EVALUATION OF METHODS FOR ESTIMATING SPECIES RICHNESS AND **ABUNDANCE OF REPTILES IN OLIVE GROVES**

ANTONIO J. CARPIO^{1,2,3}, M. CABRERA¹ AND FRANCISCO S. TORTOSA¹

¹Department of Zoology, University of Córdoba, Campus de Rabanales, 14071 Córdoba, Spain ²Instituto de Agricultura Sostenible (IAS, CSIC), Alameda del Obsinpo s/n, 14080 Córdoba, Spain ³Corresponding author, e-mail: b42carca@uco.es

Abstract.—The Mediterranean has a high diversity of herpetofauna, which continues to be understudied. Olive (Olea europaea) groves are one of the primary agroecosystems in the Mediterranean region but the effectiveness of different survey methods has yet to be tested. Therefore, we compared the effectiveness of transects and drift fences to sample terrestrial reptiles in old vs. young olive groves. We observed 857 individuals, representing 10 species (between May and July 2014). We detected 10 species (820 individuals) with transects and five species (37 individuals) using drift fences. The transect was more efficient for determining species diversity and abundance of reptiles in both young and old olive groves, but the recorded values were higher in old olives than in the young ones for both response variables. Finally, we recorded habitat features (trunks or ground) during transects where the animal was observed. We recommend the use of transect for biodiversity assessment in olive groves, where reptiles spend most of their time on trunks and avoid the ground.

Key Words.—drift fences; herpetofauna; Mediterranean; reptile surveys; species abundance; species diversity; transects

INTRODUCTION

Many reptile and amphibian populations are declining as a result of climate change, habitat loss, invasive species, disease, or agriculture intensification (Stuart et al. 2004; Foley et al. 2005). These taxa are little studied in the Mediterranean, and the status of many species is unknown (Martín and Lopez 2002, Stoate et al. 2009) including many endemic species in the Iberian Peninsula (Corbett 1989). The Iberian Peninsula has been identified as a biodiversity hotpot (Myers et al. 2000) especially for herpetofauna (Loureiro et al. 2008; García-Muñoz et al. 2010). However, due to the agricultural practices of the region, biodiversity loss is a conservation concern (Reidsma et al. 2006).

Olive (Olea europaea) groves are one example of agricultural intensification, and are one of the primary agroecosystems in the Mediterranean (Sokos et al. 2013). These groves are an impor- petofaunal species richness use only two or three

tant economical resource (Oteros et al. 2014). However, very few studies have been conducted to evaluate the impact of olive groves on reptile and amphibian biodiversity. Indeed, Fryday and Thompson (2012) identified 155 published manuscripts that associate herpetofauna from European countries with agricultural habitats, but none focused on olive groves (but see Atauri and Lucio 2001; García Muñoz et al. 2010, 2013). Although dehesas (i.e., oak woodland pastures) and pasture lands have been extensively sampled (Martín and Lopez 2002; Godinho et al. 2011; Rotem et al. 2013), very little information is available regarding diversity and abundance of reptiles in olive groves or how to sample them.

Ecological studies, including monitoring and biodiversity inventories, need survey methods that permit the most efficient and comprehensive completion of study objectives (Hutchens and DePerno 2009). However, most studies of her-



FIGURE 1. Potential ecological niche of Olive (Olea europaea) trees in the Mediterranean basin (green areas) and the distribution of olive groves in Andalusia showing the study area. (Map taken from Ministry of Agriculture, Fisheries and Rural Development of the Junta de Andalucía 2010).

sampling methodologies, which limit the relia- in other regions (e.g., Africa; Rodel and Ernst bility of estimates (Bailey et al. 2004; Hutchens and DePerno 2009). In the case of reptiles, the most widely employed techniques include drift fence arrays (with pitfall and/or funnel traps), transects, and coverboards (Willson and Gibbons 2009). The effectiveness of these methods for determining abundance and species richness varies among studies (Hutchens and DePerno 2009; Sung et al. 2011). Capture rates in coverboards and drift fences have been demonstrated to be quite high (Ribeiro-Junior et al. 2008), and these methods are common in North American studies (Hampton 2007; Hutchens and DePerno 2009), while transect are commonly used by researchers

2004).

Studies evaluating herpetofaunal sampling methods are common in North America (Hampton 2007; Hutchens and DePerno 2009), South America (Ribeiro-Junior et al. 2008), Africa (Rodel and Ernst 2004), Australia (Spence-Bailey et al. 2010), and Southeast Asia (Sung et al. 2011). However, only a few studies compare their effectiveness in Mediterranean regions. Our objectives were to compare the effectiveness of two commonly used reptile survey methods (drift fences and transect) by evaluating capture rates and observed species richness in old and young olives groves to provide managers with guidance

when choosing survey methods for future studies in woody crops.

MATERIALS AND METHODS

We conducted the study within Andalusia $(37^{\circ}30'-37^{\circ}58'N, 4^{\circ}17'-4^{\circ}56'W;$ between 159–369 m above mean sea level) located in the South of the Iberian Peninsula (Fig. 1). We selected 14 study sites in a representative range of olive groves (irrigated, unirrigated, with cover vegetation vs. bare ground, old and young olive trees). All sites were 20 km apart to ensure independence of the samples. Each site included a plantation of olive groves, which is the leading commercial tree crop in the Mediterranean area (Oteros 2014). Olive groves were 10–100 y old and we separated into young (10–20 y old) and old (90–100 y old) groves for study.

Reptiles sampling.—The Andalusian reptile fauna includes 26 autochthonous species (three chelonians, one amphisbaenian, 13 saurians, and nine ophidians), three of which have an extremely localized distribution with ranges < 2% of total survey area (Ministerio de Medio Ambiente. 2014. Spanish Vertebrate Atlas. Ministerio de Media Ambiente. Available from http://www.magrama.gob.es/es/ biodiversidad/temas/inventarios-nacionales/ inventario-especies-terrestres/

inventario-nacional-de-biodiversidad/

bdn-ieet-default.aspx. (Accessed 22 May 2014). We conducted all sampling methods from May to July 2014, a period during which reptiles are particularly active because it is their mating season (Martín and Lopez 2002; Godinho et al. 2011). We visited each plot twice (once in May and again in July)

We tested two herpetofaunal survey methods: drift fences and transect. All sampling was > 30 m inside from the edge of the olive grove to avoid edge effect (Sung et al. 2011). We used two transects at each study site for one hour (30 min for each transect) and we spaced these transects

 \geq 100 m apart (Hutchens and DePerno 2009). We counted the reptiles observed in each transect in a 10 m-wide belt, 5 m on each side of the survey line. We repeated each line transect census on three days with favorable climate conditions (warm sunny days) between 1100–1300 GMT, when reptiles were most active (Martín and Lopez 2002). We looked for reptiles at potential reptile microhabitats, including under rocks and leaf litter, woody debris, and on tree trunks (Sung et al. 2011). We noted whether the reptile was observed on the ground or on a tree trunk. We completed 168 transect (84 in each census).

Drift fences with pitfall traps and funnel traps of several designs are widely employed in reptile research (Spence-Bailey et al. 2010; Sung et al. 2011; Rotem et al. 2013). We established a drift fence array on each site (n = 14). Each array had seven pitfalls (8 L plastic buckets buried flush with the ground) spaced at 7-m intervals and three double-ended funnel traps, connected by a 50-m drift fence (similar to those of Spence-Bailey et al. 2010). We constructed drift fences by stapling 0.6 m tall transparent plastic sheeting to wooden stakes and burying the bottom (0.1-0.2 m) of the plastic sheeting in the ground to prevent reptiles from crossing underneath (see Sung et al. 2011). To prevent drowning of animals, we drilled 10 mm diameter holes in the bottom of each pitfall bucket for drainage. We constructed funnel traps using 0.3 \times 0.4 m aluminum widow screens rolled into cylinders and stapled, and we inserted two wire mesh funnels with 0.04 m diameter openings into both ends of each cylinder. We conducted trapping for four consecutive days in the spring and summer of 2014, resulting in 784 trap-nights. We checked the trap lines once per day and released individuals at the point of capture. We identified all captured animals to species.

Statistical analysis.—We evaluated the capture efficacy among capture techniques by comparing species richness (S) and the number of detections for data collected during May-July

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TABLE 1. Chi-square (χ^2) , P-values, and coefficients of the variables included in the best models to explain the number of reptiles (Model 1). The coefficients for the level of fixed factors were calculated using the reference values of 'Drift fence' in the 'Method' variable, 'Young tree' in the 'Olive age' variable and 'July' in the 'Date' variable.

Variables	χ^2	Р	P Coefficients ± SE	
Intercept			0.43 ± 0.56	
Date(May)	9.58	< 0.01	$May = -1.17 \pm 0.55$	
Olive age (old)	1.91	n.s.	Old tree = -0.36 ± 0.23	
Method	918.4	< 0.001	Survey Transect = 2.25 ± 0.23	
Date * Olive age	10.0	, 0.01	1.84 ± 0.75	
Date * Method	4.30	< 0.05	1.33 ± 0.56	
Olive age * Method	6.95	< 0.01	1.37 ± 0.45	
Olive age * Method * Date	4.05	< 0.05	-1.49 ± 0.76	

2014. We analyzed the capture rates of both methods measured as captures per trap-hour for drift fences and as capture per hour for transects. We created two Generalized Linear Mixed Models (GzLMM) to compare both methods. The response variables were the number of individuals captured (Model 1) and the number of species (Model 2). We included the method (two levels: drift fences vs. transects), the age of the grove (young vs. old), the date (May and July), and the double interactions between these variables (Date*Method; Date*Olive age; Method*Olive age) and the triple interaction (Date*Olive age*Method) as explanatory variables in these models. We considered site as a random variable. We used Poisson distribution with a log-link function for both models.

We performed the full arrangement of models (all possible combinations) and model selection by means of a best subset approach using the Akaike information criterion corrected for small sample size (AICc; Burnham and Anderson 2002). We ranked the models generated according to AICc values, in which the model with the lowest AICc is the best. Also, we reported the Δ AICc value to compare the difference between each candidate model and the best model. As a rule, a $\Delta i < 2$ suggests substantial evidence for the model (and thus for the variables included; Burnham and Anderson 2002), signifying that we eventually selected any model with $\Delta i < 2$ with regard to the model with the lowest AICc. We performed all statistical analyses using InfoStat software with $\alpha = 0.05$. Finally, we used a paired Wilcoxon test to assess the differences among the number of reptiles and number of species observed on the ground and on tree trunks (ground vs. tree) in each transect.

RESULTS

We observed 857 individuals (852 lizards, four snakes, one worm lizard) representing 10 species. We detected 10 species (820 individuals) with transects and five species (37 individuals) using drift fences for an estimated species richness (S) of 10 (Appendix 1). The most common species detected were *Podarcis hispanica* (50% of records), *Acanthodactylus erythrurus* (28%), *Tarentola mauritanica* (14%), *Psammodromus algirus* (5%), and *Podarcis vaucheri* (1%), while *Lacerta lepida*, *Bladus cinereus*, *Hemorrhois hippocrepis*, *Malpolon monsspesulanus*, or *Rhinechis scalaris* represented < 1% of the records.

The final model retained all factors because the triple interaction (Date*Olive age*Method) was significant (Table 1). In both months (May and July) the number of individuals sampled was higher when using transects than when using Carpio et al.—-Species richness and diversity in Olive groves.



FIGURE 2. Predicted mean values (\pm SE) of number of individuals sampled in May (a) and in July (b) 2014 according to the sampling method, partitioned by age of olive grove (young vs. old). Different capital letters indicate significant differences (P < 0.05) between methods.

drift fences (Fig. 2), although with the transects the number of individuals observed was higher in old olive groves (Fig. 2). The best candidate model that explained species richness included method in all models, while only retaining olive age, date, and the interaction between method and date in some of the best candidate models (Table 2). Higher values of species richness were detected using transects compared to the drift fences ($\chi^2 = 67.7$; P < 0.001), with a mean (± SE) of 1.8 ± 0.15 species for transect and $0.4 \pm$ 0.08 species for drift fences. We found more reptiles on the trunks than on the ground (Z = -2.69; P < 0.01), with a mean of 8.2 ± 2.8 individuals on trunks and 4.3 ± 2.2 individuals on the ground, although no differences were detected for species diversity (Z = -1.27; P > 0.05).

DISCUSSION

We found that transects were more effective than drift fences for detecting reptiles regardless of the age of the olive grove. Our results are similar to other studies that indicate that transects are highly effective at sampling herpetofauna species (Rodel and Ernst 2004; Hutchens and DePerno 2009; Sung et al. 2011). These results indicate

TABLE 2. The best candidate models as regards explaining species richness (Model 2). The number of estimated parameters (k), the Akaike information criteria for small sample size (AICc), the difference between each model and the best model (Δ AICc) and the Akaike weight (wi) are shown.

k	AICc	ΔAICc	Wi
1	110.37	0	0.4
2	111.23	0.86	0.26
2	112.00	1.63	0.17
3	112.27	1.9	0.15
	k 1 2 2 3	k AICc 1 110.37 2 111.23 2 112.00 3 112.27	$\begin{array}{c cccc} k & AICc & \Delta AICc \\ \hline 1 & 110.37 & 0 \\ 2 & 111.23 & 0.86 \\ 2 & 112.00 & 1.63 \\ 3 & 112.27 & 1.9 \end{array}$

transects may be a valuable tool for biodiversity assessment in woody crops (such as olive crops). Unfortunately, most studies have used only one methodology (Spence-Bailey et al. 2010; Godinho et al. 2011; Rotem et al. 2013) and comparisons between methods cannot therefore be made (Hutchens and DePerno 2009). Drift fences have been recommended by some researchers because of the ability to reveal the presence of rare species and generate significantly higher captures of common species (Garden et al. 2007; Willson and Gibbson 2009). However, in our study, drift fences were less effective at sampling reptiles than transect surveys. Moreover, the drift fence arrays were expensive to construct, maintain, and operate because traps need to be checked daily, which increasingly is required by animal care committees. Also the materials and manufacturing needed for funnel traps (three per array) contributed to a considerable portion of the total cost for arrays, while transects only require human effort (Hutchens and DePerno 2009).

One reason that might explain the differences we found between the methods is the territorial behavior of the reptiles at our sites (Haenel et al. 2003.) and the small home range of these species (e.g., 25 m² for *P. muralis* or 86 m² for *P. his*panica; Verwaijen and Damme 2008), which limits their movement to very specific areas. This might also explain the low rate of capture in drift fences. Most of the reptiles were lizards and were located on the trunks of olive trees, which likely served as shelter and foraging spots (Kerr et al. 2003). However the significant effect of tree age on the model can be explained because only old tree trunks offers adequate shelter and hunting spots, whereas young trees have homogeneous and smooth trunks lacking the microhabitat needed by lizards for refuge. Kaliontzopoulou et al. (2009) described the arboreal behavior of P. hispanica with lizards climbing the trunks of oaks to thermoregulate, find food, and escape predators. These authors relate this arboreal behavior to shortages of rocks or stones in the soil in Cork Oak (Quercus suber) forests, groves which are similar to olive groves. For Podarcis sicula, trunks of old olive trees serve as a mechanism of involuntary dispersal both within and outside its natural range (Valdeón et al. 2010; Rivera et al. 2011).

The vertical space in the woody crops used by lizards in olive groves greatly limits the usefulness and effectiveness of drift fences in this broad habitat type. We recommend the use of transect surveys to sample lizards when available time and economic resources are limited in groves of trees. Only a few snakes were detected by either drift fences with funnel traps (one capture) or along

transects (three observations). Longer periods of sampling with drift fences likely will detect more snakes, but transect surveys seem best for detecting lizards. More research into other sampling techniques in these woody crops is needed, and their effectiveness for different taxa should be compared.

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Appendices

Carpio et al.—-Species richness and diversity in Olive groves.

Species	May Drift Fence	May Transect	June Drift Fence	June Transect
Amphisbaenia				
Blanus cinereus				1
Lacertilia				
Acanthodactylus erythrurus	4	68	7	118
Lacerta lepida		2		3
Podarcis hispanica	7	97	14	233
Podarcis vaucheri			2	5
Psammodromus algirus		29	2	4
Tarentola murintanica		49	47	
Serpentes				
Hemorrhois hippocrepis		1	1	
Malpolon monsspesularus	1		1	
Rhinechis scalaris				1
Unidentified		129		32
Total	11	376	26	444

TABLE A1. Total number of captures of reptile species using different survey methods (drift fences vs. transect) in May and June 2014 in southern Spain.



ANTONIO J. CARPIO is currently a Ph.D. candidate at the University of Córdoba, and he received his Master's degree from IREC (UCLM) in 2011. His dissertation research involves the impact of different farming systems in olive agroecosystems on biodiversity (including herpetofauna, rodents, and arthropods) in the Mediterranean region. His interests include ecology and conservation. (Photographed by Esther Lopéz).



MERCEDES CABRERA is a Forestry Engineering student at the University of Córdoba. Her research focuses principally on forest restoration and assessing biodiversity in the olive groves, with special attention being paid to herpetofauna taxa. She has a long-standing interest in forest conservation and a fascination for reptiles. (Photographed by Antonio J. Carpio).



FRANCISCO S. TORTOSA is a Professor at the University of Córdoba where he is a Senior researcher. His research focus is ecological interaction, particularly in agroecosystem habitats in the Mediterranean region. His objective is to compare the effect of different forest types and crops on biodiversity. His interests include conservation biology and agriculture habitat restoration. (Photographed by José Guerrero).