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 Survey: Herring Disease Program (HDP)**
Printed Name of PI	**Paul Hershberger, Ph.D.**
Email:	**phershberger@usgs.gov**
Mailing Address	**USGS – Marrowstone Marine Field Station** **616 Marrowstone Point Road**
City, State, Zip	**Nordland, WA, 98358**
Phone:	**(360) 385-1007, Ext. 225**
Signature of PI:	_____ Date: _____
Printed Name of PI	**James Winton, Ph.D.**
Email:	**jwinton@usgs.gov**
Mailing Address	**USGS – Western Fisheries Research Center** **6505 NE 65th Street**
City, State, Zip	**Seattle, WA, 98195**
Phone:	**(206) 526-6282, Ext. 328**
Signature of PI:	_____ Date: _____
Printed Name of PI	**Maureen K. Purcell, Ph.D.**
Email:	**mpurcell@usgs.gov**
Mailing Address	**USGS – Western Fisheries Research Center** **6505 NE 65th St.**
City, State, Zip	**Seattle, WA 98195**
Phone:	**(206) 526-6282 Ext. 252**
Signature of PI:	_____ Date: _____

* www.evostc.state.ak.us/Policies/data.cfm
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FY10 INVITATION
PROPOSAL SUMMARY PAGE

Project Title: PWS Herring Survey: Herring Disease Program (HDP)

Project Period: October 1, 2009 – September 30, 2013

Primary Investigator(s): Paul K. Hershberger, USGS – Marrowstone Marine Field Station
James R. Winton, USGS – Western Fisheries Research Center
Maureen K. Purcell, USGS – Western Fisheries Research Center

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, the *PWS herring survey: Community Involvement, Outreach, Logistics, and Synthesis submitted under the BAA* (outlined in a separated proposal by Dr. Scott Pegau), that is intended to identify juvenile rearing bays, measure factors limiting the success of juvenile herring, and provide recommendations for spatial and temporal coverage of future monitoring efforts. 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 by placing special emphasis on disease processes affecting juvenile cohorts. The framework for the 2010 – 2013 *HDP* involves a combination of field surveillance efforts and laboratory-based empirical disease process 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. Empirical disease process 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.

Estimated Budget:

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

FY10	FY11	FY12	FY13	Total
$81.8 K	$284.1 K	$295.8 K	$313.5K	$975.1 K

Non-EVOS Funds to be used:

FY10	FY11	FY12	FY13	Total
$61.6 K	$97.7 K	$100.4 K	$103.2 K	$362.9 K

(NOT TO EXCEED ONE PAGE)

I. NEED FOR THE PROJECT

A. Statement of Problem

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 (10,000 tons forecast in 2008). Consequently, the PWS herring population is currently classified as an "injured resource" that is "not recovering" (EVOSTC 2002) and commercial herring fisheries have been severely curtailed or closed in recent years. 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 between inorganic nutrients (mobilized by primary and secondary production) and organic nutrients (utilized by higher trophic level predators). Therefore, the collapse and failed recovery of the PWS herring population is likely a contributing factor for the failed recovery of other major PWS resources, including harbor seals, harlequin ducks, pigeon guillemots, common loons, and 3 species of cormorants.

Definitive cause(s) of the herring population decline and failed recovery in PWS remain undetermined; however, a leading hypothesis involves epizootic mortality that resulted 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 signs associated 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 massive epizootics in populations of wild herring, pilchards, and other forage species. 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 in wild marine fishes, where it can be highly virulent. 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). Epixootics 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 in 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 possible 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 fish pathogens (Ragan et al 1996, Herr et al 1999, reviewed in Mendoza et al 2002). Currently *I. hoferi* (reviewed in McVicar 1999) 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 *In preparation*). Additional molecular phylogenetic studies are necessary to better understand the relatedness of *I. hoferi* types (Criscione et al 2002, Halos et al 2005); 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 1999). 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.

Viral erythrocytic necrosis (VEN) is a condition characterized by the presence of viroplasmic inclusion bodies within the cytoplasm of affected erythrocytes (reviewed in Dannevig and Thorud 1999). Although the etiology is not completely understood, primarily because of the refractory nature of established cell lines to infection by the causative agent (Evelyn & Traxler 1978), the condition is cause by a putative iridovirus, referred to as erythrocytic necrosis virus (ENV). In the eastern North Pacific, VEN frequently occurs in Pacific herring *Clupea pallasii* (MacMillan and Mulcahy 1979), and it has been associated with epizootics among wild herring in Alaska and Washington State (Meyers et al 1986, Hershberger et al 2009). These epizootics can occur over the period of several months and can be geographically localized events among cohorts of juvenile herring (Hershberger et al 2009).

Populations of wild herring are often infected with multiple pathogens including VHSV, ENV, and *Ichthyophonus*, and confinement of wild cohorts into laboratory tanks results in initiation of the respective diseases. Timing and progression of the three resulting diseases differ. The VHS epizootic occurs first, characterized by an initially low infection prevalence that increases quickly with confinement time, peaking at 93-98 % after confinement for 6 d, then decreases to negligible levels after 20 d. The VHS outbreak is quickly followed by a VEN epizootic that, within 12 d of confinement, progresses from undetectable levels to 100 % infection prevalence

with >90 % of erythrocytes demonstrating inclusions. The VEN epizootic persists through at least 54 d and is characterized by severe blood dyscrasias including reduction of mean hematocrit from 42 % to 6 % and replacement of mature erythrocytes with circulating erythroblasts and ghost cells. All fish having naturally-acquired *Ichthyophonus* infections at capture die within the first 3 weeks of confinement, likely as a result of the detrimental effects of the concomitant viral diseases. These results illustrate the differences in disease ecology and exacerbating effects of multiple pathogens affecting Pacific herring and highlight the difficulty in ascribing a single causation to outbreaks of disease among populations of wild fishes (Hershberger et al 2006).

A better understanding of the epidemiological principles governing herring diseases in PWS is necessary for development of adaptive management strategies designed 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 from 2007 – present. However, by lacking the capacity for empirical manipulation, the disease forecasting potential provided by field surveys is typically weak. To address this shortcoming and provide quantifiable metrics useful for an adaptive management approach, the early surveillance efforts were expanded into the Prince William Sound Herring Disease Program (HDP) in 2007 that incorporated experimental studies intended to empirically demonstrate cause-and-effect disease relationships. These relationships are currently being used to develop predictive tools that forecast the relative potential for future disease epizootics. This proposed project represents a continuation of the ongoing HDP, by maintaining disease surveys in adult herring populations, expanding disease surveys to juvenile herring populations, continuing the determination of cause-and-effect relationships specific to each disease, continuing development of disease forecasting tools, and applying novel disease forecasting tools to wild populations.

B. Relevance to 1994 Restoration Plan Goals and Scientific Priorities

This proposal addresses the "Injured Resources and Services: Evaluation and Restoration" component of the Exxon Valdez Oil Spill (EVOS) Trustee Council invitation for proposals for federal fiscal year 2010. Pacific herring are identified as one of the six resources currently categorized as "not recovered," and the other identified resources, including harbor seals, harlequin ducks, pigeon guillemonts, common loons, and 3 species of cormorants, are dependent on herring as forage during portions of their life history. This proposal addresses the specific solicitation for projects that address "…the recovery process of resources that may not be currently exposed to lingering oil but are still not recovered."

Within the current EVOS TC invitation for proposals, specific solicitation was made for projects that build on past and ongoing efforts and integrate data collection, analysis, and findings with other proposed projects. This proposed project, the HDP, is one of several individual projects that have been integrated into a proposed program to address juvenile herring in PWS rearing bays. Details of the specific projects, their integration to each other, and their involvement with the local PWS community are described in an overview proposal submitted by Dr. Scott Pegau, titled *PWS herring survey: Community Involvement, Logistics, and integration, submitted under the BAA*.

This proposal also addresses the specific solicitation for enhanced disease monitoring in the invitation for proposals; disease is one of the five factors limiting recovery that were identified by the PWS Herring Steering Committee in the Integrated Herring Restoration Program (IHRP), where disease monitoring was also listed as one of four core factors. Furthermore, disease mitigation is listed as one of the eight Restoration Options recommended for PWS herring in the IHRP. Within the EVOS TC invitation for proposals, specific solicitation was made for proposals that extend herring health surveys into Sitka Sound or the geographic expansion of current disease monitoring outside PWS. To address this specific request, we propose to continue our monitoring of the health of herring populations in Sitka Sound and Puget Sound. Additionally, this proposal seeks to determine the impact of infectious and parasitic diseases on young-of-the-year (YOY) Pacific herring juveniles in the rearing bays, another critical stage identified in the 2010 EVOS invitation for proposals.

Additionally, the EVOS TC invitation for proposals has specifically requested feasibility studies to evaluate "restoration alternatives" identified by the steering committee, which include supplemental production, carrying capacity supplementation, predator management, and competitor management. Disease will be a primary concern for all of these proposed interventions; therefore, enhanced disease surveillance efforts and better understanding of disease processes are a necessary prerequisite prior to implementing any of these options, even at the pilot scale.

II. PROJECT DESIGN

The framework for the proposed 2010 – 2013 Herring Disease Program (HDP) involves a combination of expanded field surveillance efforts for herring infection / disease prevalences and laboratory-based empirical disease process studies. As a continuation of the currently funded HDP (funded through FY'10), this proposed project would extend infection and disease surveys in adult herring from PWS, Sitka Sound, and Puget Sound through FY'13.

As an expansion to the currently-funded HDP, this proposed project would broaden the current scope of infection and disease monitoring in PWS beyond exclusive focus on adult herring cohorts to include targeted surveys on juvenile cohorts. Previous exclusion of juvenile cohorts from the PWS herring infection and disease surveys resulted in a critical gap in our understanding of the epidemiology of certain herring diseases. For example, in wild Pacific herring both VHS and VEN are typically most severe in juvenile herring cohorts (Hershberger et al 1999, Marty et al 2003, Hershberger et al 2009). In fact, VEN was detected in PWS herring (juveniles) for the first time in 2007, presumably because earlier surveys made little effort to assay juveniles for the disease (Hershberger et al 2009). Because the portion of herring that survive their first encounters with these diseases develop strong adaptive immunity that confers solid protection against subsequent epizootics (Kocan et al 1997, Kocan et al 2001, Hershberger et al 2007), surveillance efforts for infection, disease, and exposure history in juvenile cohorts are likely to provide an excellent forecaster of the potential for future epizootics in adults. Spatial difficulties inherent to sampling juvenile cohorts in PWS have prevented organized disease surveillance efforts in the past; however, we are able to overcome these sampling impediments by integrating our sampling effort with the *PWS Herring Survey* (project plan summarized in a proposal by Dr. Scott Pegau) that will be sampling juvenile cohorts in rearing bays.

Recent advances in our understanding of VHS epidemiology in herring have revealed unique characteristics regarding the epidemiology of the primary herring diseases in PWS. Novel *in vitro* and *in vivo* tools, based on these advances, are currently being developed that provide insight into the susceptibility of herring populations to future disease epizootics. These tools will be applied to the PWS herring population to determine their susceptibility to VHS and provide annual VHS risk assessments.

Empirical disease process studies will be performed to gain a further understanding of disease kinetics and environmental and host conditions that preface disease epizootics. Greater understanding of cause-and-effect relationships is required to develop additional disease forecasting tools, predict the timing of disease epizootics, and mitigate the impacts of the epizootics. Special emphasis will be placed on refining our understanding of host, pathogen, and environmental conditions associated with VHS and ichthyophoniasis.

A. Objectives

I. Field Surveillance Efforts

A. Determine the infection and disease prevalences in adult Pacific herring from PWS, Sitka Sound, and Puget Sound.

B. Determine the prevalence of infection and disease in juvenile Pacific herring from PWS.

C. Determine the disease potential in wild herring populations using newly-developed in vitro and in vivo tools.

II. Empirical Disease Process Studies

A. Production of colonies of specific-pathogen-free Pacific herring

B. Empirical disease process studies with VHS

C. Empirical disease process studies with ichthyophoniasis

B. Procedural and Scientific Methods

I. Field Surveillance Efforts

A. Determine the infection and disease prevalences in adult Pacific herring from PWS, Sitka Sound, and Puget Sound.

Disease prevalence surveys, initiated in 1994 by Dr. Gary Marty, effectively documented serious disease issues in PWS herring and demonstrated the need for long term infection and disease monitoring programs. Consequently, Alaska Department of Fish and Game incorporated a disease severity index into their stock assessment models that is based on the prevalence of clinical disease signs, including nodular lesions of the heart and internal organs (indicative of ichthyophoniasis) and focal / petechial skin reddening. This method of clinical disease surveillance does not identify subclinical infections, and questions regarding the reported etiology of these lesions and their incorporation into the disease severity index have recently come into question (Elston and Meyers 2008). As a result, we have extended the disease monitoring efforts in adult PWS herring since 2007, by expanding the disease severity index to include laboratory-validated infection and disease survey data. This proposed project will extend these validated surveillance efforts through 2013.

Pre-spawn, adult herring from PWS (60 fish / site x 3 sites), will be collected by purse seine and screened for clinical disease signs; laboratory confirmation of the suspected etiologies will be performed on all samples. To insure inter-annual consistency in sensitivity of the laboratory diagnostic techniques, samples will be processed by the ADF&G Pathology Laboratory in

Juneau, where PWS herring samples have been processed since 1994. Prevalence and severity of VHSV in the populations will be determined by plaque assay with a minimum detection threshold of 20 pfu/g. Kidney and spleen from all screened fish will be aseptically removed, diluted in MEM, and homogenized. Serial 10-fold dilutions of the homogenates will be plated onto polyethylene glycol pretreated monolayers (Batts and Winton 1989) of *Epithelioma Papulosum Cyprini* (EPC) cells (Fijan et al. 1983), overlayed with methylcellulose, incubated at 15 °C for 7 d, then fixed and stained with crystal violet - formalin solution prior to plaque enumeration. Viral identity in any positive samples will be confirmed with either serological techniques using VHSV-specific antibodies or with the polymerase chain reaction using VHSV-specific primers. Prevalence of *Ichthyophonus* will be determine by *in vitro* explant culture of heart tissues, the current gold standard technique for *Ichthyophonus* detection (Whipps et al 2006). Hearts from all sampled fish will be aseptically removed and half the heart will be placed in culture tubes containing Eagle's Minimum Essential Medium, supplemented with antibiotics and 5% fetal bovine serum (MEM). Cultures will be screened microscopically for the presence of *Ichthyophonus* after 14d. Intensity of *Ichthyophonus* infections will be determined using a combination of clinical examination and histolopathological analysis of fixed heart tissues (hematoxalin and eosin H&E or periodic acid – Schiff to stain). Prevalence of VEN will be determined by examining Giemsa-stained blood films from each fish for erythrocytic inclusions that are pathognomonic for the condition; intensity of infection will reported as the prevalence of erythrocytes demonstrating inclusions.

B. Determine the prevalence of infection and disease in juvenile Pacific herring from PWS.
Young-of-the-year (YOY) and age 1-2+ yr juveniles will be sampled for infection / disease prevalence from bays in PWS where herring rear (60 fish / site) by variable mesh gill net. The primary source of juvenile herring samples will be March surveys performed by contracted fishers through the Cordova District Fishermen United (detailed fish collection methods provided in the *PWS herring survey: Community Involvement, Outreach, Logistics, and Synthesis, submitted under the Broad Agency Agreement* proposal by Dr. Scott Pegau). Samples of juveniles for infection and disease screening will also be collected from pre-and post-winter juvenile surveys (detailed fish collection methods provided in the *PWS herring survey: Assessment of Juvenile Herring Abundance and Habitat Utilization* proposal submitted by Dr. Richard Thorne). Laboratory confirmations of VHSV, ENV, and *Ichthyophonus* prevalence and severity will be performed similarly to those described for adult herring.

C. Determine the disease potential in wild herring populations using newly-developed predictive tools.
In the context of understanding disease ecology, documentation of infection and disease prevalence is often of limited value when attempting to forecast the potential for future epizootics. For example, VHS epizootics can occur very rapidly and serious population-level impacts can easily go unnoticed. Survivors of the epizootics may clear the virus rapidly and samples collected shortly after the epizootic subsided would likely demonstrate no signs of disease nor would virus be detected in their tissues. Rather, the primary value of infection and disease survey data involves documentation of interannual changes in prevalence.

Table 1. *Ichthyophonus* prevalences in Pacific herring, based on heart explant cultures.

	Prince William Sound	Sitka Sound	Lynn Canal	Puget Sound
2007	Spring **42%** (25/60), St. Matthews Bay, adults, April 5 **15%** (9/60), Simpson Bay, juveniles (age 1+), April 19 Fall **25%** (15/60), Sawmill Bay, adults, Nov. 30 **37%** (22/60), Simpson Bay, adults, Dec. 2	Spring **28%** (17/60), S. Cannon Island, adults, April 19	Spring ND Fall **11%** (7/61), adults, Nov. 10	Spring **7%** (4/59), Johnson Point, adults, Jan. 18 **17%** (10/60), Yukon Harbor, adults, Feb. 1 **37%** (22/59), Skagit Bay, adults, Feb. 8 **25%** (15/60), Cherry Point, adults, April 30
2008	Spring **23%** (40/177), Fish and Whale Bays, adults and juveniles, March 17-24 Fall **24%** (19/80), Port Gravina, adults, Nov. 8 **0%** (0/78), Simpson Bay, juveniles, Nov. 12	Spring **28%** (17/60), N. Middle Island, adults, March 26	Spring **5%** (3/61), adults, Feb. 23 **5%** (3/61), adults, April 12 **19%** (11/59), adults, May 10	Spring **2%** (1/60), Drayton Pass, adults, Jan. 15 **7%** (4/60), Yukon Harbor, adults, Feb. 5 **23%** (14/60), Skagit Bay, adults, Feb. 2 **48%** (29/60), Holmes Harbor, adults, March 13
2009	Spring **43%** (26/60), Port Gravina, adults, March 20 **25%** (15/60), Port Gravina (immature), March 20 **10%** (6/60) , Simpson Bay, age 1+ yr, March 22	Spring **40%** (32/80), Guide Island, adults, Feb. 15-16 **27%** (12/44) adults, March 24 (*preliminary prevalence*) **27%** (18/67), St. John Babtist Bay, adults, March 26 (*preliminary prevalence*) **3%** (2/70), March 27, immature juveniles (*preliminary prevalence*)	Spring **7%** (3/44), Cohen Isl (Amalga Trench) adults, Feb 11-12 **13%** (8/60), Fritz Cove, Outer Pt, Lena Pt, adults, March 18-19	Spring **3%** (2/60), Yukon Harbor, adults, Feb. 3 **18%** (11/60), Skagit Bay, adults, Feb. 2 **22%** (13/60), Port Gamble Bay, adults, Feb. 12 **22%** (13/60), Holmes Harbor, adults, March 18

For resource management purposes, periodic risk assessments intended to describe future disease potential within a population would provide the most useful information when trying to prevent epizootics or mitigate disease impacts. Unfortunately, traditional disease research and monitoring approaches for fish and wildlife, consisting primarily of pathogen prevalence surveys, are typically not conducive to provision of predictive data. To address this shortcoming, we have taken a novel approach, involving a combination of techniques from epidemiology, population ecology, disease ecology, immunology, parasitology, virology, molecular biology, pathology, and aquaculture, to develop disease forecasting tools that can be applied to wild populations. The forecasting tools are based on epidemiological principles that are unique to each specific disease. For example, laboratory studies demonstrate that herring, naïve to VHSV exposure, are highly susceptible to the resulting disease; however, the portion of herring that survives the epizootic becomes solidly resistant (Kocan et al 1997, Hershberger et al 2007, Hershberger et al, in preparation). Therefore, knowledge of a herring population's exposure history would provide useful information in determining the potential for future VHS epizootics in that population. Based on this principle, two techniques are currently being developed at the USGS - Marrowstone Marine Field Station that are providing reliable and repeatable indications of population exposure history and susceptibility. The first technique involves *in vitro* incubation herring fin explant cultures with VHSV. Variables inherent to the assay including fin type, virus inoculation titer, number of rinse cycles required to remove unadsorbed virus, and incubation duration have been optimized and standardized. Preliminary studies using the optimized explant assay indicate that VHSV titers produced from fins donated by VHS survivors (resistant) produce approximately 10X fewer VHS virions in than do fins from naïve (susceptible) herring (Figure 1). The second technique involves a passive immunization test that was recently developed and proven effective, whereby plasma is collected from a population of herring resistant to VHS. Injection of the plasma into highly susceptible herring confers solid protection against VHS to the recipient herring; however injection of plasma from naïve (susceptible) populations does not confer resistance (Figure 2). These two techniques provide empirical evidence of the prior exposure history of herring and therefore can serve as proxy measures of population susceptibility to future VHS epizootics. Laboratory standardization studies are currently underway to assign relative population susceptibility values to a gradient of values for each assay. Once these regression values are obtained, we intend to apply the assays to wild PWS herring populations to provide an annual VHS risk assessment.

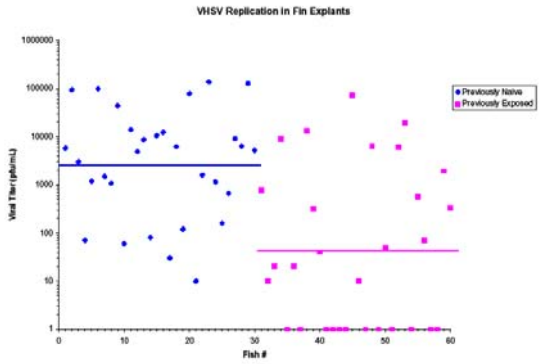

Figure 1. VHSV production in herring fin explants. Blue diamonds represent VHSV titers produced by the fins of 30 previously naïve (susceptible) herring. Magenta boxes indicate VHSV titers produced by the fins of 30 VHS survivors (resistant). Horizontal lines represent the geometric means. Note the difference in the number of fins that failed to replicate any virus between the susceptible (0/30) and resistant (12/30) groups.

Provision of annual VHS risk assessments for PWS herring will begin in FY 2011 with field application of the fin explant assay, passive immunization assay, and / or other predictive tools that are in development. Appropriate fluids and tissues for these assays will be collected during the field surveillance efforts. The fin assay will compare VHSV replication in herring fins from three groups: 1) wild herring from PWS (treatment), 2) naïve SPF herring (positive control), and 3) herring that are resistant to VHSV (negative control). The relative resistance of the wild PWS herring to both a completely susceptible population and to a completely resistant population will be determined by comparing the amount of virus that is replicated from the fins of the three groups. Similarly a passive immunization assay will consist of groups of SPF herring that are injected with one of three serum types: 1) pooled plasma from the wild population (treatment), 2) pooled plasma from naïve SPF herring (positive control), or pooled plasma from herring that are known to be resistant to VHSV (negative control). The three groups of passively immunized herring will then be exposed to VHSV (IP injection) and the relative resistance of the wild population to both a completely susceptible population and completely refractory population will be determined by comparing the cumulative mortalities between the 3 groups.

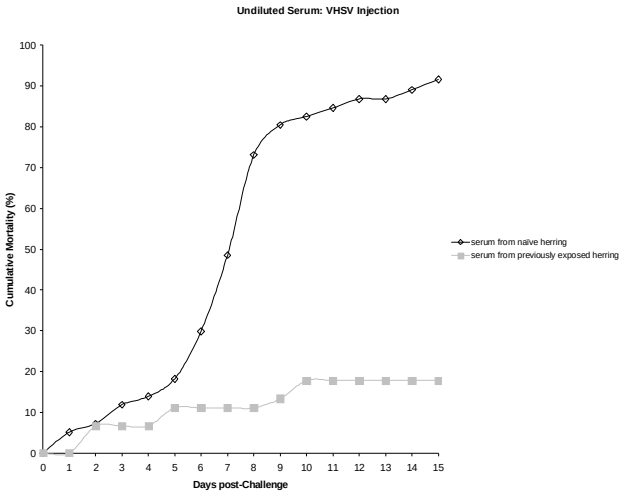

Figure 2. Cumulative mortality after VHSV exposures to SPF herring that were passively immunized with two different types of plasma: 1) plasma from previously naïve herring (black diamonds) and 2) plasma from herring that previously survived a VHS epizootic (gray squares)

II. Empirical Disease Process Studies

The primary objectives of the disease process studies will be to further understand cause-and-effect relationships between the host, pathogen, and environment that can be used to develop additional disease forecasting tools. Once developed, these tools can be used to predict the probability of future epizootics involving specific diseases. Thereafter, adaptive disease management strategies can be developed to mitigate the impacts of the diseases; the adaptive strategies can be tested and adjusted through continued disease monitoring.

A. Production of colonies of specific pathogen-free Pacific herring

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 5 age classes (age 0, 1, and 2, 3, and 5 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, including a source of control fins and serum for the predictive assays and as a source of experimental animals for additional empirical disease process studies.

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; previous studies have demonstrated that herring from Puget Sound and PWS demonstrate similar VHS susceptibilities and disease kinetics (Hershberger et al in preparation). Herring eggs and associated macrophypes 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). Larval herring are expected to metamorphose to juveniles approximately 80 d post-hatch, after which they will be weaned to non-commercial pelleted diet (51.6% protein from soy and krill, 17.4% lipid, 5.4% moisture and 6.7% ash; produced by USFWS, Abernathy Fish Technology Center). Fish meal is intentionally excluded from the diet to remove the possibility of exposure of the SPF herring to marine fish antigens through the feed.

B. Empirical disease process studies with VHS

Although VHSV is highly pathogenic to Pacific herring (Kocan et al 1997) and exposure can result in significant mortalities after exposure of naïve hosts, a portion of the exposed herring do survive the disease (Kocan et al 2001 and Hershberger et al 2007). Laboratory studies indicate that these survivors develop strong adaptive resistance to the virus that results in complete protection against the disease upon re-exposure. Therefore, if we can develop field surveillance tools that are capable of determining the resistance of wild herring populations to VHSV, based on their prior exposure histories, then we can use these relative resistance measurements as a proxy measure of the potential for future VHS epizootics. For example, low potential for future VHS epizootics would be assigned to populations that are demonstrated to be resistant to VHSV, and high potential for epizootics would be assigned to populations that are demonstrated to be susceptible (naïve) to VHSV.

A logical approach for demonstrating the resistance of a population to VHS would involve population screening for VHSV-specific neutralizing antibodies that would provide a measure of herd immunity. A similar approach, using a 50% plaque neutralization assay, has been developed and works quite well for rainbow trout and European strains of VHSV (Olesen and Jorgensen 1986, Olesen et al 1991, Jorgensen et al 1991). However, repeated attempts to adapt and apply this assay to the Pacific herring / VHS model have been unsuccessful (Hershberger and LaPatra, unpublished data). For example, herring that survive VHS exposure become completely resistant to the disease, however plasma from the protected cohorts demonstrates no detectable levels of

neutralizing antibody titer in the 50% plaque neutralization assay, even though passive transfer of the serum to naïve individuals confers protection. These results suggest that protection is conferred by either extremely low levels of circulating antibodies that are below the detection threshold of the assay, or that a humoral substance other than antibodies is responsible for the acquired resistance. Therefore, other techniques have been developed, including herring fin explant cultures and *in vivo* passive immunization assays (described above). We will continue to refine and optimize these methods during the proposed study period. Additionally, other indicators of prior exposure to VHSV will be investigated, including development of an enzyme-linked immunosorbent assay (ELISA) that would indicate whether VHSV-specific antibodies are present in wild herring. It should be noted that VHSV-specific antibodies detected in an ELISA will not necessarily be neutralizing; however, if successful, the technique would provide a reliable, high throughput, and relatively inexpensive tool to demonstrate whether sampled populations were previously exposed to VHSV. The technique will be validated using laboratory challenge studies to assign gradients of population susceptibility corresponding with different ELISA values.

Environmental conditions associated with VHS epizootics in wild herring populations are poorly understood, but field observations from VHS epizootics in British Columbia indicate that reduced temperature may be an important predisposing factor (Traxler et al 1999). Controlled laboratory studies will be performed at the Marrowstone Marine Field Station to determine the effect of temperature on the virulence of VHSV to naïve Pacific herring. Triplicate tanks (n = 30 fish / tank) containing SPF Pacific herring at each of 3 temperatures (ambient, 3°C below ambient, and 3°C above ambient) will be exposed to 10^2 pfu / mL VHSV in a standard laboratory challenge. Comparisons of cumulative mortalities will be made at the end of the epizootics between exposed herring at the three temperatures and between appropriate negative control groups (exposed to saline instead of VHSV). Additionally, to determine whether temperature changes result in relapse or increased susceptibility among herring that recovered from VHS, similar temperature studies will be performed using groups of herring that survived a previous VHS epizootic. A VHS epizootic will be induced in a tank containing several thousand SPF herring by exposing them to waterborne virus; controls will be exposed to saline in a separate tank. After the ensuing acute VHS mortality subsides, survivors will be transferred to triplicate tanks at each of three temperatures (same as described above) to determine whether the temperature change results in relapse of the disease. If relapse does not occur within 2 weeks in the temperature treatments, the treatment groups will be re-exposed to waterborne VHSV (10^2 pfu / mL) to determine whether the temperature change resulted in increased susceptibility to the disease.

C. Empirical disease process studies with ichthyophoniasis

Population implications of the *Ichthypohonus* prevalence data collected during the field surveillance efforts are difficult to interpret because the epidemiology of the disease and the disease processes in the infected host are poorly understood. For example, under appropriate host and environmental conditions, *Ichthyophonus* can be highly pathogenic and result in rapid mortality to Pacific herring (Kocan et al 1999); however, under other conditions, infected hosts appear to survive as chronic carriers for extended periods and demonstrate few adverse effects. Further, herring that survive the acute phase of an ichthyophoniasis epizootic do not clear the infection (Hershberger unpublished data); rather, they survive as carriers for extended periods (Figure 3). Therefore, population-level implications of the *Ichthyophonus* prevalence data

collected during the wild herring surveys are difficult to interpret because the stage and activity of infection remain undetermined. Even if we assume that all *Ichthyophonus* infections detected during the surveys represent long-term carriers that are not destined to immediate mortality, the impacts of these carriers to the herring population could be significant. For example if a large proportion of the adult herring population consists of *Ichthyophonus* carriers (such as currently exists in PWS and Sitka, Table 1), then it is possible that disease exacerbation could occur when these carriers encounter suboptimal environmental conditions.

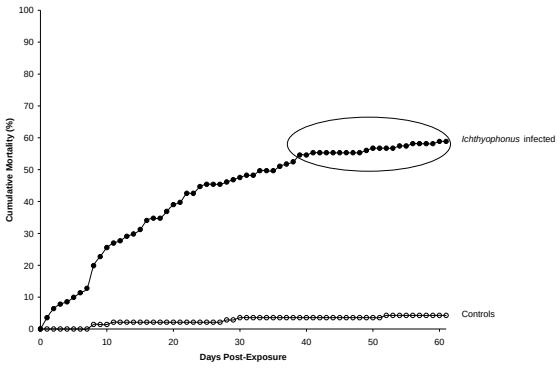

Figure 3. Cumulative mortality of Pacific herring after exposure (IP inection) to *Ichthyophonus*. Note that mortality slows after 40 d.

We propose to investigate the effects of suboptimal host and environmental conditions to herring that are chronically infected with *Ichthyophonus*. Several thousand SPF herring will be infected with *Ichthyophonus* by IP injection with ~500 macrospores / fish; a control group will be injected with a similar volume of saline and maintained in a separate tank. The resulting acute mortality phase is expected to be complete after 2 months (Figure 3); after which, the chronically-infected survivors will be exposed to several stressors including temperature fluctuations and stress hormones (corticosteroids) to determine whether additional mortality ensues. Exposures for each treatment will consist of triplicate tanks containing chronically infected herring (n = 30 fish / tank) at each of three temperatures and each of two waterborne concentrations of Dexamethasone® (synthetic corticosteroid). Cumulative mortalities will be compared among infected groups that were unmanipulated and infected groups exposed to the respective stressors. Control groups will consist of uninfected herring exposed to similar conditions. If application of nominal stressors to *Ichthyophonus* carriers results in progression of the low level infections to overt disease and mortality, then a mechanism accounting for the ichthyophoniasis epizootics in wild herring (reviewed in Sindermann 1990 and McVicar 1999) can be proposed. For example, massive population-level impacts from ichthyophoniasis could result during periods of adverse environmental conditions if the *Ichthyophonus* carrier rate is high.

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

Projects outlined in this proposal are designed to address the Pacific herring population decline and failed recovery in Prince William Sound, AK, an area encompassing approximately 3,000 miles of shoreline that is bordered by the Chugach Mountains on the north, east, and west and the Gulf of Alaska on the south. Field surveys of infection / disease prevalence in adult PWS herring will be partnered with ADF&G stock assessment surveys and are likely to include samples from Montague area and northeastern region (Port Fidalgo / Port Gravina). Field surveys in juvenile PWS herring will be partnered with the larger PWS herring survey project and are likely to include samples from Zaikoff Bay, Whale Bay, Eaglik Bay, Simpson Bay, Naked Island, or Knight Island. 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 Other Efforts

This proposed project, the PWS Herring Survey: Herring Disease Program (HDP), is one of several projects included in a proposed integrated effort that includes aerial surveys for juvenile herring (Dr. Evelyn Brown), hydroacoustic surveys for juvenile herring (Dr. Richard Thorne), oceanographic factors impacting juvenile herring (Dr. Sheldon Gay), planktonic food availability in the bays for juveniles (Dr. Robert Campbell), empirically deduced growth and bioenergeics (Dr. Ronald Heintz), field assessments of overwintering bioenergetics (Dr. Thomas Kline), seabird predation on juvenile herring (Dr. Mary-Ann Bishop), and disease (Dr. Paul Hershberger). Summaries of the individual projects and details of their proposed integration are provided in an overview proposal submitted by Dr. Scott Pegau, titled PWS herring survey: Community Involvement, Logistics, and integration, submitted under the BAA. The proposed HDP is also expected to dovetail with a herring genetics study proposed by Dr. Jeff Guyon (NOAA-Fisheries) by sharing samples of adult PWS spawning herring for both disease surveillance efforts and genetic comparisons.

The HDP is also integrated with adult herring stock assessment surveys in PWS, Sitka Sound, and Puget Sound (ADF&G and WDF&W). Previous results from the HDP (or earlier herring disease studies performed by Dr. Marty) were integrated into state herring management strategies for PWS (Marty et al 2003) and Puget Sound (Stick & Lindquist 2009).

The proposed project (HDP 2010-2012) overlaps a single year (2010) with a currently funded HDP (HDP 2007-2010) project. This overlap is necessary to fully integrate with the larger PWS Herring Survey Project outlined in a proposal by Dr. Scott Pegau. Funds are requested during this overlap year (~$75K) to expand the disease surveillance efforts to juvenile cohorts and to begin ELISA development. Full funding is requested for years 2-4 of this proposed study (FY 2011-2013) after the currently funded HDP is terminated.

III. SCHEDULE

A. Project Milestones

I. Field Surveillance Efforts

A. Determine the infection and disease prevalences in adult Pacific herring.
- To be met by June 30 each year.

B. Determine the infection and disease prevalences in juvenile Pacific herring.
- To be met by March 31 of the following year

C. Determine the disease potential in wild herring populations using newly-developed tools.
- To be met for by June 30 each year starting, in 2011.

II. Empirical Disease Studies

A. Production of colonies of specific pathogen-free Pacific herring
- SPF juveniles will be produced by Aug. 1 (2010-2013)

B. Empirical disease process studies with VHS
- To be met by March 31, 2013

C. Empirical disease process studies with ichthyophoniasis
- To be met by Sept 30, 2011

B. Measurable Project Tasks

Every Fiscal Year (FY 2010 - 2013)

1st Quarter (October 1-December 31)
- Project funding approved by TC

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
- Complete lab diagnostics on juvenile herring surveys from the previous year

3rd Quarter (April 1-June 30)
- Finish collecting and processing spring adult herring to determine infection and disease prevalence.
- Start collecting juvenile herring from PWS for disease surveys
- Participate in PI meeting in Cordova

4th Quarter (July-September 30)
- Continue sampling juvenile herring from PWS for disease surveys

Additional Quarterly Tasks

FY10, 1st Quarter (October 1 –December 31, 2009)

- Begin ELISA development

FY10, 4th Quarter (July-September 30, 2010)

- Complete ELISA development

FY11, 1st quarter (October-December 31, 2010)

- Infect herring with *Ichthyophonus* to obtain chronic carriers
- Begin lab studies to assign a gradient of protection to ELISA values

FY11, 2nd quarter (January 1-March 31, 2011)

- Expose chronic *Ichthyophonus* carriers to temperature stress

FY11, 3rd quarter (April 1 - June 30, 2011)

- Terminate *Ichthyophonus* temperature stress study and process samples

FY11, 4th quarter (July - September 30, 2011)

- Terminate *Ichthyophonus* Dexamethasone study and process samples

FY12, 1st quarter (October 1 - December 31, 2011)

- Expose SPF herring to VHSV at different temperatures (1st VHSV temperature study)

FY12, 2nd quarter (January 1 - March 31, 2012)

- Terminate first VHSV temp study

FY12, 3rd quarter (April 1 - June 30, 2012)

- Finish processing laboratory samples from first VHSV temp study
-Expose SPF herring to VHSV to obtain survivors

FY12, 4th quarter (July - September 30, 2012)

- Expose VHS survivors to temperature stress
- Re-expose VHS survivors at each temperature to VHS (2nd VHSV temperature study)

FY13, 1st quarter (October 1 - December 31, 2012)

- Terminate 2nd VHSV temperature study
- Start drafting final report
- Participate in 1st PI integration meeting

FY13, 2nd quarter (January 1 - March 31, 2013)

- Finish processing samples from 2nd VHSV temp study
- Participate in 2nd PI integration meeting

FY12, 3rd quarter (April 1 - June 30, 2013)

- Participate in 3rd PI integration meeting
- Submit draft final report

FY12, 4th quarter (July - September 30, 2013)

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

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ABBREVIATED RESUME

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

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:

Hershberger, PK, NE Elder, CA Grady, JL Gregg, CA Pacheco, C Greene, C Rice, TR Meyers. 2009. Recurring viral erythrocytic necrosis (VEN) in juvenile Pacific herring from Puget Sound, WA, USA. Journal of Aquatic Animal Health 29: 1-7.

Hershberger P.K., J Gregg, C Pacheco, J Winton, J Richard, G. Traxler. 2007. Larval Pacific herring, *Clupea pallasii* (Valenciennes), are highly susceptible to viral hemorrhagic septicemia and survivors are partially protected after their metamorphosis to juveniles. Journal of Fish Diseases 30: 445-458.

Hershberger, P.K., S.A. Hart, J. Gregg, N.E. Elder, and J.R. Winton. 2006. Dynamics of viral hemorrhagic septicemia, viral erythrocytic necrosis, and ichthyophoniasis in juvenile Pacific herring. Diseases of Aquatic Organisms 70: 201-208.

Kocan, R.M., P.K. Hershberger, N.E. Elder, and J.R. Winton. 2001. Epidemiology of viral hemorrhagic septicemia (VHS) among juvenile Pacific herring and Pacific sandlances in Puget Sound, Washington. Journal of Aquatic Animal Health 13: 77-85.

Hershberger, P.K., R.M. Kocan, N.E. Elder, T.R. Meyers, and J.R. Winton. 1999. Epizootiology of viral hemorrhagic septicemia virus in herring from the closed pound spawn-on-kelp fishery. Diseases of Aquatic Organisms 37: 23-31.

Five Additional Selected Publications

Hershberger, P.K., J.L. Gregg, C.A. Grady, R.M. Collins, J.R. Winton. *Submitted*. Virus shedding as a driver in the ecology of an acute disease of marine fish. Ecology.

Kocan, R., P. Hershberger, G. Sanders, J. Winton. *Accepted*. Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout (*Oncorhynchus mykiss*) infected with *Ichthyophonus* sp. Journal of Fish Diseases.

Hershberger, P.K., CA Pacheco, J.L. Gregg. 2008. Inactivation of *Ichthyophonus* Spores Using Sodium Hypochlorite and Polyvinyl Pyrrolidone Iodine (PVPI). Journal of Fish Diseases 31: 853-858.

Hershberger, P.K., CA Pacheco, J.L. Gregg, M. Purcell, S.E. LaPatra. 2008. Differential survival of *Ichthyophonus* isolates indicates parasite adaptation to its host environment. Journal of Parasitology 94: 1055-1059.

LaPatra, S., R. Kocan, P. Hershberger. 2008. Potential for cross-contamination of *in vitro* explant cultures initiated from *Ichthyophonus* - infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 31: 317-320.

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)

ABBREVIATED RESUME

James R. Winton, Ph.D.
Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 USA
Phone: 1-206-526-6587; FAX: 1-206-526-6654; e-mail: jim_winton@usgs.gov

Professional Interests

Infectious diseases of fish, fish cell and tissue culture, molecular taxonomy and epidemiology of fish pathogens, ecology of fish diseases in wild populations.

Membership in Professional Organizations

Phi Kappa Phi, Sigma Xi, American Association for the Advancement of Science, American Society for Microbiology, American Society for Virology, Society for General Microbiology, American Fisheries Society, American Institute of Fishery Research Biologists, European Association of Fish Pathologists, Japanese Society of Fish Pathologists, Wildlife Disease Association, World Aquaculture Association.

Current Position

Chief, Fish Health Section, Western Fisheries Research Center, Seattle, WA
Professor (Affiliate Faculty), University of Washington, School of Aquatic and Fishery Sciences

Education

1962-1964, University of Oregon, Eugene, OR.
1964-1967, B.A., University of Colorado, Boulder, CO.
1974-1981, Ph.D., Oregon State University, Corvallis, OR.

Recent Awards and Honors:

1999 - U.S. Department of Interior Meritorious Service Award
2000 - American Fisheries Society Fish Health Section S. F. Snieszko Distinguished Service Award
2002 - Award for most significant paper of 2001 in Journal of Aquatic Animal Health.
2004 Selected as Designated Expert and BKD Reference Laboratory by Office of International Epizooties
2006 - U.S. Department of Interior Distinguished Service Award.

Five Selected Publications Relevant to this Proposal

Kocan, R., P. Hershberger, G. Sanders and J. Winton. In press. Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout (*Oncorhynchus mykiss*). Journal of Fish Diseases.

Hershberger P.K., J Gregg, C Pacheco, J Winton, J Richard, G. Traxler. 2007. Larval Pacific herring, *Clupea pallasii* (Valenciennes), are highly susceptible to viral hemorrhagic septicemia and survivors are partially protected after their metamorphosis to juveniles. Journal of Fish Diseases 30:445-458.

Kocan, R., S. LaPatra, J. Gregg, J. Winton and P. Hershberger. 2006. *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids. Journal of Fish Diseases 29:521-527.

Hershberger, P.K., S.A. Hart, J. Gregg, N.E. Elder, and J.R. Winton. 2006. Dynamics of viral hemorrhagic septicemia, viral erythrocytic necrosis, and ichthyophoniasis in juvenile Pacific herring. Diseases of Aquatic Organisms 70:201-208.

Kocan, R.M., P.K. Hershberger, N.E. Elder, and J.R. Winton. 2001. Epidemiology of viral hemorrhagic septicemia (VHS) among juvenile Pacific herring and Pacific sandlances in Puget Sound, Washington. Journal of Aquatic Animal Health 13:77-85.

Five Additional Selected Publications

Naish, K.A., J.E. Taylor III, P.S. Levin, T.P. Quinn, J.R. Winton, D. Huppert and R. Hilborn. 2008. An evaluation of the effects of conservation and fishery enhancement hatcheries on wild populations of salmon. Advances in Marine Biology 53:61-194.

Batts, W.N., K. Falk and J.R. Winton. 2008. Genetic analysis of paramyxovirus isolates from Pacific salmon reveals the presence of two independently co-circulating lineages. Journal of Aquatic Animal Health 20:215-224.

Groocock, G.H., R.G. Getchell, G.A. Wooster, K.L. Britt, W.N. Batts, J.R. Winton, R.N. Casey, J.W. Casey and P.R. Bowser. 2007. Detection of viral hemorrhagic septicemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. Diseases of Aquatic Organisms 76:187-192.

Purcell, M.K., K.D. Smith, L. Hood, J.R. Winton and J.C. Roach. 2006. Conservation of toll-like receptor signaling pathways in teleost fish. Comparative Biochemistry and Physiology, Part D 1:77-88.

Elsayed, E., M. Faisal, M. Thomas, G. Whelan, W. Batts and J. Winton. 2006. Isolation of viral hemorrhagic septicemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St. Clair, Michigan, USA reveals a new sub-lineage of the North American genotype. Journal of Fish Diseases 29:611-619.

Recent Collaborators and Co-Authors:

J. Bartholomew (OSU), W. Batts (USGS), D. Beauchamp (UW), V. Blazer (USGS), D. Bouchard (Micro Technol.), R. Brunson (USFWS), D. Campton (USFWS), M-S. Chien (Taiwan), S. Chilmonczyk (France), R. Devlin (DFO), K. Einer-Jensen (Denmark), N. Elder (USGS), D. Elliott (USGS), E. Emmenegger (USGS), M. Faisal (MSU), C. Friedman (UW), K. Garver (DFO), R. Goetz (UWI), A. Goodwin (UAPB), W. Granath (UM), J. Gregg (USGS), C. Grue (UW), D. Halpenny (U. Montreal), J. Hansen (USGS), J. Hard (NMFS), R. Hardy (UI), A. Hart (UW), R. Hedrick (UCD), R. Herwig (UW), B. Hill (CEFAS), R. Hilborn (UW), L. Hood (ISB), M. House (NWIFC), C. Huang (Taiwan), S. Kaattari (VIMS), J. Kaufman (ODFW), M. Kent (OSU), B. Kerrans (MSU), C. Kim (UM), R. Kocan (UW), G. Kurath (USGS), Scott LaPatra (Clear Springs Foods), J-A. Leong (UH), J. Levin (NCS), N. Lorenzen (Denmark), E. MacConnell (USFWS), C. Mahnken (NMFS), S. McDiarmid (New Zealand), T. Meyers (ADFG), K. Miller (DFO), C. Mork (Boston University), S. Mumford (USFWS), J. Nagler (UI), K. Naish (UW), N. Nishizawa (Japan), C. O'Farrell (ORU), N. Okamoto (Japan), N. Olesen (Denmark), K. Oshima (NMSU), K. Overturf (USDA), R. Palm (ProFishent), Y. Palti (USDA) L. Park (NMFS), J. Parsons (Troutlodge), C. Patterson (USFWS), D. Powell (ProFishent), M. Purcell (USGS), T. Quinn (UW), C. Rasmussen (USGS), C. Rexroad (USDA), L. Rhodes (NMFS), J. Richard (DFO), S. Ristow (WSU), J. Roach (ISB), B. Robison (IU), J. Rolland (USDA), G. Sanders (UW/USGS), K. Smith (ISB), C. Stehr (NMFS), L. Steinbach (MSU), R. Stevens (MSU), K. Stick (WDFW), M. Strom (NMFS), P. Swanson (NMFS), E. Sweeney (UW), P. Taylor (USFWS), G. Thorgaard (WSU), G. Traxler (DFO), N. Van der Straaten (UW), M. Vijayan (Canada), P. Walsh (UI), G. Wedemeyer (USGS), P. Wheeler (WSU), G. Wiens (USDA), J. Wittouck (UW), M. Yoshimizu (Japan), W. Young (NAU), S. Yun (UCD).

ABBREVIATED RESUME

Maureen K. Purcell, Ph.D.
Western Fisheries Research Center, USGS, 6505 N.E. 65th St., Seattle, WA 98115 USA
Phone: (206) 526-6282x252, fax (206) 526-6654, e-mail mpurcell@usgs.gov

Professional Interests

Infectious diseases of fish, fish immunology, genetic basis of disease resistance

Membership in Professional Organizations

American Association for the Advancement of Science, International Society of Developmental and Comparative Immunologists, American Fisheries Society, Fish Health Section, European Association of Fish Pathologists

Education

Ph.D.	Fisheries	University of Washington	2005
M.S.	Zoology	University of Maine	1997
B.S.	Zoology	Washington State University	1993

Recent Positions

2005 to Present: Research Microbiologist; Western Fisheries Research Center, USGS (Seattle, WA)
2000 to 2005: Graduate Student; University of Washington and WFRC, USGS (Seattle, WA)
1999 to 2000: Molecular Geneticist; Conservation Biology Division, NMFS (Seattle, WA)
1998 to 1999: Professional Research Assistant; The Jackson Laboratory (Bar Harbor, ME)
1997 to 1998: Research Assistant; The Jackson Laboratory (Bar Harbor, ME)

Recent Awards and Honors

2009 U.S. Department of Interior Star Award, Biological Resources Discipline
2008 Most significant paper, American Fisheries Society, Journal of Aquatic Animal Health
2004 Faculty Merit Award; U.W. School of Aquatic and Fisheries Sciences

Five Selected Publications Relevant to this Proposal

· Peñaranda, M., **M.K. Purcell** and G. Kurath (2009) Differential virulence mechanisms of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (*Oncorhynchus mykiss*) include host entry and virus replication kinetics. Journal of General Virology.90:2172-2182.
· **Purcell, M.K.,** K.J. Laing, J. Woodson, G.H. Thorgaard and J.D. Hansen. (2009) Characterization of the interferon genes in homozygous rainbow trout reveals two novel genes, alternate splicing and differential regulation of duplicated genes. Fish and Shellfish Immunology. 26:293-304.

- Hershberger, P.K., C.A. Pacheco, J.L. Gregg, **M.K. Purcell**, S.E. LaPatra. (2008) Differential survival of *Ichthyophonus* isolates indicates parasite adaptation to its host environment. Journal of Parasitology. 94:1055-1059.
- Laing K.J., J. Zou, **M.K. Purcell**, C.J. Secombes, R. Phillips and J.D. Hansen (2006) Evolution of CD4: teleost fish possess two divergent forms of CD4 in addition to lymphocyte activation gene-3. Journal of Immunology. 177:3939-3951.
- **Purcell, M.K.,** K.M. Nichols, J.R. Winton, G.K. Kurath, G.H. Thorgaard, P. Wheeler, J.D. Hansen, R.P. Herwig and L.K. Park (2006) Comprehensive gene expression profiling following DNA vaccination against infectious hematopoietic necrosis virus. Molecular Immunology. 43:2089-2106.

Five Additional Selected Publications

- **Purcell, M.K**., A.L. Murray, A. Elz, L.K. Park, S.V. Marcquenski, J.R. Winton, S.W. Alcorn, R.J. Pascho and D.G. Elliott. (2008). Decreased mortality of Lake Michigan Chinook salmon (*Oncorhynchus tshawytscha*) following bacterial kidney disease challenge: evidence for pathogen-driven selection? Journal of Aquatic Animal Health. 20:225-235..
- Landis, E.D, **M.K Purcell**, G. Thorgaard, P. Wheeler and J.D. Hansen (2008) Transcriptional profiling of MHC class I genes following injection with live infectious hematopoietic necrosis virus or inactivated virus. Molecular Immunology. 45:1646-1657
- **Purcell, M.K**., K.D. Smith, L. Hood, J.R. Winton and J.C. Roach. (2006) Conservation of Toll-Receptor Signaling Pathways in Teleost Fish. Comp. Biochem, Phys. Section D. 1:77-88.
- Rodriguez, M.F., G. Wiens, **M.K. Purcell** and Y. Palti (2005) Characterization of Toll-like receptor 3 gene in rainbow trout (*Oncorhynchus mykiss*). Immunogenetics. 57:510-519.
- Roach, J.C., G. Glusman, L. Rowen, A. Kaur, **M.K. Purcell**, K.D. Smith, L. Hood and A. Aderem. (2005) The evolution of the Toll-like receptors. Proceedings of the National Academy of Sciences of the USA 102:9577-9582.

Recent Collaborators and Co-Authors

Alcorn, S.W. (WA DFW), Conway, C. (WFRC), Elliott, D.G.(WFRC), Elz, A.(NMFS), Foote, S. (USFWS), Garver, K.A. (DFO), Gregg, J.L.(WFRC), Hansen, J.D.(WFRC), Hard, J.J.(NMFS), Hersberger, P.K.(WFRC), Herwig, R.P.(UW), Kornfield, I.L.(U. Maine), Kurath, G.K.(WFRC), Laing, K.J.(FHCRC), Landis, E.D.(NMFS), LaPatra, S.E.(Clear Springs Food), Marcquenski, S.V.(WI DNR), Mercy, I.S.(Norway), Metzger, D.(WFRC), Murray, A.L.(Hollister-Steier), Nichols, K.M.(Purdue), Palti, Y.(USDA), Park, J.W.(Korea), Park, L.K.(NMFS), Pascho, R.J.(WFRC), Penaranda, M.(UW), Phillips, R.(WSU), Rasmussen, C.(UW), Secombes, C.J.(Scotland), Thorgaard, G.H.(WSU), True, K.(USFWS), Wheeler, P.(WSU), Wiens, G.(USDA), Winton, J.R.(WFRC), Woodson, J.C.(WFRC), Zou, J.J.(Scotland)

Budget Justification

Personnel Costs = $382.8K

FY10 ($0)
None requested: personnel are provided in the currently funded HDP.

FY11 ($118.8K)
Funding is requested to support a GS-8 laboratory technician ($56.4 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-9 post doc ($62.4K) at the Marrowstone Marine Field Station to lead the field sampling efforts and analyze and publish the large epidemiological disease data sets for PWS herring.

FY12 ($127.2K)
Continued funding is requested to support a GS-8 laboratory technician ($60.0 K) at the Marrowstone Marine Field Station and GS-9 post doc ($67.2 K) at the Marrowstone Marine Field Station; responsibilities will be the same as the previous year.

FY13 ($136.8 K)
Continued funding is requested to support a GS-8 laboratory technician ($64.8 K) at the Marrowstone Marine Field Station and GS-9 post doc ($72.0K) at the Marrowstone Marine Field Station; responsibilities will be the same as the previous year.

Travel Costs = $37.0 K

FY10 ($0)
None requested: travel costs are provided in the currently funded HDP.

FY11 ($ 11.0 K)
Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($4.0K), perform Sitka field sampling ($2.4 K), present at the annual Marine Science Symposium ($2.0K). Additional travel support is requested for the PI to participate in the annual herring integration meeting in Cordova.

FY12 ($11.0 K)
Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($4.0K), perform Sitka field sampling ($2.4 K), present at the annual Marine Science Symposium ($2.0K). Additional travel support is requested for the PI to participate in the annual herring integration meeting in Cordova.

FY13 ($15.0 K)
Round trip travel costs from Nordland, WA are requested for two pathologists to perform field sampling in PWS ($4.0K), perform Sitka field sampling ($2.4 K), present at the annual Marine

Science Symposium ($2.0K). Additional travel support ($6.0 K) is requested for the PI to participate in three herring integration meetings in Cordova.

Contractual Costs = $236.6K

FY ’10 ($75 K)
Contractual costs ($75 K), administered through WFRC, are requested to support the development of an ELISA to detect VHSV antibodies from herring. The requested funding will include contractor salary, contractor indirect costs, supplies, and production of rabbit polyclonal serum.

FY ‘11 ($82.6K)
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*. Additionally, funding is requested for two student services contracts at the Marrowstone Marine Field Station ($35.3 K, each) to assist with rearing of SPF herring and duties in the dry and wet laboratories.

FY ‘12 ($85.0 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*. Additionally, funding is requested for two student services contracts at the Marrowstone Marine Field Station ($36.5 K, each) to assist with rearing of SPF herring and duties in the dry and wet laboratories.

FY’13 (87.6 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*. Additionally, funding is requested for two student services contracts at the Marrowstone Marine Field Station ($37.8 K, each) to assist with rearing of SPF herring and duties in the dry and wet laboratories.

Commodities = $ 144.6 K

FY 11, 12, & 13, = $48.2K, each
Commodities for FY’10 are included in the currently funded HDP. Annual commodities for FY 2011-2013 include laboratory supplies for the Marrowstone Marine Station ($15.0K for fish food, $10.0K for dry lab supplies), and standard tank / bench fees at the Marrowstone Marine Station ($10.0K) to cover costs associated with seawater, plumbing, and tank maintenance. Separate funding ($13.2 K) 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.

Matching funds: $61.6 - $103.3 K/yr
Partial annual salaries and benefits for PI's and USGS support staff are offered as in kind matching funds. Rearing and feeding costs associated with existing SPF herring ($44.3 K / yr) are included as an additional match (fish are provided by an ongoing EVOS TC project and USGS).

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.