ORIGINAL ARTICLE

Habitat dependent effects of experimental immune challenge on lizard anti-predator responses

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Abstract

Lizards often respond to predators by hiding in sunless refuges, but this eliminates opportunities for thermoregulatory basking. Hiding can therefore lower body condition. Furthermore, in ectotherms basking is important to induce fever and activate an immune response. A potential trade-off therefore exists between lowering predation risk and elevating body temperature to fight infection. Such a trade-off could be habitat dependent if habitats differ in the relative risk of predation versus that of acquiring or countering an infection. Here we take an experimental approach to test whether lizard basking behavior is affected by a trade-off between predator avoidance and fighting an infection. We quantified the anti-predator behavior of male lizards (*Podarcis liolepis)* both before and after they were immune challenged (injected with LPS) or not (injected with PBS control). To test the generality of any trade-off, we tested lizards from both an urban and a natural habitat. We found that males spent less time hiding following a simulated predator attack after they had been immune challenged than before, but this decline was only significant for males from the natural habitat. We also tested whether morphological traits, body condition, and immune response level explained variation in male hiding time. In the natural habitat, but not in the urban habitat, males with relatively small heads hid for significantly longer. In conclusion, we show that lizard anti-predator behavior is affected by an immune challenge. Habitat differences in the factors that predict hiding time offers potential insights into why this might be the case.

Significance statement

There is a potential trade-off for ectotherms between remaining in a place protected from predators and countering an immune challenge. This is because hiding in sunless refuges eliminates opportunities for thermoregulatory basking that induce a fever. The optimal response to this trade-off might change depending on the habitat. Here, we compare the hiding behavior of males from natural and urban habitat following an experimental immune challenge. We found that the hiding time of immune-challenged males decreased but only for those from the natural habitat.

Introduction

How animals allocate resources and time to different activities (e.g., predator avoidance, foraging, reproduction, immune defense) is determined by trade-offs between those activities. The exact trade-off varies when the costs and benefits of each activity differ for specific individuals (e.g., McGlothlin et al. 2007). Often these costs and benefits vary depending on both an individual's phenotype (e.g., French et al. 2007a), and on the local environment. For example, larger bodied individuals

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who are less vulnerable to predators spend more time foraging. Similarly, many studies have shown that individuals spend more time foraging when predation risk is low. Quantifying variation in trade-offs between behaviors that have important fitness consequences is key to understanding how these behaviors evolve. Human-modified environments, such as urban areas, differ from natural environments in many aspects, such as predatory pressure or resource availability (Gering and Blair 1999; Ditchkoff et al. 2006). Species inhabiting both habitat types provide a unique opportunity to explore how trade-offs vary in different circumstances.

Predator avoidance usually trades-off with other fitness-enhancing activities. For instance, in many species, individuals respond to predators by retreating to a refuge (Sih et al. 1992; Martín and López 1999a, b; Cooper and Blumstein 2015), but because refuges are suboptimal habitats prolonged use of a refuge can lower body condition (Martín and López 1999a) and reduce opportunities to mate or forage (Ydenberg and Dill 1986; Sih 1997; Martín and López 2003). In ectotherms, hiding in refuges (such as rock crevices which are often cold and sunless) can also impact their thermoregulation (Polo et al. 2005) with detrimental effects for their locomotion, growth, and reproduction (Stevenson et al. 1985; Deutsch et al. 2008). Individuals must therefore balance anti-predatory responses with other life history requirements (Lima and Dill 1990; Sih 1992), and selection should favor facultative adjustment of anti-predator behavior to the prevailing conditions (Lima and Dill 1990). In support of this, there is evidence that escape decisions are affected by perceived vulnerability (Cabido et al. 2009), predator abundance (Cooper et al. 2007), and the costs of refuge use (Cooper 1997; Martín and López 1999b, 2000; Cooper 2000).

Optimal anti-predator behavior can vary among individuals because behavioral, morphological, and physiological traits influence the relative gains from other fitness-enhancing activities. For example, an elevated immune response induced by pathogens often reduces reproductive activity (French et al. 2007b), sexual coloration (López et al. 2009a), and other behaviors (Llewellyn et al. 2011), potentially affecting the optimal anti-predator response. Vertebrates usually respond to infection by elevating their body temperature and inducing fever (Llewellyn et al. 2011). In reptiles an immune challenge is expected to directly affect the use of a refuge as an anti-predator behavior. Since reptiles are ectothermic, they need to elevate their temperature behaviorally by choosing microhabitats that increase net heat gain (Deen and Hutchison 2001), and/or by basking in the sun for longer periods (do Amaral et al. 2002). An immune challenge should therefore affect the optimal response to the inherent trade-off between escaping from a predator by retreating to a cool refuge and remaining in a warm, but exposed, site to fight infection (i.e., basking).

The Catalonian wall lizard (*Podarcis liolepis*) is a small lacertid distributed along the mortheast Iberian Peninsula, from the Basque Country to Catalonia. It naturally occurs in rocky habitats, but is also found in urban habitats occupying artificial structures. Human-modified environments have distinct biotic and abiotic characteristics, which can present different challenges for animals than are found in native habitat. Urban populations experience a variety of new conditions such as lower or higher predation risk (Gering and Blair 1999; e.g., pest species Sorace 2002), and unusual food sources (Ditchkoff et al. 2006) and breeding sites (Møller 2010). In some species that do well in urban areas, lower mortality and plentiful food improve physical condition (Contesse et al. 2004), and elevate reproductive rates, and population densities (Møller 2009). However, higher population density, alongside pollutants, can also increase disease transmission, so the prevalence of diseases is sometimes greater in urban than natural habitats (Ditchkoff et al. 2006). This can result in urban populations being in worse health (Cabido et al. 2008). If being in an urban habitat affects immune system activity and predation risk, the optimal anti-predator response might differ between natural and urban habitats.

Here we test for a trade-off between the response to an immune challenge and anti-predator behavior in male lizards (*P. liolepis*). We then test whether this trade-off is habitat dependent. First, we examined whether males from an urban and a natural habitat differed in body condition, health status (immune response), and morphological features (indicative of social dominance). Then we investigated whether habitat type influenced the relationship between any of these traits and anti-predator behavior. Finally, we ran an experiment to test whether males alter their anti-predator behavior after exposure to an immune challenge and to determine whether this response differed between urban and natural habitats. We predicted that:

1) there is a difference in body condition between males from urban and rural habitats. Better body condition might occur in urban areas because of higher food availability, or due to reduced predation pressure. On the other hand, poorer body condition might occur if predators, such as cats, are more abundant so that lizards have to invest more time hiding. We also predict a poorer immune response due to pollution and higher population densities that tend to increase disease transfer.
2) morphological features, such as relative head size or color badges, which are cues of social dominance, are associated with longer male hiding time.
3) immune-challenged individuals will leave a refuge sooner than healthy ones in order to bask to combat disease by elevating their body temperature.

Material and methods

We captured (by harmless noosing) 30 adult male *P. liolepis* in May 2012 in urban parkland in the middle of the city of Donostia/San Sebastián in Northern Spain, and 30 males in nearby natural habitat in Donibane/Pasajes San Juan. The urban population is surrounded by the city and attracts large crowds of tourists. Lizards mainly occupy human constructions, like fortifications, and the occasional rocky sandstone outcrops. Due to the reduced appropriate habitat, lizards live in a limited space, so the population density is higher than in natural habitat (ICM, personal observation). The natural population is in a natural landscape along coastal cliffs visited by fewer people. Lizards occupy rocky sandstone outcrops interspersed with shrubs and grasses. Urban areas contain predators (e.g., domestic cats, jays (*Garrulus glandarius*) and magpies (*Pica pica*) (Aierbe et al. 2001; CC and ICM, personal observation), but there are fewer predatory species than occur in their natural habitat. To reduce individual variation attributable to age, sex, and fitness effects due to tail loss, we only captured adult males with intact tails (e.g., Martín and López 2003).

Lizards were individually housed in 60 indoor 36 × 42 cm PVC terraria containing rocks for refuge heated by a UV-lamp. The lamp was situated at one end of the terrarium to ensure the presence of a heat gradient so that lizards could thermoregulate. The photoperiod mimicked that of the surrounding region. Water and food (mealworm larvae and crickets dusted with multivitamin powder) were provided ad libitum. Lizards were housed for at least a week to familiarize them with their new surroundings prior to testing. Placement of terraria within the laboratory was randomized to avoid potential environmental gradients within the laboratory confounding our treatments. All the animals were healthy during the trials and maintained their original body mass. They were returned to their capture sites at the end of the trials.

Morphological characteristics and immune response

We measured several morphological characteristics that correlate with anti-predator behavior in lizards. To estimate body condition, we used the residuals from the regression of log body mass on log snout vent length (SVL). The relationship between log transformed body mass (g) and log transformed snout vent length (mm) is linear ($p < 0.001$; $r^2 = 0.85$), and this index is expected to give an accurate estimate of relative amount of fat stored (review: Green 2001; see Garrido and Pérez-Mellado 2015).

We also measured three aspects of head size using digital calipers (±0.01 mm): *depth* was the greatest vertical distance through the snout from the top of the head to the bottom of the lower jaw; *length* was the distance between the tip of the snout and the posterior side of the parietal scales and; *width* was the maximal distance between left and right parietal scales. Since head size is related to body size, we calculated the residuals of each log-transformed head measure from a regression on log-transformed SVL to get head size measurements that were independent of body size. We then used Principal component analysis based on correlation matrix to reduce the head measurements into a single axis to describe the variation in these three measurements. PC1 (hereafter '*relative head size*') showed positive loading of depth (0.80), length (0.77), and width (0.84), and explained 65.7 % of the total variance. Relative head size might affect anti-predator behavior because it is commonly associated with social status and increased aggression during male-male contests in lizards, including *P. liolepis* (Anderson and Vitt 1990; López et al. 2002). Social status has been suggested to influence anti-predator behavior in lizards (Cooper and Wilson 2007). We also noted the number of ocelli (row of small distinctive blue spots along the outer margin of the belly) on each side of each male and calculated the mean number of ocelli per male. Ocelli might be correlated with anti-predator behavior because they have previously been shown to play a role in intra-sexual social relationship (López et al. 2004), and they affect a male's perception of his risk of predation in related species (Cabido et al. 2009).

To assess a component of male immunocompetence, we used a delayed-type hypersensitivity test: the phytohaemagglutinin injection assay (PHA test). This is a reliable measure of *T* cell-dependent immunocompetence in vivo (Lochmiller et al. 1993) that has been used in many studies, including those on lizards (Svensson et al. 2001). The activation of the *T* cells induces an inflammation at the place of injection of PHA, so we use the change in thickness as a proxy for the immune response.

We measured the thickness of the left hind footpad with a pressure-sensitive spessimeter (±0.01 mm) to standardize pressure. The measure was taken five times and we used the average measurement. Immediately after, we injected 0.02 mg of PHA dissolved in 0.01 ml of phosphate-buffered saline (PBS). Lizards were released into their terraria, and after 24 h we re-measured their footpad thickness. The immune response index was calculated as the difference between pre- and post-injection measurements (Lochmiller et al. 1993; Smits et al. 1999). As the thickness of the footpad might be related to body size, we calculated relative thickness, using the residuals from a linear regression of the log transformed immune response index on log SVL (hereafter '*PHA response*'). The only detectable effect of the injection was the skin swelling which disappeared after 48 h. None of the lizards showed any sign of stress or pain after the trials (all lizards behaved and fed normally after 15 min).

Anti-predator behavior

We studied the anti-predator behavior of males within 10 days of capture, from 11:00 to 15:00 h, when the captive lizards were fully active. We allowed lizards to thermoregulate for >1 h before each trial. In each trial, we simulated a predatory attack and video recorded male behavior for the following 30 min. We rapidly approached the terraria and tapped the male near his tail to simulate an avian predator attacking from above. We are confident that we simulated a predatory attack, because the flee response was similar to that observed in field experiments (Martín and López 1999a, b; CC, personal observation). We conducted two trials before the experimental immune activation (one per day over consecutive days), and two trials after the experimental immune activation (see below), for each lizard. The same person performed all predatory attacks. To minimize observer bias, blinded methods were used whenever behavioral data were recorded and/or analyzed. The only identification of individuals was a number from 1 to 60, randomly assigned with respect to treatment and site of origin.

For the 30 min after the simulated attack, we noted the time spent in the refuge until the male's head appeared at the refuge entrance as a standard measure of 'hiding time'. Trials in which males did not entirely leave the refuge to bask ($n = 3$ of 240 trials) were discarded, because their response was ambiguous. When a lizard did not lean his head out of the refuge after 30 min ($n = 9$ of 240 trials), we assigned a hiding time of 1,800 s.

Experimental immune challenge

We challenged the immune system of males with a lipopolysaccharide (LPS) from the cell wall of the bacteria *E. coli* (serotype 0111-B4; Sigma-Aldrich, St. Louis, MO). LPS has no pathogenic effects (Janeway et al. 2001), but induces an inflammatory response by nonspecifically activating B- and T-lymphocytes, and producing specific anti-LPS antibodies. It induces immunopathological effects in reptiles (Deen and Hutchison 2001; do Amaral et al. 2002), and can reduce reproductive output and growth in female lizards (Uller et al. 2006), and sexual coloration and chemical signals of male lizards (López et al. 2009a, b). After the initial characterization of anti-predator behavior, half the lizards captured in each habitat type ('LPS-males') were injected intraperitoneally with LPS (2.5 μg per g of body weight) diluted in 0.05 ml of phosphate-buffered saline serum (PBS). This concentration was previously used to stimulate the immune system of a closely related species (*P. hispanica*; López et al. 2009a,b). The remaining control males ('C-males') were injected with the same volume (0.05 ml) of PBS, to account for any effects of handling and being injected.

Statistical analyses

To test whether males from the different habitat types differed in morphology or immune response, we used one-way ANOVAs with habitat as a fixed factor. We checked the residuals of all models to ensure that they met the assumption of normality.

To determine which male traits were associated with variation in hiding time prior to the experimental immune challenge, and whether these relationships differed depending on the habitat a male was captured in we ran a generalized lineal model (GLM) with quasi-Poisson error distribution. Hiding time was significantly repeatable before the immune challenge ($r = 0.71$, $P < 0.01$). So, in our model, we used the mean hiding time as the dependent variable. We included habitat type as a fixed factor, five covariates (body condition, PHA response, relative head size, SVL, and ocelli number), and the two-way interactions between each covariate and habitat type.

To test for the effects of immune treatment and habitat type on hiding time we ran a generalized mixed model (GLMM) in package lme4 (Bates et al. 2015). We treated the hiding time as the response variable. Treatment (LPS or PBS), habitat (natural or urban), and time (pre-, post- injection) were included as fixed factors. Since males from the two habitats differed in relative head size, which was related to mean hiding time, we therefore included this trait as covariate in our model. We also included all two-way and three-way interactions involving habitat, treatment, time, and relative head size. Although SVL differed between the two habitats, we did not include it as covariate because it was unrelated to mean hiding time in our prior analysis. We included individual as a random effect to control for individual variation in hiding time and we specified a Poisson error distribution. We corrected for overdispersion by including trial as a random effect (Harrison 2014). Following this correction, our model was underdispersed (dispersion parameter =0.1030) and our analysis should be considered conservative. Pairwise comparisons were planned using Tukey's honestly significant difference tests. All statistical tests were conducted using R 3.2.2 (R Core Team 2015).

Results

Morphological characteristics and immune response

Urban lizards were significantly bigger and had relatively larger heads than those from the natural habitat, but there was no habitat difference in PHA response, body condition, or ocelli number ($n = 60$) (Table 1).

Table 1 Habitat differences in male morphology and immune response prior to immune-challenge treatment (results from one-way ANOVAs)

Trait	Habitat (mean ± SE)		F	P
	Urban ($n = 30$)	Natural ($n = 30$)		
Body condition	0.006 ± 0.037	-0.006 ± 0.047	1.210	0.270
SVL (mm)	73.430 ± 3.201	64.33 ± 2.770	138.530	**<0.001**
Relative head size (PC1)	-0.269 ± 0.985	0.26 ± 0.955	4.610	**0.035**
Ocelli number	8.910 ± 4.620	7.33 ± 2.840	2.570	0.114
PHA response (residuals)	-0.010 ± 0.094	0.01 ± 0.101	0.668	0.416

Significant values are in bold

Anti-predator behavior

We found no overall correlation of male traits with hiding time (all p values >0.07, see Table 2). However, the effect of relative head size differed significantly between the two habitats (habitat interaction with relative head size: estimate ± SE = 0.944 ± 0.349, $t_{(48)} = 2.707$, $P = 0.009$). We therefore looked at the individual correlations in each habitat type and found that males with relatively larger heads spent significantly less time hiding in the natural ($r^2 = 0.19$; $P = 0.015$), but not in the urban habitat ($r^2 = 0.07$; $P = 0.131$) (Fig. 1). There were no habitat differences in how the other traits were associated with hiding time (interactions: all P values >0.11, Table 2).

Experimental immune challenge

There was a significant habitat difference in the change in hiding time before and after the experimental immune challenge (pre-post habitat treatment: estimate ± SE = 1.302 ± 0.532, $z = 2.446$, $P = 0.014$) (Fig. 2). Before the treatment, PBS treated males and LPS treated males from both habitats had similar hiding times

**Table 2** Effects of habitat and male traits on hiding time (results from GLM)

Factors	Estimate	SE	t	P
Habitat	7.317	7.337	0.997	0.323
Body condition	8.739	5.901	1.481	0.145
SVL (mm)	0.161	0.089	1.806	0.077
PHA response	1.779	2.055	0.866	0.390
Relative head size	−0.430	0.283	−1.519	0.135
Ocelli	0.081	0.066	1.220	0.228
Habitat*body condition	−11.894	7.445	−1.598	0.116
Habitat*SVL	−0.102	0.108	−0.943	0.350
Habitat*PHA response	−3.291	2.677	−1.229	0.224
Habitat*rel. head size	0.944	0.349	2.705	**0.009**
Habitat*ocelli	−0.107	0.078	−1.360	0.180

Significant values are in bold

(Tukey's tests: all $P > 0.36$). After the experimental treatment, however, LPS treated males in the natural habitat spent significantly less time hiding than did PBS treated males (Tukey's tests $P < 0.001$). This was not the case in the urban habitat (Tukey's test $P = 0.997$, Fig. 2). Finally, we found no significant effect of relative head size on this trade-off (estimate ± SE = 0.054 ± 0.790, $z = 069$, $P = 0.945$) (Table 3).

Discussion

Our main experiment suggests that there is an evolutionary trade-off in *Podarcis liolepis* between hiding to evade predators and responding to an immune challenge in natural habitats (Fig. 2). After an immune challenge, males from natural habitats decreased their hiding time in response to a simulated predatory attack. This was not the case for males from the urban habitat who showed no change in anti-predatory response after an immune challenge. In addition, we found that
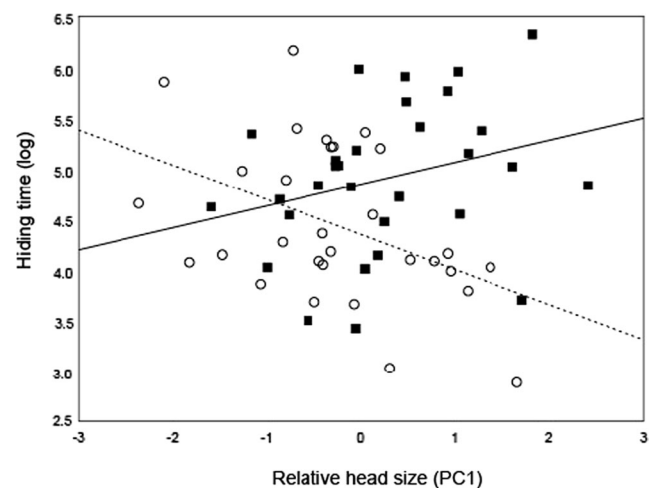
Fig. 1 Relationship between hiding time and relative head size of males from natural (*circles, dashed line*) and urban (*squares, solid line*) habitats. Relative head size was significantly correlated with hiding time in the natural but not in the urban habitat. Graph shows the raw data and bivariate regression lines, not the results of the full GLM model
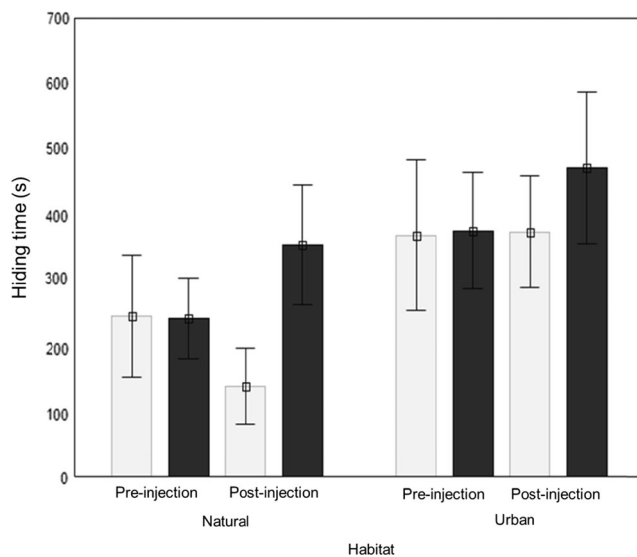
Fig. 2 Male hiding time (mean ± SE) in relation to habitat type, time, and treatment. LPS treatment is shown in *light gray*. PBS is shown in *dark gray*. Only post-treatment LPS males from the natural habitat differed significantly from the other groups (all Tukey's test $P < 0.02$)

the correlation between male relative head size and hiding time differed between our urban and natural population. When combined, our results suggest that the costs and benefits affecting the trade-off between hiding and basking (to counter an immune challenge) differ in our urban and natural habitat. Of course, future studies in other populations and/or lizard species are needed to test whether this will prove to be a general finding. When sufficient studies are published, it will then be possible to conduct a formal meta-analysis looking at

Table 3 Effects of treatment, habitat, time (pre- post- injection) and relative head size and their interactions on hiding time (results from GLMM)

Factors	Estimate	SE	z	P
Treatment	1.679	0.403	4.158	**<0.001**
Habitat	1.814	0.407	4.452	**<0.001**
Time (pre-post injection)	1.134	0.263	4.305	**<0.001**
Relative head size	−0.719	0.387	−1.858	**0.063**
Treatment*habitat	−1.496	0.578	−2.586	**0.009**
Treatment*time	−1.501	0.372	−4.034	**<0.001**
Habitat*time	−1.323	0.375	−3.521	**< 0.001**
Treatment*relative head size	0.177	0.569	0.311	0.755
Habitat*relative head size	0.792	0.636	1.245	0.213
Time*relative head size	0.333	0.357	0.933	0.350
Treatment*habitat*time	1.302	0.532	2.446	**0.014**
Treatment*habitat*rel. head size	−0.154	0.860	−0.180	0.857
Treatment*time*rel. head size	−0.158	0.524	−0.302	0.762
Habitat*time*rel. head size	−0.600	0.585	−0.103	0.918
Treat*habitat*time*rel. head size	0.054	0.790	0.069	0.945

Significant values are in bold

the effect of urbanization on the ecological trade-offs faced by individuals (e.g., Samia et al. 2015).

Habitat differences

Males from the urban population had larger bodies and larger heads (for their body size) than those from the natural population. These findings suggest that growth rates and/or survival (given indeterminate growth) might be greater in urban areas. It is possible that long-term selection has favored genotypes better suited to an urban environment. The city started to grow around this park in 1181 A.D. (Carrillo, 1979) and, although the extent to which this reduced gene flow is unknown, it is possible that the urban population has been isolated from nearby rural populations for 900 years. This is sufficient time for local adaptation to the new environment. It has been suggested that urban areas can impose similar selection to that seen on actual islands (see Marzluff 2005), since the conditions in both habitats can be quite similar. Indeed, lizard populations on islands usually tend to gigantism (Runemark et al. 2015), which might explain the between-population differences in body and head size that we observed in *P. liolepis*. Alternatively, the larger relative head size might be due to strong intra-sexual selection in the urban habitat. Relative head size is an indicator of social dominance in lizards (Braña 1996), and individuals in the urban population inhabited a limited area, so direct competition for access to females is likely to be greater (ICM pers. obs.). Finally, these morphometric differences may be due to greater survival in urban conditions of phenotypes that are linked to personality traits associated with urban tolerance (e.g., Miranda et al. 2013).

In contrast to our predictions, however, we found no habitat differences in mean male body condition or immune response to PHA. This contrasts with other studies that report that urban animals are in better body condition due to greater food availability (e.g., Contesse et al. 2004), but have weaker immune responses due to pollution and stress induced by the presence of people who increase the perceived predation risk (Amo et al. 2006; Cabido et al. 2008). The lack of differences in body condition also suggests that the predation pressure might be similar in both environments, so that animals spend similar time fleeing from predators. We also found no difference in blue ocelli number between the habitats. These ocelli appear to be sexually selected traits (López et al. 2004). Previous studies in a range of taxa have reported both increased and reduced expression of sexual traits in urban areas (Yeh 2004; Ríos-Chelén et al. 2013). To date, however, there has been no formal meta-analysis of how urbanization affects sexually selected traits. More studies such as ours are needed to permit such an analysis.

Habitat type affected the relationship between male relative head size and anti-predatory response. Males with relatively large heads spent significantly less time hiding when they were from the natural habitat, but not when they were from the urban habitat. Relative head size is associated with social dominance so a plausible reason why larger headed males emerge sooner is the greater reward of being active and engaging in territorial defense (Cooper 1999; Díaz-Uriarte 1999). More generally, habitat differences in the strength of sexual selection could alter the trade-off between predation risk and social behavior (Lima and Dill 1990; Martín and López 2003; Cabido et al. 2009). In the urban habitat, the population density appears higher (ICM pers. obs.). Changes in density often affect mate choice, mate searching (Kokko and Rankin 2006), and the size of territories (Wood et al. 2012). This yields a testable prediction: territory ownership is less important in the urban habitat, so larger headed males have less to gain from emerging sooner.

Immune challenge and hiding time

Our key finding is based on our experimental test of the relationship between an immune challenge and hiding time. Immune-challenged males from the natural habitat emerged significantly sooner than control males. No such difference was observed for males from the urban habitat. This suggests that the effect of immune activation on the trade-off between hiding in a cool, safe refuge, and basking in a warm, but exposed site to fight an infection differs between the habitats. Individuals experiencing an immune challenge should leave their refuge sooner than healthy ones to achieve a higher body temperature to fight an infection (Otti et al. 2012). Unfortunately, the need to bask conflicts with increased exposure to predators. In contrast, however, immune activation might favor re-allocation of resources to fighting infections so that individuals have a reduced locomotory performance due to lower energy availability. This could make them more vulnerable to predators and thereby increase their hiding time (Aubert 1999; Otti et al. 2012).

The net effect of an immune challenge on male hiding time is hard to predict. Our results, at least from the natural habitat, suggest that the net benefits of basking outweigh the greater predation risk that follows when a male is subject to an immune challenge. Future studies should take advantage of habitat difference in hiding behavior, such as the one reported here, in response to an immune challenge to try to pinpoint the associated costs and benefits of hiding. Specifically, it is important to understand in greater depth the effects of urban habitats on the perception of predation risk. One can also test whether fewer limitations on resource acquisition might explain the lack of change in the hiding-basking trade-off for immune-challenged males in an urban habitat.

Summary

Our experimental study showed that an immune challenge affects the trade-off between anti-predator behavior (hiding) and mounting an immune challenge (basking to elevate body temperature). Few studies to date have shown such a relationship (see Rigby and Jokela 2000). However, this trade-off differed between male lizards from urban and natural habitats. In addition, the correlation between male relative head size and hiding time differed between the two habitats. In conjunction, these twin findings allow us to better understand the relative importance of maintaining body condition, fighting infection and avoiding predators. When evolutionary trade-offs change, they affect the strength of natural selection on relevant traits. Unique ecological and social pressures in urban environments could promote changes in behavior or resource allocation that lead to local adaptation. Further studies comparing additional urban and natural habitats and a wider range of species are needed to determine whether the results we have presented apply more generally to reptiles that hide in refuge but need to bask to counter infections.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All capture and handling of lizards complied with the contemporary laws regulating the treatment of animals in Spain and was performed under license from the Bizkaia and Gipuzkoa Administrations (numbers 1695 and 2222).

Informed consent Not applicable

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