

Supplemental Materials:

Sex-Linked Changes in Biotransformation of Phenol in Brook Trout (*Salvelinus fontinalis*) over an Annual Reproductive Cycle

Richard C. Kolanczyk ^{1,*}, Laura E. Solem ^{2,†}, Mark A. Tapper ¹, Alex D. Hoffman ^{1,†}, Barbara R. Sheedy ^{1,†}, Patricia K. Schmieder ^{1,†} and James M. McKim III ^{1,‡}

¹ Great Lakes Toxicology and Ecology Division, Center for Computational Toxicology and Exposure, Office of Research and Development, United States Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804, USA

² National Research Council, 6201 Congdon Boulevard, Duluth, MN 55804, USA

* Correspondence: kolanczyk.rick@epa.gov; Tel.: +1-218-529-5152; Fax: +1-218-529-5003

† Retired.

‡ Deceased.

Table S1. Plasma Sex Steroids (Estradiol, Testosterone, and 11-Ketotestosterone as Reported Over a 7-Month Reproductive Cycle in the Brook Trout. Values are the mean \pm standard error of three fish.

	Estradiol (ng/mL plasma)		Testosterone (ng/mL plasma)		11-Ketotestosterone (ng/mL plasma)	
	Female	Male	Female	Male	Female	Male
June	5.51 \pm 1.06	0.00 \pm 0.00	1.94 \pm 0.24	2.60 \pm 0.36	0.00 \pm 0.00	3.70 \pm 0.55
July	14.77 \pm 2.65	0.00 \pm 0.00	4.28 \pm 0.55	2.68 \pm 0.28	0.00 \pm 0.00	4.20 \pm 0.39
August	24.62 \pm 5.91	0.00 \pm 0.00	9.04 \pm 0.70	5.46 \pm 1.69	1.02 \pm 0.05	6.51 \pm 1.53
September	52.64 \pm 2.69	0.00 \pm 0.00	18.86 \pm 4.46	13.51 \pm 0.35	1.91 \pm 0.30	33.71 \pm 7.53
October	52.87 \pm 5.80	0.00 \pm 0.00	42.14 \pm 4.43	27.64 \pm 1.23	1.87 \pm 0.09	66.14 \pm 3.82
November	8.20 \pm 2.67	0.00 \pm 0.00	25.02 \pm 17.72	14.35 \pm 4.98	1.79 \pm 0.29	25.97 \pm 8.93
December	6.38 \pm 3.29	0.00 \pm 0.00	3.20 \pm 1.30	14.19 \pm 1.11	0.56 \pm 0.56	26.61 \pm 0.95

Table S2. Hepatosomatic Index (HSI) and Gonadosomatic Index (GSI) as Reported Over a 7-Month Reproductive Cycle in the Brook Trout. Values are the mean \pm standard error of three fish.

	Female		Male	
	HSI	GSI	HSI	GSI
June	1.9 \pm 0.2	1.2 \pm 0.3	1.3 \pm 0.1	0.5 \pm 0.1
July	1.8 \pm 0.1	2.2 \pm 0.6	1.1 \pm 0	0.8 \pm 0.2
August	2.0 \pm 0.1	4.8 \pm 0.5	1.2 \pm 0.1	2.6 \pm 0.8
September	2.3 \pm 0.1	9.0 \pm 2.1	1.0 \pm 0.1	3.0 \pm 0.2
October	2.8 \pm 0.1	17.1 \pm 2.2	1.0 \pm 0	2.3 \pm 0.3
November	1.0 \pm 0	21.5 \pm 4.6	0.9 \pm 0.1	2.5 \pm 0.1
December	1.1 \pm 0.4	1.1 \pm 0.1	0.9 \pm 0.1	2.6 \pm 0.1

Table S3. Cytochrome P450 Protein Content Reported Over a 7-Month Reproductive Cycle in the Brook Trout. Values are the mean \pm standard error of three fish.

	(nmoles/mg microsomal protein)		(nmoles/total liver)	
	Female	Male	Female	Male
June	0.44 \pm 0.03	0.58 \pm 0.01	74.2 \pm 18.1	84.7 \pm 11.4
July	0.34 \pm 0.02	0.54 \pm 0.03	70.0 \pm 8.7	41.5 \pm 2.7
August	0.33 \pm 0.05	0.76 \pm 0.10	121.0 \pm 32.4	97.4 \pm 3.7
September	0.21 \pm 0.04	0.77 \pm 0.11	98.9 \pm 13.4	103.3 \pm 31.9
October	0.13 \pm 0.01	0.70 \pm 0.05	91.7 \pm 1.2	135.5 \pm 19.3
November	0.36 \pm 0.01	0.56 \pm 0.06	54.0 \pm 9.0	97.2 \pm 26.6
December	0.43 \pm 0.03	0.87 \pm 0.10	61.1 \pm 10.4	83.3 \pm 16.9

Table S4. EROD Activity as Reported Over a 7-Month Reproductive Cycle in the Brook Trout. Values are the mean \pm standard error of three fish.

	(pmoles/min/mg microsomal protein)		(pmoles/min/total liver)	
	Female	Male	Female	Male
June	4.83 \pm 0.95	6.28 \pm 2.08	741 \pm 18	837 \pm 224
July	2.03 \pm 0.33	12.21 \pm 2.23	409 \pm 42	958 \pm 224
August	1.47 \pm 0.18	7.21 \pm 0.78	543 \pm 134	954 \pm 144
September	0.97 \pm 0.08	5.93 \pm 0.41	460 \pm 19	769 \pm 157
October	1.19 \pm 0.17	4.51 \pm 0.84	818 \pm 92	836 \pm 102
November	3.49 \pm 0.83	4.47 \pm 0.23	493 \pm 61	755 \pm 113
December	2.83 \pm 0.32	6.83 \pm 2.10	417 \pm 109	589 \pm 83

Table S5. Rates of hydroquinone (HQ) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11 °C over a 7-month reproductive cycle from June to December. The Vmax values are fitted to the combined average rate \pm standard error of the 3 male and 3 female microsomal preparations at each Phenol concentration.

	Vmax (pmol HQ/min/mic. Protein)		Vmax (nmol HQ/min/total liver)	
	Female	Male	Female	Male
June	2244 \pm 331	938 \pm 81	352 \pm 17	138 \pm 29
July	923 \pm 92	991 \pm 404	187 \pm 14	80 \pm 38
August	1144 \pm 90	549 \pm 136	417 \pm 79	76 \pm 27
September	1512 \pm 418	300 \pm 88	702 \pm 170	40 \pm 14
October	1006 \pm 152	352 \pm 90	687 \pm 64	70 \pm 22
November	460 \pm 121	478 \pm 287	70 \pm 22	74 \pm 41
December	203 \pm 29	771 \pm 436	32 \pm 10	61 \pm 27

Table S6. Capacity parameter (Km) for hydroquinone (HQ) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11°C over a 7-month reproductive cycle from June to December. The Km values (mmol) are fitted to the combined average rate ± standard error of the 3 male and 3 female microsomal preparations at each phenol concentration.

	Km (mM)	
	Female	Male
June	173 ± 64	34 ± 12
July	87 ± 21	117 ± 68
August	45 ± 10	25 ± 5
September	127 ± 53	19 ± 3
October	72 ± 22	16 ± 9
November	64 ± 8	50 ± 17
December	4 ± 1	34 ± 18

Table S7. Rates of catechol (CAT) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11°C over a 7-month reproductive cycle from June to December. The Vmax values are fitted to the combined average rate ± standard error of the 3 male and 3 female microsomal preparations at each Phenol concentration.

	Vmax (pmol CAT/min/mic. Protein)		Vmax (nmol CAT/min/total liver)	
	Female	Male	Female	Male
June	216 ± 30	106 ± 12	34 ± 3	16 ± 3
July	192 ± 46	205 ± 80	37 ± 5	16 ± 8
August	142 ± 14	79 ± 13	51 ± 6	11 ± 3
September	123 ± 14	53 ± 7	58 ± 5	7 ± 2
October	132 ± 18	74 ± 11	91 ± 9	14 ± 3
November	111 ± 16	122 ± 32	16 ± 2	19 ± 4
December	123 ± 37	61 ± 8	20 ± 10	6 ± 2

Table S8. Capacity parameter (Km) for catechol (CAT) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11°C over a 7-month reproductive cycle from June to December. The Km values (mmol) are fitted to the combined average rate ± standard error of the 3 male and 3 female microsomal preparations at each phenol concentration.

	Km (mM)	
	Female	Male
June	37 ± 7	19 ± 5
July	37 ± 11	52 ± 20
August	17 ± 2	14 ± 1
September	20 ± 3	11 ± 1
October	25 ± 1	11 ± 1
November	19 ± 5	23 ± 7
December	15 ± 3	8 ± 2

Table S9. Rates of phenylglucuronide (PG) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11°C over a 7-month reproductive cycle from June to December. The Vmax values are fitted to the combined average rate ± standard error of the 3 male and 3 female microsomal preparations at each Phenol concentration.

	Vmax (pmol PG/min/mic. Protein)		Vmax (nmol PG/min/total liver)	
	Female	Male	Female	Male
June	1720 ± 189	1738 ± 279	274 ± 27	255 ± 55
July	1054 ± 21	2128 ± 241	217 ± 28	166 ± 30
August	669 ± 5	1938 ± 341	241 ± 31	246 ± 16
September	389 ± 90	1536 ± 169	180 ± 34	197 ± 36
October	200 ± 24	980 ± 67	136 ± 8	188 ± 23
November	735 ± 98	1124 ± 80	106 ± 8	193 ± 42
December	924 ± 77	1095 ± 249	134 ± 29	97 ± 4

Table S10. Capacity parameter (Km) for phenylglucuronide (PG) formation in adult male and female brook trout hepatic microsomes resulting from the incubation with phenol at 11°C over a 7-month reproductive cycle from June to December. The Km values (mmol) are fitted to the combined average rate ± standard error of the 3 male and 3 female microsomal preparations at each phenol concentration.

	Km (mM)	
	Female	Male
June	4.49 ± 1.49	5.97 ± 1.67
July	1.89 ± 0.26	8.61 ± 1.60
August	0.95 ± 0.18	5.98 ± 2.58
September	0.49 ± 0.25	4.60 ± 2.71
October	0 ± 0	1.62 ± 0.15
November	0.32 ± 0.10	1.29 ± 0.53
December	0.80 ± 0.13	1.25 ± 0.37