

Microhabitat selection of the Western green lizard *Lacerta bilineata*

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Abstract - We examined the characteristics of microhabitats selected by Western green lizards, *Lacerta bilineata*. Model selection was carried out with the Information-theoretic approach that focuses on the estimation of effect size and measures of its precision. Our results show that the Western green lizard selects positively sites with good shrub cover, necessary as shelter, while it avoids areas with bare soil where there are no refuges. Additionally, lizards showed a positive selection of rocks (located exclusively in artificial riverbanks) that represent a suitable habitat for thermoregulation and sheltering. The results of our work, and particularly the negative effects of bare soil and the positive effects of shrub cover, confirm the need to restore the network of hedgerows and other linear elements in cultivated landscapes in order to create suitable areas for *L. bilineata*.

Key words: habitat, information-theoretic methods, Italy, lizard, model averaging.

Riassunto - Selezione del microhabitat del ramarro *Lacerta bilineata*.

Abbiamo esaminato le caratteristiche del microhabitat selezionate dal ramarro, *Lacerta bilineata*. La selezione dei modelli è stata fatta con l'approccio *Information-theoretic* che si focalizza sulla stima del valore degli effetti e sulla misura della loro precisione. I nostri risultati mostrano che il ramarro seleziona positivamente i siti con una buona copertura arbustiva, necessaria come riparo, mentre evita le aree con suolo nudo dove non ci sono rifugi. Inoltre, i ramarri mostrano una selezione positiva delle rocce (localizzate esclusivamente nelle sponde fluviali artificiali) che rappresentano un habitat adatto per la termoregolazione e come riparo. I risultati del nostro lavoro, e in particolare l'effetto negativo del suolo nudo e l'effetto positivo della copertura arbustiva, confermano la necessità di ristabilire una rete di siepi ed altri elementi lineari del paesaggio coltivato in modo da creare aree adatte per *L. bilineata*.

Parole chiave: approccio *information-theoretic*, habitat, Italia, lucertola, model averaging.

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INTRODUCTION

The traditional agricultural environment has been modified in recent decades in order to enhance production. This model of development has led to the reduction of natural areas and especially ecotonal areas and hedgerows that may act as wildlife habitat and ecological corridors between natural and semi-natural areas. Habitat fragmentation is one of the biggest problems for the conservation of biodiversity, especially for animal groups such as reptiles, which have reduced mobility (Rubio & Carrascal, 1994).

The Western green lizard (*Lacerta bilineata*) is a large lizard (adult total length: 30-45 cm) that occupies the Northern part of the Iberian Peninsula, France, Switzerland, West Germany and Italy (Elbing *et al.*, 1997; Corti & Lo Cascio, 2002; Schiavo & Venchi, 2006). It lives in open habitats and is widespread in the uncultivated edges of woods and fields, along irrigation channels and roads (Barbieri & Gentili, 2002; Schiavo & Venchi, 2006; Meek, 2014; Pernat *et al.*, 2017).

Lacerta bilineata appeared to be still well distributed in the lowland areas of Northern Italy until a few decades ago, when it was abundant (Scali & Schiavo, 2004; Schiavo & Venchi, 2006). In recent years, however, the status of the species has become quite different because of the homogenization of the countryside, intensive cultivation and the destruction of hedgerows. In addition, the use of pesticides has greatly reduced insect abundance and thus its primary source of food. The Western green lizard is now locally threatened and the conversion of traditional agricultural habitats to intensive methods of farming is causing local population declines (Scali & Schiavo, 2004; Schiavo & Venchi, 2006). Moreover, the requirements of this species coincide with those of several other species typical of ecotones and, therefore, the Western green lizard might serve as an "umbrella species" for many other taxa, playing a key role in protecting these sensitive areas of transition. The species is listed on appendix II (strictly protected fauna species) of the Bern Convention (Council of Europe, 1979) as *L. viridis* and is considered of least concern (LC; population trend: decreasing) in the IUCN Red List of Threatened Species (Pérez-Mellado *et al.*, 2009). In Europe an important legal instrument of conservation is the EU Habitats Directive (Council of Europe, 1992); *L. bilineata*

(as *L. viridis*) is inserted in Annex IV (species in need of strict protection). To ensure a favourable conservation status, Member States shall take the required measures to establish a system of strict protection, prohibiting all forms of deliberate disturbance, capture or killing of specimens of these species in the wild and avoiding deterioration or destruction of their habitat. Nevertheless, few resources are invested in monitoring the status of species and habitats and in the absence of reliable data, it will be impossible to assess the impact of conservation measures. Therefore, studies on the ecology and status of these species are essential.

The few remaining natural areas in the plains of Northern Italy, mostly limited to floodplains, offer a unique refuge for many animal species, including the Western green lizard, which find a suitable habitat along the riverbanks. The goal of this study was to collect data on microhabitat selection by the Western green lizard in a river floodplain in North-western Italy, in order to obtain essential knowledge for developing appropriate management strategies. Since the species is found in a variety of habitats, our aim was not to describe its general habitat, but to highlight the microhabitat elements determining its presence, weighing all available microhabitats occurring in the study area.

MATERIALS AND METHODS

Study Area

The study was carried out in a natural area approximately 100 ha wide and located 70 m a.s.l., in the central part of the floodplain of the Ticino Regional Park (Fig. 1; 45°16'25"N; 8°59'05"E), in the municipality of Motta Visconti (Lombardy, North-western Italy). The climate of this area can be characterized as continental with a mean temperature of approximately 13°C. The average annual rainfall is about 700 mm and rain occur mainly during spring and autumn. The coldest month (January) has temperature ranging from 0 to 3°C, while the warmest one (July) ranges between 22-24°C (Pilon, 2004).



Fig. 1 - Location of study area. / Localizzazione dell'area di studio.

The area has a high environmental heterogeneity and includes hygro-mesophilic woods dominated by the presence of oak (*Quercus robur*) with a rich undergrowth (*Prunus padus*, *Ulmus minor*, *Crataegus monogyna*, *Cornus sanguinea*, *Ligustrum vulgare*), hybrid poplar plantations, wetlands (*Salix* spp., *Alnus glutinosa*), and open areas characterized by a low value of shrub cover and a high value of short herbaceous cover. Along a stretch of the bank of the River Ticino included in our study area, there is an artificial embankment consisting of large boulders; among the rocks a shrub vegetation has developed, which consists predominantly of bushes of *Salix alba* and *Rubus* spp..

Sampling methods

The study was conducted from July to October 2009, after the breeding season of the Western green lizard. During this period, the activity pattern of lizards and habitat characteristics were homogenous. We selected, inside our study area, most of the suitable habitats; then, in those areas, we established a sampling path (length 2574 m) along dirt roads. We walked the sampling path on days with favourable weather conditions, avoiding those with uniform cloud cover and/or rain. Surveys were conducted twice a week, covering a time band between 09:00 and 18:00 h CET. When we detected an unmarked adult in exposed positions, we captured it by noosing (Blomberg & Shine, 2006).

After capture, each individual was measured (snout-vent length, SVL), photographed (ventral photo) and immediately released at the exact location of capture. Each lizard was also individually marked by a unique colour code with acrylic paint on the dorsum for temporary identification (Rubio & Carrascal, 1994; Martín & Salvador, 1995; Blomberg & Shine, 2006). We observed an average duration of the marking of three weeks. This technique allowed long-distance identification and reduced the need of recapture (Rubio & Carrascal, 1994). Ventral photos were used to identify lizards individually using a photo-identification software, I³S Classic (Interactive Individual Identification System - vers. 2.0, available at <http://www.reijns.com/i3s>) (Speed *et al.*, 2007; Van Tienhoven *et al.*, 2007). Photo-identification uses the arrangement of scales of the chinstrap as natural marks (Sacchi *et al.*, 2010); this arrangement does not change over the lizards' lifespan (Steinicke *et al.*, 2000). This technique has the advantage of being harmless and permanent, compared to temporary marking methods (Perera & Pérez-Mellado, 2004; Sacchi *et al.*, 2010). Thanks to the use of natural marks, it was possible to recognize individuals during the study even when the paint faded away.

Microhabitat assessment

We assessed microhabitat features considering four 1 m transects, one at each of the four cardinal directions radiating from the point where each individual was first sighted (Martín & López, 1998; Amo *et al.*, 2007a,b,c). We used a scored stick standing vertically at nine sample points: the exact spot where the lizard was seen, and two points at 50 cm intervals along each transects, record-

ing the contact of the stick at ground level to different substrates (rocks or bare soil, leaf litter, grass). We noted whether there was tree cover above the sample point, the tree species and the under-canopy vegetation at each point. We classified this under-canopy vegetation into two types according to height and characteristics: A1 - shrubs (*Rubus* spp., *Hedera helix*, *Salix* spp., *Crataegus monogyna*, *Rosa canina*, *Prunus padus*, *Viburnum opulus*, *Ulmus minor*, *Cornus sanguinea*, *Ligustrum vulgare* and other species), A2 - herbaceous species that provided cover (*Ambrosia artemisiifolia*, *Solidago gigantea*, *Oenothera biennis*, *Bidens tripartita*, *Polygonum* spp.). We noted the type of vegetation (herbaceous vs. shrub), the percentage of vegetation cover and the minimum height as the height from the ground to the first contact of leaves with the stick (maximum value considered, 100 cm). This procedure allowed us to calculate for each observation the percentage cover values of each habitat variable in the area surrounding the lizard. We considered as “tree cover” trees and shrubs taller than 2.4 meters (length of the fishing rod used for noosing). Since only the microhabitats of captured lizards, which were individually recognizable, were considered, there was the certainty that all the measures were related to different individuals.

The sampling of multiple points to characterize the environment adjacent to the lizards, rather than considering only the single point of a lizard’s capture, allowed for a better characterization of the microhabitat in a lizard’s home range (for a similar sampling method see Martín & Salvador, 1992, 1995; Martín & López, 1998, 2002; Díaz *et al.*, 2006; Amo *et al.*, 2007a,b,c).

To estimate the availability of microhabitats along the standard path sampling, we recorded the same variables every 50 meters along the path; given the environmental homogeneity within each section, we considered this sampling interval wide enough to describe the micro-environmental availability. We placed the sampling point about 40 cm from the edge of the road, the position where we observed most of the lizards. Sampling was carried out both to right and left of the path.

Statistical methods

For the evaluation of microhabitat, we compared the data of captured lizards (34 individuals) with available environments along the standard path, as defined by 88 sampling points. When more than one observation was collected per individual (two cases), we averaged the data in order to have one record for each animal in the database. We also examined the possible differences between sexes, considering the data of occurrence separately, to obtain more information for a better analysis of data.

To assess the potential effect of environmental variables on the presence of lizards we used logistic regression models in which the presence of lizards was recorded as one while availability point was zero. To determine which variables should be included in the set for subsequent analysis we calculated a correlation matrix (Spearman correlation coefficient) among the environmental predictors in order to identify less-correlated variables. We considered as uncorrelated, variables with $r < 0.20$. We included in the

models at most three independent variables among the less-correlated ones according to the established threshold, so that each habitat variable was considered at least once. In the definition of the set, it is important to make a thoughtful choice based on biological hypotheses and all models considered should have some reasonable level of interest and scientific support (Anderson *et al.*, 2000, 2001; Anderson & Burnham, 2002).

In order to verify and quantify the effect of environmental variables on the presence of lizards, model selection was carried out using the Information-theoretic approach (Anderson *et al.*, 2000; Anderson & Burnham, 2002; Mazerolle, 2006). This method is more suitable in selection models of environmental variables, because it avoids all the problems of the null hypothesis approaches; it is easy to compute and understand and especially focuses on the estimation of effect size and measures of its precision, these representing the most important elements in biological questions (Anderson *et al.*, 2000, 2001; Mazerolle, 2006).

To understand which is the best model for establishing the presence and absence of lizards, we computed the modified Akaike Information Criterion (AICc; Sugiura, 1978), which measures the loss of information that results from the use of the model to explain a particular variable or pattern (Mazerolle, 2006); the “best” model will be the one with the lowest AICc, and we ranked models according to this index. To better compare the models, we calculated the differences with the minimum AICc (Δ) and Akaike weights (w_i); w_i is a measure of the probability that the model is the best among the whole set of candidate models. When models had a Δ AICc higher than three and Akaike weights smaller than 0.1, they had only a slight contribution to the data (Burnham & Anderson, 2002; Mazerolle, 2006; Pellitteri-Rosa *et al.*, 2008).

When the w value does not exceed 0.90, it is incorrect to use only the model ranked in first place for subsequent analysis; in these cases, the correct strategy is to base the inference on the entire set of models, using an approach called multimodel inference or model averaging that also provides more precise estimates (Anderson *et al.*, 2000; Mazerolle, 2006; Pellitteri-Rosa *et al.*, 2008). In model averaging, we took into account the variables in models with the highest value of w_i , considering the same number of models for each variable; we used five models containing the variables of interest. Other unused models had very low values of w_i and their use would not affect the results. The relative importance of each variable was measured by the sum of the models Akaike weights (w), where each variable appeared (Burnham & Anderson, 2002; Pellitteri-Rosa *et al.*, 2008). To quantify the effects of variables, the regression coefficients (β) for each model were weighted by the Akaike weights, obtaining an average coefficient ($\bar{\beta}$) over the entire set of models considered (Anderson *et al.*, 2000; Mazerolle, 2006; Pellitteri-Rosa *et al.*, 2008). Similarly, we computed the standard error (SE) of the model-averaging estimate, termed the unconditional SE (Mazerolle, 2006). Using the standard error, we calculated 95% confidence interval to assess the magnitude of the effect (Mazerolle, 2006); narrow intervals indicated precise estimates and when interval

within a narrow range in which performance levels are maximized (Rubio & Carrascal, 1994). Behavioural thermoregulation is achieved by means of careful selection of habitat attributes within the thermal mosaic (Avery, 1982; Huey, 1982).

From the literature, we know that *L. bilineata* lives in open and sunny habitats (Schiavo & Venchi, 2006; Pernat *et al.*, 2017). In our study area, this type of environment was located in the parts closest to the river and *L. bilineata* population was located in those areas.

Our results show that the Western green lizard selects positively sites with good shrub cover; a microhabitat fea-

ture that is primarily important to provide cover against predators (Martín & López, 1998, 2002). Another study on a large-sized lizard (*Timon lepidus*) showed a positive selection of areas with a high shrub cover, therefore suggesting that these zones may be used as refuge-connecting corridors that provide shelter to foraging and thermoregulating lizards (Díaz *et al.*, 2006). To confirm this, our data show that areas with bare soil are negatively selected and the possible reasons are the lack of refuges and a reduced number of invertebrate preys.

The cover of leaf litter was not included in the best models, because it is not a suitable refuge against preda-

Tab. 2 - Set of logistic regression models used for the Information-theoretic approach with nine microhabitat variables at 122 sampling points. / Serie di modelli di regressione logistica usati per l'approccio *Information-theoretic* con nove variabili di microhabitat in 122 punti di campionamento.

*K: number of estimated parameters; Variables: Rocks (percentage cover of rocks), Bsoil (percentage cover of bare soil), Grass (percentage cover of grass), Litter (percentage cover of litter), ShrubC (percentage cover of shrub layer), ShrubH (shrub minimum height – cm), HerbC (percentage cover of herbaceous layer), HerbH (herbaceous minimum height – cm), TreeC (percentage cover of tree layer). / *K: numero di parametri stimati; Variabili: Rocks (percentuale di copertura delle rocce), Bsoil (percentuale di copertura del suolo nudo), Grass (percentuale di copertura delle piante erbacee), Litter (percentuale di copertura della lettiera), ShrubC (percentuale di copertura dello strato arbustivo), ShrubH (altezza minima degli arbusti – cm), HerbC (percentuale di copertura dello strato erbaceo), HerbH (altezza minima dello strato erbaceo – cm), TreeC (percentuale di copertura dello strato arboreo).

Variables	AUC	-2 log-likelihood	K*	AICc	ΔAICc	w
Rocks - Bsoil - ShrubC	0.819	110.017	5	120.534	0.000	0.548
Bsoil - ShrubC	0.807	112.674	4	121.016	0.482	0.431
ShrubC	0.754	122.946	3	129.149	8.615	0.007
Rocks - ShrubC	0.764	120.817	4	129.159	8.625	0.007
Rocks - Bsoil - ShrubH	0.807	120.341	5	130.858	10.324	0.003
ShrubC - TreeC	0.749	122.913	4	131.255	10.721	0.003
Rocks - Bsoil - HerbC	0.795	125.628	5	136.145	15.611	<0.001
Rocks - Bsoil - HerbH	0.782	125.696	5	136.213	15.679	<0.001
Bsoil - ShrubH - HerbH	0.771	127.167	5	137.684	17.150	<0.001
ShrubH - Bsoil - HerbC	0.774	127.254	5	137.771	17.237	<0.001
Rocks - Grass - ShrubH	0.697	130.238	5	140.755	20.221	<0.001
Rocks - ShrubH - HerbH	0.701	130.555	5	141.072	20.538	<0.001
Rocks - ShrubH - HerbC	0.700	130.999	5	141.516	20.982	<0.001
Grass - ShrubH - HerbH	0.672	136.178	5	146.695	26.161	<0.001
Grass - ShrubH - TreeC	0.662	136.460	5	146.977	26.443	<0.001
Grass - ShrubH - HerbC	0.667	136.495	5	147.012	26.478	<0.001
HerbH - TreeC - ShrubH	0.663	136.568	5	147.085	26.551	<0.001
HerbC - TreeC - ShrubH	0.663	136.960	5	147.477	26.943	<0.001
Rocks - Grass - HerbH	0.606	137.908	5	148.425	27.891	<0.001
Rocks - Grass - HerbC	0.594	138.001	5	148.518	27.984	<0.001
Litter - HerbH	0.558	142.992	4	151.334	30.800	<0.001
Litter - HerbC	0.555	143.010	4	151.352	30.818	<0.001
Grass - HerbC - TreeC	0.521	144.170	5	154.687	34.153	<0.001
Grass - HerbH - TreeC	0.526	144.208	5	154.725	34.191	<0.001

Tab. 3 - Most important variables for the presence of lizards obtained through multimodel inference, and their effect. / Le variabili più importanti per la presenza del ramarro ottenute attraverso il *multimodel inference* e il loro effetto.

M = male, F = female; Variables: Rocks (percentage cover of rocks), Bsoil (percentage cover of bare soil), ShrubC (percentage cover of shrub layer). / M = maschio, F = femmina; Variabili: Rocks (percentuale di copertura delle rocce), Bsoil (percentuale di copertura del suolo nudo), ShrubC (percentuale di copertura dello strato arbustivo).

Variables	Sex	Σw	$\bar{\beta}$	SE	- 95%	+ 95%
ShrubC	M+F	0.996	3.072	0.955	1.200	4.944
Bsoil	M+F	0.982	-3.840	1.082	-5.961	-1.719
Rocks	M+F	0.559	4.905	1.343	2.273	7.537
Bsoil	M	0.974	-9.041	1.342	-11.672	-6.410
Rocks	M	0.684	6.588	1.908	2.849	10.326
ShrubC	M	0.474	2.488	1.038	0.454	4.521
Rocks	F	0.998	4.570	1.422	1.782	7.358
Bsoil	F	0.504	-2.154	1.100	-4.310	0.001
ShrubC	F	0.458	3.424	1.002	1.461	5.387

tors given the large size of the adult Western green lizards (unlike smaller lizards such as *Psammotromus algirus*; Díaz & Carrascal, 1991), although it may be a place rich in invertebrate preys.

Our results confirm that the Western green lizard is a species that lives primarily on the ground and rarely climbs (Saint Girons, 1977). Indeed, the important factor is the high value of shrub cover and not the vertical structure of vegetation. This aspect is confirmed by another study on *L. bilineata* in which the occurrence of the species appears to be favoured by the presence of ecotones itself, rather than by their composition and structure (Sacchi *et al.*, 2011). The minimum height of vegetation is an indication of the potential utility of vegetation as a refuge by lizards and previous studies have shown the importance of plant cover at the ground level for small lizards (Carrascal *et al.*, 1989; Martín & López, 1998). Our data show that these variables were not important in microhabitat selection, probably due to large size of *L. bilineata*. Tree cover had not a significant effect on microhabitat selection; this variable may have a major effect on macrohabitat scale.

Rocks are located exclusively in artificial riverbanks. For the conservation of floodplain ecosystems, the artificial embankments must be considered very negative, because they reduce bank erosion that make ecosystems greatly changeable and rich in biodiversity. However, the lizards found a suitable habitat in the rocky bank, where boulders are ideal for thermoregulation and cracks are excellent shelters. Indeed, rocky slopes can maximize heating rate, minimize basking time and increase time available for other activities such as foraging or mating (Carrascal *et al.*, 1992; Martín & Salvador, 1995), and rocks with several crevices might also be useful for eluding predators (Díaz *et al.*, 2006). The presence of shrubs among the rocks along the bank provides the lizards with

an ideal environment in which sunny places for basking are associated, within a short distance, with covered areas useful as shelters against predators and heat. In this study, the selection of rocky areas was probably accentuated by the fact that, being raised sites, they provided a safer area in case of flood, and a place for the winter latency.

We considered only adult lizards and so micro-environmental preferences refer to this age class; but Western green lizards shift habitat use with age: adults prefer ecotonal areas with greater cover of vegetation, while juveniles use more open and grassy areas (Angelici *et al.*, 1997; Scali *et al.*, 2001). This is confirmed by the minor importance of the herbaceous cover in our data.

L. bilineata presents variations in behaviour and activity between sexes. Males usually are the first to emerge followed, after one or two weeks, by juveniles and females, while the first to return in winter shelters are females (Saint Girons *et al.*, 1989). During the breeding season males compete for territories and mates, while females are more tolerant each other (Saint Girons, 1977; Schiavo & Venchi, 2006). Our study was not focused on sex differences, but we decided to analyse them because a small data set could be skewed by gender differences. The variables important to define the microhabitat use were the same in both sexes. There were some minor differences in their effects, but they were not enough to define a clear pattern. To analyse more in depth the variations between sexes, research should be carried out during the breeding season, when sexual differences may be more evident.

In our study area the species is still common, but considering the status of the populations in the Po Plain (Northern Italy), the abandonment of traditional farming and cattle breeding practices have probably disrupted the continuity among favourable habitats, thus limiting the opportunities for dispersal and contact among

different lizard populations. Lizards, in general, have limited dispersal ability (Boudjemadi *et al.*, 1999; Le Galliard *et al.*, 2005) making them highly sensitive to land clearance, as most species are unable to cross unsuitable habitats, such as cultivated lands or pastures interspersed among woodland remnants (Martín & López, 2002). A major problem in the Po Plain is the fragmentation and homogenization of the territory due to an unrestrained urbanization and industrial development (*e.g.*, in Lombardy the human population density is 408 inhabitants/km², one of the highest in Italy; Oprio *et al.*, 2015). In a human-dominated landscape the fragmentation of habitats and their progressive isolation are mainly related to the development of infrastructure (*e.g.*, main roads) that follow the construction of new buildings, which creates barriers often insurmountable to many animals (Wilcox & Murphy, 1985; Ficetola & De Bernardi, 2004). Quantitative data on the size of the Italian population are not presently available; although increasing habitat destruction, especially at low altitudes, and natural reforestation of previously open habitat in mountain areas, suggest that a reduction in numbers may be occurring (Schiavo & Venchi, 2006). The Western green lizard is less tolerant towards modifications of its natural habitat than other sympatric lizards (for instance those belonging to the genus *Podarcis*); in particular, the species is affected by the removal of herbaceous vegetation and of low bushes and the destruction of ecotonal belts (Venchi, 2000).

The results of our work, stress the negative effects of bare soil and the positive effects of shrub cover, confirm the need to restore, as soon as possible, the network of hedgerows and other linear elements in the cultivated landscapes dominated by intensive cultivation, in order to revert the processes which brought to population fragmentation through the loss of ecological corridors.

The study was carried out in conformity with the Italian current laws for lizard capture (Aut. Prot. DPN-2008-0003606).

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