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Selection of key features of vegetation and escape behavior in the Sand Dune Lizard (*Liolaemus multimaculatus*)

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Abstract

Vegetation structure and cover are two of the main factors which determine microhabitat preferences in lizards. The Sand Dune Lizard (*Liolaemus multimaculatus*) is a vulnerable and endemic species of the pampean coastal habitats from Argentina. We hypothesized that: a) Sand Dune Lizard prefer to perch in microhabitats that offer a good balance between visibility and refuge, and; b) lizards prefer microhabitats in which plant types allow them to resort to sand burying behavior. We recorded data of microhabitat (bunch-grasses sizes and plant types) used by lizards (males, females and juveniles) in a population at the Mar Chiquita Provincial Nature Reserve. We applied the use-availability design to assess preferences. We evaluated differences between sex and relation between sizes of lizards. Lizards preferred bunch-grasses of intermediate size. Habitats conformed only by herbaceous species were the most preferred by lizards. We did not find differences between males and females, neither relations between size of lizards and the tested variables. Adult lizards of both sexes use bunch-grasses more frequently than juvenile individuals. The preferences for herbaceous species could be related to the sand-bury behavior that lizards use to escape from predators. More studies are necessary in order to assess the processes related with habitat preferences in Sand Dune Lizard.

Keywords

Vegetation features; microhabitat use; escape behaviour; Sand Dune Lizard

Introduction

Knowledge of space use in animals is an essential element in understanding their behavior and ecology (Huey, 1991; Garshelis, 2000). In small vertebrates, the vegetation structure and the availability of shelters are key factors that determine space use and microhabitat preferences (Scott, 1976; Pianka, 1986; Huey, 1991; Lagos, et al. 1995;

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Attum and Eason, 2006; Nemes et al., 2006). Small vertebrates are generally prey to a great number of predators, and for this reason they must 'trade-off' the costs and benefits associated with avoiding predators and competitors, and with obtaining food, mates, refuge and suitable microclimatic conditions (Mc Ivor and Odum, 1988; Rocha, 1995; Converse and Savidge, 2003; Attum and Eason, 2006). Therefore, the availability of shelters with suitable thermal characteristics in a particular microhabitat has profound implications for the ecology and survival of small animals (Howard et al., 2003).

Some plant types like shrubs or bunch grasses give shelter to small vertebrates, such as certain reptiles, and for this reason it is common to find individuals perched on the base of these plants or near them (Kacoliris et al., 2008). These plants can provide protection from predators and extreme surface temperatures, and allow access to foraging areas and potential mates (Beck and Jennings, 2003). Although larger shelters provide better protection to small animals, they may also reduce the visibility of potential prey and mates. In consequence, perch site selection must be a compromise between maximizing visibility and opportunities for thermoregulation while minimizing the chance of contact with predators (Scott, 1976).

Iguanian lizards have proven useful subjects for studies of space use, because they have high site fidelity, are prey to a great number of predators and have many escape strategies to avoid predators, mainly the use of refuges (Huey et al., 1983; Galdino et al., 2006). The Sand Dune Lizard, *Liolaemus multimaculatus* (Duméril and Bibrón, 1837), is a small diurnal sand-dwelling liolaemid lizard, endemic to the Pampean coasts of the provinces of Buenos Aires and Río Negro in Argentina, and categorized as a vulnerable species (Cei, 1993; Lavilla et al., 2000). This lizard is a specialized species with respect to microhabitat selection, preferring areas with low vegetation cover and bunch grasses as shelters (Kacoliris et al., 2006; Kacoliris et al., 2008). However, it is a generalized species with respect to diet (Vega, 1997). Regarding its foraging mode, the Sand Dune Lizard is a sit-and-wait species (Vega, 2001). Males of sit-and-wait lizards are generally territorial, and for this reason differences in space use between sexes could exist (Cooper, 1994). With regard to escape behavior, two of the main features of this species are their capacity to bury into the sand (Halloy et al., 1998) and/or run to shelters (Chevez and Kacoliris, 2008) in the presence of predators. The dominant predators of this lizard in sand dune habitats (based on its abundances) are raptors (*Polyborus plancus* and *Milvago chimango*) and gulls (*Larus dominicanus*), and secondarily terrestrial animals such as foxes and cats (Molinari, ump. data). For all these reasons, we consider that this species is useful to study factors concerning space use in relation to predation risk.

Considering that the Sand Dune Lizard is prey to a wide range of predators, we hypothesized that they prefer to perch in microhabitats that offer a good balance between visibility and refuge. This hypothesis predicts that medium-sized bunch grasses will be preferred by lizards to perch, because they offer better refuge from predators than small-sized bunch grasses. At the same time, they offer better visibility to detect prey, mates and predators than large-sized bunch grasses. Because the Sand Dune Lizard is a territorial species (Chevez and Kacoliris, 2008), and considering that probably adult males actively defend territories (Vega, 1999), we hypothesized that they need more visibility than females and juveniles, to detect mates and competitors. This hypothesis predicts that a) male lizards use smaller bunch grasses than females and juveniles, and b) male lizards can perch at longer distances from shelters than females and juveniles. We also hypothesized that lizards prefer microhabitats in which plant types allow them to resort to sand burying behavior. This hypothesis predicts that microhabitats consisting mainly of herbaceous species will be preferred, because they occur in areas with the presence of patches of bare sandy soil.

Material and methods

The study site comprised two independent plots of 140 ha each, located in the Reserva Natural Integral Mar Chiquita (Mar Chiquita Integral Natural Reserve; 37° 37′ S - 57° 16′ W) in the Province of Buenos Aires, Argentina. Several habitat types, both natural and exotic, occur in this area, but our surveys were undertaken in sand grasslands, because lizards only use these habitat types (Kacoliris et al., 2006). Sand grasslands are psammophytic habitats, with medium to low vegetation cover, dominated by plant species adapted to high salinity conditions, a mobile substrate and low water availability (Cabrera, 1976).

Field activities were performed during January and February 2008. Each survey began at 11:00 a.m. and finished at 4:00 p.m., corresponding to peak daily activity for this species (Vega, Bellagamba and Fitzgerald, 2000). In order to evaluate bunch grass availability, we surveyed five random 1-ha plots recording bunch grass species and number. Although those bunch grasses do not represent a microhabitat per se, and they are included into other microhabitat categories (see below), we focused most of our efforts on this plant species, because they represent shelters for sand lizards (Chevez and Kacoliris, 2008). The length, width and height of each bunch grass were measured with a tape measure to the nearest 1 cm, in order to calculate plant size (in cm^3). For the use-availability analysis, we delineated five size categories considering: a) the degree of protection of the plants for the lizards, and b) the power of the use-availability method, following the recommendations by (Garshelis, 2000). The categories of bunch grasses considered were: 1) very small (0 to 1 m^3), in which lizards remain visible for the observer and, due to the small size of the base of the plant, on whose edge they perch; 2) small (1.1 to 2 m^3), in which lizards remain visible but they can perch under the plant; 3) medium (2.1 to 4 m^3), in which lizards remain hidden and can perch under the plant; 4) large (4.1 to 6 m^3), in which lizards remain hidden and can move around the plant; and 5) extra large (larger than 6.1 m^3), in which lizards were completely hidden unless the vegetation was actively searched.

Five observers searched for lizards by using the Visual Encounter Survey (VES), which consists in observers walking through a designated area for a prescribed time, visually searching for animals in a systematic way along transects. This method allows observers to examine extensively all microhabitat types (Crump and Scott, 1994). All surveys were carried out under similar weather conditions, resulting in 270 man-days' effort. Although the visibility is not the same across shelter types, we increased our efforts when searching in the largest shelters, in order to avoid bias.

When a lizard was found, we recorded: a) sex (whenever possible) and snout-vent length (SVL, with dial calipers to the nearest 0.05 mm); b) the length, width and height of bunch grasses when lizards were perched under a plant; c) the distance of lizards to the closest bunch grass in a 50 m radius (with a tape measure to the nearest 1 cm); and d) the escape strategy used in the presence of observers, either 1) to take refuge under a bunch grass, or 2) to bury into the sand. Variables (SVL, bunch grass size and distance) were measured always by the same observer in order to avoid bias. We considered three groups of individuals for the analysis: males, females, and all individuals. The latter group included males, females and unsexed individuals (= uncaptured).

To assess preferences in relation to plant types we categorized the plant types in any 1 m^2 plot in which lizards were detected. We recognized eight categories of plots: 1) lack of plants; 2) herbs; 3) shrubs; 4) trees; 5) herbs and shrubs; 6) herbs and trees; 7) shrubs and trees; and 8) herbs, shrubs and trees. Availability was measured by systematically sampling 156 plots (1 m^2 each) within the study area. Bunch grasses are included in the "herb plants" type, but they were considered in a separate way in both analyses (the bunch grass analysis and the microhabitat analysis).

We applied the use-availability design to assess preferences for both bunch grass size and plant types in relation to their availability. This design is based on a chi-square test for the null hypothesis of no differences between the proportion of the bunch grasses used by lizards and the proportion of used bunch grasses. This null hypothesis predicts a case in which lizards use bunch grasses in relation to their availability. Bonferroni confidence intervals were estimated to determine which bunch grass sizes were used more or less than expected (Neu et al., 1974; Byers et al., 1984; Garshelis, 2000). When the lowest value of the confidence interval is higher than the proportion of microhabitat availability, the interpretation is that individuals prefer this particular microhabitat; similarly, if the highest value of the confidence interval is lower than microhabitat availability, the result is interpreted as avoidance of the particular microhabitat by individuals. When availability values fall within the confidence limits, individuals are using the microhabitat according to its availability. We established enough habitat categories to ensure that the truly important categories were not lumped with, and thus diluted by, less important categories, while at the same time taking care not to diminish the power to discern selection due to parceling out too many categories (Garshelis, 2000). The magnitude of selection was evaluated calculating the Jacobs index: D' = (pr - gr) / (pr + gr - (2prgr). This index works with the proportion of use (pr) and availability (gr) (Manly et al., 1993), according to which an index value of -1 indicates that a particular microhabitat is completely avoided, whereas +1 indicates maximum preference. We also calculated the estimated variance (S^2) of Jacobs index, following (Strauss, 1979).

We compared the mean size of the bunch grasses used by lizards between sexes. With regard to the mean distance of individuals to bunch grasses, we considered two situations: if lizards ran and sheltered below the closest bunch grass in our presence (bunch grasses used by individuals), or if lizards ran in another direction (bunch grasses not used by individuals). We compared mean distance to closer bunch grasses by lizards, and mean distance to closer bunch grasses used by lizards, between sexes. Mean values of all the analyses were calculated through a two-sided null test with 1000 permutations, using the Rundom Express V2S application. We tested the relation between both variables (size and distance to the nearest bunch grass) and the SVL of lizards through a 1000 permutations-null test, using the Rundom Express VPC application. All statistical tests were two-tailed with a significance level of $P < 0.05$.

The relation between the patterns of shelter selection and the day temperatures was assessed in an indirect way. First, we compared the temperature data at the beginning and at the end of the surveys with a chi-square null-test with 1000 permutations through the Ecosim program, in order to detect the existence a difference along the survey period. If no differences exist, the relation between average day temperatures and the size of shelters used by lizards was assessed with a correlation null-test with 1000 permutations through the Rundom Express VPC program.

We chose random tests over parametric statistics because they are more appropriate for our data, considering that such tests do not require any a priori assumptions about the distribution of the data (Manly, 1997).

Results

Three hundred and ninety-four individuals of *Liolaemus multimaculatus* were detected, but only 52% of them were found using bunch grasses: a total of 35% of the individuals were found perched on bunch grasses and 17% were observed running to bunch grasses under our presence. Adults comprised 99% of the total number of individuals found under bunch grasses, and only five individuals were juveniles. All the bunch grasses recorded belong to the plant species *Spartina ciliata*. The mean size ± standard deviation of bunch grass used was 1.21 ± 2.05 m^3 (n = 121; range = 0.02 to 11.23 m^3) for all individuals (males, females and unsexed individuals); 1.74 ± 1.46 (n = 32; range = 0.02 to 5.64 m^3) for males; and 1.40 ± 1.30 (n = 51; range = 0.07 to 5.47 m^3) for females.

Day temperatures at the beginning and at the end of the surveys did not show differences (chi square = 8.4; p = 0.99; n = 35). A correlation null-test between average day temperatures and the size of bunch grasses used by lizards did not show any relation (r = 0.17; p = 0.14; n = 69).

The proportion in which lizards used each size category of bunch grass was different from the proportion of bunch grasses available (chi-square test, $\chi^2_4 = 17.29$; $P < 0.0001$; fig. 1) Lizards avoid bunch grasses smaller than 1 m^3, preferring larger bunch grasses (with a Bonferroni confidence interval with a significance level of 95%; see table 1). The most preferred bunch grasses were those of medium size (Jacobs Index, D′ = 0.4). The density of all the size categories of bunch grasses in the study area was 12 individuals per hectare, whereas the density of those bunch grasses larger than 1 m^3 (which are preferred by lizards) was 7 bunch grasses per hectare.

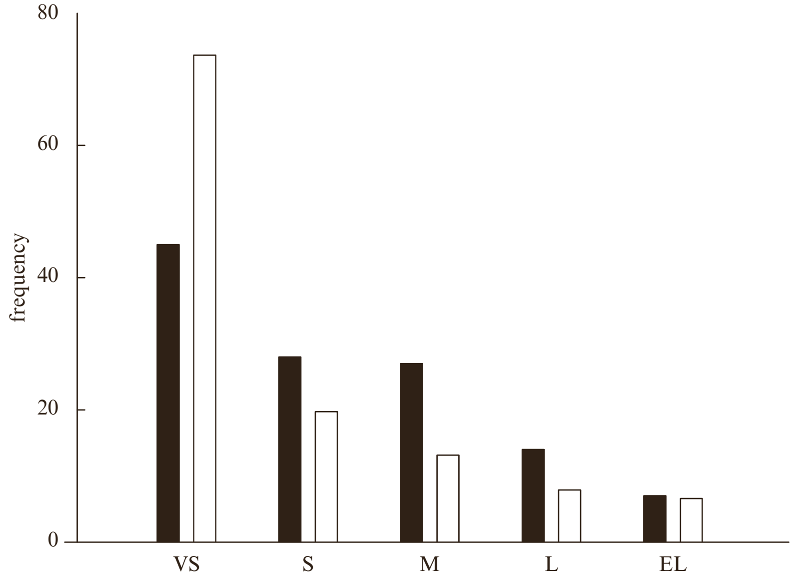

Figure 1. Bunch grass availability (white columns) and bunch grasses used by the Sand Dune Lizard (black columns). VS = very small; S = small; M = medium; L = large and EL = extra large.

Table 1.
Use-availability analysis. n = frequency of lizards; Av = bunch grass availability; BLCI = 95% lower bund of the Bonferroni confidence interval; BUCI= 95% upper bund of the Bonferroni confidence interval; D′= Jacobs Index of selectivity; S^2 D′= estimated sampling variance of D′.

Bunch grasses	n	Av	BLCI	BUCI	D′	S^2 D′
Very small	45	0.61	0.35	0.39	-0.45	0,01
Small	28	0.16	0.21	0.25	0.21	0,02
Medium	27	0.11	0.20	0.24	0.40	0,02
Large	14	0.07	0.10	0.13	0.30	0,02
Extra large	7	0.05	0.05	0.07	0.03	0,02

Mean sizes of bunch grasses used by males and females were similar (observed index = 0.34; P = 0.27). There was no correlation between SVL of lizards and size of bunch grasses (Pearson coefficient, r = 0.15; n = 83; P = 0.16).

Fifty-nine per cent of the lizards detected (n = 219) were located at distances smaller than 50 m with respect to a bunch grass. Mean distance of lizards to the closest bunch grass was 19.7 ± 21. 8 m (n = 219; range 0.5 to 50 m). In our presence, lizards ran to hide under bunch grasses located at a mean distance of 4.5 ± 3.1 m (n = 59; range 0.8 to 14.0 m). Bunch grasses located at larger distances than 14 m were avoided by lizards, and in these circumstances individuals used sand burying as escape behavior. Mean distance to bunch grasses used as shelter by males was 5.4 ± 3.7 m (n = 20; range 0.9

to 14 m; n = 20), whereas for females it was 4.2 ± 2.8 m (n = 34; range 0.75 to 13.5 m; n = 34). Mean distances to bunch grass (it includes the bunch grasses used and not used by lizards) were similar between males and females (observed index = 0.50; P = 0.12). Mean distance to bunch grass used by lizards were similar between males and females (observed index = 0.96; P = 0.06). There was no correlation between the SVL of lizards and the distance to the closest bunch grass (Pearson coefficient, r = 0.028; n = 131; P = 0.59).

Considering the habitats used by lizards in relation to plant types, significant differences exist between availability and use (chi-square test, $\chi^2_3 = 76.17$; $p < 0.0001$). Lizards prefer habitats formed only by herb plants ($D' \pm S^2 = 1 \pm 0.01$), whereas they avoid microhabitats formed by shrubs ($D' \pm S^2 = -0.93 \pm 0.01$) or trees ($D' \pm S^2 = -0.99 \pm 0.01$), as well as microhabitats formed by herbs, shrubs and trees ($D' \pm S^2 = -0.99 \pm 0.01$).

Discussion

One important function of vegetation structure for lizards is the provision of perch sites. These perches promote the effective detection of prey (Blázquez and Rodríguez-Estrella, 1997), improve the visibility of conspecific individuals for social interactions (Baird and Sloan, 2003), and offer refuge to avoid predators (Scott, 1976). Although some predators, such as snakes or small mammals, can access lizards even if they are under the vegetation, predators of this kind are depreciable in sandy habitats with low vegetation cover (Kacoliris, pers. obs.).

The Sand Dune Lizard does not use bunch grasses nor microhabitats in a random manner. Considering the intensity of selection, lizards avoid extreme size categories of plants (extra large and extra small bunch grasses) and prefer intermediate size categories (small, medium and large bunch grasses). Sand Dune Lizards also prefer microhabitats formed only by herbaceous species. These results agree with our predicted patterns for the Sand Dune Lizard. Regarding their escape strategy, lizards prefer to run to bunch grasses when plants were at a distance of less than 14 m. If plants are located at longer distances, lizards use sand burying behavior. However, is important to remark that the observed patterns are descriptive, considering that distance to shelter is not the only factor determining the escape decisions of lizards.

Despite the fact that larger bunch grasses make it difficult to catch lizards, these avoid them. These preferences could be related to the limited visibility provided by large plants. Areas with good visibility cost less to defend than those with poor visibility; territory owners of many lizard species may also prefer high-visibility territories because they reduce predation risk and/or increase foraging success (Scott, 1976; Eason and Stamps, 1992). However, if the habitat offers more visibility to lizards, they probably also offer more visibility to predators. Considering that the principal predators are birds, and that lizards are generally perched at the edge of bunch grasses and run into the plant in the presence of predators, it is probable that medium-sized and larger shelters hinder birds from catching lizards in the same proportion. Nevertheless, the degree of protection of several sizes of shelters must be evaluated in order to understand this point. In our study, in some cases in which two observers detected a lizard perched in front of a patch of large bunch grasses, one observer remained still opposite the lizard while the second observer walked behind the bunch grass (Kacoliris, pers. obs.). When the first observer moved to approach the lizard, the lizard ran away or moved into the bunch grass. But, when the second observer moved to approach the lizard, the lizard remained still and the observer was able to approach it. The lizard probably could not see the second observer due to the fact that the bunch grass between the lizard and the observer obstructed the lizard's visibility. Other studies on lizard species showed that individuals generally avoid microhabitats with visual barriers (Green et al., 2001; Tester and Marshall, 1961). This evidence supports the fact that large bunch grasses hinder visibility and therefore should be avoided by Sand Dune Lizards.

Visibility is related to the facility to detect and catch preys by insectivorous lizard species (Scott Newbold, 2007), mostly in open habitats (Green et al., 2001). However, in Sand Dune Lizards the distribution of prey does not seem to be a key factor in microhabitat selection (Vega 2001). Therefore, predation risk could be the strongest factor affecting the selection of certain plant types by lizards, as seen in Spiny-tailed Iguanas (Blázquez and Rodríguez-Estrella, 1997). Sand Dune Lizards probably prefer intermediate-sized plants because these provide more shelter than smaller plants, while at the same time still offering good visibility to detect predators, prey, mates and competitors. An alternative or complementary explanation as to why these lizards have a preference for medium-sized bunch grasses could be related to thermoregulation. Bunch grasses that are smaller might be extremely hot, whereas bunch grasses that are too large may not provide opportunities for thermoregulation. Although no relations were observed between day temperatures and the main patterns of shelter selection, microclimatic data are needed in order to precisely describe the main factors that limit perch site in this species.

The absence of relations between size of individuals and spatial use disagree with our predictions, but agree with previous research into other *Liolaemus* species (Jaksic et al., 1980; Schulte et al., 2004). However, despite the absence of relation between SVL and bunch grass size, if we considered only the age of individuals (reproductive and non-reproductive), we observed that only one percent of non-reproductive individuals was found perched on bunch grasses. If we compare this value with previous studies in the same area (Kacoliris, pers. obs.), we observe that the proportion of non-reproductive individuals (perched and not perched on bunch grasses) in a sample of 604 individuals was greater (9%). Considering that we did not find differences between males and females, results could be indicating that reproductive lizards, independently of their sex, use bunch grasses more frequently than non-reproductive individuals. However, and due to the small number of non-reproductive individuals, an alternative scenario could be that some juveniles cannot be seen by the observers because they are cryptic and hiding in the bunch grass. For this reason, conclusions about non-reproductive individuals are too vague and they must be tested in further studies.

Lizards prefer habitats formed exclusively by herbaceous species. These preferences could be related to their sand burying behavior. Habitats formed only by shrubs or trees present larger vegetation cover, which hinders this behavior due to sand compaction (Chevez and Kacoliris, 2008). Habitats consisting of only herbaceous plant species offer both little vegetation cover, which allows them to resort to sand burying behavior, and bunch grasses, which lizards can use as shelters. Further studies are necessary in order to assess the relation between social patterns and space use in the Sand Dune Lizard.

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