<u>Supplementary Material</u>

Stressed tadpoles mount more efficient glucocorticoid negative feedback in anthropogenic habitats due to phenotypic plasticity

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Table S1

Glucocorticoid disrupting chemicals analyzed in pond water samples.

Compound	Description of GC disrupting effects*	Reference†
Budesonide	synthetic GR-agonist	1,2
Clobetasol propionate	synthetic GR-agonist	1,2
Cortisone	natural GC (cortisol precursor), also used pharmaceutically	1
Dehydroepiandrosterone	natural steroid hormone, also used pharmaceutically; has anti-GC effects	1
Dexamethasone	synthetic GR-agonist	1,2
Flucinolone acetonide	synthetic GR-agonist	1
Flumethasone	synthetic GR-agonist	1,2
Fluorometholone	synthetic GR-agonist	1,2
Hydrocortisone (Cortisol)	natural GC, also used pharmaceutically	1,2
Medroxyprogesterone acetate	GR-agonist, MR-antagonist	2
Megestrol acetate	synthetic derivative of progesterone; has slight GC activity	1
6-alpha-Methylprednisolone	synthetic GR-agonist	1,2
Prednisolone	synthetic GR-agonist	1,2
Prednisone	pro-drug of prednisolone	1,2
Progesterone	natural steroid hormone, used pharmaceutically also; GR-agonist, MR-antagonist, alters GR & MR gene expression, inhibits 11β-hydroxysteroid-dehydrogenase	2
Triamcinolone	synthetic GR-agonist	1,2
Triamcinolone acetonide	a more potent derivative of triamcinolone	1
17-Hydroxyprogesterone	natural steroid hormone, also used pharmaceutically; MR-agonist, weak GR-agonist	1,2
17-alpha-Methyltestosterone	synthetic androgen; potentially GC disruptor	3
Nandrolone (19-nortestosterone)	synthetic androgen; potentially GC disruptor	3
Testosterone	natural androgen; potentially GC disruptor	3
17a-alpha-Ethinylestradiol	synthetic estrogen; potentially GC disruptor	1
17-beta-Estradiol	natural estrogen; potentially GC disruptor	1

*GC: glucocorticoid, GR: glucocorticoid receptor, MR: mineralocorticoid receptor

†References:

1. PubChem. http://pubchem.ncbi.nlm.nih.gov/

2. Macikova, P., Groh, K.J., Ammann, A.A., Schirmer, K. & Suter, M.J.F. (2014) Endocrine disrupting compounds affecting corticosteroid signaling pathways in Czech and Swiss Waters: Potential impact on fish. Environmental Science and Technology, 48, 12902–12911.

3. Mayer, M. & Rosen, F. (1975) Interaction of anabolic steroids with glucocorticoid receptor sites in rat muscle cytosol. American Journal of Physiology, 229, 1381–1386.

Table S2

Optimized and monitored MRM transitions and recovery test results obtained.

Compound name	Polarity (UniSpray™)	RT (min)	Parent mass Q1 m/z	Daughter mass Q2 m/z	Dwell time (msec)	Cone voltage (V)	Collision energy (eV)	USED FOR QUANTITATION	Mean recovery % at 5 ng/L (n=3)	RSD% at 5 ng/L	Mean recovery % at 25 ng/L (n=3)	RSD% at 25 ng/L	LOQ ng/L
Triamcinolone	US -	1.59	393.0	345.1	8	38	18						
Triamcinolone	US -	1.59	439.1	345.1	8	20	26	YES	74.5	3.4	73.0	2.4	2
Triamcinolone	US -	1.59	439.1	363.2	8	20	14						
Cortisone	US +	2.28	361.2	121.0	6	24	28						
Cortisone	US +	2.28	361.2	163.1	6	24	22						
Cortisone	US +	2.28	361.2	343.1	6	24	16						
Cortisone	US -	2.28	405.2	136.9	6	28	40						
Cortisone	US -	2.28	405.2	301.1	6	28	20						
Cortisone	US -	2.28	405.2	329.1	6	28	14	YES	117.6	19.9	111.4	1.6	1
Prednisone	US -	2.17	403.2	299.1	6	15	20						
Prednisone	US -	2.17	403.2	327.1	6	15	14	YES	106.6	4.0	107.9	2.9	2
Prednisone	US -	2.17	403.2	357.1	6	15	9						
Prednisone	US +	2.17	359.2	313.0	6	18	14						
Prednisone	US +	2.17	359.2	341.1	6	18	16						
Prednisolone	US +	2.68	361.2	343.1	6	20	16						

Prednisolone	US -	2.68	405.2	280.1	6	28	36						
Prednisolone	US -	2.68	405.2	295.1	6	28	32						
Prednisolone	US -	2.68	405.2	329.2	6	28	18	YES	127.0	1.1	130.5	1.6	1
CarbamazepineD10	US +	2.49	247.1	173.8	6	25	38						
CarbamazepineD10	US +	2.49	247.1	186.8	6	25	34						
CarbamazepineD10	US +	2.49	247.1	204.1	6	25	22	YES	101.6	3.1	101.6	3.8	0.2
Carbamazepine	US +	2.58	237.1	165.1	6	25	40						
Carbamazepine	US +	2.58	237.1	179.0	6	25	34						
Carbamazepine	US +	2.58	237.1	194.1	6	25	20	YES	See carbamazepine D10				0.2
Cortisol (Hydrocortisone)	US +	2.7	363.2	121.0	6	36	24						
Cortisol (Hydrocortisone)	US +	2.7	363.2	327.1	6	36	16						
Cortisol (Hydrocortisone)	US +	2.7	363.2	345.0	6	36	16						
Cortisol (Hydrocortisone)	US -	2.7	407.2	282.1	6	25	36						
Cortisol (Hydrocortisone)	US -	2.7	407.2	297.1	6	25	32						
Cortisol (Hydrocortisone)	US -	2.7	407.2	331.2	6	25	16	YES	124.0	14.7	127.0	2.0	1
Flumethasone	US -	3.32	455.1	305.1	10	32	38						
Flumethasone	US -	3.32	455.1	325.1	10	32	30						
Flumethasone	US -	3.32	455.1	379.2	10	32	18	YES	128.8	3.1	130.9	2.2	1
Dexamethasone	US -	3.65	437.2	292.0	10	28	38						
Dexamethasone	US -	3.65	437.2	307.1	10	28	32						
Dexamethasone	US -	3.65	437.2	325.1	10	28	32						
Dexamethasone	US -	3.65	437.2	361.2	10	28	16	YES	134.3	4.3	135.3	1.6	1
Triamcinolone acetonide	US -	3.88	479.3	337.2	10	22	19						
Triamcinolone acetonide	US -	3.88	479.3	413.3	10	22	25	YES	103.7	5.8	111.2	1.0	2
6-alpha-methylprednisolone	US -	3.91	419.2	294.1	10	32	32						
6-alpha-methylprednisolone	US -	3.91	419.2	309.1	10	32	34						
6-alpha-methylprednisolone	US -	3.91	419.2	343.2	10	32	16	YES	123.0	4.6	123.7	3.4	1
6-alpha-methylprednisolone	US +	3.91	375.2	357.1	10	20	18						
Flucinolone acetonide	US -	3.96	497.2	355.1	10	44	24						
Flucinolone acetonide	US -	3.96	497.2	373.1	10	44	20						

Flucinolone acetonide	US -	3.96	497.2	431.2	10	44	20	YES	110.3	1.4	120.6	3.0	2
Fluorometholone	US +	4.35	377.1	238.3	18	18	26						
Fluorometholone	US -	4.35	421.3	355.2	18	17	16	YES	107.6	11.1	111.2	1.6	5
Nandrolone	US +	4.72	275.0	109.0	18	20	26	YES	101.8	7.7	97.5	3.9	2
Nandrolone	US +	4.72	275.0	239.1	18	20	16						
Nandrolone	US +	4.72	275.0	257.2	18	20	16						
Dehydroepiandrosterone	US +	5.71	289.0	182.9	18	34	26						
Dehydroepiandrosterone	US +	5.71	289.0	238.4	18	34	30						
Dehydroepiandrosterone	US +	5.71	289.0	253.1	34	34	11	YES	103.5	7.4	103.2	6.2	10
Testosterone	US +	5.71	289.0	97.0	34	36	21	YES	91.3	6.5	89.9	6.0	0.2
Testosterone	US +	5.71	289.0	109.1	34	36	22						
17-alpha-hydroxyprogesterone	US +	6.26	331.1	97.0	23	42	25	YES	100.5	6.4	99.4	4.6	1
17-alpha-hydroxyprogesterone	US +	6.26	331.1	109.0	23	42	26						
17-alpha-hydroxyprogesterone	US +	6.26	331.1	313.1	23	42	14						
17-alpha-methyltestosterone	US +	6.73	303.1	97.0	23	17	25	YES	92.8	7.2	95.4	10.0	5
17-alpha-methyltestosterone	US +	6.73	303.1	109.0	23	17	27						
17-alpha-methyltestosterone	US +	6.73	303.1	285.2	23	17	14						
Budesonide	US +	7.11	431.2	413.1	23	20	18	YES	103.3	5.4	100.6	9.6	20
Budesonide	US -	7.11	475.2	357.1	23	36	16						
Megestrol acetate	US +	8.41	385.2	224.2	19	16	26	YES	93.0	9.2	94.3	8.1	1
Megestrol acetate	US +	8.41	385.2	267.2	19	16	16						
Megestrol acetate	US +	8.41	385.2	325.2	19	16	14						
Clobetasol propionate	US -	8.44	511.1	429.2	19	34	16	YES	85.4	6.2	90.1	3.9	5
Clobetasol propionate	US -	8.44	511.1	445.2	19	34	16						
Clobetasol propionate	US -	8.44	511.1	465.1	19	34	14						
Medroxyprogesterone acetate	US +	8.87	387.2	123.0	19	34	28	YES	97.2	17.6	85.1	15.1	1
Medroxyprogesterone acetate	US +	8.87	387.2	285.2	19	34	16						
Medroxyprogesterone acetate	US +	8.87	387.2	327.2	19	34	12						
Progesterone	US +	8.97	315.1	297.2	19	30	16	YES	94.9	9.6	93.8	6.4	2
Progesterone	US +	8.97	315.2	97.0	19	30	22						

Progesterone	US +	8.97	315.2	109.0	19	30	24						
17-beta-estradiol	US -	6.94	271.2	145.0	10	51	40	YES	107.7	2.9	104.2	6.2	5
17-beta-estradiol	US -	6.94	271.2	183.0	10	51	31						
17-alpha-ethinylestradiol	US -	7.42	295.0	199.0	10	45	35						
17-alpha-ethinylestradiol	US -	7.42	295.0	269.0	10	45	35	YES	113.9	2.3	100.9	6.7	5

Table S3

Reference materials purchased, supplier, purity, solvent and concentration of stock solutions.

Compound	Purchased from	Purity	Solvent	µg/ml
17-alpha-ethinylestradiol	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	methanol	3511
17-alpha-hydroxyprogesterone	TCI - Tokyo Chemical Industry (Tokyo, Japan)	≤98%	DMSO	1966
17-alpha-methyltestosterone	Dr. Ehrenstorfer GmbH (Augsburg, Germany)	≤97.7%	ethanol	2247
17-beta-estradiol	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	methanol	1745
6-alpha-methylprednisolone	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	chloroform:methanol (1:1)	7579
Budesonide	TCI - Tokyo Chemical Industry (Tokyo, Japan)	≤98%	methanol	4224
Carbamazepine	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	ethanol	1825
Carbamazepine-D10	Sigma Aldrich (St. Louis, Missouri, USA)	≤99.2%	methanol	100
Clobetasol propionate	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	methanol	2744
Cortisone	Acros Organics N.V. (Geel, Belgium)	≤98%	methanol	1697
Dehydroepiandrosterone	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	ethanol	4645
Dexamethasone	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	methanol	2008
Flumethasone	TCI - Tokyo Chemical Industry (Tokyo, Japan)	≤98%	acetic acid	3188
Fluocinolone acetonide	Alfa Aesar (Haverhill, Massachusetts, USA)	≤98%	methanol	1952
Fluorometholone	TCI - Tokyo Chemical Industry (Tokyo, Japan)	≤98%	pyridine	4703
Hydrocortisone (Cortisol)	Acros Organics N.V. (Geel, Belgium)	≤98%	methanol	2588
Medroxyprogesterone acetate	Dr. Ehrenstorfer GmbH (Augsburg, Germany)	≤99 %	acetone	1928
Megestrol acetate	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	acetone	4733
Nandrolone	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	ethanol	3026
Prednisolone	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	methanol	2619
Prednisone	Dr. Ehrenstorfer GmbH (Augsburg, Germany)	≤99 %	chloroform:methanol (1:1)	3002
Progesterone	TCI - Tokyo Chemical Industry (Tokyo, Japan)	≤98%	ethanol	5069
Testosterone	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	methanol	1797
Triamcinolone	Sigma Aldrich (St. Louis, Missouri, USA)	≤98%	DMSO	1860
Triamcinolone acetonide	Sigma Aldrich (St. Louis, Missouri, USA)	≤99 %	DMSO	3947

Table S4

Likelihood ratio (LR) tests of different correlation structures for corticosterone release rates of tadpoles in the field and in the mesocosms. The linear mixed-effects models were run with the 'lme' function in the R package 'nlme', using sample category as fixed factor and individual identity as a random factor (i.e. the three subsequent samples of each individual were used as repeated measures). Additionally, the tested models included pond identity and/or tub identity as nested random factors (individual nested within tub within pond).

Venue	Random structure	AIC	LR	*P*	Conclusion
Field	pond/individual	106			
	individual	144	40.9	<0.001	Significant pond effect in the field.
Mesocosms	pond/tub/individual	148			
	tub/individual	146	<0.001	>0.999	No pond effect in common garden.
	pond/individual	178	32.1	<0.001	Significant tub effect in common garden.

Table S5

Pairwise comparisons (*c*: linear contrasts, estimated from linear mixed model) of corticosterone release rates (log_{10} pg/g/h) between venues (tadpoles in the field and mesocosms) in each sample. *P*-values were corrected with the FDR method; df = 268.

Sample	*c* ± SE	t	*P*
Baseline	1.50 ± 0.04	35.14	<0.001
Stressed	1.05 ± 0.04	24.54	<0.001
Recovery	1.02 ± 0.04	23.66	<0.001

Fig. S1. Proportion of anthropogenic land cover (calculated as the sum of all land-use variables excepting forests in table 1) around the nine ponds (a), and its relationship with water quality (b). Water quality is the first PCA (principal components analysis) score from a PCA of the variables shown in Fig. S2, with positive loadings for all five variables.

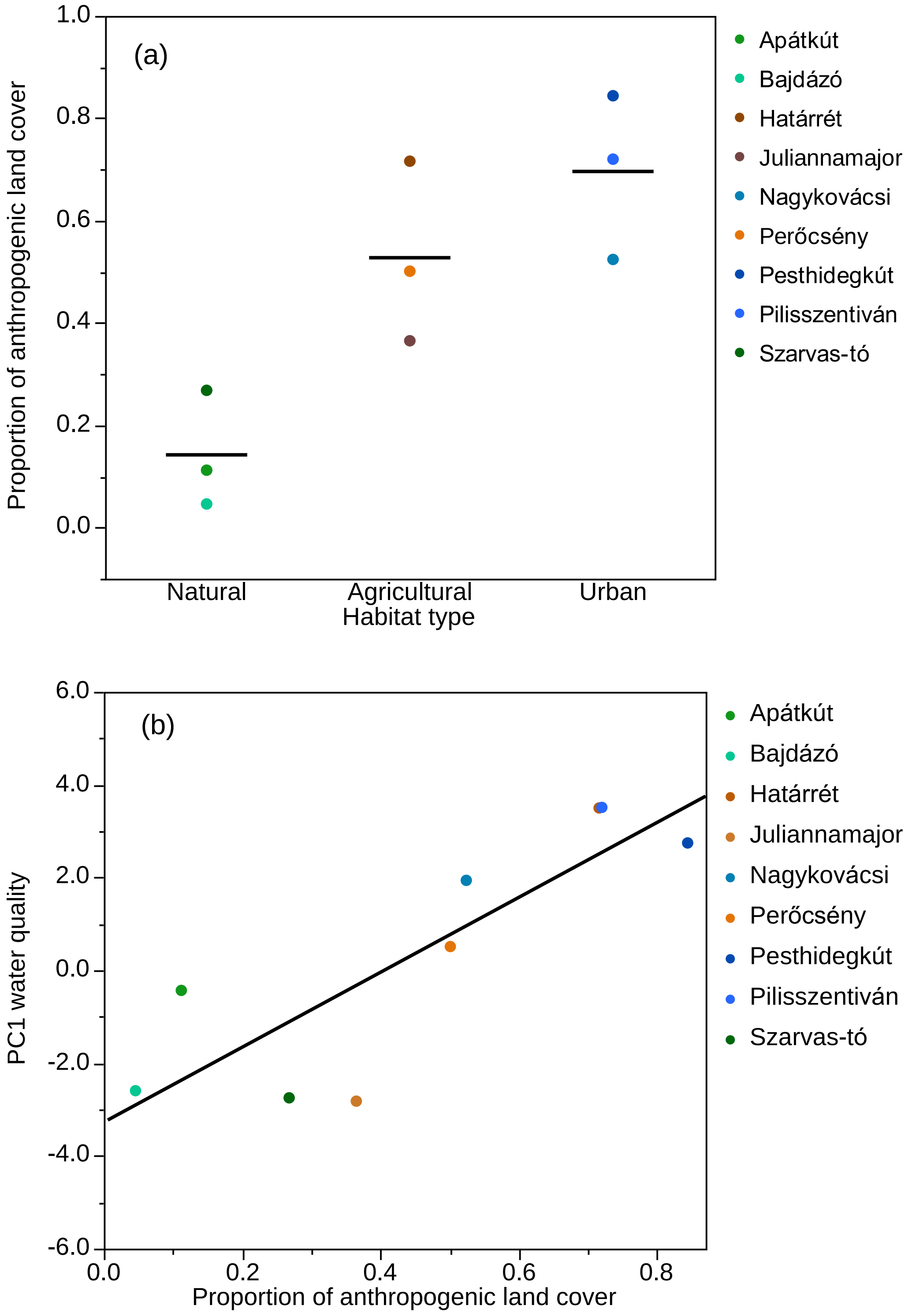

Fig. S2. The studied ponds' water-quality measurements (TDS: total dissolved solids) at the time of tadpole sampling.

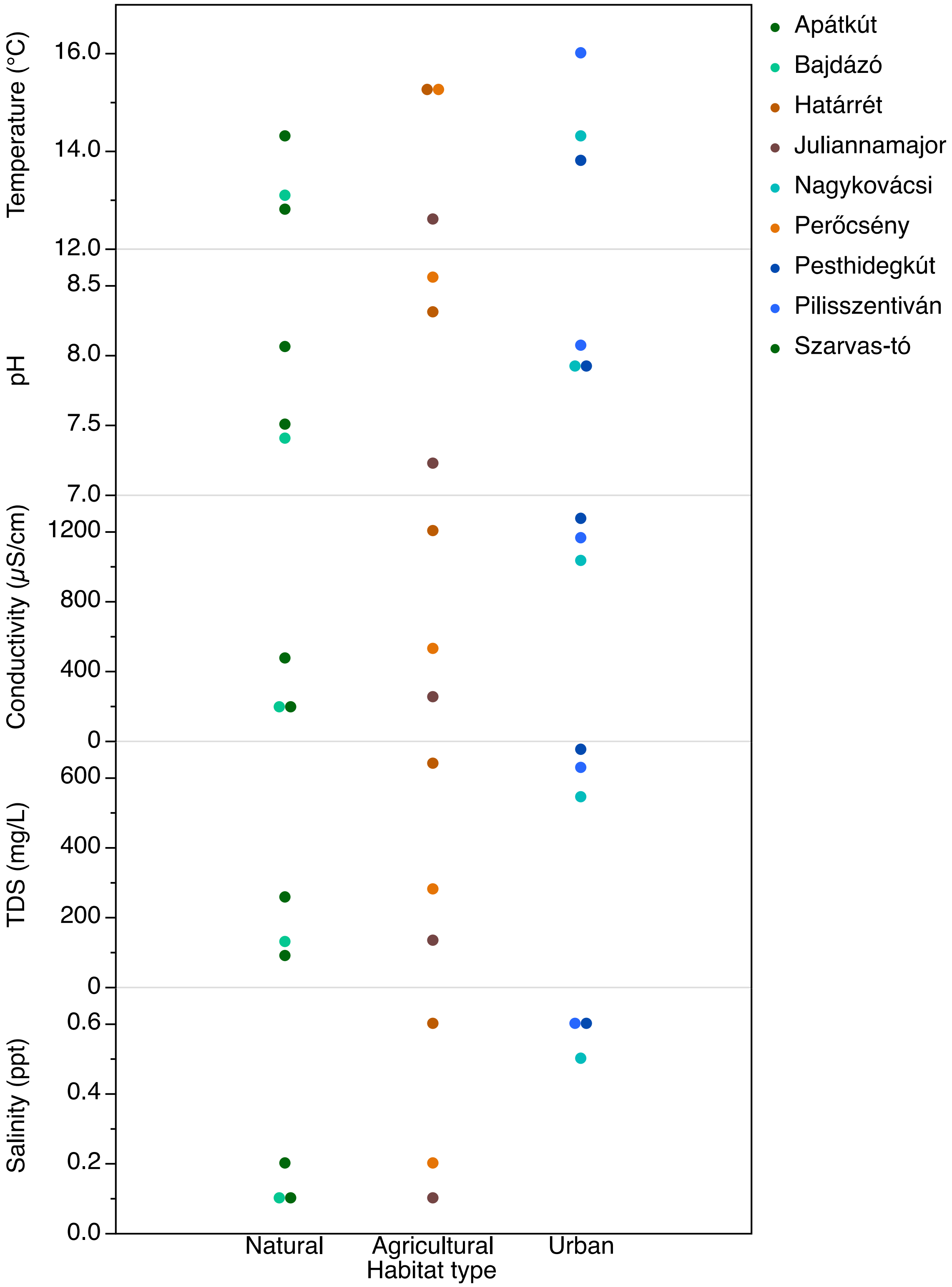

Fig. S3. Corticosterone release rates of tadpoles in relation to the concentration of corticosterone in pond water. Error bars represent means and standard errors calculated from the raw data.

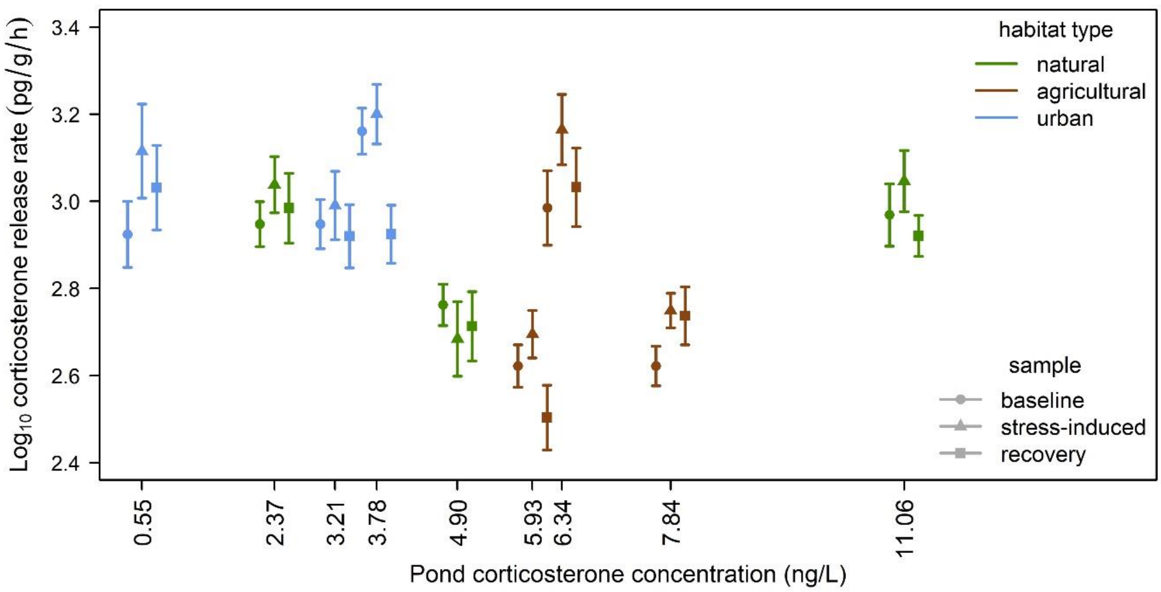

Fig. S4. Magnitude of stress response and negative feedback in free-living tadpoles in three different habitats. Error bars represent means and standard errors as predicted by the models in Table 4. Both variables were expressed as % change and transformed as described in the Methods; for illustrative purposes in this graph they were re-scaled to the same scale (i.e. mean-centered and divided by standard deviation; so the units along the Y axis are SD).