Comparing the antipredator behaviour of two sympatric, but not syntopic, *Liolaemus* lizards

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ARTICLE INFO

ABSTRACT

The microhabitat preferences of prey animals can modulate how they perceive predation risk, and therefore, their antipredator behaviour. We tested under standardized conditions how microhabitat preferences of two *Liolaemus* lizards affected their responses when confronted with two types of ambush predators (raptor vs. snake), under two levels of predation risk (low vs. high). These lizard species are sympatric, but not syntopic; *L. chiliensis* basks on bushes, a complex microhabitat that may provide protection against visual predators, while *L. nitidus* prefers open microhabitats, basking on the top of large bare rocks, highly exposed to visual predators. If microhabitat complexity modulates the antipredator response, *L. chiliensis* may perceive lower predation risk, exhibiting lower intensity of antipredator responses than *L. nitidus*. Both species reduced their activity after being exposed to both predators, but lizards differed in the assessment of predation risk; *L. nitidus* reduced its activity independently of the predation risk experienced, while *L. chiliensis* only reduced its activity in the high-risk condition. The microhabitat preferences shaped during the evolution of these species seem to modulate their perception of predation risk, which may cause interspecific differences in the associated costs of their antipredator responses.

1. Introduction

Avoiding predators is among the main challenges that most animal species experience through their life, or at least during early life stages (Caro, 2005). Some species have evolved permanent protection against predation (e.g., shells, spines), but taxa lacking such "armour" can be more vulnerable to predation while they fulfil their different requirements (e.g., search for food; Caro, 2005). There is, therefore, positive selection on prey that minimize predation risk while attending other demands, and that adjust antipredator responses according to the perceived predation risk (Lima and Dill, 1990; Eilam, 2005; Ydenberg and Dill, 1986; Endler, 1991). These strategies, further than keeping the animals alive, may allow them to maintain a positive time and energy balance with obvious fitness benefits (e.g., Lima, 1998; Cooke et al., 2003; Lima and Bednekoff, 1999; Cooper and Blumstein, 2015; Lima and Dill, 1990).

The assessment of predation risk varies depending on factors associated with predators, prey and to the conditions in which predator-prey interactions take place (Lima and Dill, 1990; Stankowich and Blumstein, 2005; Cooper and Blumstein, 2015). Factors associated with

predators include, for example, strategies used to kill the prey (e.g., venomous vs. non-venomous snakes; Sherbrooke, 2008), the speed, size and directness in approaching prey (Stankowich and Blumstein, 2005), the predator type (e.g., terrestrial vs. aereal; Ito and Mori, 2012), and visual capacities (e.g., snake vs bird; Stuart-Fox et al., 2008). Some of the factors associated with prey include size (Cooke et al., 2003), colour patterns (e.g., Carretero et al., 2006; Tan et al., 2016), proximity to a refuge (Bonnot et al., 2017), and the body parts contacted by the predator during an interaction (Ducey and Brodie, 1983; Langkilde et al., 2004). Data suggest, however, that the characteristics of the habitat in which the interaction occur may be a major driving force modulating assessment of predation risk (Endler 1980). Complex environments (e.g., high vegetation cover; Denno et al., 2005) may provide high degree of protection towards predation (Castilla and Labra, 1998), or may reduce the ability of the predator to forage (Denno et al., 2005; Warfe and Barmuta, 2004), and therefore prey may experience less predation risk (Cuadrado et al., 2001; Catano et al., 2016). Complex microhabitats do not always confer protection to prey, however, because complexity may reduce the ability to detect predators (Catano et al., 2016), and may support high predator densities (Denno et al.,

2005), among other because complexity enhances predator foraging activity, e.g., prey ambushing (Stellatelli et al., 2015; Klecka and Boukal, 2014). Therefore, microhabitat complexity per se does not allow predicting the predation risk assessment by prey, as this is highly dependent on the predator-prey system (Gorini et al., 2012). Hence, sympatric prey species exposed to the same predators, but having different microhabitat preferences, may experience different predation risk (Coleman and Hill, 2014).

We tested whether the microhabitat preferences shaped during the evolution of two sympatric, but not syntopic *Liolaemus* lizards (bush- vs. rock dweller; see below), affected their antipredator responses under standardized conditions. Both species are exposed to the same predators. The most important ones are the American kestrel (*Falco sparverius*) and the long-tailed snake (*Philodryas chamissonis*), as their diets include the highest percentage of lizards among all the sympatric predators of these lizard species (Jaksić et al., 1982; Jaksić et al., 1981; Greene and Jaksić, 1992). The American kestrel (henceforth: the raptor) most frequently uses a perch-hunting strategy, and its hunting success decreases with vegetation height (Toland, 1987); therefore, complex environments (e.g., bushes), may provide protection against this predator. The long-tailed snake (henceforth: the snake) is an ambush predator (Labra and Hoare, 2015) normally found on rocks, low vegetation pastures or open ground (Mella, 2005). This snake has rarely been observed on bushes (M. Weymann, comm. pers.) or trees (Escobar and Vukasovic, 2003), suggesting poor climbing abilities, and therefore that the complexity of bushes may provide protection against this predator.

The lizards studied were *L. chiliensis* and *L. nitidus*. The first one is a bush-dwelling species that normally perches on branches (Mella, 2005), although occasionally it is observed on the ground underneath bushes (Constanzo-Chávez, pers. obs.). The dorsal colour pattern of *L. chiliensis*, green and brown stripes and spots, provides good camouflage on branches, and also on grass (Labra, pers. obs.). This camouflage together with the structural complexity of its microhabitat may provide efficient protection against predators, and particularly to visually oriented predators, such as the raptor. The snake, however, may impose a threat to *L. chiliensis*, as it occasionally perches on the same bushes as the lizard (M. Weymann, comm. pers.). In fact, snake scents trigger a reduction of activity in *L. chiliensis*, which would increase its probability to remain undetected by the snake, which uses movements to detect prey (Labra and Hoare, 2015). The other lizard species, *L. nitidus*, is a rock dweller that usually basks on the top of large bare rocks (Mella, 2005; Jaksić and Fuentes, 1980a), a microhabitat that may cause vulnerability to visual predators, and thus, *L. nitidus* may be subjected to, and may perceive, high predation risk. *Liolaemus nitidus* has a higher proportion of autotomized tails than *L. chiliensis* (Núñez and Yáñez, 1984; and Fuentes, 1980b, 1980a;), which suggests that it may suffer a higher predation rate (Bateman and Fleming, 2009; but see Medel et al., 1988). Furthermore, the snake responds with hunting behaviour to scents of *L. nitidus*, but not to those of *L. chiliensis,* suggesting that this snake species may have under natural conditions, a higher rate of encounters with *L. nitidus* than with *L. chiliensis* (Labra and Hoare, 2015).

We compared the antipredator responses of these bush- and rock-dwelling lizards when confronted with the raptor and the snake, under standardized conditions, at two levels of predation risk, low (predator presence) and high (predator attack). If environmental complexity modulates antipredator responses, the rock dweller would be more responsive to predators, independent of the risk level, while the bush dweller would modulate its response according to the risk, i.e., stronger response with higher risk. In addition, the bush rather than the rock dweller may perceive less predation risk with the raptor, while the opposite would occur with the snake.

2. Methods

2.1. Animals and their maintenance

During the spring of 2010, we captured 20 *L. nitidus* (9 ♀, 11 ♂ mean snout-vent length: 83.2 ± 1.8 mm) in El Tabo (33°29′S, 71°37′W) and 21 *L. chiliensis* (3 ♀, 18 ♂; mean snout-vent length: 80.6 ± 1.5 SE, mm) in Melipilla (33°41′S, 71°13′W). The sex bias in *L. chiliensis* was a consequence of many gravid females not being included because pregnancy affects antipredator displays (Bauwens and Thoen 1981). Both predators were spotted in both collecting sites (Constanzo-Chávez, pers. obs.).

Lizards were transported to the laboratory in individual cloth bags moistened with water and placed in a plastic container to prevent stress, overheating, and dehydration. In the laboratory, we placed lizards inside an indoor vivarium and housed them individually in plastic enclosures (44 × 32 × 25 cm) having two windows covered with plastic mesh to provide extra climbing surface. The enclosures had a substrate of a 3-cm sand layer, and contained a small clay pot to keep water continuously, a wooden stick to be used as a perch, and an inverted clay pot to provide a refuge and a basking place. The vivarium was equipped with halogen lights to maintain similar conditions as those experienced by lizards in their habitats in normal sunny days, a photoperiod of 13:11 L:D with an associated thermal range of 33–12 °C. Lizards were fed with *Tenebrio mollitor* larvae dusted with vitamins (SERA reptimineral C), three times per week. Before beginning the experiments, lizards were allowed 1 wk of habituation to the vivarium conditions. Once all the experiments were concluded, lizards were released in healthy condition at their geo-referenced collecting points.

2.2. Experimental design

We conducted experiments in an acoustically isolated room, using an experimental arena that consisted in an acrylic enclosure (80 × 40 × 40 cm) divided in two equal halves (40 × 40 cm each) by two removable acrylic plates, a transparent and an opaque one. We placed the lizard in one section (henceforth: the experimental section), which was maintained at ∼ 35 °C, the selected body temperature of these species (Labra et al., 2009). This section floor had a thin brown carpet (40 × 40 cm) to prevent the lizard from slipping (each lizard had its own carpet), and we placed the refuge that the focal lizard had in its maintenance enclosure to provide a familiar shelter.

For the experiments, we removed the focal lizard from its maintenance enclosure and kept it in a cloth bag for 10 min (each lizard had its own bag) to reduce the handling-associated stress (Labra, 2011). Thereafter, the bag was carefully opened on the floor of the experimental section, allowing the lizard to exit freely. After that, we removed the bag and adjusted a mobile wall between the arena and the experimenter to not disturb the lizard. Suspended at about 130 cm above the experimental section, a camcorder connected to a television monitor allowed taping and control of the lizard behaviour. The record started after the lizard made the first tongue flick, the onset of the chemical exploration (Labra, 2011); if the lizard failed to tongue flick after 20 min in the experimental section, the trial was cancelled. Experiments had three stages: 1- Pre-stimulus (baseline period; 7 min), 2- stimulus (confrontation with the predator, 30 s), and 3- post-stimulus (7 min). At the end of this 14.5 min experimental period, we measured the cloacal temperature to ensure that lizards were close to the species' selected body temperature, 35 ± 2 °C (Labra et al., 2009); we did this to avoid behavioural differences due to variation in body temperature. Thereafter, we placed the lizard back in its maintenance enclosure together with its refuge. Lizards were given an inter-trial resting period of two days.

After a trial, we cleaned the experimental section with alcohol to remove potential chemical traces left by the focal lizard, and replaced the carpet for the new focal lizard. This procedure precluded that the

lizard behaviour could be influenced by the scents of the previous individual, as both *Liolaemus* species react to conspecific scents (Troncoso-Palacios and Labra, 2012; Labra and Hoare, 2015). We used disposable gloves during the whole procedure, replacing them between trials.

Using a counterbalanced design to avoid the effect of the treatment order, all lizards were subjected individually only once to each of the four treatments that resulted from the combination of the two factors, predator type (aerial –raptor– vs. terrestrial –snake–) and predation risk level (low –predator presence– vs. high –predator attack–). We used three-dimensional realistic models of the predators, considering that models provoke similar responses as real predators (e.g., Stuart-Fox et al., 2006; Carlile et al., 2006; Ito and Mori, 2012; Catano et al., 2016). In addition, models allowed determining responses to stereotyped predator behaviours as well as to avoid the risk of wounds or death that real predators could inflict to lizards. To standardize the methodology, only one person (JC-C) performed all the experiments.

2.2.1. Terrestrial predator

We used a rubber model of an adult *P. chamissonis* (68 cm snout-vent length, Greene and Jaksić, 1992), handled through transparent wires inserted in its anterior third, allowing high maneuverability of the model. The two levels of predation risk were: *A- low (predator presence)*: we placed the snake in the non-experimental section and exposed it to the lizard by raising the opaque acrylic plate for 30 s. During this period, the anterior third of the snake was lifted ∼13 cm above the floor and moved horizontally, one time to each side, and then lowered to the floor, followed by lowering the opaque barrier. *B- High (predator attack)*: the snake was introduced in the experimental section hanging from transparent wires, as in an unexpected attack. The snake "remained" quiet for 15 s on the floor, with its head at ∼10 cm from the lizard trunk. Then, it "attacked" the lizard's trunk by "touching" it twice with its snout. After that, the snake was removed in the same way as it was placed. The snake movements were based on previous observations on prey attacks (JC-C pers. obs.).

2.2.2. Aerial predator

We used a plastic model of a flying raptor painted realistically as a *Falco sparverius* attached to a rod. The model had the size of a small adult (17.5 cm long wing; Pearlstine and Thompson, 2004). The two levels of predation risk were: *A- Low (predator presence)*: at 50 cm above the experimental section, the raptor "flew" below a light projecting its shadow over the floor and the lizard, which may alert the lizard on the raptor presence (Cooper, 2009). The raptor made linear displacements, making three flights of 3 s, with breaks between flights of 7 s, simulating a flush fight. *B- High (predator attack)*: as in the previous experiment, the raptor was driven in one linear displacement above the experimental section, and then "flew" directly towards the lizard in a diagonal trajectory with a speed of ∼0.5 m/s. The raptor "touched" twice the lizard's back with its claws, and thereafter, it "flew" away at the same speed as before. The raptor movements were based on observations from videos available on the web.

2.3. Analyses

2.3.1. Behaviour

From the videos, we measured for each of the three experimental stages four variables: 1- Time in motion (s): the total time during which the lizard changed its body position, either in displacements or just moving part of its body (e.g., head movements), excluding the time when it exhibited the behaviours described below (Labra and Hoare, 2015). 2- Chemical exploration: frequency of tongue flicks to the substrate and/or air, i.e., fast protrusions and retractions of the tongue, which enable lizards to obtain chemical information (Labra and Hoare, 2015). 3-Tail waving (s): total time that the complete tail or its distal portion was moved from side to side (Troncoso-Palacios and Labra,

2012). 4- Refuge use (s). The time that the lizard was completely or partially inside the refuge. Finally, in the post-stimulus stage we recorded, 5- the latency to activity (s), i.e., the period elapsed since the end of the stimulus until the lizard performed any activity (e.g., motion; Hoare and Labra, 2013).

2.3.2. Statistics

We measured the behavioural changes induced by the predatory stimuli, by computing the pre- to post-stimulus differences of the analysed variables (i.e., post-stimulus values minus pre-stimulus values). Using General Linear Models with a partially nested three-way repeated-measures design, we determined the effect of microhabitat preferences of the two *Liolaemus* species (*L. nitidus* –simple microhabitat– vs. *L. chiliensis* –complex microhabitat–), type of predator, predation risk level, and their interactions, upon two behavioural changes (time in motion and chemical exploration), the latency to activity, and time in motion during the confrontation with predators. The within-subject variables (repeated-measurements) were the predator type and predation risk level, while the between-subject variable was species (i.e., microhabitat preference). Analyses were followed by post-hoc Fisher LSD tests. The residuals of these four variables were homoscedastic and normally distributed; however, time in motion during the stimulus was $\log_{10}$ transformed to achieve normality of the residuals. Few individuals displayed the other variables (refuge use, tail waving, and chemical exploration during the stimulus), and their residuals were not normally distributed. These variables were analysed using non-parametric, Friedman Anova and Wilcoxon Matched-Pairs tests for repeated measurements, and Mann-Whitney *U* test for independent measurements.

3. Results

The main response exhibited by both lizard species after the predatory stimuli was a decrease in two measurements of activity, i.e., time in motion and chemical exploration. The species, however, differed in the magnitude of this change (Table 1); *L. nitidus* exhibited a larger decrease in the activity than *L. chiliensis* (Fig. 1: A –time in motion–, B –chemical exploration–). In addition, both activity variables were affected by the interaction between species and predation risk level (Table 1); *L. nitidus* reduced its activity, independent of the experienced predation risk, while *L. chiliensis* only reduced its activity after high risk (Fig. 1: C-time in motion-, D –chemical exploration–).

Table 1

Results of the General Linear Models with a partially nested three-way repeated-measure design to test the effect of microhabitat preferences of two *Liolaemus* species (*L. nitidus* –simple microhabitat– vs. *L. chiliensis* –complex microhabitat–), type of predator (aereal –raptor– vs. terrestrial –snake–), predation risk level (low –predator presence– vs. high –predator attack–) and their interactions, on behavioural changes (post-stimulus minus pre-stimulus values) of two variables (time in motion and chemical exploration), the latency to activity, and time in motion ($\log_{10}$ transformed) during the confrontation with predators. Values shown are the F-statistics (*p*-value). The degrees of freedom of the tests were 1, 39. Sample size: 41 individals (20 *L. nitidus* and 21 *L. chiliensis*). Statistically-significant tests (p < 0.05) are shown in bold.

|	Change Time in motion	Change Chemical exploration	Time in motion During stimulus	Latency to activity
Species	**5.89(0.019)**	**6.88(0.012)**	3.66(0.063)	0.64(0.428)
Predator	3.49(0.069)	0.15(0.696)	0.09(0.773)	1.26(0.268)
Risk	2.05(0.161)	1.16(0.288)	**121.01(< 0.001)**	0.27(0.608)
Species* Predator	0.07(0.790)	0.01(0.907)	1.35(0.253)	0.05(0.810)
Species* Risk	**4.23(0.046)**	**5.22(0.03)**	0.01(0.910)	0.87(0.355)
Predator *Risk	0.15(0.696)	0.02(0.89)	3.05 (0.089)	**5.39(0.026)**
Species* Predator *Risk	1.29(0.262)	0.19(0.67)	**7.65(0.009)**	0.18(0.675)
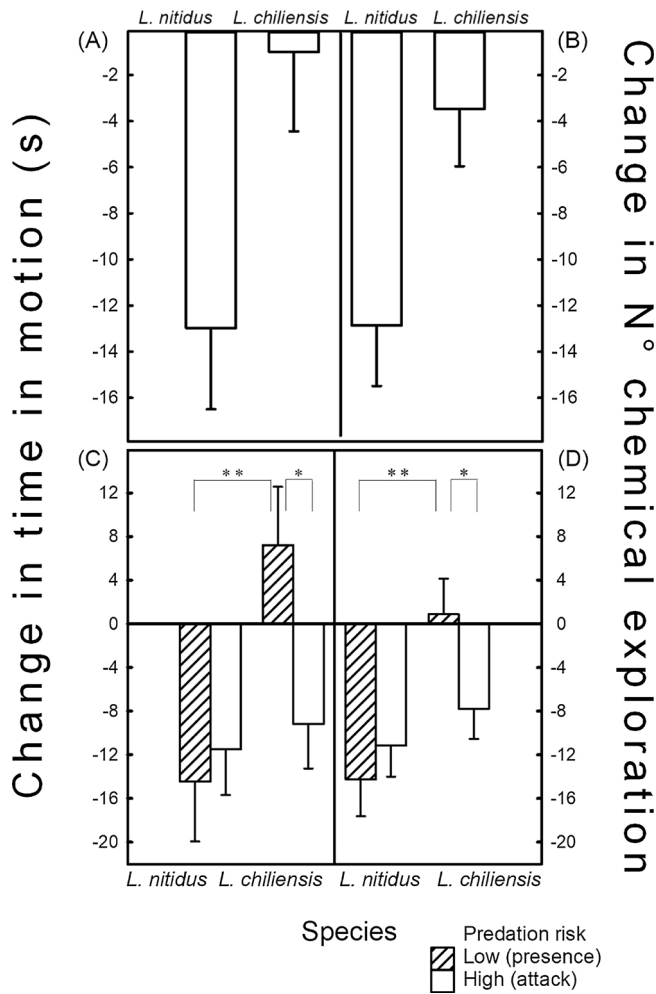
Fig. 1. Behavioral changes (mean $+/-$ SE) caused by the confrontation with two predators in two lizard species having different preferences for microhabitat complexity, *Liolaemus chiliensis* (bush dweller –complex microhabitat–) and *L. nitidus* (rock dweller –simple microhabitat–). Changes were obtained subtracting the post- from the pre-stimulus values. Negative values indicate that after the stimulus, the behaviour decreased in relation to the pre-stimulus condition. Changes in (A) time in motion and (B) chemical exploration. The figure also shows the effect of interaction between species and predation risk level upon the behavioural changes: (C) time in motion and (D) chemical exploration. Asterisks indicate significant differences between treatments obtained with post-hoc Fisher LSD tests: ** = P < 0.01 and * = P < 0.05.

A few individuals of both species used the refuge before and after the stimuli (Fig. 2A). There were, however, no significant changes in the use of this resource across the treatments (Fig. 2A; $\chi^2 = 3.93$; P = 0.27), between the two predators (Z = 1.49; P = 0.13), predation risk levels (Z = 0.19; P = 0.85), or between species in each treatment (Mann-Whitney tests, P > 0.05). Individuals of *L. chiliensis* waved their tails only during the pre-stimulus in four trials, so this species was excluded from the analyses of this behaviour. *Liolaemus nitidus* showed a tendency to increase tail waving after interacting with the snake (Fig. 2B), but there were no significant differences in tail waving among treatments ($\chi^2 = 3.00$; P = 0.39), type of predators (Z = 1.45; P = 0.15), or predation risk levels (Z = 0.05; P = 0.96).

Both species had similar latency to activity (Table 1). This variable, however, was affected by the interaction between predator type and predation risk level; both species had a longer latency after the raptor presence than after the attack. In addition, the latency was longer after the presence of the raptor than of the snake (Table 1, Fig. 3).

During the stimulus stage, the predation risk level significantly modulated the time in motion of the lizards (Table 1); both species moved more during the predator attack than during predator presence

(Fig. 4). Besides that, the interaction among the three factors affected this response (Table 1); both species moved more during the attacks than during the presence of both predators (Fig. 4). Moreover, *L. chiliensis* moved more than *L. nitidus* in the presence of the raptor than in the presence of the snake (Fig. 4). In contrast, chemical exploration did not differ among treatments ($\chi^2 = 3.62$; P = 0.31), but lizards explored chemically more during predator attacks than during predator presence (Z = 2.03; P = 0.04). Chemical exploration, however, did not differ between predation risk levels (Z = 0.03; P = 0.97), or between species in the different treatments (Mann-Whitney tests, P > 0.05). Species did not significantly use the refuge during the stimuli (Fig. 2A), which precluded further analyses. Finally, *L. nitidus* tended to show more tail waving during the predator attacks (Fig. 2B), but there were no significant differences in this behaviour among treatments ($\chi^2 = 6.08$; P = 0.11), predator type (Z = 0.10; P = 0.92), or predation risk level (Z = 1.36; P = 0.17).

4. Discussion

The main response displayed by the two *Liolaemus* species after the experimental interaction with predators, was a reduction of activity (i.e., time in motion and chemical exploration). Decreasing activity would enhance the probability of remaining undetected by the predator (Lima, 1998; Lima and Dill, 1990), because many predators have visual sensitivity to moving objects (Ruxton et al., 2004) and their prey capture rate increase when prey move (e.g., Skelly, 1994). In fact, for the two ambush predators included in this study, *Falco sparverius* and *Philodryas chamissonis*, prey movement is a key factor in detection and attack (Sarno and Gubanich, 1995; Labra and Hoare, 2015; Toland, 1987).

The two *Liolaemus* species seem, however, to differ in how they assess predation risk; the species that under natural conditions prefers open microhabitat, *L. nitidus*, decreased its activity, while *L. chiliensis,* from the complex environment, kept its activity relatively unchanged. Moreover, *L. nitidus* reduced its activity similarly after exposure to both levels of predation risk, while as predicted, *L. chiliensis* only decreased its activity after exposure to high predation risk. This suggests that, at least under these experimental conditions, *L. nitidus*, in contrast to *L. chiliensis*, may not assess predation risk level effectively, and its strategy to decrease the risk of mortality may be to reduce activity, regardless of the real predation risk involved (low vs. high). Under natural conditions, however, this apparent lack of discrimination may have costs, such as impairing the maintenance of an adequate body temperature (Polo et al., 2005), or loss of foraging or mating opportunities (Martín et al., 2003b; Martín et al., 2003a; Cooper and Sherbrooke, 2013; Catano et al., 2016). Overall, the interspecific behavioural differences in antipredator response of these *Liolaemus* species support the relevance of microhabitat preferences in the assessment of predation risk; these sympatric, but not syntopic species, may have experienced different selective pressures from the same predators (e.g., Coleman and Hill, 2014).

Both lizard species are exposed to the same predators (Jaksić et al., 1982; Jaksić et al., 1981; Greene and Jaksić, 1992), and interspecific differences in the frequency of autotomized tails most likely reflect variation in predation rate (Bateman and Fleming, 2009). Therefore, *L. nitidus* with a higher proportion of broken tails (Núñez and Yáñez, 1984; and Fuentes, 1980b, 1980a;), may suffer a higher predation rate than *L. chiliensis*. This hypothesis is supported by the positive relation between predation rate and the diversity of antipredator responses (Schall and Pianka, 1980); *L. nitidus* exhibited tail waving (see also Troncoso-Palacios and Labra, 2012), an antipredator response that is almost unobserved in *L. chiliensis*. Tail waving can deflect attacks towards the tail, an expendable body part, allowing lizards to escape (Bateman and Fleming, 2009). Therefore, tail waving and autotomy may be used by *L. nitidus* to escape predation, which may partially explain its high frequency of tail loss, even though autotomy is
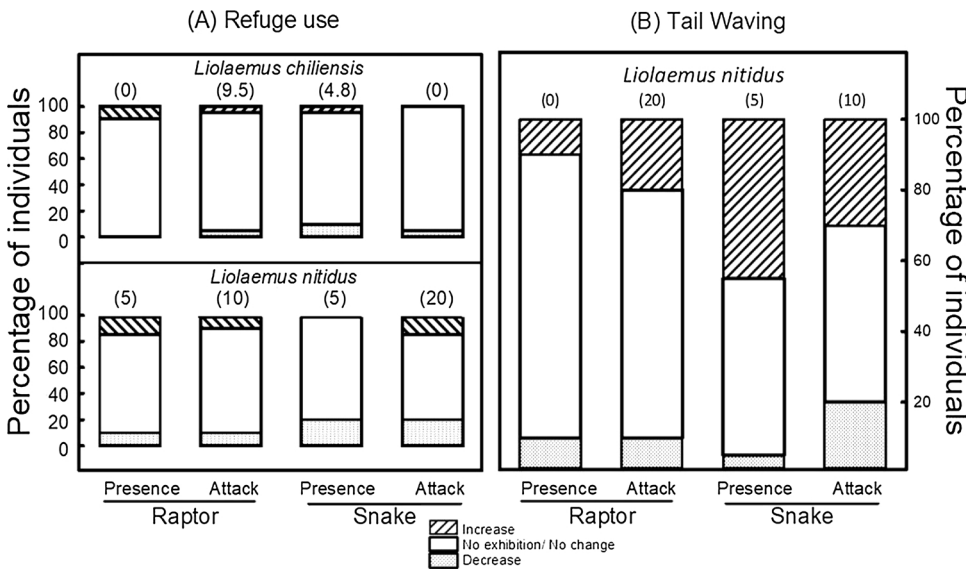
Fig. 2. Percentage of lizards that did not exhibit the behaviour across the whole experiment, and those that increased or decreased the exhibition of the behaviour after the interaction with predators, in four treatments. (A) Refuge use observed in *Liolaemus chiliensis* and *L. nitidus*. (B) Tail waving observed in *L. nitidus; L. chiliensis* did not show this behaviour (see Materials and Methods). In parentheses above the bars are the percentages of lizards that exhibited a behaviour (i.e., refuge use, tail waving) during the stimulus stage.
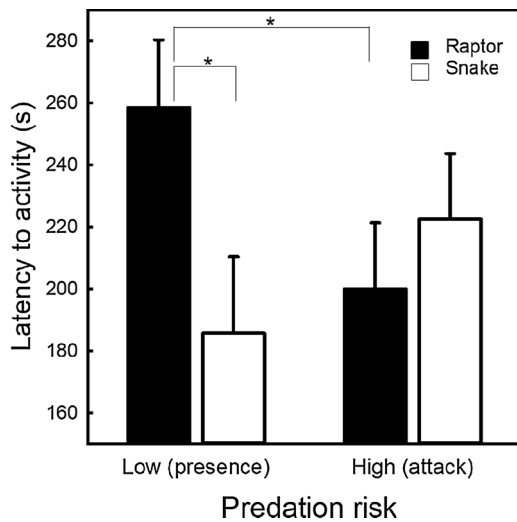

Fig. 3. Mean latency to activity (+SE) of *Liolaemus nitidus* and *L. chiliensis* after being exposed to two predators (raptor vs. snake), with two predation risk levels (low vs. high). Asterisks indicate significant differences between treatments obtained with post-hoc Fisher LSD tests: * = P < 0.05.
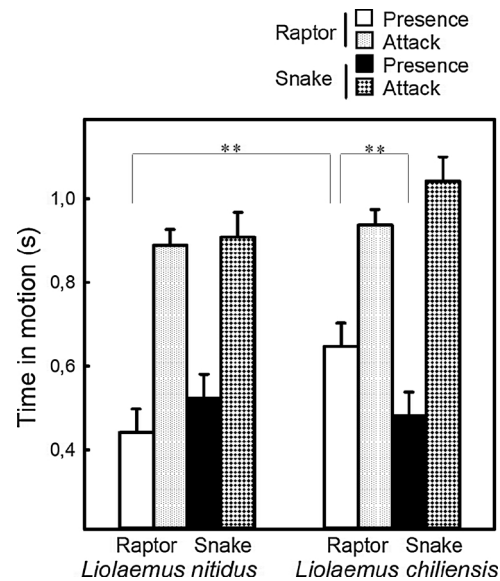

Fig. 4. Untransformed data of the mean time in motion (+SE) recorded for *Liolaemus nitidus* and *L. chiliensis* during the confrontation with two predators (raptor vs. snake), with two predation risk levels (low vs. high). Asterisks indicate significant differences between treatments obtained with post-hoc Fisher LSD tests: ** = P < 0.01.

energetically expensive for this species (Naya and Bozinovic, 2006). We cannot reject, however, that the lower occurrence of tail loss in *L. chiliensis* is less related to lower predation rates than to a higher cost, and thus threshold, for autotomy than in *L. nitidus* (e.g., Fox et al., 1998). *Liolaemus chiliensis* has a longer tail than *L. nitidus* (Jaksić et al., 1980), and regeneration would thus demand more energy than in *L. nitidus*. Furthermore, as Jaksić et al. (1980) have suggested, the tail may help *L. chiliensis* when its moves in the bushes. In fact, long tails are common in arboreal species, and their loss reduce their stability during locomotion (Gillis et al., 2009).

During the experimental conditions, lizards used the refuge infrequently, not only after been exposed to predators, but even while they were being attacked. This contrasts with the response observed in other species (e.g., Ventura et al., 2017; Polo et al., 2005). For *L. chiliensis* and *L. nitidus*, inactivity may constitute a simple but effective primary low-cost antipredator response. In fact, during the interactions with predators, lizards spent less time in motion during the presence of predators than during the attack, supporting the proposition that a reduction of activity can be an efficient defence against the nearby presence of the two tested predators. In contrast, increased movements

(e.g., run) would be a more efficient strategy under high predation risk (e.g., Cuadrado et al., 2001; Carretero et al., 2006). Remarkably, the longer time in motion went together with a higher chemical exploration, which may allow lizards obtaining information about safety areas or predator characteristics (e.g., Labra, 2006).

The time in motion during the stimulus stage was affected by all the factors considered in the study; *L. chiliensis* moved more in presence of the raptor than *L. nitidus*, and *L. chiliensis* also moved more in presence of the raptor than of the snake. This suggests that for *L. chiliensis* the snake may be a more dangerous predator than the raptor. Under natural conditions, *L. chiliensis* can easily escape from a raptor by going deeper inside the bushes. In contrast, during snake presence, movements can be risky because they increase the probability that the snake will detect and attack the lizard (Labra and Hoare, 2015), and particularly, if the lizard remains within striking distance (e.g., Clark et al., 2012).

Liolaemus chiliensis is known as the weeping lizard as it emits distress calls, a behaviour displayed mainly when it is subdued (Labra et al., 2013). It could be expected that this lizard vocalized at least

during the predator attacks or before any physical contact with the predator, as other lizard species do (e.g., Labra et al., 2007). However, none of the individuals vocalized, which suggests that the primary function of this vocal display in *L. chiliensis* is not to deter a predator attack (e.g., Hasson, 1991), but rather, that it is a warning signal for conspecifics (Hoare and Labra, 2013; Labra et al., 2016). It remains unclear, however, why there are no selective pressures for this species to vocalize before being subdued, considering that at least this snake species responds to *L. chiliensis* vocalizations with a reduction of the activity, which may decrease its hunting success or provide escape options for the lizards (Hoare and Labra, 2013).

Experimental evidence indicates that the raptor is more efficient in catching *Liolaemus* lizards than the snake, as more lizards escape by tail autotomy during a snake attack (Medel et al., 1988). Based on this information, and acknowledging that prey tend to display predator specific defences (e.g., Botham et al., 2008; Cooper et al., 2008; Ito and Mori, 2012; Ventura et al., 2017), it could be expected that these lizard species react differently to both predators. However, there was little evidence supporting this, possibly because both lizards are mainly responding to an ambush hunting strategy rather than to the specific predator. In fact, hunting strategies, particularly the ambush tactic, can have a strong effect in shaping antipredator strategies (Preisser et al., 2007). The weak evidence for predator recognition in the case of *L. chiliensis* comes by differences in motion time during the presence of the predators, as we previously discussed. Besides, we found that both lizard species had longer latency to activity after raptor presence than after snake presence or raptor attack. This suggests that lizards perceive the raptor presence as more dangerous than the snake presence, probably because it is more difficult for lizards to determine if a raptor is still perched in the surroundings after its disappearance. In contrast, lizards can visually or chemically detect the presence of a snake if it remains in distance for another attack (e.g., Labra and Hoare, 2015). Therefore, the feasibility for detecting the presence of predators after an unsuccessful attack rather than the identity per se of the predator may modulate the latency to activity observed in these two lizard species.

We conclude that these sympatric, but not syntopic, *Liolaemus* species may experience different selective pressures from their shared predators, highlighting the role of microhabitat preferences in antipredator behaviour; the species inhabiting complex microhabitats seems to better asses variation in predation risk. Moreover, the differential preference for microhabitat complexity shaped during the evolution of these species, may cause differences in the associated costs of their antipredator strategies, both direct (e.g., loss foraging opportunities, tail regeneration), and indirect (e.g., growth rate; Preisser et al., 2007). A phylogenetic study including more *Liolaemus* species that share predators, but differ in microhabitat preferences (see Mella, 2005), may contribute to clarify the strength of the present results in an evolutionary context.

Acknowledgements

References

Bateman, P.W., Fleming, P.A., 2009. To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. J. Zool. 277, 1–14.
Bauwens, D., Thoen, C., 1981. Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. J. Anim. Ecol. 50, 733–743.
Bonnot, N.C., Hewison, A.J.M., Morellet, N., Gaillard, J.M., Debeffe, L., Couriot, O., Cargnelutti, B., Chaval, Y., Lourtet, B., Kjellander, P., Vanpe, C., 2017. Stick or twist: roe deer adjust their flight behaviour to the perceived trade-off between risk and reward. Anim. Behav. 124, 35–46.
Botham, M.S., Hayward, R.K., Morrell, L.J., Croft, D.P., Ward, J.R., Ramnarine, I., Krause, J., 2008. Risk-sensitive antipredator behavior in the trinidadian guppy, *Poecilia reticulata*. Ecology 89, 3174–3185.
Carlile, P.A., Peters, R.A., Evans, C.S., 2006. Detection of a looming stimulus by the Jacky dragon: selective sensitivity to characteristics of an aerial predator. Anim. Behav. 72, 553–562.
Caro, T.M., 2005. Antipredator Defenses in Birds and Mammals. The University of Chicago Press London, pp. 591.
Carretero, M.A., Vasconcelos, R., Fonseca, M., Kaliontzopoulou, A., Brito, J.C., Harris, D.J., Perera, A., 2006. Escape tactics of two syntopic forms of the *Lacerta perspicillata* complex with different colour patterns. Can. J. Zool. 84, 1594–1603.
Castilla, A.M., Labra, A., 1998. Predation and spatial distribution of the lizard *Podarcis hispanica atrata*: an experimental approach. Acta Oecol. 19, 107–114.
Catano, L.B., Rojas, M.C., Malossi, R.J., Peters, J.R., Heithaus, M.R., Fourqurean, J.W., Burkepile, D.E., 2016. Reefscapes of fear: predation risk and reef heterogeneity interact to shape herbivore foraging behaviour. J. Anim. Ecol. 85, 146–156.
Clark, R.W., Tangco, S., Barbour, M.A., 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). Anim. Behav. 84, 183–190.
Coleman, B.T., Hill, R.A., 2014. Living in a landscape of fear: the impact of predation, resource availability and habitat structure on primate range use. Anim. Behav. 88, 165–173.
Cooke, S.J., Steinmetz, J., Degner, J.F., Grant, E.C., Philipp, D.P., 2003. Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. Can. J. Zool. 81, 699–709.
Cooper Jr., W.E., Blumstein, D.T., 2015. Escaping From Predators: an Integrative View of Escape Decisions. Cambridge University Press, Cambridge.
Cooper Jr., W.E., Sherbrooke, W.C., 2013. Risk and cost of immobility in the presence of an immobile predator. Behav. Ecol. Sociobiol. 67, 583–592.
Cooper Jr., W.E., Caldwell, J.P., Vitt, L.J., 2008. Escape responses of cryptic frogs (Anura: Brachycephalidae: *Craugastor*) to simulated terrestrial and aerial predators. Behaviour 145, 25–38.
Cooper Jr., W.E., 2009. Rapid covering by shadow as a cue to predation risk in three lizard species. Behaviour 146, 1217–1234.
Cuadrado, M., Martín, J., López, P., 2001. Camouflage and escape decisions in the common chameleon *Chamaeleo chamaeleon*. Biol. J. Linnean. Soc. 72, 547–554.
Denno, R.F., Finke, D.L., Langellotto, G.A., 2005. Direct and indirect effects of vegetation structure and habitat complexity on predator-prey and predator–predator interactions. In: Barbosa, P., Castellanos, I. (Eds.), Ecology of Predator-Prey Interactions. Oxford University Press, New York.
Ducey, P.K., Brodie, E.D., 1983. Salamanders respond selectively to contacts with snakes: survival advantage of alternative antipredator strategies. Copeia 1983, 1036–1041.
Eilam, D., 2005. Die hard: a blend of freezing and fleeing as a dynamic defense – implications for the control of defensive behavior. Neurosc. Biobehav. Rev. 29, 1181–1191.
Endler, J.A., 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34, 76–91.
Endler, J.A., 1991. Interaction between predators and prey. In: Krebs, J., Davis, N. (Eds.), Behavioral Ecology: an Evolutionary Approach. Blackwell Scientific, Oxford.
Escobar, M.A., Vukasovic, M.A., 2003. Depredación de *Philodryas chamissonis* (Serpentes: Colubridae) sobre polluelos de *Aphrasthura spinicauda* (Passeriformes: Furnariidae): ¿una culebra arborícola? Noticiario Mensual Mus. Nac. Hist. Nat. 353, 18–20.
Fox, S.F., Conder, J.M., Smith, A.E., 1998. Sexual dimorphism in the ease of tail autotomy: *Uta stansburiana* with and without previous tail loss. Copeia. pp. 376–382.
Gillis, G.B., Bonvini, L.A., Irschick, D.J., 2009. Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. J. Exp. Biol. 212, 604–609.
Gorini, L., Linnell, J.D.C., May, R., Panzacchi, M., Boitani, L., Odden, M., Nilsen, E.B., 2012. Habitat heterogeneity and mammalian predator-prey interactions. Mammal Rev. 42, 55–77.
Greene, H.W., Jaksić, F.M., 1992. The feeding behavior and natural history of two Chilean snakes, *Philodryas chamissonis* and *Tachymenis chiliensis* (Colubridae). Rev. Chil. Hist. Nat. 65, 485–493.
Hasson, O., 1991. Pursuit-deterrent signals: communication between prey and predator. Trends Ecol. Evol. 6, 325–329.
Hoare, M., Labra, A., 2013. Searching for the audience of the weeping lizard's distress call. Ethology 119, 860–868.
Ito, R., Mori, A., 2012. The Madagascan spiny-tailed iguana alters the sequence of antipredator responses depending on predator types. Afr. J. Herpetol. 61, 58–68.
Jaksić, F.M., Fuentes, E.R., 1980a. Autecological observations on *Liolaemus nitidus* (Lacertilia, Iguanidae). Stud. Neotrop. Fauna E. 15, 109–124.
Jaksić, F.M., Fuentes, E.R., 1980b. Correlates of tail losses in 12 species of *Liolaemus* lizards. J. Herpetol. 14, 137–141.
Jaksić, F.M., Núñez, H., Ojeda, F.P., 1980. Body proportions, microhabitat selection, and adaptive radiation of *Liolaemus* lizards in central Chile. Oecologia 45, 178–181.
Jaksić, F.M., Greene, H.W., Yáñez, J.L., 1981. The guild structure of a community of predatory vertebrates in central Chile. Oecologia 49, 21–28.
Jaksić, F.M., Greene, H.W., Schwenk, K., Seib, R.L., 1982. Predation upon reptiles in mediterranean habitats of Chile, Spain and California: a comparative analysis. Oecologia 53, 152–159.
Klecka, J., Boukal, D.S., 2014. The effect of habitat structure on prey mortality depends on predator and prey microhabitat use. Oecologia 176, 183–191.

Labra, A., 2006. Chemoreception and the assessment of fighting abilities in the lizard *Liolaemus monticola*. Ethology 112, 993–999.

Labra, A., 2011. Chemical stimuli and species recognition in *Liolaemus* lizards. J. Zool. 285, 215–221.

Labra, A., Hoare, M., 2015. Chemical recognition in a snake-lizard predator-prey system. Acta Ethol. 18, 173–179.

Labra, A., Sufán-Catalán, J., Solis, R., Penna, M., 2007. Hissing sounds by the lizard *Pristidactylus volcanensis*. Copeia 2007, 1019–1023.

Labra, A., Pienaar, J., Hansen, T.F., 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. Am. Nat. 174, 204–220.

Labra, A., Silva, G., Norambuena, F., Velázquez, N., Penna, M., 2013. Acoustic features of the weeping lizard's distress call. Copeia 2013, 206–212.

Labra, A., Reyes-Olivares, C., Weymann, M., 2016. Asymmetric response to heterotypic distress calls in the lizard *Liolaemus chiliensis*. Ethology 122, 758–768.

Langkilde, T., Shine, R., Mason, R.T., 2004. Predatory attacks to the head vs body modify behavioral responses of garter snakes. Ethology 110, 937–947.

Lima, S.L., Bednekoff, P.A., 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. Am. Nat. 153, 649–659.

Lima, S.L., Dill, L., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640.

Lima, S.L., 1998. Stress and decision making under the risk of predation: recent developments from behavioral reproductive, and ecological perspectives. Adv. Stud. Behav. 27, 215–290.

Martín, J., López, P., Cooper Jr., W.E., 2003a. Loss of mating opportunities influences refuge use in the Iberian rock lizard, *Lacerta monticola*. Behav. Ecol. Sociobiol. 54, 505–510.

Martín, J., López, P., Cooper Jr., W.E., 2003b. When to come out from a refuge: balancing predation risk and foraging opportunities in an Alpine lizard. Ethology 109, 77–87.

Medel, R.G., Jiménez, J.E., Fox, S.F., Jaksić, F.M., 1988. Experimental evidence that high population frequencies of lizard tail autotomy indicate inefficient predation. Oikos 53, 321–324.

Mella, J.E., 2005. Guía de Campo Reptiles de Chile: Zona Central. In: Peñaloza, A.P., Novoa, F.F., Contreras, M. (Eds.), Guía de campo reptiles de Chile. Zona central, Centro de Ecología Aplicada Ltda, Santiago, Chile.

Núñez, H., Yáñez, J.L., 1984. Colas de lagartijas de genero *Liolaemus*: autotomia e influencia en Ia predacion. Stud. Neotrop. Fauna E. 19, 1–8.

Naya, D.E., Bozinovic, F., 2006. The role of ecological interactions on the physiological flexibility of lizards. Funct. Ecol. 20, 601–608.

Pearlstine, E.V., Thompson, D.B., 2004. Geographic variation in morphology of four species of migratory raptors. J. Raptor Res. 38, 334–342.

Polo, V., López, P., Martín, J., 2005. Balancing the thermal costs and benefits of refuge use to cope with persistent attacks from predators: a model and an experiment with an alpine lizard. Evol. Ecol. Res. 7, 23–35.

Preisser, E.L., Orrock, J.L., Schmitz, O.J., 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator–prey interactions. Ecology 88, 2744–2751.

Ruxton, G.D., Sherratt, T.N., Speed, M.P., 2004. Avoiding Attack: the Evolutionary Ecology of Crypsis, Warning Signals and Mimicry. Oxford Univesity Press, Oxford, pp. 260.

Sarno, R.J., Gubanich, A.A., 1995. Prey selection by wild American Kestrels: the influence of prey size and activity. J. Raptor Res. 29, 123–126.

Schall, J.J., Pianka, E.R., 1980. Evolution of escape behavior diversity. Am. Nat. 115, 551–566.

Sherbrooke, W.C., 2008. Antipredator responses by Texas Homed Lizards to two snake taxa with different foraging and subjugation strategies. J. Herpetol. 42, 145–152.

Skelly, D.K., 1994. Activity level and the susceptibility of anuran larvae to predation. Anim. Behav. 47, 465–468.

Stankowich, T., Blumstein, D.T., 2005. Fear in animals: a meta-analysis and review of risk assessment. Proc. Roy. Soc. B-Biol. Sci. 272, 2627–2634.

Stellatelli, O.A., Block, C., Vega, L.E., Cruz, F.B., 2015. Nonnative vegetation induces changes in predation pressure and escape behavior of two sand lizards (Liolaemidae: *Liolaemus*). Herpetologica 71, 136–142.

Stuart-Fox, D.M., Whiting, M.J., Moussalli, A., 2006. Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. Biol. J. Linnean. Soc. 88, 437–446.

Stuart-Fox, D., Moussalli, A., Whiting, M.J., 2008. Predator-specific camouflage in chameleons. Biol. Letters 4, 326–329.

Tan, E.J., Reid, C.A.M., Elgar, M.A., 2016. Colour pattern variation affects predation in chrysomeline larvae. Anim. Behav. 118, 3–10.

Toland, B.R., 1987. The effect of vegetative cover on foraging strategies, hunting success and nesting distribution of American Kestrels in Central Missouri. J. Raptor. Res. 21, 14–20.

Troncoso-Palacios, J., Labra, A., 2012. Is the exploratory behavior of *Liolaemus nitidus* modulated by sex? Acta Herpetol. 7, 69–80.

Ventura, S.P., Passos, D.C., Machado, L.L., Horta, G., Galdino, C.A., 2017. Escape tactics by a neotropical montane lizard: a comparison of flight responses against natural and nonnatural predators. Acta Ethol. 20, 9–15.

Warfe, D.M., Barmuta, L.A., 2004. Habitat structural complexity mediates the foraging success of multiple predator species. Oecologia 141, 171–178.

Ydenberg, R.C., Dill, L.M., 1986. The economics of fleeing from predators. Adv. Stud. Behav. 16, 229–249.