Supporting Information for:

Sexual re-programming and estrogenic sensitization in wild fish exposed to ethinylestradiol

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Table S1. Results of two-way ANOVA analysis of effects of duration of estrogen re-challenge, EE_2 exposure concentration at early life and their interaction (duration x exposure at early life) on the expression of *esr1*, *esr2b* and *cyp19a1a* in male and female roach.

		Duration of re-challenging			Exposure at early life			Duration x exposure at early life		
Gene	Sex	F	df	p	F	df	p	F	df	p
esr1	female	2.46	1,30	0.13	8.93	2,28	< 0.001	0.47	2,26	0.63
esr2b	female	4.82	1,31	< 0.05	9.46	2,29	< 0.001	0.296	2,17	0.75
cyp19a1a	female	0.07	1,30	0.798	26.85	2,28	<0.0001	1.46	2,26	0.25
esr1	male	1.33	1,28	0.26	1.20	2,26	0.317	2.02	1,25	0.17
esr2b	male	0.097	1,28	0.76	4.42	2,26	< 0.05	1.30	1,25	0.26
cyp19a1a	male	0.78	1,28	0.38	2.63	2,26	0.09	0.27	1,25	0.61

Figure S1. (A) Standard length, (B) wet weight and (C) condition factor for male and female roach (sex confirmed by gonadal histology) exposed to environmental concentrations of EE_2 (measured concentrations: non-detectable (n.d.), 0.3 and 4 ng/L EE_2) for two years starting from the time of the fertilization of the egg. Each column represents mean±SEM and the numbers in brackets indicate the number of samples analyzed. No significant differences were observed within sexes ($p<0.05$, Dunn's test) in the EE_2 exposure. Fish exposed to 4 ng/L EE_2 were excluded from statistical analysis since all fish were phenotypic females (i.e. they included both true females and sex reversed males).

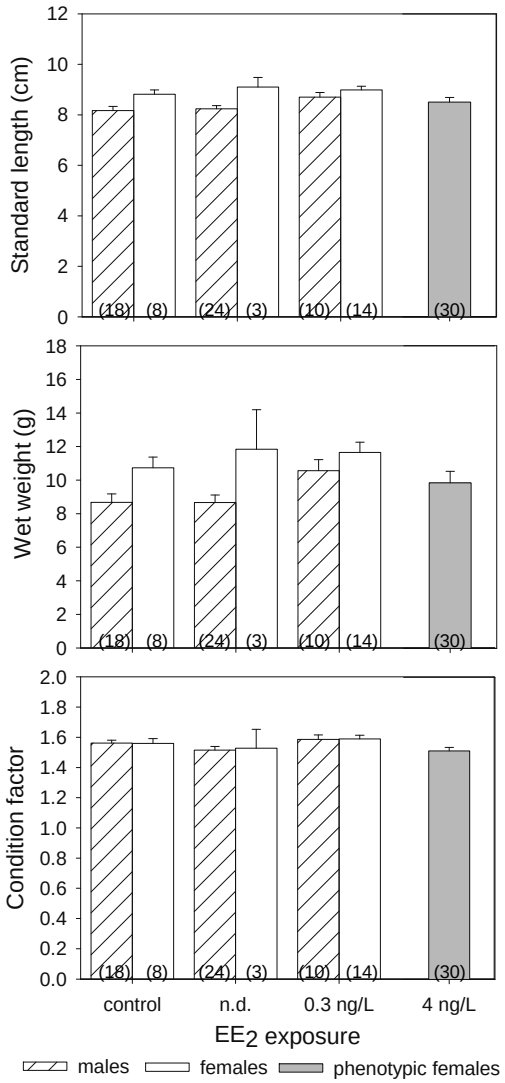


Figure S2. Gonadosomatic index (GSI) of male and female roach exposed to environmental concentrations of EE_2 for 720 days from fertilization of the egg. Data are presented as box-and-whisker plots where the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box furthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles and the dots represent the 5th and 95th percentiles. No significant differences were observed within genders ($p<0.05$, Dunn's test). Fish exposed to 4 ng/L EE_2 were excluded from statistical analysis since all fish were phenotypic females (i.e. they included both true females and sex reversed males).

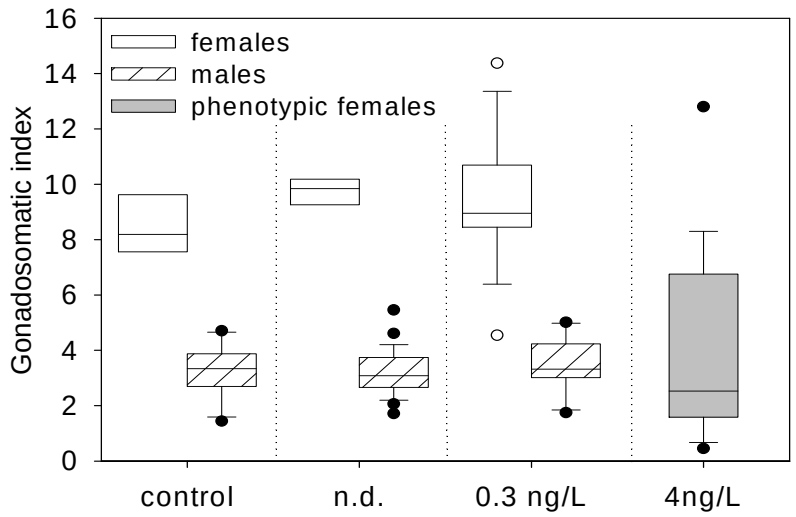


Figure S3. Histopathology of roach gonads at 250, 518 and 720 days posthatch. Rows of panels show gonads at different time points and columns represent gonads of control and expeosed fish. Control ovaries (A, E and I) and control testis (B, F and J) at different stages of development. (C&D, G&H and K&L) sections of gonads at different developmental stages in phenotypic females after exposure to measured 4 ng/L EE_2 showing representative sections for both cohorts in terms of stages of ovarian development. po, primary oocyte; so, secondary oocyte; vo, vitellogenic oocyte; sgA, spermatogonia A; sgB, spermatogonia B; sy, spermatocytes; solid scale bars: 50 μm; dashed scale bars: 250 μm.

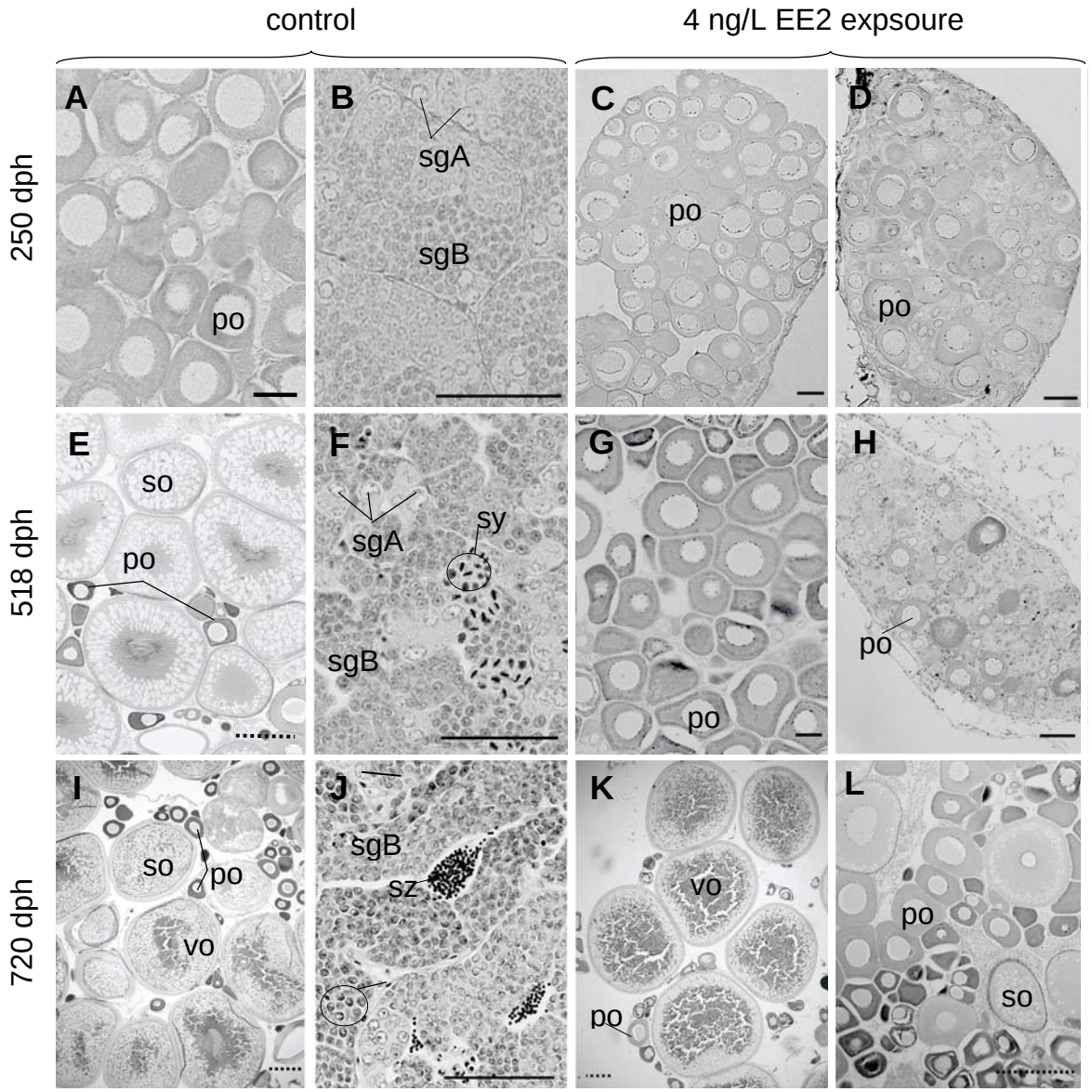
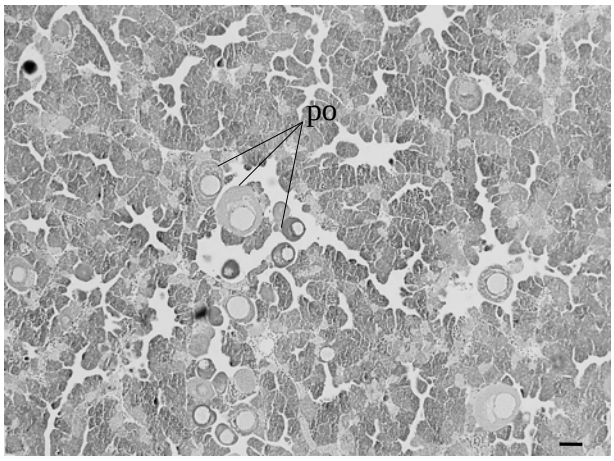
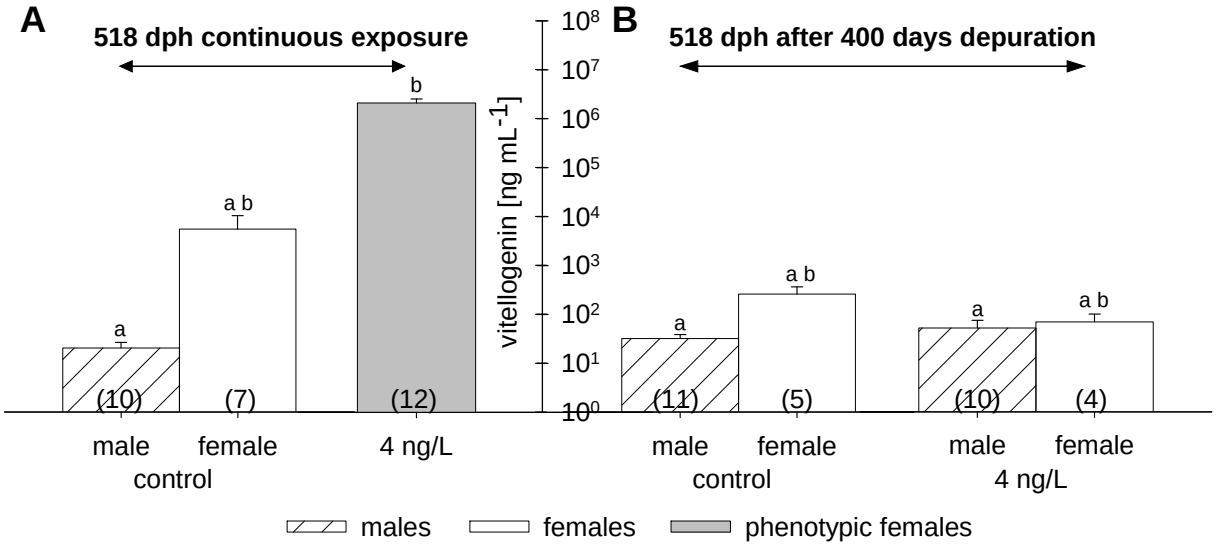


Figure S4. Testis of intersex roach exposed to 0.3 ng/L EE_2 for two years starting from the time of the fertilization of the eggs. po, primary oocyte; Scale bar: 50 μm.



Supporting Figure S5. Plasma vitellogenin concentrations in roach from the continuous and depuration experiment at 518 days posthatch (dph). Plasma vitellogenin was measured in maturing male and female roach at 518 dph exposed to water control or 4 ng/L EE_2 (A) continuously from fertilization and (B) during early life until 120 dph followed by a depuration phase for 398 days. Each column represents mean±SEM and the numbers in brackets indicate the number of samples analyzed. Different letters above bars indicate significant difference ($p<0.05$, Dunn's test).



Supporting Figure S6. Standard length (A) and wet weight (B) of roach sampled at 518 days posthatch (dph) from both, the continuous EE_2 exposure experiment and the depuration experiment. Each column represents mean±SEM and the numbers in brackets indicate the number of samples analysed. No significant differences were observed ($p<0.05$), but fish continuously exposed to 4 ng/L EE_2 were excluded from statistical analysis because all fish were phenotypic females (i.e. they included both true females and sex reversed males).