FY14 PROGRAM PROJECT
PROPOSAL FORM

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

Project Period: February 1, 2014 – January 31, 2015

Primary Investigator(s):
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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. During FY 2014 we will continue the health assessments of adult herring from Prince William Sound and Sitka Sound, we will continue to rear colonies of specific-pathogen-free Pacific herring for controlled studies in the laboratory, and we will develop a chromogenic in situ hybridization assay that will be capable of identifying *Ichthyophonus* in histological tissue sections.

Estimated Budget:
EVOSTC Funding Requested:

FY12	FY13	FY14	FY15	FY16	TOTAL
0	0	$281,900	291,900	298,000	871,800

(Funding requested must include 9% GA)

Non-EVOSTC Funds to be used:

FY12	FY13	FY14	FY15	FY16	TOTAL
		$42,100			

Includes in-kind salary and benefit contributions (20%) for P. Hershberger ($26,400) and J. Gregg ($15,700)

Date: August 9, 2013

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I. NEED FOR THE PROJECT

A. Statement of Problem

A leading hypothesis accounting for the decline and failed recovery of Pacific herring populations in Prince William Sound and other locations throughout the NE Pacific involves chronic and acute mortality from infectious and parasitic diseases including ichthyophoniasis, viral hemorrhagic septicemia (VHS), viral erythrocytic necrosis (VEN), and others (Marty et al, 1998; Marty et al. 2003; Marty et al. 2010). Here, we propose to follow up on earlier EVOS TC-funded herring disease studies by:

1) continuing surveillances of PWS herring populations for prevalence and intensity of the primary pathogens and using newly-developed disease forecasting tools to quantify the potential for future disease epizootics,
2) performing field-based disease process studies in coordination with other components of the PWS Herring Project; these observational studies will begin to address epizootiological factors including temporal and geographical patterns of pathogen exposure and resulting disease-induced mortalities that occur in wild herring populations,
3) performing laboratory-based empirical studies intended to determine cause-and effect disease relationships; these relationships will be used to develop additional disease forecasting tools and understand the fundamental disease processes

B. Summary of Project to Date (if applicable)

FY 2014 will be the first year of a new integrated herring project.

II. PROJECT DESIGN

A. Objectives

- Provision of disease prevalence data necessary for the ASA herring model
- 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.

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.

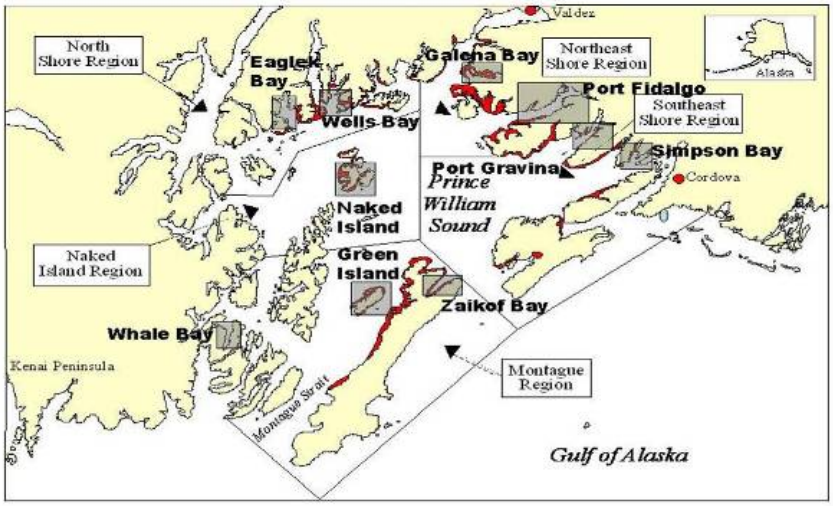

Figure 1. PWS study area, including the four SEA bays (Whale, Zaikof, Eaglek, and Simpson, as well as other bays historically important for juvenile herring.

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.

E. Coordination and Collaboration with the Program

Results from the HDP will inform the larger Herring Research and Monitoring Project by providing disease information intended to help improve predictive models of herring stocks. This will be accomplished by informing the ASA model with infection and disease values and by applying novel techniques to assess diseases-related mortality in wild herring.

III. CV's/RESUMES

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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): Current President
International Society of Aquatic Animal Epidemiology (ISAAE)
Pacific Northwest Society of Environmental Toxicology and Chemistry (PNW SETAC)

Recent Positions

2010 – Present: Affiliate Associate Professor: School of Aquatic and Fishery Sciences, University of Washington.
2004 – 2010: 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:

Lovy, J., P. Piesik, P.K. Hershberger, K. A. Garver. 2013. Experimental infection studies demonstrating Atlantic salmon as a host and reservoir of viral hemorrhagic septicemia virus type IVa with insights into pathology and host immunity. Veterinary Microbiology 166: 91-101.

Kocan, R, S. LaPatra, P. Hershberger. 2013. Evidence for an amoeba-like infectious stage of *Ichthyophonus* sp. and description of a circulating blood stage: a probable mechanism for dispersal within the fish host. Journal of Parasitology 99: 235-240.

Hershberger, P.K., M. K. Purcell, L.M. Hart, J.L. Gregg, R.L. Thompson, K.A. Garver, J.R. Winton. 2013. Influence of temperature on viral hemorrhagic septicemia (Genogroup IVa) in Pacific herring, *Clupea pallasii* Valenciennes. Journal of Experimental Marine Biology and Ecology 444: 81-86.

Lovy, J., N.L. Lewis, P.K. Hershberger, W. Bennett, K.A. Garver. 2012. Viral tropism and pathology associated with viral hemorrhagic septicemia in larval and juvenile Pacific herring. Veterinary Microbiology 161: 66-76.

Purcell, M.K., E.S. Bromage, J. Silva, J.D. Hansen, S.M. Badil, J.C. Woodson, P.K. Hershberger. 2012. Production and characterization of monoclonal antibodies to IgM of Pacific herring (*Clupea pallasii*). Fish and Shellfish Immunology 33: 552-558.

Five Additional Selected Publications

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. *In Press*. Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science.

Hershberger, P.K., L. Rhodes, G. Kurath, J. Winton. *In Press*. Infectious diseases of fishes in the Salish Sea. Fisheries.

Hart, L.M., N. Lorenzen, S.E. LaPatra, C.A. Grady, S.E. Roon, J. O'Reilly, J.L. Gregg, P.K. Hershberger. 2012. Efficacy of a glycoprotein DNA vaccine against viral hemorrhagic septicemia (VHS) in Pacific herring *Clupea pallasii*. Journal of Fish Diseases 775-779.

Glenn, J.A., E.J. Emmenegger, C. M. Conway, J. R. Winton, C.A. Grady, J.L. Gregg, S.E. Roon, P.K. Hershberger. 2012. Kinetics of viral load and erythrocytic inclusion body formation in Pacific herring artificially infected with erythrocytic necrosis virus. Journal of Aquatic Animal Health 195-200

Gregg, J.L., C.A. Grady, C.S. Friedman, P.K. Hershberger. 2012. Inability to demonstrate fish-to-fish transmission of *Ichthyophonus* from laboratory-infected Pacific herring *Clupea pallasii* to naïve conspecifics. Diseases of Aquatic Organisms 99: 139-144

Recent Collaborators and Co-Authors (Past 4 years):

S.M. Badil (USGS – Western Fisheries Research Center), **J. Beaulaurier** (Central Michigan University), **W. Bennett** (DFO – Pacific Biological Station), **N. Bickford** (University of Great Falls), **E.S. Bromage** (U. Mass – Dartmouth), **C.A. Burge** (Cornell University), **H.E. Christiansen** (Columbia River Research Laboratories), **R. Collins** (U. Hawaii), **C.M. Conway** (USGS – Western Fisheries Research Center), **E.S. Copeland** (USGS – Columbia River Research Laboratories), **H. Dolan** (University of Washington), **C.M. Eakin** (NOAA – Coral Reef Watch), **D. Elliott** (USGS – Western Fisheries Research Center), **E.J. Emmenegger** (USGS – Western Fisheries Research Center), **C.S. Friedman** (University of Washington), **B. Froelich** (University of North Carolina – Chapel Hill), **A. Gannam** (USFWS – Abernathy Fish Technology Center), **K.A. Garver** (DFO – Pacific Biological Station), **J.A. Glenn** (USGS – Western Fisheries Research Center), **T. L. Goldberg** (University of Wisconsin), **C. Grady** (USGS – Marrowstone Marine Field Station), **J.L. Gregg** (USGS – Marrowstone Marine Field Station), **S. Gutenberger** (Lower Columbia River Fish Health Center), **J.D. Hansen** (USGS – Western Fisheries Research Center), **L. Hart** (USGS – Marrowstone Marine Field Station), **C.D. Harvell** (Cornell University), **R.A. Heintz** (NOAA – Auke Bay Labs), **E.E. Hofmann** (Old Dominion University), **R.F. Goetz** (NOAA- Manchester Research Station), **A. Kagley** (NOAA – Northwest Fisheries Science Center), **R.M. Kocan** (University of Washington), **G. Kurath** (USGS – Western Fisheries Research Center), **K.L. Toohey-Kurth** (University of Wisconsin), **S.E. LaPatra** (Clear Springs Foods, Inc.), **N.L. Lewis** (DFO – Pacific Biological Station), **N. Lorenzen** (National Veterinary Institute – Denmark), **J. Lovy** (New Jersey Department of Natural Resources), **K. Lujan** (USFWS – Lower Columbia River Fish Health Center), **S.V. Marquenski** (Wisconsin Department of Natural Resources), **M.G. Mesa**, (USGS – Columbia River Research Laboratories), **T.R. Meyers** (ADF&G), **C.H. Moon** (University of Ulsan, Korea), **B.L. Norcross** (U. Alaska – Fairbanks), **W. J. Olson** (University of Wisconsin), **J. O'Reilly** (USGS – Marrowstone Marine Field Station), **M. Parsley** (USGS – Columbia River Research Laboratories), **L. E. Petes** (NOAA – Climate Program Office), **P. Piesik** (DFO – Pacific Biological Station), **M.K. Purcell** (USGS – Western Fisheries Research Center), **K. C. Prager** (UCLA), **C. Rasmussen** (USGS – Western Fisheries Research Center), **L. Rhodes** (NOAA – Northwest Fisheries Science Center), **J. Richard** (DFO – Pacific Biological Station), **S.E. Roon** (Oregon State University), **A.C. Seitz** (U. Alaska – Fairbanks), **J. Silva** (U. Mass – Dartmouth), **L. Taylor** (USGS – Marrowstone Marine Field Station), **R.L. Thompson** (USGS – Western Fisheries Research Center), **G.S. Traxler** (DFO – Pacific Biological Station), **B.K. van der Leeuw** (USGS – Columbia River Research Laboratories), **J. J. Vollenweider** (NOAA – Auke Bay Labs), **E. Weil** (University of Puerto Rico), **B. L. Willis** (James Cook University – Australia), **A.E. Wilson** (University of Wisconsin), **J.R. Winton** (USGS – Western Fisheries Research Center), **J.C. Woodson** (USGS – Western Fisheries Research Center), **S. Zuray** (Rapids Research Center)

IV. 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 Aug 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 2010 - 2013)

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 CISH development

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

- Complete CISH development

V. BUDGET

Budget Form (Attached)