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Estrogenic Compounds in New Haven Harbor

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Introduction

As more research indicates that estrogenic compounds cause many negative effects on the environment and human health, it has become extremely important to be aware of the chemicals present in our oceans, rivers, and estuaries. 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 (intersex) 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. In a previous SURF study on the investigation on plastic pollution in the New Haven Harbor, Long Wharf was shown to contain the highest concentration of plastics when compared to Lighthouse Point, Sandy Point, and Savin Rock (Dorrigo, 2013). The current study was conducted to determine if areas with a high level of plastic contamination have a higher concentration of estrogenic compounds.

Materials and Methods

Sample Collection: Glass sample bottles were cleaned with soap and water, then rinsed with deionized water and methanol. They were then baked at 450°C for 4 hours to remove any organic compounds. Samples were taken from beaches along New Haven Harbor: Long Wharf, Sandy Point, Savin Rock, and East Shore Park. Pore water samples were obtained using the sampler shown in Figure 9. Surface water samples were collected by submerging the bottles under the water.

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

Extraction: Samples were extracted according to EPA Method 1694. The sample was passed through Hydrophilic-Lipophilic-Balance (HLB-H) Oasis disks at a flow rate of 1 ml/min. The disks were rinsed with deionized water to remove salt. Methanol was then used to elute the compounds from the disks. The extracts were then dried down completely and frozen until use then resuspended in deionized water and serially diluted for use in the yeast assays.

Yeast Assays: *S. cerevisiae* BLYES and *S. cerevisiae* BLYR were grown in yeast minimal media overnight at 30°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°C for 3-4 hours and were then read using a bioluminescent 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 amount of estrogenic equivalents present. A standard solution containing 2.02×10^{-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

Table 1: Estrogenic equivalents (ng/L) in the controls.

	Controls	
	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

Table 2: The amount of estrogenic equivalents in each sample in ng/L.

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

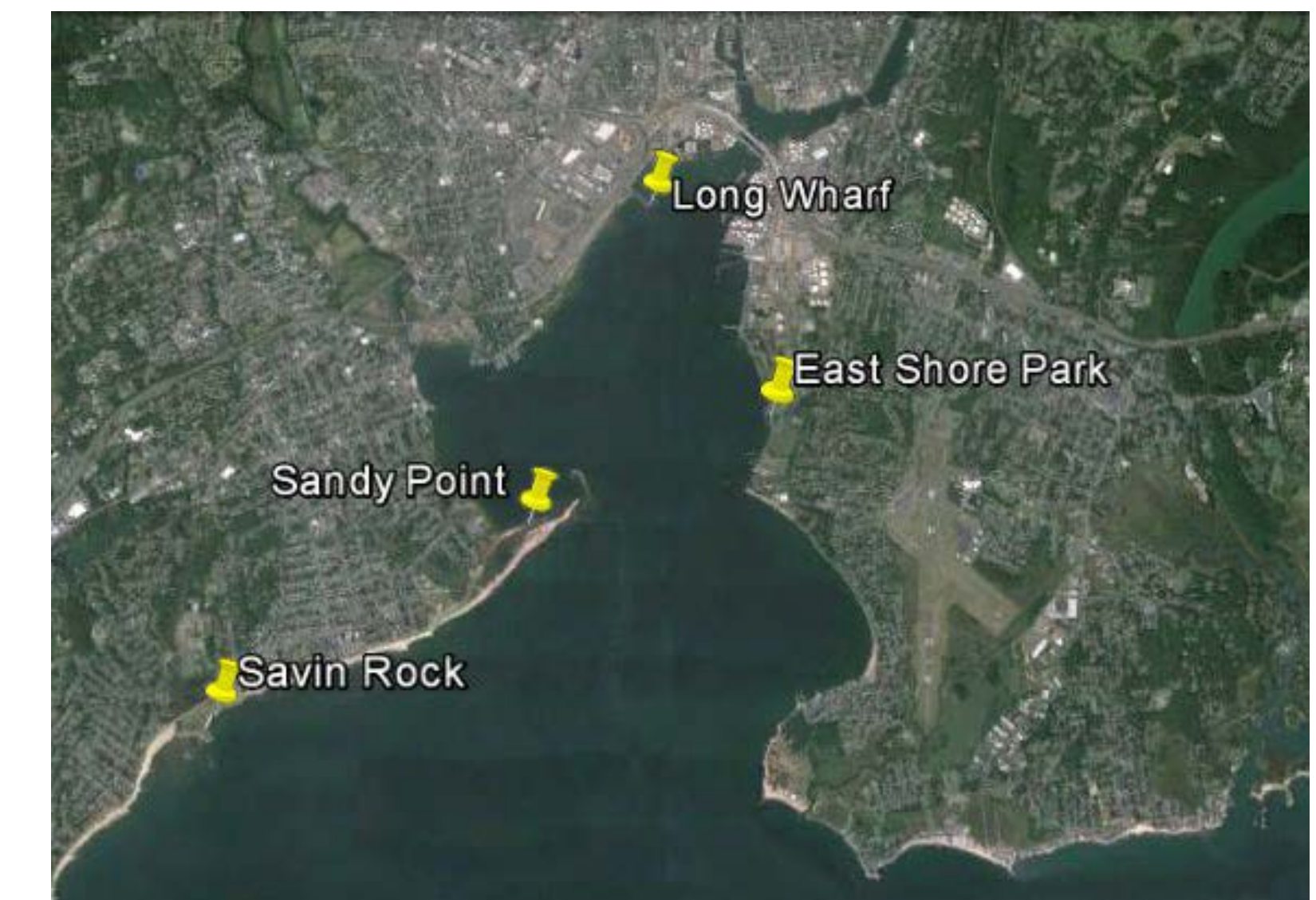
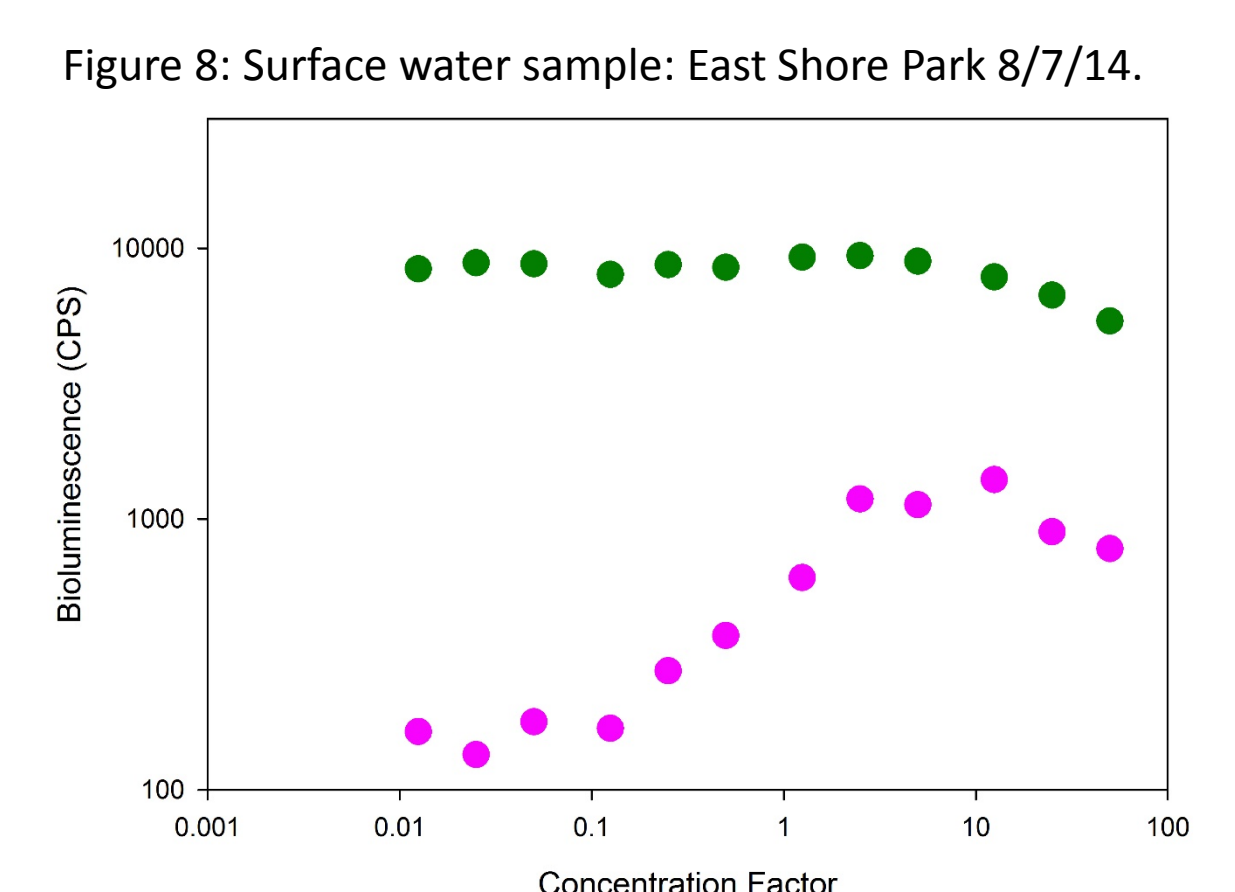
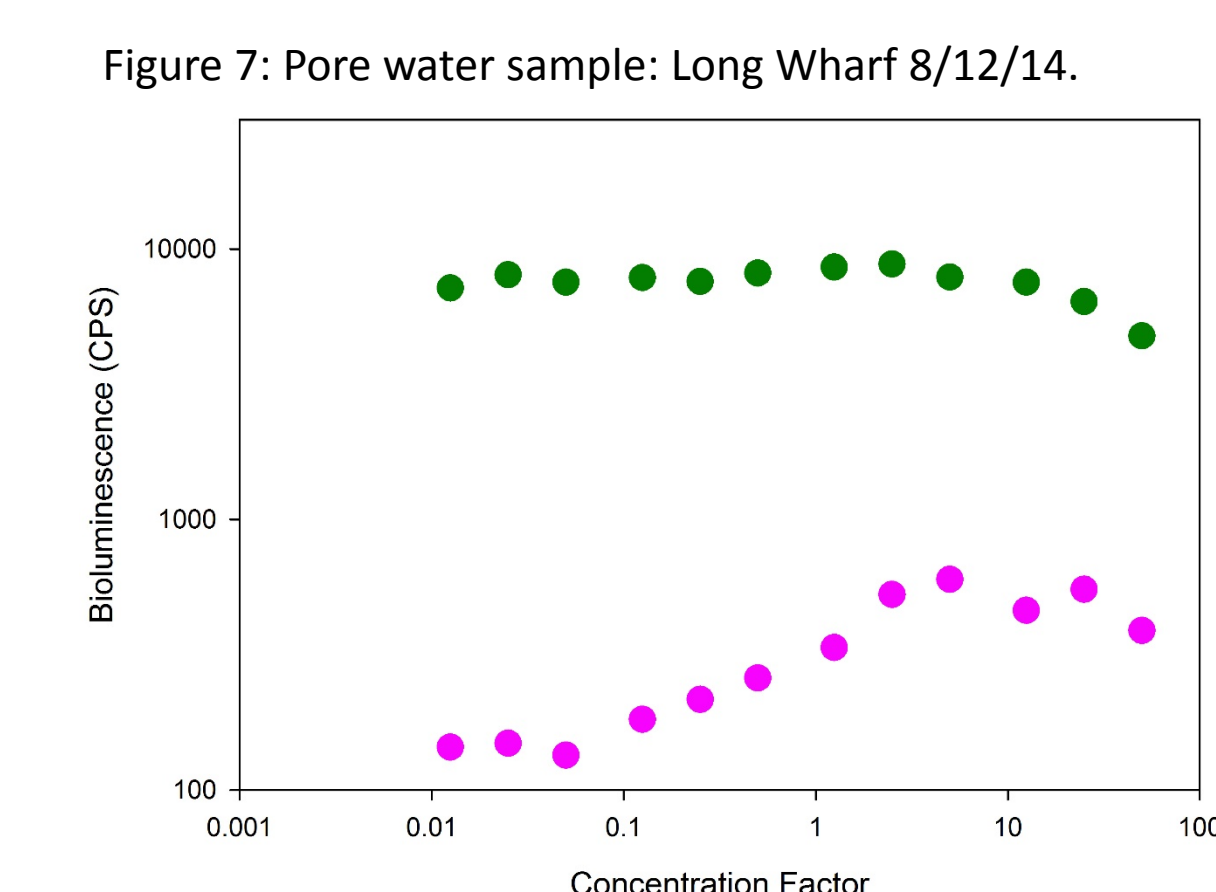
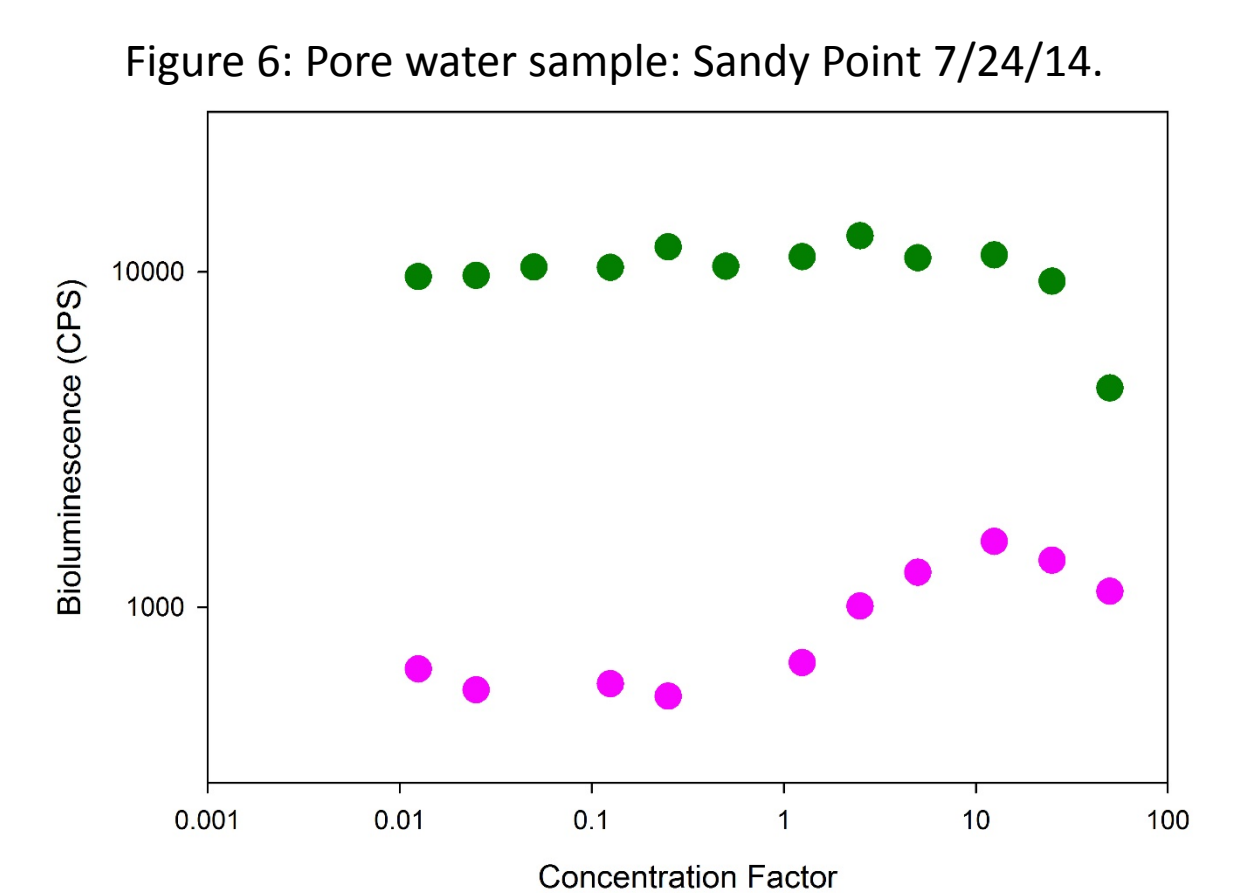
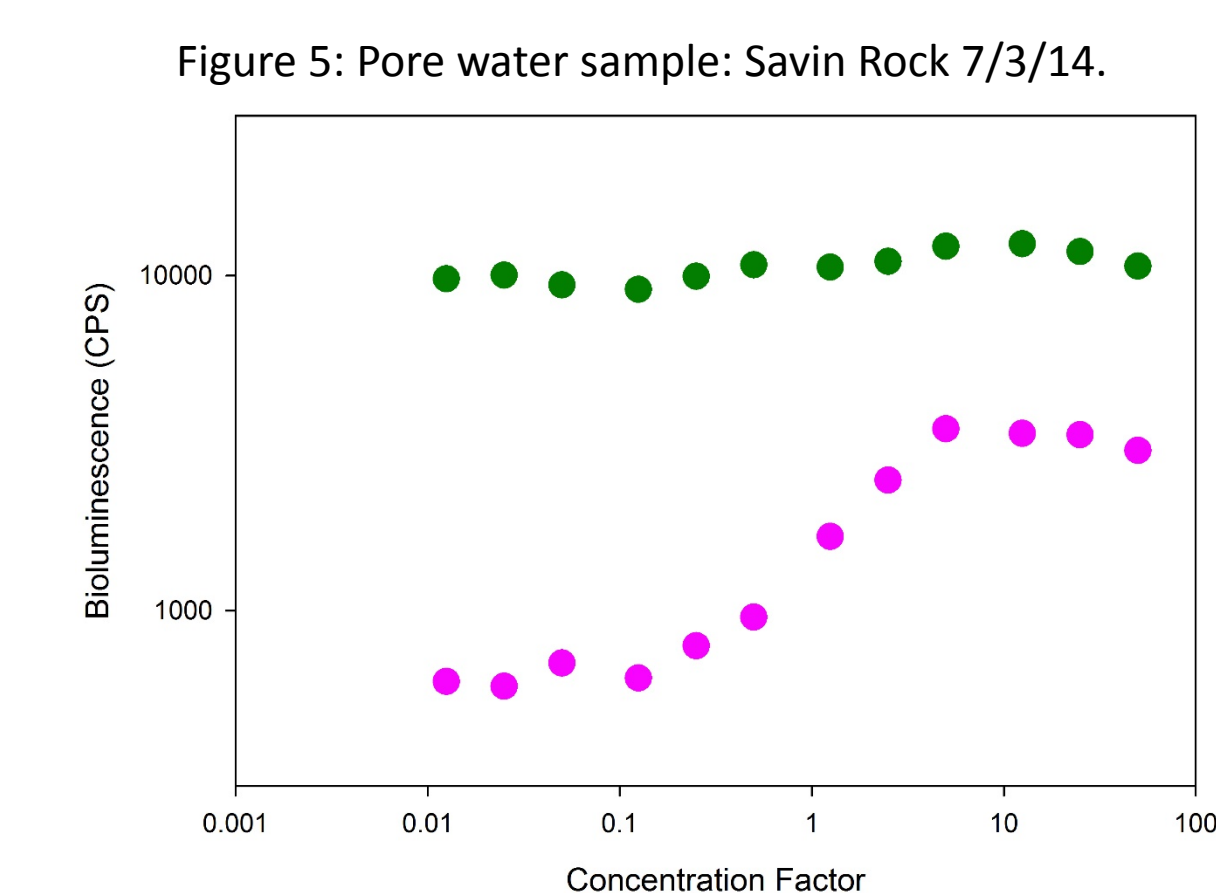
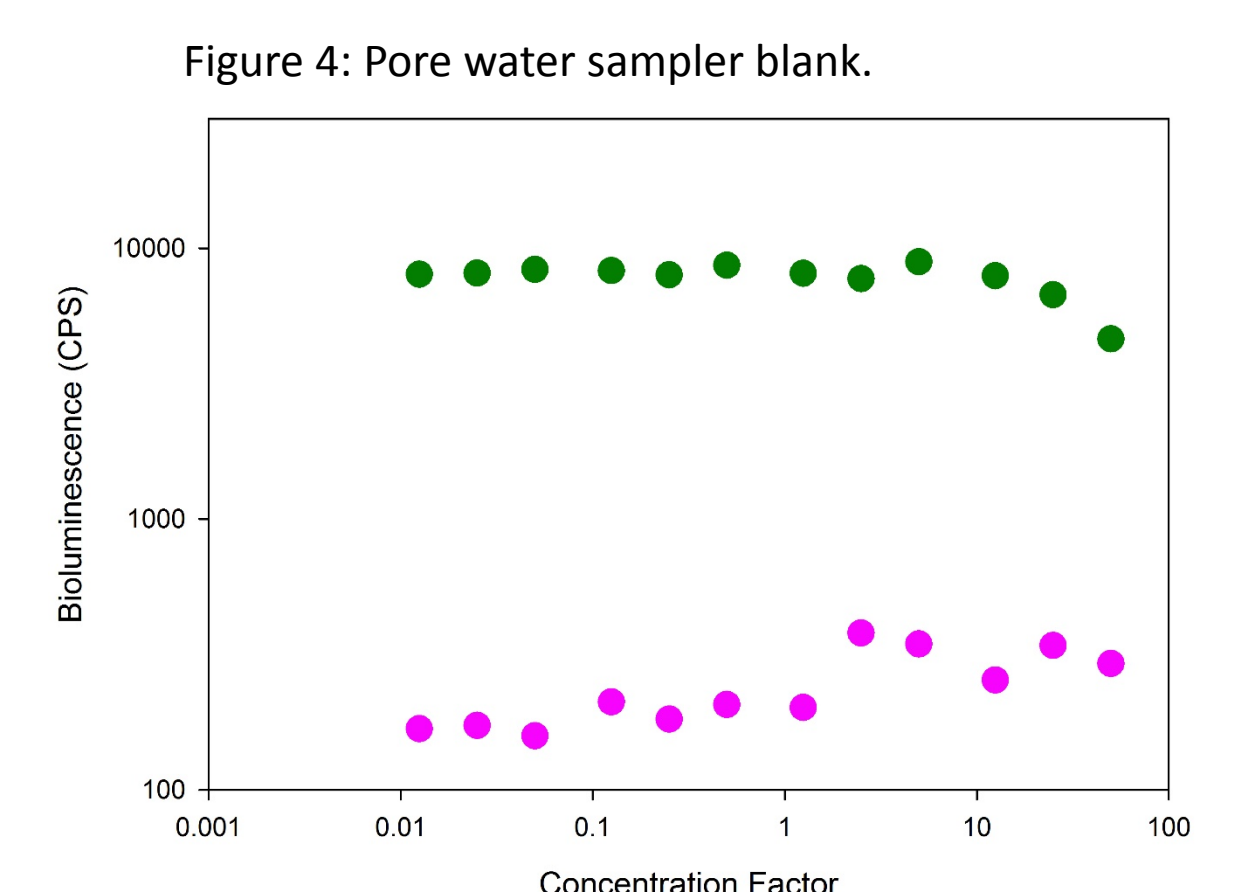
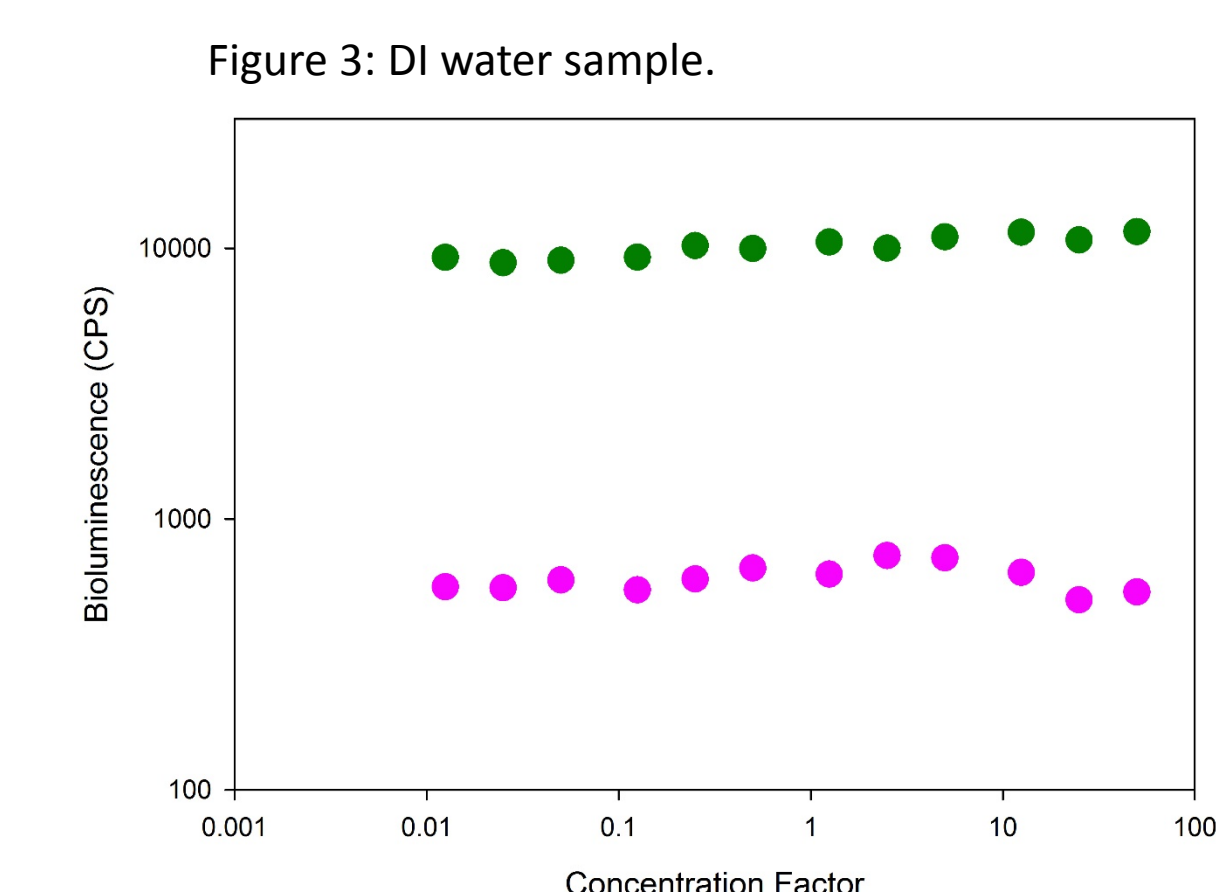
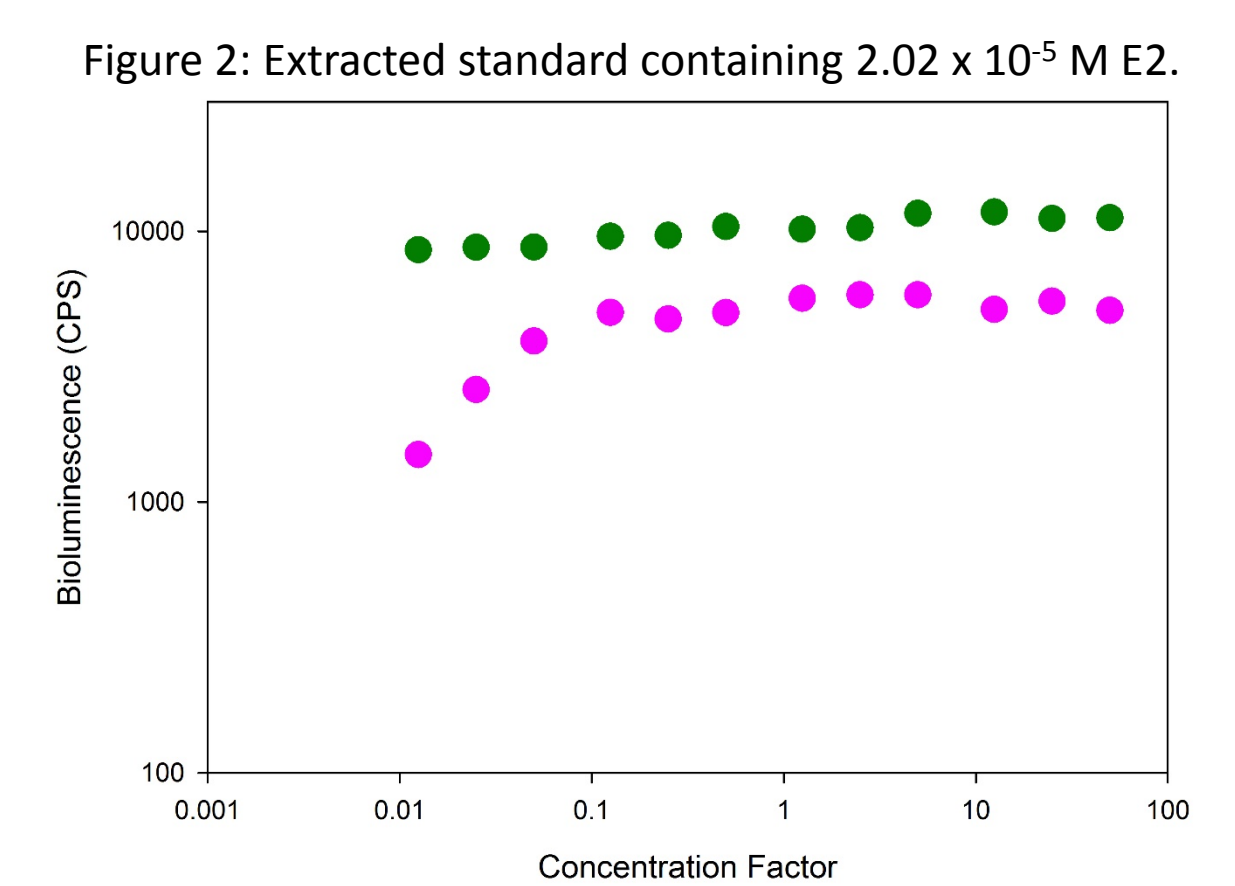
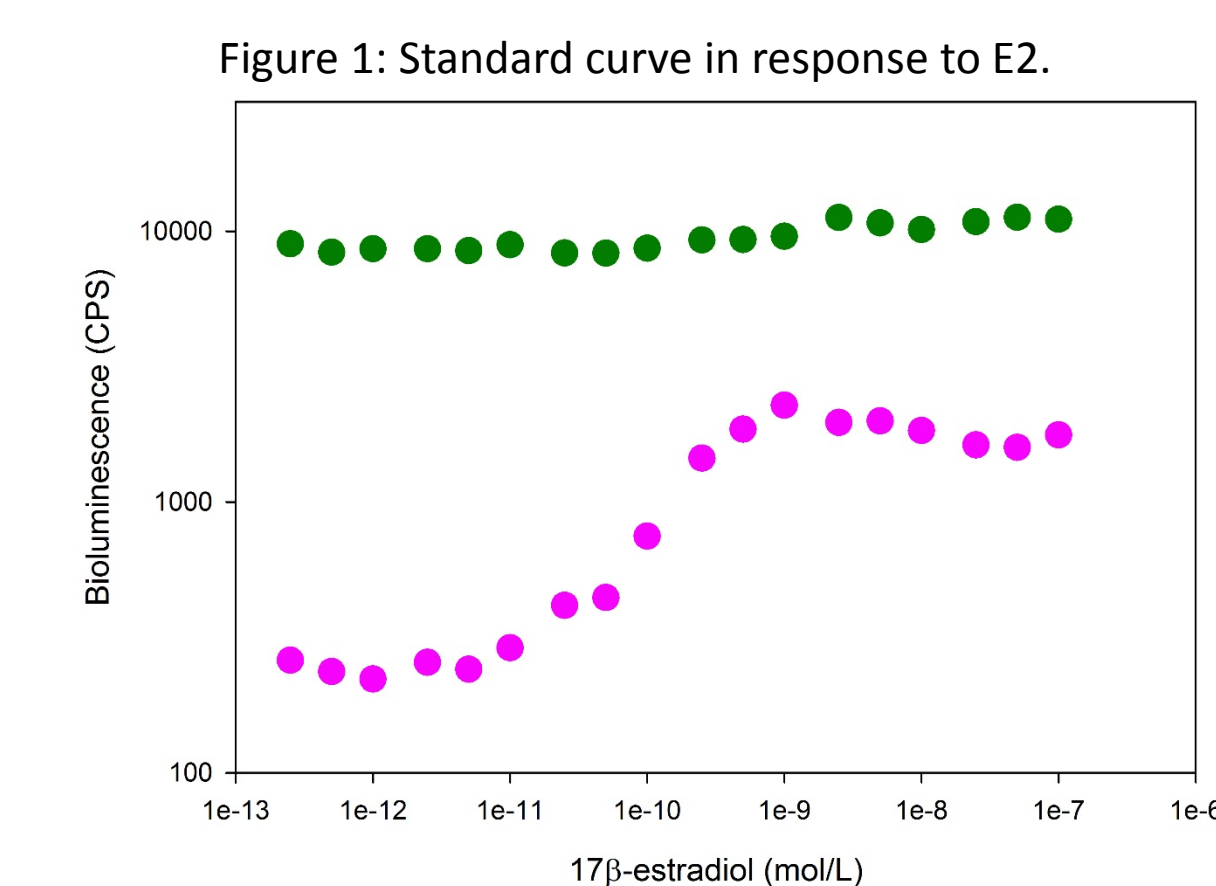


Figure 9: Four sampling locations used in this study: Savin Rock, Sandy Point, Long Wharf, and East Shore Park.

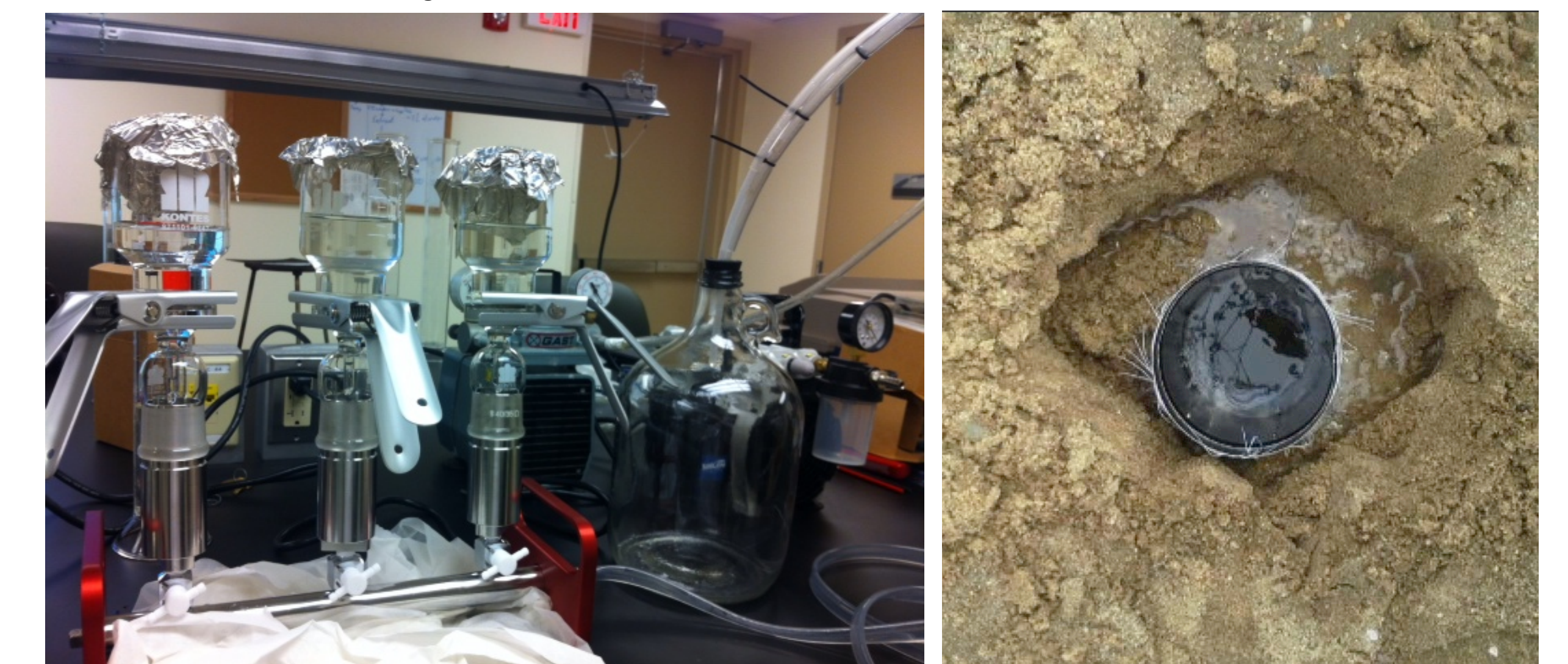


Figure 10: Solid-phase extraction equipment. This set up was used to extract and concentrate chemicals from natural samples. Figure 11: Pore water sampler. A metal tube with holes was surrounded by cloth then placed in a hole, allowing the water to be collected in the tube.

Conclusion

The results of this experiment indicate 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. 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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Acknowledgements

I would like to thank Mr. and Mrs. Carrubba and the SURF program for making this project possible. I would also like to thank both of my faculty advisors, Dr. Eldridge and Dr. Simjouw, for their help and guidance throughout this project. Also, thank you to the chemistry department for allowing us to use their furnaces.