The COMMUNITY Foundation *for* Greater New Haven
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QUINNIPIAC RIVER FUND FINAL REPORT- 2018

Please complete and submit completed form via e-mail to dcanning@cfgnh.org at The Community Foundation for Greater New Haven by March 29, 2019 (or as otherwise stated on the terms of grant).

Date: __6/29/2018_____

Group/Organization Name: _University of New Haven _____

Address: ____300 Boston Post Rd_____

City, State, & Zip: ____West Haven, CT 06510_____

Telephone #: ____203 932 1253_____

Project Name: ____Trace sources of endocrine disruptors and toxicity_____

Grant Number: ___20170146_____

Name & title of person completing this form: ___Dr. Jean-Paul Simjouw_____

E-mail address: __jsimjouw@newhaven.edu_____

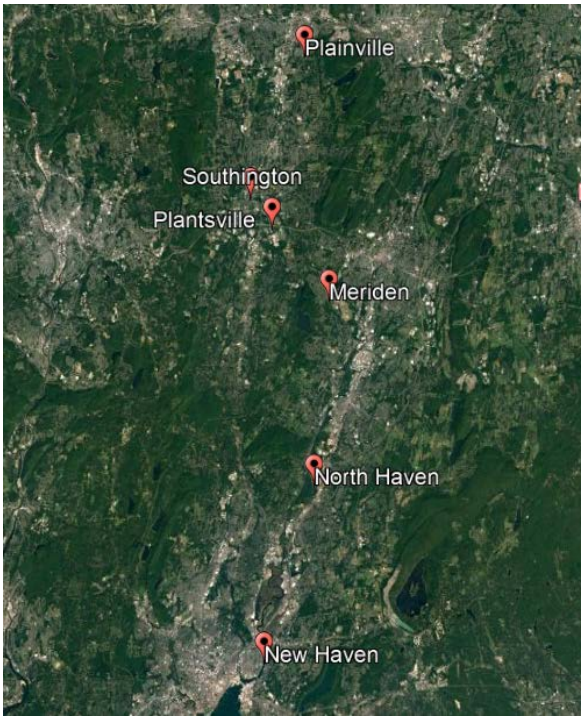
Please respond to the following statements:

1. List the specific objectives/outcomes of the project and tell how they were met during the grant period. Also, provide an update on any special conditions of the grant (if applicable). The overall objective for this project was to support the study of several sites along the Quinnipiac River for trace sources of endocrine disruptors and toxicity. More details outcomes are listed below:

- **Obtain data to determine if the sites along Quinnipiac River show a seasonal effect in estrogenic response.** Flow conditions and sediment load are factors that can impact the presence of EDCs in the river system. Water and sediment samples were collected, solid phase extracted, and processed with bioluminescent yeast bioassays to determine the concentration of bioavailable estrogens and any toxic substances.
- **Target positive sites identified by our previous study and determine potential sources of endocrine disruptors.** Sites that contained positive samples in our previous studies were monitored more intensively to attempt to track sources of the anthropogenic inputs. Sites that need more extensive study, may be studied by this research group or by other groups who can use chemical analysis to determine which particular chemical compounds are present if necessary.

- **Measure estrogenic response of sediment samples from our study sites.** Sediment samples will be collected to compliment the data obtained from water samples for a complete determination of EDCs in the river system. The sediment data will give a better long term view of EDC presence in the river (legacy pollution).

Based on previous studies we identified the following sites for further study; from north to south: Plainville, Southington, Plantsville, Meriden, North Haven, and New Haven (Figure 1). These sites were visited from June to December 2017



Water analysis

All water samples from each sampling period were extracted according to a modified version for this study of the EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. The samples were loaded at a flow rate of 5-10 mL/min through SPE Hydrophilic- Lipophilic Balanced disks. The disks were dried and the samples were eluted by adding 12ml of methanol. The extract was dried by using a nitrogen evaporator, re-dissolved in 1000 µl of deionized (DI) water and then stored in a -20°C freezer. Standard dilutions were made with 17β- estradiol and each water sample was diluted with deionized water. Both the standard dilution and the 6 water sample dilutions were added to a 96-well plate at a time. 50µl of standard dilution number 24 was added to the appropriate wells of the plate and left to dry. After completely dry, 100µl of the water sample dilutions were added to the appropriate remaining wells. 100 µl of Saccharomyces Cerevisiae BLYR (EDC toxicity reporter) was then added to all of the wells. Next, 100 µl of DI water was added only to the standard dilution wells. The plate was sealed and placed in the incubator at 30°C for 3-4 hours. After incubation time the plates were placed in a Spectra Max plate reader and bioluminescence was measured.

As stated below, we were unable to obtain concentration values for present EDCs but were able to determine toxicity potential of the samples. The toxicity potential is shown by the decrease in bioluminescence at a lower concentration factor.
Figures 2-8 show standard dilutions and water sample for the collection month of June.

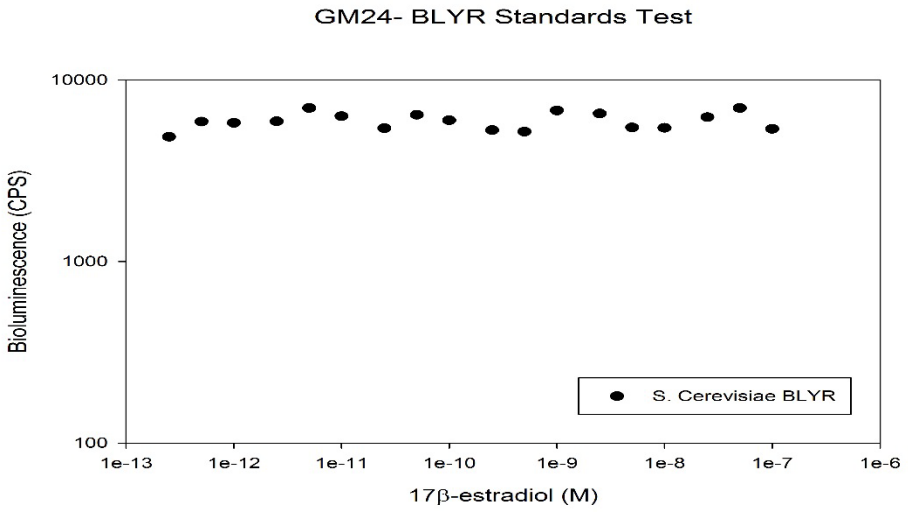


Figure 2. Standards Test 2018-04-03
(17β- estradiol incubated with 2015 BLYR for 4 hours)

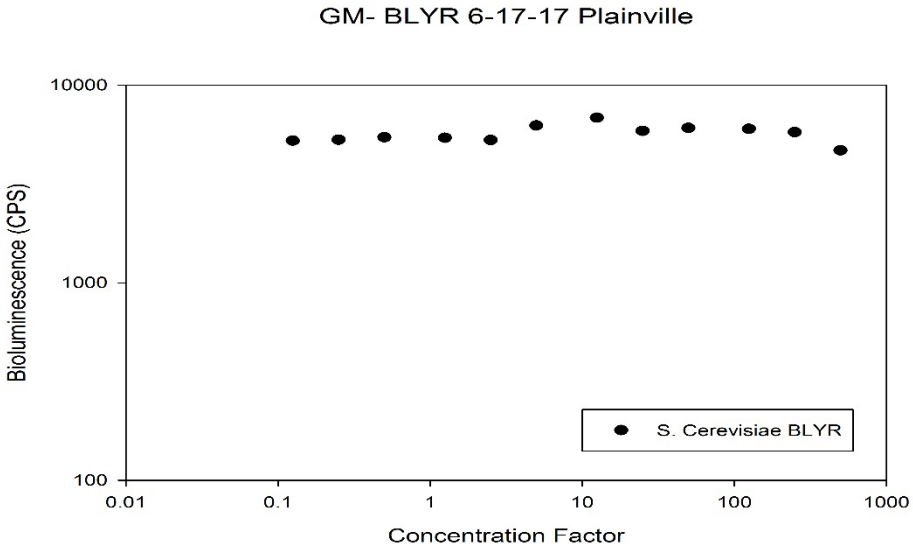


Figure 3. Sampling Test 2018-04-03
(Water Sample (Plainville- June) incubated with 2015 BLYR for 4 hours)

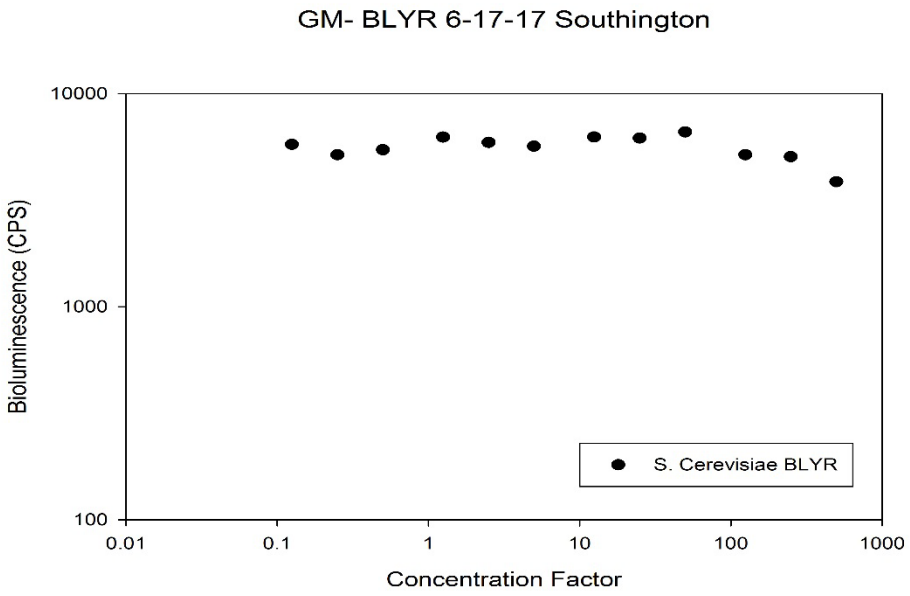


Figure 4. Sampling Test 2018-04-03
(Water Sample (Southington- June) incubated with 2015 BLYR for 4 hours)

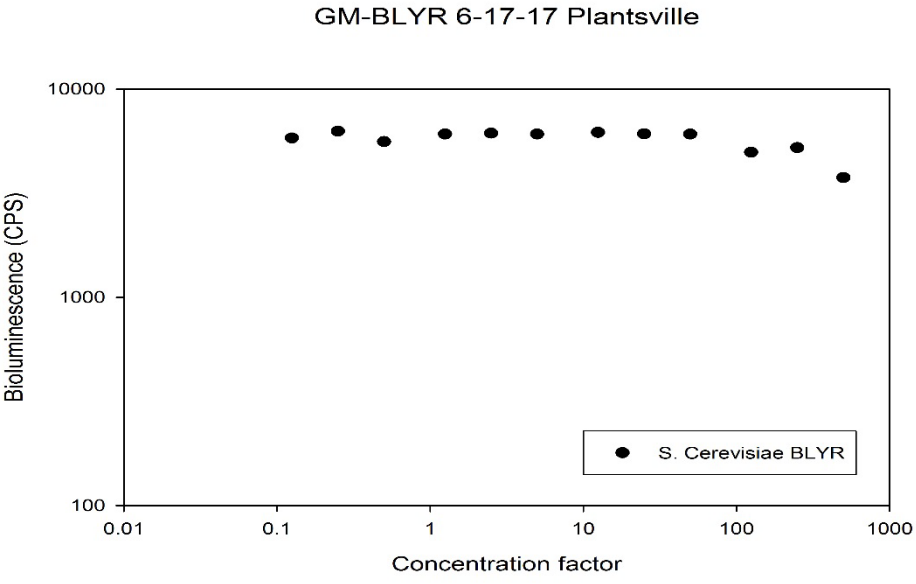


Figure 5. Sampling Test 2018-04-03
(Water Sample (Plantsville- June) incubated with 2015 BLYR for 4 hours)

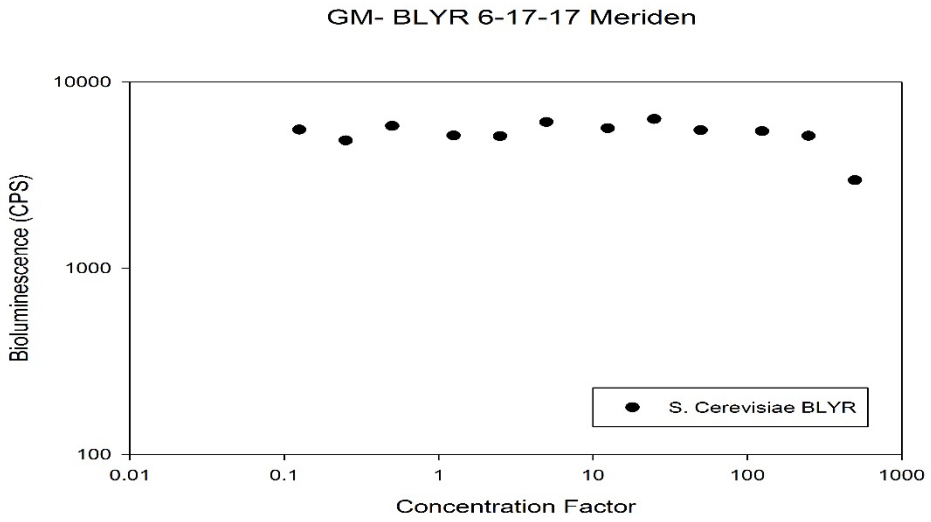


Figure 6. Sampling Test 2018-04-03
(Water Sample (Meriden- June) incubated with 2015 BLYR for 4 hours)

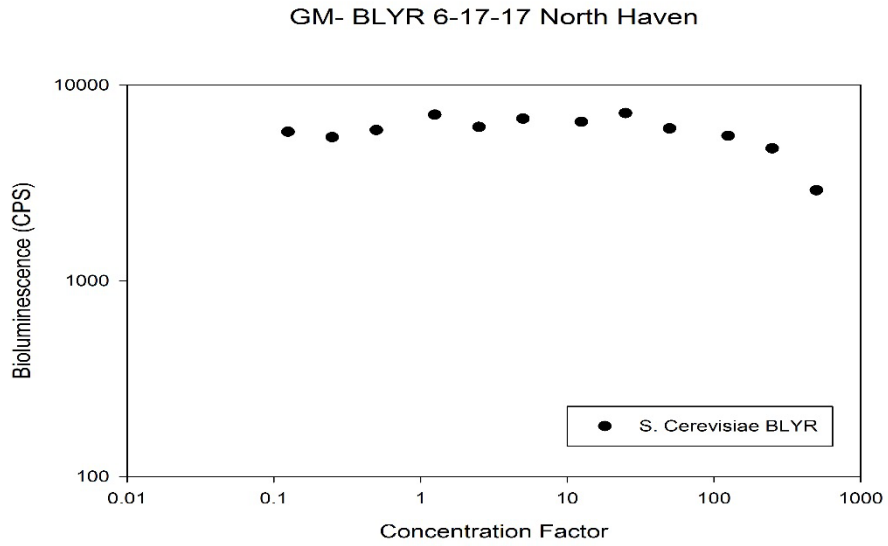


Figure 7. Sampling Test 2018-04-03
(Water Sample (North Haven- June) incubated with 2015 BLYR for 4 hours)

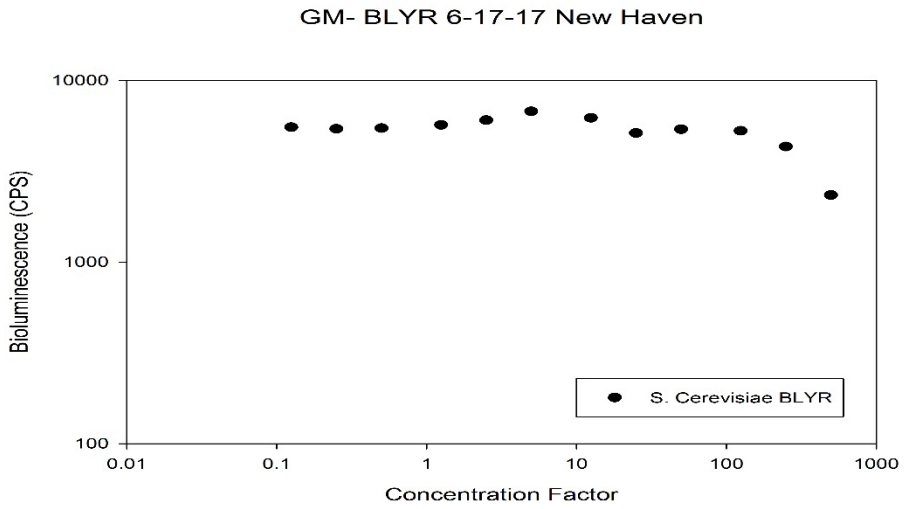


Figure 8. Sampling Test 2018-04-03
(Water Sample (New Haven- June) incubated with 2015 BLYR for 4 hours)

Contamination patterns were seen in all samples of the river compared to the blank (fig. 2) and these levels can possibly be affecting the marine life in the river in detrimental ways. To determine which areas are more concentrated than others, BLYES would have to be utilized instead of BLYES.
However, it is apparent that the North Haven sample for June shows more toxicity potential (decline in bioluminescence at lower concentration factor) than the other sites; next down would be the New Haven site followed by Plantsville.
Seasonal sampling indicates that only a few sites show more toxicity potential (Table 1):

Month 2017	Most toxicity potential
July	Meriden, Plantsville
August	North Haven
September	Meriden, North Haven, New Haven
October	Meriden
November	Plantsville

With this information it might be possible to zero in on potential sources of this potential toxicity by EDCs. River flow, rainfall, and land use along the river need to be considered.

Sediment analysis
Samples were extracted according to the modified EPA Method 1694; this modified version was developed for this study. Up to 5 g of each sample was weighed out into glass centrifuge tubes. A phosphate buffer was used to adjust the pH, to a pH of 2. The samples were then treated with three cycles of a phosphate buffer (pH 2), acetonitrile, 30 minutes of sonication, and 5 minutes of centrifugation at 3000 rpm. After each centrifuge, the aqueous layer of each sample is extracted and transferred into 250 mL glass jars. The glass jars were placed into a water bath at 50°C. The extracted solutions were then dried down to 20-30 mL with a nitrogen gas stream. Once the extracted solutions were dried, 0.5 g of EDTA and 200 mL of DI water was added. To complete the solid-phase extraction, a Hydrophilic-Lipophilic-Balance Oasis (HLB) disk at a rate

of 5-10ml/minute was treated with methanol, DI water and DI water with pH of 2. Once the disks were treated, each solution was extracted through the Oasis disk then rinsed with 20 mL of DI water, and eluted with 12 mL of methanol. Each extracted solution was then dried down with a nitrogen gas stream in a N-EVAP Nitrogen Evaporator. Once the extracted solutions were dried, they were reconstituted with 1 ml of DI water and placed in the 20°C freezer until processed in yeast assays.
Yeast analysis was done as stated before. The results indicate a higher toxicity for the sediment but for similar sites as identified by the water analysis.

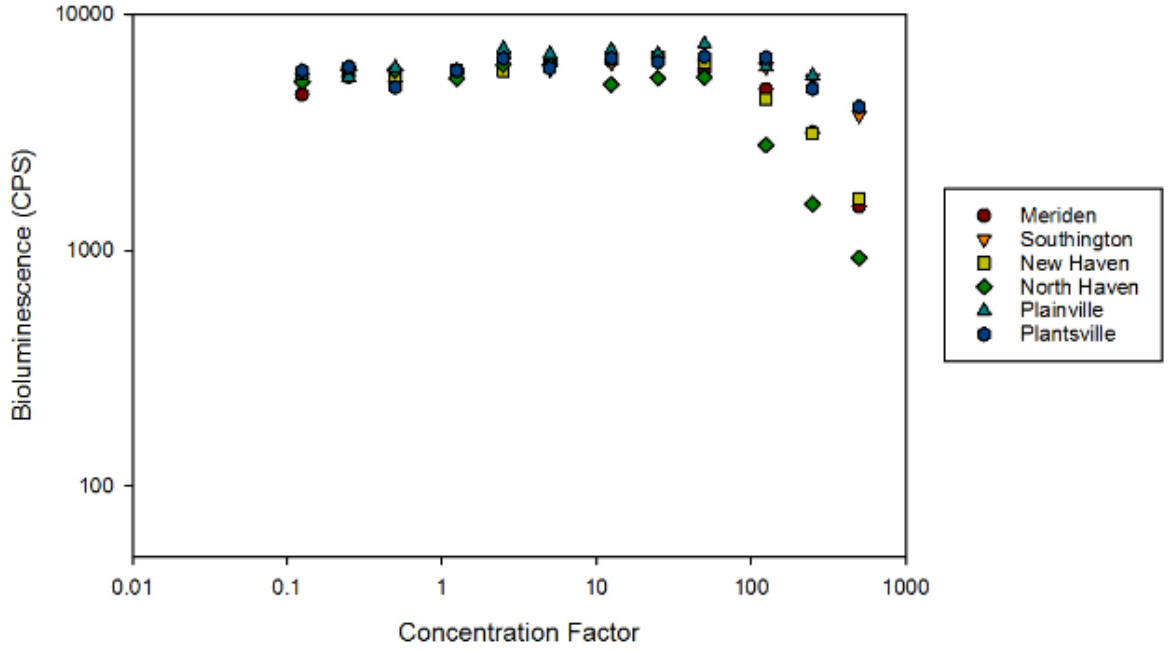


Figure 9: Bioluminescence of Quinnipiac River sediment samples collected at Meriden, Southington, New Haven, North Haven, Plainville and Plantsville on 6/17/17.

Unfortunately, without being able to determine EDCs concentration this higher toxicity potential might be artificial, but the fact that the same sampling sites are identified does strengthen the notion that these sites are more impacted by EDCs. Again, Meriden and North Haven are where we found the most toxicity potential. It is possible that legacy pollutants are the cause for the increase values of the water samples but that needs to be combined with the river flow, run off, and land use to get a more definite conclusion.

2. Please share your successes, challenges and any lessons learned through the implementation of your project. Were there any unintended consequences or lessons learned that may affect how you operate your program moving forward?

Unfortunately, at the critical moment when we needed the yeast for sample analysis, we were unable to obtain reliable standard curves for EDC concentration measurements. The yeast had lost the ability to provide luminescence at low EDC concentrations. What remained was determining toxicity of samples on the yeast (too much EDC and the yeast loses all its

luminescence in response to EDC). This is not what the study was built on; now we can only say that certain water samples and sediment samples possibly have more EDC's and/or EDC's that are more toxic. Without concentration values we cannot make this distinction. The faculty member that could have provided the modified yeast had left the university several months prior. The analysis technique working with the yeast is very tricky and needs multiple practice rounds before a good calibration curve can be expected; unfortunately the yeast was impacted before the students were ready to analyze samples. I am currently looking into revisiting this environmental toxicology study using different techniques and truly hope to submit a new proposal for 2019.

3. What are the opportunities and needs of your organization as it continues to move forward with its work to positively impact the Quinnipiac River?

As stated earlier, the technology to continue the project is no longer available at the University of New Haven. However, I am currently exploring other avenues to address the issues, e.g. what is different about the sediment at the North Haven site and what some potential sources are, using different techniques that are available.

Also, please include a photo or image that can be uploaded along with your report to The Quinnipiac River Fund website.

University of New Haven student Amy Miller collecting sediment samples. Sediment samples were collected in 40mL amber glass vials from 6 sites along the Quinnipiac River. The vial was pushed into the sediment, the sediment filled the vial and the vial was pulled out.

University of New Haven students Amy Miller and Gabrielle Montlouis collecting water and sediment samples from the Quinnipiac River (Meriden Site). Water Samples from 6 different sampling sites including New Haven, North Haven, Meriden, Southington, Plainville, and Plantsville (Figure 1) were collected in 1-liter glass bottles and immediately placed on ice. Samples were then filtered through a Whatman 0.22 Glass microfiber filter and stored for further analysis.

Collecting even if the weather is bad…