

Supporting Information for:

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

*Anke Lange, Gregory C. Paull, Tobias S. Coe, Yoshinao Katsu, Hiroshi Urushitani,*

*Taisen Iguchi, Charles R. Tyler*

8 pages

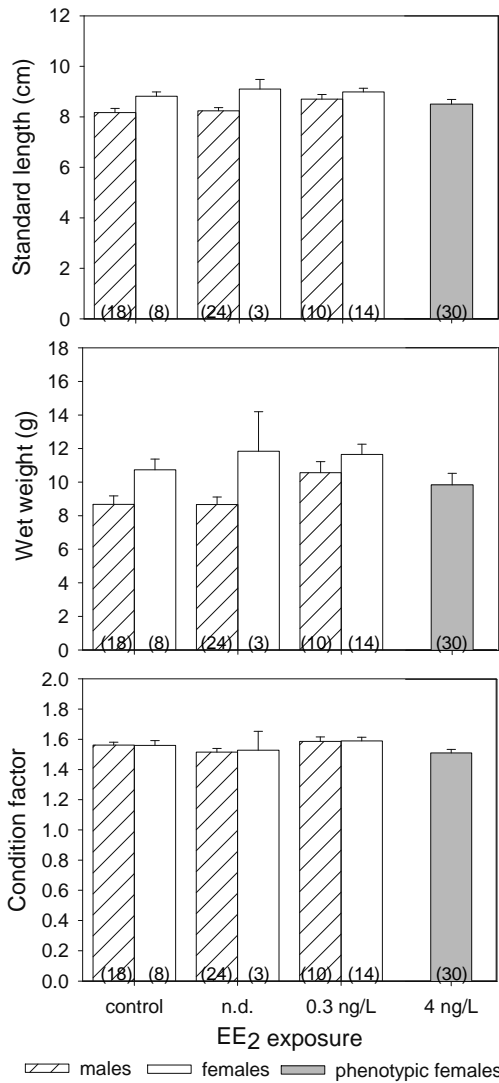
1 table

6 figures

- 1 **Table S1.** Results of two-way ANOVA analysis of effects of duration of estrogen re-challenge, EE<sub>2</sub> exposure concentration at early life  
 2 and their interaction (duration x exposure at early life) on the expression of *esr1*, *esr2b* and *cyp19a1a* in male and female roach.

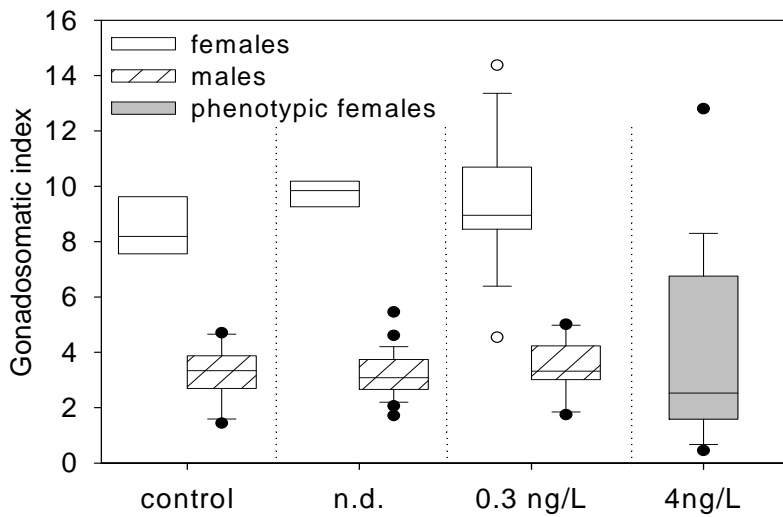
Gene	Sex	Duration of re-challenging			Exposure at early life			Duration x exposure at early life			5
		F	df	p	F	df	p	F	df	p	
<i>esr1</i>	female	2.46	1,30	0.13	8.93	2,28	< 0.001	0.47	2,26	0.63	6
<i>esr2b</i>	female	4.82	1,31	< 0.05	9.46	2,29	< 0.001	0.296	2,17	0.75	7
<i>cyp19a1a</i>	female	0.07	1,30	0.798	26.85	2,28	<0.0001	1.46	2,26	0.25	8
											9
											10
<i>esr1</i>	male	1.33	1,28	0.26	1.20	2,26	0.317	2.02	1,25	0.17	11
<i>esr2b</i>	male	0.097	1,28	0.76	4.42	2,26	< 0.05	1.30	1,25	0.26	12
<i>cyp19a1a</i>	male	0.78	1,28	0.38	2.63	2,26	0.09	0.27	1,25	0.61	

13 **Figure S1.** (A) Standard length, (B) wet weight and (C) condition factor for male and female roach  
 14 (sex confirmed by gonadal histology) exposed to environmental concentrations of EE<sub>2</sub> (measured  
 15 concentrations: non-detectable (n.d.), 0.3 and 4 ng/L EE<sub>2</sub>) for two years starting from the time of the  
 16 fertilization of the egg. Each column represents mean±SEM and the numbers in brackets indicate the  
 17 number of samples analyzed. No significant differences were observed within sexes (p<0.05, Dunn's  
 18 test) in the EE<sub>2</sub> exposure. Fish exposed to 4 ng/L EE<sub>2</sub> were excluded from statistical analysis since all  
 19 fish were phenotypic females (i.e. they included both true females and sex reversed males).



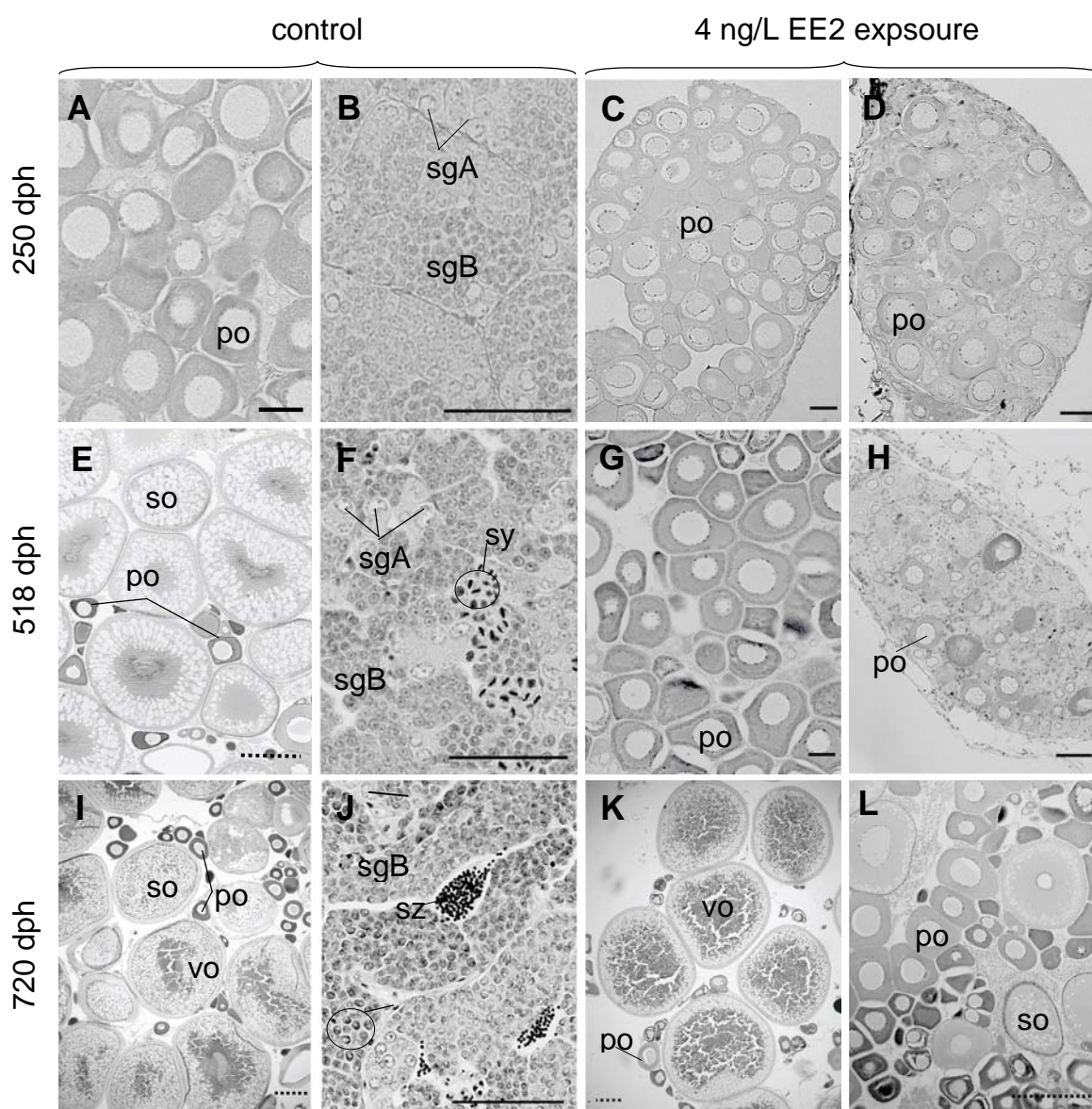
20  
21

22 **Figure S2.** Gonadosomatic index (GSI) of male and female roach exposed to environmental  
23 concentrations of EE<sub>2</sub> for 720 days from fertilization of the egg. Data are presented as box-and-whisker  
24 plots where the boundary of the box closest to zero indicates the 25th percentile, a line within the box  
25 marks the median, and the boundary of the box furthest from zero indicates the 75th percentile.  
26 Whiskers above and below the box indicate the 90th and 10th percentiles and the dots represent the 5th  
27 and 95th percentiles. No significant differences were observed within genders ( $p < 0.05$ , Dunn's test).  
28 Fish exposed to 4 ng/L EE<sub>2</sub> were excluded from statistical analysis since all fish were phenotypic  
29 females (i.e. they included both true females and sex reversed males).



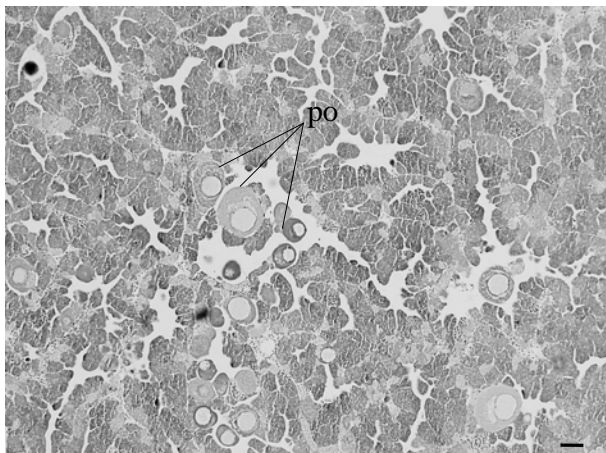
30

31 **Figure S3.** Histopathology of roach gonads at 250, 518 and 720 days posthatch. Rows of panels show  
 32 gonads at different time points and columns represent gonads of control and exposed fish. Control  
 33 ovaries (A, E and I ) and control testis (B, F and J) at different stages of development. (C&D, G&H and  
 34 K&L) sections of gonads at different developmental stages in phenotypic females after exposure to  
 35 measured 4 ng/L EE<sub>2</sub> showing representative sections for both cohorts in terms of stages of ovarian  
 36 development. po, primary oocyte; so, secondary oocyte; vo, vitellogenic oocyte; sgA, spermatogonia  
 37 A; sgB, spermatogonia B; sy, spermatocytes; solid scale bars: 50 μm; dashed scale bars: 250 μm.



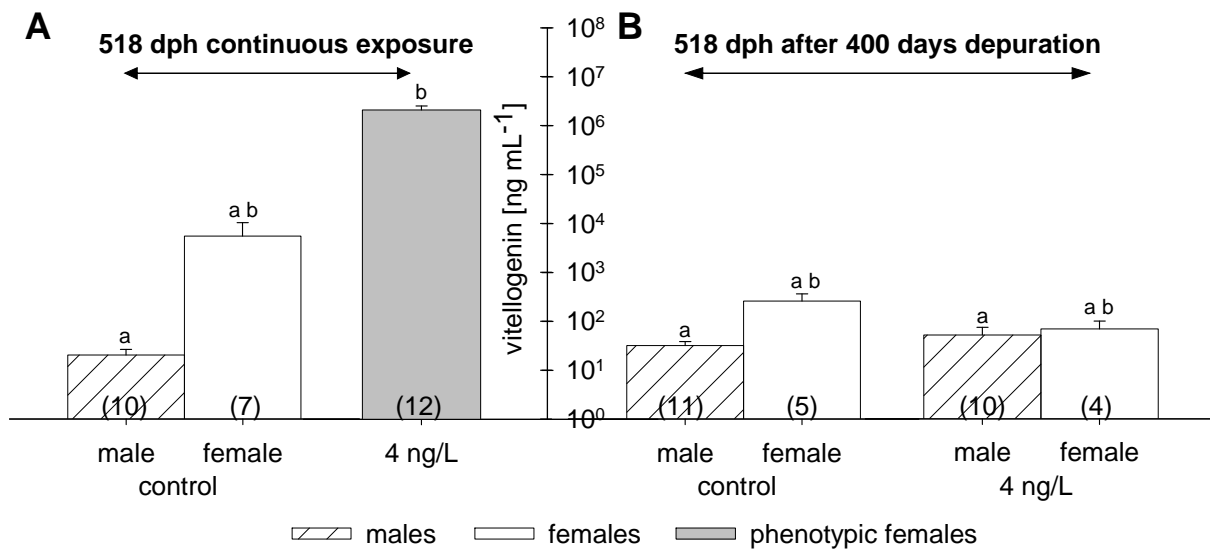
38

39 **Figure S4.** Testis of intersex roach exposed to 0.3 ng/L EE<sub>2</sub> for two years starting from the time of the  
40 fertilization of the eggs. po, primary oocyte; Scale bar: 50 μm.



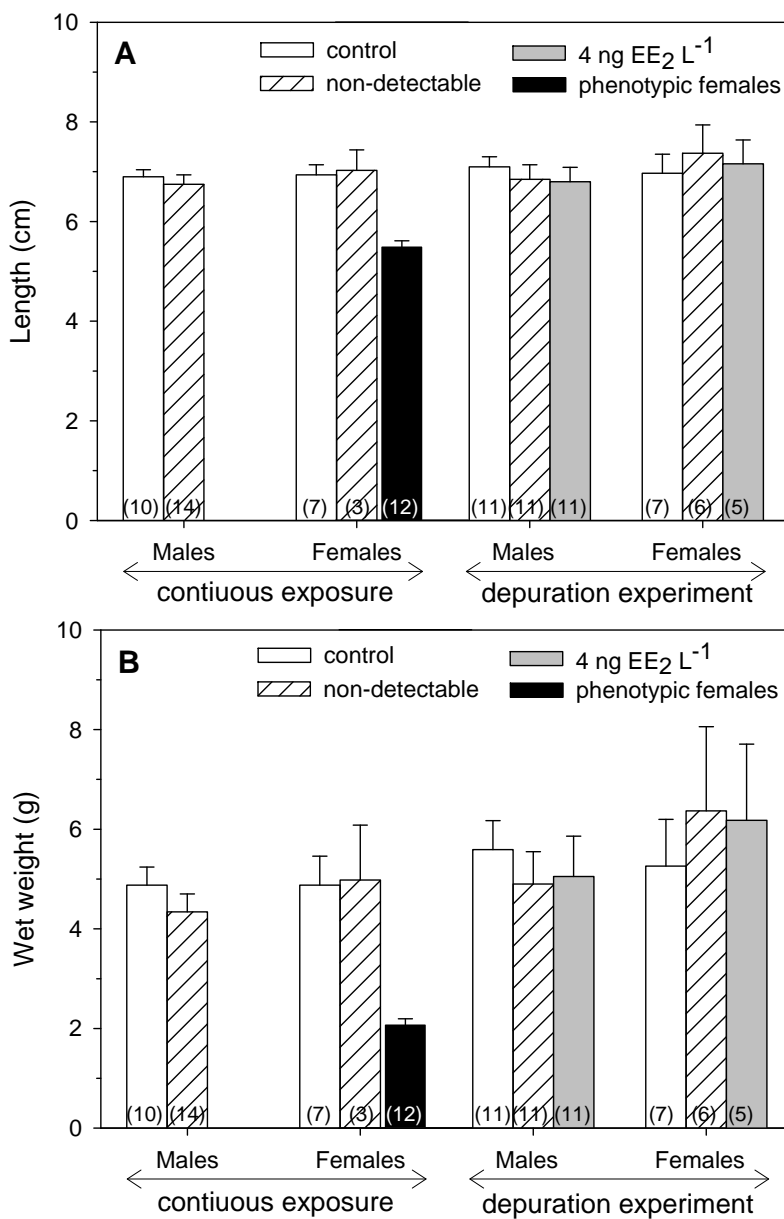
41 **Supporting Figure S5.** Plasma vitellogenin concentrations in roach from the continuous and  
 42 depuration experiment at 518 days posthatch (dph). Plasma vitellogenin was measured in maturing  
 43 male and female roach at 518 dph exposed to water control or 4 ng/L EE<sub>2</sub> (A) continuously from  
 44 fertilization and (B) during early life until 120 dph followed by a depuration phase for 398 days. Each  
 45 column represents mean±SEM and the numbers in brackets indicate the number of samples analyzed.  
 46 Different letters above bars indicate significant difference ( $p < 0.05$ , Dunn's test).

47



48

49 **Supporting Figure S6.** Standard length (A) and wet weight (B) of roach sampled at 518 days  
 50 posthatch (dph) from both, the continuous EE<sub>2</sub> exposure experiment and the depuration experiment.  
 51 Each column represents mean±SEM and the numbers in brackets indicate the number of samples  
 52 analysed. No significant differences were observed ( $p < 0.05$ ), but fish continuously exposed to 4 ng/L  
 53 EE<sub>2</sub> were excluded from statistical analysis because all fish were phenotypic females (i.e. they included  
 54 both true females and sex reversed males).



55  
56