Volume 34 (April 2024), 92–99

Herpetological Journal FULL PAPER

https://doi.org/10.33256/34.2.9299

Published by the British Herpetological Society

Seasonal variation of the relationship between body condition and fluctuating asymmetry in two sympatric ranid frogs

Enrique Cante-Bazán[1] & Ricardo Luría-Manzano[2]

[1]Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico

[2]Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Fluctuating asymmetry (FA) in organisms is an indicator of disturbances during development. This indicator has been associated with multiple causes, such as presence of metals, and organic and inorganic pollutants. However, the effect of FA on individual traits has been little explored. In this study, we examine the presence of FA in some morphological characters in two sympatric species of ranid frogs, *Lithobates spectabilis* and *Lithobates zweifeli*, predicting that *L. zweifeli* would exhibit more characters with FA and higher levels of FA. We also evaluated whether body condition in the two species differ between seasons and whether body condition is associated with FA of limb characters in two seasons. We predicted that this association would be more pronounced in the dry season. We found FA in tympanum and limb characters in both species, but *L. zweifeli* exhibited FA in one character more than *L. spectabilis*, as well as higher levels of FA in the horizontal length of the tympanum. Additionally, the characters exhibiting FA only in *L. zweifeli* are presumed to be more relevant for locomotor performance, fitness and survival than those exhibiting FA only in *L. spectabilis*. We did not find differences in body condition between seasons in either species, but we found a negative correlation between body condition and FA of the humerus in *L. zweifeli*. However, this correlation was detected in the rainy but not in the dry season, which could be related to depletion of energetic reserves associated with reproductive investment. This study highlights the importance of evaluating sensitivity of sympatric species through analyses of FA and its effects on individual traits, as well as the analysis of these effects in seasons with different environmental conditions.

Keywords: Atoyac basin, limb asymmetry, *Lithobates*, Mexico, tympanum asymmetry

INTRODUCTION

Developmental stability is the physiological process by which the expression of a predetermined phenotype is regulated by stimuli and environmental conditions during growth (Zakharov et al., 2020). This process can be altered under conditions of environmental and genetic stress, causing morphological characteristics to deviate from the standard, a phenomenon that has been called developmental instability or noise (Grillitsch & Chovanec, 1995; Glennemeier & Begnoche, 2002; Benítez & Parra, 2011). Bilateral variation has historically been measured through asymmetry, a morphometric pattern that reflects the small deviations that occur between the right and left sides in organisms with bilateral symmetry (Palmer, 1994; Niemeier et al., 2019; Gondim et al., 2020). There are three typical patterns of asymmetry: directional asymmetry (DA), antisymmetry, and fluctuating asymmetry (FA; Van Valen, 1962). Fluctuating asymmetry has been considered a useful indicator of the health of populations under conditions of anthropisation, pollution, food scarcity and increases in predators or competitors (Brown, 1996; Söderman et al., 2007; Benítez et al., 2020). Therefore, the analysis of FA is an alternative to long-term population studies. In some cases, the latter could take longer to be conducted than the time during which population declines occur, making studies of FA particularly important in groups highly susceptible to declines, such as amphibians (Stuart et al., 2004; Catenazzi, 2015).

Studies of FA in amphibians have been focused on species of the families Ranidae and Bufonidae (e.g. *Pelophylax ridibundus, Rana arvalis, Bufotes viridis*). These studies have found that deforestation, discharges from urbanised sites, heavy metals, pesticides and fertilisers affect the normal development of individuals, which is reflected in higher levels of FA in morphology. The most affected morphological traits are the limbs, digits and dorsal spot colouration patterns (Wright & Zamudio, 2002; Lauck, 2006; Söderman et al., 2007; Eisemberg & Bertoluci, 2016; Guo et al., 2017; Zhelev et al., 2017; Khattab et al., 2021). Despite the growing information about factors that cause FA, few studies have paid attention to the effects of this phenomenon on morphological, ecological and behavioural traits in amphibians. For example, FA of the tympanum in females of *Alytes obstetricans* reduces their accuracy in detecting sound sources, which could be detrimental to their survival and reproductive success (Bosch & Márquez, 2000). On the other hand, there is no effect of FA of hindlimbs on locomotor performance in *Pelophylax perezi* (Moreno-Rueda et al., 2020).

Correspondence: Enrique Cante-Bazán (cante662@gmail.com)

Another variable potentially affected by FA is body condition. For example, in *P. ridibundus* it was found that FA of the number of spots is high and body condition is low in disturbed sites (Zhelev et al., 2017). Additionally, individuals with higher levels of FA of the number of spots have lower values of body condition in the salamander *Ambystoma maculatum* (Davis & Maerz, 2007). However, in the frogs *Dendropsophus ebraccatus* and *Agalychnis callidryas* the results are not consistent. In the former species, body condition decreases in disturbed sites, but FA is higher in undisturbed sites. In *A. callidryas*, neither body condition nor FA decreases in disturbed sites (Matías-Ferrer & Escalante, 2015). Available evidence thus suggests that FA may have negative effects on body condition in some cases, but additional studies are needed in this regard. The inclusion of other variables that affect body condition in studies relating it to FA may help to identify such cases.

Since body condition is an estimate of the nutritional state of an individual, it can provide a snapshot of the physiological state (Jakob et al., 1996). Therefore, this variable is susceptible to temporal variations in environmental conditions. For example, increases in environmental temperatures and habitat disturbance over the years reduce body condition in some amphibian species (Reading, 2007; Tomašević et al., 2007; Vera-Candioti et al., 2019; Cogălniceanu et al., 2021). Additionally, it has been observed that body condition varies between seasons in amphibians such as *Lechriodus fletcheri* and *Bombina variegata*. This is possibly related to the reproductive season, when energy reserves are used and there is greater intraspecific competition for resources; alternatively, this variation may be related to the period after winter (Gould & Valdez, 2021; Băncilă et al., 2010). Therefore, seasonal variation in environmental conditions could strengthen the negative effect of FA on body condition, so it is relevant to consider this variation.

Amphibians exhibit interspecific variation in their sensitivity to environmental stressors, a variation that depends on the characteristics of each species and their ecological interactions (Angulo, 2002). This variation can be studied in amphibian species that live in sympatry, as they share the same environmental conditions (e.g. food, reproductive sites and climate), and thus may be under similar selective pressures. The anuran species *Lithobates spectabilis* and *Lithobates zweifeli* belong to the family Ranidae, are endemic to Mexico, distributed from north-east Jalisco to Guerrero, and live in sympatry in the Ameyalpa stream, which belongs to the Atoyac basin. The Atoyac river is the main water body of this basin, and has high levels of heavy metals such as Cd, Pb and Cr, which are commonly associated with FA (Mancilla-Villa et al., 2012; Ortiz-Alamilla et al., 2015; Guo et al., 2017). With this information in mind, the objectives of this study are: i) to examine the levels of FA in *L. spectabilis* and *L. zweifeli* in the Ameyalpa stream, ii) to compare body condition between seasons in both species, and iii) to evaluate the effect of FA on body condition in different seasons. Although neither species is considered to be under a risk category of extinction according to the IUCN (2022), *L. spectabilis* is commonly found in urbanised sites, whereas *L. zweifeli* is not (MacGregor-Fors et al., 2012), suggesting that the latter species is more sensitive to habitat modification. Accordingly, we predicted that *L. zweifeli* would exhibit FA in more characters and higher levels of FA than *L. spectabilis*. Furthermore, we predicted that body condition will be lower in the dry season, that it will be negatively affected by FA of the limbs, and that this latter effect will be more pronounced in the dry season. We predicted these patterns due to the increased use of energy reserves caused by resource scarcity during the dry season (Tomašević et al., 2007; Băncilă et al., 2010).

MATERIALS & METHODS

Fieldwork was conducted in the Ameyalpa stream, located in La Magdalena Cuaxixtla, municipality of Tecali de Herrera, state of Puebla, central Mexico (18.8835° N, 97.9526° W; 2041 m a.s.l). The predominant vegetation type is xeric shrubland, composed of succulent plants, some of which form colonies (Rzedowski, 2006). The study site is part of the Atoyac basin, which in turn belongs to the Río Balsas hydrological region (INEGI, 2009). The climate is temperate sub-humid with summer rains, with a mean annual temperature of 18.3 °C and an annual precipitation of 534 mm. In 2018, the mean temperature was 19.7 °C and precipitation was 155 mm in the months of the dry season covered in this study (March–May). The mean temperature was 19.7 °C and precipitation was 288 mm in the months of the rainy season covered in the study (June–September; CONAGUA, 2020).

We conducted nine three-day trips to the Ameyalpa stream, from March to September 2018. We collected postmetamorphic individuals of *L. spectabilis* and *L. zweifeli* manually or with a dip net and took them to the camp. We obtained body weight with a spring scale (± 0.1 g) and measured snout-vent length (SVL) with a digital caliper (± 0.1 mm) for each frog. Subsequently, individuals with a SVL ≥ 50 mm were anesthetised by placing them in a plastic bag with an ether-moistened cotton ball (Stetter, 2001; Zhelev et al., 2017). In *L. spectabilis*, the mean time of anaesthesia was 7.9 min and the mean recovery time was 20.21 min; in *L. zweifeli*, the mean time of anaesthesia was 4.91 min and the mean recovery time was 24.96 min. Once the frog was sedated, we measured the length of six morphometric limb characters: femur, tibia-fibula, humerus, radio-ulna, third finger and fourth toe (Matías-Ferrer & Escalante, 2015; Eisemberg & Bertoluci, 2016). We also measured the horizontal and vertical length of the tympanum (Bosch & Márquez, 2000). Additionally, we photographed the dorsum of individuals of *L. spectabilis* with a reflex camera at a standardised distance, to evaluate asymmetry in colouration pattern. It was not possible to evaluate asymmetry in colouration pattern in *L. zweifeli*, since this species does not present clearly defined spots that contrast with the background colouration. All measurements were taken with a digital calliper (± 0.1 mm), which was zeroed after each measurement, and were taken thrice by the same person (ECB) without access to values of previous measurements, to reduce

Table 1. Results of mixed-factor analyses of variance for individuals (genotype) and trait side of the two ranid frog species studied. Significant P values for the interaction of side vs. genotype are in bold, which indicates fluctuating asymmetry.

	Source of variation	*Lithobates spectabilis*			*Lithobates zweifeli*		
		MS	F	P	MS	F	P
Femur	Genotype	80.32	85.84	< 0.0001	176.06	204.46	< 0.0001
	Side	36.24	21.32	< 0.0001	72.86	48.76	< 0.0001
	Side vs. Genotype	1.7	1.82	**0.004**	1.49	1.74	**0.006**
	Error	0.935			0.86		
Tibia-fíbula	Genotype	92.46	153.7	< 0.0001	188.41	293.79	< 0.0001
	Side	5.35	6.92	0.011	0.0002	0	0.98
	Side vs. Genotype	0.77	1.29	0.135	1.57	2.46	**< 0.0001**
	Error	0.6			0.64		
Humerus	Genotype	18.26	1.18	0.237	10.09	49.29	< 0.0001
	Side	45.33	2.88	0.097	3.38	7	0.011
	Side vs. Genotype	15.72	1.01	0.46	0.48	2.36	**< 0.0001**
	Error	15.54			0.2		
Radio-ulna	Genotype	9.67	21.07	< 0.0001	28.28	88.71	< 0.0001
	Side	1.81	3.15	0.083	0.85	1.43	0.23
	Side vs. Genotype	0.57	1.25	0.164	0.59	1.88	**0.002**
	Error	0.45			0.31		
Third finger	Genotype	12.87	39.58	< 0.0001	28.99	78.83	< 0.0001
	Side	0.3	0.22	0.642	4.99	5.12	0.02
	Side vs. Genotype	1.4	4.32	**< 0.0001**	0.97	2.65	**< 0.0001**
	Error	0.325			0.36		
Fourth toe	Genotype	73.67	55.82	< 0.0001	267.51	1.69	0.01
	Side	23.38	1.68	0.202	66.88	0.43	0.517
	Side vs. Genotype	13.93	10.56	**< 0.0001**	156.85	0.99	0.495
	Error	1.31			158.3		
Tympanum horizontal length	Genotype	4	85.08	< 0.0001	4.98	49.25	< 0.0001
	Side	0.07	0.44	0.512	0.007	0.03	0.873
	Side vs. Genotype	0.17	3.65	**< 0.0001**	0.278	2.75	**< 0.0001**
	Error	0.04			0.101		
Tympanum vertical length	Genotype	3.94	49.38	< 0.0001	764.69	1.01	0.457
	Side	0.004	0.03	0.856	673.58	0.89	0.35
	Side vs. Genotype	0.131	1.64	**0.015**	757.03	1	0.473
	Error	0.079			753.93		

measurement error and increase precision (Palmer, 1994). Afterwards, the frogs were individually marked by toe-clipping to avoid pseudoreplication (Donnelly et al., 1994; HACC, 2004), vigorously washed with water after they recovered from the anaesthesia, and released at the capture site.

Data analyses

We used photographs of the dorsum of individuals to estimate total area covered by spots and number of spots on each side of the body by drawing a median line along the dorsum from snout to vent, using the Image J 1.51 program (Gallant & Teather, 2001; Wright & Zamudio, 2002). We estimated body condition with the residuals of the regression of the natural logarithm of weight against natural logarithm of SVL (residual index; Jakob et al., 1996). We classified positive values of body condition as indicating a good condition of the individuals, and negative values a poor condition (Peig & Green, 2009; Băncilă et al., 2010).

Normality of morphometric variables and body condition was verified with Kolmogorov-Smirnov tests. We evaluated the existence of FA in each morphometric character using a mixed-factor ANOVA. To determine if variation between sides (left and right) was higher than variation among replicates of the same measure (i.e. measurement error), sides and individuals were entered as fixed and random effects, respectively. A significant effect of the sides indicates the presence of DA. On the other hand, a significant effect of the interaction between sides and individuals indicates the presence of FA (Palmer & Strobeck, 1986). We estimated the levels of FA for those

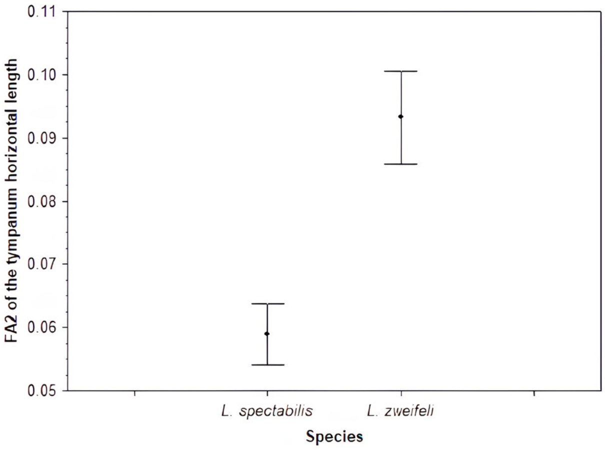

Figure 1. Mean ± SE of the level of fluctuating asymmetry (FA2; see Materials & Methods) of the horizontal length of the tympanum of the two species studied.

Table 2. Level of fluctuating asymmetry (FA10; see Materials & Methods) for both species. Cells without values correspond to morphological traits that did not show fluctuating asymmetry in a given species.

	AF10	
	Lithobates spectabilis	***Lithobates zweifeli***
Femur	0.255	0.211
Radio-ulna	-	0.093
Humerus	-	0.093
Tibia-fibula	-	0.31
Third finger	0.36	0.202
Fourth toe	4.2	-
Tympanum horizontal length	0.041	0.059
Tympanum vertical length	0.017	-

variables that exhibited this phenomenon with two indices: FA2, recommended because it corrects for dependence on body size, and FA10, because it describes the differences between sides after removing measurement error (Palmer, 1994; Villa, 2014; Matías-Ferrer & Escalante, 2015). We used the Levene test to determine if FA2 differed between species for those variables for which both exhibited FA (Palmer, 1994; Eisemberg & Bertoluci, 2016).

We tested for the presence of DA in the area covered by spots and the number of spots in *L. spectabilis* with one-sample t-tests, in which we compared R–L against a mean equal to 0. We used a linear regression of |R–L| against SVL to evaluate whether there is a relationship of the area covered by spots and the number of spots with body size. Deviations attributed to FA occur randomly and independently on each side of the body, so it is expected that if these deviations are caused by developmental noise, they would present a normal distribution. We used Kolmogorov-Smirnov tests to determine whether the frequency distributions of FA2 of the area covered by spots and the number of spots followed a normal distribution, and thus confirm the presence of FA (Palmer, 1994; Gallant & Teather, 2001).

We did not consider sex in the analyses due to the inconsistencies in the available evidence regarding the impact of this variable on fluctuating asymmetry (Zhelev et al., 2012; 2017), and since its inclusion would significantly reduce the statistical power of our tests. We compared body condition between seasons for each species with a one-way ANOVA. We conducted multiple regressions of body condition against FA2 of those variables of the limbs that exhibited FA for each species by season. We tested for multicollinearity among predictors in multiple regressions with variance inflation factor (VIF) analyses (Midi et al., 2010). The maximum VIF value we obtained was 1.36, which indicates sufficient independence among predictors. Statistical analyses were conducted in NCSS 12 and Minitab 19. Results are shown as mean ± 1 SE unless otherwise indicated.

RESULTS

Forty-two individuals of *L. spectabilis* ($\bar{x}$ = 54.48 ± 1.98 mm SVL) and 45 individuals of *L. zweifeli* ($\bar{x}$ = 58.97 ± 2.0 mm SVL) were considered for the analyses. We found DA in the length of the femur and tibia-fibula in *L. spectabilis*, and in the length of the femur, humerus and third finger in *L. zweifeli*. We found FA in the length of the femur, third finger and fourth toe, and the horizontal and vertical length of the tympanum in *L. spectabilis*; and in the length of the femur, tibia-fibula, humerus, radio-ulna and third finger, and the horizontal length of the tympanum in *L. zweifeli* (Table 1). The level of FA2 in the horizontal length of the tympanum was higher in *L. zweifeli* than in *L. spectabilis* ($F_{1,83}$ = 5.91, P = 0.02; Fig. 1); but there were no differences in FA2 of femur ($F_{1,83}$ = 0.04, P = 0.82) or third finger length ($F_{1,83}$ = 0.65, P = 0.42) between species. According to the FA10 index, the fourth toe was the most asymmetric character in *L. spectabilis*, and the tibia-fibula was the most asymmetric in *L. zweifeli* (Table 2).

We did not find DA in the variables of spots in *L. spectabilis* (t = 1.08, P = 0.28 for the area covered by spots; t = 1.17, P = 0.25 for the number of spots). We did not find a relationship between asymmetry in the variables of spots and SVL (R^2 = 0.07, P = 0.84 for the area covered by spots; R^2 = 0.005, P = 0.65 for the number of spots). The area covered by spots and the number of spots did not follow a normal distribution (P < 0.01 in both cases), so we ruled out the presence of FA in these variables.

We did not find significant differences in body condition between seasons in *L. spectabilis* ($F_{1,40}$ = 0.14, P = 0.70, n = 42), nor in *L. zweifeli* ($F_{1,43}$ = 0.86, P = 0.35, n = 45). In *L. spectabilis*, body condition was not associated with FA2 of the femur, third finger or fourth toe in either season (r = 0.34, P = 0.42, n = 25 for the dry season; r = 0.45, P = 0.45, n = 15 for the rainy season). In *L. zweifeli*, body condition was not associated with FA2 of the femur, tibia-fibula, humerus, radio-ulna, nor third finger in the dry season (r = 0.41, P = 0.40, n = 32). However, this association was significant in the rainy season (r = 0.85, P = 0.05, n = 13), particularly with body condition being negatively correlated with FA2 of the humerus (r = −0.70, P = 0.007; Fig. 2).

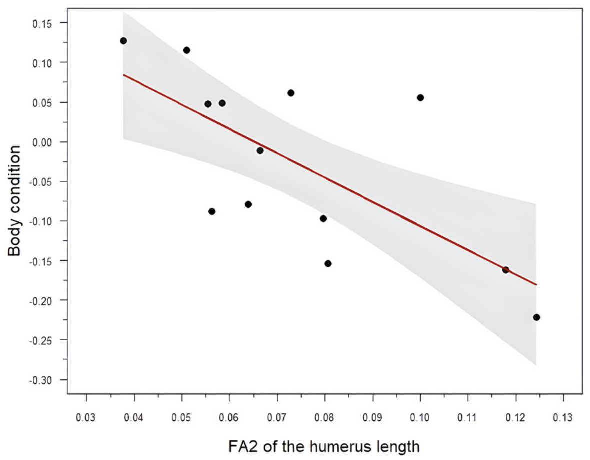

Figure 2. Linear regression between body condition and level of fluctuating asymmetry (FA2; see Materials & Methods) of the humerus length in *L. zweifeli* during the rainy season. Shadow areas represent the 95% confidence intervals.

DISCUSSION

The results of this study suggest that normal development of the two ranid frog species analysed could be affected by some stressor agent in the Ameyalpa stream. In line with our predictions, *L. zweifeli* exhibited FA in one more character than *L. spectabilis*, and also higher levels of FA in the horizontal length of the tympanum. Furthermore, we consider that the characters exhibiting FA only in *L. zweifeli* (i.e. tibia-fibula, humerus and radio-ulna) could be more relevant for locomotor performance, fitness and survival than the characters exhibiting FA only in *L. spectabilis* (i.e. fourth toe and vertical length of the tympanum). Based on these results, the response to the stress conditions is different for each of these sympatric species, with *L. zweifeli* being more susceptible to these conditions, making it a more reliable indicator of environmental health than *L. spectabilis*. We predicted that body condition would be lower in the dry season, but we did not find differences in this variable between seasons in either species. Finally, we found a negative correlation between body condition and FA of the humerus only in *L. zweifeli*, which partially supports our prediction. However, this correlation was detected in the rainy but not in the dry season, contrary to our expectation.

Origins of FA are diverse (e.g. organic and inorganic pollution, habitat modification and disturbance of ecological interactions). Many studies have evaluated the state of organic pollution in the Atoyac basin, to which the Ameyalpa stream belongs. These studies have found high concentrations of metals such as Pb, Cd, Hg, Fe, Al and Cu, as well as As, in both water and sediment (García-Nieto et al., 2011; Mancilla-Villa et al., 2012; Pérez-Castresana, 2019; Castro-González et al., 2019). Studies have found that Cd, Cr, Cu and As have negative effects on the survival and mobility of amphibians (Parris & Baud, 2004; Grillitsch & Chovanec, 1995), and that a first indicator of this detrimental effect is the appearance of FA (Khattab et al., 2021). Another detrimental effect is that organisms exposed to these metals accumulate high quantities in the kidney and liver, which may be related to high levels of FA (Guo et al., 2017; Zhelev et al., 2022). Considering that several of the contaminated localities and with high concentrations of metals in the Atoyac basin are located upstream of our study area (García-Nieto et al., 2011; Mancilla-Villa et al., 2012; Pérez-Castresana, 2019; Castro-González et al., 2019), it is possible that the Ameyalpa stream also contains high levels of metals. Therefore, it would be important to conduct further studies to evaluate whether FA of morphometric characters in *L. spectabilis* and *L. zweifeli* in this stream is related to heavy metals dissolved in the water.

Many studies have found that climate conditions cause variations in body condition (Reading, 2007; Băncilă et al., 2010; Matías-Ferrer & Escalante, 2015). However, we found that body condition did not differ between seasons in the populations studied, which suggest that environmental conditions were not inadequate for the organisms in either season. We must consider that body condition is also associated with other factors such as availability of resources and population density (Gómez-Hoyos & Maya, 2014; Unglaub et al., 2018; Vera-Candioti et al., 2019; Cogălniceanu et al., 2021). Therefore, it is possible that body condition in these populations is related to these variables, which were not considered in this study. Additionally, it has been suggested that species with generalist dietary habits are less vulnerable to disturbance than species with specialist dietary habits. Since the two species studied here have generalist diets (Mendoza-Estrada et al., 2008; Woolrich-Piña et al., 2017), this may partially explain the scant seasonal variation in body condition we found. Finally, we cannot rule out the possibility that body condition decreases in colder periods such as the winter, which was not sampled in this study.

We also predicted that FA would have a more pronounced negative effect on body condition in the dry season, because availability of resources could be lower in this season than in the rainy season. Contrary to this prediction, we found a negative association between body condition and FA of the humerus during the rainy season in *L. zweifeli*. This result may be related to the peak of the reproductive season or the period immediately following it, which presumably occurs in the rainy season as in other species of the *tarahumarae* group (Goldberg, 2020). Some anuran species decrease their food intake during the reproductive season (Díaz-Páez & Ortiz, 2003), males commonly deplete their energetic reserves at the end of this season (Eggert & Guyétant, 2003), and females of ranid frogs have large clutch sizes (Wells, 2007). Therefore, reproductive investment by both sexes is very high, which would explain our result of an effect of FA on body condition only in the rainy season. It is possible that the effort required to move between breeding sites and ultimately to clasp a female and sustain amplexus is higher in individuals with more asymmetric forelimbs, which would deplete their energetic reserves and decrease their body condition.

Amphibians in general exhibit FA in structures theoretically related to important aspects of their life, such as forelimbs, hindlimbs and tympanum (Vershinin et al., 2007; Matías-Ferrer & Escalante, 2015; Eisemberg & Bertoluci, 2016; this study). However, the consequences of this phenomenon are little known. In addition to the negative effect of FA of the humerus on body condition we found, it has been suggested that FA in the forelimbs also could negatively impact landing performance, since these structures are important to absorb the impact of jumps in ranid frogs (Petrović et al., 2017; Citadini et al., 2018; Didde & Rivera, 2019). On the other hand, there was no association between locomotor performance and FA of the hindlimbs in *P. perezi* (Moreno-Rueda et al., 2020), but this may be partially explained by the low levels of FA found. Finally, the tympanum plays a determining role in the reception of sound emissions from predators, prey and conspecifics. Therefore, FA in this structure decreases the ability of anurans to detect mates (St-Amour et al., 2010), and could even make them more susceptible to predators (Bosch & Márquez, 2000).

Although we did not find FA in the area covered by spots and the number of spots in *L. spectabilis*, previous studies have found that organisms develop FA in these variables in disturbed sites (Wright & Zamudio, 2002; Guo et al., 2017; Zhelev et al., 2019). On the other hand, studies that have found low levels of FA in colouration traits have suggested that environmental conditions during the initial stages of development of the organisms have been adequate, representing a minimal perturbation on the stability of individuals (Zhelev et al., 2021). Our finding of absence of FA in colouration variables but presence of FA in some morphometric variables in *L. spectabilis* can be explained as follows: Bone growth takes place from birth onward throughout the lifetime of an organism, unlike colouration patterns, which start to develop a few weeks before metamorphosis (Davis & Maerz, 2007; Thibaudeau & Altig, 2012). Therefore, asymmetry in traits such as colouration patterns could provide information on the stress that organisms experience during specific stages of their growth. Thus it is likely that stress factors affecting colouration (e.g. UV radiation) are different from those affecting bone growth (Gallant & Teather, 2001) or act at different stages of development.

In summary, our study revealed that the two species of ranid frogs exhibited FA in morphometric characters in accordance with the presence of a stressor agent at the study site. The species exhibited different degrees of sensitivity to the presumed stressor agent, which is reflected in differences in FA. These differences are expressed as a higher number of characters with FA and higher levels of FA in the horizontal length of the tympanum in *L. zweifeli*, but also as a negative effect of FA of the humerus on the body condition of this species in the rainy season. The latter result was unexpected due to the theoretically higher availability of resources in this season, as well as the similar body condition between seasons in the two species. Therefore, seasonality is enough to modulate the effect of FA of the humerus on body condition, but not to decrease it directly. Overall, our results highlight the importance of evaluating sensitivity of sympatric species through analyses of FA and its effects on individual traits, as well as the analysis of these effects in seasons with different environmental conditions.

ACKNOWLEDGEMENTS

We thank the authorities of La Magdalena Cuaxixtla, Tecali de Herrera, for providing us with information on potential sites to sample and logistic support. We also thank Guadalupe Méndez, Gibran Barrera, César Díaz, Adua Olvera, Fernando Licona, Uriel Reyes and Janirth Reyes for field assistance, and David Hernández for advising us on the method to anaesthetise animals. This study is part of the undergraduate research project of ECB at the Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla. We would also like to express our gratitude to the anonymous reviewer for the valuable comments.

REFERENCES

Angulo, A. (2002). Anfibios y paradojas: perspectivas sobre la diversidad y las poblaciones de anfibios. *Ecología Aplicada* 1(1), 105–109.

Benítez, H.A & Parra, L.E (2011). Asimetría Fluctuante: Una herramienta morfofuncional para Medir Estabilidad del Desarrollo. *International Journal of Morphology* 29(4), 1459–1469. Doi: 10.4067/S071795022011000400066.

Benítez, H.A., Lemic, D., Villalobos-Leiva, A., Bažok, R., Órdenes-Claveria, R., PajačŽivković, I. & Mikac, K.M. (2020). Breaking symmetry: Fluctuating asymmetry and geometric morphometrics as tools for evaluating developmental instability under diverse agroecosystems. *Symmetry* 12(11), 1789. Doi: 10.3390/sym12111789.

Bosch, J. & Márquez, R. (2000). Tympanum fluctuating asymmetry, body size and mate choice in female Midwife toads (*Alytes obstetricans*). *Behaviour* 137(9), 1211–1222.

Brown, M.E. (1996). Assessing body condition in birds. In *Current Ornithology*. Nolan, V. & Ketterson, E. (Eds.). New York, USA. 67–135 pp.

Băncilă, R.I., Smets, J., Cogălniceanu, D., Hartel, T. & Plăiaşu, R. (2010). Comparing three body condition indices in amphibians: A case study of yellow-bellied toad *Bombina variegata*. *Amphibia-Reptilia* 31(4), 558–562. Doi: 10.1163/017353710x518405.

Castro-González, N.P., Calderón-Sánchez, F., Moreno-Rojas, R., Tamariz-Flores, J.V. & Reyes-Cervantes, E. (2019). Nivel de contaminación de metales y arsénico en aguas residuales y suelos en la subcuenca del Alto Balsas en Tlaxcala y Puebla, México. *Revista Internacional de Contaminación Ambiental* 35(2), 335–348.

Catenazzi, A. (2015). State of the world's amphibians. *Annual Review of Environment and Resources* 40(1), 91–119. Doi: 10.1146/annurev-environ-102014-021358.

Citadini, J.M., Brandt, R., Williams, C.R. & Gomes, F.R. (2018). Evolution of morphology and locomotor performance in anurans: Relationships with microhabitat diversification. *Journal of Evolutionary Biology* 31(3), 371–381.

Cogălniceanu, D., Stănescu, F., Székely, D., Topliceanu, T.S., Iosif, R. & Székely, P. (2021). Age, size and body condition do not equally reflect population response to habitat change in the common spadefoot toad *Pelobates fuscus*. *PeerJ* 9, e11678. Doi: 10.7717/peerj.11678.

CONAGUA. (2020). Climatología. http://smn.conagua.gob.mx. Accessed on 29 July 2022.

Davis, A.K. & Maerz, J.C. (2007). Spot symmetry predicts body condition in spotted salamanders, *Ambystoma maculatum*. *Applied Herpetology* 4(3), 195–205.

Díaz-Páez, H. & Ortiz, J.C. (2003). Hábitos alimentarios de *Pleurodema thaul* (Anura, Leptodactylidae), en Concepcion, Chile. *Gayana* 67(1), 25–32. Doi: 10.4067/s0717-65382003000100004.

Didde, R.D. & Rivera, G. (2019). Patterns of fluctuating asymmetry in the limbs of anurans. *Journal of Morphology* 280(4), 587–592. Doi: 10.1002/jmor.20967.

Donnelly, A., Guyer, C., Juterbock, E. & Alford, R. (1994). Techniques for Marking Amphibians. In *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C. & Foster, M.S. (Eds.). Washington DC, USA. 277–284 pp.

Eggert, C. & Guyétant, R. (2003). Reproductive behaviour of spadefoot toads (*Pelobates fuscus*): Daily sex ratios and male's tactics, ages, and physical condition. *Canadian Journal of Zoology* 81(1), 46–51. Doi: 10.1139/z02-224.

Eisemberg, C.C. & Bertoluci, J. (2016). Fluctuating asymmetry in populations of the south American frog *Physalaemus cuvieri* (Leptodactylidae) in areas with different degrees of disturbance. *Journal of Natural History* 50(23–24), 1503–1511. Doi: 10.1080/00222933.2015.1130871.

Gallant, N. & Teather, K. (2001). Differences in size, pigmentation, and fluctuating asymmetry in stressed and nonstressed Northern leopard frogs (*Rana pipiens*). *Ecoscience* 8(4), 430–436. Doi: 10.1080/11956860.2001.11682671.

García-Nieto, E., Carrizales-Yañez, L., Juárez-Santacruz, L., García-Gallegos, E., Hernández-Acosta, E., Briones-Corona, E. & Vázquez-Cuecuecha, O.G. (2011). Plomo y arsénico en la subcuenca del Alto Atoyac en Tlaxcala, México. *Revista Chapingo Serie Ciencias Forestales y del Ambiente* 17(1), 7–17.

Glennemeier, K.A & Begnoche, L.J. (2002). Impact of organochlorine contamination on amphibian populations in southwestern Michigan. *Journal of Herpetology* 36(2), 233–244. Doi: 10.2307/1565996.

Goldberg, S.R. (2020). Notes on reproduction of the Tarahumara frog, *Lithobates tarahumarae* (Anura: Ranidae). *Sonoran Herpetologist* 33(1), 2.

Gómez-Hoyos, D. & Maya, J.F.G. (2014). Condición corporal de machos de *Oophaga pumilio* (Anura: Dendrobatidae) en relación con elementos del hábitat en la Estación Biológica La Selva, Sarapiquí, Costa Rica. *Revista Biodiversidad Neotropical* 4(1), 16–20.

Gondim, P.D., Rodrigues, J.F.M. & Cascon, P. (2020). Fluctuating asymmetry and organosomatic indices in anuran populations in agricultural environments in semi-arid Brazil. *Herpetological Conservation and Biology* 15(2), 354–366.

Gould, J. & Valdez, J.W. (2021). Dating on your level: Assortative mating based on body condition in an amphibian. *Ethology Ecology & Evolution*, 1–15.

Grillitsch, B. & Chovanec, A. (1995). Heavy metals and pesticides in anuran spawn and tadpoles, water, and sediment. *Toxicological & Environmental Chemistry* 50(1–4), 131–155. Doi: 10.1080/02772249509358210.

Guo, R., Zhang, W., Ai, S., Ren, L. & Zhang, Y. (2017). Fluctuating asymmetry rather than oxidative stress in *Bufo raddei* can be an accurate indicator of environmental pollution induced by heavy metals. *Environmental Monitoring and Assessment* 189(6), 189–293. Doi: 10.1007/s10661017-5991-6.

HACC. (2004). Guidelines for use of live amphibians and reptiles in field and laboratory research. American Society of Ichthyologists and Herpetologists.

INEGI. (2009). Prontuario de información geográfica municipal de los Estados Unidos Mexicanos: Tecali de Herrera, Puebla.

IUCN. (2022). The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org. Accessed on 30 May 2022.

Jakob, E.M., Marshall, S.D. & Uetz, G.W. (1996). Estimating fitness: A comparison of body condition indices. *Oikos* 77(1), 61–67. Doi: 10.2307/3545585.

Khattab, N.M., Saber, S., El-Salkh, B.A. & Said, R.E. (2021). Genotoxicity and limbs asymmetry in the Egyptian toad (*Sclerophrys regularis*) as biomarkers for heavy metals toxicity. *Egyptian Journal of Aquatic Biology and Fisheries* 25(4), 705–717.

Lauck, B. (2006). Fluctuating asymmetry of the frog *Crinia signifera* in response to logging. *Wildlife Research* 33(4), 313–320. Doi: 10.1071/wr04107.

MacGregor-Fors, I., Hernández Ordoñez, O. & Ortega-Álvarez, R. (2012). Urban croaking: Diversity and distribution of anurans in a neotropical city. *Urban Ecosystems* 16(2), 389–396. Doi: 10.1007/s11252-0120267-y.

Mancilla-Villa, O.R., Ortega-Escobar, H.M., Ramírez-Ayala, C., Uscanga-Mortera, E., Ramos-Bello, R. & Reyes-Ortigoza, A.L. (2012). Metales pesados totales y arsénico en el agua para riego de Puebla y Veracruz, México. *Revista Internacional de Contaminación Ambiental* 28(1), 39–48.

Matías-Ferrer, N. & Escalante, P. (2015). Size, body condition, and limb asymmetry in two hylid frogs at different habitat disturbance levels in Veracruz, Mexico. *The Herpetological Journal* 25(3), 169–176.

Mendoza-Estrada, L.J., Lara-López, R. & Castro-Franco, R. (2008). Dieta de *Lithobates zweifeli* Hillis, Frost y Webb 1984 (Anura: Ranidae) en un río estacional del centro de México. *Acta Zoológica Mexicana* 24(1), 169–197.

Midi, H., Sarkar, S.K. & Rana, S. (2010). Collinearity diagnostics of binary logistic regression model. *Journal of Interdisciplinary Mathematics* 13(3), 253–267. Doi: 10.1080/09720502.2010.10700699.

Moreno-Rueda, G., Requena-Blanco, A., Zamora-Camacho, F.J., Comas, M. & Pascual, G. (2020). Morphological determinants of jumping performance in the Iberian green frog. *Current Zoology* 66(4), 417–424. Doi: 10.1093/cz/zoz062.

Niemeier, S., Müller, J. & Rödel, M.O. (2019). Fluctuating asymmetry – appearances are deceptive. Comparison of methods for assessing developmental instability in European common frogs (*Rana temporaria*). *Salamandra* 55(1), 14–26.

Ortiz-Alamilla, A., Borraz, A., Hernández C. & Neri. S. (2015). Presencia de contaminantes en tres cuerpos de agua del estado de Puebla. *Revista Latinoamericana el Ambiente y* *las Ciencias* 6(12), 1590–1594.

Palmer, A. (1994). Fluctuating asymmetry analyses: A primer. In *Developmental Instability: Its Origins and Evolutionary Implications*. Markow, T.A. (Ed.). Dordrecht, Netherlands. 335–364 pp.

Palmer, A.R & Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17(1986), 391–421.

Parris, M. & Baud, D. (2004). Interactive effects of a heavy metal and chytridiomycosis on Gray Treefrog larvae (*Hyla chrysoscelis*). *Copeia* 2004(2), 344–350. Doi: 10.1643/CE-03-116R1.

Peig, J. & Green, A. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118(12), 1883–1891. Doi: 10.1111/j.16000706.2009.17643.x.

Pérez-Castresana, G. (2019). Calidad del agua del Río Atoyac en el Valle de Puebla y el riesgo en la salud de la población Emilio Portes Gil, municipio de Ocoyucan, Puebla. (PhD dissertation). Benemérita Universidad Autónoma de Puebla, Puebla, México.

Petrović, T.G., Vukov, T.D. & Tomašević Kolarov, N. (2017). Morphometric ratio analyses: Locomotor mode in anurans. *Comptes Rendus Biologies* 340(4), 250–257. Doi: 10.1016/j.crvi.2017.02.004.

Reading, C.J. (2007). Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia* 151(1), 125–131. Doi: 10.1007/s00442-006-0558-1.

Rzedowski, J. (2006). Vegetación de México. 1st digital edition, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México. 504 pp.

Söderman, F., Van Dongen, S., Pakkasmaa, S. & Merilä, J. (2007). Environmental stress increases skeletal fluctuating asymmetry in the moor frog *Rana arvalis*. *Oecologia* 151(4), 593–604. Doi: 10.1007/s00442-006-0611-0.

St-Amour, V., Garner, T.W., Schulte-Hostedde, A.I. & Lesbarrères, D. (2010). Effects of two amphibian pathogens on the developmental stability of Green Frogs. *Conservation Biology* 24(3), 788–794. Doi: 10.1111/j.1523-1739.2009.01400.x.

Stetter, M.D. (2001). Fish and amphibian anesthesia. Veterinary *Clinics of North America: Exotic Animal Practice* 4, 69–82.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. & Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702), 1783–1786.

Thibaudeau, G. & Altig, R. (2012). Coloration of anuran tadpoles (Amphibia): development, dynamics, function, and hypotheses. *International Scholarly Research Notices* 2012. Doi: 10.5402/2012/725203.

Tomašević, N., Cvetković, D., Aleksić, I. & Crnobrnja-Isailović, J. (2007). Effect of climatic conditions on post-hibernation body condition and reproductive traits of *Bufo bufo* females. *Archives of Biological Sciences* 59(3), 51–52. Doi: 10.2298/ABS070351PT.

Unglaub, B., Steinfartz, S., Kühne, D., Haas, A. & Schmidt, B.R. (2018). The relationships between habitat suitability, population size and body condition in a pond-breeding amphibian. *Basic and Applied Ecology* 27, 20–29. Doi: 10.1016/j.baae.2018.01.002.

Van Valen, L. (1962). A study of fluctuating asymmetry. *Evolution* 16, 125–142.

Vera-Candioti, J., D'andrea, M.F. & Brodeur, J.C. (2019). Body condition of *Pseudis minuta* Günther, 1858 (Anura: Hylidae) inhabiting an agroecosystem from south Santa Fe Province, Argentina. *Herpetology Notes* 12, 13–17.

Vershinin, V.L., Gileva, E.A. & Glotov, N.V. (2007). Fluctuating asymmetry of measurable parameters in *Rana arvalis*: Methodology. *Russian Journal of Ecology* 38(1), 72–74. Doi: 10.1134/s1067413607010122.

Villa, D. (2014). Asimetría fluctuante en dos especies de peces vivíparos (Poeciliidae). (Bachelor's thesis), Universidad Nacional Autónoma de México, CDMX, México.

Wells, K.D. (2007). The Ecology and Behavior of Amphibians. Chicago, USA. 451–515 pp.

Woolrich-Piña, G.A., Smith, G.R., Lemos-Espinal, J.A. & Martínez-Olguín, R.G. (2017). Resource use by adults of four species of anurans along the Río Salado, Puebla, Mexico. *Herpetological Conservation and Biology* 12, 182–191.

Wright, A.N & Zamudio, K.R. (2002). Color pattern asymmetry as a correlate of habitat disturbance in spotted salamanders (*Ambystoma maculatum*). *Journal of Herpetology* 36(1), 129–133. Doi: 10.2307/1565818.

Zakharov, V., Shadrina, E. & Trofimov, I. (2020). Fluctuating Asymmetry, Developmental Noise and Developmental Stability: Future Prospects for the Population Developmental Biology Approach. *Symmetry* 12(8), 1376. Doi: 10.3390/sym12081376.

Zhelev, Z.M, Tsonev, S.V. & Boyadzhiev, P. (2022). Using of fluctuating asymmetry in adult *Pelophylax ridibundus* (Amphibia: Anura: Ranidae) meristic traits as a method for assessing developmental stability of population and environmental quality of their habitat: industrial area in southern Bulgaria. *Turkish Journal of Zoology* 46, 220–227.

Zhelev, Z.M., Mollov, I. & Tsonev, S. (2021). Fluctuating asymmetry in meristic morphological traits of *Bufotes viridis* (Laurenti, 1768) (Anura: Bufonidae): Application for assessing environmental quality of two semi-natural habitats in Plovdiv city, Bulgaria. *Acta Zoologica Bulgarica* 73(3), 401–407.

Zhelev, Z.M., Arnaudov, A.D., Popgeorgiev, G.S. & Dimitrov, H.A. (2012). Assessment of ecological status of two rivers with different types of anthropogenic pollution in Southern Bulgaria based on the level of fluctuating asymmetry in the populations of marsh frog *Rana ridibunda* (Amphibia: Ranidae). *Acta Zoologica Bulgarica* 4, 225–231.

Zhelev, Z.M., Tsonev, S.V. & Angelov, M.V. (2019). Fluctuating asymmetry in *Pelophylax ridibundus* meristic morphological traits and their importance in assessing environmental health. *Ecological Indicators* 107, 105589. Doi: 10.1016/j.ecolind.2019.105589.

Zhelev, Z.M., Tsonev, S.V. & Arnaudova, D.N. (2017). Health status of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) in a rice paddy ecosystem in southern Bulgaria: body condition factor and fluctuating asymmetry. *Acta Zoologica Bulgarica* 69(8), 169–177.

Accepted: 9 August 2023