

Supplemental Material

Transgenic Zebrafish Reveal Tissue-Specific Differences in Estrogen Signaling in Response to Environmental Water Samples

Daniel A. Gorelick, Luke R. Iwanowicz, Alice L. Hung, Vicki S. Blazer, and Marnie E. Halpern

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Supplemental Material, Table S1. 2010 Susquehanna, Delaware, Allegheny and Shenandoah River Watershed

Passive Sampling Study.

Sample ID	Deployment date	Retrieval date	Total days	Location #	Location	N	W
1	4/21/10	6/1/10	41	SUS 5	Juniata River, Newport, Pennsylvania Susquehanna River at Mahantango	40.4910	-77.0995
2	4/21/10	6/1/10	41	SUS 6	Creek, Browns Island, Pennsylvania	40.6515	-76.9228
3	4/19/10	6/3/10	45	DE-1	Delaware River, Yardley, Pennsylvania	40.2514	-74.8414
4	4/28/10	6/7/10	40	AL-1	Allegheny River, Kittanning, Pennsylvania	40.8138	-79.5252
5	5/12/10	6/14/10	33	PSS 16	Cooks Creek, Virginia	38.3734	-78.9374
6	5/12/10	6/15/10	34	PSS 09	Naked Creek, Virginia	38.3088	-78.9226
7	5/13/10	6/15/10	33	PSS 15	Muddy Creek, Virginia	38.4308	-78.9806
8	5/13/10	6/15/10	33	PSS 10	Briery Branch, Virginia	38.3995	-79.0232
9	5/13/10	6/14/10	32	PSS 12	Long Glade Creek, Virginia	38.3773	-78.9806
10	5/13/10	6/14/10	32	PSS 11	Smith Creek (upstream), Virginia	38.5382	-78.7505
11	5/13/10	6/14/10	32	PSS 13	Linville Creek, Virginia	38.6141	-78.8004
12	5/13/10	6/14/10	32	PSS 14	Long Meadow Run, Virginia	38.6339	-78.7484
13	5/13/10	6/14/10	32	PSS 08	Smith Creek (downstream), Virginia	38.7116	-78.6416
14	5/13/10	6/14/10	32	PSS 06	Mill Creek, Virginia	38.7433	-78.6450
15	5/14/10	6/14/10	31	PSS 02	Gooney Run, Virginia	38.8669	-78.2477
16	5/14/10	6/14/10	31	PSS 07	Hawksbill Creek, Virginia	38.7083	-78.4564
17	5/14/10	6/15/10	32	PSS 03	Passage Creek, Virginia	38.8238	-78.4281
18	5/14/10	6/15/10	32	PSS 05	Stony Creek (downstream), Virginia	38.8210	-78.5550
19	5/14/10	6/15/10	32	PSS 04	Stony Creek (upstream), Virginia	38.8912	-78.6843

Supplemental Material, Table S2. Reporter activity in 3-4 day old 5xERE:GFP zebrafish in response to 2010 Susquehanna, Delaware, Allegheny and Shenandoah passive samples.

Sample ID	Location	E2Eq ^a	Dilution	Reporter activity	Total embryos (N) ^b
1	SUS 5	2.94	1:100	lethal	4
1	SUS 5	2.94	1:500	GFP heart valves, liver	5
1	SUS 5	2.94	1:1000	GFP-negative	15
2	SUS 6	3.13	1:100	lethal	4
2	SUS 6	3.13	1:500	GFP heart valves, liver	5
2	SUS 6	3.13	1:1000	GFP-negative	15
3 ^c	DE-1	1.58	1:100	lethal	4
3 ^c	DE-1	1.58	1:1000	GFP heart valves	4
3 ^c	DE-1	1.58	1:1000	GFP heart valves, liver	10
3 ^c	DE-1	1.58	1:1000 + 10 μ M ICI 182,780	GFP-negative	5
4 ^d	AL-1	0.78	1:100	lethal	4
4 ^d	AL-1	0.78	1:500	GFP-negative	5
4 ^d	AL-1	0.78	1:1000	GFP-negative	15
5	PSS 16	3.79	1:100	lethal	4
5	PSS 16	3.79	1:1000	GFP heart valves, liver	15
5	PSS 16	3.79	1:2000	GFP-negative	5
6 ^c	PSS 09	2.55	1:100	lethal	4
6 ^c	PSS 09	2.55	1:500	GFP heart valves	30
6 ^c	PSS 09	2.55	1:1000	GFP-negative	15
7 ^c	PSS 15	2.86	1:100	lethal	4
7 ^c	PSS 15	2.86	1:500	GFP heart valves, liver	5
7 ^c	PSS 15	2.86	1:1000	GFP heart valves	15
7 ^c	PSS 15	2.86	1:1000	GFP-negative	25
7 ^c	PSS 15	2.86	1:1000 + 10 μ M ICI 182,780	GFP-negative	5
8	PSS 10	1.18	1:100	GFP heart valves, liver	9
8	PSS 10	1.18	1:500	GFP-negative	5
8	PSS 10	1.18	1:1000	GFP-negative	15
9	PSS 12	5.26	1:100	lethal	4
9	PSS 12	5.26	1:500	GFP heart valves, liver	5
9	PSS 12	5.26	1:1000	GFP heart valves, liver	10
9	PSS 12	5.26	1:1000	GFP-negative	10
10	PSS 11	2.36	1:500	GFP heart valves	1
10	PSS 11	2.36	1:500	GFP heart valves, liver	3
10	PSS 11	2.36	1:1000	GFP-negative	15
11 ^c	PSS 13	2.85	1:1000	GFP heart valves, liver	10
11 ^c	PSS 13	2.85	1:2000	GFP heart valves	6
11 ^c	PSS 13	2.85	1:2000	GFP-negative	23
11 ^c	PSS 13	2.85	1:4000	GFP-negative	5
12 ^c	PSS 14	5.41	1:1000	GFP heart valves, liver	10
12 ^c	PSS 14	5.41	1:2000	GFP heart valves	13
12 ^c	PSS 14	5.41	1:2000	GFP heart valves, liver	1
12 ^c	PSS 14	5.41	1:2000	GFP-negative	15
12 ^c	PSS 14	5.41	1:4000	GFP-negative	5
13 ^d	PSS 08	0.76	1:500	GFP-negative	5
13 ^d	PSS 08	0.76	1:1000	GFP-negative	15

Sample ID	Location	E2Eq ^a	Dilution	Reporter activity	Total embryos (N) ^b
14	PSS 06	4.19	1:500	GFP liver	1
14	PSS 06	4.19	1:500	GFP heart valves, liver	3
14	PSS 06	4.19	1:1000	GFP-negative	15
15	PSS 02	1.28	1:500	GFP heart valves, liver	5
15	PSS 02	1.28	1:1000	GFP-negative	15
16	PSS 07	7.98	1:1000	GFP heart valves, liver	15
16	PSS 07	7.98	1:2000	GFP heart valves, liver	5
16	PSS 07	7.98	1:4000	GFP-negative	5
16	PSS 07	7.98	1:1000 + 10 μ M ICI 182,780	GFP-negative	5
17 ^d	PSS 03	0.54	1:500	GFP-negative	5
17 ^d	PSS 03	0.54	1:1000	GFP-negative	10
18	PSS 05	3.06	1:1000	GFP heart valves, liver	15
18	PSS 05	3.06	1:2000	GFP-negative	5
18	PSS 05	3.06	1:1000 + 10 μ M ICI 182,780	GFP-negative	5
19	PSS 04	0.82	1:500	GFP liver	1
19	PSS 04	0.82	1:500	GFP heart valves, liver	3
19	PSS 04	0.82	1:500	GFP-negative	25
19	PSS 04	0.82	1:1000	GFP-negative	15

^aEstradiol equivalents (ng/POCIS) from bioluminescent yeast estrogen receptor reporter assay (see “Methods” for details).

^bIndicates total number of embryos with indicated phenotype. ^cIndicates samples that preferentially activated the zebrafish reporter in heart valves. ^dIndicates samples that failed to activate the zebrafish reporter.

Supplemental Material, Movie S1. (NOTE: This movie is available as a separate Supplemental Material file that may be downloaded from the *EHP* website.) Estrogen receptors in heart valves respond to environmental estrogens. *Tg(5xERE:GFP)^{c262/c262}* larvae were exposed to POCIS sample 16 (Hawksbill Creek) diluted 1:500 at 2 days post fertilization. The following day, time-lapse images of GFP fluorescence in live larvae were captured for 30 seconds totaling 56 frames, 10 millisecond exposure per frame using a 20X / 0.75 NA objective. The movie plays at 5 frames per second. Note the fluorescence in the aortic and atrioventricular valves. Lateral view; anterior to the left, dorsal to the top.