Differences in escape behaviour among individuals of sand dune lizards (*Liolaemus multimaculatus*) varying in degree of research disturbance

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The goal of this work was to assess escape behaviour in individuals of sand dune lizards (*Liolaemus multimaculatus*) varying in degree of research disturbance. When searching for individuals in the field, the distance between the observer and the lizard before it started escaping (AD) and the distance between the lizards and the closest shelter (DCS), were compared in a disturbed and an undisturbed site. Disturbance was caused by three years research in which lizards were captured, marked and recaptured. The AD averaged 186 cm at the disturbed site and 330 cm at the undisturbed site, whereas the DCS averaged 1,609 cm at the disturbed site and 2,008 cm at the undisturbed site. Both variables showed significant differences between sites ($p<0.05$). This study supports the idea that research activities that involve manipulation of animals can affect their behaviour.

Key words: approach distance, distance to shelter, escape behaviour, research disturbance, Sand dune lizard.

Escape theory predicts that prey will flee when a predator approaches closer than a certain critical distance. Two basic models can explain how close the prey allows the predator to approach before starting its escape, (1) when the risk of predation is equal to the cost of escape (Ydenberg & Dill, 1986) and (2) when the expected fitness after the encounter its maximum (Cooper & Frederick, 2007). Based on these models, several studies have analyzed escape behaviour identifying some variables that can affect the approach distance in lizards: predator speed and directness of approach (Cooper, 2003; 2006), reproductive seasonality (Majláth & Majláthová, 2009), foraging opportunity (Cooper & Pérez-Mellado, 2004), the distance between predator and prey when the predator begins to approach (Cooper, 2005) and territorial aggressive encounters (Días-Uriarte, 1999). Moreover, previous researchers have shown that lizard species often differ among populations in their escape behaviour (Bulova, 1994; Lailvaux et al., 2003; Whiting et al., 2003). However, few works have analyzed the effect of human disturbances on approach distance.

Regalado (1998) studied escape behaviour in three Cuban species of *Anolis*, reporting that approach distances increased after a trial in which lizards were manipulated by an experimenter (a potential predator), showing that lizards had the ability to change their behaviour. On the other hand, Irschick et al. (2005) compared escape behaviour between two populations of *Anolis carolinensis* in habitats which differ in degree of human activity and found that approach distance decreased in the disturbed site compared to the undisturbed site.

The sand dune lizard *Liolaemus multimaculatus* (Duméril & Bibrón, 1837) is a small sand-dwelling lizard, endemic to the pampas coasts in Argentina (Cei, 1993; Avila et al., 2000). Sand dune lizards are microhabitat specialists, preferring areas with low vegetation cover and scattered bunch grasses that are used as shelter (Kacoliris et al., 2009a). They are sit-and-wait predators with a large prey spectrum (Vega, 1997; Vega, 2001). These lizards escape from a putative predator using several strategies. They rely on their cryptic colouration and remain motionless or flee by running and burying into the sand or taking refuge in a shelter (Halloy et al., 1998; Kacoliris et al. 2009b).

Between 2006 and 2008, mark-recapture field work was carried out on a population of sand dune lizards in Mar Chiquita Reserve (Buenos Aires, Argentina). As a consequence of that study a great number of individuals were captured, measured and marked (by cutting off distal phalanges). Although previous studies have shown that marks such as the ones used for this species do not have secondary effects on their locomotor performance (Huey et al., 1990; Borges-Landáez & Shine, 2003) and induce less stress than other kind of marks (Langkilde & Shine, 2006), long-term behavioural effects have not been previously assessed. The present work attempts to assess if differences exist in escape behaviour between individuals of lizards varying in degree of research disturbance.

The study area comprised two dune sites of approximately 90 ha each, located within Mar Chiquita Provincial Reserve (37º37´S, 57º16´W, Buenos Aires Province, Argentina). For a more detailed description of the habitat, see Kacoliris et al. (2009a). The studied sites are in close proximity to each other (3 km), they are equivalent in terms of general features of vegetation and microhabitat availability and prior to this research, both were free of human disturbance. Based on previous home range studies, the samples were assumed independent (Kacoliris et al., 2009c).

From 2006 to 2008, we performed a mark-recapture study in one of the sites. Due to the fact that the lizards were exposed to the frequent presence of observers and many individuals were chased, captured and marked during several sampling seasons (many of them on several

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occasions), this site was referred as the “disturbed site”. In contrast, the second site had only been visited in 2008 and no mark-recapture studies were carried out. For this reason the second site was referred as the “undisturbed site” and considered the control.

Fieldwork was performed during January and February of 2008. The surveys began at 1100 hours corresponding to the daily activity peak for this species (Vega et al., 2000) and finished at 1600. Three observers searched for lizards at each site using a visual encounter survey method (Crump & Scott, 1994). For each individual located, the following data were recorded: 1) approach distance, i.e. the minimum distance between the observer and the lizard before escape behaviour was triggered (AD; to the nearest 1 cm), and; 2) the lizard’s distance to the closest shelter (DCS; to the nearest 1 cm; shelters are bunch-grasses in all cases). The AD method assumes that a human threat mimics a natural threat at some level (Bulova, 1994). If the lizard was captured, the following information was recorded: a) sex, based on external features; (Cei, 1993), b) age class as adult or juvenile, based on the relationship between snout-vent length and reproductive state, following Vega (2001), and, c) tail state: normal or autotomized/regenerated. Tail state was considered as an indirect indicator of the pressure of predators in each site (this study did not cause autotomizations).

To test if samples were similar, the proportion of sexes, age classes and individuals with autotomized/regenerated tails were compared between sites. The AD values were compared between disturbed and undisturbed sites through a Student-t Test and a Monte Carlo Test (with 10,000 permutations). A correlation test was used in order to assess the relationship between AD and DCS at each site. Moreover, Student-t Test and a Monte Carlo Test were performed in order to test differences in DCS between sites. For a more detailed understand of escape behaviour, differences in AD were also assessed at each site comparing between sexes and individuals with normal vs. autotomized tails. Because at the undisturbed site lizards were not captured, a comparison of AD values between adults and juveniles was only possible at the disturbed site. Significance level of p was 0.05 for all tests.

The comparisons of the proportions of sexes, ages and individuals with autotomized tails did not show differences between sites ($p>0.05$ in all cases). Average AD was 186.10 cm ($n=200$; 95 % conf.=148.59 cm–223.6 cm) for the disturbed site and 330.02 cm ($n=102$; 95 % conf.=226.23 cm–435.44 cm) for the undisturbed site. A comparison of AD between sites showed significant differences (Student-t=3.079, $p=0.013$; Monte Carlo $p=0.001$). As shown in Fig. 1, the sites differed greatly at small values of AD.

The correlation test showed a positive relationship between AD and DCS in both disturbed (R=0.249; $p=0.05$), and undisturbed sites (R=0.457; $p=0.042$). However, DCS differed between sites (Student-t=2.4609, $p=0.14$; Monte Carlo $p=0.012$). Lizards were perched closer to a shelter in the disturbed site (average=1691.5 cm; 95 % conf.=1277 cm–2105.9 cm; $n=102$) in comparison to the undisturbed site (average=2406.3 cm; 95 % conf.=2008.6 cm–2803.9 cm; $n=118$).

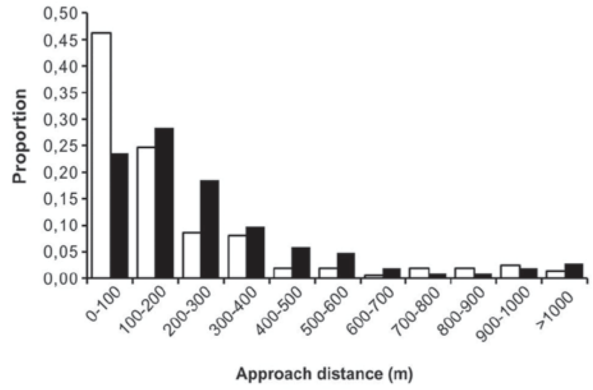

Fig. 1. Frequency of lizards recorded at several AD. White columns: disturbed site; black columns: undisturbed site.

The analysis of AD at the disturbed site did not show differences between sexes, ages or individuals with normal vs. autotomized tails ($p>0.05$, $n=200$ in all cases). Similarly, at the undisturbed site, AD was similar between sexes and individuals with normal and autotomized tails ($p>0.05$, $n=39$ in all cases).

Our results show that the lizards from the disturbed site allowed observers a closer approach before fleeing and they were perched closer to a shelter. This observation differs from that of Regalado (1998) who found that some lizards increased their AD as a consequence of experimenter actions. However, our results were in agreement with Irschick et al. (2005) in which AD appeared greater in the undisturbed site compared to the disturbed site. In summary the three studies reinforce the assumption that research activities that involve manipulation can affect lizard behaviour in some way.

One possible explanation about the differences between sites was that lizards in the disturbed site may be less wary because they are habituated and used to the presence of humans, allowing a closer approach. This suggestion would be supported by the fact that a great number of individuals at the disturbed site had been manipulated on several occasions without actual threats for their survival. As seen in Irschick et al. (2005), AD was longer in a lizard population inhabiting a typical habitat than another population inhabiting a human artificially maintained habitat. Regarding these authors, the differences may be due to the potentially higher diversity and abundance of predators at the natural habitat, although habituation to humans could have also played a role. However, in the case of sand dune lizard no differences were found between sites related to predation pressure or predators frequency.

The analysis of DCS may shed some light on this point. Correlation between AD and DCS was observed at both sites. Consequently, DCS was different between sites, being higher for the undisturbed site. Lizards allow closer AD when perched closer to shelters, probably because of the higher likelihood of escaping to the shelter. This might mean that at the disturbed site, the individuals identify the observers as a threat and vary their behaviour in order to achieve a more effective flight by perching closer to shelters. Moreover, because lizards were captured even during escape events that began from a long distance, a long AD seems to be an ineffective behaviour to avoid human predators (at least at sandy habitats).

A relationship between escape behaviour and distance to shelter was reported previously in Kacoliris et al. (2009b). When close to a shelter, lizards prefer to run to the refuge rather than bury into the sand. Running to shelters seems the preferred strategy when facing terrestrial predators. Therefore, staying close to a shelter would be a good way to avoid predation. In that case, and depending on the type and frequency of predators, the lizards could vary their location in relation to the proximity to a shelter and secondarily their AD.

This study supports the idea that research disturbances can affect the behaviour of local populations of lizards in some way. However, it is not clear which could be the potential biological or population impact of these effects. The results of this work only shown that the behaviour of some individuals in the population was altered, but this does not necessarily mean that there is a significant impact on the biology and fitness of these animals. Nevertheless, the implications of these behavioural changes of individuals must be taken into account in order to assess the possible effect of research on fitness and survival.

Acknowledgements: We would like to thank thank R. Cañete, F. Tuñon, M. Buzato, J. Mangiarotti, A. Molinari, A. Rafael, G. Sánchez Béliz, G. Guaitoli, C. Cassino, E. Guerrero, C. Ruiz de Arcaute, E. Kubish, B. Barret, and F. Cunninghame, for their help in field work. This work was funded by Rufford Small Grants, the International Reptile Conservation Foundation, the Neotropical Grassland Conservancy and the Comisión Nacional de Investigaciones Científicas y Técnicas de Argentina. The Dirección de Áreas Protegidas de la Provincia de Buenos Aires granted us the necessary permits to work in the area.

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Accepted: 10 October 2011