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Urban habitats can affect body size and body condition but not immune response in amphibians

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Abstract Does urbanization affect key life-history traits in native organisms? Some studies show that urban areas reduce diversity in certain taxa, but there is little insight into how these environments affect physiological and ecological traits. Urban areas have distinct physical structure and ecological processes compared to original habitats. The environmental changes associated with urban areas can influence the costs and benefits of different traits and behaviors of local organisms. Some of these effects have been explored in groups such as birds, but we might expect stronger effects in animals with reduced mobility, such as amphibians. Importantly, the effects of urban habitats on amphibians have not been explored, in spite that these are the most threatened vertebrate group in the world. Here, we compared three main traits related to the fitness of amphibians in urban and natural habitats: body size, body condition and immune response. To test the generality of our results, we assessed adult males of four amphibian species. We found that the body size was larger in urban environment populations in three of four studied species, while the body condition was better in the urban populations of two aquatic newt species. Finally, we found no effect of urbanization on the immune response of individuals of any species. In conclusion, we show that different species of amphibians may be affected differently by anthropogenic habitat alteration depending on their specific ecology.

Keywords Amphibian conservation · *Lissotriton helveticus* · *Pelophylax perezi* · PHA · *Salamandra salamandra* · *Triturus marmoratus*

Introduction

Urbanization of natural landscapes is occurring on an unprecedented scale worldwide. An important consequence of urbanization is the simplification of the natural landscape and the emergence of artificial structures, such as buildings and roads (Marzluff 2001). Urban environments are usually characterized by a reduced diversity of species (Shochat 2004) and altered communities, for example due to the introduction of exotic species (Marzluff 2001). Species respond differently to human-related habitat alteration, so that some species take advantage of the new characteristics while other species experience population declines. Animal populations inhabiting urban areas experience a variety of new conditions, such as different predatory pressure (lower predation risk, Gering and Blair 1999 or higher risk, Murphy et al. 2016b), increased temperatures, and unusual food sources (Ditchkoff et al. 2006). These environmental changes can influence the costs and benefits of different traits and behaviors, which in turn can alter trade-offs between traits (e.g. McGlothlin et al. 2007; Iglesias-Carrasco et al. 2016a). The investment in different traits across environments will depend on local adaptation and adaptive phenotypic responses, driven by environment-specific cues.

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The effect of urbanization has largely been studied in highly mobile animals such as birds (Møller 2009; Bókony et al. 2010; LaZerte et al. 2016), but little attention has been given to animals with reduced mobility. Critically, we might expect the effects of urbanization to be stronger on animals that have limited abilities to avoid these habitats, such as amphibians. Amphibians are one of the most threatened groups of animals in the world, affected by the loss and alteration of their habitat (Houlahan et al. 2000), pollution (Egea-Serrano et al. 2012), invasive predators (Kats and Ferrer 2003) and emerging diseases (Daszak et al. 2003). In general, alteration of the landscape with urban areas promotes reduced amphibian diversity compared to the original habitat (Rubbo and Kiesecker 2005). Amphibians are prone to the effects of urbanization for several reasons. For example, 1) they have highly permeable skin that renders them sensitive to toxic substances, both in the terrestrial and aquatic stages; 2) their ability to disperse may be hindered in urban landscapes; 3) the scarce suitable breeding habitats might constrain breeding opportunities, leading to usage of habitats that are poor or have low-suitability; 4) different stressors, such as noise, light pollution or increased temperatures in urban areas may have behavioral and ecological consequences by the disruption of acoustic signaling or the reduction of the immune response. Most studies have focused on the presence/absence of species, species richness and abundance of populations in urban habitats (Hamer and McDonnell 2008). However, the effect of urban-induced environmental changes on fitness enhancing life-history traits in amphibians remains unexplored.

We examined here whether the urban environment affects body size, body condition and immune response in four amphibian species. Body size of individuals from urban areas often differs from the surrounding natural areas, with both increases and decreases in the body size of organisms in urban habitats being reported in the literature (Ruiz et al. 2002; Murphy et al. 2016a). Differences in temperature, resource availability, or the chemistry of the water have been proposed as explanations for differences in body size across taxa (Kozłowski et al. 2004; Walters and Hassall 2006; Barrett et al. 2010). On the other hand, high food predictability and low mortality in urban habitats can elevate reproductive rates and population densities in some species (Møller 2009). Changes in population density can in turn affect the body condition of individuals, which is a crucial trait that determines fitness and is indicative of environmental stress (Peig and Green 2010). Increases in population densities are predicted to lead to poorer body condition of individuals, due to over exploitation of food that may reduce foraging success (Liker et al. 2008). However, a negative effect of urbanization on body condition is likely not to be universal (e.g. house sparrows Bókony et al. 2012), probably due to stable and abundant food supplies, such as human resources, that are exploited by many generalist species in urban areas (e.g. Herr et al. 2010). Finally, the immune response may also differ between urban and natural habitats for many reasons. For example, the poor quality (e.g. low protein intake) or reduced quantity of food available for many species in urban areas could constrain the amount of resources allocated by individuals to each physiological trait, including the immune response. Similarly, chronic stress (e.g. chronic noise or high intraspecific competence) (Padgett and Glaser 2003) and the presence of toxic chemical substances (Iglesias-Carrasco et al. 2016b) are detrimental for individual immune responses. The immune system is expensive to maintain and use, so energy deficits generated by the need to face toxins may alter the resource allocation decisions, resulting in urban populations being in worse health (i.e. lower immune responses) than natural populations (e.g. lizards Cabido et al. 2008). Contrarily, some studies have found enhanced immune responses in urban populations compared to natural ones (Audet et al. 2016) as a response to the exposure to novel pathogens.

We studied three urodele and one anuran species that are frequently found in cities. Different amphibian species may exhibit markedly different responses to urban habitats. Habitat-generalist amphibians or those with relatively low dispersal requirements appear to do better in urban areas (Hamer and McDonnell 2008). Two of our study species, the palmate newt (*Lissotriton helveticus*) and the strictly aquatic common green frog (*Pelophylax perezi*) are generalist amphibian species that breed in a wide range of aquatic environments, ranging from big lakes, to streams and flooded ditches on the roads (Montori and Herrero 2004). Both species, but mostly the common green frog, have high resistance to water pollution and show few requirements in relation to water quality (Montori and Herrero 2004). The other two of our study species, the marbled newt (*Triturus marmoratus*) and the fire salamander (*Salamandra salamandra*), are more restricted in their ecological requirements (Montori and Herrero 2004). During the terrestrial stage the marbled newt uses rocks and mammal refuges to hide, usually sited in forest or shrub areas. For reproduction, it uses several still water aquatic habitats, but usually these are ponds with high volume of water and abundant aquatic vegetation (Montori and Herrero 2004). The fire salamander is a terrestrial species that requires high humid and gloomy environments. It can be found in any type of plant community, but populations are more abundant in deciduous forests (Montori and Herrero 2004). Among urodeles, we used two species in aquatic phase (the palmate newt and the marbled newt) and a mostly terrestrial salamander (fire salamander) to test if the urban environment has different effects depending on the species ecology. We compared natural and urban populations of these four species and predicted that inhabiting the city can result in changes in 1) body size and 2) body condition, and 3) reduction of the immune response of individuals. Due to the contrasting results found in studies based on other animal groups (e.g. Liker et al. 2008;

Bókony et al. 2012) and the lack of this kind of studies in amphibians, it is difficult to make predictions about the direction of the effect of urban areas on the body size and body condition on the species studied.

Material and methods

Study area and animal maintenance

In April 2013, we captured 18–24 adult males of each of the four amphibian species (specific sample sizes shown in Fig. 1) in an urban parkland in the city of Donostia/San Sebastián in Northern Spain (43° 18′ 18.06″ N, 1° 59′ 29.97″ W), and in a nearby natural oak forest (1.7 km in a straight line to the city, 43° 18′ 8.91″ N, 2° 02′ 37.19″ W). The urban area is isolated, surrounded by the city buildings and attracts large crowds of tourists and citizens. The distance to the nearest natural habitat is approximately 1.9 km. This distance combined with several obstacles, such as roads, poses a substantial degree of isolation for the amphibian urban populations from the natural populations. Amphibians in the city occupy human constructions. In our urban population the palmate newt, the marbled newt and the common green frog breed in concrete artificial ponds, surrounded by exotic garden trees. The urban population of fire salamander studied here is viviparous and, hence, independent of the water for reproduction. This characteristic allows the survival of salamanders in isolated and somewhat extreme environments such as urban areas, where they use the walls of historic buildings as refuge. In the natural populations, individuals of the four studied species occupy naturally created ponds inside native oak forests and find shelter under rocks and logs.

It would have been interesting to look for the effect of urbanization in more than one population. However, urban populations of some of the species are very limited in numbers and restricted to the few available suitable breeding habitats that remain inside the city, so the sample size we obtained in other urban ponds was insufficient to conduct the experiment.

All the animals were captured in April and during the night, coinciding with the breeding season of the four species. To avoid potential confounding effects of weather or seasonality all the animals were captured within a single week. The aquatic newts were captured by dip netting, while the terrestrial salamander and the frog were collected by hand. Animals were individually housed in 4 L terraria with a 0.75 L water pool and moss as refuge (for terrestrial salamanders and common frogs) or in 2 L aquaria (for the aquatic newts) during the 48 h of the trials. We maintained the animals in a constant temperature of 15 °C and natural photoperiod in a laboratory. They were fed twice a day with three mealworms (for the terrestrial species) or three bloodworms (for the aquatic species) during the two days of the experiment.

Measure of morphological traits and immune response

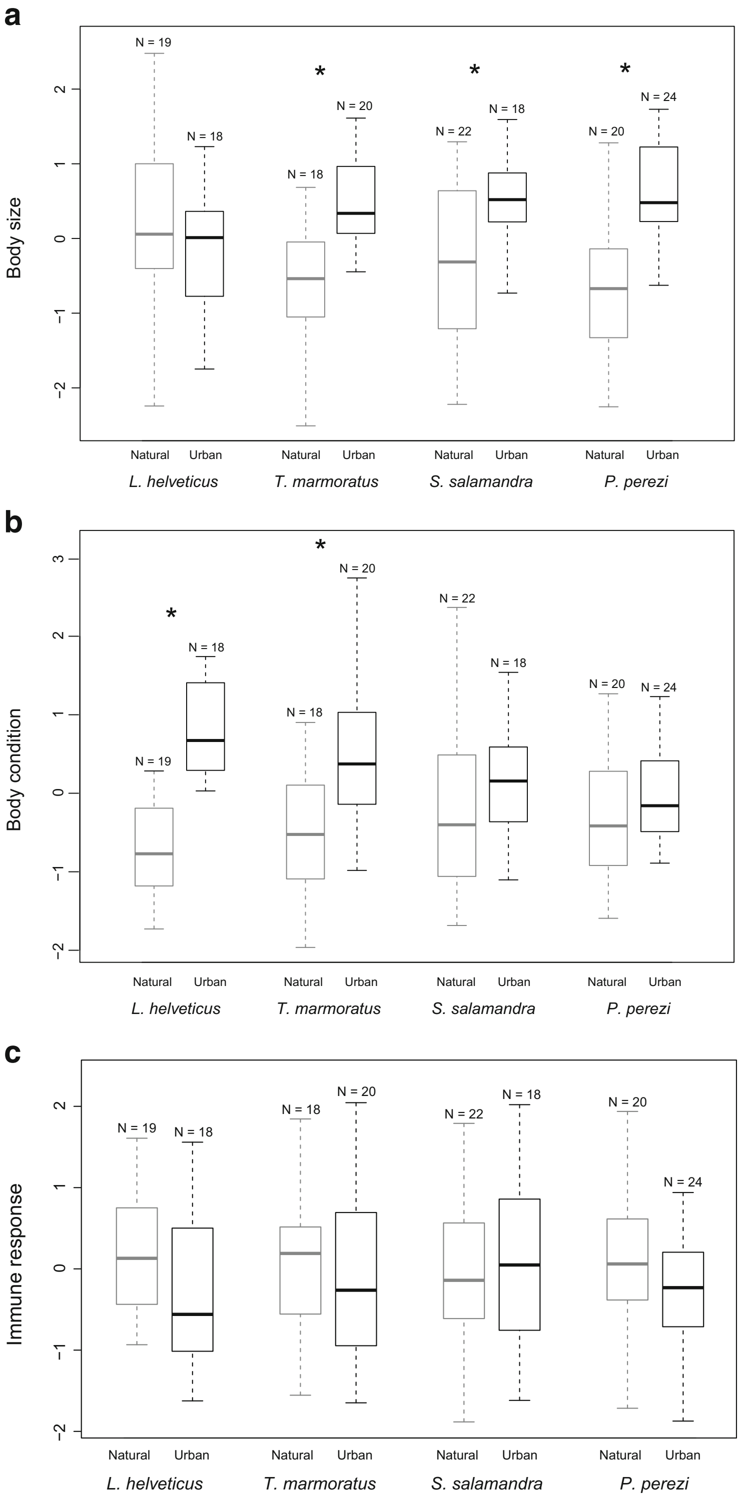
We measured three traits that are likely to be altered by urban habitats: body size, body condition and immune response. Body size was measured from photos of individuals placed on a glass board with a reference measurement scale. We used the snout-vent length (SVL: measured in mm from the tip of the snout to the ventricular opening) as a measure of the size for the anuran species, while for the urodeles we used total length (i.e. TL: measured in mm from the tip of the snout to the tip of the tail). All measures were made using Image J software (Abràmoff et al. 2004).

We calculated body condition as the residuals from the regression of log body mass (g) on log TL or log SVL. When the relationship between these variables is linear, this measure is often used as an index of the relative amount of fat stored, and hence of nutritional status (reviewed in Green 2001). This index has successfully been used to investigate the effects of differences in food availability and habitat quality in amphibians (MacCracken and Stebbings 2012; Sztatecsny et al. 2013).

Finally, to estimate the immune response we used the delayed-type hypersensitivity phytohaemagglutinin injection assay (PHA test). This assay was described as a reliable measure of T-cell dependent immunocompetence in vivo (Lochmiller et al. 1993). However, recent studies have shown that the PHA-induced swelling might instead be considered as a multifaceted index of the cutaneous immune activity (Salaberria et al. 2013). We used this test as a standard index of the immunocompetence in order to avoid differences related to the type of the immune cells involved (Kopena et al. 2014). This test has been used and validated in many studies including those on amphibians (Brown et al. 2011; Clulow et al. 2015).

All individuals were anaesthetized by immersion in Tricaine methane sulphonate (0.15 g MS-222/1 L dechlorinated water) for 5–10 min (Cakir and Strauch 2005) immediately before the measurements. Then for newts we measured the thickness of the base of the tail 5 mm from the vent (based on Iglesias-Carrasco et al. 2016b) with a pressure-sensitive spessimeter (±0.01 mm). We let the spessimeter to gently touch the skin of the animal without pressing it to avoid the post-injection inflammation caused by the PHA to disappear with the pressure. We made our measurements five times and used the average measurement for statistical analyses. Immediately after, we injected 0.01 mg of PHA dissolved in 0.01 ml of phosphate-buffered saline (PBS). Once recovered from the anaesthesia, newts were placed into plastic containers with 2 L of dechlorinated water. After 24 h, we measured the thickness of the tail base at the same point (this time without anaesthesia) to calculate the difference between pre- and post-injection measures

Fig. 1 Habitat differences in **a** body size, **b** body condition and **c** immune response of the four studied amphibian species. All measures were scaled to 0 within each species for illustration. Significant comparisons are marked with an asterisk



(inflammation). We used a similar procedure with the common green frog and the fire salamander. In this case, the measure of the thickness and the injection was made in the left hind footpad (commonly used in lizards, see e.g. Husak et al. 2016). Animals were individually maintained in a 4 L aquarium. The immune response index (hereafter 'PHA immune response') was calculated as the residuals of the regression of the inflammation against SVL or LT. The only appreciable effect of the PHA injection was a slight swelling of the skin, caused by the immune response, which disappeared after 48 h. None of the animals showed any sign of stress or pain during these tests, and all looked healthy after the trials. All individuals were returned to their capture sites 48 h after being captured.

Statistical analyses

To test whether males from the different habitat types differed in morphology or PHA immune response, we used one-way ANOVAs with habitat (natural vs urban) as a fixed factor. We ran a separate model per each species and trait, such that there were 12 models tested. We checked the residuals of all our models to ensure that they met the assumption of normality and homoscedasticity. To test whether variances in body size were statistically different between populations, we ran an F-test for each species. All statistical tests were conducted using R 3.2.2.

Results

We found significant differences between populations in body size in *T. marmoratus* ($F_{(1,36)} = 15.51$, $p < 0.001$), *S. salamandra* ($F_{(1,38)} = 5.91$, $p = 0.001$) and *P. perezi* ($F_{(1,42)} = 35.16$, $p < 0.001$), with individuals from the urban area being 6.29, 8.47 and 17.73% respectively larger in body size than the ones from the natural population (Table 1, Fig. 1a). However, we did not find any significant effect of urbanization on the body size of *L. helveticus* ($F_{(1,35)} = 1.05$, $p = 0.310$). We also found a significant difference between populations in the variances of body size of *T. marmoratus* (F test, $F = 3.508$, $p = 0.009$), so that individuals from the natural population showed a significant higher size variability than urban individuals. However, we did not find a significant effect of the population on body size variability in any of the other three species (F tests: *S.salamandra, F* = 2.282, $p = 0.088$; *L. helveticus, F* = 1.877, $p = 0.200$; *P. perezi, F* = 2.048, $p = 0.102$).

Table 1 Differences in body size, body condition and immune response between urban and natural habitats

Trait	Species	mean ± SE	
		Urban	Natural
Body size	*L. helveticus*	7.23 ± 0.28	7.35 ± 0.40
	T.marmoratus	15.23 ± 0.49	14.30 ± 0.92
	S. salamandra	15.1 ± 1.2	13.87 ± 1.87
	P. perezi	7.98 ± 0.58	6.68 ± 0.83
Body condition	*L. helveticus*	0.05 ± 0.03	−0.04 ± 0.04
	T.marmoratus	−0.04 ± 0.04	0.05 ± 0.03
	S. salamandra	−0.009 ± 0.04	0.007 ± 0.05
	P. perezi	0.01 ± 0.02	−0.01 ± 0.06
Immune response	*L. helveticus*	0.03 ± 0.11	−0.03 ± 0.11
	T.marmoratus	−0.02 ± 0.25	0.02 ± 0.21
	S. salamandra	0.01 + 0.15	−0.01 ± 0.13
	P. perezi	0.02 ± 0.17	−0.01 ± 0.18

Body condition of the two aquatic newts were significantly greater in the urban populations (*T. marmoratus*: $F_{(1,36)} = 50.24$, $p < 0.001$; *L. helveticus*: $F_{(1,35)} = 60.88$, $p < 0.001$) (Table 1, Fig. 1b). However, there were not significant differences in body condition between habitats in the terrestrial salamander ($F_{(1,38)} = 1.18$, $p = 0.283$) and the frog ($F_{(1,42)} = 2.14$, $p = 0.15$) (Table1, Fig. 1b).

Contrary to expectations, there were no significant differences between habitats in the PHA immune response in any of the species (*L. helveticus*: $F_{(1,35)} = 3.29$, $p = 0.078$; *T. marmoratus*: $F_{(1,36)} = 0.46$, $p = 0.500$; *S. salamandra*: $F_{(1,38)} = 0.47$, $p = 0.497$; *P. perezi*: $F_{(1,43)} = 0.63$, $p = 0.434$) (Table 1, Fig. 1c). Nevertheless, in *L. helveticus* the data suggest a trend for a stronger PHA immune response in the individuals from the natural population which might have reached statistical significance with a bigger sample size.

Discussion

Urbanization can affect several life-history traits of animals by imposing new evolutionary and environmental conditions. Our results suggest that some traits are more prone to be affected by urban conditions than others. Likewise, the different responses on body condition and body size among species suggest that the effect of urbanization depends on the species in question. Specific ecological requirements of each species may influence the direction of the effect, making it difficult to predict species responses under urban environmental conditions. However, it is important to take into account the limitation of our results, since we only explored one population per each species and habitat. Future studies in other populations are needed to test if our findings are general trends.

Effect of urbanization on body size

We found that in three of the four studied species, males inhabiting the urban environment had larger body sizes than those inhabiting the natural habitat. Changes in foraging behaviour, reproductive decisions, survival, and increased temperatures in urban habitats have been suggested as explanations of the body size differences between urban and natural habitats in some animal groups, such as birds or amphibians (Shochat 2004; Murphy et al. 2016a). As such, one possible explanation for our findings is that individuals inhabiting the city were older than those in the natural habitat, and thus they had had more time to grow. In the parks where we carried out the study, there are no invasive fish species that prey on amphibians, and avian and mammal predators are almost absent.

The relatively small number of predators could lead to increased survival and longer lifespan, and hence larger body size. Finally, habitat-specific costs and benefits of a specific body size may drive the adaptation of populations in different habitats. For example, strong selection due to predation may favour small adult body size (Gosler et al. 1995), so that the release from predation in the urban population leads to the evolution of larger body sizes. Interestingly, in our data the variation in body size of *T. marmoratus* individuals from the natural population was higher than in the urban population. This could be simply because in the natural habitat the population size of individuals is higher, so, despite that the captured animals were all adults, the variability in body size may be higher. However, we can not exclude that some unknown specific ecological pressure of the urban habitat is selecting for a limited body size range that might make more effective the life in this new environment.

Prey availability has also been hypothesized as an explanatory variable of larger body size in urban areas (Shochat 2004). This could also explain the differences found in our study, as exaggerated densities of some exotic invertebrate species have been observed in the studied urban habitats (MIC and CC, personal observation). On the other hand, lower abundances of conspecifics in urban streams could lead to reduced competition and increased growth rates (Petranka and Sih 1986). However, reduced competition seems unlikely in our urban population; although we did not measure the population density of adults in urban parkland, it did not seem smaller than in the natural population (MIC personal observation). Finally, Murphy et al. (2016b) showed that higher temperatures experienced in urban environments can increase metabolic rates, leading to larger amphibian larval sizes. It would be interesting to compare physiology of amphibians from the different habitats to determine whether changes in metabolic rates could explain the differences found in our study.

Effect of urbanization on body condition

We found that the body condition of the two aquatic newts was better in the urban population, but no differences were found in the anuran species or the terrestrial salamander. These findings seemingly contradict the assumption that urban areas are a low quality habitat, at least for the aquatic species. The two newt species spend a long breeding period of time in the water, during which they feed on aquatic invertebrates and other amphibian eggs and larvae (Montori and Herrero 2004). Then these newts spend several months in terrestrial phase during which they change their diet completely. These changes in the ecology of the species might explain the differences found between the terrestrial and aquatic amphibians. Some invertebrate groups are known to have lower diversity and population densities in urban environments (Weller and Ganzhorn 2004), but others can increase densities, in particular exotic ones (see e.g. ants Guénard et al. 2015). Having an amphibious diet can be beneficial when it comes to adaptation to new environments because these species can more easily take advantage of recently created exotic communities of invertebrates. However, it seems implausible that resources provided by humans drive increased body condition in amphibians. This is because, as opposed to some birds and mammals (e.g. Ross 2004; Bateman and Fleming 2012), the amphibians' diet composition (i.e. live insects, crustaceans and worms) could not be based on human resources and waste.

One alternative explanation for a better body condition in urban areas is a reduced population density or diversity of predators. Escaping from predators usually trades-off with other fitness enhancing activities, such as foraging or mating (e.g. Turney and Godin 2014). If aquatic predators of adults are scarcer in urban areas, newts inhabiting the urban pools could spend more time feeding than their natural counterparts and improve their body condition.

Effect of urbanization on the PHA immune response

Both increases and decreases of the immune function associated with urbanization have been reported in the literature in several vertebrate taxa (Bradley and Altizer 2007; French et al. 2008), but to our knowledge this has not been explored in amphibians. Novel arrays of pathogens encountered in urban habitats have been shown to enhance immunocompetence in some bird species (Audet et al. 2016), while the exposure to toxic compounds that are present in urban areas reduce the immune response of others (Lewis et al. 2013). Contrary to expectations, we did not find any effect of living in the city on the immune response of any of our four amphibian species. Amphibian populations inhabiting anthropic habitats are expected to face chemical compounds not previously encountered during the evolutionary history of the species (Croteau et al. 2008; Iglesias-Carrasco et al. 2017). Previous laboratory and field experiments show that the immune function in tadpole and adult amphibians can change rapidly when exposed to toxins as a stress-induced response (Burraco et al. 2013; Iglesias-Carrasco et al. 2016b). Other stressors derived from anthropic activities (e.g. acoustic pollution; Barber et al. 2010) may also affect the immune response of urban populations. For example, increased average temperatures in cities, the chronic light pollution or the increase in the background noise can increase physiological stress (Tennessen et al. 2014), increase the mating call effort (Sun and Narins 2005) and alter feeding behaviours of amphibians (Perry et al. 2008), which may, in turn, constrain the immunity (e.g. Raffel et al. 2006). Air pollutants are also suggested to reduce the immune function of urban populations of lizards (Cabido et al. 2008). However, the urban habitat we sampled here had low levels of pollution, potentially explaining the lack of a significant effect of habitat on immune response.

Conclusions

Unique environmental pressures in urban areas seem to promote changes in individual investment toward different traits. Intriguingly, the direction of the changes is hard to predict and appears to depend on each species' ecology, such as feeding behavior related to aquatic or terrestrial habits, or predation pressure. As eggs are more limiting than sperm, females are often the limiting factor for population growth and persistence. Comparing the studied traits in females would be also interesting and will provide information on the total effect on the specific species. Further studies comparing additional urban and natural populations are needed to determine whether the results presented here are caused primarily by urbanization and if they apply generally to amphibians.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

Ethical statement Capture and handling of amphibians complied with the contemporary laws regulating the treatment of animals in Spain. The study was performed under license from the Gipuzkoa Administrations and the City hall of Donostia/San Sebastián (numbers 2222 and 114–301). M. I-C and C.C conducted this work with Spanish accreditation to conduct experiments with animals R.D 53/2013 (ref number 10/096442.9/13).

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