August 11, 2016

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Dear EVOS Trustee Council,

I would like to thank the Science Panel for their thorough review of our proposal "Herring Disease Program IV." Their constructive comments were extremely insightful, and the revised proposal is much stronger as a result of their contributions. In addition to making the appropriate changes in the proposal, my responses to the most direct questions are included below:

1) There was a request to develop SPF herring colonies using stocks of herring from PWS, as inter-stock differences in susceptibility may exist. We were very concerned about this possibility during the first HDP several years ago; as a result, we previously compared the relative susceptibility of 3 genetically distinct herring stocks (including PWS) to VHS. The controlled studies showed no significant differences in susceptibility between the stocks. These results, an associated figure, and reference to the published article have been included in the revised proposal. Because all stocks responded similarly, there is no further need to raise PWS stocks. However, it is possible that the immune responses of these stocks may respond differently after their exposure to PAH's. Therefore, if Dr. Whitehead's new proposal in the Lingering Oil category is recommended for funding, then we will raise herring from the different stocks (including PWS) for his test animals.
2) There was a question about difference in infection prevalence and intensity between different herring populations. We have been performing a comparative assessment of pathogen prevalence and intensity between herring from PWS and Sitka for the past 10 years. Additionally, we assess the health of herring from other locations throughout the NE Pacific, including Puget Sound and Canada, as they are available. The results of these surveys are reported in the annual and final EVOS TC project reports.
3) Per the reviewer's request, the section on final validation and applications of the novel plaque neutralization test has been expanded.
4) There was a request explain why the Fluidigm Biomark technology is not employed in this proposal. The gene chip technology is an exciting tool that has some very specific applications. The real beauty of this tool is its provision of rapid screening platform for numerous microbes. Once presumed positive samples are identified using this technology, verification must occur using standard laboratory techniques; further, their pathogenic status must be confirmed. If the tool was applied to Pacific herring, a list of suspect microbes would certainly be created. However, we already know that herring are infected with dozens of microbes, the majority of which demonstrate negligible, if any pathogenicity. In the HDP study, we have limited our work to the investigation of pathogens that have a demonstrated history of fish kills with associated

population declines in Atlantic and Pacific herring. From a curiosity perspective, it would be interesting to understand more about some of these other herring microbes (many of which we already know exist), but there is certainly no indication that they have ever contributed to herring kills or population declines. In essence, the gene chip tool provides a great first step when investigating a new system containing little or no background information. However, our knowledge of the herring pathogens in PWS and their population-level impacts has advanced well beyond the ability of the Fluidigm Biomark technology to contribute meaningful information.

Again, I would like to thank the Science Panel for their time. Please let me know if I can answer any additional questions.

Sincerely,

Paul Hershberger

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EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
PROGRAM PROJECT PROPOSAL SUMMARY PAGE

Project Title

Prince William Sound Herring Restoration and Monitoring Program: Herring Disease Program IV

Primary Investigator(s) and Affiliation(s)

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Date Proposal Submitted

August 12, 2016

Project Abstract

Using an approach that involves a combination of field- and laboratory-based studies, we propose to investigate fish health factors that may be contributing to the failed recovery of Pacific herring populations in Prince William Sound. Field studies will provide infection and disease prevalence data that will inform the ASA model, serological data that will indicate the prior exposure history and future susceptibility of herring to VHS, and diet information that will provide insights into the unusually high prevalence of *Ichthyophonus* that occurs in juvenile herring from Cordova Harbor. Laboratory studies will validate the newly-developed plaque neutralization assay as a quantifiable measure of herd immunity, provide further understanding of disease cofactors including temperature and salinity, investigate the possibility of an invertebrate host for *Ichthyophonus*, and assess the virulence of other endemic pathogens to Pacific herring. Information from the field and laboratory studies will be integrated into the current ASA model, a novel ASA-type model that is based on the immune status of herring age cohorts, and a novel mixture-cure simulation model for VHS. The Herring Disease Program (HDP) is embedded within the Herring Research and Monitoring Program, and the success of the HDP relies heavily on contributions from companion projects with Principle Investigators including Steve Moffitt (platform for the collection of pathogen prevalence data), Dr. Kristen Gorman (collection of juvenile Pacific herring from Cordova Harbor), Dr. Trevor Branch (incorporation of pathogen and resistance information in to the ASA models). Additionally, this project relies on contributions from Principle Investigators in the Long Term Monitoring Program (Dr. Rob Campbell – zooplankton collections).

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

FY17	FY18	FY19	FY20	FY21	TOTAL
$197.8	$204.4	$212.2	$218.8	$226.6	$1,059.8

Non-EVOSTC Funding Available

FY17	FY18	FY19	FY20	FY21	TOTAL
$61.7	$63.6	$64.0	$65.2	$66.9	$321.4

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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

Identify the hypotheses the project is designed to address. Describe the background and history of the problem. Include a scientific literature review that covers the most significant previous work history related to the project. Please provide a summary of the project including key hypotheses and overall goals.

The biomass of adult herring in Prince William Sound (PWS) collapsed from 111,000-121,000 mt in 1988-1989 to 30,000 mt in 1993; since then, the population has remained depressed, fluctuating between 10,800-32,500 mt. Consequently, the PWS herring population is currently classified as an "injured resource" that is "not recovering" (EVOSTC 2002) and commercial herring fisheries have remained severely curtailed or closed. In addition to the human economic impacts of the population decline, the prolonged ecological impacts were devastating. In marine systems, particularly upwelling-driven systems like PWS, forage fishes, including Pacific herring, represent the primary energy link in the biological community, exerting both top-down control over primary and secondary production (phytoplankton and zooplankton) and bottom-up control over higher order predators (Rice 1995 and Currey et al 2000). The critical ecological position occupied by forage fishes is equally important in bridging the flow of inorganic nutrients (mobilized by primary and secondary production) and organic nutrients (utilized by higher trophic level predators).

Definitive cause(s) of the herring population decline and failed recovery in PWS remain undetermined; however, a leading hypothesis involves chronic and epizootic mortality that result from infectious and / or parasitic diseases (Marty et al 1998 and 2003). In 1993 only 20% of the anticipated adult herring biomass appeared in the known spawning areas. Returning fish were lethargic and demonstrated external hemorrhages consistent with viral hemorrhagic septicemia (VHS). The etiological agent, VHS virus (VHSV), was later isolated from moribund individuals. Subsequently, other suspected pathogens were identified in the PWS herring population, including *Ichthyophonus hoferi*, Anisakid worms, lymphocystis virus, *Goussia* sp (an intestinal parasite), *G. clupearum* (a liver parasite), a testicular coccidian, a myxosporean in the gall bladder, *Ortholinea orientalis*, *Ceratomyxa auerbachi*, *Gyrodactylus spp* (monogenean trematodes), branchial ciliated protozoans, a renal myxosporean, *Epitheliocystis*, gastric trematodes, intestinal trematodes, intestinal cestodes (Marty et al 1998), and erythrocytic necrosis virus (ENV; Hershberger et al 2009). Among the pathogens occurring in PWS herring, VHSV, *Ichthyophonus*, and ENV are considered the primary pathogens of concern because they have been associated with epizootics in populations of wild herring, pilchards, and other forage species (Meyers et al. 1986, Hershberger et al. 2009, Garver et al, 2013, Burge et al. 2014). Alternative and complementary hypotheses accounting for the herring population dynamics include competition with pink salmon for limited resources (Deriso et al 2008) and predation on herring populations by humpback whales and other predators.

The North American strain of VHSV (Genogroup IVa) is periodically associated with epizootics (Garver et al. 2013) in wild marine fishes, where it can be highly virulent (Kocan et al. 1997). Monospecific VHS epizootics involving wild Pacific herring were reported during 1994 in Port Fredrick (Alaska), 1993 in Prince Rupert Sound (British Columbia; Traxler and Kieser 1994, Meyers and Winton 1995), and presumably 1942 in the Strait of Georgia (British Columbia; Tester 1942). Epizootics of mixed host assemblages involving Pacific sardines and Pacific herring occurred during 1998-1999 in Queen Charlotte Strait (British Columbia) and 2001-2002 Kyuquot and Nootka Sounds (British Columbia; Hedrick et al. 2003); similar mixed assemblage VHS epizootics involving Pacific herring, Pacific hake, and walleye pollock occurred during 1998 in Lisianski Inlet (Alaska; Meyers et al. 1999). Furthermore, capture and confinement of Pacific herring, Pacific sandlance, and surf smelt routinely results in locally severe VHS epizootics among the confined populations (Hershberger et al 1999, Kocan et al 2001, Hedrick et al 2003). As larvae (Hershberger et al 2007) and juveniles (Kocan et al 1997), Pacific herring are highly susceptible to VHS, with laboratory exposures resulting in 66-100% mortality. In the wild, juvenile herring are exposed to VHSV as early as 3 months post-hatch, shortly after their metamorphosis from larvae (Kocan et al 2001). The prevalence and severity of VHSV in confined adult herring captured for spawn-on-kelp roe fisheries

decreases with age (Hershberger et al 1999), suggesting a mechanism of adaptive immunity in adults that originates from previous exposures to the virus.

Ichthyophonus hoferi is a member of the Mesomycetozoea, a monophyletic class of protists that includes several other important pathogens (Ragan et al 1996, Herr et al 1999, reviewed in Mendoza et al 2002). Currently *I. hoferi* (reviewed in McVicar 2011) and *I. irregularis* (Rand et al 2000) are the only two recognized species in the genus, but other species have likely been grouped with *I. hoferi* based on the plasticity of morphological characteristics (McVicar 1999, Rasmussen et al 2010). Recent molecular phylogenetic studies indicate that distinct genetic types of the parasite exist (Criscione et al. 2002, Halos et al 2005, Rasmussen et al. 2010, Gregg and Hershberger unpublished data); therefore, the organism will be referred to generically as *Ichthyophonus* hereafter. From 1898 through the mid 1950's, six major *Ichthyophonus*-related epizootics were described in Atlantic herring (*Clupea harengus*) from the Western North Atlantic (Sindermann 1990, McVicar 2011, Burge et al. 2014). More recently, a massive *Ichthyophonus*-related epizootic killed an estimated 300 million Atlantic herring in waters around Sweden and Denmark during the early 1990's (Rahimian and Thulin 1996), and epidemiological data implicate *Ichthyophonus* as a primary factor responsible for mortality in wild Pacific herring (*Clupea pallasii*) from estuarine waters of Washington State (Hershberger et al 2002). Unpublished reports of large *Ichthyophonus* epizootics in the waters around Iceland during the fall and winter of 2008 resulted in the capture of massive numbers of herring that were unmarketable as a result of *Ichthyophonus*-induced tissue changes.

Information gaps addressed in this proposal:

A better understanding of the epidemiological principles governing herring diseases in PWS is necessary for the development of adaptive management strategies intended to mitigate the effects of diseases to wild herring populations. Early studies of known herring pathogens in PWS were conducted by Dr. Gary Marty, and provided valuable information on trends of infection prevalence and intensity since 1994. In an effort to document changes in pathogen prevalence and severity within the PWS herring population, these surveillance efforts were continued by Hershberger et al., in the form of the Herring Disease Program (HDP) from 2007 – present. The incorporation of laboratory-based manipulations and observations in the HDP has led to the realization that some of our prior assumptions of these diseases were incorrect. For example, in a typical herring population, the prevalence of VHSV generally falls below the realistic detection threshold obtained from 60-fish subsamples of a population. Even though the endemic prevalence is typically extremely low, an epizootic can occur very quickly as a result of changing host and environmental conditions (reviewed in Hershberger et al 2016). As such, the incorporation of VHSV prevalence data into the ASA model as a forecaster of future disease potential is inconsequential from an epidemiological perspective. For example, a prevalence of 0% (0/60) in a pre-spawn herring population provides no indication of whether the population previously experienced a VHS epizootic, or an epizootic is likely to occur in the future. For this reason, we have developed a serological assay (50% plaque neutralization assay - PNT) that is capable of determining whether herring have survived previous exposure to VHSV. This knowledge is extremely important from a disease forecasting perspective because survivors of prior VHSV exposure remain refractory to the disease for a very long time; presumably for life (reviewed in Hershberger et al. 2016). Here, we will begin to apply the novel PNT assay to the level of herring populations by proceeding with a series of validation experiments intended to determine the temporal and geographic scales of serological sampling that are required to assess population herd immunity against VHS. It is anticipated that this approach can be used to assess the future potential for VHS impacts in wild herring populations - a critical piece of information that will be useful for assigning the amount of disease risk associated with opening certain herring fisheries (eg. spawn-on-kelp).

These and other required updates and possible changes to our modeling approaches will be assessed by working closely with the ASA and other modelers to begin to develop disease-based models that are more built upon biologically-relevant disease principles. For example, we will continue to provide infection and disease data for the current ASA model, provide herring antibody results to Dr. Branch who will assemble a novel VHSV-based

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ASA-type model (see his separate proposal), and provide other VHSV epizootiological data to USGS modelers who will develop a mixture cure-type simulation model for VHS in herring.

Although *Ichthyophonus* is one of the most significant parasites of wild marine fishes, causing recurring population-level impacts during the past century (reviewed in Burge et al. 2014), very little in known about its natural life cycle. From a disease forecasting perspective, the most important information gap involves unresolved routes of exposure and transmission to planktivorous fishes. Laboratory studies indicate that the parasite is not readily transmitted from herring-to-herring via direct contact or through the water (Gregg et al. 2012). Recently, we have successfully established infections in herring by habituating them to the consumption of large quantities of infected fish tissues (Hershberger et al 2015); however, the relevance of this exposure route to wild populations of Pacific herring remains questionable, as herring are generally considered planktivores. These and other results have resulted in the elevation of a hypothesis that an invertebrate, intermediate host may be involved in completing the *Ichthyophonus* life cycle. However, until recently, appropriate scientific tools did not exist for examining the possibility of an *Ichthyophonus* intermediate host. Recent work performed in the Herring Disease Program was successful in developing novel tools (quantitative PCR and chromogenic in situ hybridization) that will be useful for assessing wild zooplankters as intermediate hosts for *Ichthyophonus*. Here we will continue to assess possible natural route(s) of *Ichthyophonus* transmission by expanding laboratory studies to assess horizontal transmission, examining a particular location (Cordova Harbor) where *Ichthyophonus* infection prevalence is unusually high, and by screening common herring food items as possible intermediate hosts for the parasite.

Finally, we will continue to advance our understanding of basic epizootiological principles that govern the primary diseases of herring by continuing to employ specific pathogen-free (SPF) laboratory animals in controlled laboratory experiments. For example, we will further evaluate the importance of temperature as a VHS perpetuation cofactor factor and we will evaluate the susceptibility of herring to *Vibrio* spp. – likely the most prevalent bacterial pathogens of marine fishes in the world (Actis et al. 2011).

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2. Relevance to the Invitation for Proposals

Discuss how the project addresses the projects of interest listed in the Invitation and the overall Program goals and objectives. 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.

This proposal addresses the overall goal of the Herring Research and Monitoring Program outlined in the Invitation for Proposals:

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"The continued development and testing of an updated age structures assessment (ASA) model in collaboration with ADF&G…."

In particular, we will continue to provide infection and disease prevalence information that feed into the current version of the ASA model. Additionally, we will provide results from the novel VHSV antibody assay to Dr. Branch, who will design a novel ASA-type model that assesses past and future susceptibility of the PWS herring population to VHS. Finally, we will work with USGS modelers to provide relevant VHS data that will be incorporated into a mixture-cure visualization model that can assess the relative importance of disease cofactors in forecasting the potential for future epizootics.

Additionally, this proposal addresses a particular area of interest identified in the Invitation for Proposals:

"6. The continuation of the work to study the role of disease in herring recovery and the potential for developing tools to aid management agencies in the detection and management of disease outbreaks."

During the previous 5 year project, we successfully developed a serological tool that is capable of retrospectively assessing the exposure history of herring to VHSV. Here, we will continue the advancement of this tool by performing a series of validation tests using wild herring in the laboratory. Additionally, we will continue with laboratory-and field-based experiments intended to understand the basic principles that govern the primary diseases of Pacific herring. These principles will then be used to develop additional disease forecasting tools.

Finally, for the reasons listed above, this proposal also nests within Objectives outlined by Dr. Pegau for the proposed Herring Research and Monitoring Project (FY'17-'21):

"Expand and test the herring stock assessment model used in Prince William Sound…."

"Provide inputs to the stock assessment model…"

"Develop new approaches to monitoring…"

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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.

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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.

Five Publications Relevant to this Proposal

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.

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.

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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.

Appendix C

Five Additional Publications

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.

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.

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.Kocan, R., L. Hart, N. Lewandowski, P. Hershberger. 2014. Viability and infectivity of *Ichthyophonus sp.* in post-mortem Pacific herring, *Clupea pallasii*. Journal of Parasitology 100: 790-796.

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):

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Appendix C

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Ph.D.	2005	University of Washington	Aquatic and Fishery Sciences
M.S.	1997	University of Maine	Zoology
B.S.	1993	Washington State University	Zoology

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2007 to present: Research Microbiologist; Western Fisheries Research Center, US Geological Survey
2013 to present: Affiliate Associate Professor; School of Aquatic and Fishery Sciences, U. of Washington
2005 to 2007: Microbiologist; Western Fisheries Research Center, US Geological Survey

Five Publications Relevant to this Proposal:

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.

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Five Additional Publications

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Purcell MK, Marjara IS, Batts W, Kurath G, Hansen JD. 2011. Transcriptome analysis of rainbow trout infected with high and low virulence strains of infectious hematopoietic necrosis virus. Fish Shellfish Immunol. 30:84-93.

Metzger DC, Elliott DG, Wargo A, Park LK, Purcell MK. 2010. Pathological and immunological responses associated with differential survival of Chinook salmon following *Renibacterium salmoninarum* challenge. Dis Aquat Organ. 90:31-41.

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Hershberger PK, Pacheco CA, Gregg JL, Purcell MK, LaPatra SE. 2008. Differential survival of *Ichthyophonus* isolates indicates parasite adaptation to its host environment. J Parasitol. 94:1055-1059.

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Appendix C

4. Project Design

A. Objectives

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 and whether any changes are proposed. If the proposed project is for new work, explain why the new data is needed. Describe the anticipated final product.

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

i. Provide pathogen and disease prevalence data to inform the ASA model
ii. Produce specific pathogen-Free (SPF) Pacific herring for laboratory experiments
iii. Process new and archived herring plasma samples for indications of prior VHSV exposure
iv. Validate the novel plaque neutralization assay using wild herring
v. Contribute to novel disease modeling approaches
vi. Determine the effects of temperature on VHSV shedding
vii. Determine the susceptibility of Pacific herring to *Vibrio*
viii. Investigate the possibility of an invertebrate host for *Ichthyophonus*
ix. Determine the causes for abnormally high *Ichthyophonus* prevalence among juvenile Pacific herring that establish temporary residency in Cordova Harbor
x. Determine the impacts of salinity on fish-to-fish transmission of *Ichthyophonus*

4B. Procedural and Scientific Methods

i. Provide pathogen and disease prevalence data to inform the ASA model

Disease is a component in the Age-Structure-Analysis model for Prince William Sound; however, it is not part of the ADF&G sponsored surveys. We will provide the disease information for the ASA model by determining annual prevalence and intensity data for the most virulent pathogens occurring in the PWS herring populations, including viral hemorrhagic septicemia (VHS), viral erythrocytic necrosis (VEN), and ichthyophoniasis. Monitoring efforts will consist of the annual collection and processing of sixty adult

herring per site from each of three sites in PWS and Sitka Sound to test for infection and disease prevalence. Diagnostic techniques for these pathogens will follow standard procedures described in the "Blue Book: Standard procedures for the detection and identification of select fish and shellfish pathogens (American Fisheries Society)." Additionally, plasma samples will be collected from sampled fish in PWS and Sitka; samples will be processed using a novel serum neutralization assay that is capable of determining prior VHSV exposure history. Similar pathogen sampling will occur from Pacific herring populations in Puget Sound, WA and other locations throughout the NE Pacific as sampling opportunities become available.

ii. Produce specific pathogen-Free (SPF) Pacific herring for laboratory experiments

A critical component of both the field surveillance efforts and the empirical disease process studies involves the availability of laboratory host animals with known exposure and disease histories. We have developed techniques to rear specific pathogen-free (SPF) herring and we currently maintain several thousand SPF herring in each of 4 age classes (age 0, 1, and 2 yr) for use as experimental animals. Additional colonies will be reared to satisfy the needs described in this proposal.

As the susceptibility to VHS is similar among Pacific herring from genetically diverse stocks throughout the NE Pacific (Figure 1), SPF herring colonies will be initiated from Puget Sound herring eggs. However, SPF herring from additional stocks will be reared if the need arises. For example, we have agreed to provide SPF herring for the EVOST TC project proposed by Dr. Andrew Whitehead. This proposed project is intended to assess the effects of oil exposure on Pacific herring genes that are directly or indirectly linked to a robust immune response. This response will be evaluated using SPF herring from several distinct populations, including PWS. The functional effects of the any gene adaptations will be evaluated by performing pathogen challenge studies at the Marrowstone Marine Field Station. If funding is approved for the proposed project by Dr. Whitehead, then additional colonies of SPF herring from the appropriate populations will be reared.

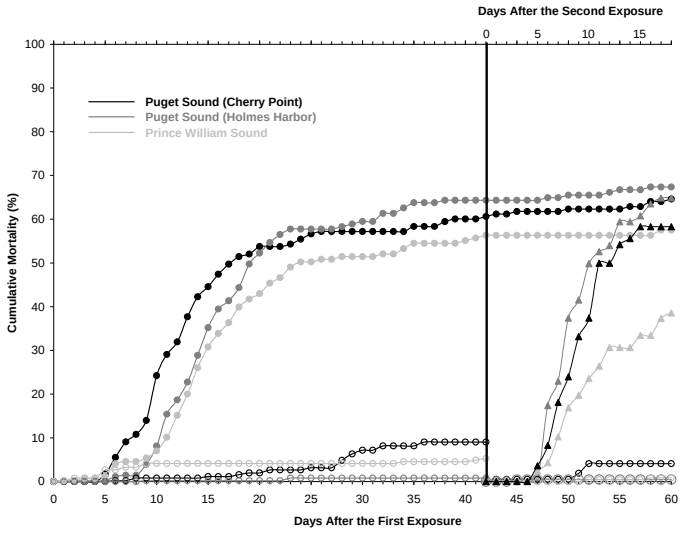

Figure 1 (from Hershberger et al. 2010). Relative susceptibilities of Pacific herring from three different stocks (Holmes Harbor, Cherry Point, and Prince William Sound) to VHS. Closed circles indicate treatment groups exposed to VHSV and open circles indicate negative control groups (exposed to saline). All survivors in the VHSV treatment groups from the first exposure were re-exposed to VHSV in the same tanks 42d later. All survivors in the negative control groups after 42 days were split into two groups (positive controls and negative controls) for the second exposure. Positive controls for the second exposure (closed triangles starting on day 42) were exposed to VHSV for the first time on day 42. Negative controls for the second exposure (open circles starting on day 42) were re-exposed to saline on day 42.

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iii. Process new and archived herring plasma samples for indications of prior VHSV exposure

In anticipation of the development of a future serological assay capable of identifying prior VHSV exposure, plasma samples have been collected from annual sampling trips of pre-spawn adult herring in Prince William Sound and Sitka Sound since 2012An effective serological assay, capable of quantifying VHSV neutralizing titers in herring plasma, has now been developed; a manuscript describing the methods of this modified plaque neutralization assay is currently in preparation. This assay has two very unique applications. First, it may provide a quantitative assessment of the potential for future VHS epizootics. For example, populations with high levels of herd immunity (i.e high antibody levels) would be assigned a low potential for future VHS impacts; conversely those with low levels of herd immunity (i.e. low antibody levels) would be assigned a high potential for future disease impacts if exposure occurs under the appropriate host and environmental conditions. Second, the assay will provide an *a priori* method to assess prior impacts of VHS to specific age cohorts within the population. For example, all archived and future plasma herring samples from PWS correspond to the ADF&G herring stock assessment database that includes age data. By pairing these age data with the PNT results, we can track the exposure history of specific year classes to determine if and when VHSV exposure occurred. When paired with population recruitment data from annual herring surveys, these temporal insights into VHSV exposures may inform the possible involvement of VHS in herring year class failures from Prince William Sound.

Here, we propose to process all the archived (2012- 2016) and newly-collected (2017-2021) herring plasma samples using the optimized PNT methods.

iv. Validate the novel plaque neutralization assay using wild herring

Although the newly-developed PNT is effective at identifying VHSV neutralizing titers and exposure histories in laboratory-reared herring (approximately 90% test sensitivity), the interpretation of PNT the values on a population scale requires further validation. For example, the optimized PNT indicates the percentage of individuals demonstrating virus neutralizing activity in their plasma and provides a quantitative assessment of these neutralizing titers. However, the application of the PNT to wild herring, with a more robust exposure history, requires further investigation. Additional vetting is also required for the interpretation of the PNT values from wild herring that correspond to demonstrated herd immunity and documented prior exposure. Further, questions of sample size and geographic scale of sampling need to be addressed.

These final PNT validation studies will be addressed using wild herring, with deduced exposure histories and demonstrated levels of herd immunity. For example, the collection of susceptible age 0 herring from bait balls and their subsequent confinement in laboratory tanks often elicits a VHS epizootic among the confined cohorts (Kocan et al. 2002). An epizootic will not occur among the confined cohorts for two possible reasons:

- The population was susceptible to VHS, but no viral carriers occurred among the captured cohorts
- The population is refractory to VHS because it survived prior exposure

To evaluate the ability of the optimized PNT methods to demonstrate herd immunity based on deduced VHS exposure history, groups of age 0 yr Pacific herring will be collected, transported alive back to Marrowstone, and maintained in tanks. Subsamples of these fish (n = 30 / day) will be collected at the following intervals:

- Day 0 (immediately after transport to the laboratory)
- Day 7
- Day 14
- Day 21

At this point, each experiment will bifurcate into one of 2 directions:

1) **If a VHS epizootic does not ensue by day 21:** then we will know that either:
   A. The fish were refractory to VHS or
   B. The fish were susceptible to VHS, but no VHSV carriers were present in the tank to serve as a source of VHSV exposure.

   To test these 2 scenarios, the remaining fish in this tank will be separated into 2 tanks on day 21. Herring in one tank will be exposed to an aliquot of VHSV isolate (5 x 10^4 PFU / mL) for 2 hr. Herring in the other tank will remain unexposed. Afterwards, fish will be subsampled from each tank (n = 30 /d) on days 28 (7d post exposure), 42 (21d post-exposure), and 84 (63d post-exposure).
   If a VHSV epizootic fails to occur in the tank of VHSV-exposed herring by day 42 (21d post-exposure), then it will be assumed that the fish were refractory.
2) If a VHS epizootic does occur by day 21, then we have determined that the fish were susceptible to VHS when captured, and that VHSV transmission occurred from positive carriers in the tank.
   If this is the case, then we will document the development of VHSV neutralizing activity in the plasma of survivors; further, we will document that those survivors are now refractory to VHS. Fish will remain in this tank until 84d post capture (63d post exposure), then subsampled (n=30) and separated into 2 tanks. Fish in one tank will remain unmanipulated, and fish in the other tank will be exposed to an aliquot of VHSV isolate (5 x 10^4 PFU / mL) for 2 hr. Mortality will be assessed through day 105 (21d post-exposure), after which the experiment will be terminated.

   Negative Controls will consist of age 0 yr SPF herring (N≈500) in a separate tank. Whenever a subsampling event occurs from the wild fish, these negative controls will also be subsampled (n= 10 fish / subsampling day). Additionally, whenever exposure to a lab aliquot of VHSV occurs, these fish will be exposed to PBS. Positive Controls, employed whenever a laboratory exposure to VHSV occurs, will consist of approximately 100 age 0 yr SPF herring that are also exposed to an aliquot of VHSV.

v. Contribute to a novel disease modeling approaches

We will work closely with Dr. Trevor Branch to provide data for a novel disease forecasting model that incorporates the serum-neutralization data, dating from 2012, into a newly-formed age-structured model for herd immunity. Additionally, we will work with USGS modelers, Drs. Russell Perry and John Plumb to develop a mixture cure model for VHS and herring. This simulation model will assess disease impacts under various scenarios that incorporate VHS covariates (including immune fraction, temperature, host aggregations, etc).

vi. Determine the effects of temperature on VHSV shedding

We previously demonstrated an inverse relationship between temperature and VHS potential in Pacific herring, with cooler temperatures resulting in elevated mortality, earlier onset and increased magnitude of viral shedding, and delayed up-regulation of genes responsible for the early anti-viral immune response (Hershberger et al. 2013). Similarly, the course of VHS epizootics tend to be longer in cooler temperatures, where the virus tends to enter a neurotropic stage and persist for extended periods in immune-privileged cells and tissues (Hershberger et al. 2010, Lovy et al. 2012).

Here, we will investigate the influence of temperature on the ability of VHS survivors to transfer the infection to naive cohorts through waterborne shedding of infectious virions. Colonies, each containing several thousand laboratory-reared SPF herring, will be established at each of two temperatures (7 and 11^{o}C). VHS epizootics will be initiated in both colonies by controlled waterborne exposure to VHSV isolates. To assess how long any shed virus from the exposed colonies can initiate VHS epizootic in sympatric groups of naïve herring, the effluent water from these donor colonies will be spilled into the supply water for other tanks containing sentinel SPF herring. If present and virulent, shed VHSV from the donor colonies will initiate VHS epizootics in the tanks containing sentinel herring. This exposure of sentinels to effluent water from the donor colonies will proceed for 3 wk; after which, all the sentinel

survivors will be euthanized and tissues will be assessed for VHSV. The sentinel tanks will then be completely disinfected, and dried for 7d; after which, another group of SPF sentinel herring will be transferred to the sentinel tanks and exposed to effluent water from the donor tank. Using this experimental approach, we will employ a new colony of sentinel herring every 4 wk, in an effort to determine how long shed virus from the donor colony remains infectious.

The experimental design will also contain donor colonies of unexposed (negative control) groups at both temperatures, the effluent of which will be spilled into analogous sentinels. All mortalities from both the donor colonies and the sentinel tanks will be assayed for VHSV titer in the tissues by plaque assay. Similarly, all surviving sentinels will be euthanized after each 3 week exposure period and tissues will be assessed for VHSV.

vii. *Determine the susceptibility of Pacific herring to Vibrio spp.*
Vibrio ordalii and *Listonella anguillarium* (formerly *V. anguillarium*) are among the most common bacterial pathogens of marine organisms in the Pacific Northwest, with the resulting diseases often causing mortality to wild and aquacultured marine fishes and shellfishes. Here, we will examine the susceptibility of Pacific herring to infection and disease from *L. anguillarium* and *V. ordalli. A* group of SPF Pacific herring will be exposed to the live *L. anguillarium* and *V. ordalii* isolates. Positive controls will consist of groups of similarly-exposed coho salmon that are known to be susceptible to the disease. Mortality will be monitored, and all fish (mortalities and euthanized survivors after 21d) will be evaluated for *Vibrio* spp. infections by culture on bacterial agar.

xi. Investigate the possibility of an invertebrate host for Ichthyophonus
Ichthyophonus is one of the most cosmopolitan marine fish parasites worldwide, with the resulting disease causing recurring epizootics in wild marine fishes, particularly clupeids (reviewed in Burge et al. 2014). Although the parasite can be easily transmitted to piscivorous fishes through the consumption of infected prey, the natural mode(s) of transmission in planktivorous fishes remains poorly understood. Recently, it was determined that Pacific herring can become infected by consuming infected tissues in the laboratory (Hershberger et al 2015); however, this route is mostly likely artificial, with the possible exception of some small herring schools that habituate to feeding around offal discharges from fish processing plants. Failure to establish the natural route(s) of transmission to Pacific herring and other clupeids has led to the prevailing hypothesis that the parasite is likely transferred trophically through an as-yet-unidentified intermediate host (reviewed in Mc Vicar 2011).

Until recently, diagnostic tools were unavailable to aid in the identification of *Ichthyophonus* from intermediate hosts. For example, the traditional means of identifying the parasite in fish tissues include explant culture of tissue explants histology with generalized stains (Hershberger 2012). However, the parasite is extremely pleomorphic, often exhibiting unique morphologies under different culture conditions. For this reason, it was feared that the appearance of the parasite in an intermediate invertebrate host would not be recognized or would be mischaracterized using these traditional techniques.

Within the past 10 years, molecular PCR-based tools with *Ichthyophonus*-specific primers have been developed (Whipps et al 2006, White et al. 2013). These tools now enable the rapid screening of large zooplankton samples for the presence of *Ichthyophonus* DNA. This tool is will be useful as an initial screening tool to assess wild zooplankton for *Ichthyophonus*. Further, cryptic or unrecognizable parasite morphologies are inconsequential for the success of this tool. However, any positive qPCR samples will need to be further evaluated with a confirmatory diagnostic technique, as a positive result could simply represent a contaminant or external adsorption of the parasite to the host carapace. Therefore,

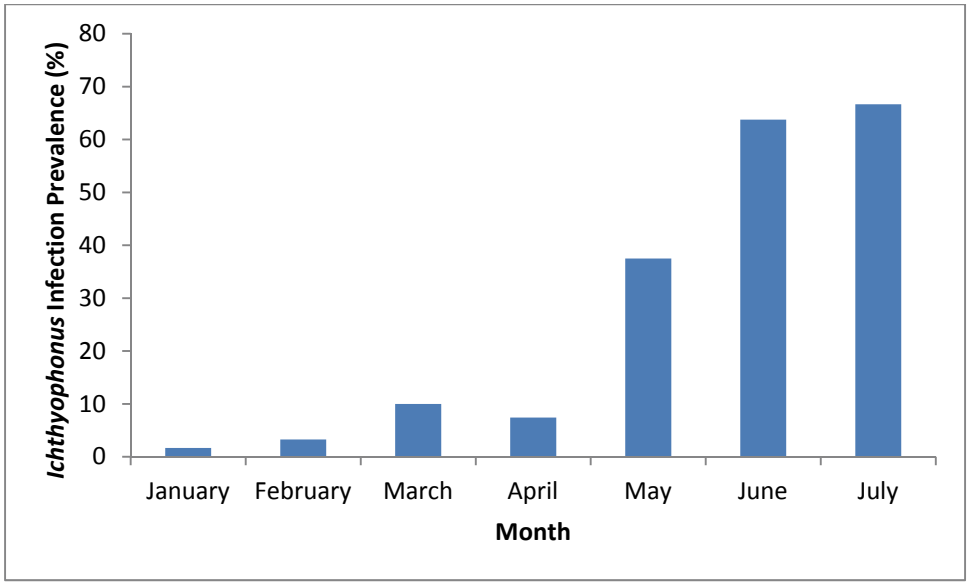
confirmation of a positive PCR sample will require further microscopic observation of the parasite within the body cavity of the suspected intermediate host.

We recently developed a chromogenic in situ hybridization assay that is capable of selectively binding to a portion of the *Ichthyophonus* genome; the tool allows for microscopic observation and confirmation of the parasite in histological sections, regardless of the parasite morphology. We propose to employ this novel CISH assay, to confirm any positive samples that are returned from the qPCR survey. Specifically, we plan to screen common herring food items, including *Neocalanus spp*. using *Ichthyophonus*-specific primers for a qPCR. If suspected positive samples are identified, then further confirmation will be made using the *Ichthyophonus*-specific CISH.

viii. Determine the causes for abnormally high Ichthyophonus prevalence among juvenile Pacific herring that establish temporary residency in Cordova Harbor

Within a population of Pacific herring, the prevalence of *Ichthyophonus* infections typically increases with age and size, with the prevalence in juvenile cohorts typically occurring below 10% (Hershberger et al 2002, Hershberger et al. 2016). However, on a number of occasions, unusually high infection prevalence has been reported from age 0-1 yr herring that established temporary residency in Cordova Harbor (Hershberger et al. 2015 and Figure 1).

Figure 1. 2015 Monthly Prevalence of *Ichthyophonus* in Juvenile Herring from Cordova Harbor



Cause(s) of this unusually high infection prevalence remain unknown, but have been hypothesized to involve exposure of juvenile herring to infected offal that is discharged from the local fish processing plant.

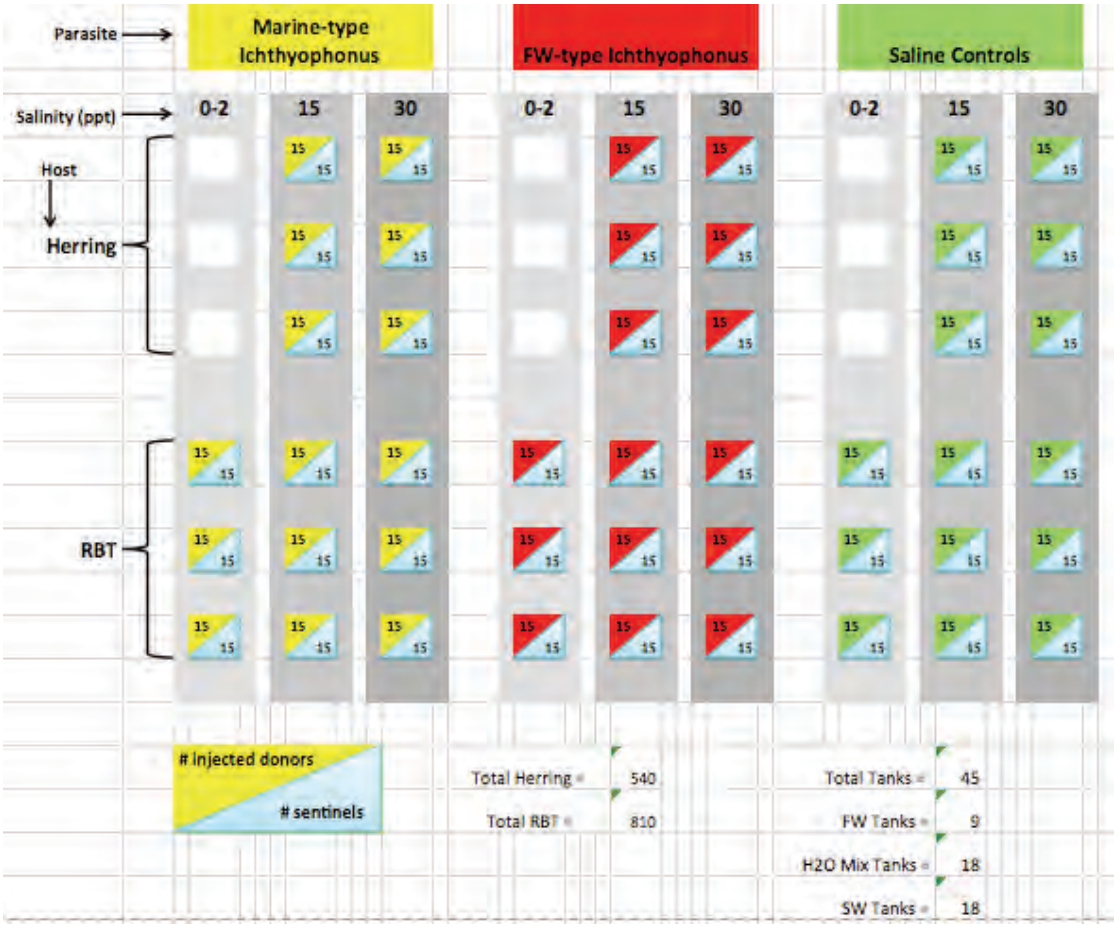
Here, we propose to collect stomach samples from herring in Cordova Harbor when the *Ichthyophonus* infection prevalence begins to increase during May and June. These stomachs will be fixed in formalin and evaluated histologically (using PAS stain and the newly-developed *Ichthyophonus* CISH assay) to determine whether infected offal was consumed by the residualized herring.

ix. Determine the impacts of salinity on fish-to-fish transmission of Ichthyophonus

There is an apparent discordance between the infection processes that maintain *Ichthyophonus* spp. infections in rainbow trout farms and those that maintain infections in wild Pacific herring. Waterborne transmission is assumed in the trout farms, and has been demonstrated using rainbow trout (Gustafson and Rucker 1956, Yokota et al. 2008); however we have been unable to demonstrate fish-to-fish

transmission through the water in herring (Gregg et al. 2012). These two infection models differ in host, parasite type (Rasmussen et al. 2010) and environment (freshwater vs seawater).

Here we will determine which of these is causing the differences we see between the two host-parasite systems. The euryhaline nature of rainbow trout and our ability to culture and molecularly identify the two parasite species allow us to design a 3-factor study design with rainbow trout in freshwater as a positive control:



Establishing Infected Donors. Four groups of infected donors (2 parasites x 2 hosts) will be established by injection with either freshwater-type or marine-type *Ichthyophonus* isolates. An uninfected donor group (negative control) for each species will be established by injections with saline. The donor groups will be held in six tanks. Rainbow trout will be held at 0 ppt salinity and herring will be held at 30 ppt salinity during incubation period. Two weeks after injections, 30 fish will be subsampled from the donor tanks to assess infection prevalence, and collect culture for DNA analysis. Three weeks post injection, infected and control donors will be moved to 45 tanks for salinity acclimation. Salinity acclimation of the donors and sentinels will occur synchronously. Sentinels will have lower lobe of caudal fin and left pectoral clipped when they are moved to these acclimation tanks, so they can be distinguished from donor herring later in the study.

Appendix C

After a 9 d acclimation, 15 sentinel fish from each salinity treatment will be cohabitated with the donor conspecifics. This will be Day-0 of the challenge. Cohabitation will be maintained for 3 months, but may be terminated sooner if culture-positive sentinel mortality occurs. Mortalities and all survivors will be assessed for *Ichthyophonus* infection by tissue explant culture.

4C. Data Analysis and Statistical Methods

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 (χ^2). Statistical significance will be assigned to all comparisons with $p \leq 0.05$. Prevalence of infection and disease in wild populations from Prince William Sound, Sitka Sound, and Puget Sound will be based on minimum sample sizes of 60 fish, sufficient to detect 5% prevalence in the population with 95% confidence.

4D. Description of the Study Area

The study area includes locations throughout Prince William Sound and Sitka Sound where pre-spawn herring aggregate.

Laboratory studies described in this proposal will be conducted at the USGS-Marrowstone Marine Field Station, and USGS-Western Fisheries Research Center where facilities ideally designed to safely and responsibly conduct experiments using endemic fish pathogens. The Marrowstone Marine Field Station represents the sole seawater-based biological research facility for the USGS. 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. The Western Fisheries Research Center (WFRC) is recognized as an international leader in fish health research. The WFRC maintains fish health laboratory facilities which are among the newest and best in the nation. The facility operates a state-of-the-art fresh water wet laboratory that is completely climate controlled and automated for disease challenges and studies in physiology and pathology. The nation's only Biosafety Level III disease containment wet laboratory for fish is also part of this facility. Additionally, the Center maintains fully equipped laboratories for molecular biology, virology, bacteriology, immunology, and histopathology.

5. Coordination and Collaboration

Within the Program

Provide a list and clearly describe the functional and operational relationships with the other program projects. This includes any coordination that has taken or will take place and what form the coordination will take (shared field sites or researchers, research platforms, sample collection, data management, equipment purchases, etc.).

With Other EVOSTC-funded Programs and Projects

Appendix C

Indicate how your proposed program 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 any local and native community involvement in the project.

5. Coordination and Collaboration

Within the Program

- Herring collections from Cordova Harbor will be provided by Drs Kristen Gorman and Scott Pegau (PWSSC). Stomachs from these fish will be assessed for indications that *Ichthyophonus*-infected offal may contribute to the unusually high infection prevalence detected among juvenile herring in Cordova Harbor.
- Serum neutralization results, to assess herd immunity by quantifying VHSV neutralizing titer, will be shared with Dr. Trevor Branch (U. Washington). These results will be used to create a novel age-structured assessment model that incorporates herd immunity by herring age class.

With Other EVOSTC-Funded Programs and Projects

- Long Term Monitoring: Yumi Arimitsu (USGS – Alaska Science Center) and John Moran (NOAA Fisheries - Auke Bay Labs) continue to send samples of suspect sick herring and other forage fish to us for diagnosis.
- Long Term Monitoring: Zooplankton collections from throughout Prince William Sound will be provided by Dr. Rob Campbell (PWSSC). Subsamples from these collections will be assessed by qPCR and CISH to look for an *Ichthyophonus* invertebrate host.
- Lingering Oil: We have partnered with Dr. Andrew Whitehead on his proposal to investigate the effects of PAH exposure to genetic pathways that directly and indirectly influence immune-competence. In kind laboratory space and SPF herring for Dr. Whitehead's projects will be provided at the USGS - Marrowstone Marine Field Station.

With Trustee or Management Agencies

- We will continue to partner with Steve Moffit (ADF&G – Cordova) to collect herring infection and disease data onboard the ADF&G seining platform used to assess pre-spawn herring biomass in PWS.
- We will continue to partner with Eric Coonradt (AFF&G – Sitka) to collect herring infection and disease data from pre-spawn aggregations in Sitka Sound.

With Native and Local Communities

- Hershberger will provide a seminar with an updated description of Herring Disease Program in Cordova.

Appendix C

-

6. Schedule

Program Milestones

Specify when critical program 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 Program Tasks

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

6. Schedule

Program Milestones (By Objective)

i. Provide pathogen and disease prevalence data to inform the ASA model
   Laboratory diagnostics for pre-spawn herring aggregations in PWS and Sitka Sound will be completed by June of each year.
ii. Rear Specific Pathogen-Free (SPF) Pacific herring
   Annual rearing of SPF herring to juveniles will be completed by August of each year.
iii. Data Archive
   Data and metadata will be submitted to the Ocean Workspace by Dec 31, each year.
iv. Process archived herring plasma samples for indications of prior VHSV exposure
   Archived herring plasma samples, dating to 2012, will be processed by December 2017; thereafter, new plasma samples for each survey year will be processed by August of the same year.
v. Validate the novel plaque neutralization assay with wild herring
   December 2021
vi. Contribute to novel disease modeling approaches
   Preliminary data for the new models will be provided by December, 2017
vii. Determine the effects of temperature on VHSV shedding
   December 2019
viii. Determine the susceptibility of Pacific herring to *Vibrio*
   December 2017
ix. Investigate the possibility of a zooplankter intermediate host for *Ichthyophonus*
   January 2022
x. Determine the causes for abnormally high *Ichthyophonus* prevalence among juvenile Pacific herring that establish temporary residency in Cordova Harbor
   December 2020
xi. Determine the impacts of salinity on fish-to-fish transmission of *Ichthyophonus*
   December 2018

Measurable Program Tasks

FY 2017

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host
- Initiate *Vibrio* challenge experiments

2nd Quarter (May 1 – Jul. 31)

- Finish processing spring adult herring to determine infection and disease prevalence.
- Collect herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus

Appendix C

- Begin studies to validate the plaque neutralization assay using wild herring
- Continue *Vibrio* challenge experiments

3rd Quarter (Aug. 1 - Oct. 31)

- Brood Year 2017 SPF herring metamorphosed to juveniles
- Continue *Vibrio* challenge experiments
- Complete analysis of 2017 plasma samples
- Continue studies to validate the plaque neutralization assay using wild herring

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Complete *Vibrio* challenge experiments
- Complete analysis of archived plasma samples from 2012-2016 and share results with modelers.

FY 2018

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host
- Initiate experiments intended to assess the effects of salinity on fish-to-fish transmission of *Ichthyophonus*

2nd Quarter (May 1 – Jul. 31)

- Continue experiments intended to assess the effects of salinity on fish-to-fish transmission of *Ichthyophonus*
- Finish processing spring adult herring to determine infection and disease prevalence.
- Continue studies to validate the plaque neutralization assay using wild herring
- Collect herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus

3rd Quarter (Aug. 1 - Oct. 31)

- Continue experiments intended to assess the effects of salinity on fish-to-fish transmission of *Ichthyophonus*
- Brood Year 2018 SPF herring metamorphosed to juveniles
- Complete analysis of 2018 plasma samples
- Continue studies to validate the plaque neutralization assay using wild herring

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Complete experiments intended to assess the effects of salinity on fish-to-fish transmission of *Ichthyophonus*

FY 2019

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host
- Initiate experiments to assess the effects of temperature on VHSV shedding

2nd Quarter (May 1 – Jul. 31)

- Continue experiments to assess the effects of temperature on VHSV shedding
- Finish processing spring adult herring to determine infection and disease prevalence
- Continue studies to validate the plaque neutralization assay using wild herring
- Collect herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus

Appendix C

3rd Quarter (Aug. 1 - Oct. 31)

- Continue experiments to assess the effects of temperature on VHSV shedding
- Brood Year 2019 SPF herring metamorphosed to juveniles
- Complete analysis of 2019 plasma samples
- Continue studies to validate the plaque neutralization assay using wild herring

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Complete experiments to assess the effects of temperature on VHSV shedding

FY 2020

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host

2nd Quarter (May 1 – Jul. 31)

- Finish processing spring adult herring to determine infection and disease prevalence.
- Continue studies to validate the plaque neutralization assay using wild herring

3rd Quarter (Aug. 1 - Oct. 31)

- Brood Year 2020 SPF herring metamorphosed to juveniles
- Complete analysis of 2020 plasma samples
- Continue studies to validate the plaque neutralization assay using wild herring

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Finish histological processing of herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus.

FY 2021

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host

2nd Quarter (May 1 – Jul. 31)

- Finish processing spring adult herring to determine infection and disease prevalence.
- Continue studies to validate the plaque neutralization assay using wild herring

3rd Quarter (Aug. 1 - Oct. 31)

- BY 2021 SPF herring metamorphosed to juveniles
- Complete analysis of 2021 plasma samples
- Complete PCR and CISH analyses of zooplankton samples for Ichthyophonus
- Complete studies to validate the plaque neutralization assay using wild herring
- Draft final report

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Respond to peer review comments, acceptance and publication of final report

7. Budget

Appendix C

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.

Annual USGS in-kind contributions (personnel contributions include salary + benefits):

	FY 17	FY 18	FY 19	FY 20	FY 21	Total
P.K. Hershberger (PI) 20%	$29,404	$30,271	$30,271	$30,518	$31,535	$151,999
M.K Purcell (PI) 10%	$14,322	$14,794	$15,256	$15,719	$16,314	$76,405
J.L. Gregg (Fish Biol) 20%	$17,971	$18,485	$18,485	$18,998	$18,998	$92,937
Total	**$61,697**	**$63,550**	**$64,012**	**$65,235**	**$66,847**	**$321,341**

Budget Justification

Personnel Costs $676.8 K

Funding is requested each year to support a GS-9 laboratory technician ($67,200 - $81,600 / yr) at the Marrowstone Marine Field Station to perform laboratory studies, process samples from laboratory studies, perform predictive disease assays, and process herring survey samples. Funding is also requested to support a GS-7 laboratory technician ($55,200 - $67,200) to provide fish husbandry for the SPF herring and assist with controlled disease experiments in the wet laboratories. Both technicians will assist with annual herring collection trips to PWS and Sitka.

Travel Costs $100.5 K

Annual round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($7.6K) and perform Sitka field sampling ($3.2K K). Additional travel costs are included for the annual meeting with the other PI's from the HRM Program.

Contractual Costs $0 K

Commodities $195.0 K

Commodities include laboratory supplies for the Marrowstone Marine Station ($17.0 K/yr for fish food, live feed production, and herring diet enrichments; $22.0 K/ yr is requested for dry lab supplies (cell culture, molecular reagents, media, histology, etc.).

New Equipment / Existing Equipment Usage: $0

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.

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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	$122.4	$128.4	$135.6	$141.6	$148.8	$676.8	
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Commodities	$39.0	$39.0	$39.0	$39.0	$39.0	$195.0	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
SUBTOTAL	$181.5	$187.5	$194.7	$200.7	$207.9	$972.3	
General Administration (9% of subtotal)	$16.3	$16.9	$17.5	$18.1	$18.7	$87.5	N/A
PROJECT TOTAL	$197.8	$204.4	$212.2	$218.8	$226.6	$1,059.8	
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	

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: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

TRUSTEE AGENCY SUMMARY PAGE

Date Prepared: 08/24/2016

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

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Ashley MacKenzie (Technician)	Herring Disease Program	12.0	5.6		67.2
Mallory Wilmot (Technician)	Herring Disease Program	12.0	4.6		55.2
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
		Subtotal	10.2	0.0	
				Personnel Total	$122.4

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
Marrowstone - Cordova (PWS sampling)	1.2	2	28	0.3	10.8
Marrowstone - Sitka (sampling)	1.2	2	14	0.3	6.6
Marrowstone - Anchorage (PI meeting)	1.2	1	5	0.3	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$20.1

FY17

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/24/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROGRAM 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
Fish food, enrichments, and live feed production for SPF herring	17.0
Laboratory supplies (cell culture, histology, molecular biology, parasitology, virology, etc.)	22.0
Commodities Total	$39.0

FY17

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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
			0.0
			0.0
			0.0
			0.0
			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: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/24/2016

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

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Ashley MacKenzie (Technician)	Herring Disease Program	12.0	5.9		70.8
Mallory Wilmot (Technician)	Herring Disease Program	12.0	4.8		57.6
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
		Subtotal	10.7	0.0	
				Personnel Total	$128.4

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
Marrowstone - Cordova (PWS sampling)	1.2	2	28	0.3	10.8
Marrowstone - Sitka (sampling)	1.2	2	14	0.3	6.6
Marrowstone - Anchorage (PI meeting)	1.2	1	5	0.3	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$20.1

FY18

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/24/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROGRAM 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
Fish food, enrichments, and live feed production for SPF herring	17.0
Laboratory supplies (cell culture, histology, molecular biology, parasitology, virology, etc.)	22.0
Commodities Total	$39.0

FY18

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY18

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/24/2016

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

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Ashley MacKenzie (Technician)	Herring Disease Program	12.0	6.2		74.4
Mallory Wilmot (Technician)	Herring Disease Program	12.0	5.1		61.2
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
		Subtotal	11.3	0.0	
				Personnel Total	$135.6

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
Marrowstone - Cordova (PWS sampling)	1.2	2	28	0.3	10.8
Marrowstone - Sitka (sampling)	1.2	2	14	0.3	6.6
Marrowstone - Anchorage (PI meeting)	1.2	1	5	0.3	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$20.1

FY19

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/24/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROGRAM 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
Fish food, enrichments, and live feed production for SPF herring	17.0
Laboratory supplies (cell culture, histology, molecular biology, parasitology, virology, etc.)	22.0
Commodities Total	$39.0

FY19

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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
			0.0
			0.0
			0.0
			0.0
			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: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/24/2016

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

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Ashley MacKenzie (Technician)	Herring Disease Program	12.0	6.5		78.0
Mallory Wilmot (Technician)	Herring Disease Program	12.0	5.3		63.6
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
		Subtotal	11.8	0.0	
				Personnel Total	$141.6

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
Marrowstone - Cordova (PWS sampling)	1.2	2	28	0.3	10.8
Marrowstone - Sitka (sampling)	1.2	2	14	0.3	6.6
Marrowstone - Anchorage (PI meeting)	1.2	1	5	0.3	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$20.1

FY20

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/24/2016

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROGRAM 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
Fish food, enrichments, and live feed production for SPF herring	17.0
Laboratory supplies (cell culture, histology, molecular biology, parasitology, virology, etc.)	22.0
Commodities Total	$39.0

FY20

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY20

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/24/2016

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

Personnel Costs:		Months	Monthly		Personnel
Name	Project Title	Budgeted	Costs	Overtime	Sum
Ashley MacKenzie (Technician)	Herring Disease Program	12.0	6.8		81.6
Mallory Wilmot (Technician)	Herring Disease Program	12.0	5.6		67.2
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
		Subtotal	12.4	0.0	
				Personnel Total	$148.8

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
Marrowstone - Cordova (PWS sampling)	1.2	2	28	0.3	10.8
Marrowstone - Sitka (sampling)	1.2	2	14	0.3	6.6
Marrowstone - Anchorage (PI meeting)	1.2	1	5	0.3	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$20.1

FY21

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
PERSONNEL & TRAVEL DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ **OIL SPILL TRUSTEE COUNCIL**
PROGRAM 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
Fish food, enrichments, and live feed production for SPF herring	17.0
Laboratory supplies (cell culture, histology, molecular biology, parasitology, virology, etc.)	22.0
Commodities Total	$39.0

FY21

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
CONTRACTUAL & COMMODITIES DETAIL

Date Prepared: 08/24/2016

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
PROGRAM 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
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY21

Project Title: Herring Disease Project
Primary Investigator: Paul Hershberger
Agency: USGS

FORM 4B
EQUIPMENT DETAIL

Date Prepared: 08/24/2016