Estrogenic Compounds in New Haven Harbor

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Abstract
Estrogen-like compounds have shown to cause negative effects to the environment and to human health. For this reason, it is important to be aware of the amount of these chemicals in the environment. Because estrogenic compounds are present in plastics, New Haven Harbor, CT is likely to contain these compounds due to the high amount of plastic contamination found there. This experiment consisted of sample collection of surface water and pore water, filtration, solid-phase extraction, and bioluminescent yeast assays. Samples were obtained from four points along New Haven Harbor: Long Wharf, Sandy Point, Savin Rock, and East Shore Park. The results indicated that there were estrogenic compounds present at each of these sites.

Introduction

Research shows that estrogenic compounds cause many negative effects on the environment and human health, therefore it has become extremely important to be aware of the chemicals present in our oceans, rivers, and estuaries. For instance, it has been demonstrated that *in utero* exposure to phthalate plasticizers is associated with a decreased anogenital distance in male infants indicating undervirilization induced by environmental levels of these endocrine disruptors (Swan et al. 2005). Phthalate exposure to girls has been correlated with an earlier onset of puberty (Colón et al. 2000), an effect that has been experimentally verified in mice in the case of the plastic component bisphenol A as well (Howdeshell et al. 1999). Additionally, epidemiological studies suggest that cumulative exposure to estrogenic chemicals is related to the incidence of reproductive cancers (Henderson, 1988). Evidence suggests that in many instances the presence of these chemicals has had deleterious effects on exposed wildlife populations (Colborn, 1993). Exposure to substances in aquatic environments that mimic hormonal activity of estrogen have been linked to widespread dysfunction of sexual development (intersexuality) in wild fish in the United Kingdom (Jobling et al. 1998) and in North America (Baldigo et al. 2006). The presence of these chemicals in the environment is specifically a concern for New Haven, as the highest concentrations would be expected to occur near urbanized or industrial areas (Jobling, 1995). Chemicals originating from the plastics and detergent industries, such as alkylphenols and bisphenol A have been discovered to be estrogenic (Jobling, 1995). Research has shown that these chemicals migrate from plastic packaging to the surrounding water. For example, one study showed that glassed bottled water had a lower estrogenicity than plastic bottled water from the same source (Wagner, 2009). Because plastics are a source of contamination in the New Haven Harbor, it is likely that estrogenic compounds are present in the water as well. In a study of the investigation on plastic pollution in the New Haven Harbor, Long Warf was shown to contain highest concentration of plastics when compared to Lighthouse Point, Sandy Point, and Savin Rock (Dorrico, 2013). This study was conducted to determine if there are estrogenic compounds in New Haven harbor and to compare each of the four sites.

Materials and Methods

To remove any residual organic material, all glassware was washed with soap and water, then rinsed with deionized water, and then rinsed with methanol. The glassware was then baked at 450^{o}C for at least 4 hours.

Sample Collection: Surface water and pore water samples were taken from 4 points along New Haven Harbor: Long Wharf, Sandy Point, Savin Rock, and Lighthouse Point Park (Figure 1). Surface water samples were obtained by using 1 liter glass bottles. Pore water samples were obtained using a metal tube wrapped in cloth. The tube was placed in a hole and water was allowed to flow into it. A glass beaker was then used to collect the water in the tube and transfer it to a 1 liter bottle.

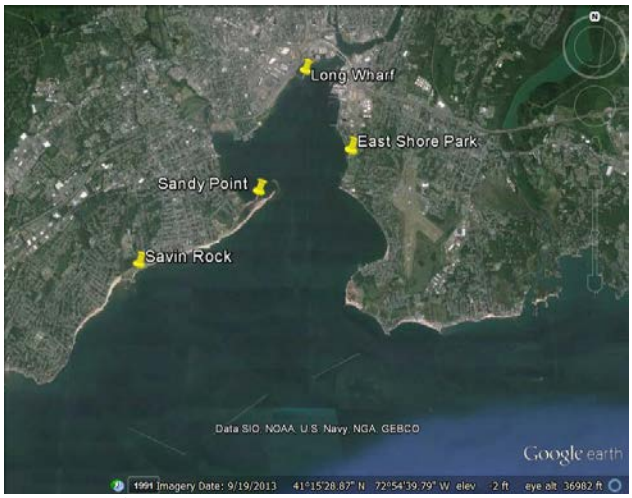

Figure 1: The four sites tested in this experiment were Savin Rock, Sandy Point, Long Wharf, and East Shore Park.

Filtration

GMF 150 multigrade filters with a 1 micrometer retention were used to filter out any large particles present in the water samples.

Solid-Phase Extraction

EPA Method 1694 was used with Hydrophilic-Lipophilic-Balance (HLB-H) Oasis disks to complete a solid phase extraction. Minor modifications were made to the method to accommodate the saltwater samples, including flowthrough of 20 ml of deionized water after the sample to remove any remaining salt on the disks. The analytes were eluted using 12 ml methanol. The methanol was then evaporated from the eluents under a gentle flow of nitrogen gas at a temperature of 50-55^{o}C. The remaining sample was then redissolved in 1 ml of deionized water and transferred into a 4 ml glass vial.

Yeast Assays

Genetically engineered *Saccharomyces cerevisiae* containing a human estrogen receptor and a plasmid-based estrogen response element was used (Sanseverino et al. 2009). Briefly, *S. cerevisiae* BLYES and *S. cerevisiae* BLYR were grown in yeast minimal media overnight at 30^{o}C and 200 rpm shaking. To make a standard curve, 17β-estradiol (E2) was serially diluted in methanol. Each of the sample solutions was added to a black 96-well plate along with the yeast. The plates were left to incubate at 30^{o}C for 3-4 hours and bioluminescence was measured using a SpectraMax plate reader.

Controls

Two trials were conducted using 1 L of deionized water to determine if any estrogenic compounds were present in control samples during the experiment. Two trials were also conducted with artificial sea water to determine if salt had an effect on the detection of estrogenic equivalents by the yeast. A standard solution containing 2.02 x 10^{-5} M 17β-estradiol was used to determine the percent recovery of the method. To determine if the pore water sampler blank introduced any estrogenic compounds to the sample, deionized water was run through the sampler and collected.

Results

The results of this study indicate that there are estrogen-like compounds present at each of the sites tested along New Haven Harbor.

Table 1: Estrogenic equivalents in ng/L.

	Surface Water		Pore Water	
	Trial 1	Trial 2	Trial 1	Trial 2
Long Wharf	No	61.0 1.6 response	5.7	
Sandy Point	5.7	No	9.7 280.1 response	
Savin Rock	39.8	15.3	26.3	5.9
East Shore Park	41.1	10.2	No response	No response

Table 2: Estrogenic equivalents in ng/L

Contro		
	Trial 1	Trial 2
D.I. Water	No response	No response
Artificial Sea Water	17.9	No response
Standard Solution	4242.8	3.9
Pore Water Sampler Blank	No response	No response

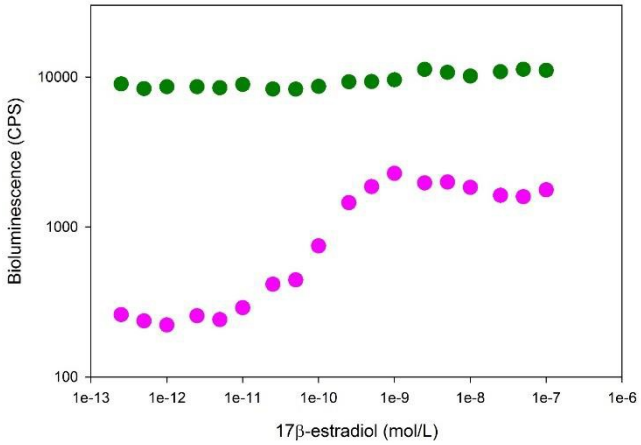

Figure 2: Standard curve of bioluminescence versus concentration of 17 β-estradiol in mol/L. The pink circles represent *S.cerevisiae* BLYES and the green circles represents *S. cerevisiae* BLYR, after exposure to the standard. This graph was used to determine the amount of estrogenic equivalents present in the samples.

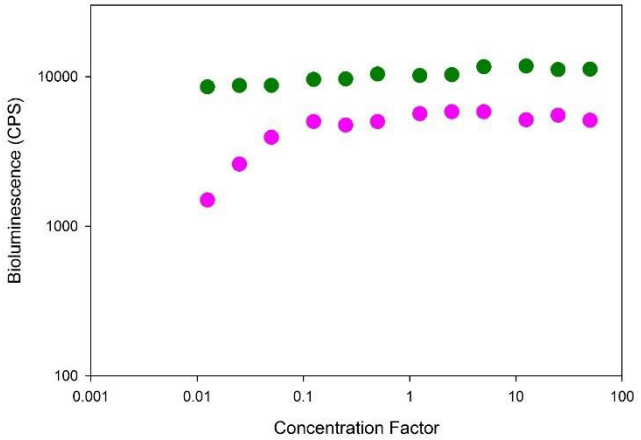

Figure 3: Bioluminescence versus concentration of a standard solution sample spiked with 17 β-estradiol. The pink circles represent *S. cerevisiae* BYLES amd the green circles represent *S cerevisiae* BLYR. This sample contains 4242.8 ng/L estrogenic equivalents.

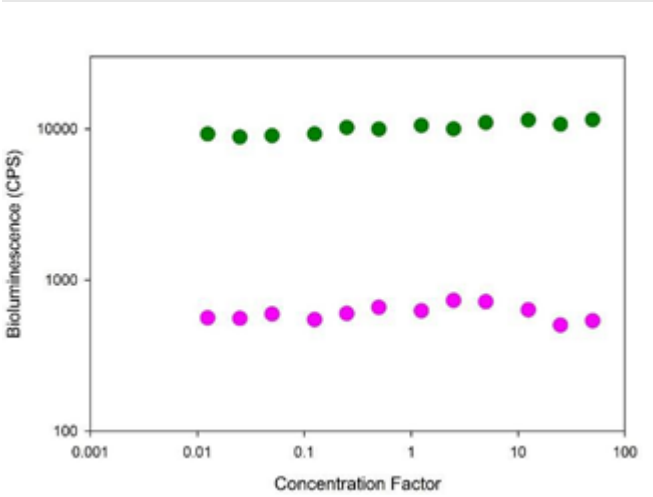

Figure 4: Bioluminescence versus concentration of the control deionized water sample. The pink circles represent *S. cerevisiae* BLYES and the green circles represent *S. cerevisiae* BLYR.

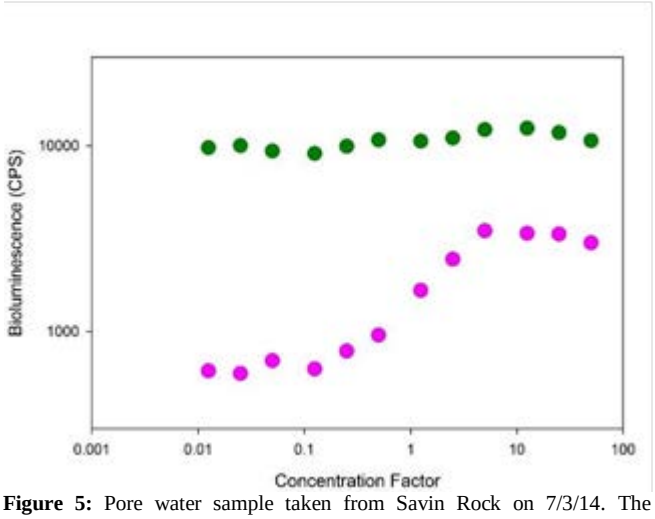

Figure 5: Pore water sample taken from Savin Rock on 7/3/14. The pink circles represent *S. cerevisiae* BLYES and the green circles represent *S. cerevisiae* BLYR. This sample contained 26.3 ng/L estrogenic equivalents.

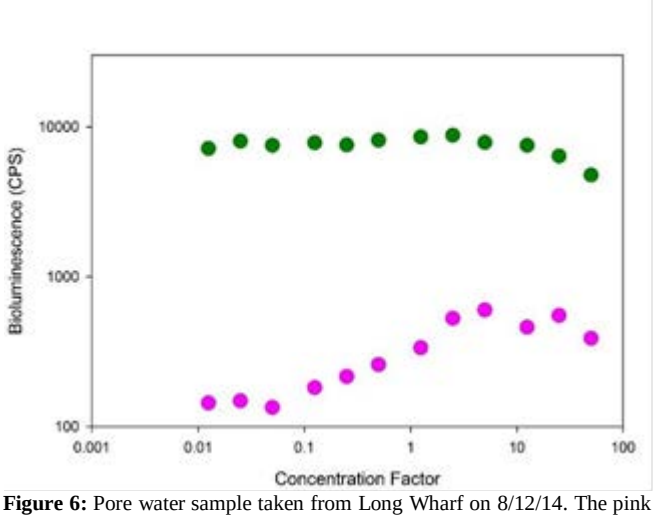

Figure 6: Pore water sample taken from Long Wharf on 8/12/14. The pink circles represent *S. cerevisiae* BLYES and the green circles represent *S. cerevisiae* BLYR. This sample contained 1.6 ng/L estrogenic equivalents.

Conclusion

The results of this experiment indicated that there are potentially estrogenic compounds present in New Haven Harbor. By comparing the curve of each sample graph to the standard curve, the amount of estrogen equivalents was determined for each sample. The control deionized water sample (figure 4) and the pore water sampler blank results indicated that no estrogenic compounds were present, or that they were below the detection limit. This shows that estrogenic compounds were not introduced into the sample by the pore water sampler. The sample spiked with 17 β-estradiol (figure 3) contained 4242.8 ng/L estrogenic equivalents for trial 1. This indicated that the method had a percent recovery of 78%. The second trial had a significantly lower value, which is likely due to the fact that the sample was not processed in the yeast assays immediately after extraction.

Estrogenic compounds were detected at all four of the sites tested. The pore water sample taken from Sandy Point contained the highest amount of estrogenic equivalents, followed by the surface water sample taken from Long Wharf. However, the concentrations of these substances were variable. A second trial indicated that the yeast did not detect estrogenic compounds in the Long Wharf surface water sample. For this reason, more trials are necessary to understand what drives the changes in concentrations of hormone-mimicking chemicals in local waterways.

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Biography

Kaitlynn is a senior majoring in biology/pre-med and forensic science with a minor in chemistry. She plans on pursuing a career in research upon graduation in May 2015.