Amphibia-Reptilia 32 (2011): 159-166

Implementation of a long-term monitoring program of the ocellated lizard (*Timon lepidus*) population on Oleron Island

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Abstract. The ocellated lizard *Timon lepidus* (Sauria; Lacertidae) has declined throughout most of its range. Habitat fragmentation and habitat loss seem to be mainly responsible for the species' decline. The ocellated lizard population of Oleron Island, confined to a longshore dune of 140 ha, is the subject of a long-term monitoring study established in 2007. The monitoring method consists of 70 plots (50 × 50 m) randomly placed within a study area divided into six distinct zones. Three surveys were conducted in the study area over the spring season of 2007. During each survey, we counted the individuals in each plot. These counts were analyzed with the PRESENCE 2.0 and the R package Unmarked software using two different modeling approaches, the 'site-occupancy model' and the 'N-mixture model'. Estimates resulting from our analyses indicated the proportion of occupied plots to be 0.76. Our results indicated that the ocellated lizard has a highly heterogeneous distribution on Oleron Island, with parts of the dune sheltering clusters of lizards, and other areas totally unoccupied. The population size was estimated to be 516 individuals (95% CI 248-783). The relative abundance of ocellated lizards on the island can be principally explained by the presence of permanent shelters (used both during winter and the lizards' active period), including rabbit and rodent burrows and artificial shelters. This monitoring survey will be replicated every three years to enable us to calculate the species' colonization and local extinction probabilities. These results will help in evaluating and guiding management and conservation measures.

Keywords: conservation, long-term monitoring, permanent shelters, *Timon lepidus*.

Introduction

Reptiles are among the most threatened terrestrial vertebrates (Gibbon et al., 2000; Araujo, Thuiller and Pearson, 2006). Today it is difficult to assess the extent of their decline and its causes. Unfortunately, no large-scale monitoring programs are in place for these species, as is the case for other zoological groups such as birds (e.g., Frances, McCulloch and Wiedenfeld, 1996; Peterjohn, Sauer and Link, 1996; Julliard, Jiguet and Couvet, 2004; Escandell, 2005), mammals (e.g., Toms et al., 1999) and butterflies (Stefanescu et al., 2008; van Swaay et al., 2008; UKBMS, 2009). In Europe, the conservation policies regarding reptiles, such as the Natura 2000 Network, rely largely on recommendations from experts (e.g., Corbett, 1989) and are thus affected by empiricism and subjectivity. The European Union has made a commitment to stop the erosion of biodiversity by 2010 in order to meet the demands of the Rio agreement. In this context, the implementation of long-term monitoring programs appears increasingly essential, not only to guide biodiversity conservation policies, but also to provide a better understanding of the mechanisms involved in biodiversity loss.

The methodological difficulties of obtaining reliable estimates of the population size of reptile species are significant, particularly for species with a small population or for which detection is difficult (Thompson, 2004; MacKenzie et al., 2006). Capture-Mark-Recapture methods (CMR) can only be used in relatively small areas with species that are easy to capture. Likewise, the use of distance-sampling methods is not suitable for species that flee in the presence of an observer, as is the case with lizards (Buckland et al., 2004). Counting individuals using

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© Koninklijke Brill NV, Leiden, 2011. DOI:10.1163/017353710X551381

the quadrat method is adapted to species with low mobility, but requires either that all individuals are exhaustively detected, or that their detectability can be estimated (Manley et al., 2004; MacKenzie et al., 2006).

The ocellated lizard is a characteristic species of open Mediterranean environments in the Iberian Peninsula and southern France, habitats which are today in marked decline (Barbero, Quezel and Loisel, 1990; Debussche, Lepart and Dervieux, 1999). Its biology is now well known (e.g., Bischoff, Cheylan and Böhme, 1984; Mateo and Cheylan, 1997; Cheylan and Grillet, 2004; Mateo, 2004) and its decline is confirmed over a large area of its distribution range (Allen, 1977; Corbett, 1989; Mateo, 2002; Grillet, Cheylan and Dusoulier, 2006). Taking into account the threats to this species, it has recently been classified by the IUCN (International Union for Conservation of Nature) in the category NT (Near Threatened) in the lists of Mediterranean and European reptiles, and is on the Red List for Spain and for France in the category VU (Vulnerable). For these reasons, national action plans are being written for this species.

Many ocellated lizard populations have disappeared in France over the last 100 years (Cheylan and Grillet, 2005). Habitat modification seems to be one of the main causes of the observed decline (Cheylan and Grillet, 2004; Grillet, Cheylan and Dusoulier, 2006). The decline is most rapid at the limits of the species' distribution range (Thirion, Grillet and Geniez, 2002; Cheylan and Grillet, 2004, 2005; Grillet, Cheylan and Dusoulier, 2006). In these areas, the populations have been split up and cover a small area, so are highly affected by habitat loss and habitat modification. The population that is most marginalized geographically is that of Oleron Island. This population has contracted over time, a process that has been partially elucidated in other studies (Cheylan and Grillet, 2005). Since 1998, the population has been the subject of thorough investigations that have led to the establishment of a conservation program to protect it. In order to assess the effectiveness of the conservation measures in place on the island, we decided to implement a long-term monitoring study of this population. The ocellated lizard is a good candidate for testing a long-term study method based on repeated observation sessions of different plots. The protocol we have selected involves establishing plots that will be surveyed three times during one spring season. The first survey was carried out in 2007 on the island's entire ocellated lizard population.

This article intends first, to describe the spatial distribution of the island's population; second, to estimate the size of this population; and third, to assess the relevance of the monitoring protocol we chose by analyzing the results obtained at the end of the first year of the study.

Materials and methods

The study area

Oleron Island is located less than a kilometer off the French Atlantic coast. It is the largest French island off the Atlantic coast (175 km^2). It benefits from aspects of a Mediterranean climate, with a relatively high number of hours of sunshine per year (Kessler and Chambraud, 1990). It is thought to have become an island quite recently (between 2000 and 3000 BP) (Brochard, 1998).

The ocellated lizard population inhabits an 8 km-long bar of sand dunes with a width ranging from 30 to 450 m (fig. 1) in the south-western area of the island (NW limit: N45°52′55.0/W001°16′13.0; SE limit: N45°49′44.0/W001°14′26.0). The population is distributed in a very linear fashion and has a small potential expansion range, limited on the west by the sea and on the east by dense forests not suitable for the species. The habitat it occupies is a fixed dune characterized by a well-developed moss layer, a relatively short grassy layer (similar to a lawn) and by the sparse presence of a few trees shaped by the wind (mainly maritime pines). The most common plants are blue hairgrass (*Koeleria glauca*), tuberous hawk's-beard (*Crepis bulbosa*) and ephedra (*Ephedra distachya*); the most abundant species is eternal flower (*Helichrysum stoechas*).

Methodology

Today, recent monitoring methods based on repeated observation sessions for which detection probability is modeled allow detection-probability estimates of a species on a sampling of sites (MacKenzie et al., 2006), as well as estimates of the species' abundance (Royle and Nichols, 2003; Royle, 2004). These methods often require less effort than others and seem well adapted to our investigation. They are becoming increasingly popular, even if currently they are rarely used for reptiles (see, however, Kery et al., 2009).

Sampling strategy. The studies of Grillet and coll. carried out between 1998 and 2002 (Grillet, 2008) on Oleron Island's ocellated lizards highlighted four spatially distinct population cores (fig. 1), linked by low population-density areas. In November 1999, Hurricane Martin opened clearings at the edge of the forest, offering new favorable habitats that the species could colonize. Taking into account these factors, we adopted a stratified sampling strategy that included six distinct sectors: the four population cores, an area of clearings opened up by the hurricane, and the remainder of the study area. These sectors did not have the same habitats available for the ocellated lizard; that is, the types of shelter (e.g., rabbit burrows) were not homogenous across all zones. A non-stratified sampling strategy would have included numerous unoccupied sites, which would have been inefficient in estimating the population size. Nor would it have been appropriate to survey only the occupied sites, since one of the objectives of the study is to measure the future evolution of the size of the population and the areas it occupies (extinctions/colonizations). Once the six sectors were chosen, the sampling within them was random. In all, 70 plots of 50 m × 50 m were established within the 140 hectares of the study area: 10 in each of the four population cores, 10 in the clearings (the surface areas of the population cores and the clearings were similar), and 20 in the remaining study area. The choice of the number of plots was a compromise between ensuring enough to be representative of the population and to allow reliable statistics and making sure the study was workable in terms of time, budget and human resources. The plots were marked on the ground by geo-referenced wooden stakes.

Survey protocol. In the spring of 2007, three one-hour surveys (in April, May and June) were conducted on each of the 70 plots, representing a total of 210 prospecting hours covering 17.5 hectares (12.5% of the total studied area). The total time required to monitor all the plots was two weeks per survey (with one principal person conducting the survey, occasionally with others, and taking into account favorable meteorological conditions), or six weeks in total for the three surveys. The first 20 minutes of each survey were dedicated to attempting to directly sight ocellated lizards with binoculars. The investigator followed a random trajectory within the 2500 m^2 of the plot, noting the total number of different individuals observed. The remaining 40 minutes were spent searching for clues of the lizards' presence (fecal droppings and tracks). The search involved inspecting five parallel transects of 10 m × 50 m (both up and down the transect) for 8 minutes per transect. Any signs of the species' presence were quantified. Each survey was conducted under a priori favorable conditions for observing the ocellated lizard: adequate temperature, limited cloud cover and no or light wind. For each plot survey, the temperature, wind speed and cloud cover were noted in order to verify that they did not have an influence on the results.

Habitat variables. Several physical and biotic variables were noted for each plot in order to determine their potential influence on the presence or abundance of the ocellated lizard in that location:

- The plot's position on the sea-forest gradient: shifting dune, grey dune, pre-forest fringe, or clearing.
- The type of plant associations: *Euphorbio-Ammophileta*, *Helichryso-Crucianelletalia*, *Koelerio-Corynephoreta*, *Artemisio lloydii-Ephedretum distachyae*, *Juncetea bufonii*, *Pino pinastri-Quercetum ilicis*.
- The percentage of groundcover vegetation: bare sand, moss, grasses, shrubs.
- The type and abundance of available shelters: rabbit burrows, rodent burrows, artificial shelters, stones, logs and branches, brambles, wind-shaped pines, yuccas, sand willows, iron sheets.

Statistical analyses. We used the method developed by Royle (Royle, 2004), the 'N-Mixture model', which models the species abundance per plot from the data provided by counts. This method assumes a Poisson distribution for the spatial variation in species abundance among plots. It is based on the hypothesis that given a certain value of local abundance N_i, the counts are assumed to be Binomial random variables with sample size N_i and success rate p_{ij}, where i is the number of the site and j the number of the field session. This method assumes site closure, i.e., that no individuals leave or enter the plot during the studied period. The studied species is territorial, so we can assume this is the case during the breeding season. Moreover, if any temporary emigration takes place during the field season, this will not be of consequence since it will be included in the detection probability, as is the case in capture-recapture methods.

The potential effects of habitat variables on abundance were explored by comparing models with and without these variables. The model was selected using the Akaike criteria (AIC) (ibidem), which is calculated by AIC = Deviance + 2*np (with np being the number of parameters). This criteria represents a compromise between a good fit of the model to the data and a limited number of parameters (parsimony). Two models are considered as different when their AIC values differ by 2 (Burnham and Anderson, 2002). The N-mixture models were analyzed using the PRESENCE 2.0 software program (Hines, 2006).

As no goodness-of-fit test is currently available for the N-mixture model in PRESENCE, we used an unmarked package (Fiske and Chandler, 2010) (available at http://CRAN.R-project.org/package=unmarked) for R 210 (R Development Core Team, 2009). The N-mixture model currently implemented in PRESENCE does not allow the modeling of potential detection variations over time. Thus, as a first step, we explored this type of variation based only on presence-absence data using the site-occupancy model (MacKenzie et al., 2002). This was used to model the species' detection probability, to estimate the proportion of occupied sites and to model the potential influence of the meteorological conditions (table 1). Other software can also be used, such as MARK, R and WinBUGS in a Bayesian mode of inference (Kery, 2008, 2010).

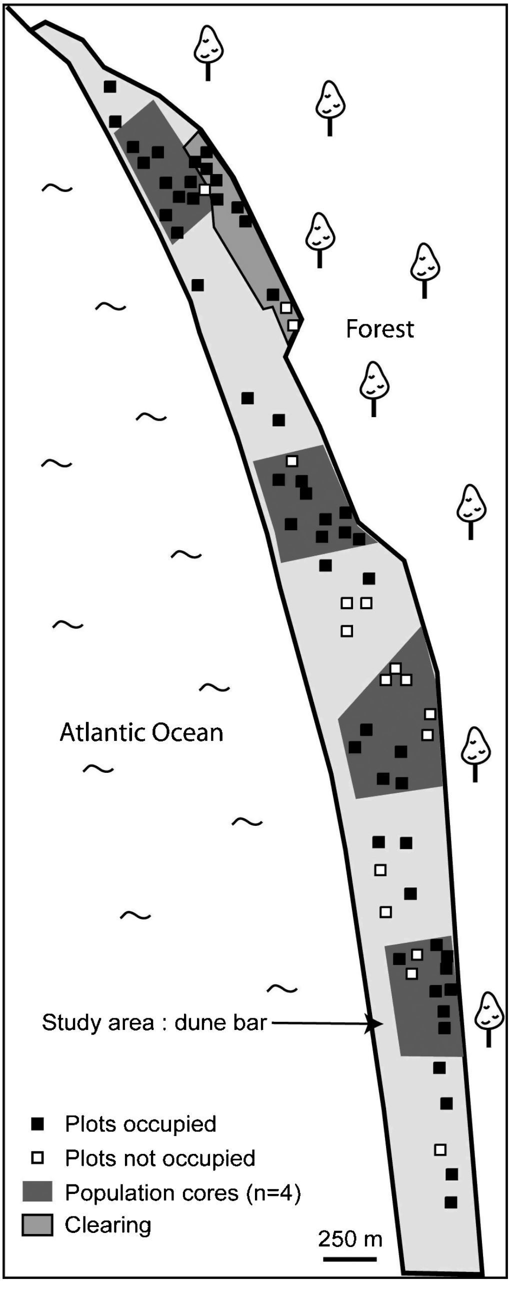

Figure 1. Location of plots occupied (black squares) and unoccupied (white squares) by the ocellated lizard.

To obtain an estimate of the total population size in the zone, we used the N-mixture model that included the number of rabbit burrows and the number of rodent burrows as explanatory covariates (see results). We were mainly interested in obtaining an estimate of population size; we did not perform a full analysis of all these habitat covariates, but only tested the one we suspected to be the most important (i.e., shelters). For this modeling step, we used data only from the last two field sessions, since detection ability increases over the course of a survey. Taking into account the first session would have led to an increase of the confidence interval by introducing heterogeneity in the detection rate. The confidence intervals were calculated in the following way: PRESENCE software was used to give an estimate of individuals per plot with a minimum and a maximum for every plot. We extrapolated estimate values for each sector's surface area, and then summed the estimates of all the sectors to calculate the total population size with a confidence interval.

Naïve occupation was calculated by $\Psi_{\text{naïve}} = n_o/N$ (with n_o the number of plots where the species was detected at least once, and N the total number of studied plots).

Results

Detection probabilities of the species

The minimal and maximal counts of individuals per plot were 0 and 1 for the first survey, 0 and 3 for the second, and 0 and 5 for the last. Mean counts of individuals per plot were respectively 0.3, 0.67 and 0.63 for each survey. The site-occupancy model allows us to conclude that the probability of detecting the species in the plots increases over the field season (0.60 in April, 0.88 in May and 0.92 in June). The deltaAIC value between a model without variation over time and with variation over time is 15.46, which is much larger than 2, the value considered to indicate a significant difference (Burnham and Anderson, 2002). Meteorological conditions have no influence on the detection probability (table 1). Compared to the reference model, the deltaAIC values of the tested models are superior to −2.

Distribution of the ocellated lizard

In the survey, 53 out of the 70 plots were observed to be occupied by the ocellated lizard.

Table 1. Modeling of the effects of meteorological variables on the detection probability using a site-occupancy model.

Covariates	AIC	deltaAIC	np
Without covariates	2006.24		2
Cloud coverage	2005.96	−0.28	7
Temperature	2013.40	+7.16	7
Wind speed	2013.57	+7.33	7

The estimated proportion of occupied sites using the single-season model is 0.76 $\pm$ SE $=$ 0.05. Naïve occupation is very close to this, at 0.757. As the map shows (fig. 1), the ocellated lizard has a highly heterogeneous distribution, with clusters living in some parts of the dune, while other parts of the dune are totally unoccupied. At the centre of the study area, no lizards were observed. The fact that, if the species is present, the detection probability after three visits is very close to 1 ($P = 0.996 \pm \mathrm{SE} = 0.002$) reinforces our conclusion that the distribution pattern we observed reflected the lizard's actual distribution. The observations made in the clearings created by the hurricane in 1999 demonstrate that these areas have now been colonized.

Importance of shelters

The N-mixture model allows us to conclude that the presence of shelters appears to be one of the main influences on the lizard's abundance (table 2). This is particularly the case for permanent shelters such as rabbit burrows, rodent burrows and artificial shelters. Temporary shelters (stones, logs and branches, brambles, wind-shaped pines, yuccas, sand willows, iron sheets) have little if any influence. Other habitat variables, such as vegetation strata, do not significantly affect the lizard's abundance.

Estimation of the population size

The parametric goodness-of-fit test performed using the unmarked package on the best model obtained (N-mixture model using permanent and artificial shelters covariates) is not significant ($p = 0.76$), which suggests that the model fits the data well. Estimated densities range between 2.96 and 7.64 individuals per hectare with a mean value of 3.69. The total ocellated lizard population size calculated from this model is estimated at 516 individuals, with a confidence interval ranging from 248 to 783 (95% CI).

Discussion

The results from the first year of our study indicate that the detection probability of the ocellated lizard increases over the spring. This is probably due to the increase in the species' activity at the beginning of its active period in March until the end of spring (Castilla, 1989; Cheylan and Grillet, 2004). This finding suggests that field work should be focused on the period from the end of April to the beginning of June in order to maximize data collection. Although the detection probability of the species is high, it is not equal to 1, confirming the need for a protocol that allows detection to be estimated. Such a protocol needs to provide reliable and precise estimates of presence/absence or abundance in the plots surveyed.

Our study found that the ocellated lizard is heterogeneously distributed over the site, with clusters living in some areas, and other areas unoccupied. The survey method used enables us to confirm that the species is not present in the unoccupied areas since the detection probability over three visits was extremely close to unity. The low standard deviations associated with the

Table 2. Modeling of the lizards' abundance using the N-mixture model.

Covariates	AIC	Dev.	deltaAIC	np	Sign.
Vegetation strata	416.76	404.76	+28.48	6	
Temporary shelters	389.45	375.45	+1.17	7	
Without covariates	388.28	384.28	–	2	
Rabbit burrows	384.92	378.92	−3.36	3	*
Artificial shelters	382.25	376.25	−6.03	3	*
Rodent burrows	379.98	373.98	−8.30	3	*
Rabbit burrows + Rodent burrows	376.06	368.06	−12.22	4	*
Rabbit burrows + Rodent burrows + Artificial shelters	369.38	359.38	−18.9	5	*

estimates suggest that the follow-up stages of the study using this protocol should enable us to monitor the evolution of the occupancy rate of the site by the species and the population's spatial variations when surveys are carried out in future years (monitoring is planned every three years – the second session was carried out during the spring of 2010). In future sessions, we will also estimate local extinction and colonization. The variations could be correlated to any future modifications in habitat characteristics to explore the mechanisms of extinction/colonization (MacKenzie et al., 2006).

In our examination of the influence of shelter on the presence of the species, we found that the number of permanent shelters has a significant impact on the abundance of the ocellated lizard. Sectors with no lizard observations were in fact sectors with the least shelters available as a resource. Between 2001 and 2006, the rabbit population declined on the island, and the number of rabbit burrows decreased dramatically (by 80%) (Grillet et al., 2010). Other habitat variables of the surveyed plots (such as the plot's position on the sea-forest gradient and its vegetation cover) were also noted. A thorough study of all these variables will enable a better understanding of the ecological requirements of this population of ocellated lizards and help to define future conservation actions to protect it. For example, we set up artificial refuges for the lizards to make up for the decline in rabbits. This was very efficient in maintaining the number of individuals (ibidem). The artificial shelters were based on the dimensions and general characteristics of natural rabbit burrows. An open-bottomed wooden box (L = 50 cm, W = 25 cm, H = 25 cm) was placed at a depth of 40 to 50 cm. The box was connected laterally to the surface via two PVC ringed pipes (diameter = 6-8 cm, length = 150 cm, slope = 30°). The whole installation was covered with sand, leaving two openings at the surface. These artificial refuges were effective in compensating for the decline in rabbit burrows in the short term.

We estimate that the size of the Oleron Island population is 516 individuals (with a confidence interval ranging from 248 to 783). Estimations from 2002, obtained from simple observation of the entire site, resulted in values between 500 and 1200 individuals (Cheylan and Grillet, 2004). The two estimates are therefore consistent, despite the use of different survey methods. The survey protocol implemented in the most recent study provides more precise population estimates than the previously used methods, which is an argument for its use in the future. However, because the confidence interval is relatively large, this estimation method is not the best choice for monitoring the evolution of the size of the population over the long term. Other methods would be more adequate for this. In future surveys, we will repeat the same protocol to calculate the rate of colonization and local extinction for each occupied plot.

The estimated density (3.69 individuals per hectare) is similar to the global density estimated for Portugal's Berlenga Island population, which is 3.3 ind./ha (Paulo, 1988). However, it is lower than the population density estimated in France's Crau area in 1992 and 1993 by Mateo and Cheylan (unpublished), which was 6.2 ind./ha on a site of 40 ha. The estimated density of ocellated lizards on Oleron Island in 2007 seems therefore relatively low for the species. As the availability of rabbit burrows appears to be a condition for ocellated lizard abundance, the latter's population growth depends on an increase of the rabbit population. The continuation of the long-term monitoring study will allow us to evaluate how the population density evolves over time and to better understand the main mechanisms that regulate the population size.

To conclude our evaluation of this new monitoring method, we found that it did not pose any technical difficulties during the survey. The estimates we obtained and their precision indicate that the protocol is appropriate for studying the species' spatial distribution as well as its future evolution. However, in terms of estimating the abundance of the species, this method has some limitations. It provides a population-size estimate that is close to those suggested in previous studies, but the relatively large confident interval limits the ability to detect a significant population change.

Acknowledgements. This study was funded by the Office National des Forêts and by the Agence Nationale de la Recherche ANR-05-BDIV-014 « Activités humaines, dynamique et gestion de la biodiversité en milieu méditerranéen ». We also thank Elise Bradbury for the translation of this manuscript.

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Received: March 10, 2010. Revised: December 10, 2010.