Morphophysiological traits of an amphibian exposed to historical industrial pollution in a Brazilian biodiversity hotspot

Erika Santana[1*,], Luis Schiesari[1,2], Fernando Gomes[3], Marcio Martins[1]

1 - Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo (USP), 05508-090, São Paulo, São Paulo, Brazil

2 - Gestão Ambiental, Escola de Artes, Ciências e Humanidades, Universidade de São Paulo (USP), 03828-000, São Paulo, São Paulo, Brazil

3 - Departamento de Fisiologia Geral, Instituto de Biociências, Universidade de São Paulo (USP), 05508-090, São Paulo, São Paulo, Brazil

[*]Corresponding author; e-mail: erika.ms@gmail.com

Supplementary Material

Additional results

We present data of body condition index of *Rhinella ornata* individuals sampled (fig. S1). We also present the results of full models' average when more than one model presented dAIC < 2.0 in the model selections conduced to analyze the effect of our predictors on our dependent variables (tables S1, S4 and S9) and the estimates resulted from the best fitted model for all analysis (tables S2, S5, S7 and S10). Additionally, we present the results of post-hoc pairwise analysis made with *pairs* function, with the marginal means estimated with *emmeans* functions for the best fitted model, in which

we compared the data collected in each site (*emmeans* package, Lenth et al., 2021; R version 1.5.4.) (tables S3, S6, S8 and S11). Finally, we present the results of the two model selection procedures conducted to assay the variation in the proportion of eosinophil counts of *Rhinella ornata* individuals, with (table S12) and without (table S13) an outlier.
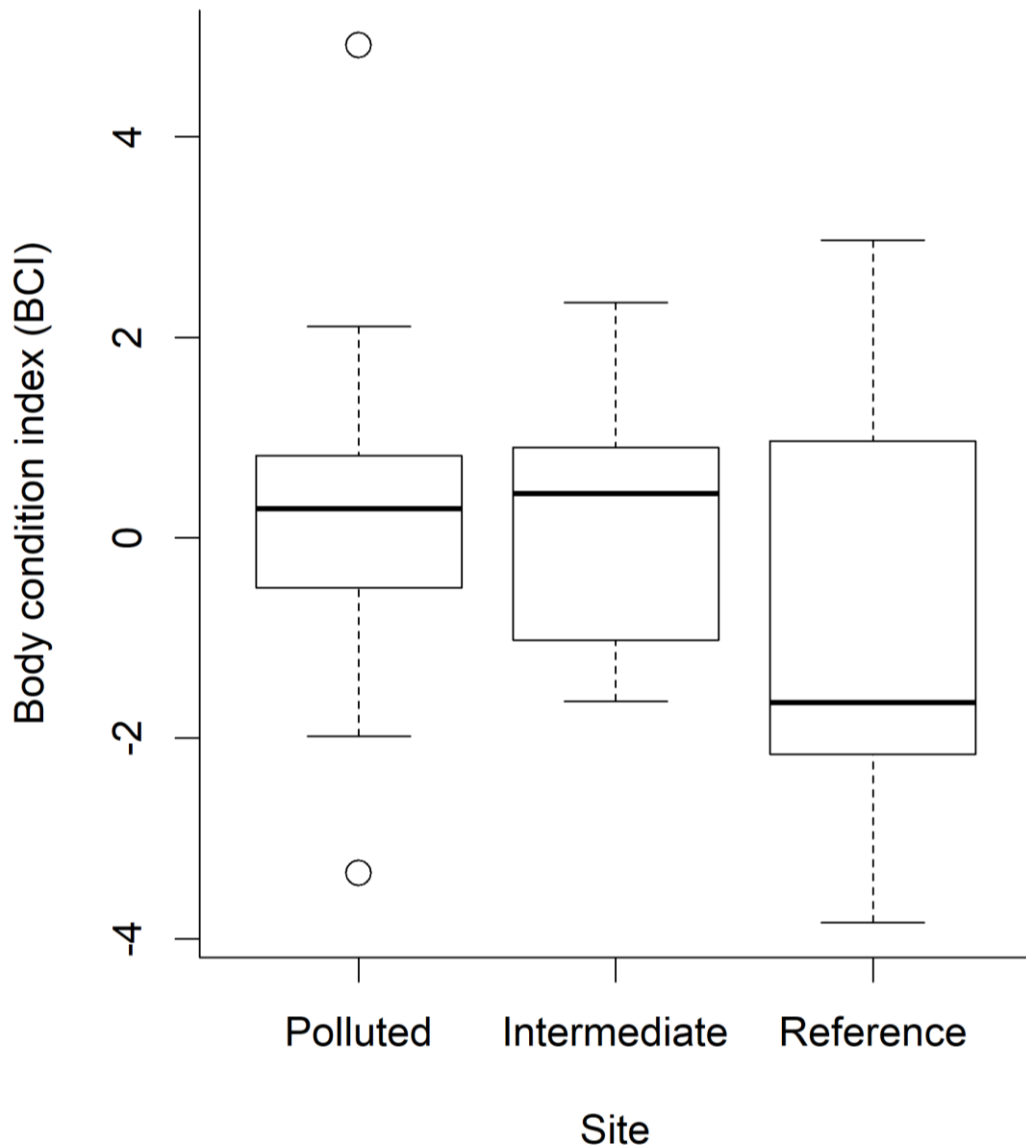
Figure S1. Body condition index (BCI) of *Rhinella ornata* populations at increasing distances from the Cubatão Industrial Complex. Polluted site n = 18, mean = 0.256, SD = 1.765; intermediate site n = 14, mean = 0.238, SD = 1.272; reference site n = 11, mean = -0.722, SD = 2.341. Boxes indicate the interval between first and third quartiles, central lines indicate the median, and whiskers indicate 1.5 times the value of the quartiles

Table S1. Full model average analysis of models with dAIC < 2.0 demonstrating the effect of body condition index (BCI) and site on organ-somatic indices of **liver** of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each predictor, the standard error (SE), values of *z* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*z*-value	CI (lower; upper)
Intercept (Polluted site)	0.0211	0.0008	24.4670	**0.0194; 0.0228**
BCI	0.0017	0.0008	2.2070	**0.0002; 0.0032**
Intermediate site	-0.0041	0.0013	3.1770	**-0.0067; -0.0016**
Reference site	-0.0051	0.0014	3.5560	**-0.0079; -0.0023**
BCI * Intermediate site	-0.0001	0.0008	0.1300	-0.0038; 0.0028
BCI * Reference site	-0.0005	0.0010	0.4420	-0.0044; 0.0005

Table S2. Results of the model with the best fit in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of liver of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each predictor, the standard error (SE), values of *t* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*t*-value	CI (lower; upper)
Intercept (Polluted site)	0.0212	0.0008	25.3250	**0.0195; 0.0228**
BCI	0.0015	0.0006	2.6510	**0.0004; 0.0026**
Intermediate site	-0.0041	0.0013	-3.2970	**-0.0067; -0.0016**
Reference site	-0.0050	0.0014	-3.6360	**-0.0079; -0.0022**

Table S3. Analysis of post-hoc pairwise contrasts made with the marginal means estimated for the best fitted model in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of liver of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each contrast, the standard error (SE), degrees of freedom (df) and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Contrast	Estimate	SE	df	CI (lower; upper)
Polluted × Intermediate sites	0.0041	0.0013	39	**0.0011; 0.0072**
Polluted × Reference sites	0.0050	0.0014	39	**0.0017; 0.0084**
Intermediate × Reference sites	0.0009	0.0015	39	-0.0027; 0.0044

Table S4. Full model average analysis of models with dAIC < 2.0 demonstrating the effect of body condition index (BCI) and site on organ-somatic indices of kidneys of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each predictor, the standard error (SE), values of z and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	z-value	CI (lower; upper)
Intercept (Polluted site)	0.0044	0.0001	28.3390	**0.0041; 0.0047**
Intermediate site	-0.0008	0.0002	3.2610	**-0.0012; -0.0003**
Reference site	-0.0010	0.0002	4.0100	**-0.0015; -0.0005**
BCI	0.0000	0.0001	0.3260	-0.0004; 0.0002
BCI * Intermediate site	0.0000	0.0001	0.2310	-0.0003; 0.0009
BCI * Reference site	0.0000	0.0001	0.2780	< -0.0001; 0.0008

Table S5. Results of the model with the best fit in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of kidneys of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each predictor, the standard error (SE), values of *t* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*t*-value	CI (lower; upper)
Intercept (Polluted site)	0.0044	0.0001	29.3700	**0.0041; 0.0047**
Intermediate site	-0.0008	0.0002	-3.3600	**-0.0012; -0.0003**
Reference site	-0.0010	0.0002	-4.1700	**-0.0015; -0.0005**

Table S6. Analysis of post-hoc pairwise contrasts made with the marginal means estimated for the best fitted model in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of kidneys of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each contrast, the standard error (SE), degrees of freedom (df) and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Contrast	Estimate	SE	df	CI (lower; upper)
Polluted × Intermediate sites	0.0008	0.0002	40	**0.0002; 0.0013**
Polluted × Reference sites	0.0010	0.0002	40	**0.0004; 0.0016**
Intermediate × Reference sites	0.0003	0.0003	40	-0.0004; 0.0009

Table S7. Results of the only model with dAIC < 2.0 in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of spleen of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each predictor, the standard error (SE), values of *t* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*t*-value	CI (lower; upper)
Intercept (Polluted site)	0.0010	0.0001	13.4670	**0.0009; 0.0012**
BCI	-0.0003	0.0001	-3.9600	**-0.0005; -0.0002**
Intermediate site	-0.0003	0.0001	-2.7090	**-0.0005; -0.00008**
Reference site	-0.0004	0.0001	-3.3280	**-0.0007; -0.0002**
BCI * Intermediate site	0.0004	0.0001	2.3920	**0.0001; 0.0006**
BCI * Reference site	0.0002	0.0001	2.2160	**<0.0001; 0.0005**

Table S8. Analysis of post-hoc pairwise contrasts made with the marginal means estimated for the best fitted model in model selection that tested the effect of body condition index (BCI) and site on organ-somatic indices of spleen of *Rhinella ornata* (see table 1 in main text). We present the estimated effects of each contrast, the standard error (SE), degrees of freedom (df) and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Contrast	Estimate	SE	df	CI (lower; upper)
Polluted × Intermediate sites	0.0003	0.0001	37	**<0.0001; 0.0006**
Polluted × Reference sites	0.0004	0.0001	37	**0.0001; 0.0007**
Intermediate × Reference sites	0.0001	0.0001	37	-0.0002; 0.0004

Table S9. Full model average analysis of models with dAIC < 2.0 demonstrating the effect of body condition index (BCI) and site on individual stress index (neutrophil / lymphocyte ratio, N/L) of *Rhinella ornata* (see table 2 in the main text). We present the estimated effects of each predictor, the standard error (SE), values of *z* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*z*-value	CI (lower; upper)
Intercept (Polluted site)	-2.4657	0.1124	20.9900	**-2.6959; -2.2354**
BCI	-0.0975	0.1346	0.7100	-0.4349; 0.1352
Intermediate site	0.9340	0.1469	6.0900	**0.6334; 1.2346**
Reference site	1.2803	0.1400	8.7570	**0.9937; 1.5668**
BCI * Intermediate site	0.0720	0.1869	0.3800	-0.0634; 0.9119
BCI * Reference site	0.0312	0.1081	0.2800	-0.2323; 0.5993

Table S10. Results of the model with the best fit in model selection that tested the effect of body condition index (BCI) and site on individual stress index (neutrophil / lymphocyte ratio, N/L) of *Rhinella ornata* (see table 2 in the main text). We present the estimated effects of each predictor, the standard error (SE), values of *t* and confidence intervals (95%). Confidence intervals that do not include zero are bold.

Predictors	Estimate	SE	*z*-value	CI (lower; upper)
Intercept (Polluted site)	-2.4642	0.1123	-21.9380	**-2.6915; -2.2506**
BCI	-0.0961	0.0535	-1.7960	-0.2018; 0.0081
Intermediate site	0.9554	0.1437	6.6510	**0.6765; 1.2402**
Reference site	1.2671	0.1390	9.1130	**0.9979; 1.5435**

Table S11. Analysis of post-hoc pairwise contrasts made with the marginal means estimated for the best fitted model in model selection that tested the effect of body condition index (BCI) and site on individual stress index (neutrophil / lymphocyte ratio, N/L) of *Rhinella ornata* (see table 2 in the main text). We present the estimated effects of each contrast, the standard error (SE), degrees of freedom (df) and confidence intervals (95%) set with asymptotic methods (see *emmeans* package documentation, Lenth et al., 2021; R version 1.5.4.). Confidence intervals that do not include zero are bold.

Contrast	Estimate	SE	df	CI (lower; upper)
Polluted × Intermediate sites	-0.955	0.144	Inf	**-1.292; -0.619**
Polluted × Reference sites	-1.267	0.139	Inf	**-1.593; -0.941**
Intermediate × Reference sites	-0.312	0.124	Inf	**-0.603; -0.020**

Table S12. Model selection approach assessing the effect of body condition index (BCI), total parasite load (P) and site on the proportion of eosinophils (E) of *Rhinella ornata*. The values of the Akaike's Information Criterion corrected for small sample sizes (AICc), the likelihood delta AICc (dAICc), the degrees of freedom (df) and the weight of evidence (Weight) of the proposed models are presented. Bold numbers are used to highlight the supported models.

Models	AICc	dAICc	df	Weight
E ~ 1	181.9	5.7	1	0.039
E ~ Site	179.9	3.6	3	0.110
E ~ P	182.1	5.9	2	0.035
E ~ BCI	181.8	5.5	2	0.042
E ~ P + Site	181.8	5.5	4	0.042
E ~ BCI + Site	**176.3**	**0.0**	**4**	**0.668**
E ~ P * Site	184.2	7.9	6	0.013
E ~ BCI * Site	181.4	5.1	6	0.051

Table S13. Model selection approach assessing the effect of body condition index (BCI), total parasite load (P) and site on the proportion of eosinophils (E) of *Rhinella ornata* (data without individual 39, an outlier). The values of the Akaike's Information Criterion corrected for small sample sizes (AICc), the likelihood delta AICc (dAICc), the degrees of freedom (df) and the weight of evidence (Weight) of the proposed models are presented. Bold numbers are used to highlight the supported models.

Models	AICc	dAICc	df	Weight
E ~ 1	169	2.3	1	0.0954
E ~ Site	170.5	3.8	3	0.0451
E ~ P	**166.7**	**0**	**2**	**0.3069**
E ~ BCI	**167.9**	**1.2**	**2**	**0.1666**
E ~ P + Site	170.4	3.7	4	0.0489
E ~ BCI + Site	**166.7**	**0**	**4**	**0.3037**
E ~ P * Site	174.2	7.5	6	0.0071
E ~ BCI * Site	171.6	4.9	6	0.0263

Reference

Lenth, RV., Buerkner, P., Herve, M., Love, J., Riebls, H., Singmann, H. (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means. Version 1.5.4.