Woods Hole Center for Oceans and Human Health

Pilot Project Proposal – 2007

# Impact of microbial community composition and temperature on *Vibrio parahaemolyticus*

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## Project Summary

The incidence of *Vibrio parahaemolyticus* infections in humans has risen dramatically over the past few years on both coasts of the US. In addition, the geographic range of V. *parahaemolyticus* is expanding northwards, suggesting that changes in water temperature associated with global climate change may further increase the incidence of infection. Biotic and abiotic factors in the sediment and water column likely influence *V. parahaemolyticus* physiology, resulting in increased pathogen abundance, alterations in potential virulence and changes in pathogen behavior, increasing likelihood of human contact**. Here we propose to examine the influence of temperature and microbial community composition on the abundance and physiology of *V. parahaemolyticus*.** The specific objectives of the current proposal are to: 1) Establish short-term laboratory microcosms that can be spiked with the bacterial human pathogen – *V. parahaemolyticus*; 2) Assess the influence of microbial community composition and temperature on *V. parahaemolyticus* abundance and physiology; and 3) Establish baseline data for a larger study of biotic and abiotic controls of *V. parahaemolyticus* physiology. We will establish laboratory microcosms with water collected from four sites (two in Massachusetts and two in Washington), which we will inoculate with *V. parahaemolyticus*. Microcosms will be incubated under two temperature treatments (ambient and elevated) and sampled at three time points. Changes in *V. parahaemolyticus* abundance and physiology will be characterized using quantitative PCR analysis. The specific gene targets for RT-PCR analysis regulate general metabolism, adaptation, swarming behavior, quorum sensing, and virulence. Variation in microbial composition found in the waters from the four specific sites will be assessed by genetic fingerprinting and 16S rDNA sequencing. Upon completion of the proposed research we will have basic information on two important factors that may influence Vp abundance, physiology, and thus incidence of disease in the coastal environment. The proposed work will establish a coordinated effort on both US coasts that will foster future scientific research and communication addressing large-scale processes, pathogen evolution, and geographic variability in human-pathogen interactions.

## Impact of microbial community composition and temperature on *Vibrio parahaemolyticus*

**Objectives**

1) Establish short-term laboratory microcosms that can be inoculated with the bacterial human pathogen - *Vibrio parahaemolyticus*.
2) Assess the influence of microbial community composition and temperature on *V. parahaemolyticus* abundance and physiology.
3) Establish baseline data for a larger study of biotic and abiotic controls of *V. parahaemolyticus* physiology.

**Background and Rationale**

Our coastal waters are increasingly becoming areas of concern for pathogen exposure (Ward and Lafferty 2004). Pathogens in the marine environment can be found dispersed in the water column and associated with components such as abiotic particles, plankton, other microbes, and aggregates. Such components have been shown to serve as important vectors and can affect pathogen biology (i.e. Lyons et al. 2007, Venkateswaran et al. 1990). Global climate change and associated increases in water temperature are also associated with increased incidence of disease, disease transmission, and host susceptibility for a number of pathogens (Harvell et al. 2002). **Here we propose to focus on the interaction among temperature, microbial community ecology and pathogen physiology of a human pathogen *Vibrio parahaemolyticus* (Vp), which is common along both coasts of the US.**

Human exposure to *Vibrio parahaemolyticus* is commonly through the consumption of seafood, though there have been instances of direct infection from other *Vibrio* species (Linkous and Oliver 1999). Vp is a leading cause of seafood-associated bacterial gastroenteritis in the United States (Mead et al. 1999). When pathogenic Vp invades the human digestive system, the organism attaches to the small intestine and secretes toxins that result in diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills. The disease is normally not life threatening unless a person is immune compromised

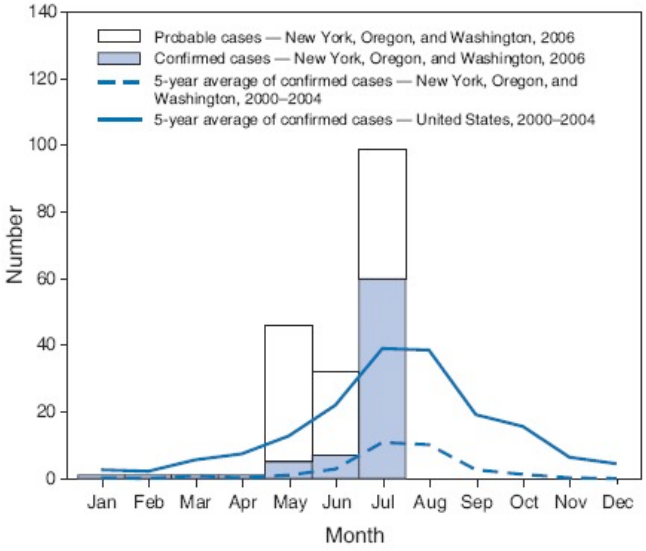


**Figure 1.** *V. parahaemolyticus* cases from NY, OR, and WA compared to with 5-year average numbers of confirmed cased nationwide, and from NY, OR, and WA during 2000-2004. (taken from CDC 2006)

The incidence of human Vp infections has risen dramatically over the past few years on both coasts of the US. In 2004, a major outbreak of Vp occurred, originating from Alaskan waters (McLaughlin, et al 2005). Prior to this outbreak, Alaskan waters were considered too cold for Vp. In 2006, New York City, New York state, Oregon, and Washington health departments reported 177 cases during May 20 to July 31 (CDC 2006). The number of confirmed cases (confirmed by isolation of the organism from a patient's stool) during this period was 72, which is more than the average number reported for the same months in the entire US during the four years prior (CDC, 2006) (Figure 1). Numerous cases of Vp infection were reported during this past summer from across the country and led to the FDA releasing a warning to consumers to avoid oysters from Hood Canal in Washington (FDA, 2007).

Proper seafood handling techniques can certainly assist in the reduction of human illness. However, increased incidence of Vp suggests environmental factors may influence Vp physiology, resulting in 1) increased abundance of Vp, 2) changes in Vp behavior increasing likelihood of human contact, and 3) alterations in potential virulence. Environmental factors likely interact to influence more than one of these parameters. **The goal of this proposed pilot project is to establish laboratory microcosm systems so that we can assess the influence of microbial community composition and temperature on abundance and physiology of a commonly occurring bacterial human pathogen - *Vibrio parahaemolyticus*.** In the remainder of this section we outline what is known about Vp biology related to pathogenicity and discuss the potential role of microbial community composition in understanding Vp dynamics.

Biology - *Vibrio parahaemolyticus* is a gram-negative, halophilic bacterium that is common in warm estuarine environments. As for many other bacteria, growth rates are directly related to temperature. Temperature can also indirectly influence Vp abundance by acting as a stressor on the oyster immune system, thus impairing the oyster's ability to mount an effective immune response.

The mechanism of Vp accumulation in shellfish is not completely understood, although it is likely that swarming behavior (i.e. biofilm formation) on oyster tissue and particulates in seawater plays a role. Quorum sensing (the process by which many bacteria coordinate gene expression according to the local density of bacteria producing signaling molecule) can regulate such behavior and is one example of the potential for microbial communities to affect Vp (for review, Donabedian 2003, Miller and Bassler 2001). Vp are somewhat unique in that they have two flagella systems associated with the different cell types (planktonic and swarming). The polar flagellum is always present while the lateral flagellum is synthesized to effectively colonize hard or viscous surfaces. The dual functional flagella system in Vp has been extensively studied and the genes involved in flagellum formation have been determined (Kim and McCarter 2000, Stewart and McCarter 2003). Environmental conditions that have been shown to be required for lateral flagella expression (and thus swarming behavior and perhaps accumulation in shellfish) include iron-limiting conditions and inhibition of polar flagella rotation (McCarter et al. 1988, McCarter and Silverman 1989).

Most of what is known regarding Vp virulence has focused on what occurs inside the human digestive system. Thermostable direct hemolysin (*tdh*) and TDH-related hemolysin have long been considered the virulence factors associated with the pathogenic strains of Vp (Joesph et al. 1982). Assay systems developed to determine whether a strain of Vp is pathogenic are often based on the presence of *tdh*. It should be noted that in the environment, less than 1% of Vp is pathogenic (Nishibuchi et al. 1985). In other words most environmental strains do not contain TDH genes and do not cause disease.

The recent sequencing of the *V. parahaemolyticus*, RIMD 2210633, (Makino et al. 2003) has revealed that this pathogen also possesses a type III secretion system (TTSS). These systems play a major role in virulence among other pathogenic bacteria including *Yersinia spp*. and *Pseudomonas aeruginosa* and are responsible for secreting and translocating virulence proteins to the host (Hueck 1998). Two distinct gene clusters were identified in Vp, TTSS1 and TTSS2, that have been suggested to be involved in cytotoxicity and enterotoxicity respectively (Park et al, 2004). Quorum sensing plays a vital role in Vp bacterial communities in regards to Vp virulence, as the expression of TTSS genes will not be expressed unless a critical concentration of specific autoinducers are detected within the community (Henke and Bassler 2004).

Ecology - Not surprisingly, on large temporal and spatial scales the prevalence of Vp and other *Vibrio spp*. has been shown to be related to temperature (Fukushima & Seki 2004). Recent detection of Vp in Alaska, Chile and Norway (Mead et al. 1999, McLaughlin et al. 2005, González-Excalona et al. 2004, Bauer et al. 2006) suggests that increasing water temperatures associated with global climate change will likely result in increased incidence of Vp related illness in both space and time.

While the simple effects of temperature on Vp abundance can be predicted, relatively little is known about how multiple environmental factors (i.e. temperature, sea water components *and* microbial community composition) may influence Vp behavior and virulence in the water column and how this might be associated with disease outbreaks. A goal of this pilot project is to begin to obtain some of the very basic information in this regard. We are interested in exploring possible links among physiological changes in organism behavior, virulence factors, and microbial community dynamics.

Biotic factors, in addition to abiotic factors, influence population and community dynamics of microbes (Horner-Devine et al. 2004). Interactions with other microbes can play important roles in structuring microbial communities and could influence abundance, behavior and virulence. As discussed above, quorum sensing may play an important role in virulence of Vp. Interspecific interactions could also influence gene expression and population dynamics of Vp. It is possible to see the signal of interactions as interspecific associations where two or more species co-occur either more or less frequently than expected due to chance alone. For example, associations regulated by competition and/or communication (i.e. interspecific quorum sensing) could influence Vp density and physiology in combination with abiotic environmental factors. One goal of the microbial community analysis proposed here is to look for signals of co-occurrence associated with Vp and Vp virulence.

We will thus examine associations that may occur as a consequence of biotic interactions such as mutualism, competition and predation. In particular, we will explore the idea that niche exclusion of Vp by other marine microbes may help explain why Vp could become a greater threat - attributable to factors such as increased

abundance, changes in behavior increasing likelihood of human contact, or alterations in potential virulence - under changing environmental conditions.

**Experimental design and methods**

Site selection and sampling We will collect water from the following four sites, two on the east coast and two on the west coast of the U.S.: i) Woods Hole, MA; ii) Plum Island Sound, MA. Plum Island is the site of ongoing work aimed at understanding the microecology and evolution of Vp by Polz and Lerczak. We thus hope our work will complement this study and leverage their results into a better understanding of Vp ecology from this pilot project; iii) Hood Canal, WA. Hood Canal was the site of a series of shellfish closures this past summer due to Vp found in oysters (resulting in the FDA warning mentioned above (FDA, 2007); and iv) Puget Sound, Main Basin. Sampling will take place in Summer 2008. Water will be collected using a Niskin bottled from each location and stored in dark 1L bottles in a cooler for transport to the lab.

Lab microcosms Water collected from these four sites will be used to establish laboratory-based, rolling tube microcosms (2L), spiked with a pathogenic strain of Vp. Vp 17802 will be obtained from American Type Culture Collection, handled according to Biosafety Level Two protocols, and cultured using standard methods. While there is the potential that Vp will be found in the samples from our four sites, it less likely that these strains will be pathogenic, and our goal is to characterize the effects on disease causing Vp. Furthermore, as described below, we will assess the composition of the water prior to addition of the Vp at an inoculation level of 6.0 log CFU/ml. Temperatures will be maintained at 1) the ambient temperature where the samples were collected and 2) at +5∞C over ambient. All treatments will be run in duplicate, and samples will be taken at two subsequent time points to assess temporal variation (i.e. 0 hour, 6 hours and 24 hours). Samples (500 ml of water) will be filtered onto a 0.2 um filter, followed with 3 ml preservation buffer (10 mM Tris pH 8.0, 100 mM EDTA, 0.5 M NaCl) and frozen at -80ºC until further analysis.

Characterizing *V. parahaemolyticus* abundance To compare Vp abundance under different environmental conditions (microbial composition, temperature, trial duration) a modification of the PCR assay developed by Takahashi et al (2005) will be used. DNA will be extracted from the filters using standard methods (Qiagen Kit). The PCR assay targets the toxR gene and will not cross-react with other species. Quantitative PCR reactions (Brilliant SYBR Green QPCR Master Mix Kit, 1-Step, Stratagene) will be carried out in an Opticon2 Continuous Fluorescence Detection System (Bio-Rad). A time zero sample will be taken and analyzed to determine if field-collected waters from any of the four sites contain Vp. For all real time assays, melting curves will be analyzed to verify that no primer dimers are formed and that CT values represent the desired amplicon. A subset of PCR products will be sequenced to verify accuracy. CT values will then be converted to relative abundance levels based on their respective standard curves and normalized to the corresponding values.

Characterizing *V. parahaemolyticus* physiology In order to determine the abiotic and biotic effects on Vp physiology related to growth rates, change in behavior, and virulence, real-time quantitative RT-PCR will be carried out. Total RNA will be extracted from the preserved filters using Tri-Reagent. A suite of 5 genes have been selected for analysis based on their role in processes of interest and the fact that for most of these genes, the PCR assays have been validated. Targets include genes associated with general metabolism (*dsdA*, Makino et al. 2003), adaptation to environmental stress (*rpoS* (alternate sigma factor of RNA polymerase) Vasudevan and Venkitanarayanan 2006, Coutard et al. 2007), lateral flagella synthesis (*lafK*, Merino et al. 2006), quorum sensing and virulence (*opaR* (*luxR* homolog) Henke and Bassler 2004), virulence (*tdh2*, Coutard et al. 2007; *spa24* (component of TTSS system), Coutard et al. 2007). The housekeeping gene *pvsA* will be used for normalizing expression levels as described in Coutard et al 2007. RT-PCR reactions will be carried and analyzed as described above except the Brilliant SYBR Green QRT-PCR Master Mix Kit, (Stratagene) will be used as RNA will be the template.

Characterizing Microbial Community Structure We will use Automated Ribosomal Intergenic Spacer Analysis (ARISA) to create a genetic fingerprint of the diverse bacterial community waters collected from the four sites. This established, rapid high throughput method uses the ribosomal RNA internally transcribed spacer (ITS), a region of DNA known to vary in size in different bacterial species (Fisher and Triplett 1999, Hewson and

Fuhrman 2004, Brown et al. 2005). This first look at microbial community differences along with the results from the gene expression analysis comparisons will inform the selection of a subset of microcosms for which we will create and sequence 16S rDNA clone libraries. For ARISA, the ITS will be amplified using universal bacterial primers (16S-1392F and 23S-R, Brown et al. 2005), including a fluorescently labeled forward primer. For each sample, four independent PCRs will be performed, pooled, ethanol precipitated to remove unincorporated primers and run on a capillary sequencer along with ROX-labeled size standards (50-1500 bp ladder, BioVentures, Inc). Fragment lengths will be sized using DAX software (http://www.dax.nl/dax.htm) using a signal to noise cutoff to verify presence of peaks.

We will use cloning and sequencing of 16S rDNA to assess bacterial community composition on a subset of the laboratory mescosms. Mesocosms will be selected based on preliminary Vp abundance and gene expression analysis results. Bacterial rDNA will be amplified using the PCR, with primer sequences corresponding to *Escherichia coli* nucleotides 1392–1406 (1392r) and 8–26 (8f). Sequences will be aligned using ARB (Ludwig and Strunk 2004), screened for chimeras and phylogenetic trees will be constructed using PAUP* (Swofford 2002).

Statistical analyses For quantitative PCR analysis, individual reaction efficiency will be incorporated into the analysis using PCR-Miner software. Expression levels will be examined in relation to specific factors by ANCOVA, Spearman Rank or Pearson Moment Correlation test and ANOVA with Tukey tests, as appropriate. For microbial populations, Canonical Correspondence Analysis (using Canoco) and SIMPER (Clarke and Warwick 2001) will be used to identify specific taxa associated with Vp expression levels. We will use an analysis of similarity (ANOSIM) to examine how community similarity varies with different our treatments (temperature, trial length) and Vp gene expression (Clarke & Warwick 2001). We will assess community structure and co-occurrence using the C-score (Stone and Roberts 1990, Horner-Devine et al. 2007) as well as other newly proposed null models (Hausdorf 2007).

**Facilities and anticipated use of Center Genomics Core facility**

Microcosm systems will be established from waters collected in Massachusetts and will be carried out by Roberts in cooperation will Smolowitz in her laboratory located in the Marine Resources Center (MBL). Dr. Smolowitz, the MBL Veterinarian routinely works with pathogens. Furthermore, Roberts and Smolowitz are currently working together on a similar design examining environmental factors influencing gene expression in the shellfish pathogen QPX (i.e. Lyons et al. in review, Roberts and Smolowitz in preparation). Dr. Horner-Devine will carry out Washington trials and ARISA fingerprinting in her lab and has extensive experience examining microbial communities. Quantitative PCR analysis will be conducted at both locations as there is a Opticon2 Continuous Fluorescence Detection System (Bio-Rad) in the Marine Resources Center and UW's School of Aquatic and Fishery Science (SAFS) has numerous models including the Opticon2. DNA sequencing for verification of Vp PCR products and for characterizing microbial communities will be performed in the Center Genomics Core Facility, located in the Josephine Bay Paul Center (MBL) using the Applied Biosystems 3730XL DNA Analyzer.

**Broader impacts of the proposed research**

The proposed pilot project is designed to develop a system to examine the complex interactions of biotic and abiotic factors affecting a human pathogen in the coastal waters. Upon completion of the proposed research we will have basic information on factors effecting *Vibrio parahaemolyticus* in the coastal environment that could lead to higher incidence of disease. Data on the relationship among Vp abundance, physiology, temperature and microbial community composition could incorporated into risk-assessment models. Future microcosm experiments based on results from the proposed work will consider other potentially important variables including artificial microbial communities, eukaryotic communities, salinity, marine aggregates, and presence of oysters. The data from this proposal will also provide base-line data for integrative field based research proposals. Furthermore, we plan to use data and funds from this project as leverage for seeking local funding from the University of Washington's School of Aquatic of Fishery Sciences. Finally, an exciting aspect of the proposed research is that it would establish a coordinated effort on both US coasts that would foster scientific research and communication addressing large-scale processes, pathogen evolution, and geographic variability in human-pathogen interactions.

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**SUMMARY**
**PROPOSAL BUDGET**
**MARINE BIOLOGICAL LABORATORY**

PRINCIPAL INVESTIGATOR: **Steven Roberts**

| **A. SENIOR PERSONNEL** | | PER-MOS | YR1 FUNDS | -I | TOTAL FUNDS |
|---|---|---|---|---|---|
| 1. PI 1 | | 0 | $0 | | $0 |
| 2. PI 2 | | 0 | $0 | | $0 |
| 3. PI 3 | | 0 | $0 | | $0 |
| 4. PI 4 | | 0 | $0 | | $0 |
| 5. PI 5 | | 0 | $0 | | $0 |
| 6. ( ) OTHERS | | 0 | $0 | | $0 |
| TOTAL SENIOR PERSONNEL | | 0 | $0 | | $0 |
| **B. OTHER PERSONNEL** | | | | | |
| 1. ( ) POST DOCTORAL ASSOC | | 0 | $0 | | $0 |
| 2. (1) OTHER PROFESSIONALS | | 0.58 | $1,399 | | $1,399 |
| 3. ( ) GRADUATE STUDENTS | | 0 | $0 | | $0 |
| 4. ( ) UNDERGRADUATE STUDENTS | | 0 | $0 | | $0 |
| 5. ( ) SECRETARIAL-CLERICAL | | 0 | $0 | | $0 |
| TOTAL SALARY AND WAGES (A+B) | | | $1,399 | | $1,399 |
| **C. FRINGE BENEFITS @ 37.8%** | 0.378 | | $529 | | $529 |
| TOTAL SALARY, WAGES AND FRINGE | | | $1,928 | | $1,928 |
| **D. PERMANENT EQUIPMENT** | | | | | |
| 1. ITEM #1 | | | $0 | | $0 |
| 2. ITEM #2 | | | $0 | | $0 |
| TOTAL PERMANENT EQUIPMENT | | | $0 | | $0 |
| **E. TRAVEL** | 1. DOMESTIC | | $500 | | $500 |
| | 2. FOREIGN | | $0 | | $0 |
| **F. PARTICIPANT SUPPORT COSTS** | | | | | |
| 1. STIPEND | | | $0 | | $0 |
| 2. TRAVEL | | | $0 | | $0 |
| 3. SUBSISTENCE | | | $0 | | $0 |
| 4. OTHER | | | $0 | | $0 |
| TOTAL PARTICIPANT COSTS | | | $0 | | $0 |
| **G. OTHER DIRECT COSTS** | | | | | |
| 1. MATERIALS AND SUPPLIES | | | $9,500 | | $9,500 |
| 2. PUBLICATION COSTS | | | $0 | | $0 |
| 3. CONSULTANT SERVICES | | | $0 | | $0 |
| 4. COMPUTER (ADPE) SERVICES | | | $0 | | $0 |
| 5. SUBCONTRACTS | | | $10,118 | | $10,118 |
| TOTAL OTHER DIRECT COSTS | | | $19,618 | | $19,618 |
| **H. TOTAL DIRECT COSTS (A THROUGH G)** | | | $22,046 | | $22,046 |
| **I. INDIRECT COSTS @ 57.8%** | | Base = | $22,046 | = | $22,046 |
| Rate = 57.8% | | Rate = | 57.8% | = | 57.8% |
| Base = | | IN CSTS = | $12,743 | = | $12,743 |
| **J. TOTAL DIRECT AND INDIRECT** | | | $34,789 | | $34,789 |

**Budget Justification**

Impact of microbial community composition and temperature on *Vibrio parahaemolyticus*

For this project to be completed, funds are requested to partially cover technician time for personnel at the MBL in the amount of $1,928. This person will assist in carrying-out microcosm trial and routine molecular work.

A total of 9,500 dollars is requested for supplies and this includes funds for DNA sequencing at the COHH Genomics Core facility at MBL ($2,500), molecular reagent kits (ie nucleic acid extraction and purification, gene discovery, SYBR green RT-PCR) ($3,900), consumable reagents and plasticware ($1,500), environmental sample collection apparatus ($500), culture of *Vibrio parahaemolyticus* from ATCC ($300) and general laboratory supplies ($800). Scientific work to be carried out at the MBL includes sample collection, incubation trials, and molecular characterization.

Funds ($500) are requested to partially cover travel costs associated with sample collection.

A subaward of $10,118 to the University of Washington (investigator: Claire Horner-Devine) is included to partially cover salary ($4,386) and supplies ($2,100) necessary for microcosm experiments to be carried out at UW to determine spatial variation in microbial communities that could influence *V. parahaemolyticus* physiology. In addition, Dr Horner-Devine, will be responsible for developing techniques required to assess microbial community structure.

Full indirect costs as determined by the MBL and UW are requested.

# CURRICULUM VITAE – STEVEN BEYER ROBERTS

***Contact Information*** University of Washington
School of Aquatic and Fishery Sciences
Fisheries Teaching and Research Building
1140 NE Boat Street
Seattle, WA 98195
phone: 206.600.4495
email: sr320@u.washington.edu

***Academic Experience***

Ph.D. – University of Notre Dame (South Bend, IN) – 2002
Integrative Cell and Molecular Physiology

B.S. – North Carolina State University (Raleigh, NC) – 1997
Natural Resources – Concentration in Marine and Coastal Resources

***Professional Experience***

*2006-Present* · Assistant Professor
University of Washington, Seattle,WA

*2006-Present* · Adjunct Assistant Scientist
Marine Biological Laboratory, Woods Hole, MA

*2003-2006* · Assistant Research Scientist
Marine Biological Laboratory, Woods Hole, MA

***Select Publications***

Roberts SB and Smolowitz R. (in preparation) Characterization of a serine protease gene in the hard clam pathogen Quahog Parasite X (QPX) and the effects of environmental conditions on transcript expression.

Lyons MM, Gast RJ, Roberts SB, Smolowitz R, Ward EJ. (2007) Growth of the hard clam pathogen Quahog Parasite X (QPX) on detrital fragments from green seaweed Diseases of Aquatic Organisms. in review

Lyons MM, Lau Y-T, Carden WE, Ward JE, Roberts SB, Smolowitz R, Vallino J, Allam B. (2007) Characteristics of marine aggregates in shallow-water ecosystems: Implications for disease ecology. EcoHealth. in press

Hodgins-Davis A, Roberts SB, Cowan D, Atema J, Avolio C, Defaveri J, Gerlach G. (2007) Characterization of SSRs from the American lobster, *Homarus americanus.* Molecular Ecology Notes. 7:330-332

Rodgers BD, Roalson EH, Weber GM, Roberts SB, Goetz FW. (2007) A Proposed Nomenclature Consensus for the Myostatin Gene Family. AJP- Endocrinology and Metabolism. 292(2):E371-2

Lyons MM, Smolowitz R, Dungan C, Roberts SB. (2006) Development of a real-time quantitative PCR assay for the hard clam pathogen, Quahog Parasite Unknown (QPX). Diseases of Aquatic Organisms. 72(1):45-52

***Select Publications continued***

Roberts SB, Romano C, Gerlach G. (2005) Characterization of EST derived SSRs from the bay scallop, *Argopectens irradians*. Molecular Ecology Notes. 5: 567-568

Biga PR, Roberts SB, Iliev DB, McCauley LA, Moon JS, Collodi P, Goetz FW. (2005) The isolation, characterization, and expression of a novel GDF11 gene and a second myostatin form in zebrafish, Danio rerio. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 141: 218-230

Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004) Transgenic salmon over-expressing growth hormone exhibit decreased myostatin transcript and protein expression. Journal of Experimental Biology. 207(Pt 21):3741-8

Kim H-W, Mykles DL, Goetz FW, Roberts SB. (2004) Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians.* BBA – Gene Structure and Expression. 1679(2):174-9

Roberts SB, Goetz FW. (2003) Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biological Bulletin. 205: 227-228.

Roberts SB, Goetz FW. (2001) Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms. FEBS Letters. Vol 491, No. 3, pp. 212-216.

***Recent Invited Presentations***

•Gene expression profiling and cellular characteristics of *Crassostrea virginica* hemocytes: evaluating interactions of physical stress and disease. Aquaculture 2007, San Antonio, TX March 1, 2007

•Characterizing myostatin function in salmonids: examining post- translational processes and protein interactions. Aquaculture 2007, San Antonio, TX. February 28, 2007

•The potential of genomics in aquaculture: current science. 2006 MIT Environmental Fellows Retreat. Woods Hole Oceanographic Institution. Woods Hole, MA. November 3, 2006,

•Genomic approaches in characterizing shellfish disease: interrelationships between animal, human and ecosystem health. Cummings School of Veterinary Medicine at Tufts University, Annual Symposium: Marine and Aquatic Medicine & Conservation. North Grafton, MA. April 22, 2006

•Characterization of differentially expressed genes from QPX: insight into possible virulence mechanisms. National Shellfisheries Association Annual Meeting, Monterey, CA. March 28, 2006

***Professional Activities***

Pan American Marine Biotechnology Association
World Aquaculture Society
National Shellfish Association
Sigma Xi Scientific Research Society
American Fisheries Society
Review Panel, National: USDA-SBIR
Proposal Reviewer, National: NOAA-ODRP, USDA-NRI

CURRICULUM VITAE - ROXANNA M. SMOLOWITZ

Office Address: Marine Biological Laboratory
7 MBL St.
Woods Hole, MA 02543

Education:

| | |
|---|---|
| 1967-69 | Indiana University |
| 1975-76 | Indiana State University, B.A. |
| 1977-81 | Purdue University, D.V.M. |
| 1982-84 | Resident in Pathology, Angell Memorial Animal Hospital, Boston, MA |
| 1985-87 | Bay Foundation Fellowship, Marine Biological Laboratory, Woods Hole, MA |

Postgraduate Training:

| | |
|---|---|
| 1979 | AQUAVET® - University of Pennsylvania (Including summer research program) |
| 1982 | Pathology of Marine Invertebrates - Marine Biological Laboratory |
| 1991 | Pathology of Laboratory Animals, AFIP, Washington, D.C. |
| 1991 | Gross Morbid Review, AFIP, Washington, D.C. |
| 1992 | Descriptive Pathology, AFIP, Washington, D.C. |
| 1992 | Toxic Pathology, C. L. Davis Foundation, Champagne, IL |

Employment:

| | |
|---|---|
| 1981-82 | Staff veterinarian, Plymouth Animal Hospital, Plymouth, MA |
| 1984-85 | Pathologist, School of Veterinary Medicine, Tufts University, Boston, MA |
| 1985-86 | Clinical Instructor in Comparative Medicine, School of Veterinary Medicine, Tufts University, Boston, MA |
| 1986-89 | Laboratory Animal Veterinarian, Marine Biological Laboratory, Woods Hole, MA |
| 1988-95 | Visiting Investigator, Woods Hole Oceanographic Institution, Woods Hole, MA |
| 1989-95 | Research Associate, School of Veterinary Medicine, UPenn |
| 1995-99 | Senior Research Investigator, School of Veterinary Medicine, UPenn |
| 1999-07 | Veterinarian, Marine Biological Laboratory |

Veterinary Licensure: Massachusetts # 2496

Honors, Awards and Organizations:

Phi Zeta Veterinary Honor Society, Graduated in the top 10%
Award for Academic Superiority in the first year of veterinary medicine
Gamma Sigma Delta
Fourth year fellowship, American Association of University Women
President, 1980-1981, Exotic Animal Medicine Club, Purdue University

Memberships in Professional and Scientific Societies:

American Fisheries Society
American Veterinary Medical Association
International Association of Aquatic Animal Medicine
Massachusetts Shellfish Officers Association
National Association of Small Ruminant Practitioners
National Shellfish Association
Sigma Xi

Bibliography: Selected Original Papers

Smolowitz, R.M. and C.L. Reinisch. 1986. Indirect peroxidase staining using monoclonal antibodies specific for *Mya arenaria* neoplastic cells. J. Invert. Path. 48:139-145.

Smolowitz, R.M., D.L. Miosky and C.L. Reinisch. 1989. Ontogeny of leukemic cells of the soft shell clam. J. Invert. Path. 52:41-51.

Smolowitz, R.M., R.A. Bullis, D.A. Abt, and L. Leibovitz. 1993. Pathologic observations on the infection of *Pagurus* spp. by plerocercoids of *Calliobothrium verticillatum* (Rudolphi, 1819; Van Benden, 1850). J. Invert. Path. 62:185-190.

Smolowitz, Roxanna M. and Carol L. Reinisch. 1993. A novel adhesion protein expressed by ciliated epithelium, hemocytes, and leukemia cells in soft-shell clams. Dev. Comp. Immunol. 17:475-481.

Wikfors, Gary H. and Roxanna M. Smolowitz. 1995. Experimental and histological studies of four life-history stages of the Eastern oyster, *Crassostrea virginica*, exposed to a cultured strain of the dinoflagellate, *Prorocentrum minimum*. Biol. Bull. 188:313-328.

Smolowitz, Roxanna. 1995. Immunochemical localization of saxitoxin in the siphon of the butter clam, *Saxidomus giganteus*. Biol Bull. 189:229-230.

Smolowitz, Roxanna and Sandra E. Shumway. 1997. Possible cytotoxic effects of the dinoflagellate, *Gyrodinium aureolum*, on juvenile bivalve molluscs. Aquaculture Inter. 5: 291-300.

P.A. Mass,S. J. Kleinschuster, M. Dykstra, R. Smolowitz and J. Parent. 1999. Molecular characterization of QPX (Quahog parasite Unknown), a pathogen of Mercenaria mercenaria. J. Shellfish Res. 18: 561-567.

Hanselmann, R., R. Smolowitz and D. Gibson. 2000. Identification of proliferating cells in hard clams. Bio Bull. 199: 199-200.

Hsieh, J.L., H.M. Chikarmane, R. Smolowitz, K. Uhlinger, W. Mebane, A. Kuzirian. 2002. Microbial analysis of ozone disinfection in a recirculating seawater system. Bio. Bull. 203: 266-267.

Hsu, A.C. and R.M. Smolowitz. 2003. Scanning Electron Microscopy Investigation of epizootic lobster shell disease in *Homarus americanus*. Biol. Bull. 205: 228-230.

Anderson, R.S., B.S. Kraus, S. McGladdery, K.S. Reece and N.A. Stokes. 2003. A thraustochytrid protist insolated from *Mercenaria mercenaria*: molecular characterization and host defense responses. Fish Shellfish Immunol: 15: 183-194.

Lyons, M.M., Roxanna Smolowitz, Kevin R. Uhlinger, Rebecca J. Gast, J. Evan Ward. 2005. Lethal marine snow: Pathogen of bivalve mollusc concealed in marine aggregates. Limnol. Oceanogr. 50: 1983-1988.

Tlusty, M.F., Halvoarson, H.O., Smolowitz, R. and Sharma. U. (eds) 2005. Lobster Shell Disease Workshop. Aquatic Forum Series 05-1. New England Aquarium, Boston, MA

Smolowitz, R., Chistoserdov, A., and A. Hsu. 2005. Epizootic shell disease in American lobster, *Homarus americanus*. IN (Tlusty, M.F., Halvoarson, H.O., Smolowitz, R. and Sharma, U., eds) Lobster Shell Disease Workshop. Aquatic Forum Series 05-1. New England Aquarium, p. 1-11.

Chistoserdov, A., Gubbala, S.L., Mirazol, F., Smolowitz R**.** and A. Hsu. 2005. A microbiological assessment of epizootic shell disease in the American lobster indicates it strictly dermal origin. In (Tlusty, M.F., Halvoarson, H.O., Smolowitz, R. and Sharma. U., eds) Lobster Shell Disease Workshop. Aquatic Forum Series 05-1. New England Aquarium, Boston, MA. Pages 12-20.

Lyons MM, Smolowitz R, Dungan C, Roberts SB. 2006. Development of a real-time quantitative PCR assay for the hard clam pathogen, Quahog Parasite Unknown (QPX). Diseases of Aquatic Organisms, 72:45-52.

Gast, R.J., E. Cushman, D.M. Moran, K.R. Uhlinger, D. Leavitt and R. Smolowitz. 2006. DGGE-based detection method for Quahog Parasite Unknown (QPX) from environmental samples and clam tissues. Journal of Shellfish Research. *Diseases of Aquatic Organisms* 70: 115-122.

Halvorson, H. and R. Smolowitz. Aquaculture, Microbiology of. In (A.I. Laskin ed., Third Edition of Encyclopedia of Microbiology) *Accepted for publication.*

Gauger, E., R. Smolowitz, K. Uhlinger, J. Casey, M. Gomez-Chiarri. 2006. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*. Aquaculture 260: 10-20.

Ford, S. and R. Smolowitz. 2006. Infection dynamics of an oyster parasite in its newly expended range. Mar. Biol. Published on line Sept 15, 2006. Springer Verlag

Smolowitz, R. 2006. Gastropods. In (G. Lewbart Ed.) Invertebrate Medicine. Blackwell Publishing, Ames, Iowa. Pp.65-78.

Berkins, I. and R. Smolowitz. 2006. Handling of Pathological Samples from Invertebrates. In (G. Lewbart, Ed.) Invertebrate Medicine. Blackwell Publishing, Ames, Iowa. Pp.263-274.

**M. CLAIRE HORNER-DEVINE**

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School of Aquatic and Fishery Sciences
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Seattle, WA 981954

Tel: (206) 897-1863
FAX: (206) 685-3275
mchd@u.washington.edu

**Academic and Professional History**

| | |
|---|---|
| 1996 | B.A., Ecology and Evolutionary Biology, Princeton University. |
| 1996-1998 | Secondary School Teacher, Westtown School, PA. |
| 2004 | Ph.D., Biological Sciences, Stanford University. |
| 2004 | Post-doctoral scholar, Stanford University (Brendan Bohannan). |
| 2004-2006 | Assistant Research Professor, School of Aquatic and Fishery Sciences, University of Washington. |
| 2006 | Acting ADVANCE Research/Program Manager, half-time. University of Washington, Center for Institutional Change. |
| 2006-present | Assistant Professor, School of Aquatic and Fishery Sciences, University of Washington. |

**Publications - 5 closely related**

**Horner-Devine, M.C.,** J. Silver, B.J.M. Bohannan, R.K. Colwell, J.A. Fuhrman, J.L. Green, C.R. Kuske, , J.B.H. Martiny G. Muyzer, S. Naeem, L. Øvreås, A.-L. Reysenbach, V.H. Smith. 2007. A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology*. 88: 1345-1353.

**Horner-Devine, M.C.** and B.J.M. Bohannan. 2006. Phylogenetic clustering and overdispersion in bacterial communities. *Ecology*. 87:S100-S108.

Martiny, J.B., B.J.M. Bohannan, J.H. Brown, R.K. Fax: 206-685-3275Colwell, J.A. Fuhrman, J.L. Green, **M.C. Horner-Devine**, M. Kane, J.A. Krumins, C.R. Kuske, P.J. Morin, S. Naeem, L. Øvreås, A.-L. Reysenbach, V.H Smith, J.T. Staley. 2006. Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*. 4: 102-112.

**Horner-Devine, M.C**., M. Lage, J. Hughes and B.J.M. Bohannan. 2004. A taxa-area relationship for bacteria. *Nature*. 432:750-753.

**Horner-Devine, M.C**., K.M. Carney, and B.J.M. Bohannan. 2004. An ecological perspective on bacterial biodiversity. *Proceedings of the Royal Society of London B*. 271:113-122.

**Publications - 5 other significant**

**Horner-Devine, M.C.** and B.J.M. Bohannan. 2006. Unifying ecology to include all creatures great and small. *Trends in Ecology and Evolutionary Biology*. 21: 473.

**Horner-Devine, M.C.,** J. Green and B.J.M. Bohannan. 2006. "Patterns in prokaryotic biodiversity" in Prokaryote Diversity: Mechanisms and Significance. pgs 19-38. Eds. N.A. Logan, H.M. Lappin-Scott and P.C.F. Oyston. The Society for General Microbiology, Reading, UK.

Cleland, E.E., M.D. Smith, S.J. Andelman, C. Bowles, K.M. Carney, **M.C. Horner-Devine**, J.M. Drake, S.M. Emery, J. Gramling, and D.B. Vandermast. 2004. Invasion in space and time: non-native species richness and relative abundance respond to interannual variability in productivity and diversity. *Ecology Letters*. 7: 947-957.

**Horner-Devine, M.C**., M.A. Leibold, V. Smith and B.J.M. Bohannan. 2003. Bacterial diversity patterns along a gradient of primary productivity. *Ecology Letters*. 6:613-622.

**Horner-Devine, M.C.**, G.C. Daily, P.R. Ehrlich and C.L. Boggs. 2003. Countryside biogeography of tropical butterflies. *Conservation Biology*. 17:168-177.

**Synergistic Activities**

Editorial Board. Environmental Microbiology (2006-2007), The ISME Journal: Multidisciplinary Journal of Microbial Ecology (2006-2007), Microbial Ecology (2006).

Proposal reviewer. California SeaGrant, Netherlands Organization for Scientific Research, National Science Foundation.

NSF panel member. Biological Oceanography.

Invited panel speaker Association of Women in Science panel discussion, Women in Academia: A Career Panel. Seattle, WA. Winter 2007. Evolution and the Intelligent Design discussion: Understanding Evolution – Science and Philosophy. University of Washington, Seattle, WA. Fall 2006.

Instructor. GEAR UP program to introduce middle and high school students to the possibility of going to college. University of Washington. 2005-2007.

Mentor-Protégée Chair and Board Member. Assoc. for Women and Science. Seattle, WA. 2005.

**Collaborators**

Dr. Karen Carney, USAID; Dr. Noah Fierer, University of Colorado, Boulder; Dr. Samantha Forde, Univ. of California, Santa Cruz; Dr. Jed Fuhrman, Univ. of Southern California; Dr. Ian Hewson, Univ. of California Santa Cruz; Dr. Jennifer H. Martiny, Univ. of California, Irvine; Dr. Gabrielle Rocap, Univ. of Washington; Dr. Joyce Yen, University of Washington.

**Graduate and Postdoctoral Advisors**

Graduate Advisor: Dr. Brendan Bohannan, Department of Biological Sciences, Stanford University.
Postdoctoral Advisor: Dr. Brendan Bohannan, Department of Biological Sciences, Stanford University.

**Thesis Advisor**

Primary advisor: Graduate students (1):
Jessica Silver – University of Washington

Graduate committee (5):
Susan Grouse, Kate Hubbard, Dave Oleyar, Mary Ramirez, Michele Wrabel – University of Washington

Primary Advisor undergraduate thesis (1):
Alison Paulson - University of Washington

**Current and Pending Support** - Roberts

Title: *Development of genetic markers to assess disease resistance in the eastern oyster*
Source of Support: USDA (if subaward) via: NRAC/University of Maryland
Total Award Amount: $54,066
Total Award Period: 9/1/2006-1/30/2008
Time Committed: 0.10 months

Title: *Production of myostatin gene knockouts in zebrafish and the effects of specific myostatin interacting proteins*
Source of Support: USDA (if subaward) via: University of Wisconsin
Total Award Amount: $195,862
Total Award Period: 1/1/2005 - 11/30/2008
Time Committed: 1.0 months

Title: *Assessing withering syndrome resistance in California Black Abalone: Implications for conservation and restoration*
Source of Support: California Sea Grant
Total Award Amount: $15,067
Total Award Period: 6/1/2007 - 5/30/2008
Time Committed: 1.0 months

Title: *Evaluation of putatively QPX-resistant strains of northern hard clams using field and genetic studies*
Source of Support: USDA/NRAC (if subaward) via: MBL
Total Award Amount: $263,490
Total Award Period: 03/01/2008 – 12/31/2010
Time Committed: 2.0 months

Title: *The spread of lobster shell disease – genetic and social barriers*
Source of Support: NOAA/RI Sea Grant (if subaward) via: University of Rhode Island/Sea Grant
Total Award Amount: $150,991
Total Award Period: 03/01/2007 – 12/31/2008
Time Committed: 1.0 months

# Current and Pending Support

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

| Investigator: Roxanna Smolowitz | Other agencies to which this proposal has been/will be submitted. |
|---|---|

Support: ☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Effect of Temperature on the Infection of Hard Clams (Mercenaria mercenaria)

Source of Support: USDA via University of Maryland
Total Award Amount: $104,353 Total Award Period Covered: 09/01/2006 - 03/14/2008
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 2.36 Acad: Sumr:

---

Support: ☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Cross-breeding and Field Trials of Disease-resistant Eastern Oysters, Crassostrea virginica

Source of Support: USDA via University of Maine
Total Award Amount: $138,519 Total Award Period Covered: 06/15/2007 - 06/15/2008
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 1.0 Acad: Sumr:

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Support: ☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Microbiology of Shell Disease: Environmental Sources and Diversity

Source of Support: NOAA, Lobster Research Initiative via University of Rhode Island
Total Award Amount: $85,380 Total Award Period Covered: 03/01/2007 - 02/29/2008
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 1.35 Acad: Sumr:

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Support: ☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: EID Collaborative Research - Linking Marine Pathogens to Molluscan Shellfish - The Ecological Role of Marine Aggregates

Source of Support: NSF, EID - NSF 03-507
Total Award Amount: $200,363 Total Award Period Covered: 09/01/2004 - 08/31/2008
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 1.5 Acad: Sumr:

---

Support: ☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Development and application of molecular methods for detection of QPX organisms in environmental reservoirs

Source of Support: NOAA, WHOI Sea Grant
Total Award Amount: $18,121 Total Award Period Covered: 03/01/2004 - 02/28/2007
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 0.75 Acad: Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

USE ADDITIONAL SHEETS AS NECESSARY

# Current and Pending Support

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

| Investigator: Roxanna Smolowitz | Other agencies to which this proposal has been/will be submitted. |
|---|---|

Support: ☐ Current ☑ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Environmental investigation and assessment of the hard clam parasite, QPX

Source of Support: NOAA (via WHOI Sea Grant)
Total Award Amount: $69,695 Total Award Period Covered: 02/01/2008-01/31/2010
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 0.09 Acad: Sumr:

---

Support: ☐ Current ☑ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Evaluation of Putatively QPX-resistant Strains of Northern Hard Clams Using Field and Genetic Studies

Source of Support: USDA (via NRAC/University of Maryland)
Total Award Amount: Total Award Period Covered: 06/01/2008-05/31/2011
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: 1.0 Acad: Sumr:

---

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project Cal: Acad: Sumr:

---

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: Acad: Sumr:

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Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project: Marine Biological Laboratory
Person-Months Per Year Committed to the Project Cal: Acad: Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

USE ADDITIONAL SHEETS AS NECESSARY

**Current and Pending Support** – Horner-Devine

| | |
|---|---|
| Project/Proposal Title: | *Collaborative Research: ADVANCE Leadership Award for Women Evolving Biological Science –WEBS Symposia for early career female scientists* |
| Source of Support: | National Science Foundation, ADVANCE |
| Total Award Amount: | $273,180 |
| Total Award Period Covered: | 11/1/2006 – 10/31/2009 |
| Location of Project: | University of Washington |
| Person-Months Per Year Committed to the Project: | Cal.: Acad.: Sumr: 1 |

| | |
|---|---|
| Project/Proposal Title: | *The impact of invasive plants on sediment microbial communities and processes* |
| Source of Support: | Royalty Research Fund, University of Washington |
| Total Award Amount: | $31,550 |
| Total Award Period Covered: | 3/1/2007 – 2/29/2008 |
| Location of Project: | University of Washington |
| Person-Months Per Year Committed to the Project: | Cal.: Acad.: 1 Sumr: |

| | |
|---|---|
| Project/Proposal Title: | *Key drivers of hypoxia: bacterial production and respiration in Hood Canal* |
| Source of Support: | Hood Canal Dissolved Oxygen Project |
| Total Award Amount: | $54,731 |
| Total Award Period Covered: | 3/1/2007 – 2/29/2008 |
| Location of Project: | University of Washington |
| Person-Months Per Year Committed to the Project: | Cal.: Acad.: .5 Sumr: |