Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Steroidal estrogen sources in a sewage-impacted coastal ocean

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Electronic Supplementary Information

Summary:

Figure S1. A selected reaction monitoring (SRM) chromatogram of Massachusetts Bay seawater spiked with 23 steroidal estrogens (500 pg L^{-1}) highlights the range of instrumental responses (where normalization level, NL, refers to the signal size at a relative abundance of 100) and the presence of matrix interferences (additional peaks) in certain SRM channels. Precursor/product transitions are shown to the right of each trace, and retention times (min) are shown at the apex of each analyte peak.

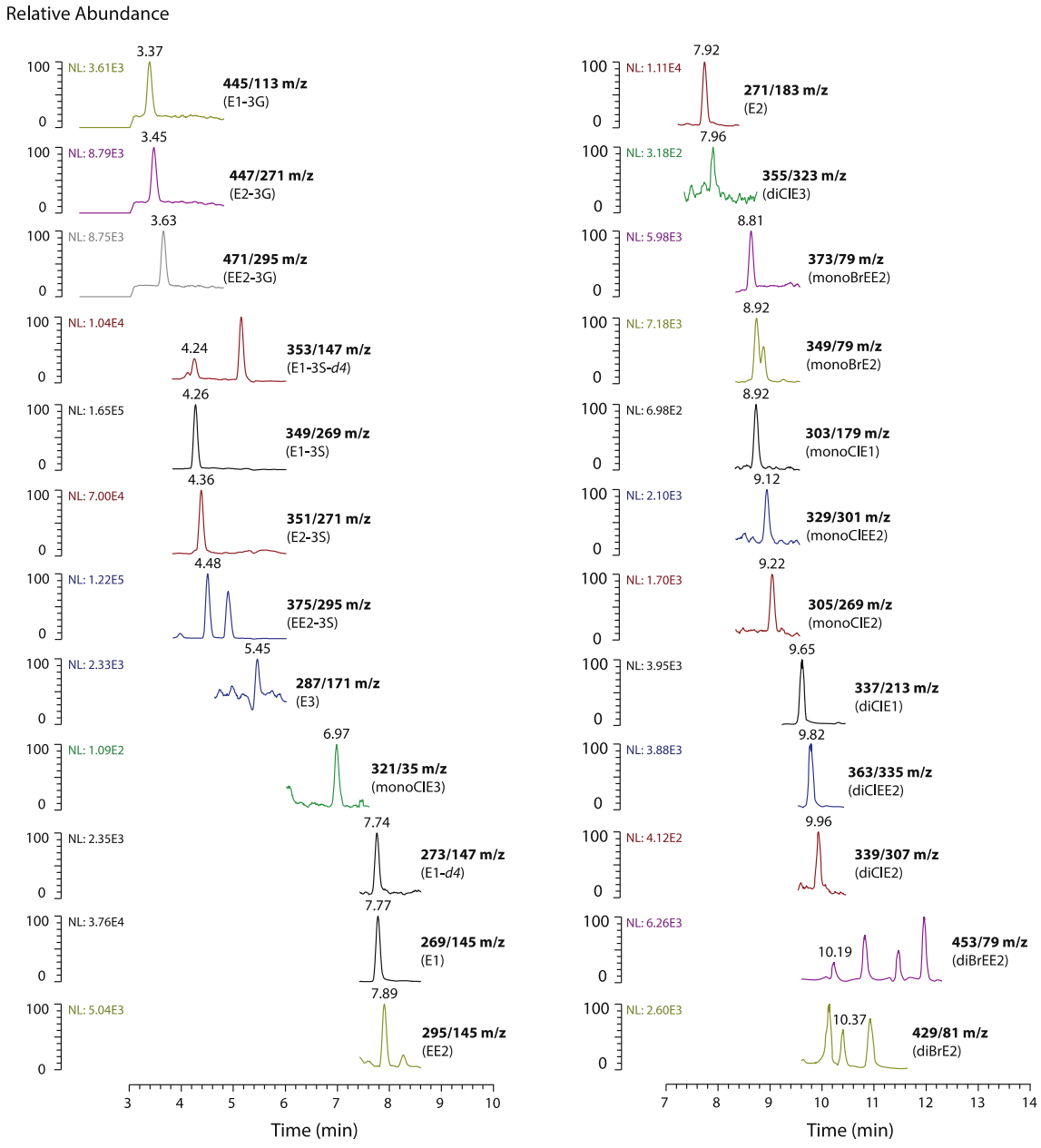

Figure S2. Depth profiles of temperature, salinity, dissolved oxygen, turbidity, and pH at the nearfield station PLM on 8 May 2013 (MB-1305a). Water samples for estrogen analysis were collected from 12 m depth at this station. Note the x-axis breaks and different horizontal scales.

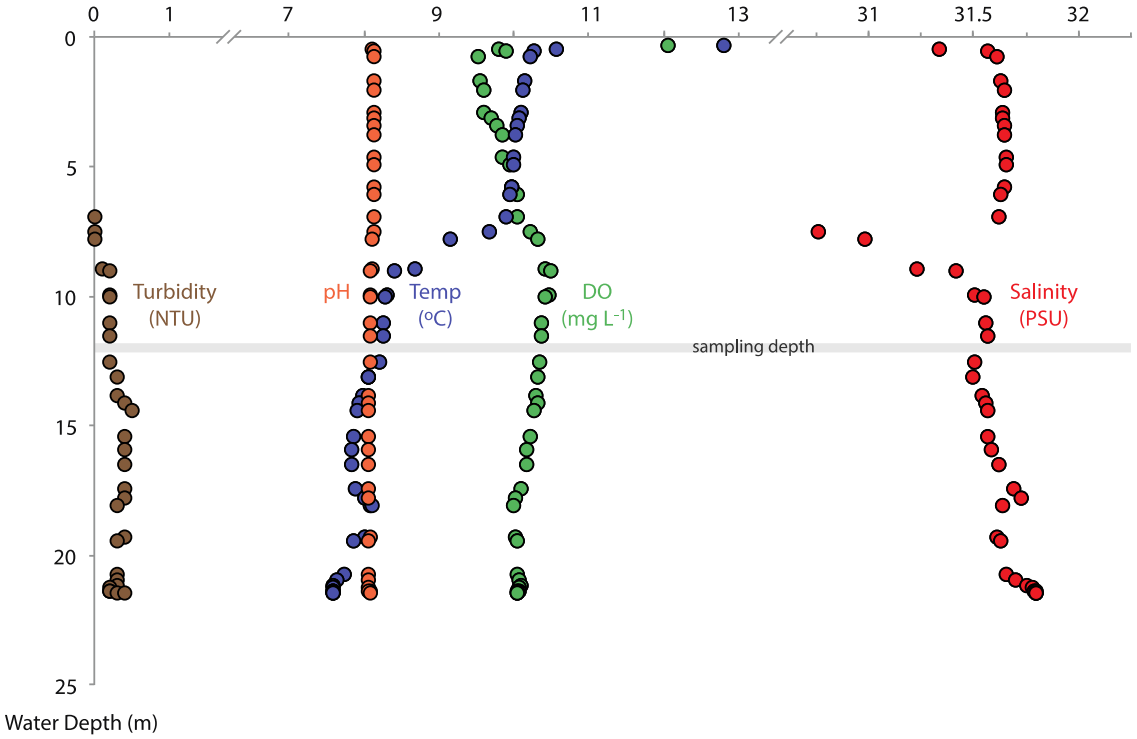

Figure S3. Estrogen concentrations in Massachusetts Bay at nearfield (PLM), down current (DS1 and DS2), up current (US), and Boston Harbor (BH) stations in May 2013 (MB-1305). Plot axis scales are identical to facilitate comparisons between stations. Note that the diClE2 at station PLM has been altered to show its full extent. The letters "nd" denote standard addition concentrations that were negative or could not be determined due to non-detects resulting in fewer than 2 spiking levels. Error bars show ± 1 standard deviation except for those that have been truncated since they extend into negative concentration space. See Table S9 for values.

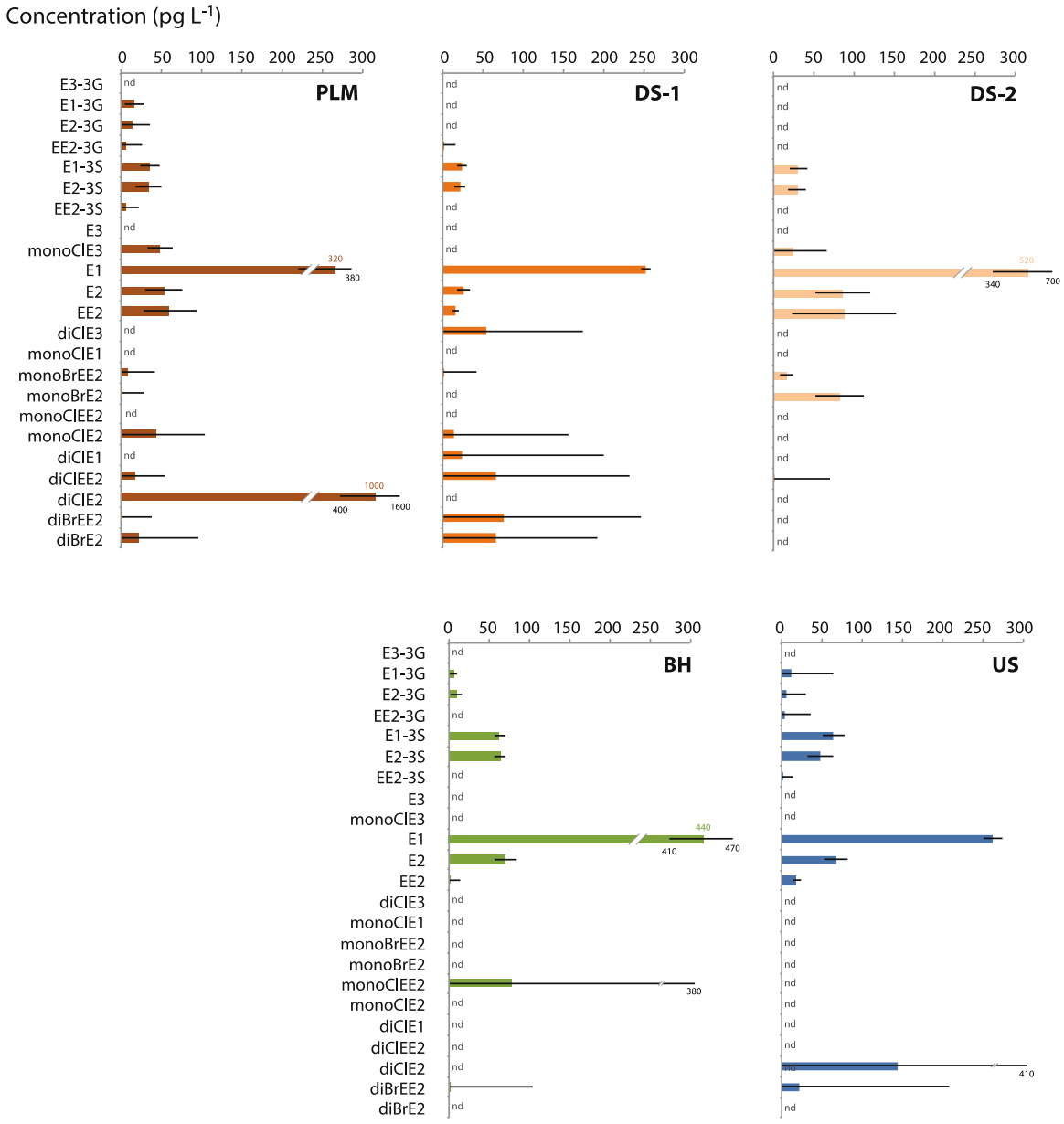

Figure S4. Sewage tracers in Massachusetts Bay (filled diamonds) were used to model dilution between station PLM and the down current stations (DS1 and DS2) in May 2013 (MB-1305). Variability within the set of four PLM samples highlights the challenge of collecting water from equivalent locations within an effluent plume discharged into a dynamic tidal system. The variability in carbamazepine and caffeine between replicate samples at DS1 and DS2 is much smaller than the size of the symbols.

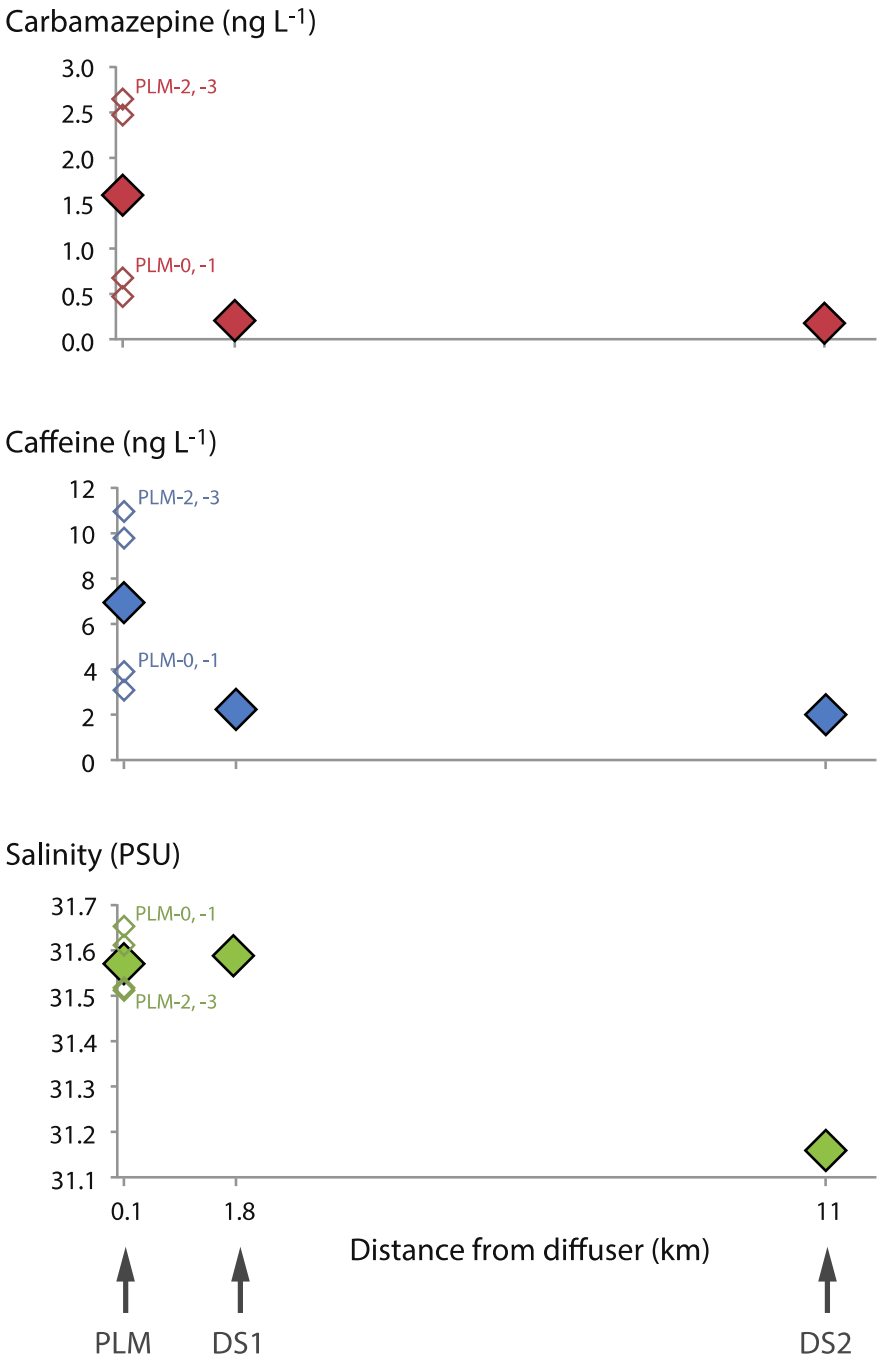

Table S1. Estrogen properties

Estrogen Name	Abbreviation	Molecular Weight	Melting Point (°C)[a]	$logK_{ow}$[a]	Aqueous Solubility (mg L^{-1})[a]	Henry's Constant (Pa m^3 mol^{-1})[a]	pK_a[b]	Estradiol Equivalents[c,i]		
								ER binding	YES	E-screen
estriol-3-glucuronide	E3-3G	464.52	294	0.56	999.7	3.00×10^{-16}	3.30	0	0	0
estrone-3-glucuronide	E1-3G	446.50	276	1.58	172.5	8.56×10^{-17}	3.30	0	0	0
17β-estradiol-3-glucuronide	E2-3G	448.52	277	2.10	61.3	8.20×10^{-15}	3.30	0	0	0
17α-ethynylestradiol-3-glucuronide	EE2-3G	472.54	285	2.27	30.7	1.79×10^{-15}	3.30	0	0	0
estrone-3-sulfate	E1-3S	350.43	214	0.95	959.8	2.06×10^{-7}	1.04	0	0	0
17β-estradiol-3-sulfate	E2-3S	352.45	216	1.46	341.4	1.98×10^{-8}	1.04	0	0	0
17α-ethynylestradiol-3-sulfate	EE2-3S	376.47	223	1.63	173.5	4.31×10^{-9}	1.04	0	0	0
estriol	E3	288.39	282[d]	2.45[e]	441[f]	1.35×10^{-7}	10.46[g]	0.3300	0.0240	0.3000
4-chloro-estriol	monoClE3	322.83	189	3.46	38.4	9.99×10^{-8}	8.97	0.0425	0.0031	0.0386
estrone	E1	270.37	255[d]	3.13[e]	30[f]	3.85×10^{-5}	10.77[h]	0.4400	0.3800	0.0100
17β-estradiol	E2	272.39	173-179[d]	4.01[e]	3.9[f]	3.69×10^{-6}	10.71[h]	1	1	1
17α-ethynylestradiol	EE2	296.41	183[d]	3.67[e]	11.3[f]	8.04×10^{-7}	10.40[i]	1.4000	1.1900	1.2500
2,4-dichloro-estriol	diClE3	357.28	198	4.10	6.7	7.40×10^{-8}	7.42	0.0007	0.0001	0.0007
4-chloro-estrone	monoClE1	304.82	170	4.08	14.5	2.85×10^{-5}	8.92	0.0567	0.0490	0.0013
2-bromo-17α-ethynylestradiol	monoBrEE2	375.31	185	5.01	2.8	3.20×10^{-7}	8.97	0.0552	0.0469	0.0493
2-bromo-17β-estradiol	monoBrE2	351.29	178	4.83	5.6	1.47×10^{-6}	8.99	0.0394	0.0394	0.0394
4-chloro-17α-ethynylestradiol	monoClEE2	330.86	180	4.76	8.5	5.96×10^{-7}	8.95	0.1804	0.1533	0.1610
4-chloro-17β-estradiol	monoClE2	306.84	170	4.59	16.7	2.73×10^{-6}	8.98	0.1288	0.1288	0.1288
2,4-dichloro-estrone	diClE1	339.26	180	4.72	2.5	2.11×10^{-5}	7.37	0.0010	0.0009	0.0000
2,4-dichloro-17α-ethynylestradiol	diClEE2	365.30	189	5.40	1.5	4.42×10^{-7}	7.40	0.0031	0.0027	0.0028
2,4-dichloro-17β-estradiol	diClE2	341.28	181	5.23	2.9	2.02×10^{-6}	7.43	0.0022	0.0022	0.0022
2,4-dibromo-17α-ethynylestradiol	diBrEE2	454.20	200	5.90	0.16	1.28×10^{-7}	7.47	0.0001	0.0001	0.0001
2,4-dibromo-17β-estradiol	diBrE2	430.18	192	5.72	0.32	5.85×10^{-7}	7.50	0.0001	0.0001	0.0001

[a](US EPA 2013);[1] [b](Hilal et al. 2003);[2] [c](Lee et al. 2008, Liu et al. 2009);[3, 4] [d](reported by manufacturer); [e](Hansch et al. 1995);[5] [f](Yalkowsky and Dannenfelser 1992);[6] [g](Eger et al. 1972);[7] [h](Lewis and Archer 1979);[8] [i](Hurwitz and Liu 1977);[9] [j](conjugates assumed to have zero estrogenicity)

Table S2. Station locations during the October 2012 (MB-1210) and May 2013 (MB-1305) field campaigns, and the location of the Deer Island Treatment Plant diffuser line.

Cruise ID	Station ID	Latitude	Longitude	Sampling Depth (m)
MB-1210	BKGD	42.3957	-70.7954	1
MB-1210	PLM-0	42.3830	-70.7995	12
MB-1210	PLM-1	42.3830	-70.7985	12
MB-1210	PLM-2	42.3841	-70.7977	12
MB-1210	PLM-3	42.3855	-70.7981	12
MB-1305a	PLM-0	42.3825	-70.8110	12
MB-1305a	PLM-1	42.3927	-70.7767	12
MB-1305a	PLM-2	42.3943	-70.7743	12
MB-1305a	PLM-3	42.3945	-70.7742	12
MB-1305b	DS1	42.3791	-70.7745	12
MB-1305b	DS2	42.3766	-70.6647	10
MB-1305c	US	42.4315	-70.8288	12
MB-1305c	BH	42.3296	-70.9734	5
DITP	Diffuser West	42.3843	-70.8038	34
DITP	Diffuser East	42.3889	-70.7801	34

Table S3. Estrogen UHPLC-MS/MS analytical method parameters.

Analyte	RT[a] (min)	Precursor (*m/z*)	Product (*m/z*)	Q = Quant ion	SRM[b] collision E	S-lens	Polarity
E3-3G	1.06	463	113		21	87	neg
		463	287	Q	42	87	neg
E1-3G	2.86	445	113	Q	21	90	neg
		445	269		40	90	neg
E2-3G	3.07	447	113		21	92	neg
		447	271	Q	42	92	neg
EE2-3G	3.21	471	113		22	78	neg
		471	295	Q	39	79	neg
E1-3S	3.83	349	145		55	79	neg
		349	269	Q	33	79	neg
E2-3S	4.07	351	145		56	79	neg
		351	271	Q	35	79	neg
E1-3S-*d4*	3.82	353	147	Q	55	73	neg
		353	273		33	73	neg
EE2-3S	4.17	375	145		58	85	neg
		375	295	Q	34	85	neg
E3	5.15	287	143		54	80	neg
		287	171	Q	37	80	neg
monoClE3	6.72	321	35	Q	31	81	neg
		321	285		30	81	neg
E1	7.4	269	143		55	50	neg
		269	145	Q	39	50	neg
E2	7.64	271	145		41	50	neg
		271	183	Q	42	50	neg
E1-*d4*	7.38	273	145		57	77	neg
		273	147	Q	39	77	neg
EE2	7.55	295	145	Q	40	50	neg
		295	159		36	50	neg
diClE3	7.68	355	323	Q	39	78	neg
		357	325		39	78	neg
monoClE1	8.46	303	179	Q	40	50	neg
		303	267		29	50	neg
monoClEE2	8.7	329	293		30	50	neg
		329	301	Q	26	50	neg
monoBrE2	8.55	349	79	Q	39	88	neg
		351	81		38	88	neg
monoBrEE2	8.4	373	79	Q	38	87	neg
		375	81		39	79	neg
monoClE2	8.83	305	35		27	76	neg
		305	269	Q	31	76	neg
diClE1	9.14	337	213	Q	41	50	neg
		339	215		40	50	neg
diClE2	9.53	339	307	Q	38	85	neg
		341	309		39	84	neg
diClEE2	9.34	363	335	Q	29	83	neg
		365	337		29	82	neg
diBrE2	9.91	429	79		44	50	neg
		429	81	Q	42	50	neg
diBrEE2	9.69	453	79	Q	46	78	neg
		453	81		45	78	neg

[a] RT: retention time
[b] SRM: selected reaction monitoring

Table S4. Sewage tracer UHPLC-MS/MS analytical method parameters.

Analyte	RT[a] (min)	Precursor (*m/z*)	Product (*m/z*)	Q = Quant ion	SRM[b] collision E	S-lens	Polarity
caffeine	3.05	195	110		22	91	pos
		195	138	Q	18	91	pos
carbamazepine	7.03	237	179		33	77	pos
		237	194	Q	18	77	pos
carbamazepine-*d10*	6.95	247	187	Q	36	83	pos
		247	204		21	83	pos

[a] RT: retention time
[b] SRM: selected reaction monitoring

Table S5. The characteristics of Massachusetts Bay water (sampled for estrogen analysis) were determined by an on-board multi-probe sensor in October 2012 (MB-1210) and May 2013 (MB-1305).

Cruise ID	Station ID	Depth (m)	Salinity (‰)	Temperature (°C)	Dissolved Oxygen (mg L^{-1})	pH
MB-1210	BKGD	1	32.45	13.00	8.60	8.20
MB-1210	PLM-0	12	32.51	13.05	8.72	8.24
MB-1210	PLM-1	12	32.49	13.06	8.09	8.25
MB-1210	PLM-2	12	32.49	13.05	11.25	8.24
MB-1210	PLM-3	12	32.50	13.05	9.36	8.23
MB-1305a	PLM-0	12	31.61	9.63	10.70	8.06
MB-1305a	PLM-1	12	31.65	8.74	10.71	8.11
MB-1305a	PLM-2	12	31.52	8.26	10.54	8.08
MB-1305a	PLM-3	12	31.51	8.20	10.35	8.08
MB-1305b	DS1	12	31.59	9.53	10.36	8.16
MB-1305b	DS2	10	31.16	10.32	10.41	8.17
MB-1305c	US	12	31.74	9.84	10.24	7.98
MB-1305c	BH	5	31.31	11.20	9.21	8.00

Table S6. Nutrient measurements in Massachusetts Bay samples collected in October 2012 (MB-1210) and May 2103 (MB-1305).

Cruise ID	Station ID	Depth (m)	NH_4^+ (μM)	SiO_4^{4-} (μM)	PO_4^{3-} (μM)	$NO_2^- + NO_3^-$ (μM)
MB-1210	BKGD	1	<0.05	3.2	0.2	0.2
MB-1210	PLM-0	12	<0.05	2.7	0.2	0.1
MB-1210	PLM-1	12	<0.05	2.4	0.1	0.1
MB-1210	PLM-2	12	<0.05	2.3	0.1	<0.05
MB-1210	PLM-3	12	<0.05	2.3	0.1	<0.05
MB-1305a	PLM-0	12	1.9	4.4	<0.05	<0.05
MB-1305a	PLM-1	12	<0.05	3.6	<0.05	<0.05
MB-1305a	PLM-2	12	9.9	4.3	0.1	0.8
MB-1305a	PLM-3	12	14.7	4.8	0.3	1.0
MB-1305b	DS1	12	<0.05	3.5	<0.05	<0.05
MB-1305b	DS2	10	<0.05	3.0	<0.05	<0.05
MB-1305c	US	12	3.0	2.7	<0.05	0.3
MB-1305c	BH	5	0.6	4.1	<0.05	0.5
Mass Bay background[a]			3.6			
DITP final effluent[a]			1860			

< indicates values below the detection limit
[a] (Hunt et al. 2010)[10]

Table S7. Measurements of organic carbon and nitrogen concentrations and isotopic ratios in Massachusetts Bay samples collected in October 2012 (MB-1210) and May 2013 (MB-1305).

Cruise ID	Station ID	Depth (m)	DOC (mg L^{-1})	POC (mg L^{-1})	$\delta^{13}C_{POC}$[a] (‰)[b]	PON (μg L^{-1})	$\delta^{15}N_{PON}$[a] (‰)[c]
MB-1210	BKGD	1	n/d	0.33	-19.9	50.1	3.7
MB-1210	PLM-0	12	n/d	0.26	-21.0	38.4	4.3
MB-1210	PLM-1	12	n/d	0.31	-19.1	45.4	5.3
MB-1210	PLM-2	12	n/d	0.40	-20.6	52.9	5.3
MB-1210	PLM-3	12	n/d	0.38	-19.4	49.4	5.2
MB-1305a	PLM-0	12	1.3	0.21	-22.4	43.4	3.7
MB-1305a	PLM-1	12	1.7	0.11	-22.2	13.2	3.9
MB-1305a	PLM-2	12	1.4	0.08	-24.4	14.1	5.0
MB-1305a	PLM-3	12	1.5	0.04	-26.1	4.2	nd
MB-1305b	DS1	12	1.7	0.14	-22.5	28.4	1.3
MB-1305b	DS2	10	1.6	0.15	-24.4	26.6	4.7
MB-1305c	US	12	1.6	0.20	-21.2	41.6	4.1
MB-1305c	BH	5	1.5	0.18	-23.5	35.9	4.9
Mass Bay background[d]			1.2	0.17			

[a] measured by gas chromatography-isotope ratio mass spectrometry at the MBL Stable Isotope Laboratory
[b] versus PDB
[c] versus AIR
[d] (Gustafsson et al. 2001)[11]
n/d: not determined

Table S8. Estrogen concentrations in Deer Island Treatment Plant wastewater effluent (grab, 24-h composite) collected on 26 October 2012 (DI-1210) and 16 May 2013 (DI-1305) to coincide with Massachusetts Bay samples (MB-1210 and MB-1305). See Griffith et al.[12] for method limit thresholds (L_C, L_D, and L_Q).

	(ng L^{-1} effluent)					
	DI-1210 GRAB		DI-1305 GRAB		DI-1305 COMP	
Analyte	AVG	STDEV	AVG	STDEV	AVG	STDEV
E3-3G	n/d	n/d	n/d	n/d	n/d	n/d
E1-3G	n/d	n/d	12.2	1.8	10.3	1.6
E2-3G	n/d	n/d	n/d	n/d	n/d	n/d
EE2-3G	0.96	0.06	n/d	n/d	n/d	n/d
E1-3S	0.78[c]	0.03	0.62[c]	0.19	1.27	0.05
E2-3S	0.22[b]	0.03	0.23[b]	0.06	0.25[b]	0.07
EE2-3S	0.26[c]	0.025	n/d	n/d	n/d	n/d
E3	0.72	0.15	5.3	0.7	4.6	0.6
monoClE3	0.32[c]	0.09	n/d	n/d	n/d	n/d
E1	12.9	0.15	18	4	13.2	1.6
E2	5.5	0.4	11	3	3.7	1.6
EE2	n/d	n/d	30.	7	24.3	2.4
diClE3	n/d	n/d	n/d	n/d	n/d	n/d
monoClE1	n/d	n/d	n/d	n/d	n/d	n/d
monoBrEE2	n/d	n/d	n/d	n/d	n/d	n/d
monoBrE2	n/d	n/d	n/d	n/d	n/d	n/d
monoClEE2	n/d	n/d	n/d	n/d	n/d	n/d
monoClE2	n/d	n/d	n/d	n/d	n/d	n/d
diClE1	n/d	n/d	n/d	n/d	n/d	n/d
diClEE2	n/d	n/d	n/d	n/d	n/d	n/d
diClE2	6.2[c]	0.9	36	12	26	4
diBrEE2	6.17[b]	0.19	n/d	n/d	n/d	n/d
diBrE2	n/d	n/d	11	4	n/d	n/d

[a] less than L_C
[b] less than L_D
[c] less than L_Q
n/d: indicates that no peak was found

Table S9. Estrogen concentrations in Massachusetts Bay at the nearfield (PLM) station on 17 October 2012 (MB-1210) and at the nearfield (PLM), down current (DS1 and DS2), up current (US), and Boston Harbor (BH) stations in May 2013 (MB-1305a,b,c). Concentrations (Qmsa) were calculated by the method of standard addition, and standard deviations reflect propagated uncertainty in the standard addition relationship.

	(pg L^{-1} seawater)											
	MB-1210		MB-1305a,b,c									
	PLM		PLM		DS1		DS2		US		BH	
Analyte	Qmsa	STDEV	Qmsa	STDEV	Qmsa	STDEV	Qmsa	STDEV	Qmsa	STDEV	Qmsa	STDEV
E3-3G	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
E1-3G	17*	13	17*	12	n/d	n/d	n/d	n/d	10*	50	5*	5
E2-3G	13*	5	14*	23	n/d	n/d	n/d	n/d	5*	24	10*	7
EE2-3G	28	6	6*	20	1*	16	n/d	n/d	4*	30	n/d	n/d
E1-3S	29	6	36	12	24	6	31	11	64	13	63	6
E2-3S	52	8	34*	17	21	7	29*	12	48	16	64	7
EE2-3S	12	4	6*	16	n/d	n/d	n/d	n/d	1*	13	n/d	n/d
E3	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
monoClE3	n/d	n/d	48*	15	n/d	n/d	20*	40	n/d	n/d	n/d	n/d
E1	86	9	320	60	253	6	520	180	263	12	440	30
E2	28*	15	53	23	26	8	90*	30	68	15	70	13
EE2	20*	9	60*	30	16*	4	90*	70	18*	5	0*	15
diClE3	n/d	n/d	n/d	n/d	55*	120	n/d	n/d	n/d	n/d	n/d	n/d
monoClE1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
monoBrEE2	41	13	10*	30	2*	40	16*	8	n/d	n/d	n/d	n/d
monoBrE2	9*	9	3*	26	n/d	n/d	80*	30	n/d	n/d	n/d	n/d
monoClEE2	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	80*	300
monoClE2	n/d	n/d	40*	60	10*	140	n/d	n/d	n/d	n/d	n/d	n/d
diClE1	15*	17	n/d	n/d	30*	180	n/d	n/d	n/d	n/d	n/d	n/d
diClEE2	21*	10	20*	40	70*	170	1*	70	n/d	n/d	n/d	n/d
diClE2	44	16	1000*	600	n/d	n/d	n/d	n/d	150*	260	n/d	n/d
diBrEE2	32*	20	2*	40	80*	170	n/d	n/d	20*	190	1*	100
diBrE2	54*	29	20*	80	70*	130	n/d	n/d	n/d	n/d	n/d	n/d

n/d: indicates that standard addition concentrations were negative or could not be determined due to non-detects resulting in fewer than 2 spiking levels.
*: indicates values that were statistically indistinguishable from zero based on a one-sample *t*-test ($\alpha = 0.05$).

Table S10. Estrone (E1) mass balance model and parameters for Massachusetts Bay

Symbol	Description	Value	Source
C_w	Steady state E1 concentration dissolved in MA Bay	Calculated	Equation S2
Q_{in}	Combined inputs of E1 to MA Bay from WWTPs, Boston Harbor, and conversions of E2 and conjugated estrogens	Calculated and variable	Sum of individual inputs (see below)
C_{DITP}	Concentration of E1 in Deer Island WWTP effluent	14.4 ng L^{-1}	This study;[12]; average of 18 grab and composite samples from March 2012 - May 2013 (SD = 2.9 ng L^{-1})
$Q_{w,DITP}$	Average flow of Deer Island WWTP effluent	360 MGD*	[13]
C_{SETP}	Concentration of E1 in South Essex WWTP effluent	10 ng L^{-1}	Estimate based on DITP effluent and literature averages
$Q_{w,SETP}$	Average flow of South Essex WWTP effluent	30 MGD	EPA Envirofacts website (www3.epa.gov/enviro/)
$C_{E1,BH}$	Concentration of E1 in Boston Harbor	0.44 ng L^{-1}	This study
$C_{E2,BH}$	Concentration of E2 in Boston Harbor (expressed as E1 equivalents)	0.070 ng L^{-1}	This study
$C_{E1conj,BH}$	Concentration of E1 conjugates in Boston Harbor (expressed as E1 equivalents)	0.107 ng L^{-1}	This study
$C_{E2,DITP}$	Concentration of E2 in Deer Island WWTP effluent (expressed as E1 equivalents)	6.4 ng L^{-1}	This study;[12]; average of 18 grab and composite samples from March 2012 - May 2013 (SD = 1.7 ng L^{-1})
$C_{E1conj,DITP}$	Concentration of E1 conjugates in Deer Island WWTP effluent (expressed as E1 equivalents)	8.5 ng L^{-1}	This study;[12]; average of 18 grab and composite samples from March 2012 - May 2013 (SD = 2.4 ng L^{-1})
V_{bay}	Volume of MA Bay	1.28×10^{11} m^3	A_{bay} and D_{avg}
A_{bay}	Surface area of MA Bay	3.2×10^9 m^2	[11]
D_{avg}	Average depth of MA Bay	40 m	[14]
V_{BH}	Volume of Boston Harbor	1.65×10^9 m^3	A_{BH} and D_{BH}
A_{BH}	Surface area of Boston Harbor	3.3×10^8 m^2	[11]
D_{BH}	Average depth of Boston Harbor	5 m	[15]
k_{flush}	Rate constant for flushing of MA Bay	12 y^{-1}	[11]
k_{deg}	Rate constant for biodegradation in MA Bay	10 y^{-1}	Estimate based on aerobic E_2 biodegradation in marine sediment[16]
$k_{flush,BH}$	Rate constant for flushing of Boston Harbor	36 y^{-1}	[11]
s	Sedimentation rate	0.31 cm y^{-1}	[17]
Φ	Porosity of surface sediments	0.73	[17]
ρ_s	Solid sediment density	2.5 g cm^{-3}	[18]
$[TSS]$	Total suspended solid concentration	1 mg L^{-1}	[19]
C_{tot}	Total concentration including both dissolved and particulate phases	nd†	$C_{tot} = C_w + C_s$
C_s	Steady state concentration on solids	nd†	$C_s = f_s C_{tot}$
f_s	Fraction of E1 in the particulate (solid) phase	5×10^{-6}	$f_s = \frac{C_s M_s}{(C_s M_s + C_w V_w)} = \frac{K_d[TSS]}{1 + K_d[TSS]}$
f_w	Fraction of E1 in the dissolved (water) phase	0.999995	$f_w = \frac{C_w V_w}{(C_s M_s + C_w V_w)} = \frac{1}{1 + K_d[TSS]}$
K_d	Solid-water distribution coefficient	~ 5 L kg^{-1}	Estimate based on upper Mississippi River sediment[20]

*MGD = million gallons per day; †nd = not determined

The following expression was used to estimate the steady state concentration of dissolved estrone (E1) in Massachusetts Bay assuming a single well-mixed box at steady state. The only input of E1 is Deer Island WWTP effluent, and removal occurs by advection (flushing into the Gulf of Maine), biodegradation, and sedimentation. Calculations suggest that removal by sedimentation will be negligible.

$$\frac{dC_w}{dt} = \frac{Q_{in}}{V_{bay}} - k_{flush}C_{tot} - \frac{s(1-\Phi)\rho_s A_{bay}}{[TSS]V_{bay}} C_s - k_{\deg}C_{tot} \qquad \text{(S1)}$$

C_{tot} is the total E1 concentration in MA bay, and C_w and C_s are those portions of E1 in the dissolved and particulate phases respectively. After C_{tot} and C_s are expressed in terms of C_w and steady state is assumed, the solution for C_w yields:

$$C_w = \frac{Q_{in}/V_{bay}}{1/f_w\,(k_{flush} + s(1-\Phi)\rho_s f_s(1/[TSS]D_{avg}) + k_{\deg})} \qquad \text{(S2)}$$

Table S11. Modeled steady state estrone (E1) concentrations in Massachusetts Bay under different scenarios.

Parameter*	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7
D_{avg} (m)	40	10	10	10	40	10	40
k_{deg} (y^{-1})	10	0	0	0	10	0	10
C_{DITP} (ng L^{-1})	14.4	14.4	200	14.4	14.4	14.4	14.4
C_{SETP} (ng L^{-1})	0	0	0	10	10	10	10
$C_{E1,BH}$ (pg L^{-1})	0	0	0	440	440	440	440
$C_{E1conj,DITP}$ (ng L^{-1})	0	0	0	8.5	8.5	8.5	8.5
$C_{E2,DITP}$ (ng L^{-1})	0	0	0	6.4	6.4	6.4	6.4
$C_{E1conj,BH}$ (pg L^{-1})	0	0	0	107	107	107	107
$C_{E2,BH}$ (pg L^{-1})	0	0	0	70	70	70	70
$Q_{additional}$ (kg y^{-1})	0	0	0	0	0	50	700
C_w (pg L^{-1})	2.5	18.7	259	134	18.3	264.2	266.9

*Those model parameters not listed above are the same for each model set, and their set values are given in Table S10.

Table S12. Sensitivity of modeled steady state E1 concentration (C_w) to changing each parameter individually by ± 50% under two different model scenarios.

Model Parameters[a]		Set 6[b] Model Result		Set 7[b] Model Result	
Symbol	% Change	C_w (pg L^{-1})	% Change[c]	C_w (pg L^{-1})	% Change[d]
A_{bay}	–50	528	+100	534	+100
	+50	176	–33	178	–33
D_{avg}	–50	528	+100	534	+100
	+50	176	–33	178	–33
$[TSS]$	–50	264	+0.0002	267	+0.0002
	+50	264	–0.0002	267	–0.0002
Φ	–50	264	–0.01	267	–0.002
	+50	264	+0.01	267	+0.002
ρ_s	–50	264	+0.004	267	+0.0006
	+50	264	–0.004	267	–0.0006
s	–50	264	+0.004	267	+0.0006
	+50	264	–0.004	267	–0.0006
k_{flush}	–50	528	+100	367	+37
	+50	176	–33	210	–21
k_{deg}	–50	264	0	345	+29
	+50	264	0	217	–19
C_{DITP}	–50	255	–4	266	–0.5
	+50	274	+4	268	+0.5
$Q_{w,DITP}$	–50	245	–7	264	–1
	+50	283	+7	269	+1
K_d	–50	264	+0.005	267	+0.0008
	+50	264	–0.005	267	–0.0008
C_{SETP}	–50	264	–0.2	267	–0.03
	+50	265	+0.2	267	+0.03
$Q_{w,SETP}$	–50	264	–0.2	267	–0.03
	+50	265	+0.2	267	+0.03
$k_{flush,BH}$	–50	217	–18	260	–2
	+50	312	+18	273	+2
$C_{E1,BH}$	–50	230	–13	262	–2
	+50	298	+13	271	+2
A_{BH}	–50	217	–18	260	–2
	+50	312	+18	273	+2
D_{BH}	–50	217	–18	260	–2
	+50	312	+18	273	+2
$Q_{additional}$	–50	199	–25	143	–47
	+50	329	+25	391	+47
$C_{E1conj,DITP}$	–50	259	–2	266	–0.3
	+50	270	+2	268	+0.3
$C_{E2,DITP}$	–50	260	–2	266	–0.2
	+50	268	+2	267	+0.2
$C_{E1conj,BH}$	–50	256	–3	266	–0.4
	+50	272	+3	268	+0.4
$C_{E2,BH}$	–50	259	–2	266	–0.3
	+50	270	+2	268	+0.3

[a] see Table S10 for a description of model parameters and their symbols
[b] see Table S11 for the parameter values associated with Set 6 and Set 7
[c] compared to C_w = 264.2 pg L^{-1} (Set 6)
[d] compared to C_w = 266.9 pg L^{-1} (Set 7)

Table S13. Summary of coastal ocean estrogen concentrations

Location	E1 (ng L_w^{-1})	E2 (ng L_w^{-1})	E3 (ng L_w^{-1})	EE2 (ng L_w^{-1})	E1-3S (ng L_w^{-1})	Reference
Kaneohe Bay, HI¥	0.04-0.6					21
N. Pacific¥	0.052					22
Biosphere 2 ocean¥	0.066					22
Fr. Polynesia¥	0.17					22
S. Molokai¥	0.12					22
Maui (n=70)¥	0.16					22
Oahu¥	0.58					22
Florida Keys¥	0.26					22
Tinian Is.¥	0.31					22
Tern Is.¥	0.35					22
Guam (resorts)¥	0.48-0.71					22
Moorea (resort)¥	0.61					22
Key Largo Shore¥	0.85					22
Maalaea Bay¥	0.69					22
Big Pine Key¥	0.66					22
Key West¥	0.81					22
Rehoboth Bay¥	1.87					22
Key West Harbor¥	1.58					22
Boston Harbor‡				<74		23
Jamaica Bay, NY‡	0.07-2.52§	0.05-0.53§				24
Tokyo Bay‡	0.05-3.60§	<0.07-0.59§			<0.03-0.05§	25
LA outfall site‡	0.6§	0.3§				26
San Diego outfall‡	<0.03§	0.3§				26
Orange Co. outfall‡	<0.03§	0.45§				26
Southern CA Bight‡	<0.03§	0.16§				26
Halifax Harbor‡	4.0-6.6	<0.10-0.57		<0.14; 0.21§		27, 28
St. Johns Harbor‡	1.4-1.5	<0.5-1.8		<1.2		28
Sydney outfall‡	0.16-1.17§	0.22-2.48§		<0.05-0.5§		29
Baltic Sea‡	0.10-0.53	<0.30	<1.0	<0.45-17.9		30, 31
Cape Cod ponds‡	ND-4.6	<2.0-2.2				32
W. Australia coral¥		0.55-4.2				33
Acushnet estuary‡	0.78-1.2	0.56-0.83		3.01-4.57		34
Biobio, Chile‡	0.06-14.5§	0.06-16.8§	0.04-53§	4.18-48.14§		35
Xiamen Bay, China‡	1.1-7.4§	1.0-2.4§		<1.3-2.2§		36
Massachusetts Bay‡	0.09-0.52	0.03-0.09		0.02-0.09	0.02-0.07	this study
Boston Harbor‡	0.44	0.07		0.0004	0.063	this study

¥ method: radioimmunoassay or bioassay
‡ method: LC-MS, GC-MS, or LC-MS/MS
§concentration reported for sediments in ng g^{-1}
see also: (Pinto et al. 2005),[37] (da Silva et al. In Press),[38] (Legler et al. 2003),[39] (Schipper et al. 2009),[40] and (Hashimoto et al. 2005)[41]

Background E1 concentrations: Two end member mixing model calculations

It is possible that dilution-based estimates of background estrogen concentrations in Massachusetts Bay would be sensitive to our assumptions about tracer concentration in ambient (background) water. Therefore, we determined the maximum range of background E1 levels possible for each dilution step (e.g., from DITP - PLM, PLM - DS1, and DS1 - DS2) by allowing background carbamazepine to vary and assuming conservative mixing according to Equations S3 and S4,

$$C_{i,\text{down}} = f_{\text{up}} C_{i,\text{up}} + f_{\text{bkgrd}} C_{i,\text{bkgrd}} \quad \text{(S3)}$$

$$1 = f_{\text{up}} + f_{\text{bkgrd}} \quad \text{(S4)}$$

where i represents either carbamazepine (CBZ) or estrone (E1), and the down current water parcel (e.g., DS2) is a binary mixture of the up current water parcel (e.g., DS1) and background water according to concentrations ($C_{i,\text{down}}$, $C_{i,\text{up}}$, and $C_{i,\text{bkgrd}}$) and fractional contributions (f_{up} and f_{bkgrd}) of each parcel. We first solved for f_{bkgrd} using measured carbamazepine concentrations at two sites ($C_{\text{CBZ,down}}$ and $C_{\text{CBZ,up}}$) and carbamazepine concentrations in background water ($C_{\text{CBZ,bkgrd}}$) that ranged from zero up to the measured concentration at the down current site. Then we used f_{bkgrd} and measured E1 concentrations ($C_{\text{E1,down}}$ and $C_{\text{E1,up}}$) to solve for the concentration of E1 in background water ($C_{\text{E1,bkgrd}}$).

Using this approach for the dilution step between DITP and station PLM ($0 < C_{\text{CBZ,bkgrd}} < 1.6$ ng L^{-1}), we calculated background E1 concentrations ($C_{\text{E1,bkgrd}}$) of 227 - 323 pg L^{-1}. Between stations PLM and DS1 ($0 < C_{\text{CBZ,bkgrd}} < 0.21$ ng L^{-1}), $C_{\text{E1,bkgrd}}$ was 240 - 250 pg L^{-1}. Both of these ranges are close to the measured E1 concentration at the "background" (up current) station US (260 pg L^{-1} E1). The story is different farther offshore between stations DS1 and DS2, where $C_{\text{E1,bkgrd}}$ was 500 - 2000 pg L^{-1}. These results suggest that ambient E1 levels in Massachusetts Bay are ~ 270 ± 50 pg L^{-1} near the outfall and potentially 2 - 7× greater in offshore waters close to Stellwagen Bank.

Estrogenicity calculations for Massachusetts Bay seawater

We determined the estrogenicity of Massachusetts Bay water at each site by multiplying the concentration of each estrogen by its particular binding affinity for an estrogen receptor (similar among vertebrates) and calculating a sum that is normalized to E2. Among the Massachusetts Bay samples, we found that the sum total E2 equivalent concentration for the suite of estrogens measured here ranged between 0.1 - 0.4 ng L^{-1} (see Table S1 for estrogenicity multipliers). These levels are near the threshold thought to cause harm to fish, and they still don't consider any of the weakly estrogenic chemicals known to persist in sewage-impacted waters. Of those estrogens detected in Massachusetts Bay waters, we found that free forms contribute between 96 – 100 % of the estrogenicity depending on the technique (e.g., estrogen receptor binding, yeast estrogen screen, and E-screen; Table S1); halogenated forms make up the remainder.

REFERENCES

1. USEPA, *Journal*, 2013.

2. S. H. Hilal, S. W. Karickhoff and L. A. Carreira, *Journal*, 2003, 158.

3. Y. Lee, B. I. Escher and U. Von Gunten, *Environmental Science & Technology*, 2008, **42**, 6333-6339.

4. Z. H. Liu, Y. Kanjo and S. Mizutani, *Science of the Total Environment*, 2009, **407**, 731-748.

5. C. Hansch, D. Hoekman, A. Leo, L. T. Zhang and P. Li, *Toxicol. Lett.*, 1995, **79**, 45-53.

6. S. H. Yalkowsky and R. M. Dannenfelser, *Journal*, 1992.

7. C. H. Eger, D. A. Norton, M. Greiner and Yarborou.C, *Steroids*, 1972, **20**, 361-381.

8. K. M. Lewis and R. D. Archer, *Steroids*, 1979, **34**, 485-499.

9. A. R. Hurwitz and S. T. Liu, *J. Pharm. Sci.*, 1977, **66**, 624-627.

10. C. D. Hunt, A. D. Mansfield, M. J. Mickelson, C. S. Albro, W. R. Geyer and P. J. W. Roberts, *Marine Environmental Research*, 2010, **70**, 150-161.

11. O. Gustafsson, C. M. Long, J. Macfarlane and P. M. Gschwend, *Environmental Science & Technology*, 2001, **35**, 2040-2048.

12. D. R. Griffith, M. C. Kido Soule, H. Matsufuji, T. I. Eglinton, E. B. Kujawinski and P. M. Gschwend, *Environmental Science & Technology*, 2014, **48**, 2569-2578.

13. M. F. Delaney and A. C. Rex, *Contaminant Monitoring of Deer Island Treatment Plant Effluent: 2000-2005*, Report ENQUAD 2007-02, Massachusetts Water Resources Authority, Boston, 2007.

14. D. Shea, *Multimedia fate model of organic contaminants in Massachusetts Bay*, Report ENQUAD 2008-12, Massachusetts Water Resources Authority, Boston, 1995.

15. R. P. Signell and B. Butman, *Journal of Geophysical Research-Oceans*, 1992, **97**, 15591-15606.

16. G. G. Ying and R. S. Kookana, *Environmental Science & Technology*, 2003, **37**, 1256-1260.

17. O. Gustafsson and P. M. Gschwend, *Geochimica Et Cosmochimica Acta*, 1998, **62**, 465-472.

18. R. P. Schwarzenbach, P. M. Gschwend and D. M. Imboden, *Environmental Organic Chemistry*, John Wiley & Sons, Inc., Hoboken, NJ, 2nd edn., 2003.

19. K. J. W. Hyde, J. E. O'Reilly and C. A. Oviatt, *Continental Shelf Research*, 2007, **27**, 1677-1691.

20. L. S. Lee, T. J. Strock, A. K. Sarmah and P. S. C. Rao, *Environmental Science & Technology*, 2003, **37**, 4098-4105.

21. A. M. Tarrant, M. J. Atkinson and S. Atkinson, *Mar. Ecol.-Prog. Ser.*, 2004, **269**, 121-129.

22. S. Atkinson, M. J. Atkinson and A. M. Tarrant, *Environ. Health Perspect.*, 2003, **111**, 531-535.

23. R. Siegener and R. F. Chen, Boston, Massachusetts, 1998.

24. S. Reddy and B. J. Brownawell, *Environmental Toxicology and Chemistry*, 2005, **24**, 1041-1047.

25. T. Isobe, S. Serizawa, T. Horiguchi, Y. Shibata, S. Managaki, H. Takada, M. Morita and H. Shiraishi, *Environmental Pollution*, 2006, **144**, 632-638.

26. D. Schlenk, Y. Sapozhnikova, M. A. Irwin, L. T. Xie, W. Hwang, S. Reddy, B. J. Brownawell, J. Armstrong, M. Kelly, D. E. Montagne, E. P. Kolodziej, D. Sedlak and S. Snyder, *Environmental Toxicology and Chemistry*, 2005, **24**, 2820-2826.

27. B. J. Robinson, J. P. M. Hui, E. C. Soo and J. Hellou, *Environmental Toxicology and Chemistry*, 2009, **28**, 18-25.

28. G. Saravanabhavan, R. Helleur and J. Hellou, *Chemosphere*, 2009, **76**, 1156-1162.

29. O. Braga, G. A. Smythe, A. I. Schafer and A. J. Feitz, *Chemosphere*, 2005, **61**, 827-833.

30. I. C. Beck, R. Bruhn and J. Gandrass, *Chemosphere*, 2006, **63**, 1870-1878.

31. I. C. Beck, R. Bruhn, J. Gandrass and W. Ruck, *Journal of Chromatography A*, 2005, **1090**, 98-106.

32. L. J. Standley, R. A. Rudel, C. H. Swartz, K. R. Attfield, J. Christian, M. Erickson and J. G. Brody, *Environmental Toxicology and Chemistry*, 2008, **27**, 2457-2468.

33. S. Atkinson and M. J. Atkinson, *Coral Reefs*, 1992, **11**, 33-35.

34. Y. G. Zuo, K. Zhang and Y. W. Deng, *Chemosphere*, 2006, **63**, 1583-1590.

35. A. Bertin, P. A. Inostroza and R. A. Quiñones, *Marine Pollution Bulletin*, 2011, **62**, 1530-1537.

36. X. Zhang, Q. Z. Li, G. X. Li, Z. S. Wang and C. Z. Yan, *Marine Pollution Bulletin*, 2009, **58**, 1210-1216.

37. B. Pinto, S. Garritano and D. Reali, *Marine Pollution Bulletin*, 2005, **50**, 1681-1685.

38. D. A. M. da Silva, J. Buzitis, W. L. Reichert, J. E. West, S. M. O'Neill, L. L. Johnson, T. K. Collier and G. M. Ylitalo, *Chemosphere*, In Press, DOI: http://dx.doi.org/10.1016/j.chemosphere.2013.04.027.

39. J. Legler, P. Leonards, A. Spenkelink and A. J. Murk, *Ecotoxicology*, 2003, **12**, 239-249.

40. C. A. Schipper, J. Lahr, P. J. van den Brink, S. G. George, P. D. Hansen, H. C. D. de Assis, R. van der Oost, J. E. Thain, D. Livingstone, C. Mitchelmore, F. J. van Schooten, F. Ariese, A. J. Murk, G. C. M. Grinwis, H. Klamer, B. J. Kater, J. F. Postma, B. van der Werf and A. D. Vethaak, *ICES J. Mar. Sci.*, 2009, **66**, 2089-2105.

41. S. Hashimoto, A. Horiuchi, T. Yoshimoto, M. Nakao, H. Omura, Y. Kato, H. Tanaka, K. Kannan and J. P. Giesy, *Archives of Environmental Contamination and Toxicology*, 2005, **48**, 209-216.