CHAPTER
FOURTEEN

Endocrine disrupting chemical (EDC) accumulation in Puget Sound sediments and the implications for native fish populations

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Abstract

Pollution derived from both natural and synthetic endocrine-disrupting chemicals (EDCs) have recently been detected in the waterways around the world. These compounds have the potential to detrimentally inhibit fish populations from reproducing successfully. To determine whether these compounds are accumulating in Puget Sound, WA, sediment samples were collected from 19 – 23 March 2007 aboard the *R/V Thomas G. Thompson* and the concentrations of these compounds were quantified using a Gas Chromatography-Flame Ionization Detector (GC-FID). Natural EDC's, such as estradiol and progesterone, had the highest concentrations ranging from 347 – 1089 ng g^{-1} and 31 – 120 μg g^{-1} respectively. There were also significant concentrations of the synthetic compounds Bisphenol A (126 – 1037 ng g^{-1}) and 19-norethindrone (419 – 890 ng g^{-1}). The highest concentrations of the EDCs were discovered at the sample station Elliott Bay #1 but the other four stations had similar quantities. These values were much higher than the values obtained from other studies performed around the world, including Glacier Bay, AK. Only the United Kingdom had similar results. It appears that the concentrations of these compounds can be directly correlated to population size and degree of urbanization. Further studies are needed to determine what concentrations of these compounds in sediment can catalyze abnormalities in fish species.

Introduction

In the past two decades, there has been increased awareness that Endocrine Disrupting Chemicals (EDCs) have negatively impacted marine waterways across the world, (Johnson and Landahl 1994; Braga et al. 2005a; Jobling et al. 2006) however public notification of this accumulation has been limited due to a lack of comprehension of the implications by the scientific community (Khanal et al. 2006). Both natural and synthetic estrogens and progestogens, which are referred to as EDCs, have the potential for altering the endocrine systems of marine life and these compounds are currently being ejected into the waters of the Northeast Pacific (Zhiqiang and Huang 2005; Malins et al. 2006). Understanding the fate and complexities of these hormones in the aquatic environment is key to calculating the degree to which human

and livestock-released estrogens can induce abnormal reproduction in the organisms that dwell in this fertile fjord (Braga et al. 2005; Khanal et al. 2006).

Estradiol (also identified as 17β-estradiol, E2 and oestradiol) is a naturally-occurring hormone and represents the major estrogen in humans. In women, estradiol acts as a growth hormone for tissue of the reproductive organs, initiates the lining of the vagina, the cervical glands, the endometrium and the fallopian tubes as well as a gamut of processes associated with bones and blood (Lascombe et al. 2000). Ethynylestradiol (17β-ethynylestradiol and EE2) is the synthetic derivation of estradiol which contains an ethynyl group on the C-17 carbon of estradiol (Table 1). This variant structure allows for ethynylestradiol to resist degradation and immediate absorption into the body and to instead be absorbed in the small intestine. In combination with progestin, a synthetic progestogen, these two compounds prevent pregnancy by ceasing ovulation and are commonly found coupled in hormonal contraceptives (Lascombe et al. 2000; Khanal et al. 2006).

While these two compounds are the most frequently investigated, they are not the only hormonal inhibitors and mimics currently being released into the environment. A few other natural estrogens (estriol and estrone), synthetic estrogens (mestranol, diethylstilbestrol, bisphenol A), natural progestogens (progesterone) and synthetic progestogens (19-norethindrone) have had detrimental impacts around the world (Lopéz de Alda et al. 2001; Ternes et al. 2002; Zhang et al. 2006). All of these chemicals are excreted in urine and feces as inactive glucuronides and sulfate conjugates which are readily hydrolyzed and activated via sewage treatment plants (Braga et al. 2005b; Williams et al. 2003).

These compounds are not removed via the current U.S. federal government-approved treatment policies (Peck et al. 2004; Zhiqiang and Huang 2005) however it has been found that the highest percentage of these compounds is from direct runoff funneled from agricultural facilities and not sewage treatment plants (Peterson et al. 2000; Khanal et al. 2006). Livestock that are fed steroids to increase fat content of meat and milk, urinate directly into the ground which contaminates aquifers and nearby streams that receive the compounds through groundwater seepage. These particular forms of water transportation are directed straight into the Puget Sound bypassing sewage treatment facilities entirely (Peterson et al. 2000). Once these compounds have been reactivated by fecal bacteria and released into waterways, they immediately adsorb onto sediment particles and accumulate in the environment as long as the sediment remains in bedload (Peck et al. 2004; Lai et al. 2000; Braga et al. 2005b).

As the accumulation of contaminated sediment particles increases, the danger to organisms increases as well. Even at low concentrations, hormone compounds can be extremely potent; less than 1 ng l^{-1} EE2 can induce vitellogenin production (an egg yolk protein associated with adult females) in male rainbow trout and 4 ng l^{-1} can cause male fathead minnows to fail to develop normal male secondary sexual characteristics (Williams et al. 2003; Kidd et al. 2007). These two types of non-sediment dwelling fish are not common to Puget Sound however they have been documented with the same abnormalities as English Sole, a common, native bottom-dwelling fish (Johnson et al. 1994; Malins et al. 2006). This type of contamination is occurring across the world and investigations of these irregularities in various fish species could prove that it is due to EDC pollution.

There have been numerous scientific studies conducted of fish populations that document a myriad of abnormalities, including: (1) lesions (Malins et al. 2006); (2) reproductive impairment and altered behavior (Peterson et al. 2000; Braga et al. 2005b; Yu et al. 2005); (3) feminization of the male fish (Lai et

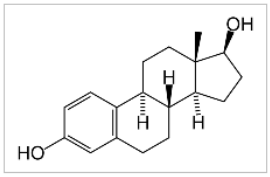
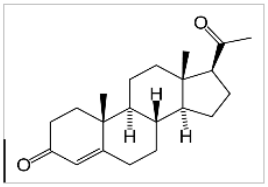
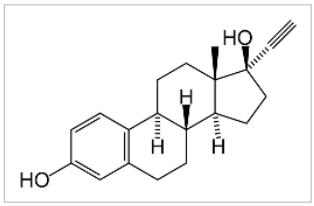
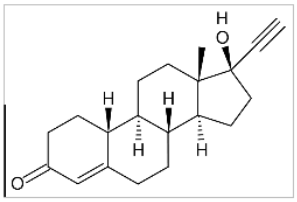
ESTROGENS	PROGESTOGENS
-*Natural hormones:* • Estradiol • Estriol •Estrone	*Natural hormones:* • Progesterone
	
- *Synthetic compounds:* • Ethynylestradiol • Diethylstilbestrol • Mestranol	- *Synthetic compounds:* • 19-Norethindrone
	

Table 14.1: A table displaying the compounds investigated in this study. They are separated into estrogens and progestogens, and then further separated into natural and synthetic. The underlined compound corresponds to the structure displayed.

al. 2000; Jobling et al. 2006); (4) depressed serum testosterone levels (Folmar et al. 2000); (5) decreased egg and larval viability (Peck et al. 2004); (6) altered enzymatic activities; and (7) decreased embryonic development and cellular damage (Braga et al. 2005b). As fish species continue to develop and grow with these irregularities, they could create a threat to the ecosystems of Puget Sound by eliminating specific fish populations and the eventual contamination of human beings via food web dynamics (Lascombe et al. 2000; Davis et al. 1998).

Estrogens and progestogens foster an immense number of vital processes of the human body such as: growth, differentiation, functioning of organs (vagina, ovary, testes, and prostate), bone maintenance, and the central nervous and cardiovascular systems (Lascombe et al. 2000). These processes require specific levels of hormones to function, and if the levels are altered due to contamination, then the processes would be compromised. In scientific studies, it has been proven that hormonal pollutants can affect human health by increasing the rate of breast cancer and other endocrinological

Station Location	Latitude (North)	Longitude (West)	Depth (meters)	Estimated Sediment Type
Elliott Bay #1	47° 37.00'	122° 23.00'	113.4	Mud and Sand
Elliott Bay #2	47° 36.05'	122° 22.00'	64.3	Muddy-Sand
West Point, Main Basin	47° 39.40'	122° 26.50'	27.4	Gravel and Mud
Dabob Bay, Hood Canal	47° 43.75'	122° 52.00'	62.2	Gravel
Great Bend, Hood Canal*	47° 22.30'	123° 08.00'	82.3	Mud
Glacier Bay, Bartlett Cove *	58° 27.02	135° 54.49	54	---
Glacier Bay, 16*	58° 53.77	136° 05.46	309	---
Glacier Bay, Head of Geike Inlet*	58° 36.35	136° 28.62	99	---
Puget Soud, Commencement Bay*	47° 17.13	122° 26.73	86	---

Table 14.2: Station locations, depths and sediment type sampled in Puget Sound, WA. At each station a Van Veen was deployed to probe the area, followed by the Multi-Corer. The '*' denotes samples that were supplied by Stefanie Keever from Glacier Bay, AK and one that was sampled from Puget Sound, WA during a University of Washington PRISM cruise.

diseases (Carnevali and Maradonna 2003; Davis et al. 1998). There is no actual measured degree of how much these compounds will affect humans in the future, or even if these compounds are presently affecting fish species of this region, but this study shows that these compounds are detectable in Puget Sound sediments and will stimulate the need for further considerations and investigations in the future.

Methods

To determine the levels of both natural (estradiol, estriol, estrone) and synthetic estrogens (ethynylestradiol, diethylstilbestrol, mestranol, bisphenol A) and both natural (progesterone) and synthetic progestogens (19-norethindrone) in Puget Sound, sediment samples were collected 19 – 23 March 2007 aboard the *R/V Thomas G. Thompson*. Six sample stations were selected in the Puget Sound (Table 2, Fig 1) based upon specific characteristics (proximity to human population, grain size of sediment, depth) of each station. Samples from Glacier Bay, AK (Fig. 2) collected by Stefanie Keever were analyzed using the same method.

Field Methods

A Van Veen grab sampler was used at each station to probe the seabed for grain size and if the sediment proved to be fine-grained (silt), then a Multi-Corer was used to collect the samples simultaneously in triplicate. If the grains were too coarse to deploy the Multi-Core, the Van Veen was deployed three times to collect samples.

The EDCs accumulating in the surface layer most impact benthic fish populations (Ylitalo,

Cooper, B. pers. comm.) so the top two centimeters were sampled and placed into Whirl-Pak sediment bags using a spatula. Samples were stored in a freezer at -20° C until analysis in Dr. Richard Keil's lab at the University of Washington, Seattle, WA.

Laboratory Methods

The laboratory analysis began by transferring the sediment with a metal spatula into combusted glass vials to prevent contamination. The samples were centrifuged for 10 minutes at 3000 rpm to remove the salt content contained within the sediment, which would alter the calculated results of the compounds. The samples were then dried in an oven at 60 °C for three days.

Solid-Phase Extraction

EDCs were extracted from the sediment samples (Zhang et al. 2006; López de Alda and Barceló 2001; Ternes et al. 2002) utilizing a combination of two known procedures; Metro King County's Environmental Lab (Walker, D. pers. comm.) and Hopmans et al. (2000). Approximately three grams of sediment were weighed into a combusted 60 ml glass vial which was then spiked with 1.5 μl of Mirex.
Fifteen ml of 100% Methanol (MeOH) was added to the sample and was vortexed, sonicated for 15 min, centrifuged at 3000 rpm for 10 min, and then decanted into a 200 ml glass receptacle three times. This process was repeated with three more solvent mixtures; (1) 50% MeOH / 50% Dichloromethane (DCM); (2) 100% DCM; and (3) 10% Acetone / 90% Hexane. All of the samples were then blown down under nitrogen using a TurboVap II to evaporate the solvents.

Clean-Up Steps

The extract needed two distinct clean-up steps to remove the excess compounds that had adsorbed to the sediment. Both of the clean-up steps used column chromatography with a single elution. The first set of columns were filled with 1.5 % deactivated silica gel, and the second with activated alumina. The samples were rinsed with specific solvent mixtures used in Metro King County's Environmental Lab, Seattle, WA (Walker, D. pers. comm.).

Derivitization

The derivitization process followed was formulated by Budzinski et al. (2006). Instead of injecting the entire sample on the Gas Chromatography – Flame Ionization Detector (GC-FID), the samples were resuspended in 1.5 ml MeOH and split into four two ml GC (gas chromatography) vials in increments of 300 μl while the last remaining 300 μl were left in the sample vial. The first GC vial was used for analysis, the second was spiked with 6 μl of an external hormone standard solution with a concentration of 0.03 mM that contained a known amount of each standard, and a third vial was spiked with 12 μl of the external solution. One μl from each of these three vials was injected on the GC-FID for analysis and the other two vials were stored in the refrigerator.

Quantification

Final analysis consisted of quantification of the compound peaks by GC-FID as formulated by Metro King County's Environmental Lab (Walker, D. pers. comm.; Zhang et al. 2006) and conversations with Dr. Richard Keil and Jacquelyn Neibauer (Keil, R. pers. comm.; Neibauer, J. pers. comm.). A standard of each of the compounds in question (Bisphenol A, diethylstilbestrol, estrone, estradiol, mestranol, 19-norethindrone, ethynylestradiol, estriol and progesterone) was injected on the GC-FID to identify their individual retention times. This information was then used to correlate the standard's peaks to their own peaks seen in the external stock standard and then later used to correlate to the peaks seen in the sample

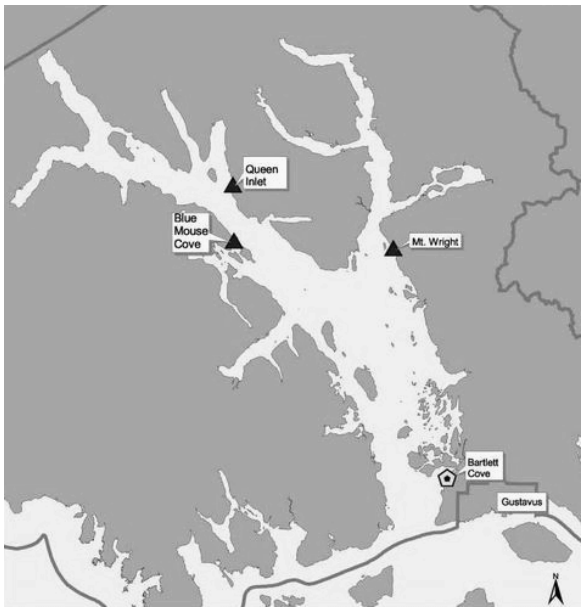


Figure 14.1: A map that denotes sample stations in Puget Sound, WA which were collected from 19-23 March 2007 aboard the *R/V Thomas G. Thompson.*

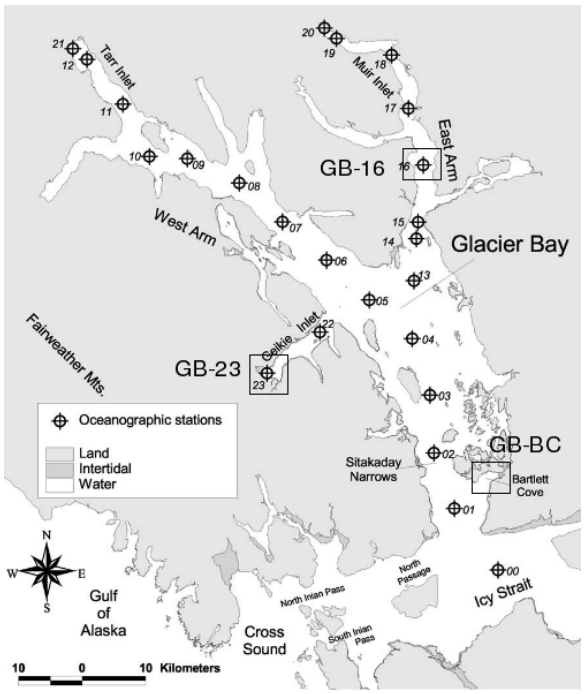


Figure 14.2: A map of Glacier Bay, AK that denotes the locations of the samples that were collected by Stefanie Keever and analyzed in this study for hormone compounds.

spectrums (Fig 3). The percentage recovery was calculated by spiking each sample with 1.5 μl of mirex. The concentrations of the compounds were determined by comparing peak areas to that of a known quantity of injected standards.

Results

The calculated concentrations for each compound in Puget Sound and Glacier Bay are displayed in Tables 3 and 4 respectively. The natural estrogens and progestogens were found in higher concentrations than the synthetic compounds. Estradiol's concentration ranged from 346.7-1089.3 ng g^{-1}. Ethynylestradiol was undetectable. For the progestogens, the concentration of natural progesterone ranged from 31.1-120.3 μg g^{-1}, and synthetic 19-norethindrone ranged from 419.4-890.0 ng g^{-1} (Fig. 4).

The highest concentrations of the synthetics Bisphenol A and 19-norethindrone were detected at the Elliott Bay Station (EB#1) while the lowest values were located in the Great Bend, Hood Canal (Fig. 5). This same relationship was seen in the concentrations of the natural compounds except that the lowest values were not seen in the Great Bend, Hood Canal station but West Point (Fig. 6). Samples collected in Glacier Bay, Alaska (AK) demonstrated a very different relationship than that of Puget Sound. The quantities of the synthetic compounds were untraceable in Glacier Bay except for the synthetic progestogen 19-Norethindrone which ranged in concentration from 611.7 ng g^{-1} to 656.6 ng g^{-1}. The main compounds that were found in Glacier Bay were natural (Fig. 7), and had similar concentrations to those detected in Puget Sound except for progesterone. In Glacier Bay, the concentration for progesterone ranged from 6.5 μg g^{-1} to 14.2 μg g^{-1} while in Puget Sound the concentration was ten times more. The quantified Puget Sound values were dissimilar to those detected in eight German rivers (Ternes et al. 2007), the United Kingdom (Jobling et al. 2006; Peck et al.

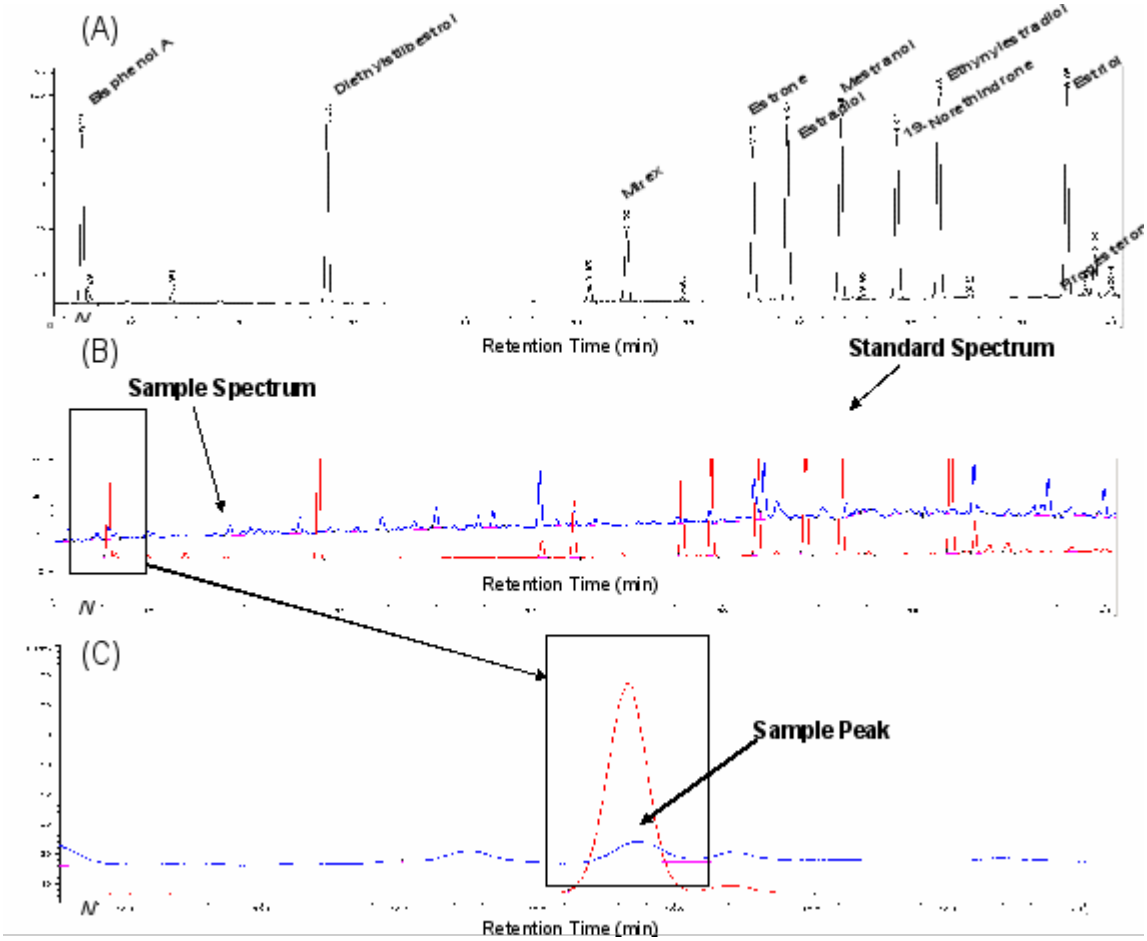


Figure 14.3: (A) A spectrum generated from the GC-FID that displays and identifies the standards peaks which were used for quantification. (B) EB#1 MC-2 sample's spectrum with the standards spectrum overlain on top. (C) A zoomed in image of the previous spectrum demonstrating the identification of the peaks in the sample by using the standards peaks; this peak is denoted as Bisphenol A.

2004; Williams et al. 2003), and Italy (Pojana et al. 2007) (Table 5).

Discussion

The two stations in Elliott Bay were chosen due to their close proximity to Seattle Proper's large population and because previous work by the National Oceanic and Atmospheric Administration (NOAA) in the area could be used to provide historical evidence of accumulation and contamination in the area (Ylitalo, Cooper, pers. comm.). The station at West Point was selected due to its propinquity to the sewage treatment facility feeding into the Main Basin and the two stations in Hood Canal were chosen because of the nearby naval facility in Dabob Bay where there is a sizeable population of human inhabitants and decreased water circulation (Carr et al. 2001). These stations were all expected to have a significant amount of accumulated EDC compounds in the sediment due to these potential sources.

The obtained concentrations of the natural compounds progesterone and estradiol were not unexpected. Due to the abundance of fish and mammals within Puget Sound whose endocrine systems function on these hormones, the high

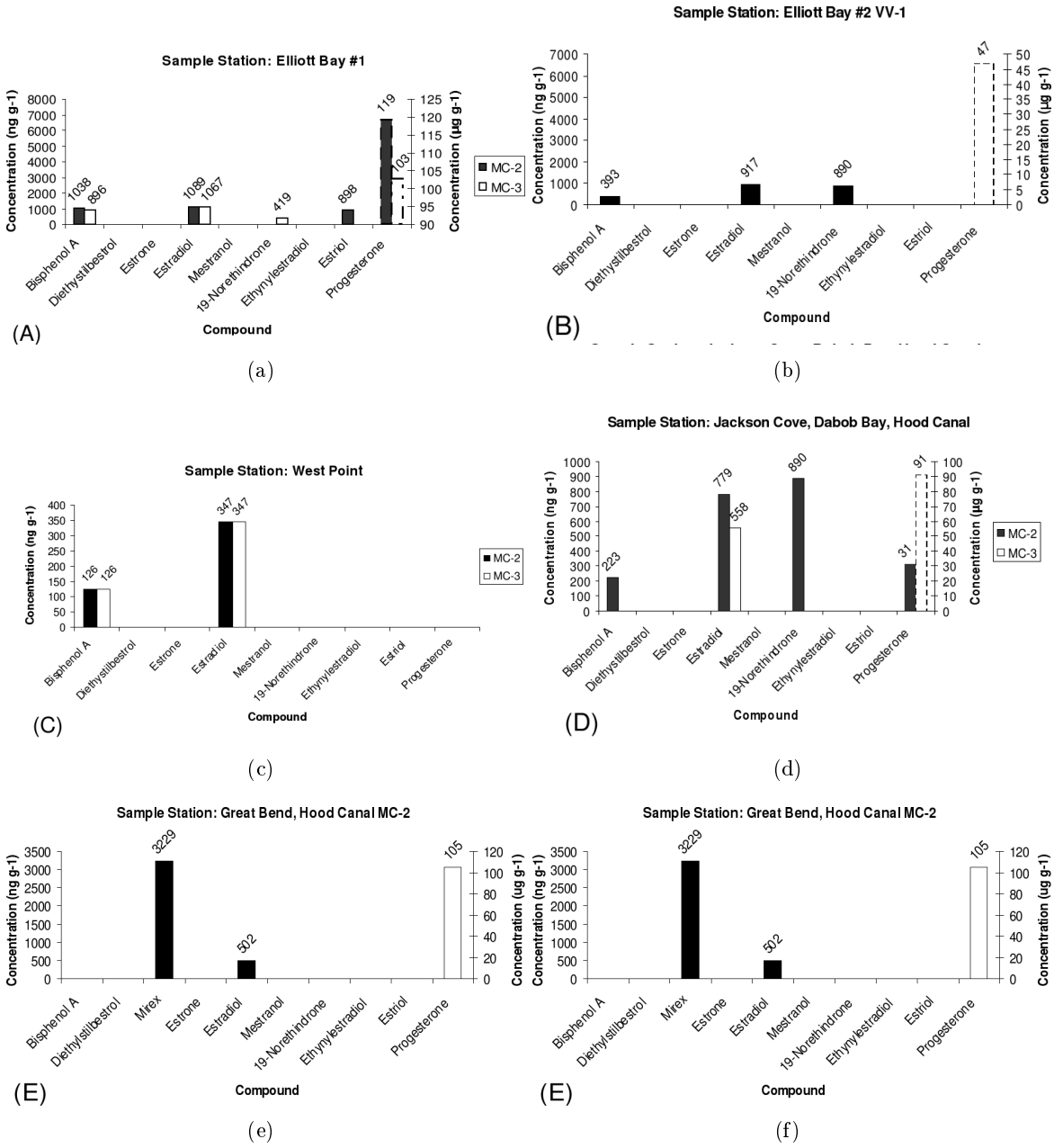


Figure 14.4: A set of six bar graphs displaying the concentrations of the compounds. (A) Elliott Bay #1 comparing the compounds to the concentrations determined. This graph has two series displayed, the first and second multi-core samples. The primary y-axis has concentrations in ng g^{-1}. The secondary y-axis is solely meant for the concentration of progesterone which was found to be in μg g^{-1}, as the dotted line denotes. (B) The second Elliott Bay station which was collected using a Van Veen. The primary y-axis has concentrations in ng g^{-1} while the secondary y-axis is in μg g^{-1} for the concentration of progesterone, again denoted by the dotted line. (C) Sample station West Point with two separate series displaying the two multi-core samples. (D) Sample station Jackson Cove in Hood Canal. The primary y-axis has concentrations in ng g^{-1} while the secondary y-axis is in μg g^{-1} for the concentration of progesterone, again denoted by the dotted line. (E) Sample station Great Bend, in Hood Canal for the second Multi-Core. The primary y-axis has concentrations in ng g^{-1} while the secondary y-axis is in μg g^{-1} for the concentration of progesterone. (F) Finally, the sample collected by Stefanie Keever from Commencement Bay. The primary y-axis has concentrations in ng g^{-1} while the secondary y-axis is in μg g^{-1} for the concentration of progesterone.

[Station Name: Concentrations reported in ng g^{-1}]									
Compound Name:	Retention Time (min):	EB#1 MC-2	EB#1 MC-3	EB #2 VV-2	WP C-2	WP C-3	JC MC-2	JC MC-3	GB MC-2
Bisphenol A	29.561	1038.0	895.9	392.8	126.0	126.3	223.0	0.0	0.0
Diethylstilbestrol	31.759	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Mirex**	34.441	5890.9	4709.2	3297.4	3274.9	2213.8	3584.3	4798.2	3257.2
Estrone	35.573	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Estradiol	35.88	1089.3	1066.5	917.3	346.7	347.3	779.0	557.6	506.8
Mestranol	36.37	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19-Norethindrone	36.865	0.0	419.4	890.0	0.0	0.0	0.0	0.0	0.0
Ethynylestradiol	37.238	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Estriol	38.386	897.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Progesterone	38.369	120341.0	103887.1	31147.7	0.0	0.0	46823.9	91087	105589.7

Table 14.3: This table displays the calculated concentrations for each compound at the Puget Sound Stations. EB stands for Elliott Bay, WP stands for West Point, JC stands for Jackson Cove, Hood Canal, and GB stands for Great Bend, Hood Canal for the sample stations and MC stands for Multi-Core and VV stands for Van Veen samples. The '*' on Mirex denotes that this compound was spiked into each of the samples in order to potentially quantify the percent recovery thus it was detected in every sample.

concentrations most likely resulted from the accumulation and degradation of their bodies on the sea floor. On the other hand, these compounds are also contained within human bodies and various pharmaceuticals. During ovulation, estradiol and progesterone are coupled in the body preparing for impregnation of the uterus (Simerly 2002). While the natural purpose of estradiol is to encourage implantation, it can and has also been combined with the synthetic estrogen ethynylestradiol to form an oral contraceptive to inhibit this process. Even though there is a noticeable concentration of estradiol at each sample station, I do not believe that the birth control pill has contributed to that quantity. In order to make such a conclusion, there would have to be at least some quantity of both compounds detected at the sample stations however ethynylestradiol was not seen in any of the samples thus the high levels cannot be attributed to anthropogenic contributions.

Progesterone has also been used in pharmaceuticals to encourage pregnancy, especially for women with a history for pre-term births (Simerly 2002). This is one of the numerous functions of this compound and the high concentrations in the sediment samples could be credited to these products. Nevertheless, this is also not conclusive and will not be proven by the data in this investigation.

The synthetic compound Bisphenol A was detected at every single Puget Sound station. This compound is mainly derived from plastic products and has been found to mimic estrogens (Fu et al. 2007). It has caused great concern to the world because it is found in baby bottles, has recently been made illegal in Europe (http://www.eubusiness.com/EUnews) and is being broadcast in the public media forum of Canada

Compound Name:	Retention Time (min):	Station Name: (Concentrations reported in ng g^{-1}) PS-VV2	GB-BC	GB-16	GB-23
Bisphenol A	29.561	521.2	0.0	0.0	0.0
Diethylstilbestrol	31.759	0.0	0.0	0.0	0.0
Mirex	34.441	0.0	0.0	0.0	0.0
Estrone	35.573	0.0	0.0	0.0	0.0
Estradiol	35.88	1551.3	878.1	986.8	993.1
Mestranol	36.36	0.0	0.0	0.0	0.0
19-Norethindrone	36.865	0.0	656.6	614.7	611.7
Ethynylestradiol	37.238	0.0	0.0	0.0	0.0
Estriol	38.386	0.0	0.0	0.0	0.0
Progesterone	38.369	94770.4	6467.4	14178.4	14070.5

Table 14.4: This table displays the calculated compound concentrations from the samples obtained by Stefanie Keever in Glacier Bay, AK and the one sample from Puget Sound, WA. PS stands for Puget Sound, and GB stands for Glacier Bay samples.

(http://www.commondreams.org/archive /2008/04/16/8330/) and the United States (http://www.usatoday.com/news/health/). Since this compound is anthropogenic, the quantities detected in these samples are directly correlated to human input.

The overall concentrations of both the natural and synthetic compounds were greatest in the most populated areas, Elliott Bay. This was an expected result due to the amount of material and people that inhabit that waterfront property. What was unexpected was that the southern Elliott Bay station did not contain the same concentrations as the northern station. This could be due to several reasons. At the southern Elliott Bay station, the sediment grains were too coarse to deploy the Multi-Core. Samples were then collected using the Van Veen which does not efficiently preserve the surface layer where the highest concentrations of EDCs are located. The Van Veen could have potentially lost the surface layer during retrieval or mixed it with the lower sediment layers decreasing the concentrations of the compounds in question. These sources of error could have diminished the overall concentrations or even eliminated any detection of them entirely. The circulation of the bay could also have attributed to the unexpected results. The Duwamish River has a strong influence on the southern portion of

Compound:	Results found in this paper (ng g-1)	German Rivers (ng g-1): (Ternes et al. 2002)	River Thames, UK (μg g-1): (Lai et al. 2007)	Venice, Italy Lagoon (ng g-1): (Pojana et al. 2007)	Botany Bay, Australia (ng g-1): (Braga et al. 2005b)	Tokyo Bay, Japan (ng g-1): (Isobe et al. 2006)
Bisphenol A	126.04-1037.97	---	---	44.89	---	---
Diethylstilbestrol	undetected	---	---	71	---	---
Mirex	Spike	Spike	---	---	---	---
Estrone	undetected	1.1	2.45 ± 0.16	water sample	0.16-1.17	0.05-3.60
Estradiol	346.66-1089.27	0.70	2.64 ± 0.05	water sample	0.22-2.48	0.07-0.59
Mestranol	undetected	undetected	3.98 ± 0.33	---	---	---
19-Norethindrone	419.39-890.02	---	---	---	---	---
Ethynylestradiol	undetected	0.80	3.37 ± 0.16	71	<0.05-0.5	0.13-0.34
Estriol	897.75	---	3.04 ± 0.27	water sample	---	---
Progesterone	6467.40-120340.95	---	---	---	---	---

Table 14.5: This table displays the concentrations of the compounds in question and then compares them to those obtained from other studies around the world (Germany, the United Kingdom, Italy, Australia, Japan).

the bay which increased the flow at this station and effectively discouraged suspended sediment from accumulating. The latter explanation is further supported by the larger grain size of the area suggesting a larger flow velocity.

The values obtained for West Point were unpredicted. This particular sewage treatment facility treats the majority of the greater Seattle area's water and sewage thus I would have expected detection of the most popular synthetics. Instead, there was absolutely no detection at all. This result supports the conclusion that these compounds are entering Puget Sound through agricultural contamination of nearby streams fluxing directly into the waterway as opposed to the material from treatment facilities.

While Elliott Bay #1 has the highest concentrations overall, it was not significant. The population of the Puget Sound region, the fish populations, and the circulation patterns must all have contributed to the accumulation of these compounds. I had expected the Great Bend station to show significant concentrations due to its decreased circulation velocity however it displayed nonexistent accumulation of the synthetics, but accurately demonstrated the natural compounds. Hood Canal has had multiple occurrences of hypoxia where blooms have destroyed fish populations, and it is evident from this data that there are still fish dying and degrading on the sea-floor. In comparison to values obtained around the world, the concentrations calculated for the Puget Sound are much larger except for the United Kingdom. This could be due to multiple sources, such as river input, agriculture, residence time, or population.

The samples collected by Ternes et al. (2002)

Compound:	Results in this paper converted to ng l^{-1}	Mississippi River, USA (ng l^{-1}): (Folmar et al. 2001)	River Ouse, UK (ng l^{-1}): (Zhang et al. 2006)
Bisphenol A	12,604- 103,797	---	72.8 ± 11.1-92.0 ±3.9
Diethylstilbestrol	undetected	---	---
Mirex	Spike	---	---
Estrone	undetected	---	72.0 ± 3.3-102 ± 5.3
Estradiol	34,666- 108,927	173.50-662.88	70.1 ± 8.9-94.2 ±9.3
Mestranol	undetected	---	---
19-Norethindrone	41939-89002	---	---
Ethynylestradiol	undetected	---	72.8 ±6.5-91.4 ± 10.1
Estriol	89,775	---	---
Progesterone	646,740-12,034,095	---	---

Table 14.6: This table compares the concentrations determined by this paper converted into ng l^{-1} in order to compare to results obtained from other studies around the world.

were gathered from eight German, relatively remote rivers unaffected by urbanization (Table 5). Pojana et al.'s (2007) samples were from a lagoon in Venice and while Venice is a populated city (271,251 people) it is not as populous as the Seattle Metropolitan Area (3.3 million) or the United Kingdom (7.5 million in London). Braga et al. (2005b)'s samples in Australia were taken directly from the ocean east of Botany Bay which explains the low calculated concentrations. The relationship displayed by these papers demonstrates that the degree of accumulation is directly correlated to the adjacent population. The eight German rivers have the lowest concentrations because they are far from urbanization. The lagoon in Venice, Italy has the next smallest values because it is near a populated city but not completely urbanized. Puget Sound is very populated and thus has much higher values than these previous examples however its values are not as high as those obtained in the United Kingdom which has the highest population.

The samples that were collected in Glacier Bay, AK efficiently acted as control stations and further support this conclusion. Three separate stations were selected to analyze for the hormone compounds and the main hormones detected at these stations were the natural ones, estradiol and progesterone. There was, however, one unexpected result. A slight concentration of the synthetic progestogen, 19-norethindrone, was discovered at each of those stations as well. This particular compound is used in progestin-only hormonal contraceptives and was not expected in the pristine environment of Glacier Bay. This particular area is devoid of human inhabitants, so there is no clear explanation as

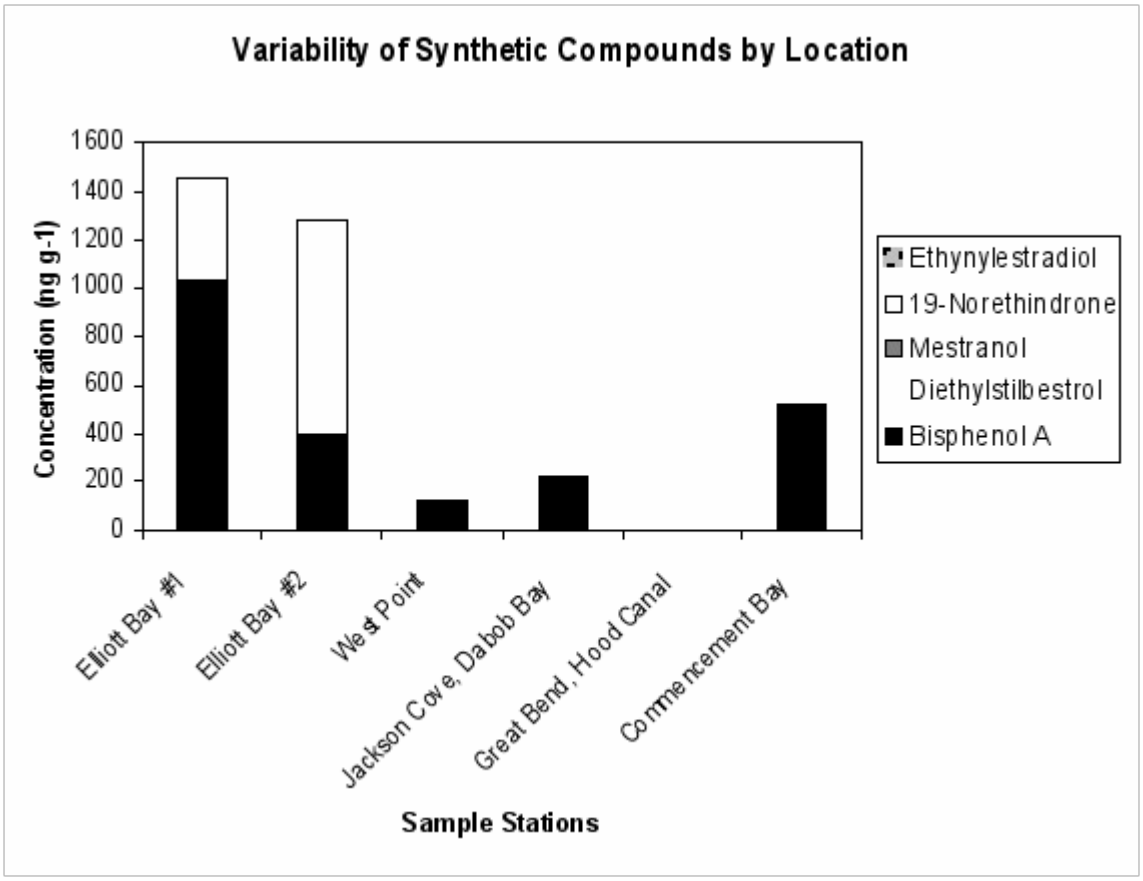


Figure 14.5: A graph displaying the variability of the synthetic compounds by location.

to why this compound was discovered in the three stations analyzed.

Nevertheless, there is the potential that the concentrations calculated for all stations analyzed are inaccurate. Based upon the current derivatization method, there are various other compounds that are displayed on the GC-FID spectrums. All of these extraneous peaks could negatively affect the sample matrixes and alter the detected compounds and concentrations. Another explanation is associated with the usage of Mirex as my recovery standard. This particular compound is not used by Metro King County's Environmental lab, but due to the requirements of SachsLab, I was unable to bring in a recovery standard with the correct isotopic signature to retrieve my compounds to their entirety. Mirex was not successful as a recovery standard however I was still able to identify the compounds in question accurately.

Despite all of these potential sources of error, it has been proven by the analysis of these samples that the identification of Bisphenol A at the Puget Sound stations is accurate. Since this particular compound is contained within many plastics, specifically plastics that were used in the method, the likelihood of contamination during the process was high however since Bisphenol A was not detected in any of the Glacier Bay samples, then it can be concluded that there was no contamination during the analysis of the Puget Sound samples.

A full assessment of the EDC sediment concentrations needed to alter the endocrine systems of fish populations are required in order to better understand and conclude whether these

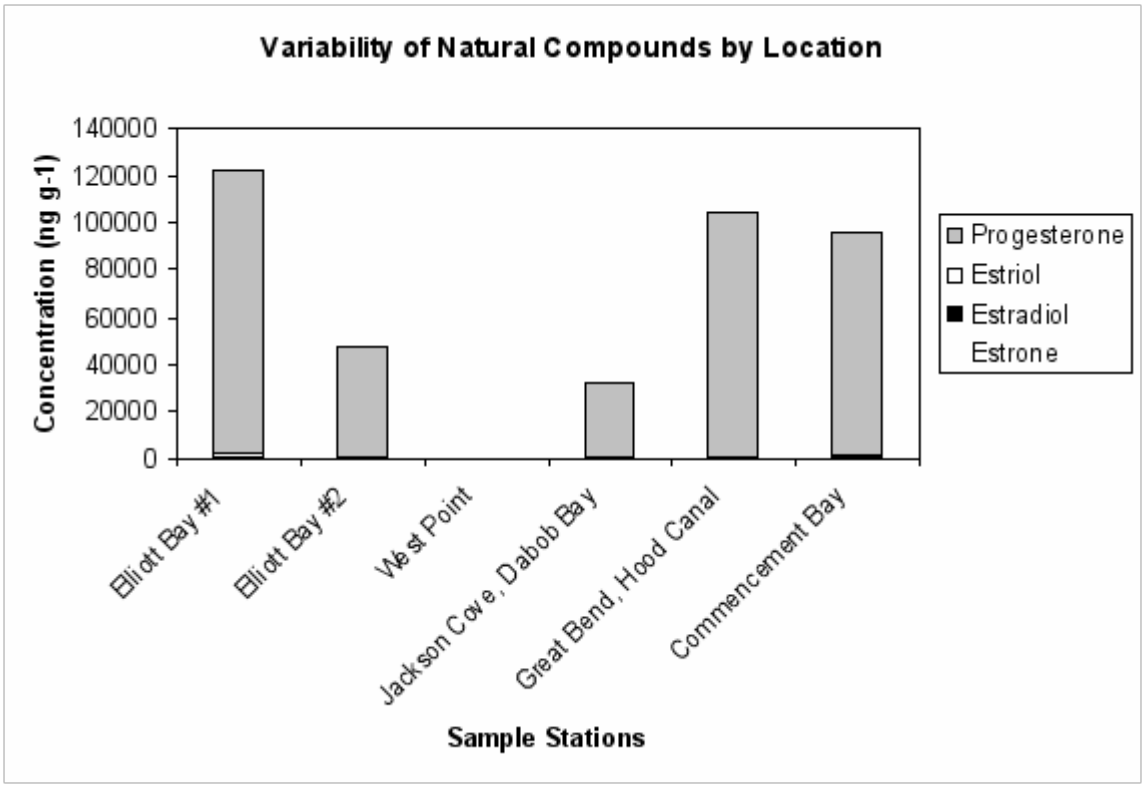


Figure 14.6: A graph displaying the variability of the natural compounds by location. The primary y-axis applies to the compounds estrone, estradiol and estriol in concentrations of ng g^{-1}, while the secondary y-axis solely represents the concentrations of progesterone which was displayed in μg g^{-1}.

compounds are indeed what is negatively impacting them. There are few studies that have been conducted to correlate sediment concentrations with fish abnormalities but Johnson et al. (1998) and Casillas et al. (1991) examined Polychlorinated biphenyl which includes Bisphenol A. They discovered that a concentration of 600-900 ng g^{-1} of these compounds, when contained within fish tissues, displayed reduced fertilization success and larval viability within fish species. The concentrations found in this study for both Bisphenol A and 19-norethindrone were much larger than those values and can suggest that these compounds are currently affecting English Sole populations however, concentrations calculated in fish tissues and those calculated in sediment are quite different.

These compounds, when activated, quickly adsorb to sediment particles after contact and in order for fish to get concentrations such as these fluxing through their bodies, they have to consume that sediment. This is why English Sole have a high probability of potential contamination since they are sediment-dwellers. The values calculated in this study were converted to ng l^{-1} in hopes to make a comparison to the few studies that have looked at the effects of these compounds on fish populations (Table 6). The converted concentrations presented in this study are much higher than those presented in others because they are based on an assumption of 90 % porosity, and are sediment concentrations, not tissue. Sediments will always have a much higher concentration than tissues since these compounds so quickly adsorb to sediment particles. It is apparent from this comparison that the concentrations calculated in this study

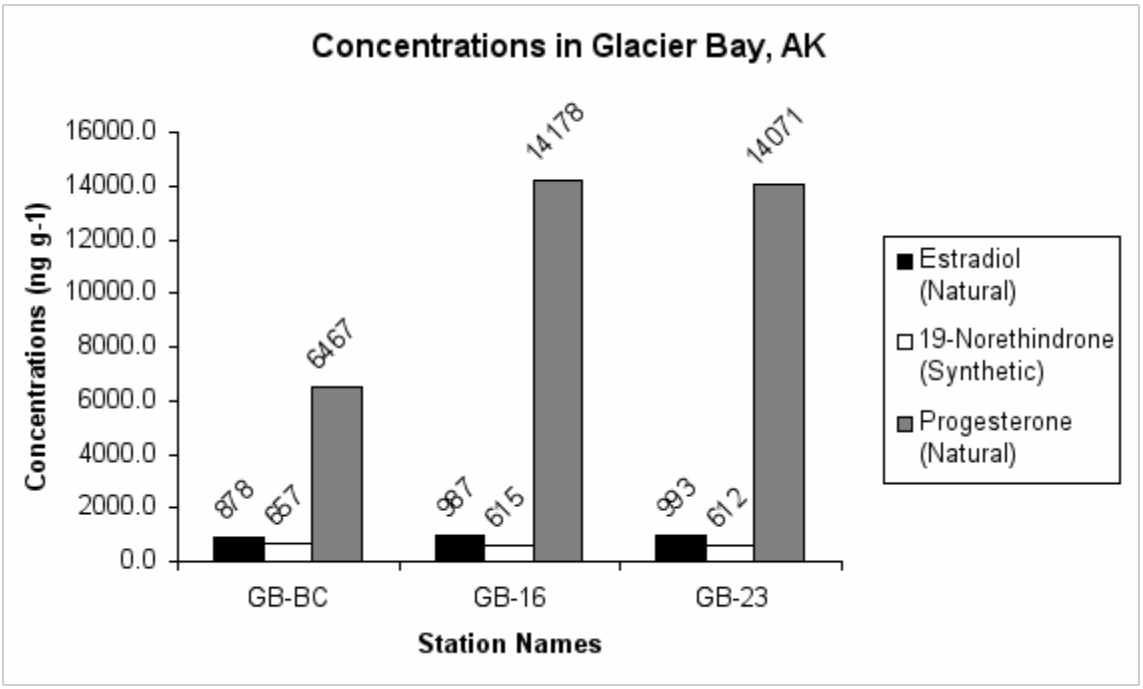


Figure 14.7: A graph displaying the concentrations obtained from the samples collected by Stefanie Keever in Glacier Bay, AK.

are substantial and thus a concern to the native fish species that dwell in the sediment. Nevertheless, no conclusive conclusions can be drawn until further research investigates the amount of concentrated sediment needed to induce these atrocious abnormalities.

Conclusions

EDC pollution is a concern for this planet. These compounds are not removed by current sewage treatment methods and thus are being deposited directly into the Puget Sound estuary. The synthetic compounds Bisphenol A and 19-norethindrone were discovered with significant concentrations at several stations in this study. It was established that the highest concentrations were found in Elliott Bay which has the largest adjacent population in comparison to the other selected locations. Glacier Bay successfully acted as a control group and presented spectrums with very few detected compounds however each of those stations did have a small concentration of the synthetic 19-norethindrone which is contained in hormonal contraceptives. Despite that this study has identified several detrimental compounds accumulating in the sediments of Puget Sound more research is needed to better determine if the concentrations of these hormones are impacting the native fish populations.

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References

Braga, O., George A. Smythe, Andrea I. Schäfer and Andrew J. Feitz. 2005a. Fate of steroidal estrogens in Australian Inland and coastal wastewater treatment plants. Environ. Sci. Technol. **39**:3351-3358.

Braga, O., G.A. Smythe, A.I. Schäfer and A.J. Feitz. 2005b. Steroid estrogens in ocean sediments. Chemos. **61**: 827-833.

Budzinski, H. M. H. Devier, P. Labadie, and A. Togola. 2006. Analysis of hormonal steroids in fish plasma and bile by coupling solid-phase extraction to GC/MS. Anal. Bioanal. Chem. **386**: 1429-1439.

Carnevali, Oliana and Francesca Maradonna. 2003. Exposure to xenobiotic compounds: looking for new biomarkers. Gen. Comp. Endocrin. **131**: 203-209.

Carr, R.S., M. Nipper, J.M. Biedenbach, R.L. Hooten, K. Miller and S. Saepoff. 2001. Sediment toxicity identification evaluation (TIE) studies at marine sites suspected of ordinance contamination. Arch. Environ. Toxicol. **41**: 298-307.

Casillas, E., D. Misitano, L. L. Johnson, L. D. Rhodes, T. K. Collier, J. E. Stein, B. B. McCain and U. Varanasi. 1991. Inducibility of spawning and reproductive success of female English sole (*Parophrys vetulus*) from urban and non-urban areas of Puget Sound, Washington. Mar. Environ. Reas. **31**: 99-122.

Davis, Devra Lee, Deborah Axelrod, Lisa Bailey, Mitchell Gaynor, Annie J. Sasco. 1998. Rethinking Breast Cancer Risk and the Environment: The Case for the Precautionary Principle. Environ. Heal. Perspec. **106**: 523-529.

Folmar, L. C., N. D. Denslow, K. Kroll, E. F. Orlando, J. Enblom, J. Marcino, C. Metcalfe, and L. J. Guillette Jr. 2001. Altered Serum Sex Steroids and Vitellogenin Induction in Walleye (*Stizostedion vitreum*) Collected Near a Metropolitan Sewage Treatment Plant. Arch. Environ. Contam. Toxicol. **40**: 392-398.

Fu, Mingzhu, Zhengyan Li and Huiwang Gao. 2007. Distribution characteristics of nonylphenol in Jiaozhou Bay of Qingdao and its adjacent rivers. Chemos. **69**: 1009-1016.

Hopmans, Ellen C., Stefan Schouten, Richard D. Pancost, Marcel T. J. van der Meer, and Jaap S. Sinninghe Damsté. 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Commun. Mass Spectrom. **14**: 585-589.

Isobe, Tomohiko, Shiegeko Serizawa, Toshihiro Horiguchi, Yasuyuki Shibata, Satoshi Managaki, Hideshige Takada, Masatoshi Morita, and Hiroaki Shiraishi. 2006. Horizontal distribution of steroid estrogens in surface sediments in Tokyo Bay. Environ. Pol. **144**: 632-638.

Jobling, Susan, Richard Williams, Andrew Johnson, Ayesha Taylor, Melanie Gross Sorokin, Monique Nolan, Charles R. Tyler, Ronny van Aerle, Eduarda Santos, and Geoff Brighty. 2006. Predicted Exposures to Steroid Estrogens in U.K. Rivers Correlate with Widespread Sexual Disruption in Wild Fish Populations. Environ. Heal. Perspec. **114**: 32-39.

Johnson, L.L., and J.T. Landahl. 1994. Chemical contaminants, liver disease, and mortality rates in English Sole (*pleuronectes vetulus*). Ecol. Applic. **4**: 59-68.

Johnson, Lyndal. L., David Misitano, Sean Y. Sol, Gregory M. Nelson, Barbara French, Gina Ylitalo and Tom Hom. 1998. Contaminant Effects on Ovarian Development and Spawning Success in Rock Sole from Puget Sound, Washington. Trans. Amer. Fish. Soc. **127**: 375-392.

Khanal, Samir Kumar, Bin Xie, Michael L. Thompson, Shihwu Sung, Say-Kee Ong and J. (Hans) Van Leeuwen. 2006. Fate, Transport, and Biodegradation of Natural Estrogens in the Environment and Engineered Systems. Environ. Sci. Technol. **40**: 6537-6546.

Kidd, Karen A., Paul J. Blanchfield, Kenneth H. Mills, Vince P. Palace, Robert E. Evans, James M. LAzorchak, and Robert W. Flick. 2007. Collapse of a fish population after exposure to a synthetic estrogen. PNAS. **104**: 8897-8901.

Lai, K. M., K.L. Johnson, M.D. Scrimshaw and J.N. Lester. 2000. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ. Sci. Technol. **34**: 3890-3894.

Lascombe, Isabelle, Dominique Beffa, Urs Rüegg, Joseph Tarradellas and Walter Wahli. 2000. Estrogenic activity assessment of environmental chemicals using *in vitro* assays: identification of two new estrogenic compounds. Environ. Heal. Perspec. **108**: 621-629.

López de Alda, Maria J. and Damià Barceló. 2001. Use of solid-phase extraction in various of its modalities

for sample preparation in the determination of estrogens and progestogens in sediment and water. J. Chromatogr. A. **938**: 145-153.

Malins, Donald C., Katie M. Anderson, John J. Stegeman, Pawel Jaruga, Virginia M. Green, Naomi K. Gilman and Miral Dizdaroglu. 2006. Biomarkers signal contaminant effects on the organs of English Sole (*parophrys vetulus*) from Puget Sound. Environ. Heal. Perspec. **114**: 823-829.

Peck, Mika, Richard W. Gibson, Andreas Kortenkamp and Elizabeth M. Hill. 2004. Sediments are major sinks of steroidal estrogens on two United Kingdom rivers. Environ. Toxicol. Chem. **23**: 945-952.

Peterson, E.W., R.K. Davis and H.A. Orndorff. 2000. 17 β-Estradiol as an indicator of animal waste contamination in mantled karst aquifers. J. Environ. Qual. **29**:826-834.

Pojana, Giulio, Alessio Gomiero, Niels Jonkers and Antonio Marcomini. 2007. Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environ. Intern. **33**: 929-936.

Simerly, Richard B. 2002. Wired for Reproduction: Organization and Development of Sexually Dimorphic Circuits in the Mammalian Forebrain. Annu. Rev. Neurosci. **25**: 507-36.

Ternes, Thomas A., Henrik Andersen, Daniel Gilberg, and Matthias Bonerz. 2002. Determination of Estrogens in Sludge and Sediments by Liquid Extraction and GC/MS/MS. Anal. Chem. **74**: 3498-3504.

Williams, Richard J. Andrew C. Johnson, Jennifer J.L. Smith and Rakesh Kanda. 2003. Steroid estrogens profiles along river stretches arising from sewage treatment works discharges. Environ. Sci. Technol. **37**: 1744-1750.

Zhang, Z.L., A Hibberd, and J.L. Zhou. 2006. Optimisation of derivatisation for the analysis of estrogenic compounds in water by solid-phase extraction gas chromatography-mass spectrometry. Analytica Chimica Acta **577**: 52-61.

Zhiqiang, Yu and Weilin Huang. 2005. Competitive sorption between 17α-Ethinyl Estradiol and Naphthalene/Phenanthrene by sediments. Environ. Sci. Technol. **39**: 4878-4885.