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Supplementary material

Oxidative stress parameters in two *Pelophylax esculentus* complex frogs during pre- and posthibernation period: Arousal vs. Heavy metals

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Table S1: Samples count for *Pelophylax esculentus* complex species for DTD canal and river Ponjavica by seasons and species with average biometric parameters (SVL- the length from the tip of the snout to the posterior end of the cloaca and BM- body mass)

Table S2: Results of determination of elements in the reference material TORT-2 (lobster hepatopancreas reference material for trace metals).

Differences between individuals from canal DTD and river Ponjavica in accumulated metals and AOS parameters (Tables S3-6)

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Table S1 Number of individuals (N), average body mass (BM) and snout-vent length (SVL) of the *Pelophylax ridibundus* and *Pelophylax esculentus* species from canal DTD and river Ponjavica during spring and autumn.

	<i>Pelophylax ridibundus</i>				<i>Pelophylax esculentus</i>			
	DTD		Ponjavica		DTD		Ponjavica	
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
N	10	10	9	10	10	9	10	9
SVL (mm)	92.7±4.6	88.6±3.1	83.9±3.8	72.5±2.8	84.0±3.2	80.6±3.0	87.4±5.1	75.0±2.3
BM (g)	63.7±3.8	62.5±3.2	58.0±4.2	37.7±3.4	57.9±3.7	52.0±4.1	67.1±5.4	39.5±3.6

Table S2 Results of determination of elements in the reference material TORT-2 (lobster hepatopancreas reference material for trace metals)

Element	TORT-2	
	Certified value±uncertainty* (mgBLDkg)	Found value±uncertainty (mgBLDkg)
As	21.6±1.8	21.0±0.9
Cd	26.7±0.6	26.65±0.05
Cr	0.77±0.15	0.8±0.2
Co	0.51±0.09	0.50±0.15
Cu	106±10	107.1±0.9
Ni	2.5±0.19	2.4±0.2
Pb	0.35±0.13	0.34±0.10
Zn	180±6	180.2±0.4
Fe	105±13	106.1±2.3
Sr	45.2±1.9	45.2±0.4
Mn	13.6±1.2	13.6±0.8
Hg	0.27±0.06	0.27±0.17

*Uncertainty for 95 % confidence level (coverage factor k = 2).

Differences between individuals from canal DTD and river Ponjavica in accumulated metals and AOS parameters

Table S3 Metal (As, Cd, Cr, Cu, Co, Fe, Hg, Ni, Pb and Zn) concentrations in skin and muscle tissues of *Pelophylax ridibundus* from DTD canal and river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between localities at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica
As	BLD	1.04±0.16	BLD	0.140±0.030	0.048±0.021	1.04±0.14	0.36 ±0.047	0.325 ±0.081
Cd	0.199±0.040	0.138±0.008	0.090±0.013	0.021±0.005	BLD	0.146±0.019	0.40±0.19	BLD
Cr	1.05±0.11	BLD	0.859±0.087	0.723±0.045	0.647±0.078	BLD	0.307±0.059	0.347 ±0.053
Cu	3.00±0.11	2.49±0.13	2.12±0.22	2.30±0.38	1.68±0.22	1.39±0.08	2.64±1.29	1.83±0.29
Co	0.029±0.013	BLD	BLD	0.101±0.071	0.157±0.023	BLD	BLD	BLD
Fe	41.3±3.2	40.4±4.8	49.8±4.7	86.3±19.0	31.7±10.6	23.4±1.9	26.9±6.4	14.26±2.21
Hg	0.064±0.008	BLD	BLD	0.030±0.006	0.138±0.016	BLD	0.080±0.017	0.046 ±0.008
Ni	1.09±0.27	0.188±0.062	0.77±0.19	1.00±0.21	0.422±0.065	0.39±0.12	0.32 ±0.04	2.35±0.71
Pb	1.47±0.39	0.406±0.198	0.417±0.117	BLD	0.294±0.076	BLD	BLD	BLD
Zn	160.2±15.8	164.9±18.7	188.4 ±21.9	179.1±19.0	28.1±3.3	21.2±1.2	21.5±1.9	19.4±2.7

Table S4 Metal (As, Cd, Cr, Cu, Co, Fe, Hg, Ni, Pb and Zn) concentrations in skin and muscle tissues of *Pelophylax esculentus* from DTD canal and river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between localities at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica
As	BLD	BLD	BLD	BLD	BLD	BLD	BLD	0.286±0.083
Cd	0.073±0.009	0.076±0.015	0.09±0.02	0.019±0.004	0.0335±0.007	0.027±0.008	0.033±0.010	0.147±0.057
Cr	0.880±0.123	0.523±0.042	1.74±0.40	2.21±0.63	0.45±0.07	0.32±0.03	1.11±0.33	1.05±0.16
Cu	2.28±0.16	2.07±0.13	3.67±1.55	2.49±0.26	1.20±0.14	0.97±0.05	1.39±0.15	1.20±0.15
Co	BLD	BLD	BLD	0.14±0.06	BLD	BLD	BLD	0.215±0.091
Fe	33.2±1.9	24.5±1.5	31.69±4.10	68.5±13.1	12.5±0.8	13.3±1.5	20.45±2.85	42.71±15.27
Hg	0.071±0.017	0.124±0.061	0.13±0.02	0.122±0.027	0.06±0.01	0.12±0.02	0.102±0.028	0.136±0.041
Ni	0.25±0.06	0.18±0.04	1.69±0.66	1.42±0.34	0.23±0.04	0.19±0.06	0.49±0.08	0.73±0.15
Pb	BLD	0.197±0.087	BLD	BLD	0.30±0.04	BLD	BLD	BLD
Zn	182.0±23.4	146.3±19.3	149.9±25.2	183.6±16.9	43.9±22.7	19.2±0.8	25.25±3.22	25.93±1.84

Table S5 Antioxidative defense system parameters in skin and muscle tissues of *Pelophylax ridibundus* from DTD canal and river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between localities at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica
SOD	3.8±0.2	3.7±0.4	3.8±0.2	6.8±0.4	2.3±0.2	2.2±0.1	5.1±0.5	8.6±0.9
CAT	7.5±0.5	5.5±0.5	7.6±0.4	8.8±0.4	7.9±1.1	2.2±0.4	6.9±0.8	8.1±0.8
GSH-Px	10.5±0.8	11.03±0.98	7.5±0.7	5.5±0.4	4.5±0.3	5.0±0.3	6.8±0.3	8.7±0.7
GR	4.32±0.27	3.64±0.24	8.18±0.47	9.71±0.82	0.86±0.10	0.80±0.05	2.0±0.2	2.3±0.2
GST	73.4±4.2	72.3±3.7	118.2±6.0	132.0±9.7	26.5±1.1	91.4±7.8	92.6±8.6	87.7±7.9
GSH	46.9±2.5	45.2±5.6	129.2±11.7	128.1±14.3	157.3±17.9	152.2±6.3	52.8±4.1	105.7±3.2
SH	222.7±8.1	227.3±14.3	822.0±2.6	674.4±17.3	271.0±1.2	273.1±1.4	649.2±16.6	563.1±22.1

Table S6 Antioxidative defense system parameters in skin and muscle tissues of *Pelophylax esculentus* from DTD canal and river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between localities at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica	DTD	Ponjavica
SOD	4.21±0.24	2.98±0.36	2.33±0.23	6.65±0.53	3.66±0.25	2.71±0.22	4.83±0.35	7.19±0.43
CAT	5.9±0.4	6.3±0.9	5.62±0.42	5.91±0.50	2.55±0.18	2.08±0.33	7.28±1.25	12.40±2.72
GSH-Px	5.7±0.6	11.5 ±1.3	5.25±0.34	6.01±0.41	4.95±0.45	4.91±0.45	4.95±0.45	11.25±1.55
GR	5.0±0.3	3.3±0.2	7.01±0.48	7.93±0.61	0.75±0.11	0.74±0.04	1.28±0.08	3.08±0.38
GST	74.4±4.9	63.8±5.8	94.0±4.8	96.0±6.8	58.4±3.3	98.6±7.99	60.8±3.6	98.6±8.9
GSH	53.7±8.8	49.2±5.4	116.5±12.4	227.5±16.8	189.1±16.1	119.8±11.6	58.0±6.0	94.3±8.5
SH	245.1±8.1	249.6±5.5	622.4±21.0	565.7±29.8	300.2±2.0	307.0±1.3	602.1±12.1	542.7±24.1

Interspecific defences in accumulated metals and AOS parameters

Table S7 Metal (As, Cd, Cr, Cu, Co, Fe, Hg, Ni, Pb and Zn) concentrations in skin and muscle tissues of *Pelophylax ridibundus* and *Pelophylax esculentus* from river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between species at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>
As	1.04±0.16	BLD	0.140±0.030	BLD	1.01±0.143	BLD	0.325±0.081	0.286±0.083
Cd	0.138±0.008	0.076±0.01	0.021±0.005	0.019±0.004	0.146±0.019	0.027±0.008	BLD	0.147±0.057
Cr	BLD	0.523±0.042	0.723±0.045	2.2±0.63	BLD	0.320±0.029	0.347±0.053	1.05±0.16
Cu	2.49±0.13	2.07±0.131	2.30±0.38	2.49±0.26	1.39±0.085	0.970±0.057	1.83±0.29	1.20±0.15
Co	BLD	BLD	0.101±0.071	0.145±0.061	BLD	BLD	BLD	0.215±0.091
Fe	40.4±4.8	24.4±1.5	86.3±19.1	68.5±13.1	23.4±1.9	13.3±1.5	14.2±2.2	42.7±15.2
Hg	BLD	0.124±0.061	0.030±0.006	0.122±0.027	BLD	0.121±0.024	0.046±0.008	0.136±0.041
Ni	0.188±0.062	0.179±0.035	1.00±0.21	1.42±0.34	0.397±0.126	0.187±0.066	2.35±0.711	0.725±0.147
Pb	0.406±0.198	0.197±0.087	BLD	BLD	BLD	BLD	BLD	2.75±0.85
Zn	164.9±18.7	146.2±19.3	179.1±19.0	183.6±16.9	21.2±1.2	19.1±0.7	19.3±2.6	25.9±1.8

BLD- below the limit of detection. Limits of detection As (). Cd (<0.002). Cr (<0.10). Co (<0.020). Hg (<0.010). Ni (<0.040). Pb (<0.020).

Table S8 Metal (As, Cd, Cr, Cu, Co, Fe, Hg, Ni, Pb and Zn) concentrations in skin and muscle tissues of *Pelophylax ridibundus* and *Pelophylax esculentus* from DTD canal during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between species at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>
As	BLD	BLD	BLD	BLD	0.048±0.010	BLD	0.361±0.047	BLD
Cd	0.20±0.04	0.073±0.009	0.090±0.013	0.094±0.027	BLD	0.034±0.007	0.402±0.190	0.033±0.010
Cr	1.05±0.12	0.880±0.123	0.859±0.087	1.74±0.40	0.647±0.078	0.446±0.070	0.307±0.059	1.11±0.33
Cu	3.00±0.12	2.28±0.16	2.12±0.22	3.67±1.55	1.68±0.23	1.20±0.14	2.64±1.29	1.39±0.15
Co	0.029±0.013	BLD	BLD	BLD	0.157±0.023	BLD	BLD	BLD
Fe	41.3±3.2	33.2±1.9	49.8±4.7	31.6±4.1	31.7±10.6	12.4±0.7	26.9±6.4	20.4±2.8
Hg	0.064±0.008	0.071±0.017	BLD	0.137±0.028	0.138±0.016	0.057±0.010	0.080±0.017	0.102±0.028
Ni	1.09±0.27	0.258±0.066	0.775±0.191	1.69±0.66	0.422±0.065	0.231±0.044	0.323±0.040	0.485±0.083
Pb	1.47±0.39	BLD	0.417±0.117	BLD	0.294±0.076	0.300±0.044	BLD	BLD
Zn	160.2±15.8	182.0 ±23.5	188.4±21.9	149.9±25.2	28.14±3.31	43.87±22.74	21.5±1.9	25.2±3.2

Table S9 Antioxidative defense system parameters in skin and muscle tissues of *Pelophylax ridibundus* and *Pelophylax esculentus* from DTD canal during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between species at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>	<i>P. ridibundus</i>	<i>P. esculentus</i>
SOD	3.84±0.24	4.2±0.2	3.80±0.21	2.33±0.23	2.28±0.15	3.6±0.3	5.09±0.54	4.83±0.35
CAT	7.5±0.5	5.9±0.4	7.61±0.44	5.62±0.42	7.9±1.1	2.5±0.2	6.92±0.85	7.28±1.25
GSH-Px	10.5±0.8	5.72±0.62	7.52±0.74	5.25±0.34	4.4±0.2	4.9±0.4	6.82±0.28	4.95±0.45
GR	4.32±0.27	5.0±0.3	8.18±0.47	7.01±0.48	0.86±0.10	0.75±0.11	2.01±0.24	1.28±0.08
GST	73.4±4.2	74.4±4.9	118.1±6.0	94.0±4.8	26.5±1.1	60.9±3.6	92.6±8.6	60.8±3.6
GSH	46.9±2.5	53.7±8.8	129.2±11.7	116.5±12.4	157.3±17.9	189.1±16.1	52.8±4.1	58.0±6.0
SH	222.7±8.1	245.1±8.1	822.0±2.6	622.4±21.0	271.0±1.2	300.2±2.0	649.2±16.6	602.1±12.1

Table S10 Antioxidative defense system parameters in skin and muscle tissues of *Pelophylax ridibundus* and *Pelophylax esculentus* from river Ponjavica during pre- (autumn) and posthibernating (spring) seasons. Significant differences ($p < 0.05$) between species at same season were written in bold.

	Skin				Muscle			
	Autumn		Spring		Autumn		Spring	
	<i>P.ridibundus</i>	<i>P.esculentus</i>	<i>P.ridibundus</i>	<i>P.esculentus</i>	<i>P.ridibundus</i>	<i>P.esculentus</i>	<i>P.ridibundus</i>	<i>P.esculentus</i>
SOD	3.77±0.45	2.98±0.36	6.82±0.38	6.65±0.53	2.16±0.12	2.71±0.22	8.60±0.89	7.19±0.43
CAT	5.45±0.54	6.38±0.91	8.80±0.41	5.91±0.50	2.14±0.41	2.08±0.33	8.09±0.84	12.4±2.7
GSH-Px	11.03±0.98	11.53 ±1.27	5.47±0.41	6.01±0.41	5.03±0.27	4.91±0.45	8.77±0.71	11.2±1.5
GR	3.64±0.24	3.33±0.21	9.73±0.82	7.93±0.61	0.80±0.05	0.74±0.04	2.34±0.23	3.08±0.38
GST	72.32±3.73	63.81±5.84	132.0±9.7	96.0±6.8	91.45±7.82	98.67±7.99	87.7±7.9	98.6±8.9
GSH	45.19±5.65	49.2±5.4	128.1±14.3	227.5±16.8	152.2±6.3	119.8±11.6	105.7±3.2	94.3±8.5
SH	227.3±14.3	249.6±5.5	674.4±17.3	565.7±29.8	273.1±1.4	307.0±1.3	563.1±22.1	542.7±24.1