

Biochemical and molecular biomarkers and their association with anthropogenic chemicals in wintering Manx shearwaters (*Puffinus puffinus*)

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Supplementary files

Table S1

Primers used for qPCR biomarkers genes of Manx shearwaters (*Puffinus puffinus*) listed in Table 1.

Gene identification and symbol	Primer sequence	Tm*	%GC**
<i>Aryl hydrocarbon receptor (AhR)</i>	F: GTCCGAATGATGAAGACAGGTGAGAGTG	60	50
	R: GGTCTTTGCGTGGCAATGATGTAATCC	60	48.1
<i>Cytochrome P450 1A-5 (CYP1A5)</i>	F: ACAGTTTGGTGATGTGGCTGCTG	59.9	52.2
	R: TCTGTAGGAAATGGAGGAATCGCTTGTTG	59.9	44.8
<i>Estrogen receptor alpha 1 (ESR1)</i>	F: GCAGAGGTGGGCGAATGATGAAAC	60.2	54.2
	R: ACAGGGCTGGACTGTTCTTCTTGTTATG	60.1	46.2
<i>Heat shock protein 70 kDa (HSP70)</i>	F: GTACGGCTTGTGAGAGGGCAAAG	59.9	56.5
	R: CGGGCACGAGTAATGGAAGTGTAGAA	59.9	50
<i>UDP-glucuronosyl-transferase (UGT1)</i>	F: CCACAGAATGATCTTCTAGCTCACCTAAG	59.3	46.7
	R: GCGTTGTCCATCTGGTCTCCAAATAAAG	59.3	46.4

* Tm = melting temperature, ** %GC = GC content.

Table S2

Matrix of association among variables related to Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil from 2016 to 2020. Linear regression was used to evaluate association between two quantitative variables (Day of the semester and Body mass). Kruskal-Wallis tests were (H) used to evaluate association between quantitative and categorical variables. Chi-Square tests and Cramer's V statistic (V) were used to evaluate association between two categorical variables.

Variable	Body mass	Region	Status	Sex	Age class	Gastrointestinal parasites	Kidney parasites	Plastics
Day of the semester	R = -0.263 $p < 0.001^{***}$	H = 5.634 df = 2 $p = 0.060$	H = 1.257 df = 1 $p = 0.262$	H = 0.367 df = 1 $p = 0.545$	H = 0.124 df = 1 $p = 0.211$	H = 0.124 df = 1 $p = 0.725$	H = 2.256 df = 1 $p = 0.133$	H = 3.555 df = 1 $p = 0.059$
Body mass		H = 2.001 df = 2 $p = 0.368$	H = 16.275 df = 1 $p < 0.001$	H = 1.757 df = 1 $p = 0.185$	H = 4.094 df = 1 $p = 0.043^*$	H = 0.241 df = 1 $p = 0.624$	H = 3.967 df = 1 $p = 0.046^*$	H = 0.033 df = 1 $p = 0.857$
Region			V = 0.261 $p = 0.005^{**}$	V = 0.035 $p = 0.909$	V = 0.186 $p = 0.068$	V = 0.316 $p < 0.001^*$	V = 0.129 $p = 0.275$	V = 0.212 $p = 0.031^*$
Status				V < 0.001 $p > 0.999$	V = 0.028 $p = 0.731$	V = 0.090 $p = 0.264$	V = 0.010 $p = 0.903$	V = 0.098 $p = 0.223$
Sex					V = 0.092 $p = 0.253$	V = 0.013 $p = 0.870$	V = 0.029 $p = 0.718$	V = 0.126 $p = 0.116$
Age class						V = 0.195 $p = 0.015^*$	V = 0.135 $p = 0.092$	V = 0.264 $p = 0.001^*$
Gastrointestinal parasites							V = 0.031 $p = 0.698$	V = 0.222 $p = 0.006^{**}$
Kidney parasites								V < 0.001 $p > 0.999$

Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table S3

Summary of General Additive Models (GAM) for the liver enzymatic activity of glutathione S-transferases (GST) and ethoxy-resorufin O-deethylase (EROD) in Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil. Coefficient estimates are provided for qualitative and non-smoothed quantitative variables. P-values (p) and significance codes are provided for all variables. The percentage of deviance explained are provided for each model.

	GST			EROD		
	Estimate	p	Signif.	Estimate	p	Signif.
Non-smoothed variables						
Region ("PR")	−0.076	0.325		0.046	0.813	
Region ("SP-RJ")	−0.001	0.989		0.124	0.449	
Status ("Beach carcass")	0.041	0.529		−0.283	0.092	(*)
Sex ("Female")	0.032	0.527		0.050	0.707	
Age class ("Adult")	−0.098	0.117		0.070	0.667	
Gastrointestinal parasites ("True")	0.033	0.576		−0.077	0.619	
Kidney parasites ("True")	0.028	0.597		0.151	0.271	
Plastics ("True")	−0.013	0.834		−0.200	0.216	
Year (integer)	0.052	0.046	*	0.242	0.001	***
Smoothed variables						
Day of the semester	1.505	0.196		0.802	0.001	**
Body mass	0.226	0.231		0.392	0.103	
PAHs	0.260	0.206		<0.001	0.918	
PCBs	0.483	0.149		<0.001	0.710	
HCB	<0.001	0.623		0.604	0.083	(*)
Drins	0.786	0.037	*	<0.001	0.410	
Mirex	1.898	0.027	*	<0.001	0.627	
Intercept	−105.320	0.044	*	−486.023	0.001	***
Deviance explained	14.0%			15.7%		

Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, (*) $p < 0.1$.

Table S4

Summary of General Additive Models (GAM) for the liver immunodetection level of the cytochrome P450 1A proteins (CYP1A) and the transcription levels of the *cytochrome P450 1A-5 (CYP1A5)* and *aryl hydrocarbon receptor (AhR)* genes in Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil. Coefficient estimates are provided for qualitative and non-smoothed quantitative variables. P-values (p) and significance codes are provided for all variables. The percentage of deviance explained are provided for each model.

	CYP1A			CYP1A5			AhR		
	Estimate	p	Signif.	Estimate	p	Signif.	Estimate	p	Signif.
Non-smoothed variables									
Region ("PR")	0.022	0.026	*	-0.497	0.021	*	-0.472	0.035	*
Region ("SP-RJ")	0.016	0.051	(*)	-0.348	0.054	(*)	-0.075	0.681	
Status ("Beach carcass")	0.009	0.312		0.308	0.096	(*)	-0.274	0.156	
Sex ("Female")	0.009	0.157		-0.196	0.172		0.064	0.665	
Age class ("Adult")	-0.002	0.800		-0.281	0.111		-0.217	0.229	
Gastrointestinal parasites ("True")	0.001	0.845		-0.108	0.519		-0.092	0.597	
Kidney parasites ("True")	0.007	0.319		-0.018	0.904		-0.293	0.056	(*)
Plastics ("True")	0.005	0.536		-0.111	0.523		0.296	0.099	(*)
Year (integer)	-0.003	0.351		-0.042	0.578		0.073	0.356	
Smoothed variables									
Day of the semester		0.011	*		0.001	**		0.198	
Body mass		0.151			0.136			0.124	
PAHs		0.310			0.149			0.082	(*)
PCBs		0.982			0.034	*		0.987	
HCB		0.243			0.001	**		0.257	
Drins		0.086	(*)		0.402			0.545	
Mirex		0.038	*		0.060	(*)		0.995	
Intercept	6.433	0.350		91.638	0.550		-141.750	0.377	
Deviance explained	18.2%			22.2%			17.4%		

Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, (*) $p < 0.1$.

Table S5

Summary of General Additive Models (GAM) for the liver transcription levels of the *estrogen receptor alpha 1* (*ESR1*), *heat shock protein 70* (*HSP70*) and *UDP-glucuronosyl-transferase* (*UGT1*) genes in Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil. Coefficient estimates are provided for qualitative and non-smoothed quantitative variables. P-values (p) and significance codes are provided for all variables. The percentage of deviance explained are provided for each model.

	<i>ESR1</i>			<i>HSP70</i>			<i>UGT1</i>		
	Estimate	p	Signif.	Estimate	p	Signif.	Estimate	p	Signif.
Non-smoothed variables									
Region ("PR")	-0.295	0.156		-0.169	0.643		-0.730	<0.001	***
Region ("SP-RJ")	-0.212	0.221		-0.147	0.627		-0.507	0.001	***
Status ("Beach carcass")	-0.012	0.945		0.239	0.453		0.224	0.147	
Sex ("Female")	-0.109	0.435		-0.324	0.184		-0.088	0.467	
Age class ("Adult")	-0.142	0.405		0.366	0.217		-0.375	0.012	*
Gastrointestinal parasites ("True")	-0.337	0.041	*	0.103	0.714		-0.225	0.114	
Kidney parasites ("True")	-0.317	0.031	*	0.032	0.899		-0.306	0.015	*
Plastics ("True")	0.089	0.599		-0.061	0.831		-0.189	0.193	
Year (integer)	-0.009	0.904		0.356	0.004	**	0.147	0.022	*
Smoothed variables									
Day of the semester		0.237			0.124			0.674	
Body mass		0.108			0.002	**		0.084	(*)
PAHs		0.831			0.157			0.953	
PCBs		0.225			0.156			0.003	**
HCB		0.048	*		0.944			0.001	***
Drins		0.404			0.502			0.517	
Mirex		0.037	*		0.876			0.669	
Intercept	21.245	0.885		-714.771	0.004	**	-289.254	0.025	*
Deviance explained	14.4%			36.7%			25.7%		

Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, (*) $p < 0.1$.

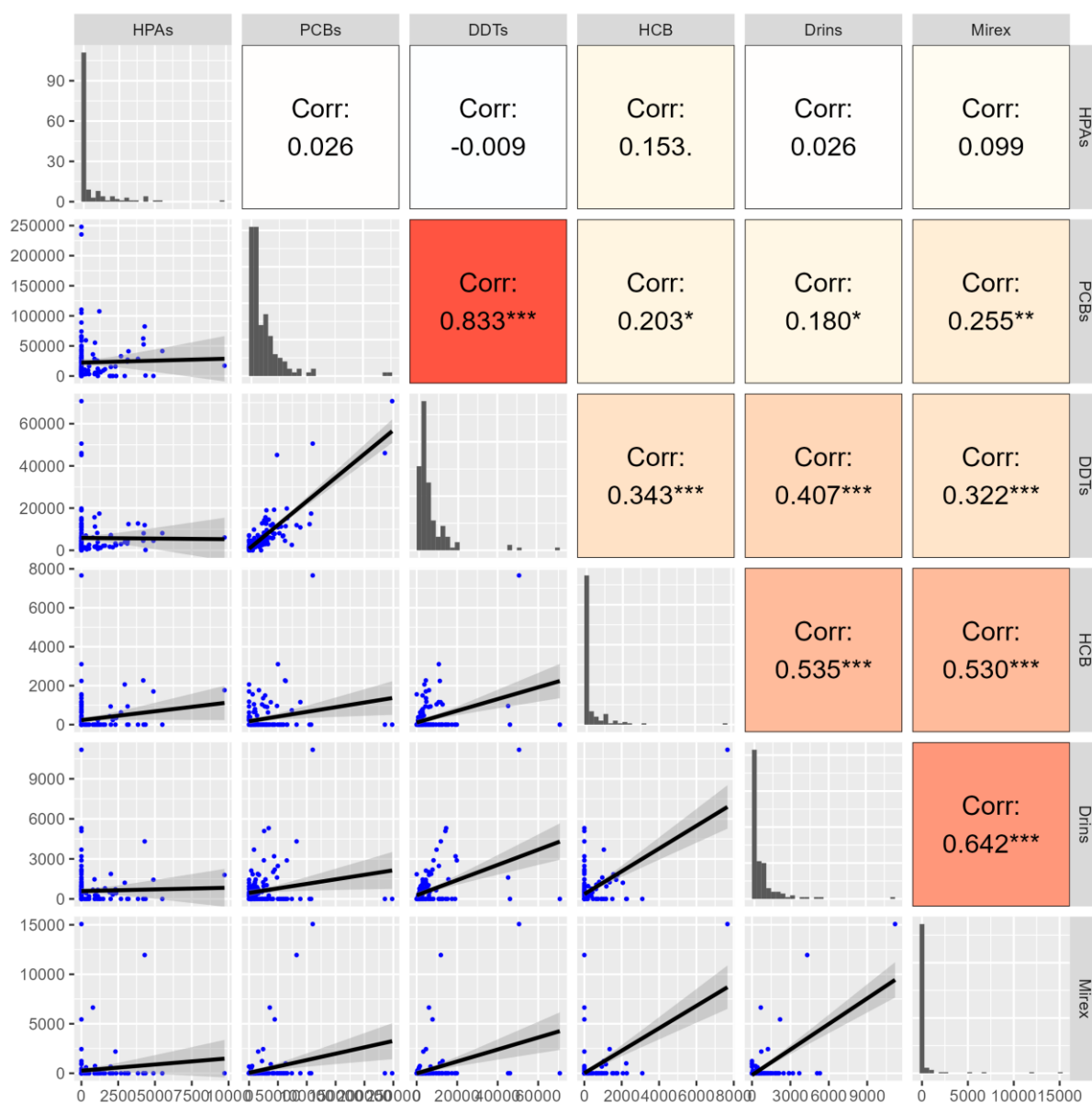


Fig. S1. Correlogram among pollutant concentrations in Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil. Diagonal plots represent the histogram of each variable. Bottom left plots represent scatter plots of each pair of variables (blue dots) as well as the corresponding linear regression (black line) and its 95% confidence interval (gray shaded area). Top right squares represent the Spearman's correlation coefficient, with background color indicating the strength of the correlation (white = 0, red = ± 1). Significance of the correlations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

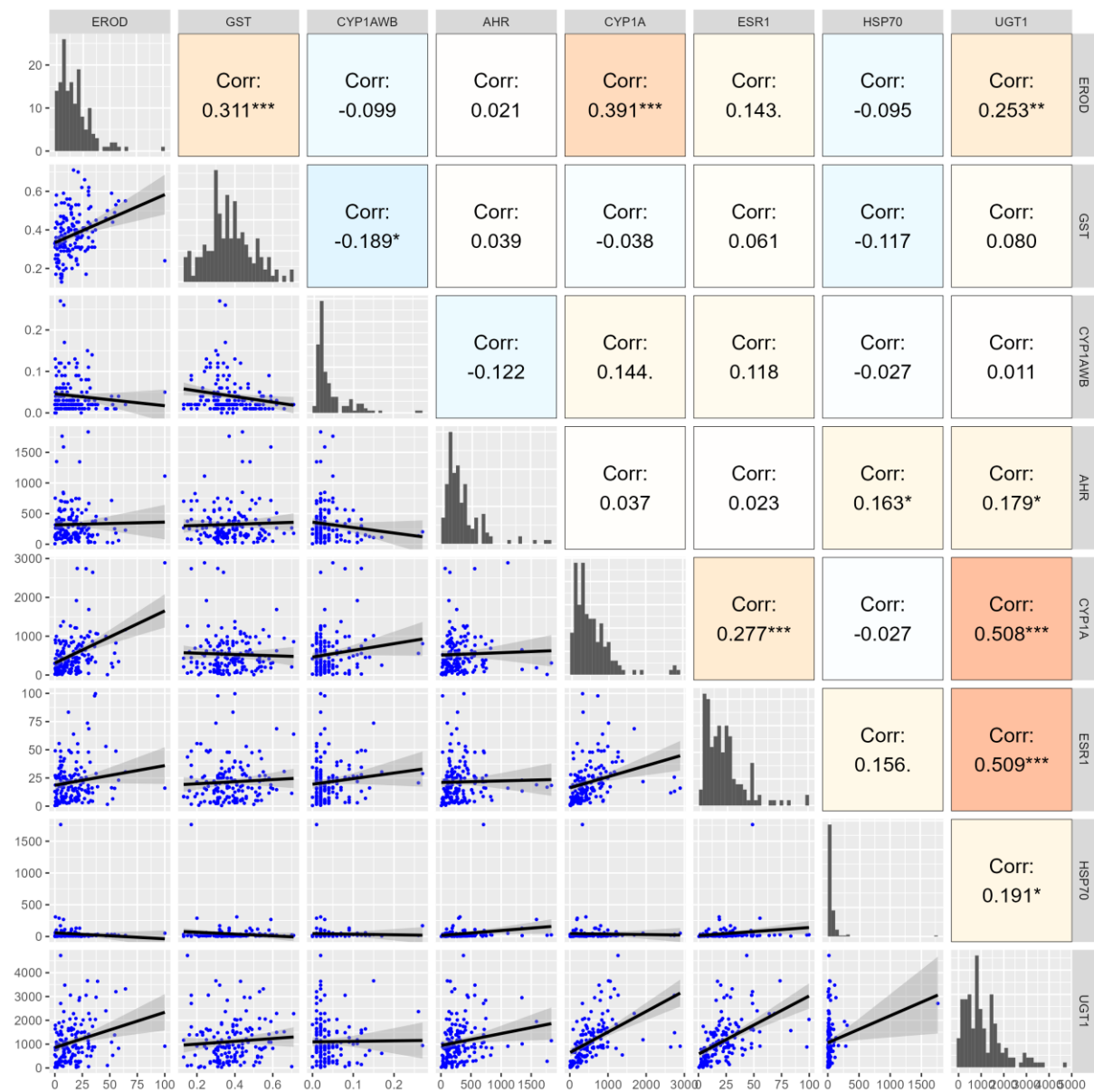


Fig. S2. Correlogram among biomarker results in Manx shearwaters (*Puffinus puffinus*) stranded along the southern and southeastern coast of Brazil. Diagonal plots represent the histogram of each variable. Bottom left plots represent scatter plots of each pair of variables (blue dots) as well as the corresponding linear regression (black line) and its 95% confidence interval (gray shaded area). Top right squares represent the Spearman's correlation coefficient, with background color indicating the strength of the correlation (white = 0, red = ± 1). Significance of the correlations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.