



Original Articles

The exposition to urban habitat is not enough to cause developmental instability in the common wall lizards (*Podarcis muralis*)

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ABSTRACT

Developing trustworthy biomarkers is of primary importance in monitoring impacts of environmental stressors on biological systems. Recently, fluctuating asymmetry in morphological traits of the common wall lizard (*Podarcis muralis*) has been proposed as useful tools for assessing environmental disturbance in urban habitats. However, the experimental design used in previous studies lacked both site replication (only one urban area was sampled) and independent assessment of the physiological conditions of lizards. Hence, we replicated the analysis involving three large cities in Northern Italy and measured lizards' health conditions as assessed by blood sampling analyses. We found a weak statistical association between fluctuating asymmetry and urban habitat. No significant differences occurred for white blood cell concentration and heterophils-to-lymphocytes ratio, whereas parasite load was significantly lower in urban than natural populations. Finally, somewhat of the variability in fluctuating asymmetry correlated with one or more latent variables not directly observed, but still not related to the habitat. Contrary to previous studies, we concluded that fluctuating asymmetry cannot be regarded as a reliable measure of urban environmental stresses. Despite this, fluctuating asymmetry in the morphology of common wall lizards has a real potential for working as biomarkers, but we need to clarify first the developmental mechanisms, the true effectors of the stress, their interactions and their effects on individual fitness in order to solve the inconsistencies occurring among different studies.

1. Introduction

Urban environments make animal populations experience a variety of new conditions, such as different predatory pressure, increased temperatures, and unusual food sources (Gering and Blair, 1999; Ditchkoff et al., 2006; Murphy et al., 2016) just to name a few. Such new conditions may stress individuals leading to homeostasis disruption and, ultimately, to individual fitness decline. However, not all species respond negatively to human-related habitat alterations, and some species may actually take advantage of the new characteristics (Martín and López, 1999a,b, 2015; Diego-Rasilla, 2003). In this scenario, conservation biologists are trying to figure out sensitive biomarkers to reliably measure exposition to various stressors, and early detect any decline in population health and consistence before it is too late (Forbes et al., 2006).

Among all possible biomarkers proposed till now (including molecular, cellular, and histological biomarkers, McCarthy and Shugart,

1990) morphological traits are particularly promising as they are cheap, low time-consuming, and not invasive. Beasley et al. (2013) suggested the use of fluctuating asymmetry (FA) as a morphological biomarker, basing on the idea that FA is a proxy for environmentally induced developmental instability. FA is defined as small, random deviations of bilateral traits from perfect symmetry due to subtle variations in the developmental environment (Palmer and Strobeck, 2003). Thus, environmental stressors interfering with the ability of individuals in maintaining precise development (resulting in negative effects on the population over time) could be detected by significantly increased FA levels. However, the initial attractiveness of FA as a potential biomarker has been weakened due to inconsistent results across species. In their meta-analysis, Beasley et al. (2013) concluded that the use of FA as a biomarker is legitimate provided that the measurement tool used to assess FA is highly sensitive (e.g., geometric morphometrics compared to linear and/or meristic measures), individuals are not able to buffer a developmental pathways against the stressor (e.g., anthropogenic

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compared to natural stressors), and the environmental stressor acting on the organism is known (e.g., the negative effects on fitness-related traits are verified). Last but not the least, stress related to FA is detected more easily in laboratory compared to field populations.

Lizards offer a good model for measuring exposition to environmental stressors since they are widespread and well adapted to urban habitats, they live at high density, move across very small home ranges (hundreds of square meters) and are easy to catch and keep in the laboratory. Accordingly, a lot of studies have used lizards for biomarker essays during the last two decades (e.g., *Gallotia galloti*, Fossi et al., 1995; Sanchez-Hernandez et al., 1997; *Diplodactylus conspicillatus* and *Rhynchoedura ornata* Read (1998); *Podarcis carbonelli*, Mann et al., 2007; *Agama agama*, Oyekunle et al., 2012; *Podarcis siculus*, Marsili et al., 2009; Verderame et al., 2016).

FA has been widely detected in lizards, and correlations with a variety of environmental conditions have been found, including population, habitat and fragmentation, temperature, pollution, and insularity (Sarre and Dearn, 1991; Sarre, 1996; Tull and Brussard, 1944; Vervust et al., 2008; Băncilă et al., 2010; Garrido and Pérez-Mellado, 2014; Urošević et al., 2015; Lazić et al., 2013, 2015). However, results are once again contradictory, and there are studies that failed to detect relationships between FA and environmental features (e.g., Crnobrnja-Isailović et al., 2005; Warner and Shine, 2006; Amaral et al., 2012). By contrast, only a few studies have investigated the effects of FA on fitness-related traits, once again with opposite results: active effects have been found on exertion (Vervust et al., 2008), escape speed (López and Martín, 2002), femoral pore secretions (Martín and López, 2000; López et al., 2002; López et al., 2006), incubation temperature (Ji et al., 2002), but not on bite force (Vervust et al., 2008), incubation temperature (Longson et al., 2007), female mating preference (Lailvaux and Irschick, 2006), testosterone plasma level (Veiga and Salvador, 1997), and hybridization (Dosselman et al., 1998).

Despite these non-univocal results, most studies have analyzed the correlation between the magnitude of FA and some environmental features without an independent validation of the potential relation linking stressors to FA (reviewed in Leung et al., 2003). That is, some environmental features have been assumed to be stressful for lizards *a priori*, without any assessment of their actual power to stress individuals settled in those habitats. A typical example of this approach concerns urban habitat: it is often considered suboptimal (i.e., more stressful) than natural ones based on the assumed negative effects of human alterations. If this reasonably applies for larger species, such mammals or birds, it becomes questionable for such small species as *Podarcis* lizards, which have been evolved in rocky and dry habitats, resulting pre-adapted to human-made environments. Hypothetically, lizards in human environments might experience lower predation risk as well as higher food availability, thus achieving higher densities than in natural habitats. Paradoxically, we could detect higher FA in human-made environments because of increased competition due to increased density rather than to human-related stressors. Without a direct measure of individuals' healthy condition, these two opposite states cannot be fully disentangled.

Recently, Lazić et al. (2013, 2015) compared the FA levels of the common wall lizard (*Podarcis muralis*) across urban and rural populations around the city of Niš (Serbia). Data collected consistently suggested that urban lizards showed higher levels of FA in four morphological traits, and this was interpreted as an evidence that anthropogenic disturbance causes an increase in FA, and hence FA can be a reliable indicator of exposition to environmental stressors following human activities. However, authors did not replicate the experiment in other cities and did not make an independent assessment of the physiological conditions of the sampled lizards. Indeed, they assumed *a priori* common wall lizards living in urban habitat being in suboptimal habitat conditions. Consequently, the experimental design did not allow to disentangle the effects of the urban environment in itself, apart from the specific features of the City of Niš. Even more, the

conclusion that fluctuating asymmetry of common wall lizards is a reliable measure of urban environmental stresses needs further experimental support.

In the present study, we analyzed the magnitude of FA in four morphological traits of the common wall lizards across urban and rural populations and we compared the degree of FA observed in urban and rural habitats with lizards' healthy conditions as assessed by blood sampling analyses. We replicated the comparison in three separate urban areas of Northern Italy in order to generalize results as representative of the effects of urbanization on the developmental instability of the species.

2. Methods

The Common wall lizard is a small lizard (snout-vent length, SVL, 45–75 mm) widespread in southern and central Europe, which mates multiply and produces two clutches per year on average (Sacchi et al., 2012) during its life (max lifetime 5 years, Barbault and Mou, 1988). Breeding season starts from late February and ends in July (Sacchi et al., 2012), and body temperature during activity is near 33 °C, being slightly higher (33–36 °C) in warmer regions (e.g., Central Italy) and lower (32 °C) in mountain areas (Tosini and Avery, 1994).

2.1. Study area and design

The study was carried out during April–July in three cities of the Liguria region in Northern-Italy at least 25 km apart from one another: Genoa (44°25'N, 8°54'E), Recco (44°21'N, 9°08'E), and Pietra Ligure (44°8'N, 8°16'E) respectively (Fig. 1). For each site we sampled three populations in as many different habitats, one in a fully urbanized area in the city center (urban habitat), one in a partially urbanized area with gardens and urban parks (sub-urban habitat), and one in a natural environment in the surrounding of the city (natural habitat). Adult common wall lizards (snout-vent length, SVL > 54 mm, Sacchi et al., 2012) of both sexes were collected by noosing, between 0900 and 1400 h, and not less than 10 individuals for each sex were caught. Overall, we collected 211 lizards (on average 23 for population, range 21–25), including 107 males and 104 females. After capture, lizards were transported to the laboratory of the University of Pavia where they were weighted and measured for snout-vent-length (SVL). For each individual we took high resolution digital images of ventral scales (VS), ocelli on both flanks (OC), femoral pores (in males only, FP), and head dorsal scales (HS) using a Nikon D50 camera at a 1.2-million-pixel resolution, equipped with a Nikkor 60 mm AF-S Micro lens, and fixed on stand at a distance of 18 cm.

2.2. Fluctuating asymmetry quantification

For each individual, one of us (MP) recorded the number of VS, OC, and FP twice from the digital images, with one week between the first and the second counting and randomizing the order of examined specimens, to ensure the independence of trait counts. We then calculated for each trait the difference between the value on the right side of the body minus that on the left side (R-L). Hence, for each individual we calculated three overall asymmetry indexes (AI_{VS} , AI_{OC} , AI_{FP} respectively) following Lazić et al. (2015) as the unsigned R-L difference between the log-transformed average of trait values across the two replicate counts as it follows:

$$AI = |\ln(R_{average}) - \ln(L_{average})|$$

Digital images of HS were analysed using geometric morphometrics. One of us (MP) digitized 36 landmarks (Fig. 2), twice for each lizard, using TpsDig2 (Rohlf, 2005; <http://life.bio.sunysb.edu/morph/>). As for previous traits, we allowed at least one week between subsequent digitalizations of the same image, and the order was randomized. Subsequently, we first reflected all landmark configurations to obtain their

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