

Effects of biotic and abiotic stressors on asymmetries and head size in two sympatric lizard species

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Abstract

Organisms face numerous environmental stressors, which can affect developmental precision, including symmetry of various physical characteristics. Fluctuating asymmetry (FA) has therefore been suggested as a simple and efficient tool for assessing sub-lethal stress levels. We analyzed FA in two sympatric lizard species (*Iberolacerta horvathi* and *Podarcis muralis*) to determine potential effects of interspecific competition and urbanization, as proxies of stress, taking into account sexual dimorphism and environmental conditions. We sampled 16 syntopic and allotopic populations and used geometric morphometrics of head morphology. We detected significant but mixed effects on the head asymmetry from the environment and the syntopic occurrence that differed between species. *P. muralis* lizards had more asymmetric heads at higher altitudes, while *I. horvathi* lizards did at mid altitudes, which may be explained by *P. muralis* experiencing environmental stress of colder conditions at higher altitudes. The mid-altitude effect on asymmetries in *I. horvathi* might be explained by a lower availability of stony walls and higher abundance of *P. muralis*, thus higher competition. The asymmetry of supraciliary granules was affected by the presence of other species. However, lizards from allotopic populations attained larger asymmetries compared to lizards from syntopic populations, which was the opposite from what was expected. There was no effect of urbanization in *P. muralis*, which could be due to

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relatively low pollution and habitat degradation in study locations. Overall, we highlighted the possibility of using lizards and FA for bioindication of environmental stressors and especially improved the knowledge gap in the research of biotic stressors.

Keywords

Competition; directional asymmetry; fluctuating asymmetry; lizards; stress; urbanization

Introduction

Negative effects of either abiotic or biotic origin, have long been the focus of research to assist biodiversity conservation (Wood et al., 2000). With the escalation of anthropogenic pressure on natural populations there is an increasing need for efficient, easy to use, inexpensive and non-invasive indicators of population disturbance to aid conservation efforts (Burger, 2006). These indicators need to be sensitive, i.e., they can detect signs of population disturbance early before components of fitness have been strongly affected and before irreversible demographic damage has occurred (Bartell, 2006; Burger, 2006).

In this context, the degree of developmental stability (DS) has been suggested as a reliable indicator, because a wide range of environmental stressors has been shown to affect developmental precision (Beasley et al., 2013). DS is a characteristic of an organism to develop along a predetermined developmental pathway and resist random disturbances, which can occur during the development (Lens & Van Dongen, 2000). The majority of organisms have one or more axes of symmetry over which the body is a mirror image. However, they are rarely perfectly symmetrical and slight differences between the repeating structures occur. These random and subtle deviations from perfect symmetry are referred to as fluctuating asymmetry (FA; Palmer & Strobeck, 1986; Leamy & Klingenberg, 2005; Graham et al., 2010), which is one of the most commonly used measures of developmental (in)stability (Van Valen, 1962). The logic behind using FA as a measurement of DS is based on the assumption that both sides of bilaterally symmetrical individuals develop in the same environment and under the effect of identical genes, so that any differences between sides have to be a result of disturbances during the development (Clarke, 1993).

FA is a non-directional asymmetry, as opposed to the directional asymmetry where one side is larger than the other side (e.g., internal organs of vertebrates, handedness and position of owl ears; Møller & Swaddle, 1997). For a given sample or population, FA is computed as the variance of bilateral differences scored between individuals, either of size or of shape. It must be distinguished from antisymmetry, another and less frequent kind of non-directional asymmetry, where signed bilateral differences do not have a Gaussian distribution (Klingenberg, 2015).

In the last two decades, there has been an increased interest in FA as a stress indicator due to its advantage over other indicators, such as direct measuring of net survival (fertility, survival), which is more expensive and impractical compared

to observations of FA (Leung et al., 2000). Some studies have shown that asymmetry levels increase with the presence of stress factors, suggesting that FA can be used as an indicator of environmental and/or genetic stress and that it also might be useful for assessing the quality and health of individuals and populations (Leung & Forbes, 1996; Clarke, 1998). Common environmental stress factors caused by anthropogenic activity are exposure to pollutants, such as heavy metals and pesticide residues, temperature changes induced by climate change and habitat destruction (often as a consequence of urbanization; Lens et al., 1999). Biotic stress factors can include competition, inbreeding (and consequently higher homozygosity) and hybridization (Garrido & Pérez-Mellado, 2014). When exposed to stress, organisms need to invest energy in order to cope with it, thus there could be less energy available for stable development, which can lead to asymmetry of physical characteristics (Leung et al., 2000).

High levels of FA in lizards have so far been described as a consequence of various disturbance factors: inbreeding in populations from small islands (Soulé, 1967; Soulé & Yang, 1973), habitat fragmentation (Sarre, 1996), suboptimal incubation temperatures of eggs (Braña & Ji, 2000) and pollution (Tull & Brussard, 2007). Furthermore, the connection between FA and the female choice showed that females preferred more symmetric males (Martín & López, 2006), indicating its potential effect of FA on individuals' and population fitness. In some lizard species a correlation between FA and performance was observed, as higher asymmetry of hind limbs negatively affected their running speed (Martín & López, 2001; López & Martín, 2002). Recent studies (Lazić et al., 2013) and general overviews of this topic (Clarke, 1995; Møller & Swaddle, 1997) established FA as a valid indicator of DS and suggested it can be used as an early warning sign.

Studying head shape asymmetry was observed to be an especially good model system for examining the effects of environmental stress on the development, as the head is a developmentally complex body part involved in several important ecological and social activities in lizards, e.g., feeding, mating, acquiring and defending territories (Pianka & Vitt, 2003). Consequently, the shape of the head varies greatly between individuals within a population and among populations, indicating that it is potentially subject to selection pressure (Lazic et al., 2015).

Lizards, as ectothermic organisms, are good models to be used as indicator species for detecting the presence of environmental stress, since their development is more sensitive to environmental and genetic changes compared to endothermic organisms (Leary & Allendorf, 1989). Their advantage as bioindicator species is also the result of the many possible metric (limbs, head dimensions) and meristic (scales, femoral pores) traits, which are relatively easy to measure (Soulé, 1967). Moreover, lizards are usually abundant, which facilitates sample collection, and they inhabit places with varying levels of potential stressors (Crnobrnja-Isailovic et ´ al., 2005; Amaral et al., 2012; Lazic et al., 2013). ´

Interspecific interactions, direct or indirect, have been identified as one of the most important processes determining the structure of natural communities (Cody & Diamond, 1975; Bonsall & Hassell, 1997; Vrezec & Tome, 2004). Interspecific competition is a relationship between species, defined as a collection of reciprocal negative effects among ecologically similar species that results in a reduction of fecundity, growth and/or survivorship due to resource exploitation or interference (Smith & Smith, 1998). Prerequisites for competition between species are: they occupy the same space (occur in a zone of sympatry), are active in the same time and their fundamental niches overlap (at least partly) (Smith & Smith, 1998; Begon et al., 2006). In communities where two or more members of the same ecological guild coexist and resources they utilize are limited, they will either segregate in one or more dimensions of ecological niche to avoid interspecific interactions or they will interact (Begon et al., 2006). As competition effects are negative for all involved in the interaction, they represent biotic stress factors that may impact DS.

However, almost no information is available so far about the effects of interspecific interactions on asymmetry of organisms as a consequence of DS, although it could play an important role as a biotic stress factor (Thornhill, 1992; Witter & Swaddle, 1994; Graham et al., 2010). For example, Mallard and Barnard (2003) performed a study testing apparent competition on the conspecific and heterospecific level between two species of crickets and observed that limb FA was significantly correlated with the competitor's presence in one, but not in the other species. Such results suggest effects of competitor species on asymmetry (one species may be more dominant than the other in the interspecific competition) that hence can shape the relationships between both species in syntopic populations (Holt, 1977).

To fill this knowledge gap, we compared FA levels between allotopic and syntopic populations of two competing lizard species, which is the first such study in lizards known to us. We used a pair of ecologically and morphologically similar species of lacertid lizards that occur in sympatry in Slovenia and Croatia: the wall lizard, *Podarcis muralis* (Laurenti, 1768) and the Horvath's rock lizard, *Iberolacerta horvathi* (Méhelÿ, 1904). The two species exhibit a spatial pattern of occurrence in alternating allotopic and syntopic populations and opposite patterns in altitudinal distribution with *P. muralis* occurring in higher abundances at lower altitudes, while *I. horvathi* is most abundant at the highest elevations (De Luca, 1989; Žagar, 2016). Due to their similarities and restricted use of common spatial resources (Žagar et al., 2012, 2017; Osojnik et al., 2013), interspecific competition occurs in syntopic populations (Žagar et al., 2015a). Competitive interactions can be a source of biotic stress, therefore we expected that species will be more asymmetric in syntopic than in allotopic populations.

In addition to potential effects of interspecific interactions, we studied the influence of urbanization (only in *P. muralis*). Higher levels of FA were expected to be found in populations from urbanized (hence degraded and polluted) sites vs natural habitat sites. Since the species occur in a relatively high altitudinal gradient (approx. 1800 m) and exhibit an opposite trend in relative densities across altitudes

Figure 1. The locations of the studied populations (see Table 1 for the legend). For better visibility the area of Kočevska, where the density of sampling points was higher, is enlarged in (a).

(*I. horvathi* is more abundant at higher altitudes and *P. muralis* at lower altitudes), we included altitude as an environmental factor, and performed an additional test on a subset of populations to exclude the effect of altitude and potential differences due to phylogenetic distances. In general, we expected that populations from colder higher altitudes will have a higher level of asymmetries than lowland populations, but the effect should be stronger in the generalist species (*P. muralis*) that has a higher occurrence in lowlands and middle altitudes. Our study should improve our understanding of the effects of biotic and abiotic stressors on the occurrence of asymmetries in vertebrates and reveal the potential of using DS as bioindicator for environmental stress.

Materials and methods

Sampling and study design

Sampling sites were distributed across the area of sympatric occurrence of the two studied species, *Iberolacerta horvathi* and *Podarcis muralis*, across Slovenia and Croatia (fig. 1; De Luca, 1989; Sillero et al., 2014; Žagar, 2016). The study area is characterized by a large altitudinal gradient, heterogeneous topography, high forest cover and a mosaic of open areas with exposed rocky areas, which represent suitable habitat for both species (Žagar et al., 2013), while *P. muralis* also occurs in urbanized areas (Krofel et al., 2009). The climate is temperate continental with an

Table 1.

Locations of studied populations of *Podarcis muralis* and *Iberolacerta horvathi* in Slovenia and Croatia as labelled on the map (fig. 1). Populations labelled with * were used in the additional analysis on a subset of populations, since they occur at close geographical proximity and are genetically closely related. Abbreviations: F, female, M, male.

alpine climatic influence from the north and influence of the Adriatic Sea from the southwest (Puncer, 1980; Perko & Orožen, 1998).

Sampled populations were distributed across an altitudinal span of approximately 1800 m (table 1). Each population was classified according to the presence of one or both species: (a) allotopic *I. horvathi*, (b) allotopic *P. muralis* or (c) syntopic (table 1, [supplementary table S2\)](https://doi.org/10.6084/m9.figshare.15657840). Synopic populations have similar abundances of both species at all sampled sites (Žagar A., pers. obs.). To test the effect of urbanization of the study site, we assigned selected sites of *P. muralis* to two categories: (a) urban and (b) natural (table 1, [supplementary table S2](https://doi.org/10.6084/m9.figshare.15657840)). This factor could only be studied in *P. muralis*, because *I. horvathi* was not sampled in urbanized areas, but only in the natural environment in our study area.

Fieldwork photography and asymmetry methods

Lizards were collected using a noose in three years between 2013 and, 2015 (table 1), measured and photographed in the field and then released at the capture location. We collected four morphometric measurements of lizard heads: pileus length, head width, length and height. Measurements were always obtained by the same person (AZ) using digital calipers to the closest 0.01 mm. We photographed the dorsal side of the head of lizards in natural light with a Nikon Coolpix camera set to the macro function. In the process of photography, the camera was mounted with the lens directed downwards to the horizontal surface, where lizard was placed in the center of the optical field to take photographs at a fixed distance (20 cm) from the individual. Using the high-resolution photos and by inspecting photographs on the computer screen, we obtained a meristic trait – the number of supraciliar granules (SCGN). SCGN are located right above the eyes, below the supraocular scales; they have no evident function (Lazic et al., 2013). The same person (AA) counted ´ the number of SCGN twice, each time in a different order with several days of rest between the first and second counting, to ensure independence of the trait counts.

Landmark-based methods of geometric morphometrics were used to analyze the variation in head size and shape (Bookstein, 1997). High-resolution photos of the dorsal side of the head were used to digitize 28 landmarks using tpsDig2 (Rohlf, 2005). Landmarks were set at the contact of big scales on lizards' heads (see Lazić et al., 2015 for landmark locations), which have a close relationship with the head bones underneath and develop according to them. Therefore, their contacts ensure a permanent position and are suitable as landmark locations. Landmarks were recorded twice by the same person (AA) in order to assess measurement error arising during digitization.

Data analysis

To test for the presence of directional asymmetry (DA) and/or FA in SCGN we used the two-way ANOVA model on log-transformed values with side as a fixed factor and individual as a random factor, as well as their interaction. In this ANOVA design, a significant effect of side alone would indicate the presence of DA and a significant interaction between side and individual would point to the existence of FA. As our results indicated the existence of FA (see *Results*), we calculated an individual asymmetry index (AI) for each trait as the unsigned right-left (R-L) difference between the log-transformed average of trait values across the two replicate counts of each individual, to account for measurement error. The values of AI did not deviate significantly from normality within each population for the studied trait (Kolmogorov-Smirnov test, *P >* 0*.*05 in all cases).

To test for the presence of FA and/or DA in lizard head shape, we used an approach similar to the one used for SCGN. Procrustes ANOVA was performed on replicated landmark configurations with individual, side and their interaction as factors. As our results showed significant individual-side interaction and thereby

indicated the presence of FA, we calculated an AI for each individual. To calculate the AI in head shape, we first calculated the difference in landmark coordinates between the left and the right side of the head by comparing original landmark configurations with the mirror-reflected copies. We used only positive originalto-reflected differences, and in cases in which these were negative, we used the reflected-to-original differences, obtaining an unsigned AI for each specimen. This matrix of unsigned asymmetry values was further used to calculate an AI index by taking the square root of sums of squared differences from all configurations of landmarks (Lazić et al., 2015).

To test for differences in the head shape asymmetry across species, sexes and while incorporating other environmental factors (altitude, presence of other species, and urbanization), we performed permutation ANOVA with 10,000 repetitions using the 'adonis' function from the *vegan* package (Oksanen et al., 2010). To test for differences in asymmetries of SCGN, head size and head shape across altitudes, allotopic/syntopic populations or urban/natural populations, we used a factorial ANOVA design including categorical factors: species, sex, and population or urbanization, and a continuous factor: altitude. For pairwise post-hoc comparisons we used the Bonferroni test. We performed an additional set of analysis only on a subsample of populations to reduce the effects of environment (altitude and geographical differences of distant locations, as well as phylogenetical differences). We used six locations from close geographical proximity (region Kočevsko, south Slovenia), at similar altitudes (700-1050 m a.s.l., specified in table 2), where we already know that individuals share common mitochondrial and nuclear haplotypes (Salvi et al., 2013; Cocca et al., 2021).

Results

We used photographs of adults of both species from 16 populations (fig. 1, table 1). In total, 436 individuals (200 of *P. muralis* and 236 of *I. horvathi*) were sampled.

Visual representation of the variation in head shape showed that most of the variation was concentrated at the base of the head in both species. Two-way ANOVA on the number of supraciliar granules (SCGN) revealed statistically significant individual variation [\(supplementary table S1](https://doi.org/10.6084/m9.figshare.15657840)). DA was not detected (insignificant side effect, [supplementary table S1](https://doi.org/10.6084/m9.figshare.15657840)), while the presence of FA was confirmed, as indicated by the significant individual-side interaction ([supplementary table S1](https://doi.org/10.6084/m9.figshare.15657840)). Similarly, Procrustes ANOVA on replicate landmark recordings revealed significant individual variation in head shape, DA and FA ([supplementary table S1\)](https://doi.org/10.6084/m9.figshare.15657840).

The results from the entire sample set showed statistically significant differences in head size across species and sexes (species-sex factor interaction, table 2). Males had bigger heads than females, but the difference in the head size between sexes was more pronounced in *P. muralis* (fig. 2A) than *I. horvathi* (fig. 2B); *P. muralis* males had significantly bigger heads than females. Head size also differed depending on the presence of other species (factor population, table 2), with individuals

Table 2.

Head size Head shape asymmetry SCGN df *F p* df *F p* df *F p* Altitude 1 7.99 **0.004** 1 0.14 0.696 1 1.51 0.205 Species 1 36.66 **0.001** 1 3.79 0.060 1 1.58 0.217 Sex 1 144.54 **0.001** 1 1.70 0.216 1 0.06 0.804 Population 1 46.00 **0.001** 1 2.85 0.110 1 4.63 **0.030** Altitude: species 1 5.71 0.019 1 8.91 0.007 1 0.01 0.906
Altitude: sex 1 20.32 0.001 1 2.93 0.094 1 0.03 0.879 Altitude: sex 1 20.32 **0.001** 1 2.93 0.094 1 Species: sex 1 7.64 **0.006** 1 0.58 0.477 1 0.02 0.890 Altitude: population 1 1.79 0.189 1 1.14 0.271 1 0.04 0.815 Species: population 1 1.32 0.264 1 0.43 0.491 1 0.74 0.392 Sex: population 1 0.20 0.660 1 1.32 0.227 1 0.24 0.646
Altitude: species: sex 1 9.07 0.004 1 0.06 0.793 1 1.17 0.216 Altitude: species: sex 1 9.07 **0.004** 1 0.06 0.793 1 1.17 0.216 Altitude: species: population 1 54.34 **0.001** 1 12.22 **0.004** 1 0.45 0.454 Species: sex: population $1 \t 0.21 \t 0.658 \t 1 \t 0.29 \t 0.578 \t 1 \t 0.01 \t 0.925$ Residuals 319 319 319 212 Total 334 334 225

Results from ANOVA comparing head size, head shape asymmetry and asymmetry of the number of supraciliar granules across species, sex, altitude and populations (allotopic/syntopic) from all sampled populations. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; *F*, *F*-test value; *p*, *p*-value; SCGN, the number of supraciliar granules.

from allotopic populations having a larger head size compared to syntopic populations in both species (fig. 2C). We also found significant effects on the head size with altitude but in combination with other factors (see altitude factor interactions, table 2), thus, head size also varied in a complex manner across altitude. Males but not females exhibited a linear negative trend of head size with altitude (fig. 3A, B), while on the species level we did not observe a linear relationship (fig. 3C, D). When we limited analysis to the six populations with similar altitudes and close geographical proximity we again observed statistically significant differences in head size between the sexes, species, sex-species interaction and presence of other species (table 3).

Head shape asymmetry differed between species; *I. horvathi* individuals had a more asymmetric head shape (table 1); however, it also depended on altitude of the locality (the interaction between species and altitude was statistically significant, table 2). Linear relationships revealed that the head shape asymmetry increased with altitude in *P. muralis* (fig. 4A), but not *I. horvathi*, where the head asymmetry was most pronounced at mid altitudes (fig. 4B). Presence of other species showed a significant effect on the head shape asymmetry only in combination with the factors altitude and species (table 2), while in the case of analysis limited to six populations, the head shape asymmetry only differed between species; *I. horvathi* had more asymmetrical heads compared to *P. muralis* (table 3).

Figure 2. Head centroid size between sexes of *Podarcis muralis* (A) and *Iberolacerta horvathi* (B), and between allotropic and syntopic populations of both species (C) . F = female, M = male.

In the case of SCGN asymmetry we found a significant difference between allotopic and syntopic populations (table 2, fig. 5), which was also confirmed in the case of analysis limited to six populations (table 3). No differences

Figure 3. Head centroid size across altitude in females (A) and males (B), and in *Podarcis muralis* (C) and *Iberolacerta horvathi* (D).

Table 3.

Results from ANOVA comparing head size, head shape asymmetry and the number of supraciliar granules asymmetry across species, sex, and populations (allotopic/syntopic) in genetically similar populations from south Slovenia. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; *F*, *F*-test value; *p*, *p*-value; SCGN, the number of supraciliar granules.

were found in SCGN asymmetry between species or sexes and across altitude (table 2).

Podarcis muralis males had larger heads than females in natural and urban populations, but we did not detect any effect of urbanization on head size, head shape asymmetry or SCGN asymmetry (table 4).

Figure 4. Levels of head shape asymmetry across altitude in *Podarcis muralis* (A) and *Iberolacerta horvathi* (B).

Figure 5. Asymmetry in SCGN across species and populations. IHOR = *Iberolacerta horvathi*, PMUR = *Podarcis muralis*.

Discussion

The interplay of various stressors can interfere with the process of an organism's development and thus lead to various phenotypic changes. These changes can be

Table 4.

Results from ANOVA comparing head size, head shape asymmetry and the number of supraciliar granules asymmetry between sexes and between natural and urban sites (urbanization). $Df =$ degrees of freedom, $F = F$ -test value, $P = p$ -value. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; *F*, *F*-test value; *p*, *p*-value; SCGN, the number of supraciliar granules.

detrimental to the organism, if they affect survival. Therefore, it is important to detect stress before changes in the environment lead to population extinction. One possible way of detecting early signs of stress is by using asymmetries of various morphological features. In this study we tested three stress effects, originating from biotic interactions (presence of competitor species) or human influence (urbanization), and the environment (colder conditions for ectotherms at higher altitudes). Two of these factors were found to affect levels of head shape asymmetry and one factor affected the scale asymmetry.

Firstly, our results showed considerable differences in head size between species and between sexes. *P. muralis* individuals had larger heads, which corresponds with previously known behavioral differences between species that showed that *P. muralis* is more dominant in the case of agonistic interactions (Zagar et al., 2015a; Žagar et al., 2017). Sexual dimorphism in head size is pronounced in many lizards, which has been attributed to sexual selection for stronger and more aggressive males (Kaliontzopoulou et al., 2007; Žagar et al., 2012). Biting is involved in territorial defense and in mating, when males grab females to hold them during copulation (Pianka & Vitt, 2003). Stronger bite force of larger-headed lizards also enables them to ingest harder prey, which can reduce intersex and interspecific competition for the same food sources (Verwaijen et al., 2002). Head size could also be related to the use of shelters (smaller, more flattened heads could allow the use of smaller shelters; Kaliontzopoulou et al., 2010).

Drop of temperature with rising altitude can represent more restricted environmental conditions for ectotherm organisms, and thus we expected that populations from colder higher altitudes will display a higher level of asymmetries than lowland populations in both species, but more pronounced in the generalist species *P. muralis*, that exhibits a predominantly lowland and middle altitude distribution and is known to be less adapted to cold conditions (i.e., has a physiology that is less adapted to shorter activity periods compared to *I. horvathi*, Žagar et al., 2015b). Indeed, altitude had a significant effect, but in a complex manner in combination with the biotic factor (presence of other species), and species. We confirmed our expectations:we found that *P. muralis* lizards had more asymmetric heads at higher altitudes, but *I. horvathi* lizards did not. A cold environment may be more stressful to the more generalist species *P. muralis*, which might experience environmental stress at higher altitudes due to its physiology being adapted to warmer conditions where activity periods are longer (Zagar et al., 2015b). On the other hand, the highaltitude specialist *I. horvathi* did not exhibit higher levels of head shape asymmetry at higher altitudes, thus confirming its adaptation to a colder environment that actually represents its optimal habitat in the study area (Žagar, 2016).

However, we detected a mid-altitude effect on asymmetries in *I. horvathi*. The highest levels of head asymmetry at mid altitude in *I. horvathi* might be related to the lower availability of favorable habitats for lizards. Namely, the lowest proportion of open areas with rock walls is found at mid altitudes compared to higher altitudes, as well as the lowest altitudes, where the species occurs only at the

entrances of karstic caves with vertical walls (Žagar, 2016). Mid altitudes also represent the area of highest overlap among the two species, as *P. muralis* is more abundant at lower and middle altitudes and *I. horvathi* is a mountain species with highest abundances at higher altitudes (Sillero et al., 2014; Žagar, 2016). The higher abundance of *P. muralis* together with limited stone wall open areas at mid altitude may also induce higher competition between the two species. And it is known that under these conditions (where sun-exposed areas as resources for basking are limited) *P. muralis* has a competitive advantage over *I. horvathi* (Žagar et al., 2015a). The higher interspecific competition for sun-exposed areas that may occur here could result in greater stress to *I. horvathi*, which possibly contributes to the higher levels of asymmetry found with our results. We assume it is also likely that other environmental factors (i.e., habitat structures used for hiding places, food availability, parasites, etc.) besides temperature are responsible for such a result, where we would need a more controlled set of populations to study the effects exempt of different potential effects that are present in natural populations.

Next, when we compared allotopic and syntopic populations on the subset of populations (to exclude the factor altitude and avoid potential effects of phylogenetically distant populations), we obtained variable results for head shape, head asymmetry and scale asymmetry. We first found that in both species the head size was affected by the presence of competitor species; lizards had larger heads when one species occurred alone (allotopy). This suggests that in areas without interspecific competitor, species may invest more energy in growth. The presence of an interspecific competitor can result in a higher resource competition (Begon et al., 2006) and can have a stressful effect on both species, which could result in smaller heads. We also observed a pronounced sex difference with males having bigger heads that females (following earlier obtained results on this species, Žagar et al., 2012). Bigger heads may be more pronounced in allotopy due to the fact that sexual selection might be stronger in allotopic populations. It is known that higher conspecific competition drives the larger size of heads, which correlates with stronger bite forces and dominance in male-male combats for females (Žagar et al., 2017).

Next, we found that in *I. horvathi* head shape was more asymmetric compared to *P. muralis*, which suggests that *I. horvathi* could be under greater stress or be more susceptible to stress effects than *P. muralis*. Such an interspecific difference suggests that the ability to buffer environmental disturbance may differ between species, and it may be connected with a more generalist character of *P. muralis*, which is very adaptable and tolerates more anthropogenic influence than most other European lizards (Arnold and Oveden, 2004). The results obtained are also interesting, because these could have an indirect effect on species' competitive interaction via the 'apparent competition' mechanism (Holt, 1977). The studied species are in fact known to potentially compete under syntopic conditions and limited microhabitat conditions connected with thermoregulation (Žagar et al., 2015a). However, in the case of SCGN asymmetries, we found – quite contrary to our expectations that competition could increase stabilizing selection – that animals tend to be more

symmetric in the presence of the other species. However, many other factors may also affect stabilizing selection, while our predictions were set to this expectation due to our study aim and sampling in syntopic and allotopic populations. Possible explanations for this finding in the light of the effects of competition might be that individuals that suffer more from interspecific competition may have a higher mortality and less reproductive success and thus lower adaptive value. Therefore, such individuals do not succeed in the population and are consequently more rare. This was also explained in some other studies (Thornhill, 1992; Møller et al., 1998; Tomkins & Kotiaho, 2001) and may also provide the explanation for why we did not detect them in our populations. Another explanation may also lie in the fact that conspecific competition exhibits higher stress pressure than interspecific competition, which is reflected in higher asymmetry in allotopic populations. We also acknowledge that this result could be due to imbalance of the sample, which is in favor of allotopic populations. Also, SCGN – as far as we are aware – have no evident functional significance, meaning that selection for DS in this character may be relaxed.

Finally, we tested if populations of *P. muralis* from urbanized (hence degraded and possibly polluted) sites show higher FA levels compared to natural habitat sites. Results provided no evidence that the urbanization influences DS of morphological traits in *P. muralis* in our study area. On one hand, this could mean that the two populations classified as urbanized in our study (railway tracks in the city of Ljubljana and the cemetery in the village of Fara) are not degraded or polluted to the level that it would disrupt developmental homeostasis and affect the developmental path of this species. To confirm this, comparison with populations from more degraded or polluted habitats would be required. To increase the detection of FA, it would also be useful to analyze a larger spectrum of physical characteristics, including already established features for measuring FA (e.g., length of hind legs, number of femoral pores, border points on lateral side of head, etc.; López & Martín, 2002; Crnobrnja-Isailović et al., 2005; Martín & López, 2006; Kaliontzopoulou et al., 2007; Lazić et al., 2013).

In conclusion, our study presents novel data on the effects of biotic stressors, such as interspecific competition, on the DS in lizards. The effects were found on the level of head shape asymmetry, but not in the scale asymmetries. Since the studied species differ in altitudinal preferences, we managed to understand the combination of effects of potential competition and environmental conditions that change across the altitude. These results emphasize the need to include variable relevant effects (biotic and abiotic) when trying to understand the occurrence of asymmetries as bioindicators of stress. We clearly highlighted the possibility of using lizards and FA for bioindication of environmental stressors.

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Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.15657840>

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Animal Biology

Effects of biotic and abiotic stressors on asymmetries and head size in two sympatric lizard species

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Supplementary material

Table S1.

Results from ANOVA testing for the presence of directional (DA) and fluctuating asymmetries (FA) in the number of supraciliar granules and head shape of lizards from all sampled populations. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; *F*, *F*-test value;*p*, *p* value, SCGN, the number of supraciliar granules.

Table S2.

Background dataset used in the analysis.

Abbreviations: IHOR = *Iberolacerta horvathi*, PMUR = *Podarcis muralis*, M = male, F =

female, SVL = snout to vent length (cm), natur = natural habitat, urban = urb