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FLUCTUATING ASYMMETRY AND ORGANOSOMATIC INDICES IN ANURAN POPULATIONS IN AGRICULTURAL ENVIRONMENTS IN SEMI-ARID BRAZIL

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Abstract.—**The influence of anthropogenic disturbance on anurans can be identified by fluctuating asymmetry (i.e., small random deviations from perfect symmetry), and by organosomatic indices (i.e., relative weights of the internal organs in relation to total body weight). The aim of this study was to investigate the occurrence of environmental stress caused by agricultural activities in *Leptodactylus macrosternum* (Miranda's White-lipped Frog) and *Scinax x-signatus* (Venezuela Snouted Treefrog) populations in the Brazilian semi-arid region in two agricultural areas and two non-agricultural areas. We used fluctuating limb asymmetry and hepatosomatic, adiposomatic, and gonadosomatic indices as indicators of disturbance. There was asymmetry only in the femur length of *L. macrosternum* and in the calcaneus-phalange length of *S. x-signatus*, but we observed no significant differences in asymmetry between the agricultural and non-agricultural areas. There was wide variation among the four studied areas in the hepatosomatic, adiposomatic, and especially gonadosomatic indices of *L. macrosternum*, but no indication of a difference between individuals from agricultural and non-agricultural areas. This suggests a possible relationship with unknown local environmental characteristics or ecological factors. Moreover, *L. macrosternum* and *S. x-signatus* are species with generalist behavior that are well adapted to disturbed areas. Thus, fluctuating asymmetry and organosomatic indices may not have been capable of detecting impacts, or the stressors that could affect them were simply not present in agricultural habitats. Future studies focusing on histological variations in the gonads and evaluations of chemical contamination of organs tissue may give a better understanding of the possible impacts of agriculture on these populations.**

Key Words.—anthropogenic disturbance; environmental stress; Leptodactylus macrosternum; Miranda's White-Lipped Frog; *Scinax x-signatus*; Venezuela Snouted Treefrog

INTRODUCTION

Anuran amphibians are among the living organisms most affected by the current biodiversity crisis (McCallum 2015), with declines and extinctions of some of their populations being reported worldwide (Gibbons et al. 2000; Stuart et al. 2004; Blaustein et al. 2011). Agriculture has been cited as a major cause of biodiversity loss due to the expansion of cultivated areas and pastures, irrigation practices, and the use of fertilizers and pesticides (Foley et al. 2011). In addition, other changes caused by agropastoral activities, such as habitat reduction and fragmentation, temperature increase, and fluctuations in the pH level of the aquatic environment, may also impact amphibians (Beebee et al. 1990; Clarke 1993; McCoy and Harris 2003; McCoy et al. 2008). Indeed, there is increasing evidence linking declining anuran populations with proximity to agricultural areas (Sparling et al. 2001; Stuart et al. 2004; Eterovick et al. 2005; Silvano and Segalla 2005; Becker et al. 2007). Amphibians are effective bioindicators of environmental health because they inhabit both terrestrial and aquatic environments and are sensitive to local factors such as water quality and microhabitat availability (Pope et al. 2000). Features such as high abundance, wide distribution, resolved taxonomy, and low dispersal ability increase the potential of an organism as a bioindicator (Hellawell 1986; Rainio and Niemelä 2003). This potential is greater in species that respond to environmental stress through changes in morphological attributes (Johnson et al. 1993).

The influence of anthropogenic disturbance on anurans can be identified, among other ways, through morphometric indicators such as fluctuating asymmetry (Zhelev et al. 2015a; Eisemberg and Bertoluci 2016; Costa et al. 2017) and organosomatic indices (Tête et al. 2013; Zhelev et al. 2014, 2015b). According to Palmer (1994), fluctuating asymmetry (FA) results from pattern of perfect bilateral symmetry variation in a sample of individuals where the mean of the right minus the left value of the bilaterally paired trait is zero and the variation has a normal distribution about that mean, and FA can serve as a biomarker of developmental instability. Fluctuating asymmetry reflects long-term changes in the body state of these organisms when extreme temperatures (Parsons 1990) or contaminants (Polak 2003) for example, cause instability during autogeny (Palmer 1994; Amaral et al. 2012). Fluctuating asymmetry may be important in evolutionary and ecological studies in providing valuable information on the adaptation of organisms or populations to particular environments (Graham et al. 2010; St-Amour et al. 2010). Several studies demonstrate the utility of FA for evaluating environmental stress in invertebrate and vertebrate species (Bonada and Williams 2002; Lens et al. 2002). At the same time, there are several studies that question the use of FA as a reliable indicator of environmental stress, and they suggest that several more questions need to be addressed before it can be used with confidence (Floate and Fox 2000; Lens et al. 2002; Longson et al. 2007; Floate and Coghlin 2010; Beasley et al. 2013).

Organosomatic indices, in turn, are the weights of internal organs relative to total body weight, and it can also be used to estimate individual fitness conditions (Norrdahl et al. 2004). The hepatosomatic index (HSI), which expresses liver size as a percentage of total body weight, may signal liver conditions, and a change in value usually indicates an effect of chemical exposure on liver function (Brodeur et al. 2011; Paunescu and Ponepal 2011) or energy production through glycogen metabolism (Barton 1987; Goede and Barton 1990). The glycogen is a storage form of energy through a very large, branched polymer of glucose residues that can be broken down to yield glucose molecules when energy is needed (Berg et al. 2002). Changes in HSI values may also indicate exposure to any kind of oxidative stress (Brodeur et al. 2012) that occurs when excess oxygen radicals are produced in cells, which could overwhelm the normal antioxidant capacity (Gagné 2014). The adiposomatic index (ASI), which expresses fat body size as a percentage of total body weight, is linked to energy reserves too, in the form of fat bodies, and it also varies in different environmental situations, mainly in pre-reproductive periods, when there is a large energy expenditure for gonadal maturation (Navarro et al. 2005; Chaves et al. 2017). The gonadosomatic index (GSI), which expresses gonads size as a percentage of total body weight, provides information on both reproductive maturity and seasonal weather changes or exogenous stress, such as exposure to contaminants (Schmitt and Dethloff 2000). Organosomatic indices are widely used in biomonitoring studies of environmental stress in fish (Adams and McLean 1985; Schmitt and Dethloff 2000; Kleinkauf et al. 2004; Dekić et al. 2016; Araújo et al. 2018), and are also studied in amphibians (Brodeur et al. 2011; Păunescu and Ponepal 2011; Zhelev et al. 2014).

The objective of this study was to evaluate the role that agricultural activities may have in influencing development for two populations of anuran species, *Leptodactylus macrosternum* (Miranda's White-lipped Frog) and *Scinax x-signatus* (Venezuela Snouted Treefrog). We used FA (for both species) and organosomatic indices (for *L. macrosternum*) as bioindicators of possible agricultural stressor exposure. We expected that nearness to agriculture would elicit stress in these organisms; thus, we also expected that this stress could be detected using FA and hepatosomatic, gonadosomatic, and adiposomatic indices.

MATERIALS AND METHODS

***Study site.*—**We conducted the study in the municipality of Tabuleiro do Norte, in the Lower Jaguaribe River region, Ceará state, Brazil (Fig. 1). The morphoclimatic domain is Caatinga, in which the vegetation consists of mosaics of thorny shrubs interspersed with seasonally dry forest (Ab'sáber 1977). The annual averages of temperature and precipitation are 26°–28° C and 794.8 mm, respectively, and the climate of the region is tropical hot semi-arid (Instituto de Pesquisa e Estratégia Econômica do Ceará [IPECE]. 2017. Perfil municipal. IPECE, Brazil. Available from https://www.ipece.ce.gov.br/wpcontent/uploads/sites/45/2018/09/Tabuleiro_do_Norte_2017.pdf [Accessed 8 September 2019]). We chose this region in Ceará because it is an important area of agribusiness, having the largest irrigation complexes in the state (Milhome et al. 2009; Gama et al. 2013).

We selected two areas that were undisturbed by agricultural activity (Area I, UTM 24M 0594570, 9415959; Area II, UTM 24M 0587910, 9409430) and two in which the agricultural activity is intense (Area III, UTM 24M 0596531, 9423803; Area IV, UTM 24M 0598188, 9422750). Areas I and II served as control areas, with herbaceous, shrubby vegetation around water bodies. Areas III and IV are environmentally impacted by agropastoral land use. The plant species grown in these areas are rice, beans, corn, bananas, and pasture grasses, the principal crops of irrigated agriculture in the Lower Jaguaribe River region (Gondim et al. 2004). We selected cultivated areas sufficiently distant from the control areas (7,697–16,829 m; Table 1).

***Study species*.**—*Leptodactylus macrosternum* is widely distributed in South America east of the Andes, extending from Colombia, Venezuela, and Guyana southwards through Brazil and Bolivia (American Museum of Natural History. 2019. Amphibian species of the world: an online reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available from http://research.amnh.org/vz/herpetology/amphibia/ [Accessed 8 September 2019]). It occurs in many habitats, including savannas, grasslands, open habitats in dry areas, forest margins, and along riverbanks in Tropical Rainforests (University of California, Berkeley. 2018. Amphibiaweb. University of California, Berkeley, USA. Available from https://

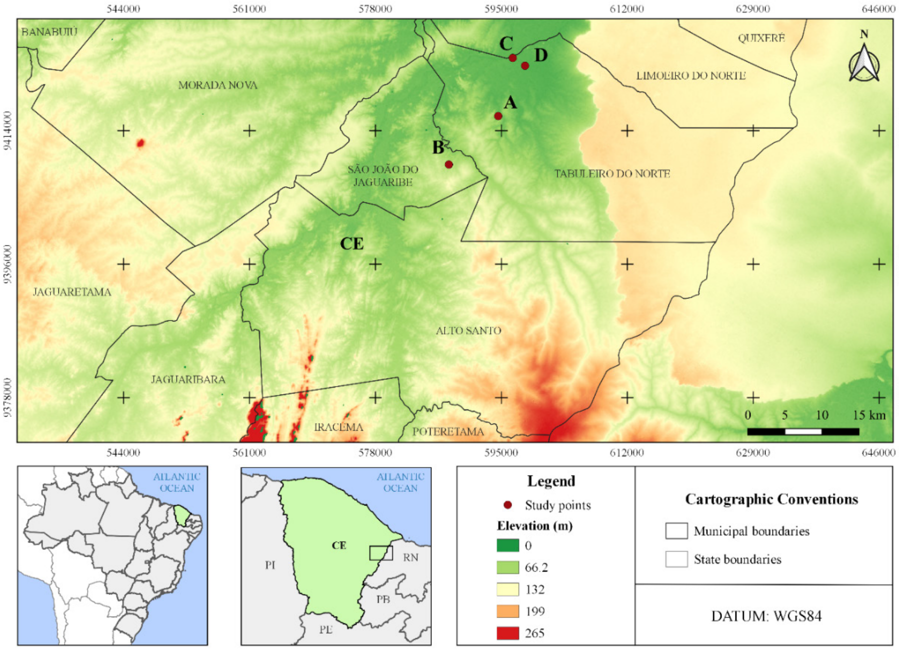

Figure 1. The location of Ceará state, Brazil (CE on inset maps), highlighting the Lower Jaguaribe River microregion (the polygon in the Ceará map) and collection areas (red-filled circles). Abbreviations are A = Area I (control), B = Area II (control), C = Area III (cultivated), D = Area IV (cultivated), and RN = State of Rio Grande do Norte. Coordinates on the border of the map are in UTM. (Map created by GeoMaps Consultoria, Fortaleza, Ceará, Brazil).

amphibiaweb.org, [Accessed 6 October 2018]). This species is extremely common, abundant, and well adapted to disturbed areas (De La Riva and Maldonado 1999). *Scinax x-signatus* is also widely distributed in South America, occurring in non-forested areas of Colombia, Venezuela, Guyana, Suriname, and Brazil (American Museum of Natural History. 2019. *op. cit.*). It inhabits tropical savannas, forest margins, and open areas (International Union for Conservation of Nature 2018). This species is also common in semi-arid environments and disturbed areas (Borges-Nojosa and Cascon 2005; Santana et al. 2015). We chose *L. macrosternum* and *S. x-signatus* based on the criteria of highest abundance (verified during pilot collections) and different habitat use (terrestrial and arboreal, respectively).

Data collection.—We conducted the fieldwork during the rainy season, over 15 d in May and June 2017. We collected specimens through active search during the night, from 1800 to 2200. We collected approximately 30 individuals per species in each area, which is considered the minimum sample number in FA studies (Palmer 1994). We then weighed the subjects with precision scales (0.01 g) and euthanized them with the following anesthetics: lidocaine ointment 50 mg/g for *S. x-signatus* and intracardiac injection of lidocaine hydrochloride for *L. macrosternum*. We used a dose of 30 mg/kg, corresponding to six times the maximum anesthetic dose cited by Chatigny et al. (2017), and as directed by the Brazilian National Council for Animal Experimentation Control - CONCEA (Animal Experimentation Control [CONCEA]. 2019. Resolução Normativa nº 13/2013. Available from https://www.mctic.gov.br/mctic/export/sites/institucional/institucional/concea/arquivolegislacao/resolucoes_normativas/Resolucao-Normativa-CONCEA-n-13-de 20.09.2013 D.O.U.-de-26.09.2012-Secao-I-Pag.-5.pdf [Accessed 21 February 2019]). We dissected these animals to determine sex (through direct observation of the gonads), and removed and weighed the gonads, fat bodies, and liver to determine the organosomatic indices. We fixed the specimens with 10% formaldehyde solution, preserved them in 70% ethyl alcohol, and added them to the Herpetology Collection of the Federal University of Ceará (CHUFC), Brazil.

Table 1. Geographical distance (m) among the four study areas sampled for *Leptodactylus macrosternum* (Miranda's White-lipped Frog) and *Scinax x-signatus* (Venezuela Snouted Treefrog), located in Tabuleiro do Norte, Ceará, Brazil. Area types are Area I: control; Area II: control; Area III: cultivated; Area IV: cultivated.

	Area I	Area II	Area III
Area I	—		
Area II	9,329 m	—	
Area III	8,087 m	16,765 m	—
Area IV	7,697 m	16,829 m	1,964 m

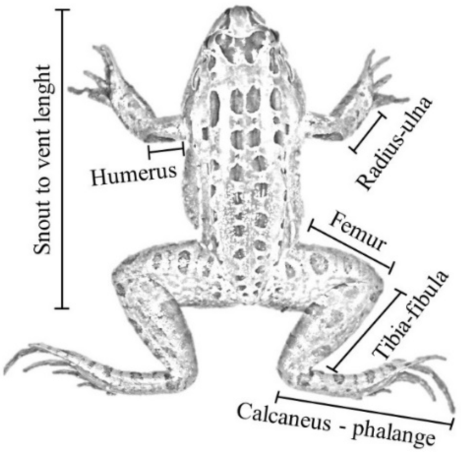

Figure 2. Morphometeric parameters analyzed for fluctuating asymmetry in *Leptodactylus macrosternum* (Miranda's White-Lipped Frog) and *Scinax x-signatus* (Venezuela Snouted Treefrog). (Schematic drawing made using the Pencil Sketch 6.7 application app by Dumpling Sandwich Software Inc., Saskatoon, Saskatchewan, Canada).

Laboratory procedures.—In the laboratory, we measured the snout-to-vent length (SVL) and the lengths on both sides of the radius-ulna, humerus, calcaneus to phalange (from calcaneus to distal tip of the largest phalange), tibia-fibula, and femur (Fig. 2) of all specimens with a digital caliper (accuracy 0.01 mm). We used these five morphometric parameters because of their visible osteological characteristics in unprepared specimens. A single researcher took each measurement three times to test whether the FA exceeded the measurement error, and we analyzed the data following the recommendations of Palmer and Strobeck (1986) and Palmer (1994).

Determination of fluctuating asymmetry.—There are three main types of bilateral asymmetry: fluctuating asymmetry (FA), directional asymmetry (DA), and anti-symmetry (AS; Palmer and Strobeck 1986, 1992, 2003; Palmer 1994). The FA is a pattern of variation of the difference between the right and left sides (R - L) where the variation is usually distributed around an average of zero. The DA is a pattern of variation (R - L) where variation is usually distributed around an average that is significantly different from zero and the longer side is usually the same. The AS is a pattern of variation (R - L) where variation is distributed around an average of zero but deviates from normality towards a platykurtic or bimodal distribution, where the larger side varies randomly among individuals (Palmer and Strobeck 1986, 1992, 2003; Palmer 1994).

We therefore conducted a set of analyses to detect DA and AS, along with FA, for each measurement of each species separately, as DA and AS can influence the FA estimate. We performed analyses to detect the measurement error (ME) and the relationship between the size of the morphometric traits and FA (Palmer 1994; Palmer and Strobeck 2003). We maintained positive and negative outlier values because they are expected in FA studies, and they may have a biological significance (Palmer and Strobeck 1986; Leung and Forbes 1996; Hardersen 2000). We determined whether the variation between R and L sides was significantly greater than the measurement error (Palmer and Strobeck 1986) using a Two-way Analysis of Variance (ANOVA) test on individual and morphological trait sides (R or L). In this analysis, we observed whether the interaction between the two factors was significant, indicating the absence of measurement error. In the subsequent analysis, we determined asymmetry by subtracting the mean of the three measurements of the right side by the mean of the three measurements of the left side of the radius-ulna (RUL), humerus (HL), tibia-fibula (TFL), femur (FL), and calcaneus to phalange (CPL) lengths. We applied the Kolmogorov-Smirnov test to determine whether the frequency distribution of the R - L measurements was normal, thereby determining the presence of anti-symmetry. We verified the presence of DA with a one-sample *t*-test, where we tested R - L scores against a predicted mean of zero. We also used Pearson's correlation to determine if there was a relationship between the mean size of the individual morphological character (R + L)/2 and the level of FA. Tests for DA and AS are important because they can determine if the asymmetry detected in a particular morphological trait has a genetic component. These types of asymmetries are related to the condition of the species, which may always have one side larger than the other (Valen 1962; Palmer 1994) and would therefore not be suitable for indicating the impact of a stressor on individuals. Finally, we used a Kruskal-Wallis test to determine whether the study areas differed in the FA modulus ($|R - L|$) for the morphological traits that presented FA, according to the previous analyses. We performed statistical analysis using the program R (R Development Core Team. 2014) and for all tests $\alpha = 0.05$. All assumptions of parametric tests were met.

Determination of organosomatic indices.—We also used the hepatosomatic (HSI), gonadosomatic (GSI), and adiposomatic (ASI) indices in the analyzes of *L. macrosternum*. The small body size of *S. x-signatus* resulted in the absence of several data and, consequently, in a very small sample, making the analysis unfeasible. We calculated the indices according to the following equations

HSI = (liver weight/total weight) × 100;

GSI = (weight of gonads/total weight) × 100;

ASI = (fat body weight/total weight) × 100.

Table 2. Adequacy of the five morphological traits of *Leptodactylus macrosternum* (Miranda's White-lipped Frog) and *Scinax x-signatus* (Venezuela Snouted Treefrog) for fluctuating asymmetry (FA) analysis. Results of statistical analysis in bold highlight traits that passed the specific test represented in the column, and bold morphological traits are those that passed all the tests. Thus, they are adequate to evaluate FA. The X represents traits not further evaluated and excluded due to measurement error. Abbreviations are FL = femur length, TFL = tibia-fibula length, CPL = calcaneus to phalange length, RUL = radius-ulna length, HL = humerus length. An asterisk (*) indicates a non-parametric RUL correlation of *S. x-signatus*: rho = -0.056, *P* = 0.546.

Species/Morphological trait	Measurement error	Size dependence	Normality	Directional asymmetry
Leptodactylus macrosternum				
FL	**$F_{122, 491} = 2.308$** **$P < 0.001$**	**$R = -0.065$, df = 121** **$P = 0.477$**	**$D = 0.040$** **$P = 0.908$**	**$t = -1.542$, df = 122** **$P = 0.126$**
TFL	$F_{122, 490} = 1.030$ $P = 0.408$	x	x	x
CPL	**$F_{122, 491} = 1.290$** **$P = 0.032$**	**$R = -0.086$, df = 121** **$P = 0.336$**	**$D = 0.049$** **$P = 0.666$**	$t = 2.335$, df = 122 $P = 0.021$
RUL	$F_{122, 490} = 1.155$ $P = 0.147$	x	x	x
HL	$F_{122, 488} = 0.846$ $P = 0.869$	x	x	x
Scinax x-signatus				
FL	**$F_{118, 476} = 1.441$** **$P = 0.004$**	**$R = -0.027$, df = 117** **$P = 0.768$**	**$D = 0.058$** **$P = 0.411$**	$t = -3.380$, df = 11 $P = 0.001$
TFL	$F_{118, 476} = 1.087$ $P = 0.271$	x	x	x
CPL	**$F_{118, 476} = 1.515$** **$P = 0.001$**	**$R = 0.102$, df = 117** **$P = 0.268$**	**$D = 0.06$** **$P = 0.324$**	**$t = -0.423$, df = 118** **$P = 0.673$**
RUL	**$F_{118, 476} = 1.474$** **$P = 0.003$**	$R = -0.212$, df = 117 $P = 0.021$*	$D = 0.106$ $P = 0.002$	$t = -1.997$, df = 118 $P = 0.048$
HL	$F_{118, 476} = 1.09$ $P = 0.252$	x	x	x

We used a Kruskal-Wallis test to compare the values of each organosomatic index among areas and to assess whether significant differences occurred at $\alpha = 0.05$. We performed *a posteriori* comparisons using Dunn tests with Benjamini-Hochberg correction method.

Results

Fluctuating asymmetry.—The variation between right and left sides was significantly greater than the measurement error for FL and CPL in *L. macrosternum*, and for FL, CPL, and RUL in *S. x-signatus*. The measurement errors for the other morphological traits did not allow asymmetry to be properly calculated (Table 2). There was no significant relationship between asymmetry and the size of the morphological character for FL and CPL in either species. These morphological traits also showed no significant deviation of R and L side differences from a normal distribution, indicating the absence of AS in both species (Table 2). The absence of DA was also confirmed by the one-sample *t*-test in *L. macrosternum* FL and *S. x-signatus* CPL. The DA was identified, however, in *L. macrosternum* CPL and *S. x-signatus* FL (Table 2). Because we only identified FA in *L. macrosternum* FL and *S. x-signatus* CPL, we only compared these two traits among the four sampled areas. We found no significant differences in these traits among areas (*L. macrosternum* FL: $H = 4.50$; df = 3; $P = 0.212$; *S. x-signatus* CPL: $H = 6.01$; df = 3; $P = 0.111$). The mean of the FA values of the individuals of *L. macrosternum* for FL were: control area I = 0.079, control area II = 0.098, cultivated area III = 0.071, and

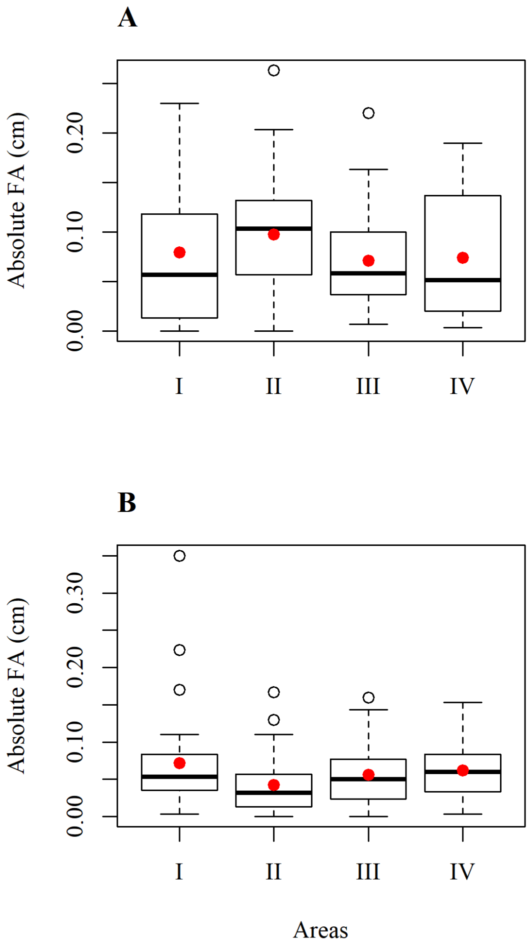

Figure 3. Variation in absolute fluctuating asymmetry (FA) for femur length in (A) *Leptodactylus macrosternum* (Miranda's White-Lipped Frog) and (B) calcaneus to phalange length in *Scinax x-signatus* (Venezuela Snouted Treefrog) for the four study sites: Area I: control; Area II: control; Area III: cultivated; Area IV: cultivated. Thick horizontal bars represent the medians of the samples. Upper and lower bounds of the boxes represent the upper and lower quartiles, respectively. Horizontal lines outside the boxes represent values within 1.5 times the interquartile range. Open circles are outliers, and solid red circles are mean values. Sample sizes of *L. macrosternum* are Area I: n = 31, Area II: n = 32, Area III: n = 30, Area IV: n = 30 and *S. x-signatus* are Area I: n = 30, Area II: n = 30. Area III: n = 30, Area IV: n = 29.

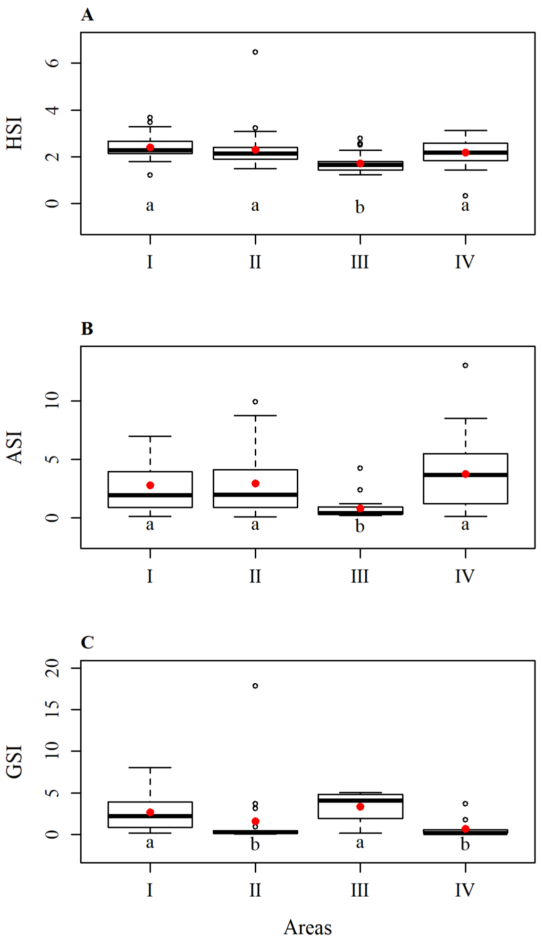

Figure 4. Variation in (A) hepatosomatic index (HSI), (B) adiposomatic index (ASI), and (C) gonadosomatic index (GSI) in *Leptodactylus macrosternum* (Miranda's White-Lipped Frog) for the study sites: Area I: control; Area II: control; Area III: cultivated; Area IV: cultivated. Horizontal lines in the boxes are the medians. Areas with the same lowercase letters are not significantly different in organosomatic indices (see Table 3 for more details). Thick horizontal bars represent the medians of the samples. Upper and lower bounds of the boxes represent respectively the upper and lower quartile. Horizontal lines outside the boxes represent values within 1.5 times the interquartile range. Open circles are outliers, and solid red circles are mean. Sample sizes of *L. macrosternum* are Area I (HSI: n = 31, ASI: n = 24, GSI: n = 12), Area II (HSI: n = 31, ASI: n = 28, GSI: n = 18), Area III (HSI: n = 30, ASI: n = 18, GSI: n = 4), Area IV (HSI: n = 30, ASI: n = 23, GSI: n = 10).

cultivated area IV = 0.074. The mean of the FA values of the individuals of *S. x-signatus* for CPL were: control area I = 0.071, control area II = 0.043, cultivated area III = 0.056, and cultivated area IV = 0.062 (Fig. 3).

***Organosomatic indices*.**—The organosomatic indices of *L. macrosternum* varied significantly among the four study sites (HSI: $H = 31.93$, df = 3, $P < 0.001$; ASI: $H = 19.34$, df = 3, $P < 0.001$; GSI: $H = 13.79$, df = 3, $P = 0.003$; Fig. 4). Values of the HSI and ASI in control areas I (mean HSI = 2.40, mean ASI = 2.76) and II (mean HSI = 2.30, mean ASI = 2.93), and cultivated area IV (mean HSI = 2.17, mean ASI = 3.74) were significantly higher than those in cultivated area III (mean HSI = 1.71, mean ASI = 0.81, Table 3; Fig. 4). Values of GSI from control area I (mean 2.67) and from cultivated area III (mean 3.35) were also significantly higher than from control area II (mean 1.60) and from cultivated area IV (mean 0.70, Table 3; Fig. 4).

Table 3. *A posteriori* comparisons among sites using Dunn tests to evaluate differences in hepatosomatic index (HSI), adiposomatic index (ASI), and gonadosomatic index (GSI) in *Leptodactylus macrosternum* for the study sites. Sites are Area I: control; Area II: control; Area III: cultivated; Area IV: cultivated. Abbreviations are = P.unadj = *P*-values unadjusted; P.adj = *P*-values adjusted according to the Benjamini-Hochberg method.

	HSI			ASI			GSI		
Comparison	Z	P.unadj	P.adj	Z	P.unadj	P.adj	Z	P.unadj	P.adj
Area I – Area II	1.508	0.131	0.158	-0.09	0.929	0.929	2.855	0.004	0.026
Area I – Area III	5.409	< 0.001	< 0.001	3.233	0.001	0.002	-0.236	0.813	0.813
Area II – Area III	3.913	< 0.001	< 0.001	3.419	0.001	0.002	-2.171	0.03	0.045
Area I – Area IV	1.526	0.127	0.191	-1.038	0.299	0.449	2.855	0.004	0.013
Area II – Area IV	0.03	0.976	0.976	-0.988	0.323	0.388	0.401	0.688	0.826
Area III – Area IV	-3.852	< 0.001	< 0.001	-4.166	< 0.001	< 0.001	2.296	0.022	0.043

Discussion

Although FA has been proposed as a biomonitoring tool for populations subjected to natural and anthropogenic stress (Parsons 1990, 1992; Sarre et al. 1994; Palmer 1996; Guillot et al. 2016), it may not be able to distinguish such stress (Tomkins and Kotiaho 2002). We found FA in only two morphological traits (*L. macrosternum* FL and *S. x-signatus* CPL), but without variation among study sites. We also found DA in the CPL of *L. macrosternum* and FL of *S. x-signatus*. Directional asymmetry and AS usually arise in studies of FA (Graham et al. 1998; Helm and Albrecht 2000; Gallant and Teather 2001; Malashichev 2002; Eisemberg and Bertoluci 2016). The FA finding in only two morphological traits may indicate that the frogs are tolerant of environmental disturbances. Species in the genera *Leptodactylus* and *Scinax* have been identified as tolerant to high levels of agricultural expansion and intensification (Silva et al. 2009; Suárez et al. 2016). This also seems to be the case for *L. macrosternum* and *S. x-signatus*, which are species very well adapted to habitat modification and anthropogenic disturbance (De La Riva and Maldonado 1999; Borges-Nojosa and Cascon 2005; Santana et al. 2015; Chaves et al. 2017). Thus, the FA may not have been able to evidence the environmental stresses related to agricultural activities. Although we have assumed their existence, there is a possibility that the stressors that could affect FA were simply not present in agricultural habitats. Levels of FA also did not vary among areas with different degrees of habitat disturbance in *Eleutherodactylus antillensis* (Puerto Rican Red-eyed Frog) and *E. coqui* (Puerto Rican Coqui; Delgado-Acevedo and Restrepo 2008) and FA was observed in only one morphological trait in *Physalaemus cuvieri* (Barker Frog; Eisemberg and Bertoluci 2016). The susceptibility to a particular stressor and the propensity to deviate from symmetry may differ among individuals and populations in different localities (Sanseverino and Nessimian 2008), and different anurans species may be associated with agricultural intensity in a variety of ways, both negative and positive (Knutson et al. 2004; Piha et al. 2007; Koumaris and Fahrig 2016; Oda et al. 2016; Suárez et al. 2016). In field studies, organisms are exposed to many environmental factors that can escape observation and detection by FA (Bjorksten et al. 2000; Floate and Fox 2000). Although there are FA studies that question its use and recommend caution (Beasley et al. 2013; Costa and Nomura 2016; Niemeier et al. 2019), various works have made efforts to evaluate the effectiveness of FA as a biomonitoring instrument in anurans (Eterovick et al. 2015; Matías-Ferrer and Escalante 2015; Eisemberg and Bertoluci 2016; Guillot et al. 2016).

The designation of populations not impacted by FA can be problematic as most habitats are in complex ecosystems and subject to multiple underlying stressors (McCoy and Harris 2003; Sanseverino and Nessimian 2008). Sanseverino and Nessimian (2008) state that choosing control areas at a distance from the studied stressor is often impractical due to the difficulty of matching the environmental characteristics (same habitat types, comparable physicochemical characteristics, etc.). The areas sampled in this study are within the same semi-arid region, and thus have similar environmental characteristics. We selected study sites seeking to ensure as much as possible that the populations sampled were independent. Anurans have limited dispersal capacity (Munguía et al. 2012) and cannot typically cover distances greater than 2 km (Smith and Green 2005; Piatti et al. 2010). Thus, we consider the cultivated areas sufficiently distant from the control areas, with a minimum distance of 7,697 m.

Several morphological traits did not meet the requirements of the statistical tests to obtain the FA index, that is, they presented measurement error, size dependence, anti-symmetry, and/or directional asymmetry. Detection of asymmetry can be hampered by the reliability of the measurements; measurements are difficult to perform on both live and preserved animals, even with all due attention to accuracy (Bjorksten et al. 2000; Helm and Albrecht 2000; McCoy and Harris 2003; Eisemberg and Jaime Bertoluci 2016). Therefore, following the recommendation by Delgado-Acevedo and Restrepo (2008) not to focus solely on asymmetric features to monitor amphibians, we also evaluated the organosomatic indices of *L. macrosternum*.

There was a tendency for the HSI and ASI values to be higher in control areas than in cultivated areas, except in area IV, where the values were similar to those in the control areas. The GSI, in turn, did not show a well-defined trend, showing higher values in control area I and cultivated area III, and also lower values in both area types, control area II and cultivated area IV. The HSI, ASI, and especially the GSI results therefore showed wide variation among the four areas studied, but with no indication of a difference between individuals from agricultural and non-agricultural areas.

The relationship between the weight of the body and the organs (such as the liver, fat bodies, and gonads), can be influenced by exposure to some pollutant (Kanamadi and Saidapur 1992; Paunescu and Ponepal 2011; Paunescu et al. 2018) and by season (Brown et al. 2011; Chaves et al. 2017), diet quality, energy dynamics (Brown et al. 2011), or reproductive status (Ebert et al. 2011; Franco-Belussi et al. 2012; Chaves et al. 2017). Although variation in the size of these organs occurs throughout the life cycle of most species (Brown et al. 2011; Sadekarpawar and Parikh 2013; Chaves et al. 2017), the differences found in this study are unlikely to be seasonal, or related to reproductive processes, as we collected all the samples at the same time. The liver is the organ where, in addition to the production and storage of glycogen as an energy reserve, xenobiotic accumulation and detoxification also occur (Fabacher and Baumann 1985; Crawshaw and Weinkle 2000; Păunescu and Ponepal 2011; Thammachoti et al. 2012). The HSI is one of the organosomatic indices most associated with exposure to contaminants (Adams and McLean 1985; Goede and Barton 1990; Sadekarpawar and Parikh 2013). This exposure usually leads to an increase in liver size (hypertrophy) or an increase in hepatocyte numbers (hyperplasia; Goede and Barton 1990; Solé et al. 2010).

In contrast, our study found higher HSI values in non-agricultural areas and a lower value in one of the areas considered disturbed by agriculture. Although we did not investigate the pesticide concentrations at the collection sites, or in the tissues of the sampled individuals, studies in fish observed a decrease in HSI in fish after exposure to contaminants in the laboratory (Barton et al. 1987; Ma et al. 2005, and Sadekarpawar and Parikh 2013). A decrease in HSI values may also occur due to the breakdown of glycogen energy reserves, stored in the liver (Barton et al. 1987; Goede and Barton 1990). The variation of ASI values among areas was equivalent to that of HSI, which also suggests the use of energy reserves, given that fat bodies provide an energy source in different environmental situations, such as food scarcity or in reproductive periods (Navarro et al. 2005; Chaves et al. 2017). Finally, GSI had the highest variation among areas. This may be explained, according to Schmitt and Dethloff (2000), because GSI can provide information on reproductive maturity, responses to environmental dynamics (e.g., seasonal changes), or exogenous stress (e.g., exposure to contaminants). Although it is one of the anuran biomonitoring tools (Păunescu and Ponepal 2011; Zaripova and Fayzulin 2012; Kitana et al. 2015; Zhelev et al. 2015b), the accuracy and reliability of the GSI has been treated with caution due to its high variation among studies (De Vlaming et al. 1981; Zhelev et al. 2014). Therefore, the organosomatic results suggest a possible relationship with unknown local environmental characteristics or ecological factors, as also reported by Tête et al. (2013).

The populations of *L. macrosternum* and *S. x-signatus* have shown, through FA and organosomatic indices (evaluated only in *L. macrosternum*), to be little impacted by areas of agricultural crops. These species, being tolerant to environmental disturbances and well adapted to habitat modification and anthropogenic disturbance, may not have been able to evidence the impacts through the morphological parameters used. Another possible explanation is that stressors that could affect these parameters were not present in agricultural habitats. Future studies focusing on histological variations in the gonads and evaluation of chemical contamination of the organs, such as liver and fat bodies, may give a better understanding of the possible impacts of agriculture on these populations.

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Literature Cited

Ab'Sáber, A.N. 1977. Os domínios morfoclimáticos na América do Sul. Geomorfologia 52:1–23.

Adams, S.M., and R.B. Mclean. 1985. Estimation of Largemouth Bass, *Micropterus salmoides* Lacépède, growth using the liver somatic index and physiological variables. Journal of Fish Biology 26:111–126.

Amaral, M.J., M.A. Carretero, R.C. Bicho, A.M. Soares, and R.M. Mann. 2012. The use of a lacertid lizard as a model for reptile ecotoxicology studies Part 1 - field demographics and morphology. Chemosphere 87:757–764.

Araújo, F.G., C.N. Morado, T.T.E. Parente, F.J.R. Paumgartten, and I.D. Gomes. 2018. Biomarkers and bioindicators of the environmental condition using a fish species (*Pimelodus maculatus* Lacépède, 1803) in a tropical reservoir in southeastern Brazil. Brazilian Journal of Biology 78:351–359.

Barton, B.A., C.B. Schreck, and L.D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile Rainbow Trout. Diseases of Aquatic Organisms 2:173–185.

Beasley, A.E., A. Bonisoli-Alquati, and T.A. Mousseau. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecological Indicators 30:218–226.

Becker, C.G., C.R. Fonseca, C.F.B. Haddad, R.F. Batista, and P.I. Prado. 2007. Habitat split and the global decline of amphibians. Science 318:1775–1777.

Beebee, T.J.C., R.J. Flower, A.C. Stevenson, S.T. Patrick, P.G. Appleby, C. Fletcher, C. Marsh, J. Natkanski, B. Rippey, and R.W. Battarbee. 1990. Decline of the Natterjack Toad *Bufo calamita* in Britain: paleoecological, documentary and experimental evidence for breeding site acidification. Biological Conservation 53:1–20.

Berg, J.M., J.L.Tymoczko, and L. Stryer. 2002. Biochemistry. 5th Edition. W.H. Freeman and Company, New York, New York, USA.

Bjorksten, T.A., K. Fowler, and A. Pomiankowski. 2000. What does sexual trait FA tell us about stress? Trends in Ecology and Evolution 15:163–166.

Blaustein, A.R., B.A. Han, R.A. Relyea, P.T.J. Johnson, J.C. Buck, S.S. Gervasi, and L.B. Kats. 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New York Academy of Sciences 1223:108–119.

Bonada, N., and D.D. Williams. 2002. Exploration of the utility of fluctuating asymmetry as an indicator of river condition using larvae of the Caddisfly *Hydropsyche morose* (Trichoptera: Hydropsychidae). Hydrobiologia 481:147–156.

Borges-Nojosa, D.M., and P. Cascon. 2005. Herpetofauna da área reserva da serra das Almas, Ceará. Pp. 243–258 *In* Análise das Variações da Biodiversidade do Bioma Caatinga. Araújo, F.S., M.J.N. Rodal, and M.R.V. Barbosa (Eds.). Ministério do Meio Ambiente, Brasília, Brazil.

Brodeur, J.C., J.V. Candioti, S. Soloneski, M.L. Larramendy, and A.E. Ronco. 2012. Evidence of reduced feeding and oxidative stress in Common Tree Frogs (*Hypsiboas pulchellus*) from an agroecosystem experiencing severe drought. Journal of Herpetology 46:72–78.

Brodeur, J.C., R.P. Suarez, G.S. Natale, A.E. Ronco, and M.E. Zaccagnini. 2011. Reduced body condition and enzymatic alterations in frogs inhabiting intensive crop production areas. Ecotoxicology and Environmental Safety 74:1370–1380.

Brown, G.P., C.M. Shilton, and R. Shine. 2011. Measuring amphibian immunocompetence: validation of the phytohemagglutinin skin-swelling assay in the Cane Toad, *Rhinella marina*. Methods in Ecology and Evolution 2:341–348.

Chatigny, F., C. Kamunde, C.M. Creighton, and E. Don Stevens. 2017. Uses and doses of local anesthetics in fish, amphibians, and reptiles. Journal of the American Association for Laboratory Animal Science 56:244–253.

Chaves, M.F., C.M.A.T. Fernanda, L.V.L.S. Igor., J.C.L.N. Clovis., W.T. Valeria, J.B.M. Geraldo, and A.C.T. Álvaro. 2017. Correlations of condition factor and gonadosomatic, hepatosomatic and lipo-somatic relations of *Leptodactylus macrosternum* (ANURA: Leptodactylidae) in the Brazilian Semi-arid. Anais da Academia Brasileira de Ciências 89:1591–1599.

Clarke, G.M. 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. Environmental Pollution 82:207–211.

Costa, R.N., and F. Nomura. 2016. Measuring the impacts of Roundup Original on fluctuating asymmetry and mortality in a Neotropical tadpole. Hydrobiologia 765:85–96.

Costa, R.N., M. Solé, and F. Nomura. 2017. Agropastoral activities increase fluctuating asymmetry in tadpoles of two neotropical anuran species. Austral Ecology

42:801–809.

Crawshaw, G.J., and T.K. Weinkle. 2000. Clinical and pathological aspects of the amphibian liver. Avian and Exotic Pet Medicine 9:165–173.

Dekić, R., S. Nebojša, M. Maja, G. Dragojla, and P. Jerko. 2016. Condition factor and organosomatic indices of Rainbow Trout (*Onchorhynchus mykiss*, Wal.) from different brood stock. Biotechnology in Animal Husbandry 32:229–237.

De La Riva, I., and M. Maldonado. 1999. First record of *Leptodactylus ocellatus* (Linnaeus, 1758) (Amphibia, Anura, Leptodactylidae) in Bolivia and comments on related species. Graellsia 55:193–197.

Delgado-Acevedo, J., and C. Restrepo. 2008. The contribution of habitat loss to changes in body size, allometry, and bilateral asymmetry in two *Eleutherodactylus* frogs from Puerto Rico. Conservation Biology 22:773–782.

De Vlaming, V.L., G. Grossman, and F. Chapman. 1981. On the use of gonadosomatic index. Comparative Biochemistry and Physiology Part A Physiology 73:31–39.

Ebert, T.A., J.C. Hernandez, and M.P. Russell. 2011. Problems of the gonad index and what can be done: analysis of the Purple Sea Urchin *Strongylocentrotus purpuratus*. Marine Biology 158:47–58.

Eisemberg, C.C., and J. Bertoluci. 2016. Fluctuating asymmetry in populations of the South American frog *Physalaemus cuvieri* (Leptodactylidae) in areas with different degrees of disturbance. Journal of Natural History 1:1–9.

Eterovick, P.C., L.F.F. Bar, J.B. Souza, J.F.M. Castro, F.S.F. Leite, and R.A. Alford. 2015. Testing the relationship between human occupancy in the landscape and tadpole developmental stress. PLoS ONE 10(3): e0120172. https://doi.org/10.1371/journal.pone.0120172.

Eterovick, P.C., A.C.O.Q. Carnaval, D.M. Borges-Nojosa, D.L. Silvano, M.C. Segalla, and I. Sazima. 2005. Amphibian declines in Brazil: an overview. Biotropica 37:166–79.

Fabacher, D.L., and P.C. Baumann. 1985. Enlarged livers and hepatic microsomal mixed-function oxidase components in tumor-bearing Brown Bullheads from a chemically contaminated river. Environmental Toxicology and Chemistry 4:703–710.

Floate, K.D., and P.C. Coghlin. 2010. No support for fluctuating asymmetry as a biomarker of chemical residues in livestock dung. Canadian Entomologist 142:354–368.

Floate, K.D., and A.S. Fox. 2000. Flies under stress: a test of fluctuating asymmetry as a biomonitor of environmental stress. Ecological Applications 10:1541–1550.

Foley, J.A., N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston, N.D. Mueller, C. O'Connell, D.K. Ray, P.C. West, et al. 2011. Solutions for a cultivated planet. Nature 478:337–342.

Franco-Belussi, L., L.R.S. Santos, R. Zieri, C.A. Vicentini, S.R. Taboga, and C. Oliveira. 2012. Liver anatomy, histochemistry and ultrastructure of *Eupemphix nattereri* (Anura: Leiuperidae) during the breeding season. Zoological Science 29: 844–848.

Gagné, F. 2014. Biochemical Ecotoxicology: Principles and Methods. 1st Edition. Academic Press, Amsterdam, Netherlands.

Gallant, N., and K. Teather. 2001. Differences in size, pigmentation, and fluctuating asymmetry in stressed and non-stressed Northern Leopard Frogs (*Rana pipiens*). Ecoscience 8:430–436.

Gama, A.F., A.H.B. de Oliveira, and R.M. Cavalcante. 2013. Inventário de agrotóxicos e risco de contaminação química dos recursos hídricos no semiárido cearense. Química Nova 36:462–467.

Gibbons, J.W., D.E. Scott, T.J Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Greene, T. Mills, Y. Leiden, S. Poppy, et al. 2000. The global decline of reptiles, déjà vu amphibians. Bioscience 50:653–666.

Goede, R.W., and B.A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pp. 93–108 *In* Biological Indicators of Stress in Fish. Adams, S.M. (Ed.). American Fisheries Society, Bethesda, Maryland, USA.

Gondim, R.S., A.S. Teixeira, M.F. Rosa, M.C.B. Figueiredo, P.M. Pereira, C.A.G. Costa, and K.V. Sabino. 2004. Diagnóstico da agricultura irrigada no Baixo e Médio Jaguaribe. Revista Econômica do Nordeste 35:424–430.

Graham, J.H, J.M. Emlen, D.C. Freeman, L.J. Leamy, and J.A. Kieser. 1998. Directional asymmetry and the measurement of developmental instability. Biological Journal of the Linnean Society 64:1–16.

Graham, J.H., S. Raz, H. Hel-Or, and E. Nevo. 2010. Fluctuating asymmetry: methods, theory, and applications. Symmetry 2:466–540.

Guillot, H., A. Boissinot, F. Agelier, O. Lourdais, X. Bonnet, and F. Brischoux. 2016. Landscape influences the morphology of male Common Toads (*Bufo bufo*). Agriculture, Ecosystems and Environment 233:106–110.

Hardersen, S. 2000. Effects of carbaryl exposure on the last larval instar of *Xanthocnemis zealandica*. Fluctuating asymmetry and adult emergence. Entomologia Experimentalis et Applicata 96:221–230.

Hellawell, J.M. 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier, New York, New York, USA.

Helm, B., and H. Albrecht. 2000. Human handedness causes directional asymmetry in avian wing measurements. Animal Behaviour 60:899–902.

International Union for the Conservation of Nature (IUCN). 2018. IUCN Red List of Threatened Species, 2018. http://www.iucnredlist.org.

Johnson, R.K., T. Weiderholm, and D.M. Rosenberg. 1993. Freshwater biomonitoring using individual organisms, populations and species assemblages of benthic macroinvertebrates. Pp. 40–105 *In* Freshwater Biomonitoring and Benthic Macroinvertebrates. Rosenberg, D.M., and V.H. Resh (Eds.). Chapman and Hall, New York, New York, USA.

Kanamadi, R.D., and S.K. Saidapur. 1992. Effects of exposure to sublethal mercuric chloride on the testis and fat body of the frog *Rana cyanophlyctis*. Journal of Herpetology 26:499–501.

Kitana, J., O. Achayapunwanich, P. Thammachoti, M.S. Othman, W. Khonsue, and N. Kitana. 2015. Cadmium contamination and health assessment in frog *Microhyla fissipes* living downstream of zinc mining area in Thailand. EnvironmentAsia 8:16–23.

Kleinkauf, A., L. Connor, D. Swarbreck, C. Levene, P. Walker, P.J. Johnson, and R.T. Leah. 2004. General condition biomarkers in relation to contaminant burden in European Flounder (*Platichthys flesus*). Ecotoxicology and Environmental Safety 58:335–355.

Knutson, M.G., W.B. Richardson, D.M. Reineke, B.R. Gray, J.R. Parmelee, and S.E. Weick. 2004. Agricultural ponds support amphibian populations. Ecological Applications 14:669–684.

Koumaris, A., and L. Fahrig. 2016. Different anuran species show different relationships to agricultural intensity. Wetlands 36:731–744.

Lens, L., S. Van Dongen, S. Kark, and E. Matthysen. 2002. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? Biological Reviews of the Cambridge Philosophical Society 77:27–38.

Leung, B., and M.R. Forbes. 1996. Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. Ecoscience 3:400–413.

Longson, C.G., K.M. Hare, and C.H. Daugherty. 2007. Fluctuating asymmetry does not reflect environmental stress during incubation in an oviparous lizard. New Zealand Journal of Zoology 34:91–96.

Ma, T., X. Wan, Q. Huang, Z. Wang, and J. Liu. 2005. Biomarker responses and reproductive toxicity of effluent from a Chinese large sewage treatment plant in Japanese Medaka (*Oryzias latipes*). Chemosphere 59:281–288.

Malashichev, Y.B. 2002. Asymmetries in amphibians: a review of morphology and behaviour. Laterality 7:197–217.

Matías-Ferrer, N., and P. Escalante. 2015. Size, body condition, and limb asymmetry in two hylid frogs at different habitat disturbance levels in Veracruz, México. Herpetological Journal 25:169–176.

McCallum, M.L. 2015. Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation 24:2497–2519.

McCoy, K.A., and R.N. Harris. 2003. Integrating developmental stability analysis and current amphibian monitoring techniques: an experimental evaluation with the salamander *Ambystoma maculatum*. Herpetologica 59:22–36.

McCoy, K.A., L.J. Bortnick, C.M. Campbell, H.J. Hamlin, L.J. Guillette, and C.M. St Mary. 2008. Agriculture alters gonadal form and function in the toad *Bufo marin*us. Environmental Health Perspectives 116:1526–1532.

Milhome, M.A.L., D.O.B. de Sousa, F.A.F. Lima, and R.F. do Nascimento. 2009. Avaliação do potencial de contaminação de águas superficiais e subterrâneas por pesticidas aplicados na agricultura do Baixo Jaguaribe, CE. Engenharia Sanitária e Ambiental 14:363–372.

Munguía, M., C. Rahbek, T.F. Rangel, J.A.F. Diniz-Filho, and M.B. Araújo. 2012. Equilibrium of global amphibian species distributions with climate. PLoS ONE 17:1–9. https://doi.org/10.1371/journal.pone.0034420.

Navarro, R.D., O.P.R. Filho, G.S. Yasui, E.C.S. Maciel, and L.C. Santos. 2005. Efeito do hormônio 17-α-metil-testosterona nos índices somáticos de *Rana catesbeiana*. Zootecnia Tropical 23:319–325.

Niemeier, S., J. Müller, and M.O Rödel. 2019. Fluctuating asymmetry - appearances are deceptive. Comparison of methods for assessing developmental instability in European Common Frogs (*Rana temporaria*). Salamandra 55:14–26.

Norrdahl, K., H. Heinila, T. Klemola, and E. Korpimaki. 2004. Predator-induced changes in population structure and individual quality of *Microtus* voles: a largescale field experiment. Oikos 105:312–324.

Oda, F.H., V.G. Batista, P.G. Gambale, F.T. Mise, F. Souza, S. Bellay, J.C.G. Ortega, and R.M. Takemoto. 2016. Anuran species richness, composition, and breeding habitat preferences: a comparison between forest remnants and agricultural landscapes in southern Brazil. Zoological Studies 55:1–14.

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

Palmer, A.R. 1996. Waltzing with asymmetry. Bioscience 46:518–532.

Palmer, A.R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual

Review of Ecology and Systematics 17:391–421.

Palmer, A.R., and C. Strobeck. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:55–70.

Palmer, A.R., and C. Strobeck. 2003. Fluctuating asymmetry analysis revisited. Pp. 279–319 *In* Developmental Instability (DI): Causes and Consequences. Polak, M. (Ed.). Oxford University Press, New York, New York, USA.

Parsons, P.A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews 65:131–145.

Parsons, P.A. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity 68:361–364.

Păunescu, A., and C.M. Ponepal. 2011. Effect of Roundup herbicide on physiological indices in Marsh Frog *Pelophylax ridibundus*. Scientific Papers University of Agronomic Sciences and Veterinary Medicine of Bucharest - UASVM Bucharest, Series A 54:269–274.

Paunescu, A., L.C. Soare, R.C. Fierascu, I. Fierascu, and M.C. Ponepal. 2018. The influence of six pesticides on physiological indices of *Pelophylax ridibundus* (Pallas, 1771). Bulletin of Environmental Contamination and Toxicology 100:376–383.

Piatti, L., F.L. Souza, and P.L. Filho. 2010. Anuran assemblage in a rice field agroecosystem in the Pantanal of central Brazil. Journal of Natural History 44:1215–1224.

Piha, H., M. Luoto, M. Piha, and J. Merila. 2007. Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Global Change Biology 13:300–311.

Polak, M. 2003. Developmental Instability: Causes and Consequences. University Press, New York, New York, USA.

Pope, S.E., L. Fahrig, and H.G. Merriam. 2000. Landscape complementation and metapopulation effects on Leopard Frog populations. Ecology 81:2498–2508.

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Rainio, J., and J. Niemelä. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation 12:487–506.

Sadekarpawar, S., and P. Parikh. 2013. Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. World Journal of Zoology 8:110–118.

Sanseverino, A.M., and J.L. Nessimian. 2008. Assimetria flutuante em organismos aquáticos e sua aplicação para avaliação de impactos ambientais. Oecologia Brasiliensis 12:382–405.

Santana, D.J., S. Mângia, R.R. da Silveira-Filho, L.C.S. Barros, I. Andrade, M.F. Napoli, F. Juncá, and A.A. Garda. 2015. Anurans from the Middle Jaguaribe River region, Ceará State, northeastern Brazil. Biota Neotropica 15:1–8.

Sarre, S., J.D. Dearn, and A. Georges. 1994. The application of fluctuating asymmetry in the monitoring of animal population. Pacific Conservation Biology 1:118–122.

Schmitt, C.J., and G.M. Dethloff. 2000. Biomonitoring of Environmental Status and Trends (BEST) Program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems. Information and Technology Report USGS/BRD-2000–0005, U.S. Geological Survey, Biological Resources Division, Columbia, Missouri, USA. 81 p.

Silva, F.R., R.S. Santos, M.A. Nunes, and D.C. Rossa-Feres. 2009. Anuran captured in pitfall traps in three agrossystems in northwestern São Paulo State, Brazil. Biota Neotropica 9:253–255.

Silvano, D.L., and M.V. Segalla. 2005. Conservation of Brazilian amphibians. Conservation Biology 19:653–658.

Smith, M.A., and D.M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110–128.

Solé, M., M. Antó, M. Baena, M. Carrasson, J.E. Cartes, and F. Maynou. 2010. Hepatic biomarkers of xenobiotic metabolism in eighteen marine fish from NW Mediterranean shelf and slope waters in relation to some of their biological and ecological variables. Marine Environmental Research 70:181–188.

Sparling, D.W., G.M. Fellers, and L.L. McConnell. 2001. Pesticides and amphibian population declines in California, USA. Environmental Toxicology and Chemistry 20:1591–1595.

St-Amour, V., T.W.J. Gamer, A.I. Schulte-Hostedde, and D. Lesbarrères. 2010. Effects of two amphibian pathogens on the developmental stability of Green Frogs. Conservation Biology 24:788–794.

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

Suárez, R.P., M.E. Zaccagnini, K.J. Babbitt, N.C. Calamari, G.S. Natale, A. Cerezo, N. Codugnello, T. Boca, M.J. Damonte, J. Vera-Candioti, et al. 2016. Anuran responses to spatial patterns of agricultural landscapes in Argentina. Landscape Ecology 31:2485–2505.

Tête, N., C. Fritsch, E. Afonso, M. Coeurdassier, and

J.C. Lambert. 2013. Can body condition and somatic indices be used to evaluate metal-induced stress in wild small mammals? PLoS ONE 8:1–9. https://doi.org/10.1371/journal.pone.0066399.

Thammachoti, P., W. Khonsue, J. Kitana, P. Varanusupakul, and N. Kitana. 2012. Morphometric and gravimetric parameters of the rise frog Fejervarya limnocharis living in areas with different agricultural activity. Journal of Environmental Protection 3:1403–1408.

Tomkins, J.L., and J.S. Kotiaho. 2002. Fluctuating asymmetry. *In* Encyclopedia of Life Sciences. John Wiley and Sons. Ltd., Chichester, UK. http://www.els.net doi: 10.1038/npg.els.0003741.

Valen, L.V. 1962. A study of fluctuating asymmetry. Evolution 16:125–142.

Zaripova, F.F., and A.I. Fayzulin. 2012. Characteristic of morphophysiological parameters of population of the Marsh Frog *Rana ridibunda* (Anura, Amphibia) in urban areas in the republic of Bashkotorstan. Proceedings of the Samara Scientific Center of Russian Academy of Sciences 14:145–149.

Zhelev, Z.M., G.S. Popgeorgiev, A.D. Arnaudov, K.N. Georgieva, and N.H. Mehterov. 2015a. Fluctuating asymmetry in *Pelophylax ridibundus* (Amphibia: Ranidae) as a response to anthropogenic pollution in South Bulgaria. Archives of Biological Sciences 67:1009–1023.

Zhelev, Z.M., G.S. Popgeorgiev, and N.H. Mehterov. 2014. Changes in the basic morphophysiological parameters in the populations of *Pelophylax ridibundus* (Amphibia: Ranidae) from anthropogenically polluted biotopes in southern Bulgaria. Part 1. Bulgarian Journal of Agricultural Science 20:1202–1210.

Zhelev, Z.H.M., G.S. Popgeorgiev, and N.H. Mehterov. 2015b. Changes in the hepatosomatic index and condition factor in the populations of *Pelophylax ridibundus* (Amphibia: Ranidae) from anthropogenically polluted biotopes in Southern Bulgaria. Part II. Bulgarian Journal of Agricultural Science 21:517–522.

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