OMB Number: 4040-0004
Expiration Date: 04/31/2012

Application for Federal Assistance SF-424

Version 02

*1. Type of Submission	*2. Type of Application	*If Revision, select appropriate letter(s):
☐ Preapplication	☑ New	280932
☑ Application	☐ Continuation	* Other (Specify) 280932
☐ Changed/Corrected Application	☐ Revision	

*3. Date Received: 4. Application Identifier:

5a. Federal Entity Identifier:	*5b. Federal Award Identifier:

State Use Only:

6. Date Received by State:	7. State Application Identifier:

8. APPLICANT INFORMATION:

* a. Legal Name: University of Washington

* b. Employer/Taxpayer Identification Number (EIN/TIN):	*c. Organizational DUNS:
91-6001537	605799469

d. Address:

*Street1: 4333 Brooklyn Ave NE
Street 2:
*City: Seattle
County: King
*State: WA
Province:
Country: US *Zip/ Postal Code: 98195-9472

e. Organizational Unit:

Department Name:	Division Name:
School of Aquatic and Fishery Sciences	

f. Name and contact information of person to be contacted on matters involving this application:

Prefix: First Name: Steven
Middle Name:
*Last Name: Roberts
Suffix:

Title: Principal Investigator

Organizational Affiliation:

*Telephone Number: 206-600-4495 Fax Number: 206-600-4495

*Email: sr320@uw.edu

OMB Number: 4040-0004
Expiration Date: 04/31/2012

Application for Federal Assistance SF-424 Version 02

9. Type of Applicant 1: Select Applicant Type: H. Public/State Controlled Institution of Higher Education

Type of Applicant 2: Select Applicant Type: - Select One -

Type of Applicant 3: Select Applicant Type: - Select One -

*Other (specify):

*10. Name of Federal Agency: U.S. Environmental Protection Agency, Region 10

11. Catalog of Federal Domestic Assistance Number:
66.123

CFDA Title:

*12. Funding Opportunity Number: EPA-R10-PS-1004

*Title: Puget Sound Scientific Studies and Technical Investigations Assistance Program

13. Competition Identification Number:

Title:

14. Areas Affected by Project (Cities, Counties, States, etc.):
Puget Sound Watershed

*15. Descriptive Title of Applicant's Project:
Integrated Approach to Assess Emerging Threats to Puget Sound Ecosystem Health

Attach supporting documents as specified in agency instructions.

OMB Number: 4040-0004
Expiration Date: 04/31/2012

Application for Federal Assistance SF-424

Version 02

16. Congressional Districts Of:

*a. Applicant WA-007 *b. Program/Project: WA-007

Attach an additional list of Program/Project Congressional Districts if needed.

17. Proposed Project:

*a. Start Date: 7/1/2010 *b. End Date: 6/30/2012

18. Estimated Funding ($):

*a. Federal	$614,828.00	*d. Local	
*b. Applicant		*e. Other	
*c. State		*f. Program Income	
*d. Local		*g. TOTAL	$614,828.00

***19. Is Application Subject to Review By State Under Executive Order 12372 Process?**

☐ a. This application was made available to the State under the Executive Order 12372 Process for review on
☐ b. Program is subject to E.O. 12372 but has not been selected by the State for review.
☑ c. Program is not covered by E.O. 12372

*20. Is the Applicant Delinquent On Any Federal Debt? (If "Yes", provide explanation.)
☐ Yes ☑ No

21. *By signing this application, I certify (1) to the statements contained in the list of certifications** and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances** and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 218, Section 1001)

☒ **I AGREE

** The list of certifications and assurances, or an internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

Authorized Representative:

Prefix: *First Name: Lynne

Middle Name:

*Last Name: Chronister

Suffix:

*Title: Asst Vice Provost for Research & Director of Sponsored Programs

Grant & Contract Administration
Attn: Program Income Check

*Telephone Number: 206-543-4043 Fax Number: 206-685-1732

*Email: osp@u.washington.edu

*Signature of Authorized Representative: Date Signed: 03-04-10

OMB Number: 4040-0004
Expiration Date: 04/31/2012

Application for Federal Assistance SF-424

Version 02

*Applicant Federal Debt Delinquency Explanation

The following field should contain an explanation if the Applicant organization is delinquent on any Federal Debt. Maximum number of characters that can be entered is 4,000. Try and avoid extra spaces and carriage returns to maximize the availability of space.

OMB Approval No. 0348-0044

BUDGET INFORMATION - Non-Construction Programs

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Puget Sound S	66.123	$	$	$ 614828	$	$
2.						
3.						
4.						
5. Totals		$	$	$ 614828	$	$ 614828

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY				Total (5)
	(1)	(2)	(3)	(4)	
a. Personnel			139386		139386
b. Fringe Benefits			34254		34254
c. Travel			2700		2700
d. Equipment					
e. Supplies			68000		68000
f. Contractual			200227		200227
g. Construction					
h. Other			19151		19151
i. Total Direct Charges (sum of 6a-6h)	0	0	463718	0	463718
j. Indirect Charges			151110		151110
k. TOTALS (sum of 6i and 6j)	$ 0	$ 0	$ 614828	$ 0	$ 614828
7. Program Income	$	$	$	$	$

SECTION C - NON-FEDERAL RESOURCES				
(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8.				$
9.				$
10.				$
11.				$
12. Total (SUM OF LINES 8-11)				$

SECTION D - FORECASTED CASH NEEDS					
13. Federal	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
	$333897	$83475	$83474	$83474	$83474
14. Non-Federal					
15. TOTAL (sum of lines 13 and 14)	$333897	$83475	$83474	$83474	$83474

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT				
(a) Grant Program	FUTURE FUNDING PERIODS (years)			
	(b) First	(c) Second	(d) Third	(e) Fourth
16. Puget Sound Scientific Studies and Technical Investigati	$280932	$	$	$
17.				
18.				
19.				
20. TOTAL (sum of lines 16-19)	$280932	$	$	$

SECTION F - OTHER BUDGET INFORMATION	
21. Direct Charges: 463718	22. Indirect Charges: 151110
23. Remarks:	

Authorized for Local Reproduction

EPA United States Environmental Protection Agency

EPA Project Control Number

CERTIFICATION REGARDING LOBBYING

CERTIFICATION FOR CONTRACTS, GRANTS, LOANS AND COOPERATIVE AGREEMENTS

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all sub-awards at all tiers (including sub-contracts, sub-grants, and contracts under grants, loans, and cooperative agreements) and that all sub-recipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31 U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

Lynne Chronister
Executive Director
Office of Sponsored Programs

Typed Name & Title of Authorized Representative

Cristie Schmitt 03-04-10

Cristie Schmitt
Grant & Contract Administrator
Acting for Lynne Chronister

Signature and Date of Authorized Representative

Form Approved OMB No:2030-0020 Approval Expires 04/2012

KEY CONTACTS FORM

Authorized Representative: *Original awards and amendments will be sent to this individual for review and acceptance, unless otherwise indicated.*

Name: Lynne Chronister
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4333 Brooklyn Ave NE, Seattle, WA, 98195-9472
Phone Number: 206-543-4043

Payee: *Individual authorized to accept payments.*

Name: Tamy Sadusky
Title: Executive Director, Grant and Contract Accounting
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12455 Collections Dr, Chicago, IL, 60693
Phone Number: 206-543-8454

Administrative Contact: *Individual from Sponsored Program Office to contact concerning administrative matters (i.e., indirect cost rate computation, rebudgeting requests etc.)*

Name: Christin Schmitt
Title: Administrator
Mailing Address: 4333 Brooklyn Ave NE
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FAX Number:
E-Mail Address: schmittc@u.washington.edu

Principal Investigator: *Individual responsible for the technical completion of the proposed work.*

Name: Steven Roberts
Title: Associate Professor
Mailing Address: 1122 NE Boat St., Rm. 116
Seattle, WA, 98195
Phone Number: 206-600-4495
FAX Number: 206-600-4495
E-Mail Address: sr320@uw.edu
Web URL: http://www.fish.washington.edu/people/roberts/

EPA Grant Application: Scientific Studies and Technical Investigation Assistance Program

PROJECT TITLE - Integrated Approach to Assessing Emerging Threats to Puget Sound Ecosystem Health

APPLICANT INFORMATION - Steven Roberts
University of Washington, Box 355020, Seattle WA, 98105, 206-600-4495, sr320@uw.edu

DUNS NUMBER – 605799469 **TOTAL FEDERAL FUNDS REQUESTED** - $614,828

- Informed of RFA through EPA's Website
- Not a subsidiary of the Association of Community Organizations for Reform Now

Abstract This effort will provide valuable information to assess emerging contaminants while simultaneously broadening the scope of environmental assessment tools using a non-biased genomic approach with key species in Puget Sound. The specific aims of this proposal are to: 1) Implement an integrated approach to characterize the occurrence of endocrine disruptive contaminants and 2) Measure indicative physiological responses of aquatic organisms to conditions in Puget Sound. This interconnected effort will generate critical metrics for monitoring and assessing the health of the Puget Sound ecosystem. To carry out Aim 1 a select suite of contaminants in sediment, water column, and benthic invertebrate tissue will be measured at several locations. To complement these activities, the physiological response of benthic invertebrates, anadromous fish, and marine mammals will be measured using innovative genomic approaches (Aim 2). This project directly supports the Puget Sound Action Agenda's primary goal of achieving fresh and marine waters and sediments of sufficient quality that are safe for drinking, swimming, shellfish harvest and consumption.

Summary for the Proposed Investigation - Urbanization, coastal population growth, agricultural and industrial practices, and climate change have significantly altered water quality and aquatic ecosystems within the Puget Sound Region. Indeed, the Puget Sound Partnership's Action Agenda (PSP 2008) acknowledges that ongoing pollution input is one of the top two immediate threats to the Puget Sound ecosystem, and has called for enhanced monitoring activities to establish baselines and trends. Many chemicals contaminating aquatic ecosystems, including Puget Sound (King County 2007, Johnson et al 2008), are capable of disrupting endocrine function, potentially leading to impaired development and reproduction of both humans and wildlife (Colborn et al. 1993, Tyler et al. 1998, Damstra et al. 2002). Chemicals with reported endocrine disrupting activity include pharmaceuticals (e.g. anti-seizure, antibiotics, oral contraceptives, anti-depressants, and blood-lipid regulators), pesticides, surfactants, personal care products (PCPs), plastics (e.g. pthalates, bisphenol-A), persistent organics (e.g. polychlorinated biphenyls [PCBs] polybrominated diphenlyethers [PBDEs]), plant compounds (e.g. phytoestrogens), and anti-fouling agents (tributyltin) (McLaughlan 2001). Some of these chemicals are persistent and bioaccumulate in tissues (e.g. PCBs, PBDEs), while others are not persistent, but are continually discharged, and referred to as pseudopersistent (e.g. pharmaceuticals in sewage effluent). In addition to chemical contamination, environmental conditions (e.g., moderate hypoxia) induced by human activities can cause endocrine disruption in fish leading to reductions in fertility (Wu 2002, Wu et al. 2003, Raloff 2004, Thomas & Rahman 2009) or sex reversal (Shang et al. 2006).

Endocrine disruption associated with contaminants and environmental conditions is of particular regional concern given recent evidence of exposure to estrogenic chemicals and reproductive impairment in a variety of fish species in Puget Sound and the Columbia River Basin (Lomax et al. 2003, Feist et al. 2005, Johnson et al. 1998, 2006, 2008) as well as the unknown sublethal effects of

hypoxic conditions in Hood Canal to local marine species (Newton et al. 2002). Furthermore, Pacific salmon and orcas in Puget Sound contain very high levels of PCBs and PBDEs, yet little is known about potential impacts to their health and reproductive success (Collier et al. 1998, O' Neil et al. 1998, West et al. 2001, Puget Sound Water Quality Action Team 2002, Missldine et al. 2005, Stone et al. 2006, Sloan et al. 2010). Levels of a variety of PBDEs, which have known toxic and thyroid disruptive effects in both mammals (Darnerud 2003, Tomy et al. 2004, de Wit 2002) and finfish (Lema et al. 2007, 2008; Arkoosh et al. in press), are increasing in marine organisms in the Pacific Northwest Region (Ikonomou et al. 2006). *Given the potential for adverse effects, an accurate assessment of the exposure and effects of these endocrine disrupting contaminants is needed. Knowledge of exposure and impacts of these compounds to native Puget Sound species are necessary to assess the current status of ecosystem health and any changes associated with environmental rehabilitation and stock recovery efforts*.

Approach - An integrated approach will be used to characterize the presence of emerging contaminant threats to the Puget Sound ecosystem while monitoring biological effects of these contaminants using a genomic approach across multiple trophic levels. This effort will provide valuable metrics for accurately detecting and assessing the effects of endocrine disruptors while simultaneously broadening the scope of environmental assessment methods by using a non-biased multi-species genomic approach. Results from this study will also provide valuable data to expand the utility of existing contaminant food web models of Puget Sound. The Washington Department of Ecology (Ecology) recently developed numerical models to predict the loading and concentrations of toxic contaminants in water, sediment, and biota of Puget Sound (Pelletier & Mohamedali 2009). Several key recommendations of this report (in bold below) are directly addressed by the proposed project: 1) "[the need for] additional measurements of toxic contaminants at various locations within Puget Sound. **The scarcity of water column data using methods that are capable of detecting the low concentrations that exist is a major data gap**" 2) "Data gaps exist concerning the concentrations of toxics in various species of biota of the Puget Sound region, including several trophic layers within the food web…. **Paired measurement of concentrations in biota and sediment would also be useful**" 3) "Modeling of other endpoints in nearshore biota, such as reduced fecundity and reduced age to sexual maturity, **or other endpoints specific to endocrine disruptors, is also needed**" (p. XIV in Pelletier & Mohamedali 2009).

This project aims first to quantify levels of endocrine disrupting chemicals (EDC) of concern in paired measurements of marine sediment, water and shellfish in various locations in Puget Sound. Contaminant surface water concentrations will be measured using passive sampling devices (PSD) to overcome detection limitations imposed by low environmental concentrations. The second aim of this project is to evaluate potential impacts of these chemicals at three trophic levels in shellfish (oysters), finfish (salmon) and marine mammals (orcas) using both targeted and global gene expression methods. The advantage of large-scale gene expression methods over individually monitoring specific biomarkers of exposure (e.g. EROD activity for polycyclic aromatic hydrocarbons [PAH] exposure, vitellogenin induction for xenoestrogen exposure) is that the degree of exposure to multiple chemical classes and toxic impacts can be monitored simultaneously. Global methods for quantifying messenger RNAs (transcriptomics) can be used to characterize a vast array of biochemical pathways within a cell, providing a comprehensive assessment of the functional state of that cell/tissue. Using the more cost effective next-generation sequencing technology, the entire transcriptome of a cell in any organism can be characterized as described below in Aim 2. Thus, analysis of the transcriptome is not limited to only those organisms for which DNA microarrays have been developed or to the limited set of genes on the arrays.

Justification of bioindicator species- **Shellfish** have been used effectively as bioindicators of coastal pollution (e.g. Scanes et al. 1996, Blaise et al. 2002). NOAA's Mussel Watch Program, one of the longest running national coastal monitoring programs, has used bivalves to monitor contaminant status and trends in coastal water for over 20 years. The attributes that make bivalves exceptional environmental monitors are 1) they are common; 2) widely distributed; 3) immobile with high site fidelity; 4) reasonably resistant to contaminants; and 5) continuously filter the water in which they live. In addition, they have important economic and socio-cultural roles in the Pacific Northwest. Two key goals of the Puget Sound Action Agenda (PSAA) are maintenance of 1) adequate marine resources to sustain cultural, spiritual and subsistence needs and economic endeavors of Puget Sound tribal communities and 2) an ecosystem that supports thriving natural resource and marine industry uses such as aquaculture and tourism. Pacific oysters were selected for this study because they are present in high numbers as a result of significant aquaculture efforts as well as robust natural sets. Thus, harvesting samples for research will not have a significant impact on the population and industry participants can provide genetically similar oysters to minimize genetic influences on organismal responses.

Salmon, a keystone species, will also be used as a bioindicator of environmental stressors and ecosystem health. Pacific salmon are iconic in the Pacific Northwest and a critical ecological, economic, and social component of the Puget Sound ecosystem. Coho salmon will be used because 1) many of the molecular tools that can be exploited as bioindicators of sensory and reproductive health in salmon have been developed in this species, and 3) coho salmon display a simple and ubiquitous life history pattern (one year old smolts, 2-3 year old spawners) that minimizes confounding gene expression complexities associated with plasticity in smolting or reproductive timing. Coho salmon are also an excellent surrogate for other Puget Sound salmon species (e.g. Chinook) because many of the physiological systems (e.g. olfaction, smolting, imprinting, maturation) and target bioindicator genes are shared by most salmon species in Puget Sound and, as a non-threatened species, they are present and available for sampling from most Puget Sound watersheds.
Furthermore, a key goal of the PSAA is to implement the Puget Sound Salmon Recovery Plan (2007) calling for enhanced "monitoring, modeling, assessment and applied research capacities" and "baseline studies and ecosystem monitoring activities to demonstrate measurable environmental changes and/or trends in condition." Development and implementation of bioindicator monitoring for Puget Sound salmon as outlined in this proposal will help facilitate these goals. Initially, lab studies exposing coho salmon to PBDE mixtures found in Puget Sound fresh and marine waters (Sloan et al. 2010; State of the Sound 2009) will be used to identify endocrine physiology changes and target tissue gene expression in these critical physiological systems.

Orca whales occupy the highest trophic level in the Puget Sound ecosystem and have achieved an equally high position in public attention. Characterizing the response of this species will complement work with oysters and salmon and allow for a better understanding of contaminant effects across the ecosystem with a comparative perspective. While there has been significant research on tissue contaminants in orcas (e.g. Hickie et al. 2007, Rayne et al. 2004, Ross et al. 2000) there are limited transcriptomic studies on this species. A primary goal for this aspect of the project is to develop those resources using next-generation sequencing technology. Analysis of gene expression patterns in resident orcas in relation to tissue contaminant loads (data concurrently collected by NOAA/NWFSC biopsy sampling program) will provide important insight by revealing the physiological pathways that are implicated in contaminant exposure.

Project Components: This project will characterize the presence of emerging threats associated with endocrine disruptors to the Puget Sound ecosystem by monitoring biological effects (as measured by gene expression) across multiple trophic levels. Though often recognized as a concern, the extent of contamination by endocrine disrupting compounds in Puget Sound is largely unknown. This effort will

provide valuable metrics that allow accurate assessment of select compounds, in addition to expanding the scope of environmental assessment tools by using a non-biased genomic approach on model organisms paired with environmental measurements of levels of chemical contamination.
The specific aims of this proposal are to:

1) Implement an integrated approach to characterize the occurrence of endocrine disruptive contaminants
2) Measure indicative physiological responses of aquatic organisms to conditions in Puget Sound

To carryout Aim 1, a combination of sediment and surface water (using PSDs) sampling and analysis of oyster tissue will be used to assess the environmental and biological occurrence of EDCs identified at nine sites in Puget Sound. To complement these activities, the physiological response of oysters, anadromous fish, and marine mammals will also be measured (Aim 2). In addition to providing tools to guide conservation efforts, the results will provide data to determine the biomagnification potential of the monitored contaminants. The results will also be valuable for enhancement of existing and future development of contaminant food web models due to the use of sentinel organisms representing multiple trophic levels with diverse life histories. The remainder of this section describes details of the ‘asks and activities associated with the two project aims.

Aim 1: Integrated approach for characterizing presence of emerging contaminants
The key tasks associated with Aim 1 are collection and analysis of sediment samples along with the simultaneous deployment of PSDs and caged oysters at nine locations. The sediment, PSD extracts and oyster tissue will be analyzed for the contaminants listed in Table 1. Contaminant selection was based on the following 5 principles. 1) The established toxicity of PBDEs (reviewed in de Wit 2002, Darnerud 2006, Muirhead et al. 2007, Hallgren and Darnerud 2002, Lema et al. 2008) and the recent detection of elevated PBDEs levels in downstream migrating salmon in Puget Sound and the Columbia River (Sloan et al. 2010). 2) The discovery of the natural formation of PBDE derivatives occurs in marine invertebrates (Vetter 2006) and the high levels of these compounds have been found in marine mammals, including whales (Vetter et al. 2002, Pettersson et al 2004). Mounting evidence indicates that PBDE hydroxylated metabolites are the most potent (relative to other PBDEs) at disturbing thyroid hormone transport and are hypothesized to be potent agents of neurological effects (Legler and Brouwer 2003, Ucann-Marin et al. 2009). 3) Exposure to estrogen or xenoestrogens at levels below one part-per-trillion is a well documented to cause of endocrine disruption (Brown et al. 2007) and recent evidence suggests the widespread presence of estrogenic material in Puget Sound (Johnson et al. 2008). 4) Of the many pharmaceuticals released into the environment, anti-depressants such as selective-serotonin-reuptake inhibitors (SSRIs) are of high concern due to their environmental persistence and effects on aquatic animals (Lister et al. 2009, Kreke and Dietrich 2008, Brooks et al. 2005). Schultz et al. (2009) (Project Co-PI) recently demonstrated the occurrence of two SSRIs, Fluoextine and Sertraline in the White River near Enumclaw, WA 5) Other contaminants (triclosan, bisphenol-A [BPA], nonylphenol [NP]) are known to impact the thyroid (Crofton et al. 2007) and/ or are estrogenic (Kwak et al. 2001). In addition, several UV blockers such as benzophenone and analogues, are environmentally persistent and have estrogenic properties (Kunz and Fent 2006; Fent et al. 2008). Other pharmaceuticals such as carbamazepine have recently been found to bioaccumulate in fish (Ramirez et al. 2009). For many of these contaminants, the experimental approach will allow an initial assessment of their potential for bioaccumulation in Puget Sound through comparisons of oyster and sediment / water concentrations.

Table 1. Contaminants to be monitored as part of Aim 1

PBDEs	Pharmaceuticals		Misc / Personal Care Products
PBDE Congeners:	**Steroids**	**SSRI / SNRI**	
17,28,33,47,49,66,75,85,99,100,	Estrogen	Fluoxetine	Triclosan[1]
138, 153, 154, 183, 190, 209	Estrone	Norfluoxetine	Bisphenol-A[2]
Hydroxylated PBDEs:	Estriol	Sertraline	Nonnylphenol[2]
4' OH-BDE-17, 3 OH-BDE-47	Ethynylestradiol	Paroxetine	Homosalate[4]
6 OH-BDE-47, 5 OH-BDE-47	Testosterone	Venlafaxine[3]	4-methylbenzy-lidenecamphor[4]
6 OH-BDE-100	Trenbolone[5]	Citalopram	Benzophenone[4]
Methoxylated PBDEs :	**Anti-Seizure**	Mirtazapine	Octocrylene[4]
3 MeO-BDE-47,2' MeO-BDE-66	Carbamazepine	Viloxazine	Butylmethoxydibenzoylmethane[4]
5' MeO-BDE-47, 4' MeO-BDE-49			Ethylhexylmethoxy-cinnamate[4]

[1]Anti-microbial; [2]Alylphenol; [3]SNRI (selective norepinephrine reuptake inhibitor); [4]UV blockers used in sunscreens; [5]Anabolic steroid .

*Aim 1, Task 1: Deployment of passive samplers***.** Exclusive reliance on "grab" water samples for contaminant monitoring of wastewater or stormwater discharges is problematic due to low environmental levels of some compounds and unpredictable or episodic release (Zabiegala et al. 2010). The use of PSDs that effectively sequester contaminants such as pharmaceuticals, PCBs and persistent organic pollutants such PBDEs improve monitoring efforts due to their ability to concentrate contaminants and improve detection. PSDs are also advantageous because of their structural simplicity, lack of heterogeneity, and ease of adaptation as a screening tool. When properly deployed and analyzed, differences in contaminant loading on PSDs can be more readily correlated to temporal or spatial differences at various sites, thus facilitating assessment of seasonal and regional differences in contaminant loading (Lohmann and Muir 2010). In this task, PSDs will be used to determine the time-weighted-average (TWA) concentration of each measured contaminant. Samplers will be deployed for up to 28 days, during three distinct time periods representing dry conditions (late summer), heavy rainfall (Fall early winter) and spring runoff conditions.

Two types of PSDs will be used; the polar organic integrative sampler (POCIS) and semipermeable membrane devices (SPMDs). The POCIS uses a solid phase matrix contained within a semi-permeable membrane and is better suited to sequester more hydrophilic contaminants such as many pharmaceuticals (Alvarez et al. 2004). The SPMDs are made from low density polyethylene tubing that contains triolein as the receiving phase. SPMDs are now well established for their ability to sequester more lipophilic contaminants such as PBDEs (Harman et al. 2008). POCIS samplers, recently used in Puget Sound streams, were found to effectively sequester estrogens such as estradiol (E2) and estrone (E1) along with pharmaceuticals such as SSRIs from natural waters (Schultz et al. 2009). Because contaminant release is typically episodic, it is unlikely a true equilibrium is reached and a kinetic approach to passive sampling needs to be employed. The mathematical basis for relating the total amount of adsorbed contaminant trapped in the sampler to the TWA of the water concentration has been previously described (Huckins et al. 1999, Luellen and Shea 2002; Vrana et al. 2002).

Aim 1, Task 2: Sediment sampling. Surface sediment samples will be collected at all sites adjacent to where the caged oysters / PSDs are deployed using a van Veen grab sampler. Sampling methods will be consistent with the Puget Sound Estuary Program (PSEP) (PSEP1997), and EPA (EPA 2001) sampling protocols.

Aim 1, Task 3: Deployment of caged oysters. Pacific oysters (*Crassostrea gigas*) will be placed in steel cages and deployed at 9 sites (See MAP). The cages (24"L x24"W x 3"H) will be placed 12" off the bottom, anchored to concrete blocks. The PSDs (Task 1) will be attached to the outside of the cages. Oysters with the same genetic background (siblings) will be placed at each site to better delineate genetic vs environmental factors. Water quality parameters (temperature, DO, salinity, and pH) will be measured during each deployment. The cages and the PSDs (Task 1) will be retrieved after 28 days and whole oyster soft tissue collected. Samples to be used for transcriptomics (details provided in next section) will be immediately preserved in RNAlater (Ambion). Samples to be used for contaminant exposure will be frozen at -80^{o}C for later analysis.

Site Selection. Inputs of pollution into the Puget Sound come from a variety of point and non-point sources. The nine sites selected for this study represent areas impacted by a variety of potential contamination sources including urban and agricultural stormwater runoff, wastewater discharges, and industrial contamination (see MAP). A number of the sites selected have been previously characterized as having high amounts of contaminant input (Elliot Bay sites, Hylebos Waterway and Sinclair Inlet sites) (Berbells 2006, Hart Crowser 2007) and others have not been previously characterized but are likely to be affected due to proximal pollution input sources (e.g. agricultural runoff and septic system failures at Drayton Harbor and wastewater discharge at Solo Point). Given concern in Elliot Bay, three sites have been selected and include; Myrtle Edwards Park, Bell Harbor, and Duwamish Waterway. A relatively low impact site with minimal impact is also being monitored for reference (Big Beef Creek). One of the challenges in characterizing inputs and associated effects is that aquatic systems are affected by multiple sources of contaminanats and understanding of synergistic effects are poorly understood, therefore, one of the benefits of the bioindicator approaches outlined here is that they will allow for intepretation of synergistic effects of multiple stressors in Puget Sound biota.

Quantification of environmental contaminants in passive samplers, sediment and biological matrices. Sediment and homogenized oyster soft tissue will be extracted using an accelerated solvent extractor (Dionex ASE model 300). The SPMDs will be first dialyzed in hexane to recover bound contaminants. The ASE / SPMD extracts will next undergo gel permeation chromatography to remove lipids (or co-extracted triolein from the SPMDs). For the POCIS samplers, the receiving phase is extracted with methanol to recover bound contaminants. Subsequent chemical analysis will rely on GC-MS using previously established methods in co-PI Schultz's laboratory for steroid, SSRI and PBDE analysis (Schultz et al. 2001, Muirhead et al. 2006, Lema et al. 2008, Schultz et al. 2009). For other contaminants listed in Table 1, the GC-MS methods described by Sebok et al. 2009 will be used.

Aim 2: Physiological responses of aquatic organisms to conditions in Puget Sound

The second aim of this project is to quantifiy the physiological response of organisms at multiple trophic levels. Specifically, a transcriptomic approach will be used to provide a comprehensive analysis of the gene expression patterns in organisms from the wild as well as under controlled laboratory conditions. The use of gene expression patterns as biomarkers has proven useful in evaluating responses to various anthropogenic environmental contaminants such as; benzene, heavy metals, PCBs and EDCs (Forrest et al 2005, Venier et al 2006, Tabuchi et al 2006, Quiròs et al 2007). The coupling of genomic approaches with state of the art water quality assessment is a core component of the research and will allow us to *directly relate contaminant levels to the respective biological impact.* For instance, the molecular analysis will allow us to detect disturbances in fundamental biological processes such as reproduction, immune response and growth by comparing gene expresssion patterns from organisms from different environments. This information will allow us to

better predict effects realized at the population level. In addtion, the transcriptomic approach *will provide prime 'markers' for contaminant exposure and ecosytem health*. Regardless of its function, any gene's expression pattern that is significantly correlated with the presence of a contaminant quantified as part of Aim 1, could be used as a simple, indirect assessment of water quality when direct measurements are not feasible. Finally, one of the greatest advantages of examining the response of aquatic organisms to environmental conditions *is that this is one of the only ways to identify new environmental threats and/or unrealized impacts of contaminants in our waterways*. The remainder of this section will detail the specific tasks associated with Aim 2. These include transcriptomic analysis of 1) oysters from field sites, 2) salmon exposed to contaminants under controlled conditions, and 3) archival and current orca biopsy samples.

Aim 2, Task 1: Pacific oyster gene expression analysis. We will use gene expression analysis to evaluate biological responses and generate cost-effective markers of exposure to EDCs in Pacific oysters. This transcriptomic approach is well supported by the literature as previous studies have characterized differential gene expression in bivalves exposed to various stressors such as; heavy metals and hydrocarbons (Boutet et al., 2004; Manduzio et al, 2004), sewage (Medeiros et al., 2008), EDCs (Canesi et al, 2008), pathogen exposure (Roberts et al, 2008, Tirapé et al, 2007), and temperature (Tirapé et al, 2007, Farcy et al, 2008, Ivanina et al, 2008). In addition, a pilot study has been underway at two of the study sites (Big Beef Creek and Drayton Harbor) since April of 2009. Through this investigation, we have been able to identify a number of target genes that are differentially expressed in oysters at a minimally impacted site and a site characterized by urbanization, agricultural runoff and failed septic systems. Next-generation sequencing technology (ABI SOLiD System 3) is being used for this effort. Genes found to be upregulated include steroid 17 alpha-hydroxylase and a serine protease. Assays for these target genes have already been developed and are ready to use. In addition to the genes identified during the pilot study, we will also include genes shown to be regulated in oysters in response to EDCs including vitellogenin, cytochrome P450, and multi-drug resistance protein. For this study, caged, sibling oysters will be sampled at three time-points (described above Aim 1, Task 3) and gill tissue will be collected and stored until RNA can be isolated. Analysis of a minimum of seven genes will be carried out using quantitative RT-PCR as previously described (Roberts et al 2008).

Aim 2, Task 2: Salmon gene expression analysis We will examine salmon indicator genes involved in olfactory signaling, reproduction and the thyroid endocrine axis. These physiological systems are ideal as bioindicators because they are extremely sensitive to environmental cues and stressors, are critical for survival of the organism, are relatively well-characterized, and are susceptible to a number of known chemicals of concern (e.g. EDCs, pharmaceuticals, metals, pesticides) found in Puget Sound waters (Tierney et al. 2010, Hutchison et al. 2006, King County 2007, Schmutzler et al. 2007, Lubliner et al. 2010). The salmon olfactory system in particular provides an extremely sensitive model for detecting the effects (and therefore the presence) of environmental stressors, including EDCs. Salmon have an acute sense of smell that plays a critical role in almost every aspect of their lives (e.g. feeding, reproduction, migration, predator avoidance) (Dittman and Quinn 1996, Hasler and Scholz 1983, Dittman et al. 1996, Dittman et al. 1997; Nevitt and Dittman 2007, Dittman, May and Johnson unpublished). Earlier studies have indicated that salmon smolts exposed to xenoestrogens, at concentrations found in Puget Sound waters, demonstrated changes in a number of gonadal and pituitary genes that can be developed as indicators of juvenile exposure to these compounds (Swanson et al 2003, Swanson et al. unpublished).

Smolting coho salmon will be exposed to mixtures and concentrations of PBDEs previously detected in smolts collected from Puget Sound waters (Sloan et al. 2010). One-year old coho salmon reared at

the NWFSC will be tagged with passive integrated transponder (PIT) tags and transferred in January 2011 to a single freshwater tank at the Batelle Laboratories for acclimation. Beginning in February 2011, pre-treatment samples will be collected, fish will be weighed and measured to allow monitoring of individual growth, and then divided into duplicate tanks (100 fish/tank) for each treatment. Fish will be fed either control food or PBDE-treated food prepared with two concentrations of PBDE congeners: 1) a low dose designed to achieve levels of PBDE congeners found in Puget Sound smolts (Sloan et al 2010) or 2) a high dose (same congeners) at 5x concentration. Other PBDE congeners or hydroxylated and methoxylated may also be included based on results from chemical sampling outlined in Aim 1. Dr Schultz's lab has extensive experience in preparing PBDE-treated foods and oral delivery of these foods to achieve desired concentrations of PBDEs in experimental fishes. After the initial sampling, treatments will be initiated and 12 fish per tank will be sampled every three weeks until the conclusion of the experiment at 15 weeks. Beginning in week 13, fish will be gradually converted to saltwater and the final samples will be collected from saltwater-acclimated fish. For each sampling, fish will be euthanized using MS-222 according to Batelle IACUC procedures and measured for body mass and fork length. Blood will be collected for plasma hormone assays of T4 and T3 (Dickhoff et al. 1978), gill tissue will be collected for Na^+/K^+ ATPase assays (McCormick 1993) and the olfactory rosette, pituitary gland, gonad, liver and brain will be frozen in liquid nitrogen and stored at –80° C for later RNA analysis. Indicator genes for PBDE–exposure will be identified by conducting transcriptome analysis of control and PBDE-treated fish using next-generation sequencing of salmon olfactory libraries. Specifically, the ABI SOLiD System 3 will be used and sequencing performed at the University of Washington Genomics Core Facility. The PIs (Roberts and Swanson) have used this technology with success in both oysters and salmon. Ultimately, indicator genes identified in these studies will be used to examine expression patterns in naturally outmigranting smolts to monitor for physiological stressors (e.g. contaminants) in watersheds throughout Puget Sound.

Aim 2, Task 3: Orca transcriptomic analysis. Sampling marine mammals with skin and blubber biopsies is a well-established and effective method of monitoring the physiology of wild animals. Biopsies allow for sampling of a large number of animals over an extensive geographic range; acquiring sequential samples from the same individual; accurate and environmentally-representative measurement of PAHs, organochlorines (OCs) and heavy metals; and biomarker analysis (Fossi et al. 2003). Since different tissues demonstrate different gene expression profiles in response to stresses (Mancia et al. 2007), it is important to establish a consistent and informative tissue-sampling technique. Through a pre-established Puget Sound orca biopsy program accomplished jointly by the Northwest Fisheries Science Center of NOAA (NWFSC) and Cascadia Research Collective (Olympia, WA), we have access to current and archival blubber samples (Dr. Brad Hansen; NWFSC/NOAA). Blubber is an essential tissue for insulation and thus survival of marine mammal species and is the site of extensive bioaccumulation of contaminants (Tabuchi et al. 2006, Ross et al. 2004, Fossi et al. 2006). Thyroid receptor genes, the expression of which are inversely correlated with PCB load, are expressed in blubber (Tabuchi et al. 2006). Changes in thyroid function would also have significant effects on a variety of other physiological mechanisms, such as growth, reproduction, and immune function. For the current proposal we will examine genes in this pathway as well as taking a more global approach. Preliminary work in the lab of the PI has shown RNA can be successfully isolated from archival samples and stress related genes (e.g. HSPs) have been amplified. Just as with salmon, next-generation sequencing will be used, however in this case it will be utilized as a gene discovery effort. For samples collected during the project period, a single, pooled animal library will be constructed and sequenced using the ABI SOLiD System. In addition, select genes will be quantified using qRT-PCR to evaluate individual organism expression in both current and archival samples. These data will be correlated with contaminant levels as determined by separate efforts.

Ultimately, bioindicator genes identified from these keystone species' (oyster, salmon, and whale) will be interconnected to develop ecosystem-wide monitoring indicators that can be used to assess food web and general ecosystem health. These results will also provide key data for development and enhancement of existing food web based models of Puget Sound ecosystem dynamics. Finally, as compounds of concern are identified under specific Aim 1 (e.g. pharmaceuticals, phthalates), we will develop further indicators for biomonitoring of ecosystem health in subsequent years.

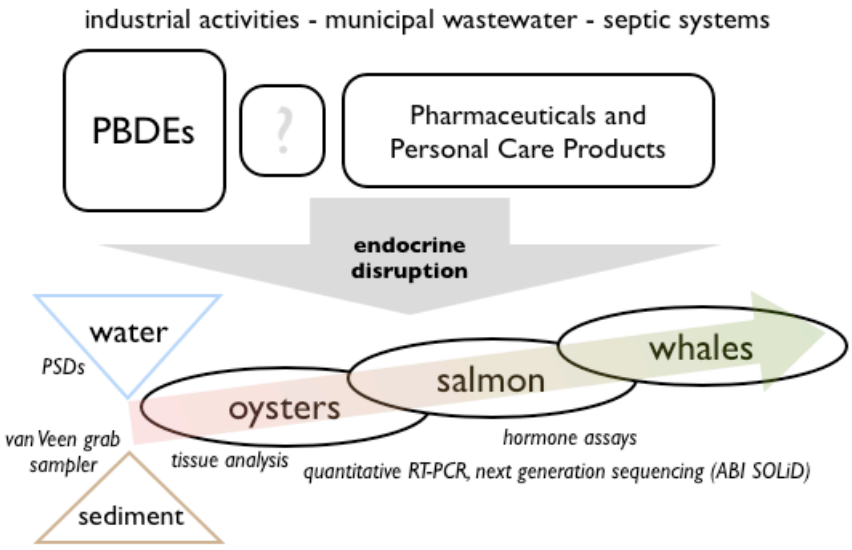

Figure 1. Ecosystem conceptual model showing emerging contaminants and aquatic organisms examined as part of this project. Methodologies used are shown in italics.

Environmental Significance: The Puget Sound Partnership's Action Agenda identified ongoing pollution input as one of the top immediate threats to the Puget Sound ecosystem (PSP 2008), and has called for enhanced monitoring and research activities to establish baselines, trends, and biological effects. Although some of the potential contaminants that contribute to these threats have been identified, there are many emerging chemicals of concern and environmental stressors that are only now being identified and assessed for impacts on the Puget Sound ecosystem. The PSP's Action Agenda and Science workplan calls for efforts to "develop a better understanding of the Puget Sound Ecosystem" and "building capacity for conducting strategic science for ecosystem recovery. " This study will utilize an integrated approach to simultaneously monitor both the presence of emerging chemicals of concern and biological responses to these chemicals and other anthropogenic stressors at multiple trophic levels. The products of this proposed work will allow for identification of specific contaminant threats and geographic areas of concern and provide tools for comprehensive monitoring of previously unidentified ecosystem stressors. Specifically, the proposed work will inform managers about the presence of endocrine disruptors and their biological effects and facilitate development of specific mitigation strategies that can be effectively monitored for recovery from baseline levels. Results from this study will also help fill data gaps (Pelletier & Mohamedali 2009) that confound development of accurate contaminant food web models of Puget Sound called for under the Action Agenda.

Anticipated Outputs and Outcomes: Outputs of the current research effort will be the quantification of emerging contaminants in Puget Sound. This includes both direct measurements as well as characterizing specific physiological responses of aquatic organisms. Accurate assessement of contaminants and their biological effects is essential to evaluate future efforts to clean up Puget Sound. One outcome of this project will be development of tools to monitor ecosystem health improvement. In addition, the biological aspects of the project provide a better understanding of how organisms are

impacted and the magnitude of effects from EDCs. These data will assist in predicting population and ecosystem interactions. Together this information will be extremely useful for understanding to what degree contaminants are problematic so that funding and effort can be focused on appropriate actions. For instance, when deciding on wastewater treatment options, ecosystem impacts can be discussed in the context of finacial costs of improvement. Furthermore, one of the most profound outcomes of this project will be the future development of environmental system models that incorporate fundamental biological information from organims at multiple trophic levels.

Monitoring and Measuring: The PI will be responsible for overall monitoring of project progress. More specifically, Dr. Schultz will be primarily overseeing water quality analysis, Dr. Dittman will be overseeing the characterization of salmon responses, while Dr. Roberts will be overseeing analysis on oyster and orca samples. One of the primary means by which we will be monitoring progress of the technical investigation will be the use of an *external review panel* of stakeholders (see collaboration section below). In addition, personnel will have monthly discussions to make sure the project is on track. Successful progress will also be assessed by our ability to disseminate information. This will be measured by publications, presentations, and traffic on the project website.

Innovation: This project takes an integrated approach to characterizing emerging threats to the environment in a manner that will provide essential tools for monitoring conservation efforts. The simultaneous measurement of contaminants in sediment-water-biota is a novel approach, although highly needed for Puget Sound. While limited research has been done on the presence of PBDEs, pharmaceuticals, and personal care products, there has yet to be a comprehensive analysis of the these threats along with direct biological responses. Biological responses to environmental stressors will be analyzed using the most recent molecular approaches involving whole trancstriptome analysis of affected tissues and high throughput next-generation sequencing. This innovative technology will provide a wealth of information that has significant importance in regards to monitoring but also population and ecosystem level effects.

Collaboration: This project is developed with expertise from federal government agencies (NOAA and DOE: Marine Sciences Laboratory), an educational institution (University of Washington - School of Aquatic and Fishery Sciences - College of Environment), and local government (King County Department of Natural Resources). In order to ensure success of our technical investigation we will develop a review panel of project stakeholders to provide feedback on project design and implementation and improve information transfer. This external panel will be composed of representatives of the shellfish industry, wastewater management, government, and academia.

Outreach and Information Transfer: The outreach and information transfer component of this project will be focused on three groups; 1) the scientific community (e.g. modelers, toxicologists, biologists), 2) regional government (e.g. PSP, resource managers, wastewater treatment agencies, Ecology, WRIA's) and (3) the interested public (shellfish growers, NGOs [e.g. People for Puget Sound]). All project updates and activities, data, and reports will be hosted on a website developed specifically for the project, similar to other ongoing projects of the PI (fish.washington.edu/genefish/robertslab/Research.html). The lab of the PI is also actively involved in open science practices with all personnel keeping open access electronic notebooks (genefish.wikispaces.com) that are publicly accessible. Through these web-based, open access methods of sharing data, the public can follow the efforts and results of this research in near-real time throughout the duration of the project. Data from all aspects of the project (water quality, pollution sources, gene expression) will be presented in a visual, geographical-based manner. This will include generation of Federal Geographic Data Committee (FGDC) compliant metadata which can be uploaded to various state and federal GIS data clearinghouses for other institutions, federal agencies,

public, and private entities to view and download. This research will also be integrated into courses at the University of Washington including; FISH310: Biology of Shellfishes, FISH441 Integrative Environmental Physiology, and FISH546 Bioinformatics. This will include a field trip in FISH310 where the students will be involved in sampling.

The scientific community will use these data in a variety of modeling applications. To facilitate this, we will make regular presentations to the Puget Sound Marine Environmental Modeling (PSMEM) group, which is a partnership of five academic, governmental and private non-profit organizations that seek to develop predictive modeling tools for Puget Sound. In addition to project reports to the EPA, the results will be published in peer reviewed journal articles.

A key element of the outreach and information task will be dissemination of project results to local government and regional resource mangers. The project outputs will be useful for stormwater and wastewater agencies as most of the chemicals to be monitored by this project are present in wastewater and stormwater but not routinely measured. Project results will provide an indicator of the level of exposure to aquatic species and provide managers with additional information on the presence and potential effects of these chemicals. This project will add to the limited regional data available regarding the presence and exposure potential and effects of EDCs to aquatic life and provide data to guide future monitoring efforts. Local NGOs and the public have a high interest in the presence and potential effects of EDCs in Puget Sound. A lay person summary (for the non-scientist) of the project results will be prepared for dissemination to the interested public. Project results will also be presented at public forums to provide the public with an opportunity to interact with project participants.

Programmatic Capability and Past Performance:

University of Washington: *Threats to bivalve aquaculture and fisheries: the influence of emerging diseases and environmental change*. PI: S. Roberts. Funded by NOAA Saltonstall-Kennedy Program. Total cost: $243,000. Period of performance 2009-2011 with all reports to date and budget spend plan completed on time. This project focuses on how multiple environmental factors impact physiology of Pacific oysters and *Vibrio tubiashii*. Similar transcriptomic approaches will be used for the current project.

Battelle MSL: *An Integrated Laboratory and Field Assessment of Select Endocrine Disruptors in the Puget Sound Region*. PI: Irv Schultz. Funded by Battelle's Independent Research & Development program. Total cost $130K. Period of performance 2007-2008, with semi-annual progress reports and budget spend plan completed on time with final report accepted in October 2008. This project assessed the utility of grab samples vs. POCIS samplers for detecting pharmaceuticals in Puget Sound area streams and tested the feasibility of deploying live, adult caged salmonids in streams for effects assessments.

Battelle MSL: *Reconstructing Trends in Hypoxia Using Multiple Paleoecological Indicators Recorded in Sediment Cores from Puget Sound, WA*. PI: J. Brandenberger and E. Crecelius. Funded by NOAA Coastal Hypoxia Research Program Grant Number: NA05NOS4781203. Total cost 550,000. Period of performance: 2005-2008 with annual progress reports submitted and schedule completed on time with a final report submitted and accepted in July 2008. The sediment cores continue to support research on historical trends for Puget Sound with funding provided for additional reconstructions by U.S. EPA for PBDEs and triclosan, Texas A&M University for lead isotopes and PAHs, and the U.S. Navy for mercury.

NWFSC NOAA: *Research to Support Hatchery Reform, including Captive Broodstock Programs*. PIs: B. Berejikian, P. Swanson, A. Dittman, B.R. Beckman, PIs. Funded by Bonneville Power Administration Grant Number: 199305600. Total Cost 2.5 million. Period of performance 2007-2011, with Annual progress reports, Statements of Work, quarterly reports, and budget spend plan completed on time and several peer reviewed publications. This project developed methods to improve artificial

production of salmon and developed some of the molecular tools for olfactory and reproductive physiological assessment that will be utilized in this proposed study.
University of Washington/NWFSC NOAA: *Development of Biomarkers of Fish Reproductive Health*. PIs: G.Young, P.Swanson, J.A. Luckenbach and F.W. Goetz. Washington Sea Grant, Total cost 600,000. Period of performance 2006-2011, with annual progress reports, Statements of Work, quarterly reports, and budget spend plan completed on time. This project is developing genomic tools that can comprehensively assess reproductive status of salmon and be used to examine effects of a variety of environmental stressors on salmon reproduction.

Key Personnel:
Steven Roberts (Ph.D Biological Sciences, University of Notre Dame) is an Assistant Professor at the University of Washington. His research program focuses on the physiological response of shellfish to environmental stress. Dr. Roberts has extensive experience in the application of genomic approaches to study biology of fish and shellfish, including current use of short-read sequence technology to characterize gene expression patterns in oysters and salmon. Roberts will be responsible for overall project coordination and tasks associated with characterizing the physiological response of oysters and orca tissue analysis. *Relevant publications:* Roberts et al 2009, Roberts et al 2008, Goetz et al 2010.
Irv Schultz (Ph.D, Pharmacology/Toxicology, Washington State University) is a staff scientist with Batelle's Marine Science Lab ecotoxicology group. Dr. Schultz has been involved in toxicological research since 1986 with research interests covering both ecological and human health issues. For the past 10 years his research activities have focused on the bioaccumulation and adverse effects of endocrine disruptors in aquatic organisms. Schultz will be responsible for tasks associated with direct measurements of emerging contaminants. *Relevant publications:* Lema et al 2007, Lema et al 2008, Schultz and Walters 2009, Muirhead et al 2006.
Deb Lester (MS, University of Vermont) manages the Toxicology and Contaminant Assessment Group in WLRD and has over 28 years of experience working on various aspects of water quality and sediment contamination related projects including project management, bioaccumulation of PCBs in large lake systems, ecological effects, risk assessment; routine monitoring and emerging contaminants. Lester serves on the Water Environment Research Foundation Committee on Trace Organic Compounds. Lester will be responsible for coordinating the review panel, have a key role in outreach, and assist Schultz with water quality aspects. *Relevant publications:* King County 2007, Lester and McIntosh 1994|
Andrew Dittman (Ph.D, Fisheries/Pharmacology, University of Washington) has been a Fishery Biologist with the Physiology Program at the NWFSC, NOAA Fisheries for the last 10 years. Dr. Dittman has been studying the olfactory physiology of fishes, particularly salmonids, for over 20 years. His research has focused on the molecular and cellular basis of olfactory imprinting and homing in salmon and endocrine control of olfactory-mediated behaviors. Dittman will be responsible for characterizing the response of salmon to contaminant exposure. *Relevant publications:* Dittman and Quinn 1996, Dittman et al. 1996, 1997, Nevitt et al. 2004, Speca et al. 1999.
Penny Swanson (Ph.D. Zoology, University of Washington) is the Manager of the Physiology Program Program at the NWFSC, NOAA Fisheries. Dr. Swanson has studied fish endocrinology for over 30 years and is an internationally recognized leader in this field. Her recent research has focused on the reproductive endocrinology of salmonids and development of genomic biomarkers of fish reproductive health. Swanson will be working with Dittman to characterize the response of salmon. *Relevant publications:* Campbell et al. 2003, 2006, Swanson et al 2003, 2007, Kutsukabe et al. 2006, Lema et al. 2007, 2008, Luckenbach 2008.

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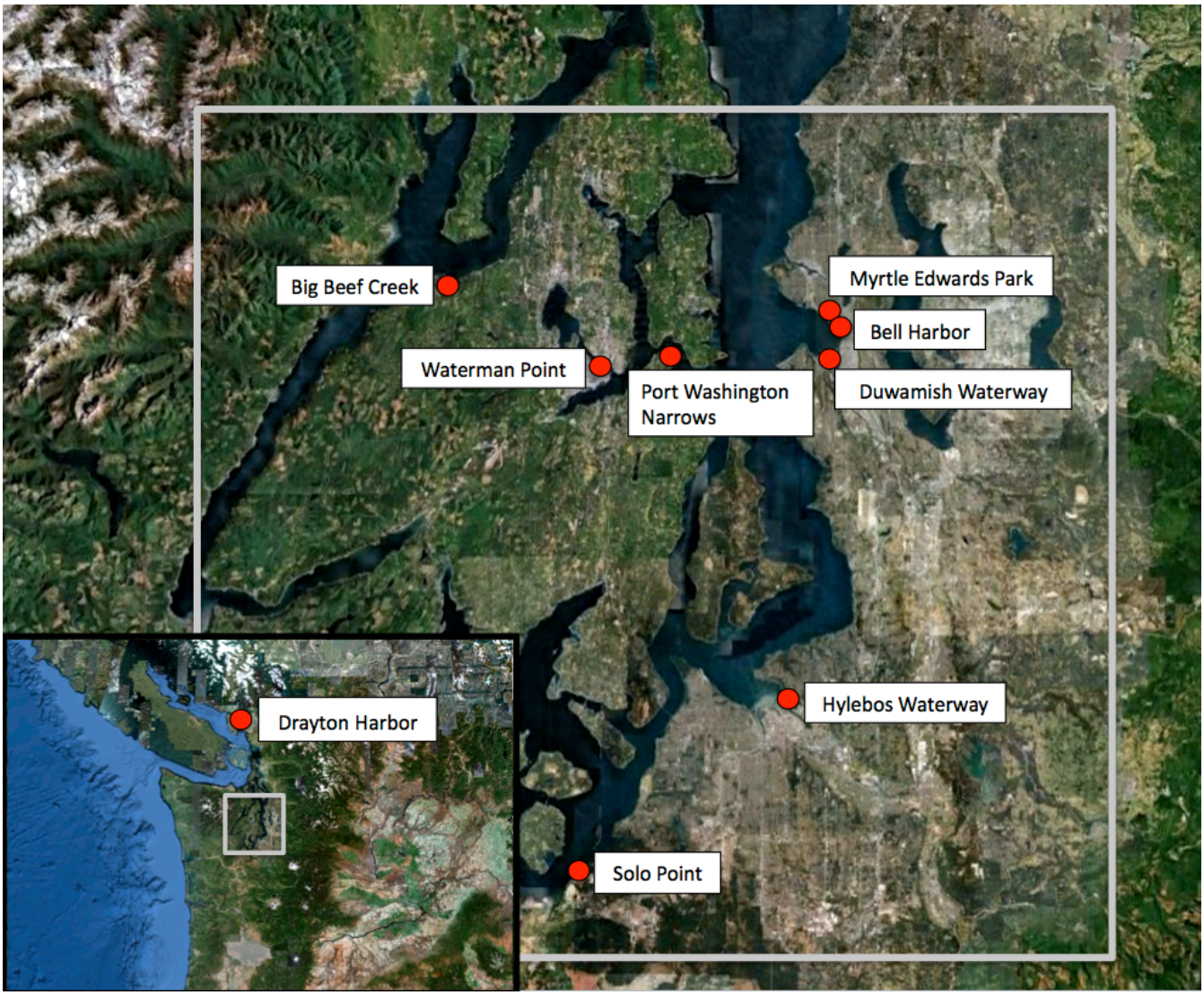

Map of field sites. Inset at left shows regional perspective with site located farthest north (Drayton Harbor). Primary view shows other eight sites. At each of these sites, oysters will be deployed and contaminants will be measured in water, sediment, and oyster tissue. In addition, gene expression analysis will be carried out on oyster samples to examine physiological responses.

Detailed Budget

Personnel

For the completion of the specific aims outlined in the proposal we are requesting over the 2 years, salary for S. Roberts (PI), a graduate student, and professional staff. Roberts requests 2 months of salary and his primary responsibilities will include supervising gene expression analysis, as well as overall management of the project. Given the large amount of sample processing and routine molecular procedures, a total of 22 months of support are requested for professional staff during years 1 and 2. This includes 15 months for a Fish Biologist 1 in year 1 and 8 months in year 2. This person will participate in set-up and initiation of salmon exposure studies. In addition they be responsible for running all salmon quantitative PCR, plasma hormone and gill Na+K+ ATPase assays. A total of 7 months is requested for a Research Scientist to assist in molecular assays and library construction associated with oysters

A graduate student will be supervised by the PI on this proposal and will be committing 18 months on this project. Support is requested from the EPA ($34,518) to cover his/her salary and the student will be responsible for experimental procedures, processing and analysis.

Fringe Benefits

These monies are budgeted at rates set by the University of Washington: 23.6% for faculty, 12.9% for graduate students and 29.3% for professional staff. Total funds requested from the EPA to cover fringe benefits are $34,253.

Travel

$2700 is requested to fund local travel for sampling activities ($700) and to partially cover the costs of personnel air travel and per diem for presenting research results.

Supplies

A total of $68,900 is requested to cover material and supplies necessary to carry out the research aims of the current proposal. During year 1, funds ($7500) are needed for collection, processing, extracting nucleic acids, and analyzing (i.e., performing quantitative PCR) the oyster and salmon samples collected as well as archival whale samples and any new samples taken. Specifically this will include collection supplies (e.g. tubes, buffers, vials, instruments, dry ice, sample boxes), molecular lab reagents (e.g. TriReagent, primers, Amplitaq DNA Polymerase Kit, Perkin & Elmer; TOPO 2.1 TA Cloning Kit, Invitrogen, Quantitative PCR Master Mix kits, DNase), lab consumables (e.g., 96-well plates, gels, covers, tips, agar plates, media), and molecular reagents and plasticware needed for sequencing. $10,000 is requested to cover costs of limited quality analysis including analysis of alkylphenol compounds as well as for purchasing of water quality sampling devices. Note most of water quality costs are included in subcontract (see below) $4000 is requested in year one to carryout deep sequencing (ABI SOLiD System 3) in order to take a non-biased, complementary approach to the targeted qPCR effort with whale samples. $2800 is needed to pay for chemicals and food required for salmon exposures. The remaining $4700 requested during year 1 will cover costs of T4/T3 assays, ATPase assays, and associated consumables.

During year 2, $39,000 is requested to cover supplies needed for analysis of samples. Primary research activities in year will include qPCR assays targeting stress response genes in all three species and $25,000 will be used for this. Specifically this includes enough reagents to complete analysis on approximately 15 target genes in each species includes; collection supplies (e.g. tubes, buffers, vials, instruments, dry ice, sample boxes), molecular lab reagents (e.g. TriReagent, primers, Amplitaq DNA Polymerase Kit, Perkin & Elmer; TOPO 2.1 TA Cloning Kit, Invitrogen, Quantitative PCR Master Mix kits, DNase), lab consumables (e.g., 96-well plates, gels, covers, tips, agar plates, media).

$10,000 is requested to cover costs of a portion of water quality analysis including analysis of alkylphenol compounds. $4000 will be used to cover the cost of construction and sequencing of salmon deep sequencing libraries on an ABI SOLiD System 3.

Contractual

There is one subcontract ($200,227) within this proposal to Battelle Pacific Northwest National Laboratory. Battelle will provide analysis of selected contaminants (as identified in the proposal) in sediment, water, passive samplers and biological tissues collected or generated during the study. Biological tissues may consist of oysters, marine mammal tissues and Coho salmon. For this project, Battelle has estimated costs based on the number of staff days required to perform the analysis. Depending on the type of analysis and matrix being analyzed, between 10 and 25 sample extracts can be processed in one work ay along with associated method performance standards and instrument calibrating standards. The Battelle budget provides support for 56 workdays in Year 1 for Drs. Schultz and Kuo, who will be doing the analysis. This level of support allows for the analysis of 560 – 1400 sample extracts depending on matrix and analyte. Note that some samples such as a passive sampler extracts may be analyzed thrice, for different classes of contaminants requiring different analytical detection methods. Multiple analysis of a sample is included in the total number estimate of samples analyzed. Thus, although there may be only 300 – 475 actual samples generated by the project, this may still require up to 1400 analysis of extracts. In Year 2 of the project, 31 and 38 work days have been budgeted for Drs. Schultz and Kuo. This level of support provides for the additional analysis of approximately 625 sample extracts. A summary of effort is provided:
I Schultz – 0.25 FTE Year 1 and 0.14 FTE Year 2
L Kuo – 0.25 and 0.20 FTE Years 1 and 2
J Brandenberger – 0.05 FTE Year 1.

I Schultz's responsibility will be overseeing all analytical measurements for contaminants in sediment, water, passive samplers and biological samples. Dr. Schultz will also provide technical support for laboratory exposures of salmon to contaminants. Dr. Schultz will be assisted in these activities by Dr. Li-jung Kuo, who will be working as a Post-doctoral researcher at Battelle MSL in Sequim. Dr. Kuo's background is in analytical chemistry and is currently specializing in environmental analysis of PBDEs. Ms Jill Brandenberger will provide technical support in maintaining equipment and assist with collating and data reduction.

Other

The proposed project will be the primary thesis material of a graduate student. He/she will commit 18 months towards this project and therefore funds are requested ($18,650) to cover tuition during this time.

A total of $500 is requested in YR2 to cover publication costs associated with all outreach publications (e.g. fact sheets, posters, website) and for publication of results in scientific literature.

Indirect Charges

Indirect costs are calculated as 56.0% of all modified total direct costs excluding tuition and subcontract.

Guiding Objectives

Action Agenda

• Development of monitoring approaches, information and/or analyses which support the understanding and communication of ecosystem conditions, status and rates of change.

Puget Sound Science Work Plan

• Improving the application of science to support priority ecosystem protection and restoration strategies and outcomes.
• Enhancing collaborative monitoring, modeling, assessment and applied research capacities
• Using science support and technical studies to improve the effectiveness of both local and regional management activities and initiatives
• Conducting baseline studies and ecosystem monitoring activities to demonstrate measurable environmental changes and/or trends in condition

Other objectives of RFA

• Provide strategically valuable analytical tools, information and/or technical approaches that will enable and catalyze measurable outcomes in Puget Sound.
• Help to fill critical program implementation needs leading to significant environmental results in Puget Sound.
• Produce scientifically defensible products in support of major Puget Sound initiatives and planning and regulatory decisions.
• Promote adaptive management approaches by providing information, analysis and objective guidance to programs and policies seeking to protect and restore Puget Sound.

EPA Strategic Plan

• Goal 2 - Clean and Safe Water
  o Objective 2.1 Protect Human Health
    2.1.2 Fish and Shellfish Safe to Eat
  o Objective 2.2 Protect Water Quality
    2.2.1 Protect and Improve Water Quality on a Watershed Basis

Specific Aims

AIM 1: Implement an integrated approach to characterize the occurrence of endocrine disruptive contaminants

Tasks
• Passive Sampling
• Sediment Sampling
• Benthic Invertebrate tissue sampling

AIM 2: Measure indicative physiological responses of aquatic organisms to conditions in Puget Sound

Tasks
• Oyster gene expression (field)
• Juvenile Salmon experiment (lab)
• Whale gene discovery
  • archival and current samples

Participants

University of Washington - School of Aquatic and Fishery Sciences

Battelle Pacific Northwest National Laboratory

King County Department of Natural Resources

National Oceanic and Atmospheric Administration

Outputs

• Baseline quantification of emerging toxins in Puget Sound
• Data on comparison of single target in solution, sediment and biological tissue
• Gene expression panels for several critical species
• Essential data for ecosystem monitoring

Outcomes

Short term
• Increased public awareness of anthropogenic pollution
• Better understanding of basic physiological processes in key species

Medium term
• Integrated framework for continued biomonitoring across watershed
• Improved decision making for resource allocation

Long term
• Recommendations to policy makers
• Important ecosystem model development

Roberts et al. LOGIC MODEL

Statement on use of animals: Roberts et al. proposed project, “Integrated Approach to Assess Emerging Threats to Puget Sound Ecosystem Health”. This study will involve the euthanization of salmon in accordance with Batelle IACUC protocol 2007-01.

COLLEGES AND UNIVERSITIES RATE AGREEMENT

EIN #:

DATE: October 13, 2009

INSTITUTION:
University of Washington
Management Accounting and Analysis
UW Box 354165

FILING REF.: The preceding Agreement was dated November 5, 2008

Seattle WA 98195

The rates approved in this agreement are for use on grants, contracts and other agreements with the Federal Government, subject to the conditions in Section III.

SECTION I: FACILITIES AND ADMINISTRATIVE COST RATES*

RATE TYPES: FIXED FINAL PROV.(PROVISIONAL) PRED.(PREDETERMINED)

TYPE	EFFECTIVE PERIOD FROM	TO	RATE(%)	LOCATIONS	APPLICABLE TO
PRED.	07/01/04	06/30/05	51.6	(1) & (A)	(G)
PRED.	07/01/05	06/30/07	55.5	(1) & (A)	(G)
PRED.	07/01/07	06/30/09	56.0	(1) & (A)	(G)
PRED.	07/01/04	06/30/09	26.0	(1) & (B)	(H)
PRED.	07/01/04	06/30/05	55.0	(1) & (A)	(I)
PRED.	07/01/05	06/30/09	58.0	(1) & (A)	(I)
PRED.	07/01/04	06/30/09	26.0	(1) & (B)	(I)
PRED.	07/01/04	06/30/05	30.0	(1) & (C)	(J)
PRED.	07/01/04	06/30/05	60.0	(1) & (C)	(K)
PRED.	07/01/05	06/30/09	44.0	(1) & (C)	(J)
PRED.	07/01/05	06/30/09	75.0	(1) & (C)	(K)
PRED.	07/01/04	06/30/09	17.0	(1) & (D)	
PRED.	07/01/04	06/30/09	25.0	(2) & (F)	
PRED.	07/01/05	06/30/09	66.0	(1) & (L)	(G)
PROV.	07/01/09	UNTIL AMENDED	Use same rates and conditions as those cited for fiscal year ending June 30, 2009.		

(A) On-Campus
(B) Off-Campus
(C) Regional Primate Center
(D) Applied Physics Laboratory
(E) (Intentionally Blank)
(F) Vessel Operations
(G) Organized Research
(H) Organized Research & General Clinical Research Center
(I) Instruction
(J) Core Grant Only
(K) Regional Primate Center Research except Core Grant
(L) Lake Union Campus

*BASE:
(1) Modified total direct costs, consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel, and subgrants and subcontracts up to the first $25,000 of each subgrant or subcontract (regardless of the period covered by the subgrant or subcontract). Equipment, capital expenditures, charges for patient care and tuition remission, rental costs, scholarships, and fellowships as well as the portion of each subgrant and subcontract in excess of $25,000 shall be excluded from modified total direct costs.

(2) Direct salaries and wages including vacation, holiday and sick pay and other paid absences but excluding other fringe benefits.

INSTITUTION:
University of Washington
Management Accounting and Analysis

AGREEMENT DATE: October 13, 2009

SECTION I: FRINGE BENEFITS RATES**

RATE TYPES: FIXED FINAL PROV.(PROVISIONAL) PRED.(PREDETERMINED)

TYPE	EFFECTIVE PERIOD FROM	TO	RATE(%)	LOCATIONS	APPLICABLE TO
FIXED	07/01/09	06/30/10	23.6	(1) & (B)	Faculty
FIXED	07/01/09	06/30/10	25.7	(1) & (A)	Auxiliary Teaching
FIXED	07/01/09	06/30/10	24.5	(1) & (A)	Residents
FIXED	07/01/09	06/30/10	12.9	(1) & (A)	Graduate Students
FIXED	07/01/09	06/30/10	19.4	(1) & (A)	Post Doctorate
FIXED	07/01/09	06/30/10	38.3	(1) & (B)	Classified Staff
FIXED	07/01/09	06/30/10	29.3	(1) & (B)	Professional Staff
FIXED	07/01/09	06/30/10	15.9	(1) & (B)	(D)
FIXED	07/01/09	06/30/10	20.8	(1) & (B)	(E)
FIXED	07/01/09	06/30/10	7.4	(1) & (B)	(F)
FIXED	07/01/09	06/30/10	13.0	(1) & (A)	Hourly
FIXED	07/01/09	06/30/10	10.3	(1) & (A)	Pre-Doctoral Fellows
FIXED	07/01/09	06/30/10	62.8	(2) & (C)	Classified Staff
FIXED	07/01/09	06/30/10	51.2	(2) & (C)	Professional Staff
FIXED	07/01/09	06/30/10	39.6	(2) & (C)	Faculty

(A) Entire University
(B) All except Applied Physics Laboratory
(C) Applied Physics Laboratory
(D) Professional Staff - Global (No Health)
(E) Professional Staff - Global (No Retirement)
(F) Professional Staff - Global (No Health or Retirement)

**BASE:
(1) Direct salaries and wages including vacation, holiday, and sick pay but excluding other fringe benefits.

(2) Direct salaries and wages excluding vacation, sick leave, holidays, other paid absences and all other fringe benefits.

INSTITUTION:
University of Washington
Management Accounting and Analysis

AGREEMENT DATE: October 13, 2009

SECTION II: SPECIAL REMARKS

TREATMENT OF FRINGE BENEFITS:
This organization uses a fringe benefit rate which is applied to salaries and wages for both budgeting and charging purposes for Federal projects.

TREATMENT OF PAID ABSENCES:
Vacation, holiday, sick leave pay and other paid absences are included in salaries and wages and are claimed on grants, contracts and other agreements as part of the normal cost for salaries and wages. Separate claims for the costs of these paid absences are not made.

Beginning October 1, 1996 the Applied Physics Laboratory (APL) has separate fringe benefit rates from the remainder of the University of Washington. These rates include paid absences. Therefore, charges for direct salaries and wages from APL must exclude charges for paid absences, including vacation, sick leave, holidays, and other paid absences.

DEFINITION OF EQUIPMENT
Equipment is defined as tangible nonexpendable personal property having a useful life of more than one year, and an acquisition cost of $2,000 or more per unit.

The following fringe benefits are included in the fringe benefit rate(s):
TIAA/CREF, HEALTH INSURANCE, MEDICAL AID, INDUSTRIAL INSURANCE, WORKERS COMPENSATION, STATE RETIREMENT, SOCIAL SECURITY, AND UNEMPLOYMENT COMPENSATION.

DEFINITION OF ON-CAMPUS, OFF-CAMPUS AND SPECIAL RATES:
DEFINITION OF OFF-CAMPUS RATE
a. An off-campus program is one that is conducted (1) in leased facilities where space related costs (e.g. rent, utilities and maintenance) are charged directly to the program, or (2) in facilities made available (at no cost) to the program by a non-University organization, or (3) away from the University over an uninterrupted period of time in excess of 30 days for field work. The Off-Campus rate is not to be used as a substitute for the Vessel Operations rate or the Applied Physics Laboratory rate. Even though Pack Forest, Big Beef Creek, and Olympic Natural Resource Center are owned and operated by the University, these facilities are considered to be off campus.

b. Projects conducted at two or more locations:
There are instances where a project supported by a single grant or contract is conducted at two or more locations, thus requiring special consideration in determining the appropriate indirect cost provision. The following should be observed in such circumstances:

(1) Where the total annual amount of the grant or contract direct costs is less than $250,000, a single indirect cost rate will be applied. This rate will be the one currently applicable to the location where the preponderance of project salaries is located.

(2) Where the total annual amount of the grant or contract direct costs is $250,000 or more, the appropriate rate for each location will be applied to the modified total direct costs specifically assigned to the respective location. In the absence of the institution's ability to specifically identify and assign costs to each location, the appropriate rate for each location will be applied to total project costs in the same ratio as direct salary costs incurred at each location during the period covered by the project billing or accounting.

The Regional Primate Center changed to a dual rate structure beginning July 1, 1997. When applying the rate for RPCR except Core Grant, the difference in recoveries between this rate and the Core Grant only rate shall be retained by the Core Grant.

This rate agreement updates the fringe benefits only.

INSTITUTION:
University of Washington
Management Accounting and Analysis

AGREEMENT DATE: October 13, 2009

SECTION III: GENERAL

A. LIMITATIONS:
The rates in this Agreement are subject to any statutory or administrative limitations and apply to a given grant, contract or other agreement only to the extent that funds are available. Acceptance of the rates is subject to the following conditions: (1) Only costs incurred by the organization were included in its facilities and administrative cost pools as finally accepted: such costs are legal obligations of the organization and are allowable under the governing cost principles; (2) The same costs that have been treated as facilities and administrative costs are not claimed as direct costs; (3) Similar types of costs have been accorded consistent accounting treatment; and (4) The information provided by the organization which was used to establish the rates is not later found to be materially incomplete or inaccurate by the Federal Government. In such situations the rate(s) would be subject to renegotiation at the discretion of the Federal Government.

B. ACCOUNTING CHANGES:
This Agreement is based on the accounting system purported by the organization to be in effect during the Agreement period. Changes to the method of accounting for costs which affect the amount of reimbursement resulting from the use of this Agreement require prior approval of the authorized representative of the cognizant agency. Such changes include, but are not limited to, changes in the charging of a particular type of cost from facilities and administrative to direct. Failure to obtain approval may result in cost disallowances.

C. FIXED RATES:
If a fixed rate is in this Agreement, it is based on an estimate of the costs for the period covered by the rate. When the actual costs for this period are determined, an adjustment will be made to a rate of a future year(s) to compensate for the difference between the costs used to establish the fixed rate and actual costs.

D. USE BY OTHER FEDERAL AGENCIES:
The rates in this Agreement were approved in accordance with the authority in Office of Management and Budget Circular A-21 Circular, and should be applied to grants, contracts and other agreements covered by this Circular, subject to any limitations in A above. The organization may provide copies of the Agreement to other Federal Agencies to give them early notification of the Agreement.

BY THE INSTITUTION:
University of Washington
Management Accounting and Analysis
(INSTITUTION)
V'Ella Warren
(SIGNATURE)
V'Ella Warren
(NAME)
Senior Vice President &
(TITLE) Treasurer Board of Regents
11/2/09
(DATE)

ON BEHALF OF THE FEDERAL GOVERNMENT:
DEPARTMENT OF HEALTH AND HUMAN SERVICES
(AGENCY)
Wallace Chan
(SIGNATURE)
Wallace Chan
(NAME)
DIRECTOR, DIVISION OF COST ALLOCATION
(TITLE)
October 13, 2009
(DATE) 2129

HHS REPRESENTATIVE: Patrick Smith
Telephone: (415) 437-7820