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August 23, 2016

Catherine Boerner
Exxon Valdez Oil Spill Trustee Council
4210 University Drive
Anchorage, AK 99508-4626

Proposal Entitled: "Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse"
UC Davis Principal Investigator: Dr. Andrew Whitehead
UC Davis Amount Requested: $1,521,838
USGS Amount Requested: $17,810
General Administration: $91,042
Total Funds Requested: $1,630,690
Project Period: 02/01/2017 – 01/31/2022

Dear Dr. Boerner:

On behalf of The Regents of the University of California, Davis Campus it is our pleasure to present for your consideration the above-referenced proposal.

Please contact me with any administrative questions. We request correspondence pertaining to this proposal be sent via email to proposals@ucdavis.edu or mailed to the Office of Research Sponsored Programs Office, 1850 Research Park Drive, Suite 300 Davis, CA 95618-6153.

We look forward to working with you on this important project.

Sincerely,

Shanna Nation Jose
Contracts and Grants Analyst
Phone: (530) 754-8318
snation@ucdavis.edu

**Please refer to SPO #201700574 on all future correspondence.*

Send Award Notice to:

Office of Research, Sponsored Programs
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University of California Davis
PO BOX 989062
West Sacramento, California 95798-9062

Appendix E

EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
LINGERING OIL PROJECT PROPOSAL SUMMARY PAGE

Project Title

Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse

Primary Investigator(s) and Affiliation(s)

Dr. Andrew Whitehead, University of California Davis

Date Proposal Submitted

08/24/2016

Begin and End Dates of Proposed Project

02/01/2017 to 01/31/2022

Project Abstract

The causes of the collapse of the Prince William Sound (PWS) Pacific herring stock are controversial, and the reasons for the lack of recovery remain a mystery. In the research proposed here we interrogate the genome structure and genome function of PWS fish to test hypotheses about the causes and consequences of the collapse, by revealing ecological, evolutionary, and genetic mechanisms governing the demographic trajectory of PWS fish over the past ~30 years. Conspicuous events that coincided with the dramatic PWS collapse include the Exxon Valdez oil spill (EVOS) four years previous, and the emergence of disease. We test hypotheses concerning the effects of oil exposure, the effects of disease challenge, and the potential interactive effects of oil exposure and disease challenge, on herring health and fitness. Since oil is exquisitely toxic to developing fish embryos at concentrations that were experienced in PWS following the EVOS, we predict that this exposure presented a significant selective event with the side effect of impaired immune function (as evidenced by our recent studies in killifish) leaving fish susceptible to disease and subsequent decline. Alternatively, the oil spill may not have been a significant selective force, but genetic attributes of the PWS stock may have made them susceptible to disease outbreak. In either scenario (and others), we predict that the collapse resulted in significant erosion of genetic diversity in PWS fish, perhaps particularly in immune system genes, which may be limiting their recovery. We will test these predictions and hypotheses by reconstructing genome-wide genetic change through time (pre-EVOS and pre-collapse, post-EVOS soon after collapse, post-EVOS 10 years post-collapse, and contemporary) in PWS fish, and compare this to population genetic change through time in a reference site population. Furthermore, a series of laboratory-based experiments will test for population differences in their response to oil exposure in early life and subsequent resilience to pathogen exposures. Physiological measurements and patterns of genome-wide gene expression will serve to reveal similarities and differences in mechanisms of response to these stressors between PWS and reference population fish. These studies should provide novel insights into the causes and consequences of recent dramatic demographic changes in PWS fish, potentially inform novel intervention strategies, and provide modern genomic resources for management and conservation of Pacific herring.

Appendix E

EVOSTC Funding Requested *(must include 9% GA)*

FY17	FY18	FY19	FY20	FY21	TOTAL
$217,968.00	$385,968.00	$392,244.30	$310,923.10	$323,590.60	$1,630,689.40

Non-EVOSTC Funding Available

FY17	FY18	FY19	FY20	FY21	TOTAL

Appendix E

Please refer to the Invitation for the specific proposal requirements for each Focus Area. The information requested in this form is in addition to the information requested in each Focus Area and by the Invitation.

1. Executive Summary

Please provide a summary of the project including key hypotheses and overall goals. Describe the background and history of the problem.

1A. GOALS and HYPOTHESES

Genetic attributes unique to the PWS population, that either pre-existed or emerged in the years following the EVOS, may help explain the lack of recovery in the PWS stock following the 1993 collapse, and may also illuminate the causes of the collapse. Diseases are key variables that help explain the population dynamics of PWS herring since the 1990s decline (Marty et al. 2010). The contribution of the EVOS to the PWS decline is more controversial (Pearson et al. 1999; Carls et al. 2002). However, recent studies have shown that herring embryos are sensitive to fitness impacts at very low concentrations of oil (Incardona et al. 2015), these low-level exposures can affect fitness in the field (Heintz et al. 2000), and natural selection from pollutants can quickly drive complex genetic change in PAH-exposed populations (Reid et al. in review-b). Our **overarching question** is: *Are there functional connections that link the PWS herring collapse and lack of recovery with disease impacts and the EVOS?*

Our **hypotheses** are:

H1: Natural selection following EVOS exposure came at the cost of compromised immune function.

H1-alternate: No evidence for oil-induced selection, but population collapse resulted in erosion of genetic diversity, especially in immune system genes, which impairs protective innate and adaptive immune responses.

H2: Exposure to oil during development compromises the ability in later life to mount an effective immune response to pathogen exposure.

To test our hypotheses, we propose the following **approaches**: 1) Retrospective population genomics; 2) Experimental/comparative physiology; 3) Experimental/comparative functional genomics.

1B. BACKGROUND and HISTORY

The causes of the herring fishery collapse in PWS in the early 1990's are complicated and controversial, as is the relationship of the collapse to the 1989 EVOS (Pearson et al. 1999; Carls et al. 2002; Thorne and Thomas 2008). We posit that contaminating oil in 1989, and perhaps lingering oil in subsequent years, acted as a selective agent on developing herring, the consequences of which may have contributed to their subsequent decline and/or lack of recovery. Support for this hypothesis emerges from recent discoveries about how exposure to pollutants may drive genetic change in populations of Atlantic killifish (*Fundulus heteroclitus*) (Reid et al. in review-b): a forage fish with very large population sizes. In particular, some populations of killifish have rapidly evolved within an environment contaminated with creosote, which is chemically and toxicologically similar to crude oil. Creosote pollution acted as a strong selective agent, primarily for survivorship during embryogenesis. Favored were genetic variants with impaired function of the aryl hydrocarbon receptor (AHR) signaling pathway. Normal AHR signaling interacts with immune system signaling, such that impairments in AHR function could come at the cost of normal immune system function. Indeed, in killifish populations we find evidence for

subsequent selection on immune system genes, which supports this prediction. These discoveries were achieved through population genomics and comparative transcriptomics and physiological analyses, similar to what we propose here for Pacific herring.

Given these findings in Atlantic killifish, and more generally that pollutants can act as powerful selective agents (Monosson 2012), that evolutionary genetic change can occur very rapidly (Hendry et al. 2008), that rapid adaptation often incurs physiological or life history costs (Gassmann et al. 2009; Hochmuth et al. 2015; Qi et al. 2016), that crude oil can interact with immune function (Reynaud and Deschaux 2006), and that crude oil impairs herring development at extremely low concentrations (Incardona et al. 2015), we posit the following: 1) Exposure to contaminating oil during 1989 (and perhaps to lingering oil in subsequent years) selected for genetic variants with impaired AHR function in developing embryos. Alternatively (or in addition), exposure to oil in 1989 and perhaps to lingering oil in subsequent years impaired immune function. 2) The frequency of individuals with impaired immune function increased within the PWS population. 3) By 1993, fish from the PWS 1989 year class are recruiting into the fishery, contributing fish with impaired resilience to pathogens, enabling an epizootic, that contributed to the population collapse. 4) Recovery is slow in PWS because compensatory adaptations for immune function is slow, possibly because the collapse significantly eroded genetic diversity available for subsequent natural selection on immune system genes. We design our proposed experiments and contrasts to test this set of hypotheses, as well as to test complementary and alternative hypotheses.

Our discoveries about the nature of pollutant-induced genetic change in Atlantic killifish were enabled through an integrated combination of population genomics, comparative transcriptomics, and comparative physiology studies (Whitehead et al. 2010; Whitehead et al. 2012; Reid et al. in review-b). Comparative transcriptomics and physiology revealed the functional changes that were induced in pollutant-exposed populations. Population genomics revealed the genetic variants that were under selection, thereby exposing the toxicological mechanisms that were relevant to fish living in polluted sites, and exposing the pathways that were also perturbed following the primary adaptation for survival. We propose to deploy a similar toolkit and experimental design to shed light on the causes and consequences of the PWS herring collapse.

2. Relevance to the Invitation for Proposals

Discuss how the project addresses the Lingering Oil Focus Area as described in the Invitation. Describe the results you expect to achieve during the project, the benefits of success as they relate to the topic under which the proposal was submitted, and the potential recipients of these benefits.

2A. RELEVANCE

In the decades following the EVOS, it has become increasingly apparent that oil can be toxic at extremely low concentrations to developing fish embryos including herring (Incardona et al. 2015), where some toxic phenotypes may be apparent during embryogenesis but some are delayed until later in life (Hicken et al. 2011). Therefore acute and lingering oil may act as an insidious selective force within populations. Much nearshore herring spawning habitat was oiled following the EVOS, and oiling coincided with herring spawning (Carls et al. 2002). In subsequent years, lingering oil, which accumulated in nearshore environments (Short et al. 2004; Short et al. 2006; Short et al. 2007) and was at least partially bioavailable (Fukuyama et al. 2000), may have contributed to impacts in subsequent year classes of developing herring in PWS. These exposures may have contributed to the herring collapse either through direct delayed toxicity, through the interactive effects of oil and pathogen

exposures, or through evolved tolerance to toxicity that came at the cost of compromised immune function. Alternatively, the PWS herring population collapse could have been unrelated to the oil spill, but erosion of genetic diversity associated with the collapse could compromise recovery, compromise resilience to pathogens, or may limit the ability of the population to adapt to future environmental change. Knowledge of the dynamics of population genetic change through time will help inform management activities for Pacific herring, and could contribute novel considerations for the complexity through which populations respond to oil spills.

2B. EXPECTED RESULTS

Detailed characterization of the genetics of PWS fish (and reference stocks), coupled with comparative studies in genome function and physiology, should yield important insights into the characteristics of the PWS stock that may help explain their dramatic collapse and unexpectedly slow recovery. These insights could include, but are not limited to, the roles of disease and oil exposure. We present four scenarios (that are not necessarily mutually exclusive) where different results would support different hypotheses.

Expected results, Scenario 1: Retrospective population genomics shows signatures of natural selection on the AHR signaling pathway compatible with the timing of the EVOS. This is coupled with refractory response of AHR signaling genes in following experimental oil exposure (similar to Atlantic killifish). This should result in impaired ability to mount protective immune responses in pathogen-exposed fish (as evidenced in Atlantic killifish). Comparative physiology studies would confirm this. Furthermore, genes that are transcriptionally involved in mounting a protective immune response should be those known to interact with normal AHR signaling. These results would support the hypothesis that evolutionary change from oil exposure came at the cost of impaired immune function. Persistence of impaired immune function could be because insufficient generations have elapsed, or because little allelic diversity in relevant genes remained following the collapse, to enable compensatory adaptation. These results would link the ultimate (EVOS) and proximate (pathogen) causes of the collapse, and explain the lack of recovery (genetic change, erosion of allelic diversity).

Expected results, Scenario 2: Comparative physiology studies indicate that PWS fish have impaired ability to mount protective immune responses, but population genomics does not show signatures of selection compatible with the timing of the EVOS. Population genomics does show erosion of genetic diversity coincident with the PWS collapse, particularly in immune system genes, including those that are transcriptionally activated upon experimental pathogen exposure. This result (lack of genetic diversity in key genes) would help explain the slow recovery of a population decimated by pathogens, but would not explain the cause of the collapse. However, if exposure to field-relevant concentrations of crude oil in early life impairs immune function in later life, this result could help explain the collapse.

Expected results, Scenario 3: There is no unusual genetic change in the PWS stock compared to other Alaskan populations (e.g., no selection or erosion of genetic diversity associated with the EVOS or the collapse), but PWS fish show an impaired ability to mount protective immune responses compared to other populations. Population genetics indicates significant allele frequency differences for genes involved in immune function. These results would suggest that PWS fish were predisposed to the epizootic.

Expected results, Scenario 4: No interesting genetic differences between PWS fish and reference populations, including for immune system genes. Oil exposure during early life impairs the ability of fish to mount protective immune responses in later life. This result would link ultimate (EVOS) and proximate (pathogen) causes of the collapse, but would not explain the lack of recovery.

One may imagine a number of alternative scenarios that would emerge from a combination of any of the results outlined in the above four scenarios (and others). Of course it is also possible that we detect no genetic change in PWS fish, and no differences between PWS and other populations in their response to oil or pathogens. In this case our data would still be useful. We would have a detailed characterization of population genetic change through time for multiple populations of an economically important fish. This could provide key insights into the dynamics of effective population size change over time, and the dynamics of allele frequency and nucleotide diversity change through time. This could provide useful information for fisheries management. These data would also reveal any local adaptation between Alaskan populations of Pacific herring, which could influence fisheries management and future stocking decisions. We would also gain insight into the genes and pathways that are important for mounting protective immune responses following pathogen exposure in Pacific herring, and into the mechanisms that underpin the immunotoxicity of oil. These discoveries could improve predictions of the consequences of future oil spills and inform novel intervention strategies for future spills and/or epizootics. Our research activities would also provide a reference genome for this economically and culturally important species, where genomics resources are providing new tools for conservation and fisheries management (Wenne et al. 2007; Ouborg et al. 2010; Benestan et al. 2016). Our activities would also provide a detailed characterization of recent and contemporary genetic variation in Pacific herring, which would serve as a benchmark for tracking future population genetic change following predicted environmental change (ocean warming, acidification, harvest pressure).

2C. EXPECTED BENEFITS

If we discover that PWS fish are compromised in their ability to recover from population decline because of their genetic characteristics, then this might inform novel recovery management activities, such as supplementing the population with genotypes from other populations. Genetically informed management has contributed to the successful recovery of other species in decline such as wolves and panthers (Hedrick and Fredrickson 2010). Furthermore, understanding the interactive effects of oil and sensitivity to disease and pathogens could increase ability to predict biotic responses to future oil spills.

Knowledge of how neutral and selective forces shape genetic diversity within Pacific herring, for one population that experienced the EVOS and a recent collapse and one that did not, should provide important insights into the demographic forces that shape this important species. This could provide useful information to stock managers. These data will also provide a detailed characterization of contemporary genetic diversity, such that the genetic characteristics of this important species may be tracked though future important environmental change, including ocean warming, acidification, and continued harvest, or (perish the thought) oil spills.

Pacific herring is a key resource for the Alaskan commercial fishing economy and for many Native American groups, and is a species of special focus for the EVOSTC. Genomes have been sequenced for most of the most important terrestrial animal food species (e.g., cow, pig, chicken). Similarly, several genomes have been recently sequenced from fish species that are commercially and culturally important (e.g., Atlantic herring, Atlantic cod, Pacific Bluefin tuna, Atlantic salmon, European and Japanese eel). Genome sequences can provide an important resource for understanding the characteristics (life history, physiology, behavior, morphology) that are unique to species, and as a tool for management (identification of stock structure) and conservation (characterization of genetic diversity, local adaptation) (Wenne et al. 2007; Ouborg et al. 2010; Benestan et al. 2016). The reference genome sequenced in the proposed studies will serve as a similar resource for Pacific herring research, conservation, and management, and will thus serve the many stakeholders invested in the long-term health of Pacific herring.

Appendix E

3. Project Personnel

The CV's of all principal investigators and other senior personnel involved in the proposal must be provided. Each resume is limited to two consecutively numbered pages and must include the following information:

- A list of professional and academic credentials, mailing address, and other contact information (including e-mail address)
- A list of up your most recent publications most closely related to the proposed project and up to five other significant publications. Do not include additional lists of publications, lectures, etc.
- A list of all persons (including their organizational affiliations) in alphabetical order with whom you have collaborated on a project or publication within the last four years. If there have been no collaborators, this should be indicated.

ANDREW WHITEHEAD

Associate Professor. Department of Environmental Toxicology, University of California, Davis, CA. 530-754-8982, awhitehead@ucdavis.edu, https://whiteheadresearch.wordpress.com/

PROFESSIONAL PREPARATION

- University of Miami, Rosenstiel School of Marine and Atmospheric Science: Post-doctoral Research Associate, Evolutionary Genomics (2003-2005).
- University of California at Davis: Ph.D., Pharmacology and Toxciology (2003).
- University of Guelph: Bachelor of Science, Honors Environmental Toxciology (1996).

APPOINTMENTS

July 2014 – present Associate Professor, Department Environmental Toxicology, University of California, Davis, CA
July 2012 – June 2014 Assistant Professor, Department Environmental Toxicology, University of California, Davis, CA
August 2011 – June 2012 Associate Professor, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA
August 2005 – August 2011 Assistant Professor, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA

5 most relevant publications

Reid, N.M., D.A. Proestou, B.W. Clark, W.C. Warren, J.K. Colbourne, J.R. Shaw, S.I. Karchner, M.E. Hahn, M.F. Oleksiak, D.L. Crawford, and A. Whitehead (in review). The genomic landscape of rapid repeated evolutionary rescue from toxic pollution in wild fish.
Cherr, G.N., E. Fairbairn, and A. Whitehead (in review). Impacts of petroleum-derived pollutants on fish development. *Annual Review of Animal Biosciences*.
Reid, N.M., C.E. Jackson, D. Gilbert, P. Minx, M.J. Montague, T.H. Hampton, L.W. Helfrich, B.L. King, D. Nacci, N. Aluru, S.I. Karchner, J.K. Colbourne, M.E. Hahn, J.R. Shaw, M.F. Oleksiak, D.L. Crawford, W.C. Warren, and A. Whitehead (in review). The Atlantic killifish (*Fundulus heteroclitus*) genome and the landscape of genome variation within a population.
Whitehead, A., W. Pilcher, D. Champlin, and D. Nacci (2012). Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings of the Royal Society B,* 279(1728): 427-433.
Whitehead, A., B. Dubansky, C. Bodinier, T. Garcia, S. Miles, C. Pilley, V. Raghunathan, J. Roach, N. Walker, R. Walter, C.D. Rice, and F. Galvez (2012). Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes. *PNAS*. 109(50): 20298-20302.

Appendix E

5 additional relevant publications

Pilcher, W., S. Miles, S. Tang, G. Mayer, and A. Whitehead (2014). Genomic and genotoxic responses to controlled weathered-oil exposures confirm and extend field studies on impacts of the Deepwater Horizon oil spill on native killifish. *PLoS One,* 9(9): e106351.

Dubansky, B., A. Whitehead, J.T. Miller, C.D. Rice, and F. Galvez (2013). Multitissue molecular, genomic, and developmental effects of the Deepwater Horizon oil spill on resident Gulf killifish (*Fundulus grandis*). *Environmental Science and Technology*, 47: 5074-5082.

Whitehead, A., J. Roach, S. Zhang, and F. Galvez (2011). Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. *PNAS,* 108(15): 6193-6198.

Whitehead, A. (2012). Comparative genomics in ecological physiology: Toward a more nuanced understanding of acclimation and adaptation. *Journal of Experimental Biology*, 215: 884-891.

Whitehead, A, and D.L. Crawford (2006). Neutral and adaptive variation in gene expression. *PNAS,* 103: 5425-5430.

Collaborators and Co-Authors:

Ken Able (Rutgers), Celia Chen (Dartmouth), Gary Cherr (UC Davis), Bryan Clark (US EPA), John Colbourne (Birmingham), Douglas Crawford (U. Miami), Benjamin Dubansky (UNT), David Duvernell (SIUE), Joel Fodrie (UNC), Rebecca Fuller (U Illinois), Fernando Galvez (LSU), Christopher Green (LSU), Mark Hahn (WHOI), Olaf Jensen (Rutgers), Benjamin King (MDIBL), Genevieve Kozak (U Illinois), Seth Kullman (NCSU), Greg Mayer (TTU), Scott Miles (LSU), Diane Nacci (US EPA), Roger Nisbet (UCSB), Marjorie Oleksiak (U. Miami), Charles Rice (Clemson), Patricia Schulte (UBC), Joseph Shaw (Indiana U), Bruce Stanton (Dartmouth), Richard Stevens (TTU), Eugene Turner (LSU), Ronald Walter (TSU), Wes Warren (Washington U)

Paul K. Hershberger, Ph.D.
U.S. Geological Survey - Marrowstone Marine Field Station
616 Marrowstone Point Road, Nordland, WA 98358
Telephone: (360) 385-1007, Ext 225, Email: phershberger@usgs.gov

Education:

Ph.D. Fisheries, University of Washington: 1998
M.S. Fisheries, University of Washington: 1995
B.S. Chemistry & Biology, Northland College (Manga Cum Laude): 1993

Recent Positions

2003 - Present: Station Leader & Research Fishery Biologist
USGS Marrowstone Marine Station
2010 – Present: Affiliate Associate Professor
School of Aquatic and Fishery Sciences, University of Washington
2014 – 2015: Past-President, American Fisheries Society, Fish Health Section
2013 – 2014: President, American Fisheries Society, Fish Health Section
2012 –2013: President Elect, American Fisheries Society, Fish Health Section
2011 – 2012: Vice President, American Fisheries Society, Fish Health Section
2004 – 2010: Affiliate Assistant Professor:
School of Aquatic and Fishery Sciences, University of Washington.

Appendix E

Five Publications Relevant to this Proposal:

Gregg, J.L., R.L. Powers, M.K. Purcell, C.S. Friedman, P.K. Hershberger. 2016. *Ichthyophonus* parasite phylogeny based on ITS rDNA structure prediction and alignment identifies six clades, with a single dominant marine type. Diseases of Aquatic Organisms 120: 125-141.

Hart, L.M., C.M. Conway, D.G. Elliott, P.K. Hershberger. 2016. Persistence of external signs in Pacific herring *Clupea pallasii* with ichthyophoniasis. Journal of Fish Diseases 39: 429-440.

Hershberger, P.K., K.A. Garver, J.R. Winton. 2016. Principles Underlying the Epizootiology of Viral Hemorrhagic Septicemia in Pacific Herring and other Fishes throughout the North Pacific Ocean. Canadian Journal of Fisheries and Aquatic Sciences. 73: 853-859.

Hershberger, P.K., J.L. Gregg, L.M. Hart, S. Moffitt, R. Brenner, K. Stick, E. Coonradt, T. Otis, J. J. Vollenweider,K. A. Garver, J. Lovy, T.R. Meyers. 2016. The parasite *Ichthyophonus* sp. in Pacific herring. Journal of Fish Diseases 39: 309-410.

Conway, C.M., M.K. Purcell, D.G. Elliott, P.K. Hershberger. 2015. Detection of *Ichthyophonus* by chromogenic *in situ* hybridization. Journal of Fish Diseases 38: 853-857.

Five Additional Publications

Purcell, M.K., S. Pearman-Gillman, R.L. Thompson, J.L Gregg, L.M. Hart. J.R. Winton, E.J. Emmenegger, P.K. Hershberger. 2016. Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of quantitative real-time PCR assays for quantification of ENV DNA. Journal of Veterinary Diagnostic Investigation 28: 382-391.

Friend, S.E., J. Lovy, P.K. Hershberger. 2016. Disease surveillance of Atlantic herring: molecular characterization of hepatic coccidiosis and a morphological report of a novel intestinal coccidian. Diseases of Aquatic Organisms 120: 91-107.

Fuess, L.E., M.E. Eisenlord, C.J. Closek, A.M. Tracy, R. Mauntz, S. Gignoux-Wolfsohn, M.M. Moritsch, R. Yoshioka, C.A. Burge, C.D. Harvell, C.S. Friedman, I. Hewson, P.K. Hershberger, S.B. Roberts. 2015. Up in Arms: Immune and Nervous System Response to Sea Star Wasting Disease. PLoS ONE 10(7): e0133053. doi:10.1371/journal.pone.0133053.

Hershberger, P.K., L.M. Hart, A.H. MacKenzie, M.L. Yanney, C. Conway, D. Elliott. 2015. Infecting Pacific herring with *Ichthyophonus* sp. in the laboratory. Journal of Aquatic Animal Health 27: 217-221.

Burge, C. A., C. M. Eakin, C. S. Friedman, B. Froelich, P. K. Hershberger, E. E. Hofmann, L. E. Petes, K. C. Prager, E. Weil, B. L. Willis, S.E. Ford, C. D. Harvell. 2014. Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science 6: 249-277.

Recent PI Collaborators and Co-Authors (Past 5 years):

E. Bromage (U. Mass – Dartmouth), C. Burge (U. Maryland), C. Closek (Penn State U.), D. Elliott (USGS), M. Eakin (NOAA Coral Reef Watch), E. Emmenegger (USGS), B. Foelich (UNC – Chapel Hill), S. Ford (Rutgers U.), C. Friedman (U. Washington),L. Fuess (U. Texas – Arlington), A. Gannam (USFWS), K. Garver (DFO), F. Goetz (NOAA – Fisheries), J. Hansen (USGS), C.D. Harvell (Cornell U.), I. Hewson (Cornell U.), E. Hofmann (Old Dominion U.), R. Kocan (UW-SAFS), G. Kurath (USGS), S. LaPatra (Clear Springs Foods), N. Lorenzen (Danish National Veterinary Institute), J. Lovy (New Jersey F&W), M. Mesa (USGS), T. Meyers (ADF&G), M. Moritsch (Northeastern U.), K. Prager (UCLA), M. Purcell (USGS), L. Rhodes (NOAA – Fisheries), S. Roberts (U. Washington), K. Toohey-Kurth (U. Wisconsin), E. Weil (U. Pureto Rico), J. Willis (James Cook U.), J. Winton (USGS).

4. Project Design

A. Objectives

Appendix E

List the objectives of the proposed research and briefly state why the intended research is important. If your proposed project builds on recent work, provide detail on why the data set needs to be continued. If the proposed project is for new work, explain why the new data is needed. Describe the anticipated final product. Include a brief scientific literature review that covers the most significant previous work history related to the project.

B. Procedural and Scientific Methods

For each objective listed in A. above, identify the specific methods that will be used to meet the objective. In describing the methodologies for collection and analysis, identify measurements to be made and the anticipated precision and accuracy of each measurement and describe the sampling equipment in a manner that permits an assessment of the anticipated raw-data quality.

If applicable, discuss alternative methodologies considered, and explain why the proposed methods were chosen. In addition, projects that will involve the lethal collection of birds or mammals must comply with the EVOSTC's policy on collections, available on our website www.evostc.state.ak.us

C. Data Analysis and Statistical Methods

Describe the process for analyzing data. Discuss the means by which the measurements to be taken could be compared with historical observations or with regions that are thought to have similar ecosystems. Describe the statistical power of the proposed sampling program for detecting a significant change in numbers. To the extent that the variation to be expected in the response variable(s) is known or can be approximated, proposals should demonstrate that the sample sizes and sampling times (for dynamic processes) are of sufficient power or robustness to adequately test the hypotheses. For environmental measurements, what is the measurement error associated with the devices and approaches to be used?

D. Description of Study Area

Where will the project be undertaken? Describe the study area, including, if applicable, decimally-coded latitude and longitude readings of sampling locations or the bounding coordinates of the sampling region (e.g., 60.8233, -147.1029, 60.4739, -147.7309 for the north, east, south and west bounding coordinates).

4A. OBJECTIVES

Aim 1 objective: *Generate insight into the genetic changes that contributed to, or resulted from, the Pacific herring collapse in PWS*. Over the past two decades it has become increasingly appreciated that population genetic change can happen over very short time scales, including adaptive evolution and changes in genetic diversity. Population genetic characteristics (e.g., allelic diversity, signatures of selection) can illuminate historical processes, can indicate phenotypic attributes that matter for contemporary environments, and can influence the future evolutionary trajectory of populations. By characterizing patterns of genetic change through time in PWS fish, and reference population fish, we can both test and generate hypotheses about the causes and consequences of Pacific herring population crash following the EVOS. Furthermore, a reference genome will serve as an important research tool for the next generation of conservation and fisheries management research in Pacific herring (Wenne et al. 2007; Ouborg et al. 2010; Benestan et al. 2016). Evidence uncovered for EVOS/collapse associated natural selection or contraction of allelic diversity in PWS could help guide restoration efforts.

Aim 2 objective: *Test whether early-life exposure to oil compromises the ability of subsequent life stages to mount an effective immune response to common pathogens endemic to PWS herring*. Much research has focused on the direct effects of crude oil, including low concentrations that may persist as lingering oil for long periods of time, on fish development (e.g., (Cherr et al. in review)). However less

is known of how oil may interact with other stressors (Whitehead 2013), including those normally encountered by natural populations such as exposure to pathogens and disease.

Aim 3 objective: *Test whether the PWS population varies from other populations in their ability to tolerate oil exposure during early life and/or to mount a robust protective immune response to viral pathogen*. 21st century toxicology and public health science is starting to acknowledge the importance of genetic variation in determining individual and population variation in sensitivity to environmental toxicants and disease (National Research Council 2007). 21st century evolutionary biology is recognizing how swiftly genetic characteristics may evolve within populations (Hendry et al. 2008; Alberti 2015), especially those in human-altered environments. The comparative studies proposed here will provide key insights into how recent eco/evolutionary dynamics have shaped population differences in response to environmental stress, and therefore provide a mechanistic basis for understanding variation in contemporary population health.

This research is important because the causes of the Pacific herring collapse in PWS, and reasons for lack of recovery, remain a mystery. Much information about individual and population characteristics, and historical demographic processes, are archived within the genome. Modern high-throughput genomics and mature population genetic theory offer the tools to shed light on the historical phenomena the have influenced the evolutionary and demographic trajectories of populations. In our research group, these tools have revealed striking genetic, evolutionary, and demographic mechanisms that have recently shaped killifish populations living in human-altered environments (Reid et al. in review-b), some of which were predicted and some of which were not anticipated. We expect similar insights, including some unexpected surprises, to emerge from the multi-population retrospective population genomic and functional genomic analyses proposed here.

4B. PROCEDURAL and SCIENTIFIC METHODS

Aim 1: A key resource for all genomics analyses is a high-quality reference genome. The state-of-the art for complex eukaryote genome sequencing and assembly is using Pacific Biosciences SMRT sequencing technology (PacBio). We will extract high molecular weight DNA in the Whitehead laboratory and make libraries appropriate for PacBio sequencing. The sequencing core facility at UC Davis will do the sequencing. We will collaborate with the UC Davis Bioinformatics Core for genome assembly. This group is highly experienced in using the latest technologies and algorithms to assemble complex eukaryote genomes.

For population genomics, frozen tissue collections maintained by the Alaska Department of Fish and Game (personal communication with collection curator Chris Habicht) include sufficient numbers of well-preserved (frozen) tissue samples from at least four relevant time points over the past 27 years, such that we can track genetic change through time. Adult tissues (e.g., muscle, liver) are available from 1989 (pre-collapse; fish born pre-EVOS), 1996 (post-collapse; many fish born post-EVOS), 2006 (post-collapse; most fish born post-EVOS), and contemporary samples. We will sequence genomes of 50 individuals per population (10X coverage per individual) per time point for the PWS population and a population of herring outside of PWS that was not exposed to the EVOS and did not experience a collapse during the past 27 years (reference population – from Sitka Sound). Modern high-throughput and massively parallel sequencing technologies make this task feasible. Indeed, our group sequenced, assembled, and annotated a high-quality reference genome and whole genomes of nearly 400 additional killifish in 2012, and the technology has more than doubled in throughput in just the past year. The Whitehead lab has also mastered high-throughput and low-cost techniques for sequencing library preparation. In addition, long-read technologies now available are vastly improving the *de novo*

assembly of large and complex eukaryotic reference genomes (Gordon et al. 2016) at very low cost. We propose to sequence and assemble a reference genome of Pacific herring using Pacific Biosciences SMRT sequencing technology (Falcon assembler coupled with consensus algorithm Quiver (Chin et al. 2013)), and re-sequence population samples using Illumina HiSeq4000 technology. We will map Illumina reads to the reference genome using BWA-MEM (Li and Durbin 2009), call variants using GATK (McKenna et al. 2010), filter SNPs that have minor allele frequencies <0.05, and calculate population genetics statistics (e.g., $F_{ST,}$ nucleotide diversity, Tajima's D) for each locus and in 5 kb and 50 kb sliding windows.

We will scan population genomes for genetic changes associated with the location and timing of the EVOS and subsequent rapid population decline. Specifically, we will test for signatures of selection including allele frequency differentiation, local reduction in nucleotide diversity, and shifts in the allele frequency spectrum. We will also test for impacts on genome-wide genetic diversity, with particular focus on immune system genes. We will test whether population genetic change appears across the pre-to-post collapse sampling times in the PWS population, but with no changes in the control population, to establish strong scope for inferring cause and consequence. The population genetic theory for detecting selection in time series data is mature (Schraiber et al. 2016).

Aim 2: We will collect naturally deposited embryos from wild herring (Puget Sound) to generate pathogen-free groups of animals. We will expose embryos to sub-lethal concentrations of weathered oil during embryogenesis at two doses ("high" = concentrations comparable to peak field exposures in 1989; "low" = concentrations comparable to lingering oil concentrations in years subsequent to 1989) plus control (no oil). Embryos will be exposed from shortly after fertilization through to post-organogenesis to the water soluble fraction of Alaska North Slope crude oil using oil-coated gravel generator columns as previously described (Marty et al. 1997) and recently used for herring studies (e.g., (Incardona et al. 2012; Incardona et al. 2015)). Heart rate and heart morphology will be measured pre-hatch as sensitive measures of the developmental impacts of crude oil (e.g., (Dubansky et al. 2013; Incardona et al. 2015)). Hatched fish from these three exposure treatments will be raised in clean water. We will then test responses of three subsequent developmental stages to pathogen challenge (larvae, juvenile, adult). The effect of embryonic exposure to oil on susceptibility to common herring pathogens of PWS will be assessed using standardized exposure procedures for VHSV and *Ichthyophonus* (Hershberger et al. 2010; Hershberger et al. 2015). We will measure ability to mount robust protective antibody response following exposure to pathogens. We will also measure genome-wide gene expression in blood and liver in response to pathogen challenge to infer molecular pathways and systems that are functionally important during pathogen exposure.

Aim 3: Methods and procedures will be the same as for Aim 2, but repeated with multiple populations. We will then compare responses to oil and pathogen exposures between populations. Populations to include are PWS (focal population), Sitka Sound (reference population, closely related to PWS), and Puget Sound (reference population, more distantly related to PWS). We are particularly interested in responses that differ between the PWS population and the two reference populations. Population-dependent responses will be detected using 2-way ANOVA with source population as one main effect and exposure response variable (e.g., cardiac measurements, immune measurements, gene expression) as the second main effect.

4C. DATA ANALYSIS and STATISTICAL METHODS

Reference genome: The genome sequence will be assembled using the Falcon assembler coupled with consensus algorithm Quiver (Chin et al. 2013), and error corrected with Illumina data. Gene models will

be characterized using an evidence-based approach, which uses Illumina RNA-seq data and orthology analysis, and will be implemented using Augustus (Stanke et al. 2006). For these purposes RNA-seq data will be assembled into a reference transcriptome using Trinity (Haas et al. 2013) and mapped to the reference genome using PASA (Haas et al. 2003). Orthology to other fish gene model sets (e.g., Atlantic herring, stickleback, zebrafish, killifish, pufferfish, Japanese medaka, Amazon molly) will be determined using OrthoMCL (Li et al. 2003). Functional annotation of genes will be achieved through orthology with genes in the UniProt database.

Population genomics: We will map Illumina reads to the reference genome using BWA-MEM (Li and Durbin 2009), call variants using GATK (McKenna et al. 2010), filter SNPs that have minor allele frequencies <0.05, and calculate population genetics statistics (e.g., $F_{ST,}$ nucleotide diversity) for each locus and in 5 kb and 50 kb sliding windows. We will use the software package ANGSD (Korneliussen et al. 2014) to estimate the summary statistics π, Tajiima's D and F_{ST}. We will estimate demographic models for each population using the Python module dadi and folded allele frequency spectra estimated using ANGSD as input. To identify candidate regions associated with collapse-associated or EVOS-associated events, we will scan the PWS genome for canonical signals of selective sweeps that distinguish pre-EVOS/collapse from post-EVOS/collapse populations: reduction in genetic diversity (measured by π), a skew in the allele frequency spectrum (measured by Tajima's D) and high allele frequency differentiation (F_{ST}). Genomic regions that show time-course patterns associated with the EVOS/collapse in the PWS population, but not in the reference population, will be considered key candidates. Reductions in genome-wide or gene-specific allelic diversity that temporally correlates with the EVOS/collapse in the PWS population but not the reference population will also provide evidence to test our hypotheses.

RNA-seq from exposure experiments: We will quality trim reads using Trimmomatic (Bolger et al. 2014) according to recommendations in (MacManes 2014). We will align reads to the reference genome using TopHat (Trapnell et al. 2009) and count reads falling in annotated gene regions using featureCounts (Liao et al. 2013) and test for differential expression using the quasi-likelihood method (Lund et al. 2012) implemented in edgeR (Robinson et al. 2010) and retain as differentially expressed genes with p-values that put their false discovery rate below 5%. We will test for genes that show treatment effects associated with oil exposure, and treatment effects associated with pathogen challenge, with particular focus on genes that show population-dependent responses to these experimental variables (significant interaction).

Physiological effects from exposure experiments: Standard statistical comparisons for pathogen virulence studies will be employed in all experiments. For example, percent cumulative mortalities in replicate tanks / aquaria will be arc sin transformed and transformed means from all groups will be statistically compared using Student's T-test (1-tailed) or ANOVA followed by the Tukey test for multiple comparisons. In non-replicated tanks, percent mortality in control and treatment groups will be statistically compared using the Chi Square statistic ($\chi 2$). Statistical significance will be assigned to all comparisons with $p < 0.05$.

4D. DESCRIPTION of STUDY AREA

Newly deposited and fertilized herring eggs will be collected from actively spawning aggregations in Prince William Sound, Sitka Sound, and Puget Sound. Fertilized eggs will be chilled to 4C to delay development and shipped to the USGS Marrowstone Marine Field Station where they will be separated into respective exposure treatment groups. Collaborator Hershberger has procured and worked with

these populations currently and in the past. As such, results from proposed experiments will be comparable to those that are already published.

We will perform population genomics analysis on two Pacific herring populations, one from Prince William Sound and the second reference population is Sitka Sound. Tissues from PWS were collected from the Montague Island area (e.g., Stockdale Harbor, 60°18'52.00"N, 147°11'27.00"W; Zaikof Bay, 60°18'22.27"N, 147° 0'39.39"W). Tissues from the reference population were collected from Sitka Sound (57° 7'12.00"N, 135°28'12.00"W).

5. Coordination and Collaboration

With Other EVOSTC-funded Programs and Projects

Indicate how your project relates to, complements or includes collaborative efforts with other proposed or existing programs or projects funded by the EVOSTC.

With Trustee or Management Agencies

Please discuss if there are any areas which may support EVOSTC trust or other agency work or which have received EVOSTC trust or other agency feedback or direction, including the contact name of the agency staff. Please include specific information as to how the subject area may assist EVOSTC trust or other agency work.

If the proposed project requires or includes collaboration with other agencies, organizations or scientists to accomplish the work, such arrangements should be fully explained and the names of agency or organization representatives involved in the project should be provided. If your proposal is in conflict with another project or program, note this and explain why.

With Native and Local Communities

Provide a detailed plan for local and native community involvement in the project if applicable.

5. COORDINATION and COLLABORATION

This project is to be conducted in close collaboration with EVOSTC funded investigator Dr. Paul Hershberger (USGS). In particular, PI Whitehead and Hershberger will coordinate laboratory exposure experiments, which are to be conducted at Dr. Hershberger's research facilities (USGS-Marrowstone Marine Field Station), which are ideally designed to safely and responsibly conduct experiments using fish pathogens. Marrowstone facilities include three large wet laboratory buildings with approximately 10,000 square feet of wet laboratory space, replicated with approximately 60,000 liter tank capacity, and supplied with 400 gpm of high quality filtered and UV irradiated seawater. Back-up, redundant water treatment systems are incorporated into the supply water for each wet laboratory. Separate laboratory buildings are designated as specific pathogen-free nursery zones and experimental pathogen manipulation zones. Laboratory effluent water is disinfected with chlorine and treated to insure safe and responsible handling of endemic pathogens. Dr. Hershberger will collect the herring for these experiments from Alaska and Puget Sound in coordination with his ongoing EVOSTC-funded disease research activities.

PI Whitehead has contacted, and sought feedback from, established toxicologists and herring biologists with extensive experience and expertise on the EVOS, including Ron Heintz (NOAA, Auke Bay), Mark Carls (NOAA, Auke Bay), and Steve Moffitt (Alaska Department of Fish & Game). Initial contact with these researchers sought input and advice, and they were all enthusiastic and very helpful for formulating ideas and tracking down research resources. PI Whitehead plans to share data and findings with these and other researchers as the program unfolds. PI Whitehead has also sought input from Sharon Wildes (NOAA, Auke Bay) who is an expert in

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Pacific herring population genetics. She has been enthusiastic about the research ideas presented here, and has provided input on populations to use as a reference population for PWS and information on sourcing of tissues. PI Whitehead will provide periodic updates to Sharon Wildes and share population genomics data and findings. PI Whitehead has contacted Chris Habicht who is director for the Alaska Department of Fish & Game Gene Conservation Laboratory, which is the primary resource for historical and contemporary tissues preserved from Pacific herring from the sites and time points that make the retrospective comparative population genomics study possible. Habicht, and Judy Berger who is the tissue archivist in his group, have been very helpful in locating preserved tissue samples for this study, and they have communicated that the tissues would be available to PI Whitehead if this project were to proceed. PI Whitehead has established initial contact with other potentially interested parties to make them aware of project goals and objectives, including the Sitka Sound Science Center (Lisa Busch), and will cultivate relationships with these groups and others as the project unfolds.

6. Schedule

Project Milestones

Specify when critical project tasks will be completed. Reviewers will use this information in conjunction with annual program reports to assess whether the program is meeting its objectives and is suitable for continued funding.

Measurable Project Tasks

Specify, by each quarter of each fiscal year (February 1 – January 31), when critical project tasks will be completed.

6A. PROJECT MILESTONES

- *Production of a reference genome for Pacific herring (Clupea pallasii), including comparative analysis with Atlantic herring and other fish genomes*. To be started in FY1 and completed in FY2.
- *Provision of comparative oil and disease interaction exposure studies for multiple Pacific herring populations*. To be completed in FY2.
- *Production of sequenced genomes for population comparative time-course*. To be completed in FY3.
- *Production of functional genomics data coupled with comparative oil and disease interaction studies*. To be completed in FY3.
- *Population and functional genomics analyses to test hypotheses*. To be completed in FY4 and beginning of FY5.

6B. MEASURABLE PROJECT TASKS

FY1, Q1: Acquire tissue samples from Alaska Fish & Game (contact: Chris Habicht) for population genomics and for reference genome.
FY1, Q2: Extract genomic DNA and prepare libraries for reference genome sequencing.
FY1, Q3: Extract genomic DNA and prepare libraries for population genomics. Sequence reference genome.
FY1, Q4: Continue preparing libraries for population genomics. Assemble reference genome.

FY2, Q1: Collect herring eggs for laboratory exposure experiments. Sequence population genomics libraries.
FY2, Q2: Conduct laboratory exposure experiments (at Marrowstone).
FY2, Q3: QA/QC of population genomics data. Extract RNA from laboratory exposure experiments and prepare libraries for functional genomics (RNA-seq). Analyze developmental physiology and disease data from exposure experiments (Marrowstone).
FY2, Q4: Finish reference genome assembly and validation, and upload reference genome to NCBI.

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FY3, Q1: Start read mapping and variant calling for population genomics. Start annotation and comparative analysis of reference genome.
FY3, Q2: Sequence RNA-seq libraries.
FY3, Q3: QA/QC of population genomics data.
FY3, Q4: Draft and submit reference genome release manuscript.

FY4, Q1: RNA-seq and population genomics data analysis.
FY4, Q2: RNA-seq and population genomics data analysis.
FY4, Q3: RNA-seq and population genomics data analysis.
FY4, Q4: RNA-seq and population genomics data analysis.

FY5, Q1: RNA-seq and population genomics data analysis.
FY5, Q2: Draft and submit population genomics and exposure and RNA-seq manuscripts, start drafting final report.
FY5, Q3: Prepare and upload all data to online repositories (NCBI).
FY5, Q4: Respond to peer review comments, acceptance of publications and final report.

7. Budget

Budget Forms (Attached)

Please provide completed budget forms. Please note that the following items will not be considered for funding:

- Costs associated with international travel for meetings, symposia, or presentations.
- Costs associated with attendance at meetings, symposia, or presentations outside of those required to coordinate with project members.
- Costs associated with outreach or education efforts.

Sources of Additional Funding

Identify non-EVOSTC funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

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BUDGET JUSTIFICATION

Personnel Costs

FY17
One month summer salary plus benefits is requested for PI Andrew Whitehead ($10,353 + $1,815). Funding is requested to support a technician (Staff Research Associate Jennifer Roach) at 75% time including benefits ($42,482 + $22,165). Ms. Roach is a highly trained and experienced molecular biologist and laboratory manager. She is highly trained in the advanced molecular biology techniques crucial for the success of this project, including the preparation of hundreds of sequencing libraries. Ms. Roach's work in the Whitehead lab in the past 2 years has been to dramatically increase the throughput and decrease the cost of library preparation. This has led to an order-of-magnitude decrease in cost (and parallel increase in throughput) for library preparation compared to what is charged by genome centers. Since the proposed work includes preparation of many genomic DNA and RNA libraries, Ms. Roach's contribution is important. Feasibility of our methods for the work proposed here is evidenced by our recent sequencing of a high-quality reference genome plus 400 additional killifish genomes. In addition, our lab regularly prepares hundreds of RNA-seq libraries for multi-factor and highly replicated experiments. Therefore the advanced molecular biology skills and experience contributed by Ms. Roach is important for the proposed research. Ms. Roach is also an experienced laboratory manager. Her key responsibilities will be to assist with laboratory studies, process samples from studies, extract and purify DNA and RNA from samples, and prepare DNA libraries for reference genome and population genomics sequencing and RNA libraries for RNA-seq sequencing. She will also contribute to training of students and postdocs, assist in data QA/QC, data management, and data archiving, and sample archiving. Salaries are increased by 3% each fiscal year.

FY18
One month summer salary plus benefits is requested for PI Andrew Whitehead ($10,663 + $1,922). Funding is requested to support a technician (Staff Research Associate Jennifer Roach) at 75% time including benefits ($43,756 + $23,511). Ms. Roach's responsibilities will be the same as the previous year. Funding is requested to support a UC Davis Ph.D. student stipend plus benefits ($24,687 + 321). The proposed exposure experiments, which include the effects of oil and pathogens on development and immune function, and which integrate both physiological, developmental, immunological, and functional genomic endpoints, will provide the foundation for a robust Ph.D. thesis. The Ph.D. student's responsibilities will be to help plan, coordinate, and conduct animal exposure experiments, and conduct resulting data analysis, and contribute to authoring manuscripts. Funding is requested to support 25% of a technician salary + benefits for three months ($12,810) for assistance with animal exposure experiments. This technician is an employee of collaborator Dr. Paul Hershberger (USGS, Marrowstone Marine Field Station) who is currently funded by EVOSTC. Since these funds are to be sent to Dr.

Hershberger's group they are included in the "Trustee Agency Sheets" portion of the budget.

FY19
One month summer salary plus benefits is requested for PI Andrew Whitehead ($10,983 + $2,035). Funding is requested to support a technician (Staff Research Associate Jennifer Roach) at 75% time including benefits ($45,069 + $24,939). Ms. Roach's responsibilities will be the same as the previous year. Funding is requested to support a UC Davis Ph.D. student stipend plus benefits ($26,191 + 341), whose responsibilities will be the same as the previous year. Funding is requested to support at postdoctoral research associate salary and benefits ($51,249 + $9,528). The population genomics sequence data will be available by this point in the project, and a post-doctoral research associate will be recruited to carry out the sophisticated population genomics analysis. This will be a data analysis task on par with our recent analysis of 400 killifish genomes, which required 2 years of full-time dedicated analysis by a well-trained post-doctoral research associate, including an additional year to summarize, write up, and publish results. The post-doctoral research associate's responsibilities will be to contribute to genome annotation and finishing and comparative genome analysis and population genomics data analysis and interpretation and writing of manuscripts.

FY20
One month summer salary plus benefits is requested for PI Andrew Whitehead ($11,313 + $2,158). Funding is requested to support a technician (Staff Research Associate Jennifer Roach) at 75% time including benefits ($46,421 + $26,457). Ms. Roach's responsibilities will be the same as the previous year. Funding is requested to support a UC Davis Ph.D. student stipend plus benefits ($26,191 + 341), whose responsibilities will be the same as the previous year. Funding is requested to support at postdoctoral research associate salary and benefits ($52,786 + $10,109) whose responsibilities will be the same as the previous year.

FY21
One month summer salary plus benefits is requested for PI Andrew Whitehead ($11,652 + $2,292). Funding is requested to support a technician (Staff Research Associate Jennifer Roach) at 75% time including benefits ($47,813+ $28,062). Ms. Roach's responsibilities will be the same as the previous year. Funding is requested to support a UC Davis Ph.D. student stipend plus benefits ($26,976 + 351), whose responsibilities will be the same as the previous year. Funding is requested to support at postdoctoral research associate salary and benefits ($54,370 + $10,738) whose responsibilities will be the same as the previous year.

Travel Costs

FY17
No funds requested

FY18
Funding is requested to support travel of a UC Davis Ph.D. student to collaborator Paul Hershberger's lab (Marrowstone Marine Field Station, Nordland, WA) to conduct animal exposure experiments. Travel costs included mileage reimbursement from UC Davis to Marrowstone lab return ($0.56 per mile * 1,576 miles = $882.60) and lodging ($10 per day at Marrowstone dorms for 30 days = $300).

FY19
No funds requested

FY20
No funds requested

FY21
No funds requested

Contractual Costs

FY17
No funds requested

FY18
No funds requested

FY19
No funds requested

FY20
No funds requested

FY21
No funds requested

Commodities

FY17
Funds are requested to support reference genome sequencing, including PacBio SMRT library preparation ($470) and sequencing 64 SMRT cells ($20,081) for ~75X coverage of the expected 0.85 Gb genome. Funds are requested to support the first stage of the population genomics project, which includes DNA extraction and Illumina library preparation for ~400 tissue samples ($32,000; $80 per sample is considerably less than what genome centers would charge). Funds are requested for assistance in genome assembly by the UC Davis Genome Center Bioinformatics Core analysis group. These funds will support a bioinformatics analyst's time (estimated 20 hours analyst time, at $97 per hour UC Davis preferred rate) and their

responsibilities include read processing, de novo assembly, error correction, scaffolding, gap filling, error correction with Illumina data, and assembly cleaning and submission to NCBI.

FY18
Funds are requested for Illumina sequencing of the 400 libraries prepared in FY17 for the population genomics data collection (38 lanes; $94,444). The UC Davis Genome Center per-lane sequencing costs (for UC Davis faculty) are among the lowest available, and the Whitehead lab has a good working relationship with the UC Davis Genome Center – they produce high sequencing yields and rapid turn-around times. Funds are requested for RNA-seq library preparation following animal exposure experiments (360 samples; $10,800). The Whitehead laboratory has optimized RNA-seq library preparation methods such that we can prepare libraries at a fraction of the cost of what is charged by most genome centers (e.g., $30 per library compared to nearly $300 per library). Funds are requested for UC Davis graduate student tuition and fees ($14,509) which do not contribute to the base for indirect costs. Funding is requested for Dr. Paul Hershberger's group for laboratory supplies to support animal exposure experiments ($5,000 for fish food and dry lab supplies). Since these funds are to be sent to Dr. Hershberger's group, and he is a USGS employee (Marrowstone Marine Field Station) currently funded by EVOSTC, they are included in the "Trustee Agency Sheets" portion of the budget.

FY19
Funds are requested for Illumina sequencing of the 360 RNA-seq libraries prepared in FY18 (23 lanes; $56,250). Funds are requested for UC Davis graduate student tuition and fees ($15,960) which do not contribute to the base for indirect costs.

FY20
Funds are requested for UC Davis graduate student tuition and fees ($17,555) which do not contribute to the base for indirect costs.

FY21
Funds are requested for UC Davis graduate student tuition and fees ($19,311) which do not contribute to the base for indirect costs.

New Equipment / Existing Equipment Usage

No new equipment with a life span of more than one year and a unit value greater than $1,000 is needed or requested for this project.

INDIRECT COSTS
Per the University of California, Davis' Federally approved Indirect Cost Rate for on-campus research, the rate of 57% Modified Total Direct Cost (MTDC) has been applied to the project.

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Appendix E

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***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$76,815.0	$117,670.0	$169,562.0	$175,776.0	$182,254.0	$722,077.0	
Travel	$0.0	$1,182.6	$0.0	$0.0	$0.0	$1,182.6	
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Commodities	$54,491.0	$124,753.0	$72,210.0	$17,555.0	$19,311.0	$288,320.0	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
SUBTOTAL	$131,306.0	$243,605.6	$241,772.0	$193,331.0	$201,565.0	$1,011,579.6	
Indirect Costs (*will vary by proposer*)	$74,844.4	$120,433.3	$128,712.8	$100,192.3	$103,884.8	$528,067.7	
General Administration (9% of subtotal)	$11,817.5	$21,924.5	$21,759.5	$17,399.8	$18,140.9	$91,042.2	
PROJECT TOTAL	**$217,968.0**	**$385,963.4**	**$392,244.3**	**$310,923.1**	**$323,590.6**	**$1,630,689.4**	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	N/A

COMMENTS:
This summary page provides an five-year overview of proposed funding and actual cumulative spending. The column titled 'Actual Cumulative' must be updated each fiscal year as part of the annual reporting requirements. Provide information on the total amount actually spent for all completed years of the project. On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

FY17-21

Project Title:
Primary Investigator:

PROJECT SUMMARY PAGE

Date Prepared: xx/xx/xxxx

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$76,815.0	$104,860.0	$169,562.0	$175,776.0	$182,254.0	$709,267.0	
Travel	$0.0	$1,182.6	$0.0	$0.0	$0.0	$1,182.6	
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Commodities	$54,491.0	$119,753.0	$72,210.0	$17,555.0	$19,311.0	$283,320.0	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
SUBTOTAL	$131,306.0	$225,795.6	$241,772.0	$193,331.0	$201,565.0	$993,769.6	
Indirect Costs (*will vary by proposer*)	$ 74,844.4	$ 120,433.3	$ 128,712.8	$ 100,192.3	$ 103,884.8	$ 528,067.7	$ -
General Administration (9% of	$11,817.5	$20,321.6	$21,759.5	$17,399.8	$18,140.9	$89,439.3	N/A
PROJECT TOTAL	**$217,968.0**	**$366,550.5**	**$392,244.3**	**$310,923.1**	**$323,590.6**	**$1,611,276.5**	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	

COMMENTS:
This summary page provides an five-year overview of proposed project funding and actual cumulative spending. The column titled 'Actual Cumulative' must be updated each fiscal year as part of the annual reporting requirements. Provide information on the total amount actually spent for all completed years of the project. On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

FY17-21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

NON-TRUSTEE AGENCY SUMMARY PAGE

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Andrew Whitehead	Principal Investigator	1.0	12168.0		12,168.0
Jennifer Roach	Staff Research Associate	9.0	7183.0		64,647.0
					0.0
					0.0
		Subtotal	19351.0	0.0	
				Personnel Total	$76,815.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Principal Investigator: Andrew Whitehead

FORM 3B
PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
Reference genome sequencing	20,551.0
Population genomics (DNA extraction and Illumina library preparation)	32,000.0
Genome sequence assembly (UC Davis Genome Center Bioinformatics Core)	1,940.0
Commodities Total	$54,491.0

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Principal Investigator: Andrew Whitehead

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Principal Investigator: Andrew Whitehead

FORM 3B
EQUIPMENT DETAIL

Personnel Costs: Name	Project Title	Months Budgeted	Monthly Costs	Overtime	Personnel Sum
Andrew Whitehead	Principal Investigator	1.0	12585.0		12,585.0
Jennifer Roach	Staff Research Associate	9.0	7474.1		67,267.0
TBD	Graduate Student	12.0	2084.0		25,008.0
					0.0
					0.0
					0.0
		Subtotal	22143.1	0.0	
				Personnel Total	$104,860.0

Travel Costs: Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
UCD Ph.D. student to Marrowstone lab for exposure experiments (dri	882.6	1	30		882.6
Student stay at Marrowstone in dorms ($10 per day for 30 days)	10.0	30	30		300.0
					0.0
					0.0
					0.0
				Travel Total	$1,182.6

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Principal Investigator: Andrew Whitehead

FORM 3B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
UC Davis graduate student tuition and fees	14,509.0
Population genomics (Sequencing)	94,444.0
RNA-seq (RNA extraciton and Illumina library preparation)	10,800.0
Commodities Total	$119,753.0

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
EQUIPMENT DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Andrew Whitehead	Principal Investigator	1.0	13018.0		13,018.0
Jennifer Roach	Staff Research Associate	9.0	7778.7		70,008.0
TBD	Postdoctoral Resarcher	12.0	5064.8		60,777.0
TBD	Graduate Student	12.0	2146.6		25,759.0
					0.0
					0.0
		Subtotal	28008.0	0.0	
				Personnel Total	$169,562.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
UC Davis graduate student tuition and fees	15,960.0
RNA-seq (sequencing)	56,250.0
Commodities Total	$72,210.0

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
EQUIPMENT DETAIL

Personnel Costs: Name	Project Title	Months Budgeted	Monthly Costs	Overtime	Personnel Sum
Andrew Whitehead	Principal Investigator	1.0	13471.0		13,471.0
Jennifer Roach	Staff Research Associate	9.0	8097.6		72,878.0
TBD	Postdoctoral Resarcher	12.0	5241.3		62,895.0
TBD	Graduate Student	12.0	2211.0		26,532.0
					0.0
					0.0
					0.0
		Subtotal	29020.8	0.0	
				Personnel Total	$175,776.0

Travel Costs: Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY20

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Contractual Costs: Description	Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
UC Davis graduate student tuition and fees	17,555.0
Commodities Total	$17,555.0

FY20

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY20

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
EQUIPMENT DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Andrew Whitehead	Principal Investigator	1.0	13944.0		13,944.0
Jennifer Roach	Staff Research Associate	9.0	8430.6		75,875.0
TBD	Postdoctoral Resarcher	12.0	5425.7		65,108.0
TBD	Graduate Student	12.0	2277.3		27,327.0
					0.0
					0.0
					0.0
		Subtotal	30077.5	0.0	
				Personnel Total	$182,254.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
UC Davis graduate student tuition and fees	19,311.0
Commodities Total	$19,311.0

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Andrew Whitehead

FORM 3B
EQUIPMENT DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$0.0	$12,810.0	$0.0	$0.0	$0.0	$12,810.0	
Travel	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Commodities	$0.0	$5,000.0	$0.0	$0.0	$0.0	$5,000.0	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
SUBTOTAL	$0.0	$17,810.0	$0.0	$0.0	$0.0	$17,810.0	
General Administration (9% of	$0.0	$1,602.9	$0.0	$0.0	$0.0	$1,602.9	N/A
PROJECT TOTAL	**$0.0**	**$19,412.9**	**$0.0**	**$0.0**	**$0.0**	**$19,412.9**	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	

COMMENTS:
This summary page provides an five-year overview of proposed project funding and actual cumulative spending. The column titled 'Actual Cumulative' must be updated each fiscal year as part of the annual reporting requirements. Provide information on the total amount actually spent for all completed years of the project. On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

FY17-21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

TRUSTEE AGENCY SUMMARY PAGE

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
					0.0
					0.0
					0.0
		Subtotal	0.0	0.0	
				Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
Commodities Total	$0.0

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY17

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B
EQUIPMENT DETAIL

Personnel Costs: Name	Project Title	Months Budgeted	Monthly Costs	Overtime	Personnel Sum
TBD	Technician	3.0	4270.0		12,810.0
					0.0
					0.0
					0.0
		Subtotal	4270.0	0.0	
				Personnel Total	$12,810.0

Travel Costs: Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
laboratory supplies for the Marrowstone Marine Station (fish food + dry lab supplies)	5,000.0
Commodities Total	$5,000.0

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B CONTRACTUAL & COMMODITIES DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY18

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B EQUIPMENT DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
					0.0
					0.0
					0.0
					0.0
		Subtotal	0.0	0.0	
				Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
Commodities Total	$0.0

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY19

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B
EQUIPMENT DETAIL

Personnel Costs: Name	Project Title	Months Budgeted	Monthly Costs	Overtime	Personnel Sum
					0.0
					0.0
					0.0
		Subtotal	0.0	0.0	
				Personnel Total	$0.0

Travel Costs: Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY20

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Contractual Costs: Description		Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	**Contractual Total**	$0.0

Commodities Costs: Description	Commodities Sum
Commodities Total	$0.0

FY20	**Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse** **Primary Investigator: Paul Hershberger**	**FORM 4B** **CONTRACTUAL & COMMODITIES DETAIL**

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY20	**Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse** **Primary Investigator: Paul Hershberger**	**FORM 4B** **EQUIPMENT DETAIL**

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
					0.0
					0.0
					0.0
		Subtotal	0.0	0.0	
				Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B PERSONNEL & TRAVEL DETAIL

Contractual Costs:	Contract
Description	Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$0.0

Commodities Costs:	Commodities
Description	Sum
Commodities Total	$0.0

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/02/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY21

Project Title: Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse
Primary Investigator: Paul Hershberger

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/02/2016