for the protection of this species. For distance-sampling surveys, we used a systematic line-transect design; for mark-recapture sampling, we performed exhaustive surveys and captured, marked, and recaptured lizards manually. Based on distance-sampling, populations were estimated at 3.6 and 5.4 individuals per ha in 2007 and 2008, respectively; corresponding estimates based on mark-recapture data were 5.2 and 4.1 individuals per ha in 2007 and 2008, respectively. Detection probabilities were 0.23 in both 2007 and 2008 distance-sampling analyses and capture probabilities were 0.02 and 0.05 in 2007 and 2008 mark-recapture analyses. Based on these estimates, the Mar Chiquita Reserve contains a population of at least 10,000 individuals. Both methods were adequate for estimating populations of sand dune lizards, given the facility with which individuals can be detected and captured. The distance-sampling method requires less effort, but the mark-recapture method allows estimates of survival as well as density. Results of this work provide the baseline for developing a monitoring program for this lizard, and we suggest that the distance-sampling method be used to monitor all populations of sand dune lizard.

Key words: Conservation; Density; Monitoring; Sand dune lizard

Possibly the greatest obstacle to the detection of species declines is the paucity of long-term data on population trends (Pechman and Wilbur, 1994), especially for many threatened or rare species (Funk et al., 2003; Gaston, 1994). Thus, well-designed population monitoring programs that estimate parameters of interest over time are needed (Thompson et al., 1998). One of the main parameters of interest in population monitoring programs is usually abundance (the absolute number of individuals) or density (the number of individuals per unit area). Accurate estimation of animal densities is a basic requirement both for ecological research and wildlife management (Krebs, 1999; Zug et al., 2001) because density is linked to genetic variability and to the susceptibility of a population or species to extinction (Pough et al., 1998; Soulé, 1976; Zug et al., 2001). Consequently, choosing an appropriate method for estimating density is important. The best density estimates are those that are both precise and unbiased (Thompson et al., 1998). A lack of precision, manifested as high sampling variance, standard error, and coefficients of variation, reduces power. An estimate that is consistently biased will not reduce power but will simply be an overestimate or underestimate of the true density.

No information on population size exists for most South American species of lizards (Rocha, 1998), and little is known about which methods are best to estimate abundance or density. Previous studies on lizards have used a variety of methods to estimate numbers, including counts of lizards or lizard scats (Beauchamp et al., 1998; Rorabaugh et al., 1987; Turner and Medica, 1982), mark–recapture analyses (Boyarski, 2001), and, more recently, distance-sampling methods (Grant and Doherty, 2005). Counts based on scats did not produce estimates that were correlated with counts of lizards (Beauchamp

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et al., 1998), suggesting that estimates of abundance that are based on lizard detections are more likely to produce accurate information than estimates based on scat detections. Raw counts, however, fail to account for detection probability, often resulting in biased estimates and misleading results (Anderson, 2003; Thompson et al., 1998; Williams et al., 2002). Closed mark-recapture (Otis et al., 1978) and distance-sampling (Buckland et al., 2001) density estimates, in contrast, can account for differences in detection.

The sand dune lizard, *Liolaemus multimaculatus*, is a small, diurnal, sand-dwelling Liolaemid lizard, which is endemic to the pampean coasts of Buenos Aires and Río Negro Provinces in Argentina, where it occurs only in dune habitats (Cei, 1993). Populations currently are restricted to six small and isolated dune areas (Kacoliris et al., 2006). Four of these areas are considered as "Grassland Valuable Areas" for South America (Bilenca and Miñarro, 2004) because they are important for conservation of Pampean biodiversity. These areas encompass 80% of the current distribution of sand dune lizards, but in only one of these areas, the "Mar Chiquita Provincial Reserve", have conservation management actions been established to protect an important population of this species (Kacoliris et al., 2006). Lavilla et al. (2000) considered the sand dune lizard as a vulnerable species because of its low abundance and because anthropogenic disturbances have greatly affected its habitat. Previous research has documented reduction in abundance, possibly an effect of road construction (Vega et al., 2000). At present, however, there is no accurate estimate of density and no established monitoring programs for the sand dune lizard.

We had two basic objectives for this study. First, we compared mark-recapture and distance-sampling methods to estimate density of sand dune lizards based on samples conducted in Mar Chiquita Reserve. Second, we used these results to estimate population size of this lizard in the Mar Chiquita Provincial Reserve and to make recommendations for a monitoring program for this vulnerable species.

Methods

The study was conducted at Mar Chiquita Provincial Reserve (37° 37′ S–57° 16′ W) within the Central Dunes, the largest coastal dunes sector of Buenos Aires province, Argentina (Bilenca and Miñarro, 2004). In addition to the Mar Chiquita Reserve (~3,080 ha of dunes), the Central Dunes also include the Faro Querandi Municipal Reserve (~5,757 ha of dunes). Vegetation in the study area consists of patches of sand-grasslands and inter-dunes in a matrix of dunes without vegetation (Cabrera, 1976). Sand-grasslands are characterized by plant species adapted to high salinity conditions, mobile substrate and low water availability. Inter-dunes correspond to humid lowlands and show a mix of grasslands and aquatic plants. Exotic forests of *Pinus* sp. and *Acacia* sp. also exist at the study area.

We estimated density of sand dune lizards using distance-sampling and mark-recapture methods. Both techniques have been used successfully in previous studies of terrestrial lizards (Boyarski, 2001; Dickinson and Fa, 2000; Dickinson et al., 2001; Grant and Doherty, 2005). We conducted surveys simultaneously between January and February in two consecutive years (2007 and 2008). We searched for lizards from 1000 to 1600 h, the peak of activity for this species (Vega et al., 2000).

For distance-sampling surveys, we used a systematic segmented grid design on a 1300-ha area. We established seven grids separated from each other by 1 km. Each grid had 4–6 1000-m parallel transects, with 38 total transects. Transects were at least 250 m apart to ensure independence. During each survey, two observers walked along the centerline of each transect and recorded perpendicular distance (in cm) from the centerline to each lizard detected, using a measuring tape. A third observer walked 15 m behind the first two, dragging feet in the sand to help detect any lizards that were buried in the sand and not detected by the first two observers. The starting point for the systematic transects was located randomly. The total effort was, on average, 30 person days each year.

Distance-sampling has three main assumptions that should be verified (Buckland et al., 2001). The most important is that detection probability of individuals at the centerline is equal to one ($g_{(0)} = 1$). The other two assumptions are that individuals were detected at their original position and that measurements were exact. The escape behavior of this lizard promotes its detection because individuals tend to run in the presence of observers, even if they are buried (F. P. Kacoliris, personal observation). In our surveys, the responsibility of the third observer was to ensure that all lizards on the centerline were detected. We defined the centerline as the area contained within a distance less than 100 cm from the center of distance transects. Following Funk et al. (2003), we verified the $g_{(0)} = 1$ assumption considering a density value obtained by an independent sample. We used the density value obtained from the mark-recapture analysis (see below) and compared it with the density value obtained by counting all lizards detected at the centerline. We assume that mark-recapture yielded an unbiased estimate. Under this assumption, if all lizards at the centerline are detected, density values must be similar. We compared both density values, and results did not indicate differences (t-test $= -0.54$, df $=$ 37, 14; $P > 0.05$), which supports the $g_{(0)} = 1$ assumption. To corroborate the assumption that all individuals were detected at their original position, we used the shape criterion (Buckland et al., 2001). We constructed histograms to determine if there was evidence for a lack of observations to the zero distance. Histograms for both seasons showed the typical shouldered curve, suggesting that lizards were not detecting observers and moving away before their original position was noted (Fig. 1a,b). We also identified outlying observations and performed a right data truncation (discarding 5% of outlier values) to obtain a better fit of our data to distance models.

We tested the fit of the three main models in distance methods (half-normal, uniform and hazard-rate) to our data, using a chi-square goodness-of-fit test. Significant P-values would indicate that the model might be a poor fit. We selected the best model based on Akaike's Information Criterion (AIC), which evaluates models in terms of parsimony and fit to the data (Akaike, 1973). For variance estimation, we consider transects separate. Sampling design and data analyses were performed using Program Distance 5.0 (Buckland et al., 2001; Thomas et al., 2006).

Mark-recapture surveys were carried out by a four-person team during 15 days in 2007 (60 person days effort) in an 85 ha plot area, and by a six-person team during 15 days in 2008 (90 person days effort) in a 75 ha plot area. Both plots were placed randomly at the study area. Plots size was based on the greatest area that could be exhaustively surveyed in one day. We chose to work in one large plot instead of several smaller plots because large areas with short sampling periods usually mean that the closed-population assumption is met (Krebs, 1999). Effort was increased in 2008 to obtain more recaptures than in 2007. We searched for lizards, captured them manually, and marked them using an individual toe-clipping code (Woodbury, 1956) by removing the distal one third of it with sharp scissors. After marks were cauterized, we released lizards near to the point of capture. These marks do not have secondary effects in terrestrial lizards (Borges-Landáez and Shine, 2003; Huey et al., 1990; Paulissen and Meyer, 2000), they are permanent, and they cannot be overlooked by the observer, satisfying two mark-recapture assumptions.

Mark-recapture includes two kinds of models depending on whether the population is assumed to be open or closed during the survey period (Krebs, 1999). Closed-capture models assume that no individuals enter (through births or immigration) or leave (through deaths or emigration) the population during the study, with the ultimate goal of estimating population size. In many cases, closed models have higher precision than open models but they assume that populations are closed geographically and biologically (Otis et al., 1978). Closed-population capture-recapture models are preferred for population estimation over the open-population models, which do not assume closure, because heterogeneity in detection probabilities can be accounted for, leading to improved estimates (Stanley and Richards, 2005). We used the "Otis" and the "Burnham and Stanley" tests through the CloseTest Program to evaluate

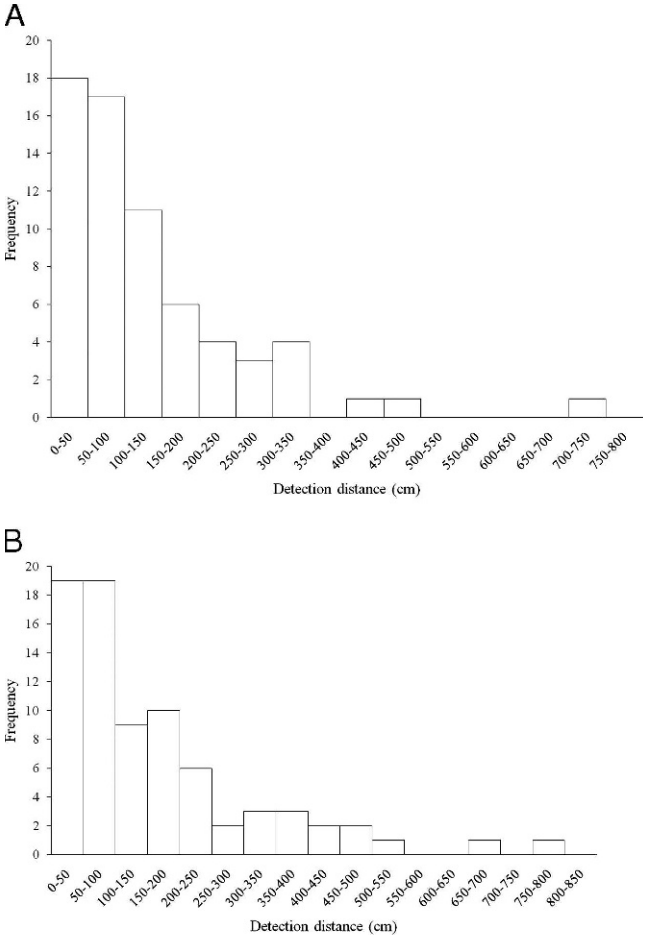

FIG. 1.—Histogram of perpendicular distance data of 2007 (A) and 2008 (B).

the key assumption of closure for 2007 and 2008. Both tests indicated that, during the survey period, the sampled population acted as a closed population for recruitment and loss of individuals in both years (Z = 0.48 for 2007, and Z = 0.89 for 2008; $\chi^2 = 16.58$ df = 18 for 2007, $\chi^2 = 28.83$ df = 21; $P > 0.05$ in all cases). A problem with these tests is that a failure of closure cannot be distinguished from behavioral changes in capture probabilities or from certain patterns of time-trends in capture probabilities. Thus, if the best model based on AIC is the behavioral response model (see below), then the closure test will not be valid. Abundances were estimated using Program MARK (Cooch and White, 2004; White and Burnham, 1999) and extrapolated to the effective plot area to obtain density values.

Our analytic method dealt with possible effects of individual heterogeneity, behavioral response to capture, and time-related variations on capture probabilities (Otis et al., 1978; Williams et al., 2002). For this aim, we constructed several mark-recapture models through MARK Program, for the lizards' data: M_0 (constant capture probability), M_t (capture probability varying by time), M_b (behavioral response in capture probability), M_h (capture probability heterogeneous among individuals), M_{tb} (capture probability varying by time and behavioral response), M_{th} (capture probability varying by time and heterogeneous among individuals), M_{bh} (behavioral

TABLE 1.—Density values obtained using distance-sampling models. AIC = Akaike's values. Data are presented as number of individuals per hectare (lower and upper 95% confidence intervals).

Model	2007			2008		
	AIC	Delta AIC	Indiv/ha	AIC	Delta AIC	Indiv/ha
half normal	726.12	0.00	3.6 (2.5–5.1)	973.27	5.15	4.3 (3.3–5.8)
hazard rate	729.41	3.29	4.0 (2.6–6.1)	968.12	0.00	5.4 (3.6–8.1)

response in capture probability with heterogeneity among individuals), M_{tbh} (capture probability affected by time, behavioural response and heterogeneity). Heterogeneity was modelled using a finite mixture model (Pledger, 2000) with two groups of animals. We used AIC to select the most appropriate model for each year data set.

We estimated the sand dune lizard population size for the dunes sector of Mar Chiquita Provincial Reserve by multiplying the obtained densities by dune area of the Reserve.

RESULTS

We obtained 63 sightings in 2007 and 79 in 2008 distance-sampling surveys. In both cases, the numbers of sightings were adequate to obtain robust models (Buckland et al., 2001). Of the total lizards detected in both years, 96% (n = 136) were detected by the first observers; the rest were detected by the third observer. Our data fit the half-normal and the hazard-rate models ($P > 0.05$ in all cases). Based on AIC, the half normal was the best model for 2007 and the hazard rate was the best for 2008 (Table 1). Considering each transect as a survey unit, coefficients of variation ranged between 18% (half normal 2007) and 20% (hazard rate 2008). Detection probability was 0.23 (95% Confidence interval of 0.20–0.26) for 2007 distance data and 0.23 (95% Confidence interval of 0.17–0.32) for 2008 distance data.

Regarding Mark-recapture, the M_t model was the best in both years (Table 2A, B). During 2007, 152 lizards were marked and 25 (16%) recaptured on 30 occasions (i.e., 1.2 ± 0.5 recaptures per recaptured individual). Estimated density for 2007 was, based on the M_t model, 5.2 ± 0.8 individuals per ha (95% Confidence intervals 4.0–7.2). During 2008, 159 lizards were marked and 77 (48%) recaptured on 143 occasions (1.86 ± 1.28 recaptures per recaptured individual). The M_t model estimated 4.1 ± 0.4 individuals per ha (95% Confidence intervals 3.5–5.0). Average ± DS of capture probability was of 0.03 ± 0.02 for the 2007 data set and 0.05 ± 0.02 for the 2008 data set. Based on these density estimates, the Mar Chiquita Provincial Reserve could be protecting a population of approximately 13,300 (between 10,000 and 17,000) individuals of sand dune lizard (Fig. 2).

DISCUSSION

Our results are the first density and population size estimates for sand dune lizards. Given that estimated density ranged

TABLE 2.—Closed capture models for the 2007 and 2008 data sets. AIC = Akaike's value; Par = parameters; M_0 = constant capture probability; M_t = capture probability varying by time; M_b = behavioural response in capture probability; M_h = capture probability heterogeneous among individuals; M_{tb} = capture probability varying by time and behavioural response; M_{th} = capture probability varying by time and heterogeneous among individuals; M_{bh} = behavioural response in capture probability with heterogeneity among individuals; M_{tbh} = capture probability affected by time, behavioural response and heterogeneity.

Model	AIC	Delta AIC	Weight	#Par
A. 2007				
M_t	−188.12	0.00	0.94	16
M_{tb}	−182.02	6.10	0.04	29
M_{htb}	−179.97	8.15	0.02	30
M_{ht}	−156.30	31.82	0.00	17
M_0	−120.99	67.13	0.00	2
M_b	−119.27	68.85	0.00	3
M_h	−118.22	69.91	0.00	4
M_{hb}	−115.55	72.57	0.00	6
B. 2008				
M_t	25.19	0.00	0.88	16
M_{ht}	29.66	4.47	0.09	17
M_{tb}	32.63	7.44	0.02	29
M_{hb}	37.51	12.32	0.01	5
M_h	41.65	16.46	0.00	2
M_0	41.65	16.46	0.00	2
M_b	43.09	17.91	0.00	3
M_{htb}	62.12	36.93	0.00	55

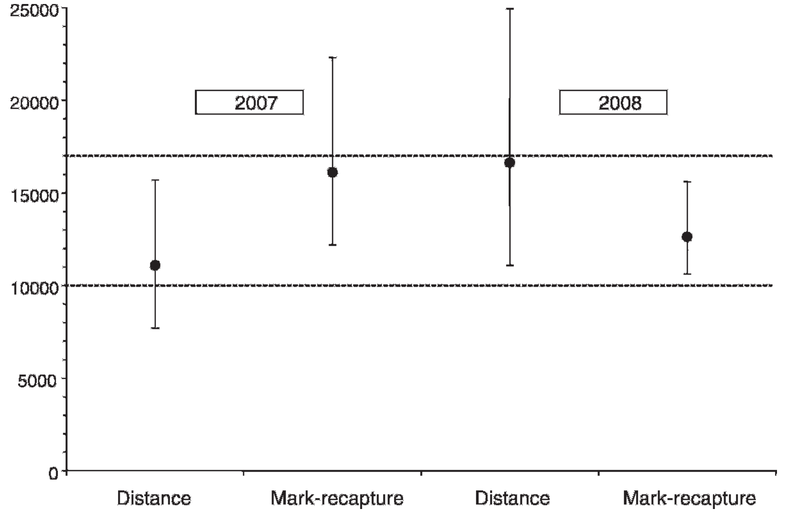

FIG. 2.—Estimated population size for sand dune lizards in 2007 and 2008 at Mar Chiquita Provincial Reserve.

from 3.6 to 5.4 individuals per ha, the Mar Chiquita Provincial Reserve should contain a sand dune lizard population of at least 10,000 individuals.

The distance-sampling method was simple to perform at dunes, because good visibility allowed the detection of lizards. The tendency of the lizard to bury itself in the sand did not appear to affect the probability of detecting individuals along the centerline of the transect, and the $g_{(0)} = 1$ assumption was met. An advantage of this method in relation to mark-recapture was that it required less effort (1/2 and 1/3 with respect to the total effort of mark-recapture in 2007 and 2008 respectively). We only needed 10 survey-days to obtain enough sightings (more than 60) to allow the construction of robust models for density estimates. Another advantage is that distance methods avoid causing any stress to individuals by the capture and marking process.

A mark-recapture approach also was easy to perform with sand dune lizards, because of the facility with which lizards can be detected and captured. This is related to the sand dune lizards' escape behavior and microhabitat use, because lizards mostly bury into the sand or shelter on the basis of the shrubs, which facilitates their rapid capture. Mark-recapture analyses also allow assumptions to be verified and the best models to be selected through use of readily available software such as MARK and CloseTest. Tests for closure, for example, helped us to confirm that our survey plots were acting as closed populations. However, closed tests can be difficult because, if there was a behavior effect, then the closure test will not be valid. A major advantage of mark-recapture is that it allows survival estimation over multiple years.

We consider that both mark-recapture and distance-sampling methods are adequate to estimate sand dune lizard population size. We recommend mark-recapture methods for long-term studies on lizard population dynamics, primarily because they provide estimates of survival as well as density. Future studies could utilize multiple plots in order to obtain a better representation of the entire area of interest. In contrast, we recommend distance-sampling if the objectives are to obtain a rapid density measure, and/or to compare abundance over several sites. One problem with distance methods is the verification of the $g_{(0)} = 1$ assumption. In the case of sand dune lizards, if individuals are under the sand and not detectable the mark-recapture estimate could be an underestimate. If this is a problem, the distance-sampling and mark-recapture estimates are estimates of the available lizards. In our study we used two methods to test the $g_{(0)} = 1$ assumption: (a) the third observer dragging feet in the sand (in our surveys), and (b) a comparison with the mark-recapture results. Both methods bring evidence that support the $g_{(0)} = 1$ assumption. However, future studies should use ancillary information to determine if a proportion of lizards are unavailable for detection, in order to avoid bias if it exists.

Densities for small lizards, those less than 110 (mm) snout–vent length (similar to sand dune lizard size) vary between one and more than 1000 individuals per ha in some insular species (Zug et al., 2001). Most previous studies have, however, focused on temperate lizard species, whereas in tropical or subtropical regions, which harbor the largest lizard diversity, research on population dynamics (e.g., studies on density) is scarce (Rocha, 1998). Zug et al. (2001) considered densities of less than 10 individuals per ha to be low. Following this criterion, our estimates indicate low densities for sand dune lizards, corroborating the low abundance assumed by Lavilla et al. (2000).

We compare our results with three Liolaemid lizards: *Liolaemus wiegmanii*; *Liolaemus lutzae* and *Liolaemus huacahuasicus*. *Liolaemus wiegmanii* is a small lizard that inhabits a wide range of habitats, including sandy habitats. This lizard is sympatric with the sand dune lizard, and both species are included in the "*wiegmanii*" group. A mark-recapture study of *L. wiegmanii* (Martori et al., 1998) indicated a density of 100 individuals per ha, higher than sand dune lizard. However, this research was carried out on grasslands habitats and no data exist about *L. wiegmanii* densities on sandy habitats. The Brazilian sand lizard (*L. lutzae*) is an endemic and vulnerable species that inhabits a small area of dunes (200 km of coast). Mark-recapture studies on Brazilian sand lizards determined that densities varied from 41 to 114 individuals per ha (Rocha, 1998). In contrast, *L. huacahuasicus* (also endemic and vulnerable) inhabits mountains in western Argentina, and its density has been estimated at 4 individuals per ha (Cei, 1993). Our estimates were similar to those for *L. huacahuasicus*, but Cei (1993) did not show the dispersion of their estimates and did not describe which method was used. Concerning *L. lutzae*, it is important to consider that, although the area that *L. lutzae* inhabits is half that of the sand dune lizards (approximately 400 km of coast), density of the Brazilian sand lizard is much higher than that of sand dune lizards. Thus, the total number of individuals of both species should not be very different. In this case, and considering that risks are the same in both areas, the sand dune lizard should be included with the Brazilian sand lizard in the IUCN Red List.

An accurate estimation of population size in each Grassland Valuable Area is necessary to assess the population status and to develop management recommendations for this species and its habitat. Results of this work provide the baseline for implementing a monitoring program for sand dune lizards. Based on our results, we propose that distance-sampling be used to monitor all populations of sand dune lizards through space and time. Further, we recommend that mark-recapture studies continue in a core area to assess survival and population growth rate. We also recommend the use of both methods for density estimates in the other three sand lizard species that inhabit coastal dunes in Argentina (*Liolaemus wiegmanii*, *Liolaemus gracilis* and *Stenocercus pectinatus*), and more generally, for other terrestrial lizards that inhabit open habitats.

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