



Size, body condition, and limb asymmetry in two hylid frogs at different habitat disturbance levels in Veracruz, México

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Habitat disturbance is one of the main factors contributing to population declines. Changes in the amount and quality of available habitat can affect body condition and morphology. In this study we evaluated the effect of habitat disturbance (deforestation, urbanisation and land-use change) on body size, body condition and fluctuating asymmetry in two species of arboreal tropical frogs (*Agalychnis callidryas* and *Dendropsophus ebraccatus*) at Los Tuxtlas Biosphere Reserve, Veracruz, México. We did not find significant differences in body condition or body size associated with habitat disturbance in *A. callidryas*, although the species reduced its local distribution in line with habitat disturbance. In *D. ebraccatus*, on the other hand, we observed a significant relationship between body condition and size with habitat disturbance. It is unknown whether these changes are adaptive or compromise the permanence of populations. Fluctuating asymmetry indices for both species exhibited no significant differences except for the tibia-fibula length of *D. ebraccatus*, which was significantly higher in undisturbed habitat.

Key words: *Agalychnis callidryas*, body condition, body size, *Dendropsophus ebraccatus*, fluctuating asymmetry, habitat disturbance, Hylidae

INTRODUCTION

Habitat loss and fragmentation are the two most important factors causing biodiversity loss (Henle et al., 2004a). Land use changes have led to the decline and deterioration of habitats, resulting in the reduction of habitat quality (Henle et al., 2004b). Changes in microclimate and food availability affect the physiology of organisms. These changes may increase mortality or interrupt dispersal processes, and thereby increase the risk of decline and local extinction. Habitat changes may also affect the abundances of predators and prey as well as parasites and pathogens (Lips, 1999).

Changes in the environment can lead to changes in the morphology of organisms (Sumner et al., 1999). For example, size and body condition may decrease in response to changes in habitat quality (Lauk, 2006; Delgado-Acevedo & Restrepo, 2008; Henríquez et al., 2009). Transformed habitat conditions can also cause stress in resident populations, inducing changes during development that result in morphological asymmetry (Lens et al., 2002; Wright & Zamudio, 2002). Fluctuating asymmetry (FA) is a pattern of random deviations from symmetry of bilateral characters (Palmer & Strobeck, 1992), and has been used as an indirect measure of developmental instability in populations subjected to different environmental stressors (Hoffmann & Woods, 2003; Söderman et al., 2007). In some species, asymmetry

increases with the degree of habitat disturbance (Sarre, 1996; Wright & Zamudio, 2002).

Rainforests are the most diverse terrestrial ecosystems, and are rapidly disappearing due to high rates of deforestation. In Mexico, 80–90% of rainforests have been cleared or severely altered by human activities such as logging, livestock production, farming and urbanisation (Guevara et al., 2004). The most important Mexican boreal remnant rainforest is situated in Los Tuxtlas, Veracruz. Currently, Los Tuxtlas consists of forest, pastures, and crop fields in the lowlands (Dirzo & Garcia, 1992; Guevara et al., 2004). The region, encompassing an area of 155,122 ha, was declared a Natural Protected Area and a Biosphere Reserve in 1998 (CONANP & SEMARNAT, 2006).

The red-eyed tree frog (*Agalychnis callidryas* Cope, 1862) and the hourglass tree frog (*Dendropsophus ebraccatus* Cope, 1874) are both members of the family Hylidae and have a wide distribution stretching from Southern México to Costa Rica and Colombia, respectively (Duellman, 1970). *Agalychnis callidryas* is 40–60 mm in snout vent length (SVL), and *D. ebraccatus* is <30 mm in SVL (Duellman, 1970; Rodríguez-Mendoza & Pineda, 2010). They are frequently found in the rainy season on vegetation overhanging permanent and temporary ponds where they breed (Duellman, 1970; Warkentin 2000). Their eggs are deposited on leaves, and tadpoles fall into the water for development to metamorphosis

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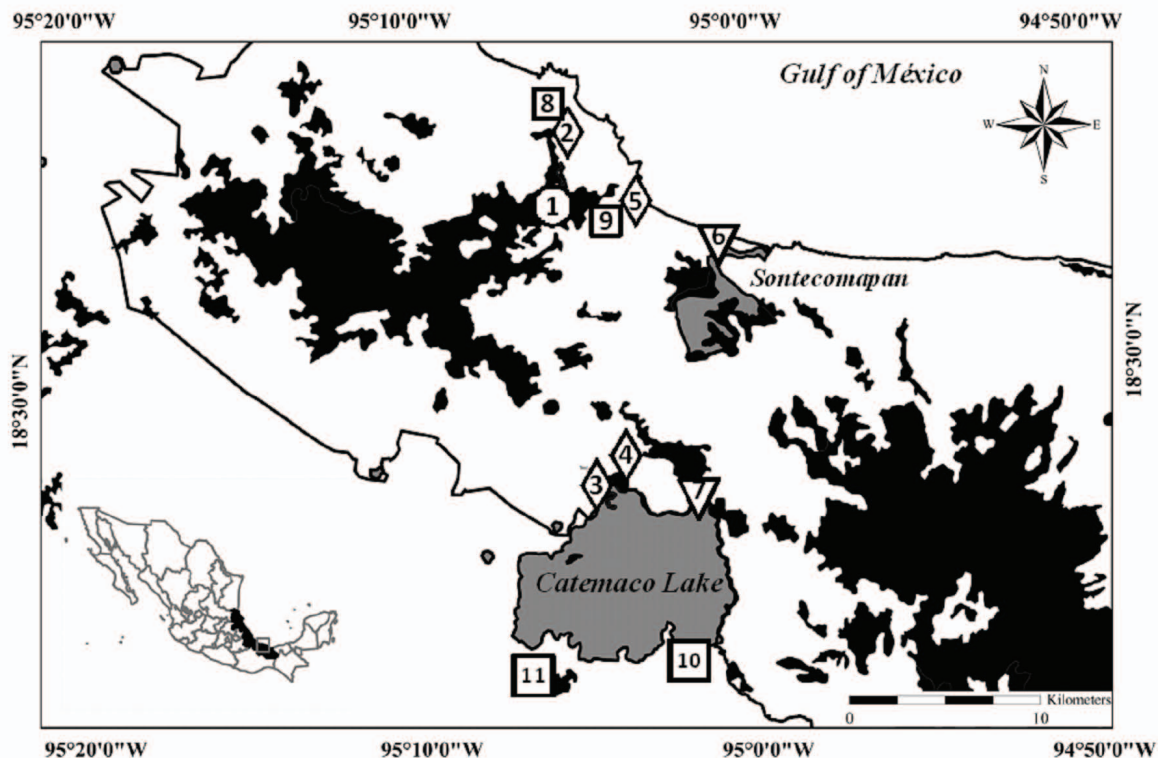


Fig. 1. Collection sites for sampling of *Agalychnis callidryas* and *Dendropsophus ebraccatus* for length, body condition and limb asymmetry. The area corresponds to the northern region of the Biosphere Reserve Los Tuxtlas, Veracruz (México). Black areas are primary vegetation remnants, located mainly above 600 m. White areas are livestock, urban, and agricultural areas; circles: undisturbed habitat (UD), diamonds: low disturbance habitat (LD), triangles: intermediate disturbance (ID) and squares: high disturbance (HD). Within the symbols are localities numbers.

(Vogt, 1997; Cedeño-Vázquez et al., 2006). Both species have an extended breeding season (Donnelly & Guyer, 1994). Land use changes, global climate change, and pollution are considered risk factors for both species in Mexico (Solís et al., 2008; Jungfer et al., 2008), and records for both species are rare compared to other frogs (Urbina-Cardona & Reynoso, 2005; Rodríguez-Mendoza & Pineda, 2010). While no data are available on population trends (Frias-Álvarez et al., 2010), both species may be tolerant of habitat disturbance, as they have been found in orchards, secondary vegetation, pastures and habitat edges (Duellman 1970; Cedeño-Vázquez et al., 2006). The aim of this study is to evaluate the effect of habitat disturbance on both tree frog species. We test the following predictions: i) habitat disturbance influences adult size and body condition and ii) the asymmetry of bilateral characters will increase with the degree of environmental disturbance.

MATERIALS AND METHODS

This study was conducted in the northern part of Los Tuxtlas Biosphere Reserve, an area with a complex topography. Original forest vegetation is found primarily on hills and ridges, whereas the lowlands have been converted into grasslands, crop fields and villages. The main remaining patch of lowland rainforest is situated in the Biological Station of UNAM (Universidad Nacional Autónoma de México, Fig. 1).

The study area was surveyed with the aid of local guides until a total of 11 sites with permanent and semi-permanent breeding ponds were found and included in our sampling design; a random design was impossible because the most pristine areas are in the highlands and are not suitable for the formation of breeding ponds for *A. callidryas* and *D. ebraccatus* (Fig. 1). The degree of habitat perturbation was categorised as Undisturbed (UD), Low Disturbance (LD), Intermediate Disturbance (ID) or High Disturbance (HD) based on knowledge and experience (Table 1). We manually collected individuals at night during the rainy season between July and December 2009–2011 (125 days in total). Each site was visited only once, with the exception of sites 7 and 10 (Table 1) which are represented by individuals collected on two different years. At each site, we collected at least 10 calling males per species, which had well-developed nuptial pads as described by Duellman (1970). After capture, each individual was weighed with a digital scale (± 0.01 g), and snout vent length (SVL) was measured with a digital caliper (± 0.01 cm). Because asymmetry analyses require high accuracy (Palmer, 1994), captured animals were euthanised with 5% Xylocaine and fixed with 10% formaldehyde. The length of the tibia-fibula was measured from the knee to the ankle joint, and femur length was measured from the knee to the tight joint (following Lauk, 2006; Delgado-Acevedo & Restrepo, 2008; Plăiașu et al., 2012). Three replicate measurements were taken to reduce measurement error (ME). All measurements were made by the same person.

SVL was used as a measure of body size. Body condition was obtained using the scaled mass index (SMI, Peig & Green, 2009; 2010; MacCracken & Stebbins, 2012). We used an ordinary least squares regression to estimate SMI parameters. SMI and body size were averaged by category of habitat disturbance for each species. The total number of specimens was 65 and 196 individuals of *A. callidryas* and *D. ebraccatus*, respectively. The normality distribution and homogeneity of variances of the sample data were tested before constructing a general linear model. SVL and SMI were considered response variables, and the disturbance category a fixed factor. Each response variable was evaluated in a separate analysis.

Limbs were assessed in terms of fluctuating asymmetry (FA) following Palmer (1994) and Palmer & Strobeck (2003). We obtained data on the tibia-fibula and femur lengths from 63 and 105 individuals of *A. callidryas* and *D. ebraccatus*, respectively. Factors that obscure the analysis of FA (i.e., directional asymmetry, antisymmetry and trait size) were ruled out as follows. First, we performed a two-way model ANOVA with individual as a random factor and side as a fixed factor (Palmer & Strobeck, 2003). Significant interaction terms (side x individual) suggested the presence of antisymmetry or FA (Palmer & Strobeck, 2003). Then, we calculated measurement error as $M_{SE} / MS_{SI} \times 100$, with M_{SE} and MS_{SI} being mean squares of error and interaction, respectively. We estimated repeatability with the ME5 index ($MS_E / MS_{SI} + (n-1) MS_E$). To confirm that directional asymmetry (DA) was absent, we performed a one-sided *t* test comparing the mean of (R-L) to zero for each trait in each habitat for each species. Finally, we assessed antisymmetry with the Kolmogorov-Smirnov test for differences from normality for the difference between of sides ([R-L]) of the tibia-fibula and femur lengths (Parris & Cornelius, 2004). A

non-normal distribution is indicative of antisymmetry, whereas a normal distribution with a mean of zero is indicative of FA (Polak, 2003). Trait size may also confound estimates of FA. To check for size dependence of trait size in trait variation, we performed a linear regression between the asymmetry absolute value of R-L ($|R-L|$) and trait size $((R+L)/2)$ as independent variable for each habitat disturbance category.

We calculated three FA indices described in Palmer & Strobeck (2003) for each species, trait and category of disturbance: FA1, FA4a, and FA10a. FA1 (mean $|R-L|$) is the recommended index because it is easy to interpret, but for small sample sizes ($n < 30$) has low statistical power. FA4a ($0.798 \sqrt{\text{var}(R-L)}$) has higher statistical power and represents the contribution of FA measurement error (ME, Bechshoft et al., 2008); FA10a ($0.798 \sqrt{2\sigma_i^2}$) is recommended because it describes the difference between the sides after ME has been removed (Palmer & Strobeck, 2003). Given that the FA1 ($|R-L|$) in each category did not deviate from a normal distribution, the data were analysed using a general linear model with categories of habitat disturbance as an explanatory factor and FA1 as the response variable. All statistical analyses were performed in R v.2.1.3.1 (The R Foundation for Statistical Computing, 2011) and Statistica for Windows v.7.0 (StatSoft, Inc. 2004).

RESULTS

Body size and condition

Agalychnis callidryas was found only in the three least-disturbed categories (UD, LD and ID). Individuals from UD and LD sites were larger compared to ID individuals (Fig. 2a), a differences which was however not statistically significant ($F_{3,195} = 1.068, p = 0.349$). In *D. ebraccatus*, significant differences in body size were found between

Table 1. Sampling sites, habitat type and degree of disturbance. (UD: undisturbed, LD: low disturbance; ID: intermediate disturbance and HD: highly disturbed). Site numbers correspond to each location in Fig. 1. Species: A=*Agalychnis callidryas* and D=*Dendropsophus ebraccatus*.

Locality	Habitat type	Species	Disturbance Category
1 Laguna El Zacatal	continuous rainforest	A, D	UD
2 Potrero Ejido 2 de Abril	rainforest fragment <1 ha.	A	LD
3 Nanciyaga Ecological Park	rainforest fragment >40 ha.	D	LD
4 La Jungla Park, Catemaco	rainforest fragment >40 ha. (artificial pond)	A	LD
5 Playa Escondida	reed patch	A, D	LD
6 Laguneta de El Real, Sontecomapan	disturbed mangrove, urban zone	A	ID
7 La Cruz, near Tebanca	macrophyte patch near pasture	D	ID
8 Villa El Cariño. Ejido 2 de Abril	pasture	D	HD
9 Road to Playa Escondida	pasture	D	HD
10 Las Margaritas	flooded plot, urban zone	D	HD
11 Deviation to San Juan Seco	Pasture, highway on one side	D	HD

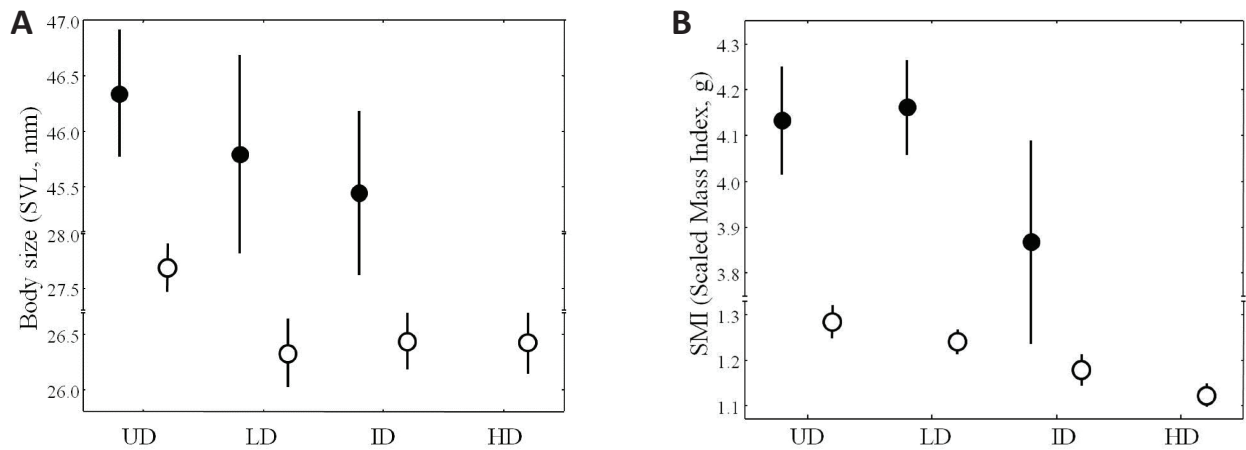


Fig. 2. Mean (circles) and standard error (bars) for (A) body size (mm, Snout Vent Length) and (B) body condition (g, Scaled Mass Index) for *Agalychnis callidryas* (black circles) and *Dendropsophus ebraccatus* (open circles) in relation to different levels of habitat disturbance in the Biosphere Reserve of Los Tuxtlas, Veracruz (México). UD: undisturbed, LD: low-disturbance, ID: intermediate disturbance and HD: high disturbance. The horizontal lines join habitat categories not significantly different from one another.

the UD habitat and the other categories ($F_{3,195}=23.4$, $p<0.001$; Fig. 2a). We did not find significant differences in *A. callidryas* body condition among categories of habitat disturbance ($F_{2,64}=2.783$, $p=0.069$; Fig. 2b). In *D. ebraccatus*, we found significant decreases in SMI depending on habitat disturbance (UD-LD vs. ID-HD, $F_{3,195}=22.485$, $p<0.001$).

Limb asymmetry

The interaction term was significant ($p<0.001$), demonstrating that non-directional asymmetry could be distinguished from measurement error. Measurement errors were $>10\%$ for all characters except femurs length of *D. ebraccatus* (15–30%). The repeatability of FA was 0.6–0.8 for *A. callidryas*, and 0.08–0.31 for *D. ebraccatus* (Table 2).

The mean (R-L) was significantly different from zero ($t=2.8$, $p=0.048$) in *D. ebraccatus* femur lengths in the UD habitat, demonstrating the presence of directional asymmetry (DA). Therefore, DA for this character was removed by estimating the differences between right and left sides, subtracted from the larger measurement and added to the shorter measurement (Table 2; following Palmer, 1994). The Kolmogorov-Smirnov test did not suggest a significant departure from normality for any trait or species (all d -KS >0.08 , $p>0.05$), demonstrating that non-directional asymmetry can be used as an estimate for FA. The relationship of trait size with FA1 ($|R-L|$) provides no evidence of a size-dependent component of FA ($\beta=-0.2-0.3$, $p>0.05$, Table 2).

No significant differences were found in FA1 limb traits with respect to habitat disturbance in *A. callidryas* (tibia-fibula, $F_{2,60}=0.020$, $p=0.98$; femur, $F_{2,59}=0.577$, $p=0.57$, Table 3), while significant differences were observed for *D. ebraccatus* ($F_{3,101}=4.843$, $p=0.003$). A *post hoc* comparison using Tukey's HSD test showed that UD exhibited a higher tibia-fibula FA1 than the remaining categories (Mean=0.067, SE=0.008, $p<0.001$). The values of FA4 and FA10 displayed similar asymmetry results

(Table 3). For femur length, FA1 was higher in UD but the difference was not significant ($F_{3,101}=1.5216$, $p=0.214$, Table 3).

DISCUSSION

Morphological changes due to habitat transformations have been proposed as indicators of habitat deterioration and the health status of individuals in transformed environments (Alford et al., 1999; Delgado-Acevedo & Restrepo, 2008). In this work, we quantified the effects of habitat disturbance in two species of tropical frogs, *A. callidryas* and *D. ebraccatus*, in a protected area. Our results suggest that *A. callidryas* is not significantly affected by habitat disturbance for the measured morphological parameters. However, frogs were not found in the highly disturbed habitat, and the lack of statistical evidence is linked with a small sample size obtained in the more disturbed categories. The size and body condition of *D. ebraccatus* decreased with habitat disturbance, consistent with studies on other anuran species (Neckel-Oliveira & Gascón, 2006; Lauk, 2006; Delgado-Acevedo & Restrepo, 2008). Reduced body size and poorer body condition might be responses to environmental changes related to increased solar radiation, decreased humidity, pond desiccation, and/or restricted food availability, all of which are outcomes of altered habitats (Palkovacs, 2003; Buckley et al., 2005; Lowe et al., 2006). Predation may also influence size and body condition, as habitat disturbance might require higher energy expenditure for predator avoidance (Camargo & Kapos, 1995; Ball & Baker, 1996). Alternatively, larger individuals might be more conspicuous, which could explain why small-sized individuals prevail in areas with higher disturbance (Martín & Lopez, 1998; Delgado-Acevedo & Restrepo, 2008). Moreover, predation might accelerate the times of hatching and metamorphosis, resulting in a smaller adult body size (Warkentin, 1995; 2000; Ball & Baker, 1996; Jessop et al., 2006; Touchon

Table 2. Analysis of variance (ANOVA) to test the variation regarding trait measurement error (side x individual interaction) in *Agalychnis callidryas* and *Dendropsophus ebraccatus* by disturbance category (UD: undisturbed habitats; LD: low disturbed habitats; ID: intermediately disturbed habitats and HD: highly disturbed habitat). *n*=sample size. TI: tibia-fibula, FE: femur, ME3: measurement error expressed as MS_e/MS_{si} x 100 where MS_e and MS_{si} are the Mean Squares of error and side x individual interaction of the mixed model ANOVA, respectively. ME5: repeatability (Palmer & Strobeck, 2003), the values range from -1 to 1. df: degrees of freedom of the side x individual interaction. *F*: *F* statistic for the side x individual interaction. R, L: sides; (R+L)/2: Trait size. DA: Directional asymmetry. %DA:(mean |R|-mean |L|)x100. *A. callidryas* was not captured at HD sites, therefore no data for this habitat category. * Value of (R-L) before to remove the DA, which is made by subtracting the long side and by adding on the shorter side, the mean (R-L). After correction, the final values are mean(R-L)=0.03 (SE=0.02). † Slope of linear regression between |R-L| and trait size ((R+L)/2). ‡ t-test of one-side analysis of mean (R-L) respect to zero.

Habitat/ trait	<i>n</i>	ME3 (%)	ME5	df	<i>F</i>	<i>p</i> -values	R (Mean±SE)	L (Mean±SE)	(R+L)/2 (Mean±SE)	Slope† ±SE	DA=R-L (Mean±SE)	%DA	(t-test)‡
<i>A. callidryas</i>													
UD/TI	29	1.75	0.66	28	62.58	<2.2e-16	26.4 (2.6)	26.5 (2.6)	23.9 (-0.2)	-0.02	-0.02 (0.00)	2.2	1.2
LD/TI	22	1.44	0.72	25	67.45	<2.2e-16	22.8 (0.2)	22.8 (0.2)	23.0 (-0.2)	0.01	-0.03 (0.00)	2.7	1.4
ID/ TI	7	1.83	0.88	6	55.22	3.1 e-14	23.4 (0.2)	23.5 (0.2)	23.1 (-0.1)	0.02	-0.02 (0.00)	2.1	0.5
HD/TI	-	-	-	-	-	-	-	-	-	-	-	-	-
UD/FE	30	1.94	0.63	29	57.52	<2.2e-16	24.6 (0.2)	24.6 (0.2)	24.5 (-0.2)	-0.01	-0.04 (0.00)	3.9	2.4
LD/FE	22	1.68	0.69	25	62.05	<2.2e-16	24.0 (0.3)	24.0 (0.3)	24.2 (-0.2)	0	-0.01 (0.00)	1.3	0.6
ID/ FE	7	4.13	0.79	5	24.11	1.2e-08	31.7 (7.0)	31.7 (7.0)	24.5 (-0.1)	0.01	-0.02 (0.00)	1.5	0.5
HD/FE	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>D. ebraccatus</i>													
UD/TI	22	5.46	0.19	21	18.52	1.9 e-15	14.6 (0.1)	14.6 (0.1)	14.6 (0.1)	0.02	0.01 (0.02)	8.4	1.3
LD/TI	20	9.01	0.12	23	10.99	3.1 e-12	14.1 (0.1)	14.1 (0.1)	14.0 (0.1)	-0.01	0.00 (0.01)	0.5	0.9
ID/ TI	25	6.56	0.16	26	15.14	<2.2e-16	14 (0.1)	14 (0.1)	14.0 (0.1)	-0.01	0.01 (0.01)	3.2	0.5
HD/TI	31	7.27	0.15	30	13.85	<2.2e-16	14.3 (0.1)	14.3 (0.1)	14.3 (0.1)	0.01	0.02 (0.01)	1.1	0.4
UD/FE	21	29.8	0.05	20	3.36	0.000047	14.1 (0.1)	14 (0.1)	14.1 (0.1)	0.03	-0.08 (0.01)*	5.4	2.8
LD/FE	26	19.97	0.06	28	4.99	1.2 e-07	13.8 (0.1)	13.8 (0.1)	13.8 (0.1)	0.02	0.01 (0.01)	0.5	2.1
ID/ FE	22	21.09	0.08	21	4.74	6.9 e-06	13.6 (0.1)	13.6 (0.1)	13.6 (0.1)	0	-0.03 (0.02)	1.0	0.7
HD/FE	36	16.54	0.06	35	6.05	6.2 e-11	13.8 (0.1)	13.8 (0.1)	13.8 (0.1)	-0.02	-0.01 (0.01)	1.6	0.4

et al., 2013). Paternal effects may also influence the size of individuals, as small males may sire smaller froglets (Briggs, 2013). In some anurans, body condition is lower at the end of the breeding season, due to the energy consumed in reproductive activities (Reading & Clarke, 1995). In our study, the samples were obtained during breeding, with the exception of one *D. ebraccatus* population which comprised smaller individuals without lowered body condition. Our observations might however also be influenced by a relationship of age with size and body condition (Ormerod & Tyler, 2009; Iturra-Cid et al., 2010), and a lower average age in areas of higher disturbance (Semlitsch, 2008; VanderWerf, 2004).

Habitat disturbance did not strongly influence limb asymmetry. In *A. callidryas*, FA did not exhibit a correlation with habitat disturbance, a finding similar to those of other studies (Labrie et al., 2003; Henríquez et al., 2009). For *D. ebraccatus*, the results were unexpected because the FA of tibia-fibula length was significantly higher in undisturbed areas (but see also Floate & Fox, 2000; Lauk, 2006; Delgado-Acevedo & Restrepo, 2008; Hopton et al., 2009). The lack of an effect of habitat disturbance on FA has previously been attributed primarily to high measurement error, higher mortality of more asymmetrical individuals (Møller, 1997; Floate & Fox, 2000; Henríquez et al., 2009), the traits selected for measurement (Lens et al., 2002; Vishalakshi & Singh, 2008; Henríquez et al., 2009), and sample size (Palmer, 1994; Delgado-Acevedo & Restrepo, 2008). In this study, the measurement error was low (<30%). We do not know whether the measured traits are linked to individual fitness (Lauk, 2006), and the possibility of differential mortality at sites with higher disturbance is difficult to test. Floate & Fox (2000) considered that, under lower stress or a lack of environmental disturbance, the presence of individuals with extreme asymmetry can significantly skew the normal frequency distribution of FA. In this study, all samples were normally distributed, which makes increased mortality in sites with higher disturbance unlikely. However, differential mortality could also occur in earlier life stages (see for example Gagliano et al., 2008).

Small sample sizes can be translated into low statistical power (Delgado-Acevedo & Restrepo, 2008), and uneven sample size might have contributed to the differences found between *A. callidryas* and *D. ebraccatus*. Most ponds in disturbed areas were temporary, and migration to less disturbed areas after metamorphosis is possible. It is however unknown to what extent FA is retained between different stages in amphibians. Growth and metamorphosis influence adult size (Halliday & Verrell, 1988), whereas developmental stability is related to growth rate (Danzmann et al., 1986; McKenzie & O'Farrell, 1993). At a higher stability of development, FA will be lower (Palmer & Strobeck, 1992). In disturbed or high stress environments, metamorphosis and growth can be accelerated (Lowe et al., 2006; Crump, 1989), leading to both lower body size and FA (Møller & Manning, 2003).

Agalychnis callidryas was previously more abundant in undisturbed areas (Vogt, 1997), and local declines were reported (Urbina-Cardona & Reynoso, 2005;

Urbina-Cardona et al., 2006; Rodríguez & Pineda, 2009; Cabrera-Guzmán & Reynoso, 2012). In this work, sites with intermediate and high disturbance are located in deforested areas with practically no tree cover or ground vegetation above 2 m in height, vegetation features which are essential to the breeding habitat of *A. callidryas*. Also, illegal markets in México City are supplied with individuals from Los Tuxtlas (personal observation), which might put the remaining populations at greater risk.

Dendropsophus ebraccatus was found across the entire gradient of habitat disturbance, despite an influence on body size and condition. We do not know to what extent the morphological changes caused by habitat disturbance are adaptive, and lack data on population trends. Habitat disturbance can exert long-term pressure on a population's demography due to the continual loss of particular individuals through direct mortality (Dodd & Smith, 2003). We consider *D. ebraccatus* as a suitable indicator of environmental change (following Caro & O'Doherty, 1999).

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REFERENCES

- Alford, R.A., Bradfield, K. & Richards, S. (1999). Measuring and analyzing developmental instability as a tool for monitoring frog populations. Pp. 34–43. In: A. Campbell, editor. *Declines and disappearances of Australian frogs. Environment Australia, Canberra.*
- Ball, S.L. & Baker, R.L. (1996). Predator-induced life history changes: Antipredator behavior costs or facultative life history shifts? *Ecology* **77**, 1116–1124.
- Bechshoft, T.O., Riget, F.F., Wiig, O. & Sonne, C. (2008). Fluctuating asymmetry in metric traits: A practical example of calculating asymmetry, measurement error, and repeatability. *Annales Zoologici Fennici*, **45**, 32–38.
- Briggs, V.S. (2013). Do big dads make big babies? Paternal effects on larval performance in red-eyed treefrogs of Belize (*Agalychnis callidryas*, *A. moreletti*). *Herpetological Journal* **23**, 131–138.
- Buckley, C.R., Michael, S.F. & Irschick, D.J. (2005). Early hatching decreases jumping performance in a direct-developing frog, *Eleutherodactylus coqui*. *Functional Ecology* **19**, 67–72.

- Cabrera-Guzmán, E. & Reynoso, V.H. (2012). Amphibian and reptile communities of rainforest fragments: minimum patch size to support high richness and abundance. *Biodiversity Conservation* 21, 3267–3268.
- Camargo, J.L.C. & Kapos, V. (1995). Complex edge effects on soil moisture and microclimate in central Amazonian forest. *Journal of Tropical Ecology* 11, 205–221.
- Caro, T.M. & O’Doherty, G. (1999). On the use of surrogate species in conservation biology. *Conservation Biology* 13, 805–814.
- Cedeño-Vázquez, J.R., Calderón-Mandujano, R. & Pozo, C. (2006). Anfibios de la Región de Calakmul Campeche, México. México: CONABIO/ECOSUR/CONANP/PNUD-GEF/SHM A.C.
- Comisión Nacional de Áreas Naturales Protegidas & Secretaría del Medio Ambiente y Recursos Naturales (CONANP & SEMARNAT). (2006). Programa de Conservación y Manejo Reserva de la Biosfera Los Tuxtlas. México: *Comisión de Áreas Naturales Protegidas/Secretaría del Medio Ambiente y Recursos Naturales*.
- Crump, M.L. (1989). Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. *Copeia* 1989, 794–797.
- Danzmann, R.G., Ferguson M.M., Allendorf, F.W. & Knudsen, K.L. (1986). Heterozygosity and developmental rate in a strain of rainbow trout (*Salmo gairdneri*). *Evolution* 40, 86–93
- Delgado-Acevedo, J. & Restrepo, C. (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.
- Dirzo, R. & García, M.C. (1992). Rates of deforestation in Los Tuxtlas and neotropical area in south east México. *Conservation Biology* 6, 84–90.
- Dodd C.K Jr. & Smith L.L. (2003). Habitat destruction and alteration. In: Semlitsch, R.D (ed.). *Amphibian Conservation*, pp. 94–110. R. Smithsonian Institution, Washington, D.C.
- Donnelly, M.A & Guyer, C. (1994). Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* 98, 291–302.
- Duellman, W.E. (1970). The hylid frogs of Middle America. *Monograph of the Museum of Natural History, the University of Kansas* 1970, 1–753.
- Frías-Álvarez P., Zúñiga-Vega, J J. & Flores-Villela, O. (2010). A general assessment of the conservation status and decline trends of Mexican amphibians. *Biodiversity and Conservation* 19, 3699–3742.
- Floate, K D. & Fox, A.S. (2000) Flies under stress: A test of fluctuating asymmetry as a biomonitor of environmental quality. *Ecological Applications* 10, 1541–1550.
- Gagliano, M., Depczynski, M., Simpson S.D., & Moore, J.A. (2008). Dispersal without errors: symmetrical ears tune into the right frequency for survival. *Proceedings of the Royal Society Biological Sciences* 275, 527–34.
- Guevara, S.S., Laborde, D.J. & Sánchez-Ríos, G. (2004). La Deforestación. In *Los Tuxtlas. El Paisaje de la Sierra*, pp. 85–110. Guevara, S.S., Laborde D.J. & Sánchez-Ríos, G. (eds.). México: *Instituto de Ecología A. C.-Unión Europea*.
- Halliday, T.R. & Verrell, P.A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* 22, 253–265
- Henle, K., Lindenmayer D.B., Margules, C.R., Saunders, D.A. & Wissel, C. (2004a). Species survival in fragmented landscapes: where are we now? *Biodiversity and Conservation* 13, 1–8.
- Henle, K., Davies, K.F., Kleyer, M., Margules, C. & Settele, J. (2004b). Predictors of species sensitivity to fragmentation. *Biodiversity Conservation* 13, 207–251.
- Henríquez, P., Donoso, D.S. & Grez, A.A. (2009). Population density, sex ratio, body size and fluctuating asymmetry of *Ceroglossus chilensis* (Carabidae) in the fragmented Maulino forest and surrounding pine plantations. *Acta Oecologica* 35, 811–818.
- Hoffmann, A.A. & Woods R.E. (2003). Associating environmental stress with developmental stability: problems and patterns. In: *Developmental Instability. Causes and Consequences*. 387–401. Polak, M. (ed.). New York: OXFORD
- Hopton M.E., Cameron G.N., Cramer, M.J., Polak, M. & Uetz G.W. (2009). Live animal radiography to measure developmental instability in populations of small mammals after a natural disaster. *Ecological Indicators* 9, 883–891.
- Iturra-Cid, M., Ortiz, J.C., & Ibarguengoytia, N.R. (2010). Age, size, and growth of the Chilean frog *Pleurodema thaul* (Anura: Leiuperidae): latitudinal and altitudinal effects. *Copeia* 2010, 609–617.
- Jessop, T.M., Madsen, T., Sumner, J., Rudiharto, H., et al. (2006). Maximum body Size among insular Komodo dragon populations covaries with large prey density. *Oikos* 112, 422–429.
- Jungfer, K.H., Lynch, J., Morales, M., Solís, F., et al. (2008). *Dendropsophus ebraccatus*. In: IUCN 2008. *2008 IUCN Red List of Threatened Species*. Available from: <<http://www.iucnredlist.org>>.
- Labrie, G., Prince, C., Bergeron, J.M. (2003). Abundance and developmental stability of *Pterostichus melanarius* (Coleoptera, Carabidae) in organic and integrated pest management orchards of Quebec, Canada. *Environmental Entomology* 32, 123–132.
- Lauk, B. (2006). Fluctuating asymmetry of the frog *Crinia signifera* in response to logging. *Wildlife Research* 33, 313–320.
- Lens, L., Van Dongen, S., Kark, S. & Matthysen, E. (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.
- Lips, K. (1999). Mass mortality and population decline of anurans at an upland site in western Panama. *Conservation Biology* 13, 117–125.
- Lowe, W.H., Likens, G.E., & Cosentino, B.J. (2006). Self-organization in streams: The relationship between movement behaviour and body condition in a headwater salamander *Freshwater Biology* 51, 2052–2062
- MacCracken, J.G. & Stebbings, J.L. (2012). Test of a body condition index with amphibians. *Journal of Herpetology* 46, 346–350.
- McKenzie, J.A. & O’Farrell, K. (1993). Modification of developmental instability and fitness: malathion resistance in the Australian sheep blowfly, *Lucilia cuprina*. *Genetica* 89, 67–76.
- Martín, J. & López, P. (1998). Shifts in microhabitat use by the lizard *Psammotromus algirus*: responses to seasonal changes in vegetation structure. *Copeia* 1998, 780–786.

- Møller, A.P. (1997). Development stability and fitness: a review. *American Naturalist* 149, 916–932.
- Møller, A.P. & Manning, J. (2003). Growth and developmental instability. *The Veterinary Journal* 166, 19–27.
- Neckel-Oliveira S. & Gascón C. (2006). Abundance, body size and movement patterns of a tropical treefrog in continuous and fragmented forests in the Brazilian Amazon. *Biological Conservation* 28, 308–315.
- Ormerod, S.J. & Tyler, S.J. (1990). Assessments of body condition in dippers *Cinclus cinclus*: potential pitfalls in the derivation and use of condition indices based on body proportions. *Ringing & Migration* 11, 31–41.
- Palkovacs, E.P. (2003). Explaining adaptive shifts in body size on islands: a life history approach. *Oikos* 103, 37–44
- Palmer, A.R. (1994). Fluctuating asymmetry analyses: a primer. In: *Developmental Instability: its origin and Evolutionary Implications*. pp 335–364. Markow, T.A. (ed.). *Dordrecht The Netherlands: Kluwer Academic*.
- Palmer, A.R. & Strobeck, C. (1992). Fluctuating asymmetry as a measure of developmental stability: Implications of non normal distributions and power of statistical tests. *Acta Zoologica Fennica* 191, 57–72.
- Palmer, A.R. & Strobeck, C. (2003). Fluctuating asymmetry analyses revisited. In: *Developmental Instability. Causes and Consequences*. Pp. 279–319. Polak, M. (ed.). *New York: Oxford*.
- Parris, M.J. & Cornelius, T.O. (2004). Fungal pathogen causes competitive and developmental stress in larval amphibian communities. *Ecology* 85, 3385–3395
- Peig, J. & Green, A.J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118, 1883–1891.
- Peig, J. & Green A.J. (2010). The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology* 24, 1323–1332
- Plăiașu, R., Vörös, J. & Băncilă, R. (2012). Fluctuating asymmetry as a tool in identifying population stress in Hungarian populations of *Bombina bombina*, *B. variegata* and their hybrids. *Acta Zoologica Academiae Scientiarum Hungaricae* 58, 361–368.
- Polak M. (2003). *Developmental Instability. Causes and Consequences*. New York: Oxford Univ. Press
- Reading, C.J. & Clarke, R.T. (1995). The effects of density, rainfall and environmental temperature on body condition and fecundity in the common toad, *Bufo bufo*. *Oecologia* 102, 453–459.
- Rodríguez-Mendoza, C. & Pineda, E. (2010). Importance of riparian remnants for frog species diversity in a highly fragmented rainforest. *Biology Letters* 6, 781–784.
- Sarre, F. (1996). Habitat fragmentation promotes fluctuating asymmetry but not morphological divergence in two geckos. *Researches on Population Ecology* 38, 57–64.
- Semlitsch, R.D. (2008). Differentiating migration and dispersal processes for pond-breeding amphibians. *The Journal of Wildlife Management* 72, 260–267.
- Solís F., Ibáñez, R., Santos-Barrera, G., Jungfer, K.H., et al. (2008). *Agalychnis callidryas*. In: IUCN 2008. *2008 IUCN Red List of Threatened Species*. Available from: <<http://www.iucnredlist.org>>.
- Söderman F.S., Van Dongen, S., Pakkasnaa, S. & Merilä, J. (2007). Environmental stress increases skeletal fluctuating asymmetry in the moor frog *Rana arvalis*. *Oecologia* 151, 593–604.
- StatSoft, Inc. (2004). STATISTICA (data analysis software system), version 7. Available from: <<http://www.statsoft.com>>.
- Sumner, J., Moritz, C. & Shine, R. (1999). Shrinking forest shrinks skink: morphological change in response to rainforest fragmentation in the prickly forest skink (*Gnypetoscincus queenslandiae*). *Biological Conservation* 91, 159–167.
- The R Foundation for Statistical Computing. (2011). R version 2.13.1 Copyright (C). ISBN 3-900051-07-0. ISBN 3-900051-07-0.
- Touchon, J.C., McCoy, M.W., Vonesh, J.R. & Warkentin, K.M. (2013) Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. *Ecology* 94, 850–860.
- Urbina-Cardona, J.N. & Reynoso, V.H. (2005). Recambio de anfibios y reptiles en el gradiente potrero-borde-interior en Los Tuxtlas, Veracruz, México. In *Sobre Diversidad Biológica: El significado de las Diversidades Alfa, Beta y Gamma*. 191–207. Halffter, G., Soberón, J., Koleff, P. & Melic, A. (eds.). México: CONABIO/SEA/CONACYT/ DIVERSITAS.
- Urbina-Cardona, J.N. Olivares-Pérez M. & Reynoso, V.H. (2006). Herpetofauna diversity and microenvironment correlates across a pasture–edge–interior ecotone in tropical rainforest fragments in the Los Tuxtlas Biosphere Reserve of Veracruz, Mexico. *Biological Conservation* 32, 61–75.
- VanderWerf, E.A. (2004). Demography of Hawai'i 'elepaio: variation with habitat disturbance and population density. *Ecology* 85:770–783
- Vishalakshi, C. & Singh, B.N. (2008). Effect of developmental temperature stress on fluctuating asymmetry in certain morphological traits in *Drosophila ananassae*. *Journal of Thermal Biology* 33, 201–208.
- Vogt, R.C. (1997). Las ranas de Laguna del Zacatal. In *Historia Natural de Los Tuxtlas*, 500–503. González-Soriano E., Dirzo, R. & Vogt, R.C. (eds.). México: UNAM.
- Warkentin, K.M. (1995). Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences* 92, 3507–3510.
- Warkentin, K.M. (2000). Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Animal Behaviour* 60, 503–510.
- 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, 129–133.

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