Item 4. Statement of Objectives

I first became interested in the aquatic sciences and the environment while at Blakely Island in the San Juan Islands of Washington State. It was there I took undergraduate summer courses in *Marine Ecology* and *Phycology* and had my first experiences in developing and carrying out exciting research projects. These experiences were the foundation for a fascination with the fragility of estuarine systems and the plants and animals that live there. It was at this time that I also began to see the great potential of using a variety of laboratory techniques to study how environmental conditions could have significant, long-lasting, though not necessarily lethal, impacts on organisms. Furthermore, I began to see links between what was going on in these environments and human health, particularly in regards to those plants and animals we consume. Following graduation, I was offered a position as a quality control analyst at a biomedical company and accepted this position. Several factors were considered in making this decision, one of which was that I knew this would provide a stimulating environment for me to put into practice laboratory techniques I had become familiar with, and allow me to continue to learn a broader range of cellular, molecular and biochemical approaches applied in a drug development setting. While I have been very pleased with my work experiences over the past few years, I have the desire to continue on with a graduate-level education to examine environmental issues affecting aquatic systems and human health using some of these same laboratory techniques.

By pursuing a master's degree in the School of Aquatic and Fishery Science (SAFS) at the University of Washington, I will be able to combine my enthusiasm for the study of aquatic environments with my experience in state of the art analytical cellular and molecular biology. The master's of science program at the SAFS will help me achieve the academic and career goals I have set by allowing me to perform research under the guidance of the best in the field, and by developing the skills necessary to communicate results to members of the scientific community as well as to those outside of the field. I plan to study the physiology of marine invertebrates in graduate school. Specifically, my academic goal is to examine the relationships between marine invertebrates' physiological functions and the impacts of environmental stresses, such as pollution and climate change. At the time of matriculation, my goal will be to have gained enough knowledge and experience, as a confident research scientist, to effectively communicate research findings to diverse audiences. The process of having to present and defend a thesis project will enable me to conduct and communicate research as a teacher, presenter and as a steward of local marine ecosystems.

My specific career goals are to conduct research with a focus on understanding the anthropogenic impacts on marine organisms, and how this in turn will affect humans. My ideal job will combine research and education in either a traditional classroom setting or in a community outreach setting. Science is one of the best tools we have towards understanding the natural world. However, scientific knowledge in the hands of the few will not make the best use of this understanding. Scientists need to be able to share research findings and collaborate with those outside of their field of expertise. It is my goal and my responsibility to become a scientist who not only performs quality research, but one who inspires others to ask questions about their environment and seek answers. Given the opportunity to study at the graduate level, I believe that I could make an important contribution in these aspects. Obtaining a Master of Science degree in Aquatic Science will help me achieve these academic and career goals by allowing me to become a member of an exciting scientific community and to perform the course work and research necessary to become an expert in the field.

Item 4. Narrative Statement

The research I wish to carry out in graduate school and propose in the following section, will hopefully assist the EPA and other management agencies make informed decisions regarding conservation and restoration. It is my intention that this research will further our collective understanding of the complex relationships between environmental changes caused by natural and anthropogenic events and the organisms that rely on the health of the environment for well-being. I am committed to using scientific knowledge as a tool to protect marine animals and their habitat. Similar to The Clean Water Act which is a tool that agencies use to protect the marine environment. The Clean Water Act is a comprehensive statute aimed at restoring and maintaining the chemical, physical and biological integrity of the nation's waters. This act imposes baseline pollution standards and holds businesses and individuals legally accountable for the amount of pollutants discharged into national surface waters. The Clean Water Act also authorizes research and studies on the prevention, reduction and elimination of pollution. The research proposal outlined below falls into this category, as the purpose of the study is to elucidate overlooked effects of pollution on molluscs.

Introduction

As the human population continues to increase in coastal communities, water quality has become an increasing concern. Poor water quality is routinely associated with habitat deterioration, species decline, and can have deleterious impacts to human health. Coastal marine and estuarine environments face additional, unique challenges caused by pollution such as: eutrophication, pathogen and toxic chemical contamination, and alteration of freshwater inflow. Among other organisms, bivalve molluscs are dangerously impacted by such environmental problems. Bivalves are filter feeders, and therefore will easily accumulate toxins present in their environment. Unlike many other marine animals, sedentary bivalves, such as oysters, are at a unique disadvantage because they cannot move from an area if it becomes polluted.

Pollution, loss of habitat, overharvesting, and disease have decimated oyster populations in the United States in recent years. Native oyster species such as the Eastern and Olympia oyster have been particularly devastated and have all but been replaced by non-native species intentionally introduced to replenish the declining populations. One of the species introduced in the U.S. was the Japanese or Pacific oyster (C*rassostrea gigas*). The Pacific oyster was introduced to the United States from Japan in the 1920's and has since become the most important commercial oyster species in the Pacific region, constituting 99% of all west coast production. However, both native and non-native species are threatened by pollution and disease. Increasing our scientific understanding of the complex relationships between environmental stresses and physiological responses in oysters will provide valuable information that can be used to restore and conserve oyster populations and habitat. The following paragraphs highlight the environmental and societal importance of oysters and will illuminate why it is so important to deepen our scientific understanding of this invaluable organism and how poor water quality affects them, and in turn can impact human well-being

Oysters improve water quality and provide habitat. Poor water quality in marine and estuarine systems due to eutrophication and the presence of toxins have negative impacts on benthic communities and can be toxic to fish and shrimp. Oysters are filter feeders and can remove excess toxins from the water. Simply increasing the number of bivalves in open waters can have a considerable environmental effect by removing harmful pollutants. In addition to their filtering

capabilities, oyster reefs are the primary source of hard bottom habitat in nearshore ecosystems that are otherwise dominated by soft sediments. Reef habitat is integral not only for the oysters, but for other organisms such as worms, snails, crabs and fish that utilize oyster reefs as habitat.

Oysters are economically important. More than seventy percent of the seafood consumed in the United States is imported, and about 40% of these imports are aquaculture products. Domestic production could help decrease the reliance on imported seafood supply and increase regional food supply and security, provide jobs for coastal communities and maintain the maritime heritage of these communities. Shellfish aquaculture provides the added benefit of improving water conditions through water filtration and habitat creation.

Oysters are an important public health issue. As evidenced by the strong shellfish aquaculture industry, seafood popularity is high. However, concerns have been raised worldwide related to health risks from shellfish contaminated with human waterborne pathogens. Like other filter-feeding bivalves, oysters harbor and concentrate pathogens that occur in their marine habitat and transfer them to humans when the oysters are eaten. Consumption of oysters, which are typically prepared raw or minimally cooked, can cause outbreaks of human diseases, especially if the oysters have been harvested from polluted waters (Rippey, 1994). This has become very evident in Washington state this past year as deaths across the United States have been attributable to oysters harvested from the state (US Food and Drug Administration, press release, 2007).

Oysters are an indicator species. Marine organisms, such as molluscs, may accumulate environmental contaminants in their tissues that are at or above ambient levels in the environment. This allows such species to be used as indicator or sentinel organisms, reflecting relative levels of environmental contamination in a manner suited to short and long term monitoring. Oysters are commonly used as estuarine and marine indicator species as they are easily obtained, likely to survive test conditions and accumulate the contaminants of interest to concentrations proportional to ambient waters (Phillips and Rainbow, 1993). For example, oysters have been used to track the abundance of trace metals and organochloride compounds from nearshore to offshore waters after a change to offshore disposal of sewage. In Sidney, Australia, data collected from oyster tissues allowed researches to demonstrate that the diversion of sewage from nearshore to offshore areas resulted in a significant drop of organochlorides in oysters deployed near sewage outfalls in the nearshore regions (Scanes, 1996). In estuarine systems, the abundance of macrobenthic taxa has been negatively correlated with annual sewage nitrogen loads (Savage et al., 2002). Stable isotope ratios of carbon and nitrogen can trace the movement and assimilation of nutrient matter sources, such as sewage effluent, within food chains of coastal systems. Stable isotope ratio studies using oysters as indicators of nutrient loads have been used to investigate anthropogenic nutrient enrichment over large spatial-temporal scales (Fry and Sherr, 1984). Stable isotope studies using oysters as indicators have also been used to study community ecology and food webs. Ruesink *et al* (2006) studied how secondary productivity varies across a spatial scale by evaluating the stable isotope signature of oysters outplanted across Willapa Bay, Washington State. The diferent carbon signatures of marine and terrestrial plants allowed researchers to determine how much of what the oysters were eating was coming from marine versus riparian food sources.

Not only are oysters an important indicator species, but they are also used to test for various biomarkers. A biomarker is a biological response to an environmental chemical, which gives a

measure of exposure and sometime also an toxic effect. For example, an oyster will produce metallothionein, a protein involved in detoxification, in response to metal exposure (Jenny *et al* 2006). Therefore, the amount of metallothionein in oysters indicates that metal contamination has taken place and to what extent. Consequently, biomarker responses that involve synthesis of biochemicals involved in detoxification may utilize energy that would otherwise be spent on growth, reproduction and immune function, which may directly impair the fitness of the animal. It is for this and other reasons that physiological responses to pollution should be further investigated in mollusc populations.

While oysters have been studied and used extensively to characterize the environment, there are far fewer studies dedicated to understanding how reduced water quality influences subsequent normal physiological responses of the organism such as reproductive functions and immune responses. The goal of the proposed research is to increase this body of knowledge by characterizing the immune function of oysters at a molecular level in order to understand potential long-term effects of pollution on population and ecosystem processes. When we as humans change the environment enough to kill a population, I believe it is generally understood that something went wrong. However, I think we might have a false sense that everything is fine when high mortality events are not observed. Some examples of scientific questions that might be overlooked included: Has the potential reproductive output been compromised? Are growth rates influenced that open opportunity for new predators? Is shell strength weakened? Can a host effectively invoke an appropriate immune response when faced with a common pathogen? Will the pathogen proliferate above safe levels for human consumption?

It would be beyond the scope of a Master's thesis to answer all these questions and therefore I hope to focus on how pollution affects the immune system of oysters. Specifically, the two specific objectives of my proposed research are to:

1. *Analyse real-time physiological effects on oysters grown in polluted environment*
2. *Evaluate potential long-term implication of poor water quality on oyster populations by assessing effects on immune function*

Proposed Methods

In order to carry out these objectives I propose to spawn and set oyster from a common group of broodstock and place them polluted and pristine environments. While the oysters are in the field I plan to indirectly examine the environment and the real-time effects using molecular tools as describe above. For the second objective, I plan to take a novel approach to assess the potential lasting effects of pollution on oyster immune function. With the assistance of cooperating hatcheries I would spawn oysters and set them onto square tiles which would allow them to be easily transported and quantified. When oysters were in their first year, they would be placed out into at least two sites that are representative of pristine and polluted areas. If I were to be accepted at the University I would attempt to get oysters spawned during the Spring 2008 so they could be deployed at the end of my first year of study. Once deployed, every month tiles would be checked and oysters counted and measured. Environmental parameters (temperature, light) would be monitored with continuous logger systems. For the polluted environment I hope to find a site that is actively being monitored. Every two months oysters will be sampled and tissues samples archived for genetic analysis. The entire time oysters will be in the field is estimated to be approximately 6 months. In Fall, oysters will be from all sites will transported back to SAFS and placed into one of the recirculating systems. A group of the young oysters will be exposed to

the bacterial pathogen *Vibrio* in a challenge experiment. Briefly, the oysters will be exposed to a bath of the pathogen for 6 and 24 hours. From other research it is evident that will active an immune response in the oysters easily characterized by gene expression analysis (see below). In addition, a remaining group of tiles (oysters) will be communally held for about 2 months and then exposed to the same treatment. The rationale for this second exposure would be to determine if effects of the environment are realized at some time after exposure. At the end of each exposure trial, tissue will be taken and stored at -80C for quantitative RT-PCR analysis. In addition, tissue samples will be taken for histological analysis to determine the level of infection. For the exposure experiments samples will also be taken from control oysters not exposed to bacteria.

For gene expression analysis, quantitative RT-PCR will be carried out using SYBR green technology. Messenger RNA (the expressed genes) will be reverse-transcribed and subjected to PCR in the presence of SYBR green (a dye that binds to double-stranded DNA) in order to allow for accurate quantitation of products. As described above, some sampling will occur during deployment of oysters in to the field (pristine and polluted sites). Samples will be taken for RT-PCR analysis and levels of specific genes will be quantified. Based on work by other researchers, genes will be selected that have been shown to be differentially expressed in response to environmental stressors. Examples of genes that would be selected include peroxiredoxin 6 (David et al 2007), aspartate aminotransferase (Boutet et a 2005) and estrogen receptor (Matsumoto et al 2007). The first two examples are from studies that have shown a significant difference in gene expression related to pollution, while the latter example is selected to see if pollution might have an indirect effect on reproduction. Upon completion of this objective I hope to have a better understanding on how the environment influences physiology while in a given environment.

To obtain a better understanding of how environmental conditions affect immune function (objective 2), a separate suite of genes will be characterized that will provide the basis for comparing relative differences in immune function from oysters at different sites. This analysis will be performed following controlled bacterial challenges in tanks as opposed to the characterization of genes being expressed when in polluted and non-polluted environments (above). These genes will be selected from Expressed Sequence Tags (ESTs) generated in Dr. Roberts' lab from a cDNA library generated from hemocytes. Dr. Roberts and collegues have used some of these genes in the past to characterize the physiological response of oysters from different locations to mechanical and disease stress (Roberts et al. in preparation). Examples of some of the novel genes that will be used in the current proposal to better understand the differences associated with environmental impacts on immune status include: cathepsin C (Acc# EW778914), interleukin 17 (Acc# EW779217), tumor necrosis factor receptor associated factor 3 (Acc# EW779535) and C-type lectin (Acc# EW778826) (Roberts et al. in preparation). By comparing relative gene expression along with histological profiles I hope to better understand how water quality influences the ability of the oysters ability to function and survive in the face of common pathogens. Upon completion of this objective, I will learn more about the potential lasting implications pollution can have on oyster immune function, which good ultimately have significant implications at the population and ecosystem level as well as effecting human health.

Project Support

As mentioned in the *Statement of Objectives*, I plan to continue on with a graduate-level education to examine environmental issues affecting aquatic systems and human health using state of the art laboratory techniques. The success of such a project as described here is dependent on the support received from the academic institution where the research will take place. The reason I would like to develop and carry out such a research project is that it combines scientific discovery using cutting edge scientific techniques wish a concern for ecological relationships between human population pressures, aquatic organisms and their environments. University programs dedicated to aquatic sciences not only generate new research, but also focus on educating and training the next generation of scientists, which has a lasting impact on the environment. For example, at the University of Washington School of Aquatic and Fishery Science (SAFS), researchers have studied many aspects of physiology, toxicology, and fishery science, published in peer-reviewed journals and presented at national and international conferences. Many of their graduating master's students go on to work for public and private environmental agencies or in environmental education.

It is for these reasons that I am applying to the SAFS graduate program to obtain a Master of Science degree. This is the ideal place to undertake research in the interactions of environmental stresses and physiological changes in marine animals. The graduate program at SAFS is highly respected and the faculty is dedicated to educating future environmental scientists. To obtain a Master of Science in Aquatic & Fishery Sciences requires 45 credits (27 coursework credits and 18 thesis credits), a thesis, and successful Final Examination in defense. The coursework requirements are designed to aid students in writing proposals, conducting sound research, using appropriate statistical analysis and preparing reports and presenting and defending research. The thesis project is conducted under a supervisory committee made of appropriate faculty based on the approach.

If accepted into SAFS at the University of Washington, I would like to work under the advisement of Dr. Steven Roberts. The research in Dr. Roberts' lab is primarily involved with growth, disease and genetics in shellfish a regularly uses gene expression analysis to study oyster physiology and disease resistant mechanisms. This will ensure that the project has the appropriate guidance to secure the greatest likelihood of success. Dr. Roberts' lab is also set up to accommodate this project with minimal set up which will help to keep the research moving forward in a timely manner.

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Item 5. Education and Experiences

Educational Experience
Seattle University, B.S. 2001 Biology (*magna cum laude*)

- Relevant Coursework: Marine Biology, Invertebrate Physiology, Genetics
- Senior Thesis Project: Investigated the viability of Nori aquaculture in the Puget Sound. Research review included analyzing environmental impact statements, farming techniques and water quality data of the Puget Sound.

Seattle Pacific University, Blakely Island Field Station, summer 1999 and 2000

- Relevant Coursework: Marine Ecology, Phycology
- Research Experience: Performed beach transects and algae identification. Performed water quality measurements such as disolved oxygen content, salinity, and conductivity in multiple bays with different productivities and examined data for trends. Performed grazing preference experiments in aquaria using sea urchins. Coordinated and led journal discussion groups.

Professional Experience
Seattle Genetics Corporation, Bothell WA (July 2006 – Present)

Quality Control Analyst III
·Prepare concise technical reports supporting toxicology studies and clinical trials.
·Responsible for the transfer and troubleshooting of various HPLC methods.
·Responsible for the transfer and qualification of cell-based test methods.

Targeted Genetics Corporation, Seattle WA (2001 – July 2006)

Senior Quality Control Analyst - Stability Lead 2005 - July 2006
·Responsible for development, training and mentoring the stability group staff.
·Represent the QC department on various project teams.

Quality Control Analyst II – Stability Lead 2003-2005
·Responsible for stability program maintenance, scheduling and meeting timelines.
·Led stability meetings to disseminate stability data to various department heads.
·Provided technical expertise in response to product investigations.

Quality Control Analyst II 2003-2004
·Led development efforts, transfer and qualification of new test methods.
·Lead investigator and trainer for Bacterial Endotoxin Test.

Quality Control Analyst I 2001-2003
·Performed release, and stability testing of raw materials and rAAV product candidates

Proficient in the following laboratory skills utilized exstensively in aquatic environmental sciences:

- HPLC: Reverse-Phase, Ion Exchange, Size Exclusion
- Real-time quantitative PCR
- DNA restriction digests
- UV/Vis Spectrophotometry
- DNA radiolabeling/hybridization
- Gel Electrophoresis
- Western Blotting
- SDS-PAGE
- ELISA
- Cell-based Bioassays

Volunteer Activities

- Volunteer, GEMS Program (Girls in Engineering, Math and Science), 2007
- Steering Committee Member, Ballard Corners Park, 2006-2007
- Biology tutor, Seattle University, 1999-2001