The Community Foundation *for* Greater New Haven

QUINNIPIAC RIVER FUND FINAL REPORT- 2014

Please complete and submit completed form via e-mail to dcanning@cfgnh.org at The Community Foundation for Greater New Haven.

Date: 11 January, 2015

Group/Organization Name: University of New Haven

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Project Name: Using vitellogenin and choriogenin gene expression in wild mummichog (*Fundulus heteroclitus*) as bioassays for the presence of endocrine disrupting chemicals in the lower Quinnipiac River

Grant Number: 20140144

Name & title of person completing this form: John T. Kelly, Associate Professor

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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 goal of this study was to look for evidence of the deleterious effects of endocrine disrupting chemicals (EDCs) in wild fish within the lower Quinnipiac River. Endocrine disruptors are a broad class of pollutants that interfere with the normal functioning of the endocrine system in animals. They can have harmful impacts on reproduction, growth, and development of animals, including humans, who are exposed to polluted waters. One of the most common types of EDCs are those that mimic sex steroids, particularly estrogens. These chemicals include natural or synthetic estrogens from pharmaceutical sources (e.g. therapeutic medicines, birth control pills), as well as a host of chemical pollutants with estrogenic effects, including PCB's, pesticides, and plasticizers such as phthalates and bisphenols. These pollutants often enter aquatic ecosystems in wastewater treatment effluent or industrial discharge. Endocrine disrupting chemicals (EDCs)

can be difficult to test for directly, so fish have often been used as a bioassay since they are directly and continuously exposed to the water, and are vertebrates sharing a very similar endocrine system to humans.

In this study, a common local species of killifish, mummichog (*Fundulus heteroclitus*), was captured in the lower Quinnipiac River. The length and weight of each fish was measured, and sex and reproductive status confirmed by examination of the gonads, which were removed and weighed to determine gonadosomatic index (the ratio of dried gonad mass/body mass). Using qRT-PCR, liver tissue from male fish were tested for the activity of specific genes associated with reproductive proteins that should be found only in female fish. The presence of two gene products were looked for – vitellogenin mRNA, which has been used in this species in other regions as a bioassay for EDCs, and choriogenin mRNA, which has been identified as an candidate for use as a bioassay in Japanese medaka (*Oryzias latipes*), but has not yet been developed in this species. This was one of the few studies of EDCs ever conducted in the Quinnipiac River, and the first study conducted on vertebrates.

Field collection of liver and gonadal tissue was conducted in August 2014. After some initial trials to identify the best methods for capturing fish, sampling was successfully conducted at five sites in the New Haven Harbor-Quinnipiac River system (Fig. 1) using baited minnow traps. Samples were collected from 1) Savin Rock, West Haven, 2) East Shore Park, New Haven, 3) Clifton Street Boat Ramp, New Haven, 4) Quinnipiac River Marsh Wildlife Area, North Haven, 5) Tilcon Boat Ramp, North Haven.

Table 1. Sampling results for male and female mummichogs in the New Haven Harbor-lower Quinnipiac River system. N = number captured; GSI = Gonadosomatic Index, a ratio of dried gonad mass/body mass. All measurements ±SEM.

Site	Date	N	Mass (g)	TL (cm)	GSI
Female					
1	8/5/14	16	8.7 (0.5)	8.7 (0.1)	1.4 (0.2)
2	8/6/14	14	6.8 (0.2)	8.0 (0.2)	1.1 (0.1)
3	8/4/14	15	6.9 (0.6)	8.0 (0.2)	1.4 (0.4)
4	8/19/14	13	8.0 (0.7)	8.3 (0.3)	0.6 (0.1)
5	8/12/14	16	10.0 (1.1)	8.9 (0.2)	1.6 (0.6)
Male					
1	8/5/14	13	7.0 (0.4)	8.2 (0.1)	1.0 (0.1)
2	8/6/14	14	5.6 (0.4)	7.6 (0.2)	0.9 (0.1)
3	8/4/14	13	5.9 (0.8)	7.6 (0.3)	1.4 (0.3)
4	8/19/14	13	5.9 (0.4)	7.7 (0.1)	0.2 (0.0)
5	8/12/14	14	8.2 (0.7)	8.5 (0.2)	0.4 (0.1)

Figure 1. Fish capture locations (red circles) in the New Haven Harbor/lower Quinnipiac River system. White circles indicate wastewater treatment facilities.

The specific objectives of this study were to:

a) Determine the effectiveness of measuring vitellogenin mRNA expression in mummichogs as a bioassay of EDCs in the lower Quinnipiac River.

Vitellogenin (Vtg) is a protein that is produced by the liver and cleaved into critical yolk proteins in the gonads. In most fishes, there are multiple paralogs of the vitellogenin gene that produce different protein products, many of which may be responsive to EDCs. We tested for the products of the two most common variants, Vtg I and Vtg II. In most cases, Vtg I is found at higher levels naturally in females and is more responsive to EDCs in males. The Vtg assays worked well and were easy to perform. As in previous studies, Vtg I activity in females was markedly higher than Vtg II (Figures 2 and 3, respectively), suggesting it would be more useful in biomonitoring studies in the species.

b) Develop a choriogenin mRNA expression assay in mummichogs and compare effectiveness to the more common Vtg assays.

Choriogenin is a protein produced in the liver that forms part of the egg envelope. As with vitellogenin, there are multiple paralogs producing slightly different proteins. We tested for the products of the two most common variants, Chg L and Chg H, both of which are reported to be sensitive to EDCs. The female fish tested should definitely have displayed Chg gene activity, but we were unable to produce consistent results with Chg assays. Given the success with the Vtg assays that were conducted at the same time, it is likely that the primers we developed were faulty and failed to amplify the region of interest. Choriogenin may still have value in EDC bioassays, but additional development and testing would be necessary to address this.

c) Conduct the first screening of wild vertebrates in the lower Quinnipiac River to determine if there is any indication of endocrine disruption.
and
d) Determine if there are broad spatial patterns to any observed endocrine disruption activity in the lower Quinnipiac River.

One hundred and forty one fish (67 males, 74 females) were captured at five locations spanning the approximately 19 river kilometers from the mouth of New Haven harbor to the end of saline influence in North Haven. Females were captured to provide a baseline comparison and to validate the assay. Although water quality studies in the area have noted measurable levels of individual EDCs (e.g. phthalate plasticizers, H. Pylypiw, Final Report QRF grant #20140051), there was no evidence that estrogenic chemicals were influencing the physiology of male fishes. Vitellogen I and II gene activity were not significantly elevated in males at any site (Figs. 2 and 3), though elevated levels were found in females at a number of locations. High vitellogenin gene activity is a normal component of female reproductive biology and is not considered indicative of EDC activity. GSI did not differ in female fish at any site, though males from sites 4 and 5 had lower GSI than fish sampled at other locations (Table 1). The variation in female expression between sites is likely related to when in the spawning cycle the fish were sampled. Mummichogs typically spawn at high tide associated with the full moon, which occurred on 10 August during this study. Fish sampled at sites 1-3 were approaching ovulation, whereas fish at sites 4-5 were sampled post-spawn. Similarly, variations in GSI are likely to be associated with spawning stage rather than the presence of EDCs.

Based on the samples analyzed to date in this study, there is no evidence that EDCs are present in the saline reaches of Quinnipiac River and harbor at levels sufficient to cause biological impacts to male fishes.

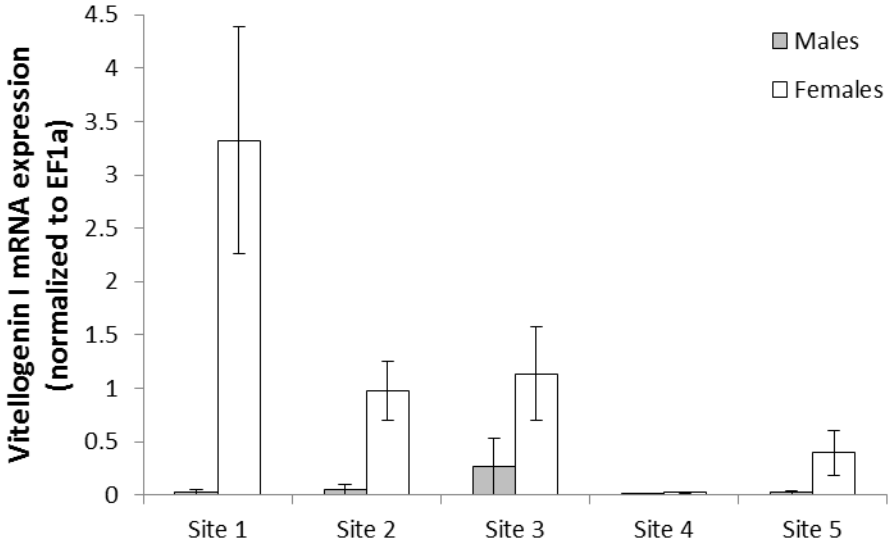

Figure 2. Relative expression of vitellogenin I mRNA (normalized to EF1a activity) in liver tissue of mummichog collected in the New Haven Harbor/lower Quinnipiac River system.

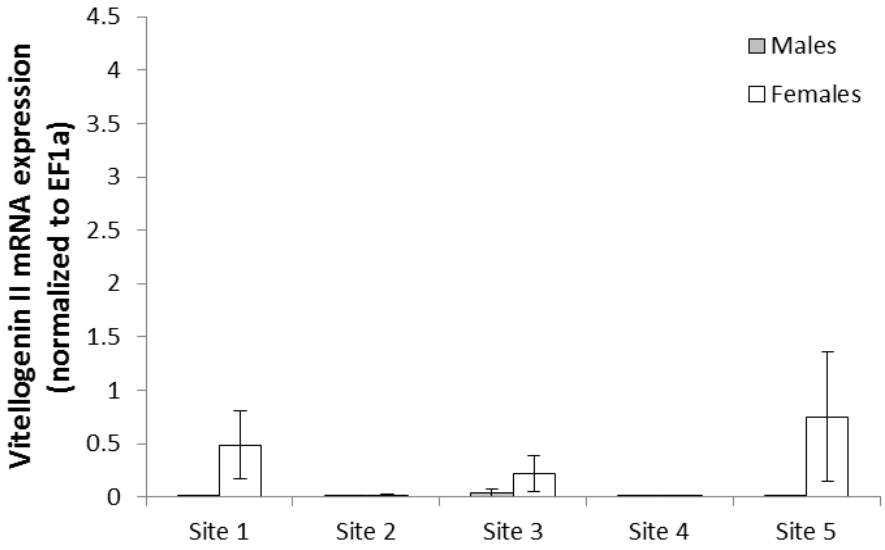

Figure 3. Relative expression of vitellogenin II mRNA (normalized to EF1a activity) in liver tissue of mummichog collected in the New Haven Harbor/lower Quinnipiac River system.

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**?

The most significant challenge we faced was that we found no actual evidence of impacts from EDCs on fishes, though this is certainly good news for the river and associated ecosystems. We were successfully able to validate and adapt the vitellogenin bioassay for use in the saline reaches of the Quinnipiac River and harbor. Using the assay, we collected a robust data set that allowed us to assess EDC impacts in the river at this time and that will serve as a baseline for comparisons in the future. Having now validated the vitellogenin bioassay, we would feel confident utilizing this bioassay in coastal and estuarine sites throughout Long Island Sound, but we would focus future sampling on male fishes and collect only a few females as internal controls. We were not successful in developing and validating a similar bioassay for choriogenin. It is still possible that choriogenin can be used as a useful bioassay; however, additional development and testing would be necessary and it is not clear at this point if this tool would be better than existing techniques. Additionally, we were unable to conduct one originally proposed activity. We had intended to run a small cage exposure trial, in which ten male fish from an unimpacted site were tranlocated to a cage at the most impacted location in order to determine the sensitivity of the assays to EDC exposure; however, since we found no evidence of impacts anywhere in the river system, we were unable to conduct this trial.

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?**

The Fish Ecophysiology and Behavior Lab at the University of New Haven, along with the labs of our colleagues, maintains significant expertise in fields such as ecotoxicology, water quality and pollution, and fish, algal, and invertebrate biology. We will continue to develop projects that utilize our strengths to assist The Community Foundation for Greater New Haven in its mission to restore, conserve, and protect the Quinnipiac River and its surrounding watershed.