PROPOSAL SIGNATURE FORM

THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL. If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (Trustee Council Data Policy*, adopted March 17, 2008) and reporting requirements (Procedures for the Preparation and Distribution of Reports**, adopted June 27, 2007).

PROJECT TITLE:	**PWS Herring Research and Monitoring: Herring Disease Program (HDP)**
Printed Name of PI	**Paul Hershberger, Ph.D.**
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* www.evostc.state.ak.us/Policies/data.cfm
** www.evostc.state.ak.us/Policies/reporting.cfm

FY10 INVITATION
PROPOSAL SUMMARY PAGE

Project Title: PWS Herring Research and Monitoring: Herring Disease Program (HDP)

Project Period: October 1, 2011 – September 30, 2016

Primary Investigator(s): Paul K. Hershberger, USGS – Marrowstone Marine Field Station

Study Location: Field components will be performed in Prince William Sound (adult herring spawning aggregations and juvenile rearing bays), Sitka Sound (adult spawning aggregations), and Puget Sound (adult spawning aggregations). Laboratory components will be performed at the USGS – Marrowstone Marine Field Station.

Abstract:
The *Herring Disease Program (HDP)* is part of a larger integrated effort, Prince William Sound Research and Monitoring (outlined in a separated proposal by Dr. Scott Pegau). Within this integrated effort, the *HDP* is intended to evaluate the impact of infectious and parasitic diseases on the failed recovery of the PWS herring population. The framework for the 2012 – 2016 *HDP* involves a combination of field surveillance efforts, field-based disease process studies, and laboratory-based controlled studies. Field surveillance efforts will provide continued and expanded infection and disease prevalence data for herring populations in Prince William Sound (PWS), Sitka Sound, and Puget Sound. Additionally, samples from field surveillance efforts will be processed using newly developed disease forecasting tools to provide annual risk assessments that quantify the potential for future disease epizootics. Laboratory-based empirical studies will provide an understanding of cause-and effect epidemiological relationships between the host, pathogen, and environment; understanding of these relationships represents a first step towards developing additional disease forecasting tools. Specific emphasis will be placed on refining our understanding disease processes specific to viral hemorrhagic septicemia (VHS) and ichthyophoniasis, two primary diseases of herring in PWS. Additionally, a novel diagnostic tool for Ichthyophonus, a fluorescent in situ hybridization (FISH) probe, will be developed.

Estimated Budget:
EVOS Funding Requested *(must include 9% GA)*

FY12	FY13	FY14	FY15	FY16	Total
$0 K	$0 K	$267.5K K	277.5K	$283.6K	$828.6 K

(NOT TO EXCEED ONE PAGE)

I. NEED FOR THE PROJECT

A. Statement of Problem

Robust Pacific herring (*Clupea pallasii*) populations, suitable for exploitation by commercial fisheries, are typically sustained by periodic recruitment of strong year classes into the adult spawning population. However, the Prince William Sound (PWS) herring population has not had a strong recruitment class since 1989, when the *Exxon Valdez* Oil Spill (EVOS) occurred. In the EVOS settlement herring were identified as an injured resource and they remain listed as an unrecovered species by the EVOS Trustee Council (EVOSTC). Understanding why herring have not recovered in Prince William Sound requires understanding potential bottlenecks in the herring life cycle. The identification of the limiting conditions to herring recovery requires a series of focused process studies combined with monitoring of the natural conditions that affect herring survival.

Described here are projects for a program that will enhance the current monitoring efforts of the Alaska Department of Fish and Game (ADF&G), and examine aspects of particular life stages to allow better modeling of herring populations. **The long-term goal of the program is to improve predictive models of herring stocks through observations and research.** While we do not anticipate that there will be a major change in our modeling ability in the next five years, we expect that the combination of monitoring and focused process studies will provide incremental changes over the next twenty years and result in a much better understanding of herring populations by the end of the program.

B. Relevance to 1994 Restoration Plan Goals and Scientific Priorities

The proposed program addresses the goals and priorities outlined in the 1994 Restoration Plan (http://www.evostc.state.ak.us/Universal/Documents/Publications/IHRP%20DRAFT%20-%20July%202010.pdf) and in the FY 2012 invitation for proposals. In particular our program addresses the need to "Conduct research to find out why Pacific herring are not recovering" and "Monitor recovery", listed on page 48 of the 1994 Restoration Plan. It will lead to the development of new tools to improve herring management. The latter will be accomplished by providing the information needed to develop or test biological and physical models of herring growth.

In November 2006, a Herring Steering Committee was formed and tasked with developing a focused Restoration Program that identifies strategies to address recovery and restoration of herring, recognizing that activities in the program must span an ecologically relevant time frame that accounts for herring population dynamics and life history attributes. A draft Integrated Herring Restoration Program (IHRP) was completed in the fall of 2008 and was further refined in July of 2010. The main goal of the program is to determine what, if anything, can be done to successfully recover the Pacific herring in PWS. In order to determine what steps can be taken, the program examines the factors limiting recovery of herring in PWS, identifies and evaluates potential recovery options, and recommends a course of action for achieving restoration.

Based on the recommendations of the IHRP the Trustee Council has stated in the FY12 request for proposals that they have chosen Restoration Option #2, Enhanced Monitoring, as the focus for their research interests. The program described below aims to meet the goals of this option by utilizing a combination of monitoring efforts to provide more information about the existing stock and process studies to elucidate aspects of the herring life cycle necessary to move us towards an analytical modeling approach.

II. PROJECT DESIGN

A. Objectives

During FY 2012-2016, the Herring Disease Program (HDP) will address the following objectives:

- Provision of disease prevalence data necessary for the ASA herring model
- Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS
- Collection of novel disease forecasting data
- Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships
- Development of a novel diagnostic technique (fluorescent in situ hybridization) intended to provide confirmatory diagnosis of *Ichthyophonus* from histology sections.

B. Procedural and Scientific Methods

Provision of disease prevalence data necessary for the ASA herring model

Disease is now 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 that are currently endemic 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 and sixty juvenile herring per site from three sites in PWS to test for disease. 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)." We will also examine efficacy of newly-developed procedures that may forecast the potential for future disease mortalities and simplify the disease surveillance efforts.

Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS

Mortality from infectious and parasitic diseases has been identified as a leading hypothesis accounting for the decline and failed recovery of PWS herring (Marty et al. 1998; Marty et al. 2003; Marty et al. 2010); unfortunately, the location and timing of the acute and / or chronic mortalities remain unaddressed because of difficulties inherent to sampling in marine systems. However, recent empirical studies provide insights into seasonal periods that are critical to disease processes, based on water temperatures and herring behavioral patterns. For example, the probability of viral hemorrhagic septicemia (VHS) epizootics increase as water temperature decreases, because virulence, magnitude and duration of viral shedding, and VHSV persistence in infected hosts increase as the temperature decreases (Hershberger unpublished data). Similarly, the infectivity of *Ichthyophonus* to Pacific herring is inversely related to temperature, with infection prevalence decreasing from 76%, 54%, and 24% at temperatures of 9.3°C, 12°C, and 15.3°C, respectively (Gregg et al 2011).

In association with sampling from other components of this program, we will investigate the seasonality of these diseases by focusing disease surveys during the coldest periods of the year,

when *Ichthyophonus* infectivity is highest and VHS is likely to have its greatest impacts. An additional risk factor for VHS mortality includes periods of high aggregation when effective fish-to-fish transmission is most likely to occur (Hershberger et al. 2010; Hershberger et al. submitted); this risk factor is enhanced during cold water periods, when viral shedding from carrier individuals is greatest. Therefore, field disease surveillance efforts will be focused on the overwinter and spring-spawning periods. Additionally, controlled laboratory studies will be performed to further understand cause-and-effect disease relationships and to further develop predictive tools that forecast the potential for disease-related mortality.

Collection of novel disease forecasting data

High-throughput techniques intended to forecast the potential for future herring mortalities caused by viral hemorrhagic septicemia are currently being developed, optimized, and validated. The techniques are based on the well-demonstrated concept that survivors of prior VHS exposure demonstrate resistance to the disease after subsequent exposure to the virus. Therefore, the potential for future VHS epizootics and resulting fish kills can be enumerated if we can determine the prior exposure history and subsequent levels of herd immunity conferred to wild herring populations; whereby previously-exposed populations would have high immunity and a resulting low potential for future VHS impacts. We have successfully developed an enzyme-linked immunosorbent assay (ELISA) that quantifies the prior exposure history of herring populations by detecting levels of circulating antibodies that are specific to VHSV. We are in the final phases of ELISA optimization and validation. This tool will be incorporated into the annual herring assessments to determine the potential for future VHS epizootics in the PWS populations. Additionally, we will continue to develop further disease forecasting tools for VHS and other primary diseases of PWS herring, including ichthyophoniasis and viral erythrocytic necrosis.

Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships

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 thousands of SPF herring in each of 4 age classes (age 0, 1, and 5 and 6 yr) for use as experimental animals. These laboratory animals are the only SPF herring known to exist and are offered as an in-kind contribution to the proposed project. Additional colonies need to be developed and maintained to satisfy the needs described in this proposal.

Colonies of specific pathogen-free (SPF) Pacific herring will be reared at the USGS - Marrowstone Marine Field Station each year, taking special precautions to prevent their exposure to marine pathogens or antigens of marine pathogens through the rearing water or feed. As a source of SPF Pacific herring, naturally deposited herring eggs attached to submerged macrophytes will be collected from locations in Puget Sound, WA. Herring eggs and associated macrophytes will be transported to the USGS, Marrowstone Marine Field Station, where they will be incubated in 260 L tanks supplied with single-pass, processed seawater. Ambient seawater will be processed by double sand-filtration, 100 µm particle filtration, and double UV-irradiation prior to delivery to culture facilities where SPF herring will be reared and live feeds will be produced. Submerged macrophytes will be removed from the tanks after yolk sac larvae have emerged. Early larvae will be fed live rotifers (*Brachionus plicatilis*) and later weaned to

Artemia nauplii (*Artemia franciscana*, instar 1-2). Live rotifer colonies will be maintained on concentrated algae, (*Isochrysis* sp., *Nannochloropsis* sp.) and Artemia will be hatched daily from chlorine-decapsulated cysts; both live feed items will be enriched with Super Selco® (INVE Aquaculture; Dendermonde, Belgium), Protein HUFA (Salt Creek Inc., Salt Lake City, Utah), or Algamac 3050 (Aquafauna Bio-Marine, Hawthorne, California) for 12 hr prior to use. The enrichments will be rotated daily. Herring larvae will later be weaned onto Cyclop-eeze™, a product of frozen copepods harvested from a freshwater Arctic lake (Argent Laboratories, Redmond, WA).

Development of a novel diagnostic technique (fluorescent in situ hybridization) intended to provide confirmatory diagnosis of Ichthyophonus from histology sections.
Fluorescent *in situ* hybridization (FISH) allows specific nucleic acid sequences to be identified in morphologically preserved cells or tissues. FISH is often used for specific identification of a pathogen in host tissues, but has also been used for a wide range other applications, including the identification (using epifluorescence microscopy) or quantification (using flow cytometry) of microbial and fungal communities in aquatic environments (Amann and Fuchs 2008; Jobard, Rasconi et al. 2010). The most common nucleic acid targets are regions within the ribosomal gene complex; this gene region is widely used for phylogenetic analyses. The fluorescently-labeled oligonucleotide probes diffuse into permeabilized cells and hybridize to homologous DNA or RNA sequences. A major drawback of the technique can be low sensitivity due to the ribosome content in the cells or high background due to autofluorescence (Jobard, Rasconi et al. 2010). However, assay sensitivity can be improved using probes labeled with horseradish peroxidase (HRP) which catalyze multiple fluorescent labeled tyramides (Catalyzed reporter deposition (CARD)-FISH) (Schmidt, Chao et al. 1997).

There are currently no FISH assays available for the detection of *Ichthyophonus* but methods have been developed for other members of the Class Mesomycetozoea. ISH has been used to successfully to identify *Rhinosporidium seeberi* in human tissues and lake water (Fredericks, Jolley et al. 2000; Kaluarachchi, Sumathipala et al. 2008) and *Anurofeca richardsi* spores in frog feces (Baker, Beebee et al. 1999),

Ichthyophonus-specific oligonucleotide probes will be designed to conserved portions of the 18S small subunit (SSU) ribosomal gene; the SSU gene has been sequenced in a range of *Ichthyophonus* isolates (Criscione, Watral et al. 2002; Rasmussen, Purcell et al. 2010). Heart and skeletal muscle tissue from *Ichthyophonus* infected herring will subjected to routine processing and paraffin embedding using published procedures (Garver, Conway et al. 2005). Serial 5 µm tissue sections will be subjected to ISH using previously described methods (Carnegie, Meyer et al. 2003) (Fredericks, Jolley et al. 2000). Briefly, fluorescently-labeled oligonucleotide probes will be purchased commercially. Sections will de-paraffinized, re-hydrated and digested with proteinase K and/or lysozyme. Probes will be hybridized to the sections, washed and slides will be examined by epifluorescence microscopy. A variety of parameters will be evaluated for optimal assay performance, including (1) probe design, (2) fluorochrome choice, (3) tissue fixation procedures, (4) hybridization conditions and (5) use of tyramide signal amplification (CARD-FISH) to enhance sensitivity.

Assay development and validation will be performed using tissues sampled from laboratory-challenged Pacific herring and *Ichthyophonus* culture. Assay sensitivity will be compared to

tissue explant culture and histopathological examination. Specificity will be tested using fish infected with the freshwater form of *Ichthyophonus* (Hershberger, Pacheco et al. 2008; Rasmussen, Purcell et al. 2010) as well as tissue samples infected with other mesomycetozoeans (obtained from various collaborators).

C. 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 $p \leq 0.05$. Prevalences 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% population prevalence with 95% confidence.

D. Description of Study Area

The study area includes all of Prince William Sound. However, most of the projects will focus on the four bays (Zaikof, Whale, Eaglek, and Simpson) that were extensively studied during the Sound Ecosystem Assessment study and PWS Herring Survey program (Figure 1). This allows the work to build upon the historical research completed in those bays. These bays also cover four different quadrants of the Sound. We anticipate a potential build out to include other bays or contraction based on the results from the synthesis. As part of the synthesis effort we will be reviewing the question "What is the appropriate sampling distribution?" as applied to the questions of juvenile herring condition and providing an index of juvenile abundance.

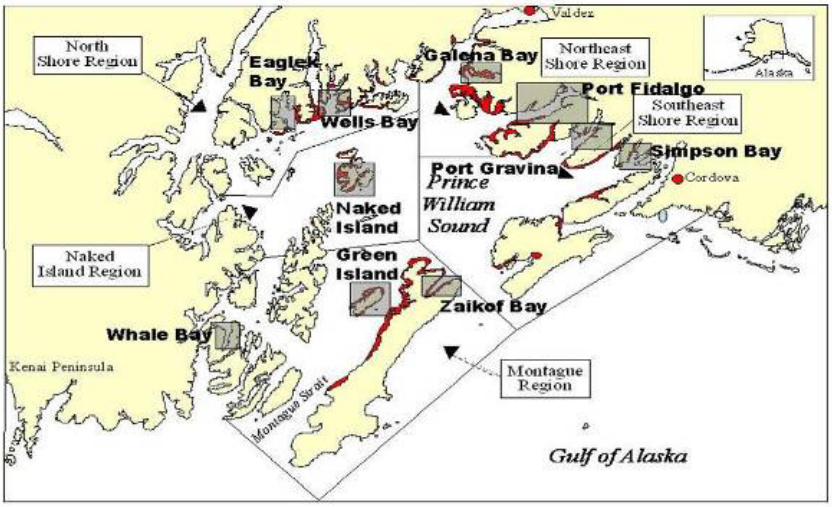

Figure 1. PWS study area, including the four SEA bays (Whale, Zaikof, Eaglek, and Simpson, as well as other bays historically important for juvenile herring.

Herring collection sites in Sitka Sound and Puget Sound will be determined by the respective management authority in each region (ADF&G and WDF&W, respectively), but are likely to include locations similar to those described in Table 1.

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.

III. SCHEDULE

A. Project Milestones

- ***Provision of disease prevalence data necessary for the ASA herring model***
  To be met by June 30 each year.
- ***Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS***
  Laboratory diagnostics will be completed <8 weeks after sample collections in the field
- ***Collection of novel disease forecasting data***
  Laboratory diagnostics will be completed <4 weeks after the sample collections in the field
- ***Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships***
  SPF juveniles will be produced by July 15 each year
- ***Development of a novel diagnostic technique (fluorescent in situ hybridization) intended to provide confirmatory diagnosis of Ichthyophonus from histology sections.***
  Will be developed by Sept 30, 2014.

B. Measurable Project Tasks

Every Fiscal Year (FY 2014 - 2016)

1st Quarter (October 1-December 31)

- Project funding approved by TC
- Perform empirical disease studies in the laboratory

2nd Quarter (January 1-March 31)

- Attend Alaska Marine Science Symposium and present results

- Collect herring eggs for rearing SPF colonies
- Begin collecting adult herring to determine infection and disease prevalence
- Perform empirical disease studies in the laboratory

3rd Quarter (April 1-June 30)

- Finish collecting and processing spring adult herring to determine infection and disease prevalence.
- Participate in PI meeting in Cordova
- Perform empirical disease studies in the laboratory

4th Quarter (July 1- Sept. 30)

- Perform empirical disease studies in the laboratory

Additional Quarterly Tasks

FY14, 1st quarter (October-December 31, 2013)

- Begin FISH development

FY14, 4th quarter (July 1 – Sept 30, 2014)

- Complete FISH development

FY16, 1st quarter (October 1 - December 31, 2015)

- Start drafting final report
- Participate in 1st PI integration meeting

FY16, 2nd quarter (January 1 - March 31, 2016)

- Participate in 2nd PI integration meeting

FY16, 4th quarter (July - September 30, 2016)

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

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Marty, G.D., E.F. Freiberg, T.R. Meyers, J. Wilcock, T.B. Farver, D.E. Hinton. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* in Prince William Sound, Alaska, USA. Diseases of Aquatic Organisms 32: 15-40.

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ABBREVIATED RESUME

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Professional Interests

Disease ecology and processes affecting the health and survival of wild fishes
Effects of multiple stressors on the health and survival of wild fishes
Climatic/oceanic factors affecting populations of wild fishes

Membership in Professional Organizations

American Fisheries Society (AFS), and Fish Health Section (FHS)
International Society of Aquatic Animal Epidemiology (ISAAE)
Pacific Northwest Society of Environmental Toxicology and Chemistry (PNW SETAC)

Recent Positions

2004 – Present: Affiliate Assistant Professor: School of Aquatic and Fishery Sciences, University of Washington.
2003 – Present: Research Fishery Biologist and Station Leader: USGS- BRD, Marrowstone Marine Field Station
1999-2003: Faculty Research Associate - University of Washington
2003: Co-Instructor, UW – Friday Harbor Labs: FISH-499B "Emerging Diseases and Latent Infections in Aquatic Organisms"
2001: Instructor, UW – School of Aquatic and Fishery Sciences: FISH 404 "Diseases of Aquatic Organisms"
2001: Co-Instructor, UW – Friday Harbor Labs: FISH 499B: "Latent Viruses in Marine Fish,"
2000: Co-Instructor, UW – Friday Harbor Labs: FISH-499B: "Marine Fish Disease Research"

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 Awards and Honors:

2008: USGS STAR Award
2004: USGS Exemplary Act Award
2004: USGS STAR Award
2001: Most significant paper of the year 2001: Journal of Aquatic Animal Health

Five Selected Publications Relevant to this Proposal:

Vollenweider, J.J., J. Gregg, R.A. Heintz, P.K. Hershberger. 2011. Energetic cost of *Ichthyophonus* infection in juvenile Pacific herring (*Clupea pallasii*). Journal of Parasitology Research.doi:1155/2011/926812, 10 pp

Gregg J, J Vollenweider, C Grady, R Heintz, P Hershberger. 2011. Effects of environmental temperature on the dynamics of ichthyophoniasis in juvenile Pacific herring (*Clupea pallasii*). Journal of Parasitology Research. doi: 10.1155/2011/563412, 9pp.

Grady, C.A., J.L. Gregg, R.M. Collins, P.K. Hershberger. 2011. Viral Replication in Excised Fin Tissues (VREFT) corresponds with prior exposure of Pacific herring, *Clupea pallasii* (Valenciennes), to *viral haemorrhagic septicaemia virus* (VHSV). Journal of Fish Diseases 34: 34:-12.

Hershberger PK, JL Gregg, CA Grady, L Taylor, JR Winton. 2010. Chronic and persistent viral hemorrhagic septicemia virus infections in Pacific herring. Diseases of Aquatic Organisms 93: 43-49.

Hershberger P, J Gregg, C Grady, R Collins, J Winton. 2010. Kinetics of viral shedding provide insights into the epidemiology of viral hemorrhagic septicemia in Pacific herring. Marine Ecology Progress Series 400: 187-193.

Five Additional Selected Publications

Kocan R, H Dolan, P Hershberger. 2011. Diagnostic methodology is critical for accurately determining the prevalence of *Ichthyophonus* infections in wild fish populations. Journal of Parasitology 97: 344-348.

Hart L, GS Traxler, KA Garver, J Richard, JL Gregg, CA Grady, G Kurath, PK Hershberger. 2011. Larval and juvenile Pacific herring *Clupea pallasii* are not susceptible to infectious hematopoietic necrosis under laboratory conditions. Diseases of Aquatic Organisms 93: 105-110.

Hershberger, P.K., B.K. van der Leeuw, J.L. Gregg, C.A. Grady, K. Lujan, S. Gutenberger, M. Purcell, J.C. Woodson, J.R. Winton, M. Parsley. 2010. Amplification and transport of an endemic fish disease by an invasive species. Biological Invasions 12: 3665-3675.

Hershberger PK, JL Gregg, CA Grady, RM Collins, JR Winton. 2010. Susceptibility of three stocks of Pacific herring to viral hemorrhagic septicemia. Journal of Aquatic Animal Health 22: 1-7.

Kocan, R. M., J. L. Gregg, P. K. Hershberger. 2010. Release of infectious cells from epidermal ulcers in *Ichthyophonus* sp. – infected Pacific herring (*Clupea pallasii*): evidence for multiple mechanisms of transmission. Journal of Parasitology 96: 348-352.

Recent Collaborators and Co-Authors:

W. Batts (USGS-WFRC), B. Bui (UW-FHL), E. Emmenegger (USGS), N. Elder (USGS), D. Elliott (USGS), J. Gregg (USGS), J. Hansen (USGS), R. Kocan (UW-SAFS), G. Kurath (USGS), S. LaPatra (Clear Springs Foods), M. Purcell (USGS), J. Richard (DFO), N. Sholtz (NMFS – NW Center), K. Stick (WDFW), G. Traxler (DFO), N. Van der Straaten (UW-FHL)

Budget Justification

Personnel Costs = $382.8K

FY12 ($0)
None requested: personnel are provided in the currently funded HDP.

FY13 ($0)
None requested: personnel are provided in the currently funded HDP.

FY14 ($170.4K)
Funding is requested to support a GS-7 laboratory technician ($60 K) at the Marrowstone Marine Field Station to perform laboratory studies, process samples from laboratory studies, perform predictive disease assays, and process Puget Sound herring survey samples. Funding is also requested to support a GS-7 laboratory technician ($60K) at the Western Fisheries Research Center, where development of the FISH will occur. Funding is also requested for student interns ($50.4K total) who will assist with the rearing of larval herring and with empirical studies.

FY15 ($186.6K)
Continued funding is requested to support a GS-7 laboratory technician ($62.4 K) at the Marrowstone Marine Field Station. A GS-9 post doc ($72.0 K) will be hired to assist with empirical laboratory studies. Funding is also requested for student interns ($52.2K total) who will assist with the rearing of larval herring and with empirical studies.

FY16 ($190.8)
Continued funding is requested to support a GS-8 laboratory technician ($63.6 K) and a GS-9 post doc ($73.2K) at the Marrowstone Marine Field Station; responsibilities will be the same as the previous year. Funding is also requested for student interns ($54.0K total) who will assist with the rearing of larval herring and with empirical studies.

Travel Costs = $37.0 K

FY12 ($0)
None requested: travel costs are provided in the currently funded HDP.

FY13 ($0)
None requested: travel costs are provided in the currently funded HDP.

FY14 ($ 17.0 K)
Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($7.6K), perform Sitka field sampling ($3.2K K), present at the annual Marine Science Symposium ($3.4K). Additional travel support is requested (2.8K) for the PI to participate in the annual herring integration meeting in Cordova.

FY15 ($17.0 K)

Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($7.6K), perform Sitka field sampling ($3.2K K), present at the annual Marine Science Symposium ($3.4K). Additional travel support is requested (2.8K) for the PI to participate in the annual herring integration meeting in Cordova.

FY16 ($18.4 K)
Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($7.6K), perform Sitka field sampling ($3.2K K), present at the annual Marine Science Symposium ($3.4K). Additional travel support is requested (4.2K) for the PI to participate in the annual herring integration meeting in Cordova.

Contractual Costs = $236.6K

FY ’12 ($0 K)
None Requested.

FY ‘13 ($0 K)
None Requested

FY ‘14 ($12.0 K)
Funding for a subcontract ($12.0K), administered through WFRC, is requested to include two months salary for a fish health professional with expertise working with VHS and *Ichthophonus*.

FY’15 ($12.0 K)
Funding for a subcontract ($12.0K), administered through WFRC, is requested to include two months salary for a fish health professional with expertise working with VHS and *Ichthophonus*.
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FY’16 ($12.0 K)
Funding for a subcontract ($12.0K), administered through WFRC, is requested to include two months salary for a fish health professional with expertise working with VHS and *Ichthophonus*.

Commodities = $124.0 K

Commodities for FY 2014-2016 include laboratory supplies for the Marrowstone Marine Station ($15.0-17.0K/yr for fish food, $20.0 -22.0K for dry lab supplies). Separate funding ($13.2 K/ yr) is requested for ADF&G Pathology Laboratory in Juneau to process PWS and Juneau herring samples.

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.

Data Management and Quality Assurance / Quality Control (QA/QC) Statement:

The USGS, Marrowstone Marine Field Station and Western Fisheries Research Center comply with all data management and QA/QC policies described in the USGS-Survey Manual describing Fundamental Science Practices, particularly:

- SM 502.1, Fundamental Science Practices Foundation Practices, http://www.usgs.gov/usgs-manual/500/502-1.html
- 502.2 - Fundamental Science Practices: Planning and Conducting Data Collection and Research, http://www.usgs.gov/usgs-manual/500/502-2.html
- 502.3 - Fundamental Science Practices: Peer Review, http://www.usgs.gov/usgs-manual/500/502-3.html
- 502.4 - Fundamental Science Practices: Review, Approval, and Release of Information Products, http://www.usgs.gov/usgs-manual/500/502-4.html
- 205.18 - Authority to Approve Information Products, http://www.usgs.gov/usgs-manual/200/205-18.html
- Part 1100 – Publishing, http://www.usgs.gov/usgs-manual/t500.html#pubs

Additionally, both laboratories maintain accreditation with the Association for Assessment of Laboratory Animal Care (AALAC) through semiannual inspections and certifications with the University of Washington Institutional Animal Care and Use Committee (IACUC), overseeing laboratory animal welfare and human health issues associated with utilizing live animals for experimental purposes.

The USGS Marrowstone Marine Field Station and Western Fisheries Research Center maintain semiannual certification with the University of Washington Institutional Animal Care and Use Committee that oversees both laboratory animal welfare and human health issues associated with utilizing live animals for experimental purposes. Additionally, both facilities are inspected twice annually by an internal Institutional Animal Care and Use Committee, and both laboratories conform to Title 21 Code of Federal Regulations: Good Laboratory Practice for Nonclinical Laboratory Studies.

Budget Justification

Personnel Costs = $382.8K

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FY13 ($0)
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