Abundance, microhabitat selection and conservation of eyed lizards (Lacerta lepida): a radiotelemetric study

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Keywords
census methods; detectability; lizard; radiotracking; refuge selection.

Abstract
The utility of radiotelemetry as a tool for estimating the size and microhabitat requirements of a population of Lacerta lepida, the largest European lacertid, was investigated in central Spain. Population density estimates based on repeated marking and recapture (3.2 lizards ha\(^{-1}\)) were much higher than those based on line transects (0.22 lizards ha\(^{-1}\)). The probability of sighting lizards before they could retreat into a refuge was largely increased by our ability to radiolocate them. Rocks were selected as refuges 96% of the times, and the locations of radiotracked lizards were much closer to rocks than randomly expected. Rocks used as retreat sites were larger and had more crevices than those available at random, which suggests that refuge selection was primarily determined by the need to find shelter from predators. Rocky patches, which were positively selected, might be used as refuge-connecting corridors that combine shelter with opportunities to forage and thermoregulate. Our results emphasize the need for using radiotelemetry to establish baseline information on abundance and to clarify the actuality, extent and pattern of the population declines experienced by species that may function as key links in their ecosystems, but the wariness of which poses a serious problem for monitoring their conservation status.

Introduction

The ectothermy and insectivorous diet of lizards allow them to attain high densities in low productivity ecosystems because they can successfully exploit a large prey base (small arthropods) that most endothermic predators cannot energetically afford to feed on (Regal, 1983). These high densities, in turn, provide a resource for a myriad of higher level predators such as raptors and diurnal carnivores. Thus, over the long term, lizards are key links between trophic levels in unpredictable environments, and changes in lizard population densities can have cascading effects on other trophic levels. This high ‘caloric capacitance’ may be particularly important in the case of large-sized species such as the eyed lizard Lacerta lepida, the largest European lacertid (snout–vent length and total length may reach 242 and 754 mm, respectively) and possibly the flag-ship species for the conservancy of European reptiles and amphibians (Corbett, 1989). This lizard is mainly found in the Iberian Peninsula, where it is an important part of the diet of several raptors that could optimize their energy intake by actively selecting this large reptile prey (Martín & López, 1996). It has even been argued that the generalized decline of L. lepida, pointed out by several authors (Allen, 1977; Corbett, 1989; Mateo, 2002), may partly be due to its increased importance in predators’ diets after the dramatic decrease in rabbit Oryctolagus cuniculus populations because of myxomatosis and viral pneumonia epidemics (Martin & López, 1996).

However, quantitative data providing support for the suspected declines of L. lepida populations are very few or non-existent, whereas data collected during unsystematic surveys may present a misleading picture of the status of the species. In some instances, the methodological information provided may be insufficient to allow reliable repetition of population counts. Allen (1977) reported significant decreases in population density between 1969 and 1975 at degraded habitats subject to human pressure, and Mateo (2002) reported densities that have fallen dramatically (from more than 50 lizards ha\(^{-1}\) to a vestigial presence) in sectors of the species range, but these authors give no information about the census methods used. Line transects along a well-conserved open Mediterranean forest yielded a much lower density of 1.5 lizards ha\(^{-1}\) (Cano, 1984). Thus, reliable field data are needed to establish baseline information on abundance and to clarify the actuality, extent and pattern of population declines.

Habitat requirements of L. lepida are also unclear, despite their importance for developing appropriate management strategies. Although widespread in a variety of both uncultivated and man-made habitats, it prefers sites with a complex vertical structure of vegetation and with some rocks (Castilla & Bauwens, 1992), and it avoids densely
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vegetated forest tracts with no clearings (Llorente et al., 1995). However, it may be abundant at sites with hardly any cover of vegetation (Cheylan, Megerle & Resch, 1990) as long as there are refuge-providing structures such as rocks, boulders or stone piles. In fact, the removal of stone refuges in fields during agricultural intensification exerts a further pressure upon the species (Corbett, 1989), suggesting that the availability of refuges may be an important factor for the maintenance of viable populations.

The objective of this paper is to take advantage of radiotelemetry to accomplish a double goal: (1) to compare different methods to estimate the abundance of an L. lepida population; and (2) to examine its microhabitat and refuge selection patterns. Radiotelemetry provides detailed information about habitat use by active individuals (Salvador, Veiga & Esteban, 2004) and retreat-site selection by hidden ones. This, in turn, allows us to compare various census methods by considering how they are affected by the reduced detectability of hidden individuals. Our aim was to identify the most suitable methods to estimate the population size and microhabitat requirements of species that may function as key links in their ecosystems, but the wariness of which poses a serious problem for the design of monitoring and management programs.

Materials and methods

Study site, radiotracking procedure and census methods

Our study site was a deciduous Pyrenean oak Quercus pyrenaica forest near Navacerrada (Sierra de Guadarrama, central Spain: 40° 44’ N, 4° 00’ W; 1250 m altitude), in which shrub patches (mainly of oak saplings and rockroses Cistus laurifolius) are interspersed with grasslands and rocky outcrops. We established a 7 ha plot (400 × 175 m) that we visited 5 days a week, weather permitting, from 15 April until 15 July 2004.

We restricted our study to adult lizards because subadults and juveniles could not be fitted with radiotransmitters, and because their detectability was extremely low (only two subadults and one juvenile were observed). Lizards were noosed using a fishing pole between 27 April and 17 June. Capture efficiency was high and we were able to capture most adult lizards at first encounter. Only two individuals, which were occasionally seen on different sampling days, could not be noosed. Immediately after capture, the lizards were weighed and measured [snout–vent length (SVL)]. We outfitted lizards with 2.5 g radiotransmitter collars (Biotrack Ltd, Wareham, UK) and released them at the site of capture after ensuring that they had recovered from the stress because of capture and handling, and that their escape behavior was normal.

Between 3 June (when we had already captured, radio-collared and released 12 individuals) and 17 June, we performed seven mark-recapture sessions to obtain population estimates. We chose the Schnabel method of repeated marking and recapture because it is particularly appropriate when the study animals are scarce, difficult to detect or capture, and can be obtained only in small numbers (Cox, 1985). On each new sampling day, we walked throughout the study plot and we noted, for each lizard seen, whether it was marked or not. If not, we captured and radiocollared it, so that the total number of marked animals increased from 12 to 18 between 3 and 17 June. Because all marked individuals could be radiotracked, we were certain that no death or emigration occurred during the study period, thus fulfilling the main conditions for the population estimate to be valid.

We also considered the number of different individuals that, according to radiotracking data, were located within a 1 ha section of the plot that had been censused in 2000 and 2001 in the course of a study of the home ranges of radiocollared lizards (Salvador et al., 2004). This was done to obtain comparable data about population density that could be used to assess its temporal variation.

As an alternative method of estimating lizard abundance, we also walked a number of parallel transects that were regularly distributed across the study plot. Transects were walked on 8 sunny days between 08:00 and 12:00 h (Mean European Time). Each observer walked slowly, at a constant velocity of c. 1 km h⁻¹, counting all adult lizards detected within a 10 m wide band. In addition, we also recorded the lizards seen perching on rocks above the mean height of vegetation within a 50 m wide band. Overall, we walked a total amount of 36 km of transects.

Microhabitat and refuge selection

We used an RX-8910HE (Televilt, Lindesberg, Sweden) radio receptor to locate radiocollared individuals between 08:00 and 15:00 h, and we registered their position with a Garmin® (Garmin Ltd, Romsey, UK) GPS 12 Personal Navigator®. We obtained data about lizard activity by radiotracking marked individuals and noting whether they were active or hidden. Thus, our estimates are of minimum activity, because some lizards may have sought refuge before being detected.

Locations of active lizards were used for the analyses of microhabitat selection. When we spotted a radiocollared lizard, four 10 m lines were laid out radiating from the lizard location into the four cardinal directions. We registered the presence or absence of grass, leaf litter, rockrose shrubs, oak saplings and rocks at 2 m intervals along these lines. We also used a calibrated stick to note the presence or absence of vegetation at different heights above the ground. This procedure allowed us to calculate the per cent cover values for each habitat variable. Because lizards used rocks as refuges with few exceptions, we noted the distance to the nearest rock that had a maximum diameter > 40 cm and at least one crevice that could be entered by an adult lizard. We also noted the mean distance to the nearest potential refuge in each of the four quadrants defined by the plant-cover lines.

We characterized refuges by noting the maximum length, width and height of each rock or rocky outcrop where
hidden lizards were radiotracked. We also noted the number of crevices that could be entered by an adult lizard. Although some refuges were used several times by the same or different individuals, we counted these refuges only once to avoid pseudoreplication.

To measure the availability of microhabitats and refuges, we selected 33 points using a random number table applied to latitude and longitude values within the ranges set by the GPS locations of radiotracked lizards. We used the GPS to determine the exact location of these points, and we measured all habitat variables as described previously. We characterized the rocks >40 cm with at least one crevice that were closest to each random point, to obtain a null hypothesis of refuge availability.

When necessary, habitat and refuge variables were arcsine- or log-transformed to fulfill the requirements of parametric tests. We performed a principal components analysis to reduce the number of habitat variables, rotating the factors with eigenvalues greater than one (Varimax rotation) to obtain a clearer pattern of loadings.

**Results**

**Population density and detectability of lizards**

During the study period, we captured 18 adult lizards (eight males and 10 females); the mean SVL (±1 se) was 158.3 ± 3.3 mm (range = 142–190), and the mean body mass was 94.8 ± 7.0 g (range = 60–155).

According to the Schnabel method, the adult population size (±1 se) was 22.5 ± 1.3 lizards (Fig. 1). Because the estimate of population size remained relatively constant after the third mark-recapture day, had a relatively small standard error and was close to the actual number of lizards observed, we are confident that this figure is a robust approximation to the number of adult lizards present at the study plot during the census period. Thus, we obtained a population density of 3.21 ± 0.19 lizards ha⁻¹. The 1 ha section of the plot previously censused in 2000 and 2001 included radiolocations of seven individuals, i.e. less than in 2000, when Salvador et al. (2004) captured 10 individuals ≥130 mm in SVL at the same plot, but more than in 2001, when only five individuals were captured (partly because of the difficulty in recapturing lizards with the fishing pole).

These results contrast with the ones obtained by means of transect counts. Considering the 10-m wide census band, the mean lizard abundance (±1 se) was 0.22 ± 0.08 lizards ha⁻¹, whereas considering the 50-m wide census band, it was 0.12 ± 0.03 lizards ha⁻¹. Thus, although some lizards could be seen perching on rocks, the overall detectability decreased between 5 and 25 m each side of the progression line. We conclude that the transect method was able to detect only a small fraction of the lizards known to be present. This could be because of a high proportion of inactive individuals, but our data indicate that 69% of the observations of radiocollared lizards corresponded to active animals, and that the visual detectability of active lizards must therefore be regarded as very low.

**Microhabitat and refuge selection**

Lizards were selective in their use of microhabitats as most variables showed significant differences between the locations of active lizards and the sample of randomly selected sites (Table 1). The principal components analysis with the habitat variables produced four principal components (PCs) that accounted for 72.4% of the variance (Table 2). The first component (PC-1) was positively correlated with rock cover and negatively correlated with the distance to the nearest refuge. The second component (PC-2) showed a positive correlation with the cover of oak saplings and with plant cover 25 and 50 cm above the ground, and a negative correlation with plant cover 5 cm above the ground. The third component (PC-3) gave high scores to sites with high values of rockrose cover leaf litter cover and plant cover 75 and 100 cm above the ground. The fourth component (PC-4) gave high scores to grasslands with a high cover of herbs 5 cm above the ground and with a low cover of rocks.

Lizards actively selected sites with high scores in PC-1 (\(r_{xy} = 13.22, P < 0.001; \text{Fig. 2}\)), and, to a lesser extent, also in PC-3 (\(r_{xy} = 2.37, P = 0.020; \text{Fig. 2}\)). Thus, lizards tended to remain close to refuges and to select sites with a high rock cover or a high cover of rockrose patches that could also provide some shelter. This is basically consistent with the results of univariate tests after applying the sequential Bonferroni correction (Table 1).

Interindividual differences in microhabitat use were significant along all axes except for PC-1 (Table 3; one female was excluded because it only had two observations). This probably reflects structural and/or floristic differences among the home ranges of different individuals. Thus, interindividual differences were particularly clear for the gradient associated with rockrose cover (PC-3), which was negatively correlated with longitude for the randomly selected sites (\(r = -0.433, n = 33, P = 0.012\)); the mean PC-3 scores of radiotracked lizards were negatively correlated with longitude for the randomly selected sites (\(r = -0.433, n = 33, P = 0.012\)).
Loadings with absolute values greater than 0.5 are shown in bold.

Explained variance 0.216 0.188 0.180 0.140
Eigenvalue 2.59 2.25 2.16 1.68
Plant cover 100 cm in height (%) 0.214/C0 0.652/C0
Plant cover 75 cm in height (%) 0.082 0.368 0.793/C0
Plant cover 50 cm in height (%) 0.256/C0
Plant cover 25 cm in height (%) 0.604/C0
Plant cover 5 cm in height (%) 0.387/C0
Cover of oak saplings (%) 0.760/C0
Cover of herbs (%) 0.384/C0
Cover of rocks (%) 0.134/C0
Cover of rockrose (%)/C0
Mean distance to four nearest rocks (m) 3.4±2.0

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Cover of oak saplings (%) 0.760/C0
Cover of herbs (%) 0.384/C0
Cover of rocks (%) 0.134/C0
Cover of rockrose (%)/C0
Mean distance to four nearest rocks (m) 3.4±2.0

Results of t-tests and associated significance levels are also shown; asterisks indicate differences that remain significant after applying the sequential Bonferroni correction.

Table 2 Principal component (PC) analysis with the microhabitat data described in Table 1

<table>
<thead>
<tr>
<th>PC-1</th>
<th>PC-2</th>
<th>PC-3</th>
<th>PC-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance to nearest rock (m)</td>
<td>-0.890</td>
<td>0.151</td>
<td>-0.167</td>
</tr>
<tr>
<td>Mean distance to four nearest rocks (m)</td>
<td>-0.855</td>
<td>-0.095</td>
<td>-0.210</td>
</tr>
<tr>
<td>Rockrose cover (%)</td>
<td>0.076</td>
<td>-0.384</td>
<td>0.720</td>
</tr>
<tr>
<td>Leaf litter cover (%)</td>
<td>-0.497</td>
<td>-0.070</td>
<td>0.571</td>
</tr>
<tr>
<td>Cover of herbs (%)</td>
<td>-0.197</td>
<td>0.151</td>
<td>-0.107</td>
</tr>
<tr>
<td>Cover of rocks (%)</td>
<td>0.760</td>
<td>-0.059</td>
<td>-0.180</td>
</tr>
<tr>
<td>Cover of rockrose saplings (%)</td>
<td>-0.134</td>
<td>0.712</td>
<td>0.015</td>
</tr>
<tr>
<td>Plant cover 5 cm in height (%)</td>
<td>0.210</td>
<td>-0.567</td>
<td>0.220</td>
</tr>
<tr>
<td>Plant cover 25 cm in height (%)</td>
<td>-0.151</td>
<td>0.654</td>
<td>-0.312</td>
</tr>
<tr>
<td>Plant cover 50 cm in height (%)</td>
<td>0.256</td>
<td>0.793</td>
<td>-0.039</td>
</tr>
<tr>
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<td>0.082</td>
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</tr>
</tbody>
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Loadings with absolute values greater than 0.5 are shown in bold.

Mean scores (± 95% confidence interval) of lizard locations and randomly selected sites on principal component (PC)-1 (a gradient of proximity to rock cover) and PC-3 (a gradient of development of the shrub layer, mainly of rockroses Cistus laurifolius, 75–100 cm above the ground). Factor loadings are given in Table 2.

**Discussion**

Our data produced two important results. First, the estimates of lizard abundance were strongly dependent on the
Capturing and radiotagging lizards were expensive and time-consuming (c. 80 person-hours walking transects, and more effort would not have increased the effectiveness of our censuses). This method may be useful for obtaining indexes that allow to compare the population densities of different species in open habitats (Germaine & Wakeling, 2001; Garcia & Whalen, 2003) or to estimate the abundance of common species across different habitats (Díaz & Carrascal, 1991) or microhabitats (Martin & Salvador, 1997), but it is of limited utility for censusing scarce, elusive animals such as eyed lizards. Thus, abundance estimates based on line transects were much lower than those based on the Schnabel method. Transects carried out during occasional visits, such as the ones that could take place in the context of a larger scale survey, could even suggest that lizards were absent from the study area (no lizards were detected within the 10-m wide census band in 3 of the 8 census days).

Capturing and radiotagging lizards were expensive and time-consuming (c. 80 person-hours distributed over 20 days, with a cost of 150€ per radiotransmitter) because of the complexity of the habitat and the wariness of the animals. However, mark-recapture statistical methods were necessary to obtain a reliable estimate of population size. This can be attributed to an extremely low detectability, shown by the fact that only radiotagged lizards could be located with some ease, and by the decrease in the number of sightings between the 10 and 25-m wide census bands. Radiotracking may be replaced by other marking procedures, but at the cost of losing information on retreat sites and of not assessing the fulfillment of the assumption that the population remains constant throughout the study period. We therefore encourage the use of the Schnabel method, combined with radiotelemetry, as a reliable procedure to measure population size. We also emphasize several precautions. First, data should be collected until standard errors are approximately coincident with the number of lizards seen but not captured. Second, population counts should be carried out on areas large enough (e.g. ≥5 ha) to counteract the large size of lizard home ranges (Salvador et al., 2004) and the patchy distribution of lizard locations (with sectors where no observations were made). Finally, repeated censuses aimed to estimate population trends should not be undertaken at very short intervals, because lizards are very difficult to noose the second time, and 3–4 years are approximately the time needed to have most of the population replaced (only a 7-year-old male captured in 2004 was a 2000/2001 survivor).

The population density obtained in this study was lower than those reported for Mediterranean dehesas with a well-developed undergrowth of shrubs (Martin & López, 2002; Mateo, 2002). This may be because of the lack of tree management and scarce livestock grazing supported by Pyrenean oaks at our study site, whereas habitat suitability for L. lepida is expected to be highest at intermediate stages of forest degradation (Santos & Telleria, 1989; Lorente et al., 1995). In agreement with this hypothesis, radiotracked individuals selected sites with a high cover of rockrose shrubs, which are dominant in south-facing forest clearings. Thus, although agricultural intensification is deleterious for L. lepida populations (Corbett, 1989; Cheylan & Grillet, 2005), the increase in forest cover associated with the abandonment of traditional agro-silvo-pastoral techniques may also have negative effects (Cheylan & Grillet, 2005). Similar direct relationships between anthropogenic forest clearance and population density or habitat use have been reported for other large-bodied heliothermal lizards (Vitt et al., 1998; Sartorius, Vitt & Colli, 1999).

Microhabitat and retreat-site selection

Our data suggest that the availability of refuges was the most important single factor determining the quality of a habitat for L. lepida. Thus, rocks were selected as refuges by a vast majority (>95%) of hidden lizards, and lizards remained on average 8.5 times closer to rocks than expected at random. The importance of retreat-site selection for ectotherms has been emphasized by several studies (Christian, Tracy & Porter, 1984; Huey et al., 1989; Huey, 1991; Schlesinger & Shine, 1994; Webb & Shine, 2000; Sabo, 2003) that have pointed out that most ectotherms actually spend longer periods in retreats than above ground (Huey, 1982).

Table 3 ANOVAs with interindividual differences in microhabitat use, as measured by the scores of radiotracked lizards on the principal components shown in Table 2

<table>
<thead>
<tr>
<th>PC</th>
<th>SS effect (among individuals)</th>
<th>SS error (within individuals)</th>
<th>F_{16, 46}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-1</td>
<td>7.66</td>
<td>12.58</td>
<td>1.75</td>
<td>0.070</td>
</tr>
<tr>
<td>PC-2</td>
<td>29.69</td>
<td>42.06</td>
<td>2.03</td>
<td>0.031</td>
</tr>
<tr>
<td>PC-3</td>
<td>30.70</td>
<td>32.92</td>
<td>2.68</td>
<td>0.005</td>
</tr>
<tr>
<td>PC-4</td>
<td>29.18</td>
<td>35.05</td>
<td>2.39</td>
<td>0.011</td>
</tr>
</tbody>
</table>

PC, principal component.
Thus, refuge selection can have a major impact on the thermal physiology and ecology of ectotherms (Huey et al., 1989; Huey, 1991; Goldsborough, Hochuli & Shine, 2003).

Nevertheless, we suspect that refuge selection by *L. lepida* was not primarily related to the thermal properties of retreats. Huey *et al.* (1989), in a detailed study of the thermal consequences of retreat site selection by garter snakes (*Thamnophis sirtalis*), carried out during midsummer at a latitude and altitude similar to the ones reported here, found that rock height, rather than shape or mass, was the primary determinant of the daily thermal cycles under rocks. Moreover, thick boulders (>43 cm) offered temperatures that never reached the lower limit of the snakes’ preferred range (28°C; Scott, Tracy & Pettus, 1982), and were used much less frequently than warmer rocks of intermediate thickness (20–40 cm). This is in contrast with our results, because 88% of the rocks used as retreat sites were ≥45 cm thick. Not unexpectedly, animals captured soon after emergence from their refuges were fairly cool (personal observation), despite the high body temperatures (30–35°C) exhibited by field-active lizards (Busack & Visnaw, 1989). We therefore hypothesize that the selection of large rocks (length, width and thickness were highly correlated, all *P* < 0.001) was primarily determined by the need to find shelter from predators. This would be consistent with the high predation pressure to which *L. lepida* is exposed (Martín & López, 1996; see Salvador et al., 2004 for data on predation at the study site), and that has been hypothesized as one of the causes of its suspected decline. The importance of refuges has also been noted at the Crau steppe in southern France, a hard soil plain where eyed lizards were restricted to specific areas with stone piles (built during the Second World War to impede the landing of Allied aircraft), which were the only retreat sites available for adult lizards (Mateo, 2004). The preference of lizards for rocks with several crevices should also be useful for eluding predators, because it could facilitate both entry and exit from retreat sites. Larger rocks provide deeper crevices, which may offer a more stable microenvironment (Huey *et al.*, 1989; Kearney, 2002; Beck & Jennings, 2003). Also, the positive selection of rockrose patches with a high plant cover 75–100 cm above the ground suggests that these patches may be used as refuge-connecting corridors that provide shelter to foraging and thermo-regulating lizards.

**Concluding remarks**

Radiotelemetry was an invaluable tool to assess the population density and spatial ecology of *L. lepida*. Radiotransmitters allowed us to confirm the assumptions of most mark-recapture methods, to achieve reliable estimates of population size, and to obtain data on retreat-site selection (Huey *et al.*, 1989; Beck & Jennings, 2003; Whitaker & Shine, 2003). Moreover, radiotelemetry was also essential for an adequate characterization of microhabitat preferences, because the probability of sighting marked lizards before they could seek shelter in a nearby refuge was largely increased by our ability to radiolocate them. An additional advantage of radiotracking is that it facilitates assessment of interindividual differences in microhabitat or retreat-site selection, which may be useful for understanding the patterns of space use (Whitaker & Shine, 2003; Salvador *et al.*, 2004). In our study, for instance, interindividual differences in microhabitat use, which were significant for most variables, were seemingly related to differences in habitat structure within the study area. However, there were no significant interindividual differences in PC-1, the habitat axis on which selection was the strongest. This suggests that all individuals, independent of other characteristics of their home ranges, remained as close as possible to rocks, which reinforces the role of retreat-sites as a limiting factor for eyed lizards (Mateo, 2004).

We can therefore conclude that adequate management of retreat sites is crucial for the conservation of *L. lepida*, and that the removal of stone and boulder refuges in fields associated with agricultural intensification should be avoided (Corbett, 1989). We suggest adding artificial shelter sites to restore degraded habitats, a procedure that has been successful for several species of endangered reptiles (Heenan & McCloskey, 1998; Webb & Shine, 2000; Mateo, 2004; Souther, Bull & Hutchinson, 2004). We also encourage the use of radiotelemetry to monitor the demographic trends of the largest European lacertid, because its wariness, rapidity, and close association with retreat sites make its visual detectability unexpectedly low. Although we found no clear evidence of decline at our relatively well-conserved study area, it is important to confirm the extent to which human pressure has led to a substantial decrease of its populations over most of its distribution range (Allen, 1977; Mateo, 2002).

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