

## **Supplementary information**

### **Background fish feminization effects in European remote sites**

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Table S1. Averaged data of the different parameters analyzed in this study, grouped by fish population

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Zone	Lake	Altitude	Calculated T																	
				n	Lenght	Weight	CF	RS	Age	Vtg mRNA levels	Zrp1mRNA levels	ER mRNA levels	Cyp1A mRNA levels	HCB	g-HCH	Sum PCB	SUM DDE			
		(m)	(°C)		(cm)	(g)	(cg cm <sup>-3</sup> )	(a)	(yr)	(b)	(b)	(b)	(b)	(pg/g)	(pg/g)	(pg/g)	(pg/g)			
Pyrenees	Llebreta	1620	6.11	All	14 32 ± 3	347 ± 100	11 ± 0.1	2.6 ± 18	6.2 ± 14	20 ± 38	2400 ± 4600	2.2 ± 10	270 ± 120	0.36 ± 0.15	10 ± 11	4.2 ± 10	5.1 ± 15			
	42.5508N 0.8903E			Males	1 36	560		12	10		6.0	b.d.l.	590	1.1	.	440	0.13	0.7	5.9	6.9
				Females	13 31 ± 3	330 ± 83	11 ± 0.1	2.8 ± 18	6.2 ± 15	21 ± 39	2600 ± 4800	2.3 ± 0.9	260 ± 110	0.37 ± 0.14	11 ± 12	4.0 ± 0.9	5.0 ± 15			
				Cavallers	1800	5.50	All	14 27 ± 4	266 ± 104	12 ± 0.1	2.7 ± 14	4.5 ± 12	15 ± 25	1200 ± 1500	19 ± 0.9	550 ± 700	0.49 ± 0.13	10 ± 0.5	4.9 ± 11	4.3 ± 11
	42.5926N 0.8578E			Males	4 30 ± 2	346 ± 74	13 ± 0.0	10 ± 0.0	4.5 ± 10	0.8 ± 12	87 ± 100	15 ± 0.9	1000 ± 1300	0.46 ± 0.11	11 ± 0.3	5.1 ± 10	4.0 ± 0.9			
				Females	10 26 ± 5	235 ± 99	12 ± 0.1	3.4 ± 1.1	4.5 ± 14	19 ± 28	1600 ± 1600	2.5 ± 0.9	370 ± 200	0.50 ± 0.14	10 ± 0.6	4.8 ± 12	4.5 ± 12			
				Llong	2000	4.10	All	10 27 ± 4	254 ± 116	12 ± 0.1	1.7 ± 13	8.0 ± 2.4	19 ± 42	2100 ± 4200	2.9 ± 0.7	490 ± 360	0.29 ± 0.10	13 ± 0.6	5.7 ± 4.3	4.9 ± 2.2
	42.5743N 0.9506E			Males	2 24 ± 1	165 ± 35	12 ± 0.0	10 ± 0.0	7.0 ± 2.8	b.d.l.	77 ± 42	2.1 ± 0.4	150 ± 120	0.40 ± 0.11	17 ± 0.2	4.9 ± 2.3	5.4 ± 2.8			
				Females	8 28 ± 4	276 ± 120	12 ± 0.1	1.9 ± 1.4	8.3 ± 2.4	24 ± 47	2600 ± 4600	3.2 ± 0.6	580 ± 350	0.26 ± 0.08	12 ± 0.6	5.9 ± 4.8	4.8 ± 2.2			
				Redo	2235	3.18	All	7 27 ± 5	204 ± 81	10 ± 0.1	n.d.	9.9 ± 3.8	7.6 ± 8.1	2100 ± 2500	3.0 ± 0.4	1700 ± 700	0.52 ± 0.11	2.6 ± 2.1	18 ± 13	16 ± 14
	42.6421N 0.7795E			Males	5 26 ± 7	195 ± 95	0.9 ± 0.1	n.d.	9.0 ± 4.2	3.8 ± 5.1	1900 ± 2900	3.0 ± 0.4	1900 ± 500	0.56 ± 0.10	3.4 ± 16	22 ± 13	19 ± 15			
				Females	2 27 ± 2	228 ± 41	11 ± 0.1	n.d.	12 ± 14	17 ± 7	2800 ± 1600	n.d.	1100 ± 1000	0.39 ± 0.00	0.2 ± 0.0	6.3 ± 0.0	7.6 ± 0.0			
				Xic de Colomina	2425	2.50	All	10 24 ± 2	148 ± 36	11 ± 0.2	17 ± 0.9	6.2 ± 0.8	3.0 ± 4.1	5500 ± 13900	15 ± 0.7	1400 ± 800	0.40 ± 0.14	16 ± 0.8	8.2 ± 4.5	9.9 ± 5.0
	42.5215N 0.9956E			Males	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
				Females	10 24 ± 2	148 ± 36	11 ± 0.2	1.7 ± 0.9	6.2 ± 0.8	3.0 ± 4.1	5500 ± 13900	15 ± 0.7	1400 ± 800	0.40 ± 0.14	16 ± 0.8	8.2 ± 4.5	9.9 ± 5.0			
				Vidal d'Amunt	2688	1.00	All	9 29 ± 3	279 ± 55	11 ± 0.2	2.8 ± 1.4	5.4 ± 12	52 ± 91	3000 ± 7100	3.8 ± 0.9	840 ± 350	0.65 ± 0.30	2.2 ± 0.5	9.8 ± 8.0	19 ± 8
	42.5328N 0.9935E			Males	3 29 ± 2	266 ± 28	11 ± 0.2	2.0 ± 0.0	5.0 ± 0.0	0.02 ± 0.01	400 ± 300	3.6 ± 0.7	640 ± 270	0.54 ± 0.12	2.1 ± 0.3	6.5 ± 17	16 ± 4			
				Females	6 29 ± 3	282 ± 62	11 ± 0.2	3.1 ± 1.5	5.6 ± 13	69 ± 100	4200 ± 8500	4.1 ± 1.2	930 ± 330	0.70 ± 0.33	2.2 ± 0.5	11 ± 9	20 ± 8			
Tatras	Morskie Oko	1395	2.40	All	13 21 ± 10	245 ± 649	11 ± 0.7	2.9 ± 2.4	7.1 ± 3.7	48 ± 106	2100 ± 4600	1.1 ± 0.7	910 ± 890	0.18 ± 0.18	1.2 ± 0.7	55 ± 170	110 ± 360			
	49.1978N 20.0722E			Males	10 22 ± 12	295 ± 741	10 ± 0.3	2.3 ± 2.0	7.5 ± 4.1	0.10 ± 0.23	73 ± 82	1.1 ± 0.7	1200 ± 900	0.17 ± 0.19	11 ± 0.7	70 ± 193	140 ± 410			
				Females	3 18 ± 4	75 ± 8	17 ± 1.3	5.0 ± 2.6	5.7 ± 0.6	175 ± 152	7600 ± 6600	n.d.	77 ± 24	0.22 ± 0.17	1.4 ± 0.5	2.8 ± 18	14 ± 9			
				Popradské Pleso	1494	2.00	All	13 19 ± 3	73 ± 42	0.9 ± 0.1	3.4 ± 2.1	7.2 ± 2.4	5.1 ± 117	450 ± 1100	1.5 ± 0.7	2700 ± 1800	0.40 ± 0.17	4.0 ± 1.7	240 ± 130	16 ± 13

CF, Conditioning factor

### RS. Reproductive status

b.d.l., below detection limits; n.d., no data available

a) 1: undifferentiated gonads, 7: post-spawning

b) mRNA copies per 100 copies of b-Actin mRNA

Table S2. Spearman rank correlations ( $\rho$ ) between hepatic mRNA levels for different genes and physical and biological parameters and pollutant content.

	Cyp1A		Vtg				Zrp1				ERa			
	Males and females		Males		Females		Males		Females		Males		Females	
	$\rho$	n	$\rho$	n	$\rho$	n	$\rho$	n	$\rho$	n	$\rho$	n	$\rho$	n
Cyp1A	1	101												
Vtg	0.334 *	39	1	39	1	51								
Zrp1	-0.013	39	0.172	37	0.659 ***	49	1	39	1	50				
ERa	-0.208	38	0.050	37	-0.395	24	0.584 ***	36	0.779 ***	25	1	38	1	25
Temperature	-0.555 ***	101	0.041	39	-0.171	51	0.150	39	0.026	50	0.125	38	-0.020	25
Condition factor	-0.283 **	101	0.126	39	-0.089	51	-0.007	39	-0.149	50	-0.028	38	0.314	25
Reproductive status	-0.094	94	-0.102	34	0.669 ***	49	-0.364 *	34	0.787 ***	48	-0.107	35	0.243	25
Age	0.017	100	-0.175	38	0.121	51	0.278	38	0.128	50	0.005	37	-0.187	25
a-HCH	-0.323 **	96	0.333	35	0.153	50	0.244	35	0.180	49	0.226	36	0.152	25
HCB	-0.108	98	-0.013	37	0.285 *	50	0.211	37	0.376 **	49	0.590 ***	38	0.267	25
g-HCH	0.385 ***	98	-0.175	37	0.078	50	0.066	37	-0.041	49	0.274	38	-0.054	25
PCB28	0.385 ***	98	0.250	37	0.272	50	-0.048	37	0.145	49	0.002	38	-0.002	25
PCB52	0.295 **	94	-0.049	34	0.168	50	0.123	33	0.218	49	0.252	35	-0.044	25
PCB101	0.506 ***	95	-0.013	35	0.111	50	-0.017	34	0.162	49	-0.013	35	0.106	25
PCB118	0.432 ***	98	0.034	37	0.150	50	0.267	37	-0.150	49	0.143	38	-0.328	25
PCB153	0.575 ***	98	-0.191	37	-0.024	50	0.019	37	-0.245	49	0.005	38	-0.219	25
PCB138	0.579 ***	98	-0.130	37	-0.044	50	0.071	37	-0.303 *	49	0.120	38	-0.161	25
PCB180	0.578 ***	98	-0.153	37	-0.021	50	-0.095	37	-0.265	49	-0.157	38	-0.387	25
Sum PCB	0.633 ***	98	-0.156	37	0.022	50	0.021	37	-0.259	49	0.009	38	-0.217	25
pp-DDE	0.437 ***	98	0.141	37	0.054	50	0.150	37	-0.177	49	0.043	38	-0.210	25
pp_DDT	0.428 ***	95	0.053	34	0.128	50	0.210	34	-0.052	49	0.220	35	-0.322	25
SUM DDE	0.459 ***	98	0.137	37	0.050	50	0.141	37	-0.191	49	0.057	38	-0.215	25
PC1	0.583 ***	98	-0.089	37	0.170	50	0.032	37	-0.129	49	0.084	38	-0.213	25
PC2	-0.194	98	0.108	37	0.228	50	0.341 *	37	0.212	49	0.669 ***	38	0.071	25

\*, p&lt;0.05; \*\*,p&lt;0.01; \*\*\*, p&lt;0.001

PC1, PC2, principal components 1 and 2