**Mandatory Application Information**

Regarding: Gavery, Mackenzie (FON: EPA-F2011-STAR-K1)

**Item 3. Front Page**

1. Name: Gavery, Mackenzie, R
2. EPA-F2011-STAR-K1, Pesticides and Toxic Substances (K1)
3. Current University: University of Washington, College of the Environment, School of Aquatic and Fishery Sciences, Seattle WA.
4. Intended University: University of Washington, College of the Environment, School of Aquatic and Fishery Sciences, Seattle WA.
5. Title: An epigenomic and transcriptomic framework for identifying novel modes of action and physiological effects of endocrine disrupting compounds in shellfish.
6. Degree Sought: Ph.D.: expected completion date Dec 2013
7. Environmental Discipline: Aquatic & Fishery Sciences
8. Educational Level: Entering Doctoral Student (ED)

**Item 4. Personal Statement**

This August, my interest in coastal ecosystems and the oysters that inhabit them inspired a personal trip to the Gulf Coast. As a shellfish biologist and educator, I felt it was important to meet with researchers and oystermen to listen, observe, and learn. I explored coastline through four states affected by the Deepwater Horizon disaster, but I never *saw* any oil. This simple observation had a powerful effect on me. It provided a mirror, albeit on a larger scale, of a similar problem we face in the Puget Sound. How do we solve, mitigate and ultimately prevent environmental problems that we cannot see, smell or taste? I have come to recognize the difficulties of managing and regulating problems that occur in places where on the surface, everything looks just fine. My research focuses on interactions between shellfish and their environment. Specifically, I am interested in the *unseen* or sub-lethal effects of contaminants on shellfish. As an environmental scientist, I will contribute by raising awareness of these issues and providing the best science to decision makers, regulatory agencies and the public. I will also do my best, through education and outreach, to inspire others to protect our natural resources.

My career path may not have been direct, but each step has been critical to lead me to where I am today. As an undergraduate, I focused on marine biology and ecology, spending summers studying marine ecology at a field station in Washington’s San Juan Islands. After earning my B.S. in Biology, I entered the biotech industry as a Quality Control Analyst at a company that develops gene therapy products to treat inherited diseases. I mastered a wide range of molecular and cellular techniques and was promoted to lead the department’s stability program. Stability studies are required by the FDA to ensure the safety and quality of products over time; my job was to manage this testing and present the results to management. It was extremely rewarding to compile and trend complex data over time to detect minute changes that could then be used to predict future product performance. Although I enjoyed this work, I was driven to merge the skills I learned in biotech with my passion for marine biology and aquatic science. I began searching for a lab where I could utilize molecular and cellular techniques to address pressing environmental issues. I found the perfect fit in the lab of Dr. Steven Roberts at the School of Aquatic and Fishery Sciences (SAFS) at the University of Washington.

While earning my Master’s degree at SAFS, I have applied methods such as quantitative PCR and next-generation sequencing to promote a greater understanding of how oysters respond to their environment. Recently, my research has focused on characterizing epigenetic mechanisms in the Pacific oyster. I first encountered epigenetics or things ‘above the genome’ in a seminar class. We learned how xenobiotic compounds can disrupt epigenetic patterns and how many of these disruptions affect phenotypes including disease susceptibility. It appeared likely that these mechanisms could play an important role in how shellfish respond to their environment. Moreover, the influence of environmental signals on these mechanisms meant potentially large implications for shellfish living in contaminated or stressful habitats. I was immediately fascinated. As I investigated what little is known about DNA methylation in invertebrates, I was particularly struck by the diversity of methylation and the implications for different functional roles across taxa. I was especially interested in the literature demonstrating the importance of diet on DNA methylation patterns in honey bees1,2,3. There, researchers demonstrated that DNA methylation regulates genes that determine whether a bee becomes a worker or a queen. I was extremely influenced by this research - could we be overlooking such an important mechanism in molluscs? This question inspired me to initiate the first characterization of oyster DNA methylation. The culmination of my research suggests an important functional role of DNA methylation in oysters, and these findings have recently been published in *BMC Genomics*. This characterization provides a basis to address how environmental signals, such as the presence of endocrine disrupting compounds, influence epigenetic mechanisms and ultimately phenotypes in oysters. This research is outside of the scope of my Master’s project, which is why I would love to continue this research as a PhD student.

Outside of research, mentoring and teaching are the most rewarding parts of my academic and professional career. Last summer, I volunteered to develop and lead an activity for GEAR UP, a program encouraging high school students from low income and underserved communities to attend college. I designed the lab to demonstrate the role that bivalves play in mitigating the effects of pollution. The students performed an experiment to see how quickly a beaker of clams could filter bright green algae out of the water. They watched the water clear as we opened and observed live mussels filter feed on yeast particles placed on their gills. In order to foster scientific understanding, I felt it was important for students to actually *see* this process. I knew by their excitement that the lessons learned that day would have a lasting effect and foster environmental awareness and stewardship.

Invisible threats to coastal environments, such as the presence of endocrine disrupting compounds, ocean acidification and long-term effects of oil spills, can have sweeping consequences. In order to mitigate and predict the risk of these threats, we must improve our understanding of how organisms and populations are currently being affected and develop the tools to accurately monitor and trend changes to predict risk. My background and experience have prepared me to contribute valuable research and expand our understanding of how shellfish respond to stress. Through my PhD project I will provide insight into how endocrine disrupting compounds affect DNA methylation patterns and physiological responses in oysters. I will also continue to look for ways to teach and inspire students through hands-on experiences with natural processes as I continue my career as student, scientist and educator.

1Wang Y et al., *Science* 2006, **314:645 (2006)**

2Elango N. et al., *PNAS*, **106**:11206 (2009)

3Foret S et al., *BMC Genomics* 2009, **10**:472 (2009)

**Item 5. Proposal Description**

**An epigenomic and transcriptomic framework for identifying novel modes of action and physiological effects of endocrine disrupting compounds in shellfish**

**1. Project Summary**

Concern over human and wildlife health has brought increased attention to a group of emerging environmental contaminants referred to as endocrine disrupting compounds (EDCs). While progress has been made in describing the effects of these compounds in vertebrates, there are still gaps in our understanding of alternative modes of action and physiological effects outside of the reproductive axis. There is little known regarding the physiological impact and mode of action of EDCs in benthic macroinvertebrates. The research proposed here aims to characterize alternative modes of action of endocrine disrupting compounds by utilizing molecular tools to examine epigenetic and physiological changes in Pacific oysters (*Crassostrea gigas*) exposed to EDCs in the laboratory. A novel and important aspect of this project is the focus on epigenetics, a discipline that is increasingly becoming an important component in ecology and toxicology. This research will facilitate not only the characterization of endocrine disruption in shellfish, but will also provide important information on the mechanisms by which these compounds alter physiological processes.

**2. Introduction**

Endocrine disrupting compounds are emerging environmental contaminants that threaten water quality and health of humans and wildlife worldwide. EDCs are broadly defined as “exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis, reproduction, development, and/or behavior”1. These compounds, many with structural similarities to hormones such as estrogen, affect endocrine pathways and can cause reproductive perturbations. These compounds enter aquatic ecosystems though a variety of sources including wastewater treatments facilities, septic systems, and industrial effluents. The Environmental Protection Agency (EPA) and other agencies monitor the presence and concentrations of such compounds and have found EDCs in wastewater effluents, drinking water, rivers, streams, sediments and coastal areas nationwide. While physiological effects of endocrine disruption have been described, it is becoming evident that our understanding of the biological pathways affected are incomplete and that alternative modes of action need to be explored. Considering the widespread presence of EDCs, it is of paramount importance to increase our understanding of both the modes of action and the physiological effects of these compounds in order to best evaluate impacts to organismal health, population resilience, and ecosystem structure.

The most well studied mode of action of EDCs is through their interaction with sex steroid receptors. Specifically, these compounds can inappropriately bind to nuclear estrogen and androgen receptors leading to activation or antagonism of the receptor signaling pathway. In fact, this mechanism has been used to screen compounds for endocrine disrupting activity *in vitro* using ligand binding assays since the late 1980s. 2 However, additional modes of action, such as receptor protein degradation, hormone metabolism and altered DNA methylation have been proposed or identified.3 Indeed, some compounds that show weak activity *in vitro* nevertheless show potent estrogen activity *in vivo*.4 It is becoming clearer that endocrine disrupting compounds induce biological effects outside of the canonical nuclear-receptor dependent pathways. One way that EDCs may elicit these changes is through disruptions to normal epigenetic mechanisms.

Epigenetics refers to heritable processes that alter gene activity without manipulating the underlying DNA sequence.5 Common epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNA activity. The best studied of these is DNA methylation, which refers to the enzymatic addition of a methyl group to a cytosine residue in DNA. DNA methylation is an important mechanism of gene regulation in both plants and animals. In vertebrates, DNA methylation plays important roles in providing genomic stability6, genetic imprinting7, and X-chromosome inactivation8. It has been shown that DNA methylation patterns are susceptible to disruption by a range of environmental factors including diet9,10, xenobiotic chemicals 11, as well as endocrine disrupting compounds12. Increasingly, studies have linked these disruptions with various disease states such as cancer13, diabetes14 and asthma15. Furthermore, DNA methylation, like many epigenetic marks, may be heritable; therefore environmentally-induced changes can be passed on for multiple generations.12 ***Considering the important role of DNA methylation in gene regulation, the susceptibility to be influenced by environmental signals, and potential for heritability, DNA methylation analysis is a prime candidate for providing new insights into how EDCs impact wildlife.***

The relationship between epigenetics and endocrine disruption was first explored in mammalian systems where it was shown that exposure to bisphenol A in pregnant mice induces DNA hypomethylation in offspring with a distinct phenotype.16 Exposure to cadmium, a trace metal with known endocrine disrupting effects, has been shown to disrupt DNA methyltransferase activity and induce global hypermethylation *in vitro* (rat liver cells) after prolonged exposure17. Very recently, DNA methylation has been evaluated in aquatic organisms. A recent study by Stromqvist et al. (2010)18 reported significant hypo-methylation of the vitellogenin gene promoter in male zebrafish exposed to EE2, suggesting an epigenetic basis for the induction of vitellogenin (decreased DNA methylation is typically associated with increased transcription). Similarly, Wang and colleagues (2010)19 reported global hypo-methylation in the liver tissue of false kelpfish (*S. marmoratus*) exposed to environmentally relevant concentrations of tributyltin. Although the mechanism of DNA methylation has been less explored in invertebrates, there is evidence that DNA methylation is affected by similar compounds. In the water flea, *Daphnia magna*, compounds such as zinc, vinclozolin, and 5-azacytidine (a pharmaceutical compound) have been shown to alter global DNA methylation in a dose-dependent manner.20,21In addition, transgenerational effects of nonylphenol, an aquatic pollutant and known EDC, have been reported in Pacific oysters, although the underlying mechanism remains unclear.22

Until recently, there has been limited research regarding the impacts of EDCs in aquatic invertebrate species such as molluscs. This is in part due to the fact that less is known about the endocrine system in these organisms. For example, evidence for a functional estrogen receptor,

the canonical target of EDCs, has only been recently identified in bivalve molluscs.23 Nevertheless, a number of studies have recently described reproductive and developmental

disruption in molluscs exposed to EDCs.22,24 There is a tremendous amount to be gained by extending our knowledge of both the modes of action and the physiological effects of EDCs in molluscan taxa, especially bivalves. First, molluscan models, by the nature of their relatively simple endocrine systems (compared to vertebrate systems), provide a streamlined model to investigate novel modes of action of endocrine disruption outside of the traditional nuclear-receptor binding pathway. Second, bivalves are commonly used as bioindicators of aquatic health and research in this area will likely elucidate new biomarkers of exposure. Third, bivalves are key structural and functional components of estuarine ecosystems and play important roles in biogeochemical cycling as well as serving as food supply for humans and other wildlife. Finally, bivalves and other estuarine organisms are increasingly under pressure from new environmental and anthropogenic threats. Unfortunately, 2010 provided stark examples of these pressures from coast to coast including the Deepwater Horizon oil spill in the Gulf Coast to increasing concern of ocean acidification in the Pacific Northwest. In order to protect these ecologically and economically important species, we need to better understand the stressors they face and be able to predict how they will respond to environmental change and anthropogenic pressures such as the presence of endocrine disrupting compounds. To that end, my recent research has focused on the characterization of DNA methylation as an epigenetic mechanism of gene regulation in the Pacific oyster.

The results of this characterization indicate that DNA methylation plays an important role in regulating gene expression in *C. gigas*.25 *In silico* analyses show that genes associated with developmental processes and immune function (e.g. cell adhesion, cell signaling and stress response) are likely to have the greatest epigenetic flexibility, and therefore, highest regulatory control. These findings have significant implications for impacts of environmental disruption in shellfish. First, disruptions to DNA methylation are likely to affect sensitive gene classes such as those involved in development and immune responses, which could have detrimental impacts to organismal health, population resilience, and ecosystem structure. Second, these results highlight an important difference between invertebrate and vertebrate DNA methylation patterns. Namely, invertebrates, such as oysters, show significant variation in intragenic methylation. This is in contrast to vertebrates where the majority of intragenic DNA is heavily methylated and variation is exhibited primarily in promoter regions. Thus, it cannot be assumed that the mechanism of regulation is conserved and conclusions drawn from vertebrate models such as zebrafish may not be appropriate to describe responses in invertebrates. Based on this evidence, it is clear we need to increase our understanding of how EDCs disrupt DNA methylation patterns in oysters. It is also apparent from these findings that *C. gigas* will be valuable as a model organism for studying the impacts of epigenetic disruptions by EDCs in invertebrates. The proposed project will capitalize on this initial research as it aims to characterize epigenetic disruptions by EDCs in this important estuarine bioindicator.

The specific objectives of the proposed research are to:

1. *Investigate DNA methylation disruption as an alternative mode of action of EDCs in oysters.*
2. *Characterize the physiological effects of EDCs in oysters.*
3. *Develop an educational project to engage middle school girls and undergraduate students and encourage their participation in environmental science.*

In order to carry out the first two objectives, oysters will be exposed to EDCs in the laboratory and epigenetic and physiological (morphological and molecular) changes will be characterized. In order to carry out the third objective, a hands on activity will be designed to present important biological concepts of water quality, emerging contaminants and bioindicators (shellfish) to middle school girls enrolled in Girls in Science Math and Engineering (GEMS) a science enrichment program led by the Seattle chapter of the Association of Women in Science. In addition, undergraduate students will be recruited to assist with molecular analysis of this project. The successful completion of the first two objectives will result in the elucidation of previously unexamined mechanisms and physiological effects of endocrine disruption in shellfish. In addition, it is anticipated EDC exposure will produce unique epigenetic and gene expression ‘signatures’ that are indicative of exposure and can therefore be utilized as early detection biomarkers. The completion of the third objective will promote involvement in environmental science and stewardship in students. The following sections of the proposal will outline the general approaches taken to accomplish the research objectives (section 3), provide detailed methodologies (section 4), and discuss expected results (section 5).

**3. Approach**

The proposed research will consist of laboratory-controlled exposures of Pacific oysters to 17α-ethynyl estradiol (EE2) and cadmium. Oysters will be exposed to one of two concentrations for each compound; one that represents concentration currently observed in the environment and a second that represents a ‘worst-case’ scenario (details below). To evaluate temporal effects, oysters will be exposed for two different exposure times to evaluate both acute and chronic responses. Gonad and hemocytes will be sampled and DNA and RNA will be isolated for DNA methylation and gene expression analysis. Morphological measurements will also be performed.

EE2 and cadmium are two relatively well-characterized EDCs. EE2, the active constituent of the female contraceptive pill, exhibits greater potency and is more resistant to degradation and metabolism than endogenous estrogens.26 Not only is EE2 persistent, but has also been reported at high concentrations in aquatic environments. Data collected in 1999 – 2000, from US streams in 30 states, reported concentrations of EE2 between 5 and 273 ng/L.27  Effects of EE2 exposure include delayed sexual development, induction of vitellogenesis and feminization of males in both fish and bivalves.28, 29 Cadmium is a persistent pollutant found in coastal and estuarine environments. Historically, the presence of this trace metal has been associated with industrial effluents. In fish, cadmium exposure has been shown to alter hormone synthesis30 and inhibit steroidogenesis, ovarian maturation and oogenesis31,32. Both EE2 and cadmium can disrupt endocrine function by binding to estrogen receptors.33,34 In addition, EE2 has been previously shown to impact DNA methylation in zebrafish models.18

**3.1 DNA methylation and EDCs**

In order to address the first research objective and test the hypothesis that ***DNA methylation patterns will be altered upon exposure to EDCs in oyster****s,* a high resolution, genome wide DNA sequencing approach will be used. This will allow for the identification of multiple targets, and therefore multiple pathways, being affected. Specifically next-generation sequencing technology will be used to quantify DNA methylation (specific methods provided in section 4). While these methods have been applied widely in biomedical research, they have only recently begun to be used in ecotoxicology and wildlife ecology studies. To my knowledge, this is the first time a high resolution genome-wide methylation analysis will be applied in a non-mammal in response to an EDC.

**3.2 Physiological Effects of EDCs**

An integration of traditional morphological analysis and gene expression analysis will be used to determine physiological effects of EE2 and cadmium on oysters.The hypotheses to be tested are that EDC exposure will **a) *change sex ratios in adult oysters* and b) *alter expression patterns for genes involved in reproduction, stress, and detoxification.***

*3.2.1 Morphological Analysis*

*C. gigas* have protandric alternative sexuality with a low frequency of hermaphroditism.35,36 Oysters exposed to EDCs in the laboratory show an increase in female:male sex ratio, number of intersex individuals and temporal differences in gonad development.22,29 Similar observations will be made here. At each time-point, gonad tissue samples will be taken for sex identification and gonad development. Condition Index will also be determined to evaluate overall health. Statistical analysis will be performed to determine significant differences in sex ratio, percent intersex and stage of gonad development between treatment and control at each time point.

*3.2.2 Transcriptomics*

Transcriptomics-based approaches are providing some of the most interesting data regarding alternative modes of action of endocrine disruptors. For example, a recent study conducted by Wang et al (2010)37 identified alternative mechanisms of endocrine disruption utilizing a transcriptomics-based framework to identify pathways and transcription factors impacted by EDCs in zebrafish. The results indicated that a large number of cellular functions are impacted during endocrine disruption including those involved in stress response, cell cycle, and apoptosis. Similarly, gene expression profiling in male fathead minnows exposed to EE2 revealed an upregulation of genes involved with 39 different biological processes.38 Although not as extensive in nature, gene expression studies in mussels exposed to the bisphenol A, a known EDC, showed altered patterns of gene expression and activity of enzymes involved in redox balance.39 These studies and others illustrate that EDCs influence multiple pathways outside of the reproductive axis.

Numerous studies have characterized gene expression in bivalves exposed to various stressors, such as heavy metals and hydrocarbons40,41, sewage42, EDCs39, pathogen exposure43,44, and temperature45,46. For this study, a suite of genes involved in various stress responses including EDC exposure and detoxification will be analyzed for expression (see Table 1). Additionally, gene expression analysis will take place after DNA methylation data has been analyzed so that genes identified as being differentially methylated in response to the exposures can be assayed for changes in gene expression.

Transcriptomic analysis will also be used to confirm a treatment effect of EE2 and cadmium exposures. *Vitellogenin* expression will be used to verify effect of treatment of EE2 in oysters as it is significantly upregulated in both male and female blue mussels exposed to 17β-estradiol.47 Likewise, *metallothionein* is upregulated in oysters exposed to cadmium at concentrations similar to those used in this trial.48

Table 1. Candidate genes to be characterized including functional description and NCBI Accession number. Differences in gene expression will be assessed to better understand physiological impacts of EDCs. Asterisks indicate genes that will be serve as confirmation of EE2 and cadmium exposure.

|  |  |  |
| --- | --- | --- |
| Functional Category | Gene | Accession # |
| Reproduction | vitellogenin\* | AB084783 |
| estrogen receptor | AB259818 |
| General Stress Response | heat shock protein 70 | AB122063 |
| Detoxification | metallothionein IV\* | AJ243263 |
| cytochrome P450 | EF645271 |
| multi-drug resistance protein | EU073425 |

**3.3 Outreach**

An important part of understanding and protecting the environment and our natural resources is awareness, participation and collaboration between scientists, students and the public. To this end, I will develop a hands-on activity based on this research project for middle school girls enrolled in GEMS. This activity will include both a half-day field component and a three hour laboratory based activity where students will learn about xenobiotics and how they affect wildlife such as shellfish. We will also discuss simple things they can do to reduce their impact on the marine environment. In addition, portions of the project will be incorporated into a workshop for Expanding Your Horizons, an annual conference where women scientists volunteer to host workshops that introduce 6th – 8th grade girls to careers in math, science and computer technology.

**4. Detailed Materials & Methods**

*Experimental Design*

One hundred and eighty adult *C. gigas* will be used in this experiment. Oysters will be obtained from hatchery stock (Taylor Shellfish Inc., Dabob Bay, WA). For this analysis, full siblings will be used to limit genetic variability, which could confound epigenetic and transcriptomic results. Oysters will be brought into the lab during a ‘resting’ condition and acclimated at 18ºC in seawater (22 ppt salinity) for several days. Temperature will be raised at 1 degree per day to 22ºC to initiate gonadal development to be synchronous with the commencement of the trial. Twenty-eight static, aerated, 8L aquaria will be used. Each aquaria will hold 5 oysters. Seawater will be completely changed and treatments maintained 3 times per week. Oysters will be fed an algal diet at 1 x 109 cells/day. Oysters will be exposed to one of two nominal treatments for EE2 (6.25 ng/L, 50 ng/L in seawater) and cadmium (50 ug/L, 100 ug/L) and a seawater control. Oysters will be removed (3 aquaria per treatment/time point) from the experiment at 2 sampling time-points: 4 days (acute exposure) and 14 days (chronic exposure).

*DNA Isolation & Methylation Enrichment*

DNAzol (MRC) will be used to isolate DNA from gill and gonad tissue according to the manufacturer’s protocol. Methylation enrichment will be performed using the Methyl-Miner Kit (Invitrogen) which binds fragmented double-stranded genomic DNA using biotin labeled Methyl Binding Domain 2 protein (MBD2), and can be eluted using a salt gradient. DNA will first be fragmented to ~150 bp, then incubated with methyl-CpG binding domain of human MBD2 protein, coupled to paramagnetic bead via a biotin linker. The bound fraction (methylated fraction) will be eluted with a high salt concentration buffer. The moderately methylated and unbound fraction will also be retained.

*Library Construction – Bisulfite Sequencing*

The bisulfite treated library will be constructed using the SOLiD DNA Fragment Library Kit (Life Technologies) with the following exceptions: the top strand of the P1 adaptor will be synthesized with 5-methyl cytosine instead of cytosine to prevent modification during bisulfite conversion and during nick-translation 2′-deoxycytidine-5′-triphosphate (dCTP) will be replaced with 5-methyl-2′-deoxycytidine-5′-triphosphate (5mC-dNTP) in the original dNTP mixture (standard dNTPs for A, G, and T will be used). Bisulfite conversion will be performed in solution according to Ranade et al (2009)49. Initially, an octect (1/8 of a slide) will be sequenced from each library. Sequencing will be carried out at the University of Washington high-throughput sequencing facility.

Sequencing data will be analyzed using CLC Genomics WorkBench (CLC Bio) along with publicly available databases (NCBI, SWISS-PROT, GigasBase) and our own unpublished *C. gigas* RNA-Seq libraries. Analysis will include quality trimming, de novo assembly, and BLAST. Comparisons among libraries will be made within CLC Genomics Workbench (ChIP-Seq, RNA-Seq), Microsoft Access, and Galaxy tools (http://main.g2.bx.psu.edu/).

*Morphological Analysis*

Sex will be determined by taking 5 separate samples along the length of the gonad using a glass pipette. Samples will be examined under a microscope using oocytes or spematozoa as indicators. Gonad samples will be processed for histological examination and gonad development stage will be assessed based on methods and criteria described by Robinson (1992)50. Condition Index is determined by soft tissue dry weight/(total weight-shell weight)\*100). Statistical analysis will be performed using SPSS Software (Somers, NY).

*Reverse-transcription Quantitative PCR*

Gene expression analysis will be carried out as described in Roberts et al. (2008)43. A minimum of 8 genes will be examined including those listed in Table 1 as well as genes identified from sequencing efforts. RNA will be reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen), and quantitative PCR will be carried out in an Opticon 2 thermocycler (BioRad) as previously described (Roberts et al., 2008). Analysis of PCR data will be carried out based on the kinetics of individual PCR reactions using Real-time PCR Miner v2.151. All qPCR data will be normalized to a corresponding reference gene (18s rRNA) and expressed as fold increase over minimum.

**5. Expected Results**

Accurate and thorough understanding of risks associated with endocrine disrupting compounds in aquatic systems requires greater knowledge of their modes of action and their physiological effects. These compounds are present in our water sources, therefore, the best science should be made available so that the EPA can accurately assess and manage the potential risks associated with these contaminants. The research outlined here will provide a unique and integrated view of the epigenetic and transcriptomic landscape of an aquatic invertebrate in response to EDC exposure. This robust analysis will increase the science of understanding of the mode of action of these contaminants and the risks associated with exposure. The benefit of this holistic approach is that it not only examines a novel pathway of disruption (epigenetic disruption), but will also provide a mechanistic understanding of the response through morphological analysis and transcriptomics.

The successful completion of this research will be able to provide (1) a deeper and broader understanding of the modes of action of endocrine disrupting compounds, (2) the first integrative study using epigenetics, transcriptomics and phenotypic analyses to evaluate the effects of EDCs in an aquatic invertebrate species, and (3) provide a proof of concept framework which can be applied to advance the study of the biological effects of EDCs in other aquatic species. This research will also provide data that can be utilized in modeling approaches to predict risks and hazards of environmental contaminants and the identification of novel targets for biomarkers of exposure.

The research outlined here aims to compliment the research being done on endocrine disruptors by the EPA. For example, the National Health and Environmental Effects Research Laboratory (NHEERL) has identified three focus areas for EDC research: Methods Development & Screening Studies, Wildlife and Extrapolation Studies, Low-Dose Studies and Effects of Multiple EDCs. The project outlined here can benefit all three of these target areas. In terms of Methods Development & Screening, this project will complement transcriptomics assays currently being developed by providing additional epigenomic biomarkers that can be analyzed with only slight modifications to traditional PCR methods providing additional markers at a low cost. The benefits to Wildlife and Extrapolation Studies will be realized by elucidating novel modes of action and effects in an invertebrate species with ecological and economic importance. Additionally, the biomarkers identified through this research can be used to determine baseline endocrine status in wild oyster populations. This research will also foster a fuller understanding of comparative endocrinology and help to identify similarities and differences in physiological responses and effects between species. In terms of Low Dose studies, this proposal includes testing at environmentally relevant concentrations to investigate effects at low doses. This project will compliment and enrich the research currently being performed at or supported by the EPA without duplicating research efforts.

The results of this project will be disseminated through publication in peer-reviewed journals and presentations at national conferences. Research progress will be made publicly available in real-time via electronic laboratory notebooks (http://genefish.wikispaces.com/Mac's+Notebook) and a dedicated project website (*e.g*. http://goo.gl/tR8v). The project will also will be incorporated into an activity for GEMS and workshop for Expanding Your Horizons to engage and motivate girls in environmental science and protecting their environment. In addition, undergraduates will be recruited to assist in the laboratory in order to facilitate mentoring, teaching and learning between graduate and undergraduate students in the department.

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**Item 6. Background Information**

Education and Relevant Experiences……page 18

Publications & Presentations……..…..….page 19

Coursework……………….……..…….....page 20 - 25

**Education**

University of Washington-**M.S.**School of Aquatic & Fishery Sci., Summer 2011 (expected)

Seattle University-**B.S.** Biology (*magna cum laude*), received February 2001

Seattle Pacific University-coursework, Blakely Island Field Station, Summer 1999 & 2000

**Research & Laboratory Experience**

***Graduate Research Assistant, SAFS*** – University of Washington, Seattle, WA (2008 – present)

* Characterize Pacific oyster response to environment using differential gene expression analysis, DNA methylation profiling and transcriptome analyses.

***Quality Control Analyst III*** *–*Seattle Genetics Corporation, Bothell,WA (2006 – 2008)

* Designed and performed assay validation to assess quality of clinical phase monoclonal antibody therapies.

***Quality Control Analyst –*** Targeted Genetics Corporation, Seattle, WA (2001 – 2006)

*Senior Quality Control Analyst - Stability Lead (2005 - 2006)*

* Analyzed data and prepared technical reports for long-term stability studies for clinical phase products.

*Quality Control Analyst I(2001-2003) / II (2003-2005)*

* Performed release and stability testing of recombinant AAV product candidates. Methods utilized: qPCR, Western blotting, LAL assays, cell-based infectivity assays, ELISA.

**Teaching Experience**

***Graduate Teaching Assistant*** –University of Washington SAFS

* Spring 2010 & Spring 2009 *Biology of Shellfish (FISH 310)*
* Fall 2009 *Integrative Environmental Physiology (FISH 441/541)*
* Winter 2008 *Integrative Environmental Physiology (FISH 441/541)*

***Volunteer Lab Instructor*** –GEAR UP WASHINGTON

* Summer 2009 *Summer Institute Session -* *Puget Sound Threats and Processes*

***Mentor*** -ASSOCIATION OF WOMEN IN SCIENCE, Seattle Chapter

* 2007-2010 *Girls in Engineering, Math and Science (GEMS)*

***Undergraduate Teaching Assistant*** - SEATTLE UNIVERSITY

* Winter 1999 *General Biology II* (BIOL166)
* 1999 – 2001 *Biology Tutor, Seattle University Learning Center*

**Honors & Awards**

* Student Scholarship Award for Applied Science, Pacific Coast Shellfish Growers Association (PCSGA), 2009
* Best Grad Student Presentation, PCSGA, Portland, OR, 2009
* Student Endowment Travel Award, National Shellfisheries Association, 2009 & 2010
* Victor and Tamara Loosanoff & John G. Peterson Scholarship, UW SAFS 2009 & 2010
* William H. Pierre Sr. Fellowship, UW SAFS, 2008/2009

**Memberships**

* National Shellfisheries Association
* Association of Women in Science – Seattle Chapter

**Presentations & Posters**

* DNA Methylation Patterns & Epigenetic Regulation in the Pacific Oyster. PCSGA Annual Meeting. September 2010. Oral Presentation.
* Pacific oysters & ecosystem health. Aquaculture 2010 / National Shellfisheries Association, 102nd Annual Meeting. March 2010. San Diego, CA. Oral Presentation.
* Pacific oysters and ecosystem health. SAFS Graduate Student Symposium. Nov 2009. Seattle, WA. Oral Presentation.
* Pacific oysters as indicators of ecosystem health. PCSGA Annual Meeting. September 2009. Oral Presentation.
* Characterization of prostaglandin pathway genes of the Pacific oyster (*Crassostrea gigas*): Evidence for a role in immune response. National Shellfisheries Association 101st Annual Meeting. March 2009. Savannah, GA. Poster Presentation.
* Characterization of prostaglandins in the Pacific oyster *Crassostrea gigas*: evidence for a role in the immune response. SAFS Graduate Student Symposium. Nov 2008. Seattle, WA. Oral Presentation.

**Publications**

Gavery M, Roberts SB: DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). *BMC Genomics* 2010, 11:483.

Credits Obtained at University of Washington, Seattle, Washington:

**Fall Quarter 2008**

*09/24/08 – 12/5/08*

Subject Number Title Credits Grade

FISH 510 Topics Genet & Phys 2.00 CR

FISH 522 Hot Topics in Aqua & Fish 2.00 CR

FISH 700 Masters Thesis 2.00 CR

GRDSCH 615 TA Prep 2.00 CR

Q SCI Statistical Inference in Ecol 5.00 3.9

Grade Points: 19.5 Term Credits Attempted 13.00

Term GPA: 3.9 Term Credits Earned 13.00

**Winter Quarter 2009**

*01/05/09 – 3/13/09*

Subject Number Title Credits Grade

FISH 507 Topics Fisheries (Mol Method) 5.00 4.0

FISH 521 Rsrch Prop Writing 4.00 3.9

FISH 700 Masters Thesis 2.00 CR

Grade Points: 35.6 Term Credits Attempted 15.00

Term GPA: 4.0 Term Credits Earned 15.00

**Spring Quarter 2009**

*3/30/09 – 6/05/09*

Subject Number Title Credits Grade

FISH 700 Masters Thesis 5.00 CR

GENOME 599 Topic Gen Sci (Epigenetics) 5.00 3.5

-

Grade Points: 5.2 Term Credits Attempted 10.00

Term GPA: 3.5 Term Credits Earned 10.00

**Fall Quarter 2009**

*09/30/09 – 12/11/09*

Subject Number Title Credits Grade

FISH 700 Masters Thesis 10.00 CR

Grade Points: 0.0 Term Credits Attempted 10.00

Term GPA: NA Term Credits Earned 10.00

**Winter Quarter 2010**

*01/04/10 – 03/12/10*

Subject Number Title Credits Grade

FISH 700 Masters Thesis 10.00 CR

FISH 546 Topics Bioinformatics 3.00 4.0

Grade Points: 12.00 Term Credits Attempted 13.00

Term GPA: 4.0 Term Credits Earned 13.00

**Spring Quarter 2010**

*03/29/10 – 06/04/10*

Subject Number Title Credits Grade

FISH 700 Masters Thesis 10.00 CR

Grade Points: 00.00 Term Credits Attempted 10.00

Term GPA: N/A Term Credits Earned 10.00

**Fall Quarter 2010**

*09/29/10 – 12/10/10*

Subject Number Title Credits Grade

FISH 700 Masters Thesis 10.00 CR

Grade Points: 00.00 Term Credits Attempted 10.00

Term GPA: N/A Term Credits Earned 10.00

**Total UW Credits: 71.5**

**Cumulative GPA: 3.91**

Credits Obtained at Seattle University, Seattle, Washington:

**Fall Quarter 1996**

*09/25/96 – 12/14/96*

Subject Number Title Credits Grade

FA-120 Experiencing the Arts 5.00 A

HS-121 Studies in Modrn Civ 5.00 B+

EN-110 Freshman English 5.00 A

Grade Points: 56.50 Term Credits Attempted 15.00

Term GPA: 3.767 Term Credits Earned 15.00

**Winter Quarter 1997**

*01/06/97 – 3/22/97*

Subject Number Title Credits Grade

PL-110 Intr Pl/critic think 5.00 A-

EN-120 Masterpieces of Lit 5.00 A

CSC-103 Intro Computer Appl 5.00 B

Grade Points: 53.50 Term Credits Attempted 15.00

Term GPA: 3.567 Term Credits Earned 15.00

**Spring Quarter 1997**

*3/31/97 – 6/14/97*

Subject Number Title Credits Grade

MT-118 College Algebra-Bus 5.00 A

HS-120 Origins Western Civ 5.00 A-

EN-258 Creative Writing 5.00 A-

Grade Points: 57.00 Term Credits Attempted 15.00

Term GPA: 3.800 Term Credits Earned 15.00

**Fall Quarter 1997**

*09/24/97 – 12/13/97*

Subject Number Title Credits Grade

BIOL-165 General Biology I 5.00 A

PHIL-220 Phil of Human Person 5.00 A

SPAN-115 Spanish Language I 5.00 A-

Grade Points: 58.50 Term Credits Attempted 15.00

Term GPA: 3.900 Term Credits Earned 15.00

**Winter Quarter 1998**

*01/05/98 – 03/21/98*

Subject Number Title Credits Grade

PSYC-120 Intro Psychology 5.00 A

SPAN-125 Spanish Language II 5.00 A-

BIOL-166 General Biology II 5.00 A

Grade Points: 58.50 Term Credits Attempted 15.00

Term GPA: 3.900 Term Credits Earned 15.00

**Spring Quarter 1998**

*03/30/98 – 6/13/98*

Subject Number Title Credits Grade

TRST-293 Women & the Gospel 5.00 A

BIOL-167 General Biology III 5.00 A-

SPAN-135 Spanish Language III 5.00 A-

Grade Points: 57.00 Term Credits Attempted 15.00

Term GPA: 3.800 Term Credits Earned 15.00

**Fall Quarter 1998**

*09/23/08 – 12/12/98*

Subject Number Title Credits Grade

MATH-121 Precalculus: Trignometry 2.00 A

CHEM-121 General Chemistry I 4.00 A

CHEM-131 General Chemistry Lab I 1.00 A

PSYC-201 Statistics I 5.00 A

PHIL-345 Ethics 5.00 B+

Grade Points: 64.50 Term Credits Attempted 17.00

Term GPA: 3.794 Term Credits Earned 17.00

**Winter Quarter 1999**

*01/04/99 – 03/20/99*

Subject Number Title Credits Grade

CHEM-122 General Chemistry II 4.00 A-

CHEM-132 General Chemistry Lab II 1.00 A

BIOL-240 Genetics 5.00 A

BIOL-325 Anatomy of the Vertebrates 5.00 A-

Grade Points: 57.30 Term Credits Attempted 15.00

Term GPA: 3.820 Term Credits Earned 15.00

**Spring Quarter 1999**

*03/29/99 – 6/12/99*

Subject Number Title Credits Grade

MATH-131 Calculus for Life Sciences 5.00 A

BIOL-275 Marine Biology 5.00 A

CHEM-123 General Chemistry III 4.00 A-

CHEM-133 General Chemistry Lab III 1.00 A

Grade Points: 58.80 Term Credits Attempted 15.00

Term GPA: 3.920 Term Credits Earned 15.00

**Fall Quarter 1999**

*09/21/99 – 12/11/99*

Subject Number Title Credits Grade

CHEM-335 Organic Chemistry I 3.00 B+

CHEM-345 Organic Chem Lab I 2.00 A

NURS-481 Stress/Surv/Adapt 3.00 A-

PHYS-105 Mechanics and Sound 5.00 A

Grade Points: 49.00 Term Credits Attempted 13.00

Term GPA: 3.769 Term Credits Earned 13.00

**Winter Quarter 2000**

*01/03/00 – 03/18/00*

Subject Number Title Credits Grade

PHYS-106 Electr.Magnetism.Thermody 5.00 A-

CHEM-336 Organic Chemistry II 3.00 A

CHEM-346 Organic Chem Lab II 2.00 A

TRST-338 Human Sexuality: Chal Love 5.00 A-

Grade Points: 57.00 Term Credits Attempted 15.00

Term GPA: 3.800 Term Credits Earned 15.00

**Spring Quarter 2000**

*3/27/00 – 06/10/00*

Subject Number Title Credits Grade

CHEM-337 Organic Chemistry III 4.00 A

CHEM-347 Organic Chem Lab III 2.00 B+

PHYS-107 Survey of Modern Physics 5.00 A

BIOL-235 Invertebrate Zoology 5.00 A

BIOL-488 Biology Senior Synthesis: Sem 1.00 A

Grade Points: 66.60 Term Credits Attempted 17.00

Term GPA: 3.918 Term Credits Earned 17.00

**Fall Quarter 2000**

*09/19/00 – 12/09/00*

Subject Number Title Credits Grade

ANTH-230 Cultural Anthropology 5.00 A-

BIOL-388 Animal Physiology 5.00 A-

BIOL-485 Cell Physiology 5.00 A

Grade Points: 57.00 Term Credits Attempted 15.00

Term GPA: 3.800 Term Credits Earned 15.00

**Winter Quarter 2001**

*01/03/01- 03/17/01*

Subject Number Title Credits Grade

BIOL-496 Independent Study 2.00 A

Grade Points: 8.00 Term Credits Attempted 2.00

Term GPA: 4.000 Term Credits Earned 2.00

**Total Seattle University Credits: 199.00**

**Cumulative GPA: 3.82**

**Degree Obtained: B.S. Biology**

Credits Obtained at Seattle Pacific University, Seattle, Washington:

**Summer Quarter 1999**

Subject Number Title Credits Grade

BIO-4981 Maine Ecology 5.00 A

**Summer Quarter 2000**

Subject Number Title Credits Grade

BIO-4744 Marine Botany 5.00 A

**Total Seattle Pacific University Credits: 10.00**

**Cumulative GPA: 4.00**