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 the 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 July 9, 2002) and reporting requirements (*Procedures for the Preparation and Distribution of Reports***, adopted July 9, 2002).

PROJECT TITLE: Prince William Sound Herring Disease Program

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Printed Name of co-PI Diane Elliott, Ph.D.

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Signature of co-PI: _____ Date _____

* www.evostc.state.ak.us/Policies/data.htm

**www.evostc.state.ak.us/Policies/Downloadables/reportguidelines.pdf

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FY07 INVITATION
PROPOSAL SUMMARY PAGE
(to be filled in by proposer)

Project Title: Prince William Sound Herring Disease Program

Project Period: FY 2007-2010

Proposer(s): Paul Hershberger, Richard Kocan, John Hansen, Diane Elliott, Eveline Emmenegger, Gael Kurath, Scott LaPatra, Jim Winton

Study Location: Prince William Sound, AK

Abstract:
A leading hypothesis accounting for the decline and failed recovery of the herring population in Prince William Sound involves epizootic mortality resulting from infectious and parasitic diseases. Ongoing and past surveillance of herring diseases in PWS, initiated by Dr. Gary Marty and continued by ADF&G through the herring disease index, is extremely valuable and necessary to document changes in disease prevalence, but field surveys are unable to unequivocally demonstrate epidemiological relationships that modulate disease cycles. This proposed multi-year Herring Disease Program (HDP) consists of three components intended to provide predictive metrics that forecast future disease epidemics and offer empirical relationships useful in developing adaptive management policies to mitigate the effects of epizootic and chronic diseases. The first component involves laboratory validation of the ongoing PWS herring disease index. Long-term continuation of the herring disease index, paired with laboratory validation, is necessary to confirm the efficacy of future adaptive disease management strategies. The second component involves empirical studies intended to determine the basic epidemiological relationships between environmental and biological factors influencing infection / disease prevalence. The final component involves development of immunological and molecular tools that will be useful in predicting the potential for future disease epidemics. Combined, this three-tiered approach will provide the basic epidemiological information necessary to develop and validate adaptive management techniques intended to mitigate the effects of future herring disease outbreaks in PWS.

Funding: (including 9% GA)

EVOS Funding Requested:

FY 07	$246.5K
FY 08	$257.1K
FY 09	$258.6K
FY 10	$272.8K
TOTAL =	$1,035.0K

Total Non-EVOS Funds: $1,132.5K

Date: August 1, 2006

NEED FOR THE PROJECT

Statement of Problem

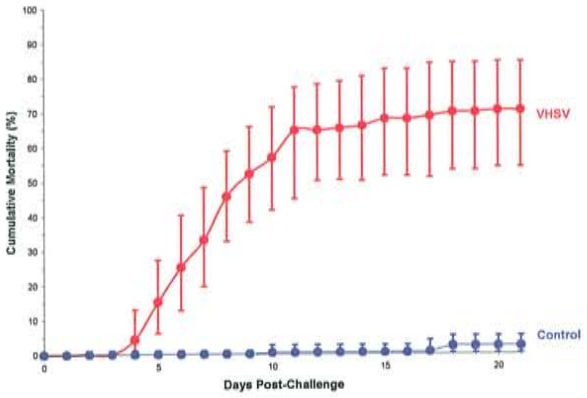
The biomass of adult herring in Prince William Sound (PWS) collapsed from 111,000-121,000 mt in 1988-1989 to 30,000 mt in 1993; since then, the population has remained depressed, fluctuating between 10,800-32,500 mt (15,800 mt in 2005). Consequently, the PWS herring population is currently classified 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). Similarly, 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 responsible 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 remains 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) and, the causative 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, and intestinal cestodes (Marty et al 1998). Among the pathogens occurring in PWS herring, VHSV, *Ichthyophonus*, and erythrocytic necrosis virus (ENV) are considered the primary pathogens of concern because they have been previously associated with massive epidemics in wild herring populations. Virulence of these agents to Pacific herring, both individually and in combination, remains largely uninvestigated and represents a significant information gap to epidemiological understanding.

Pacific herring demonstrating classic signs of VHS. Note hemorrhages around the eyes, mouth, and fins.

The North American strain of VHSV (Genogroup IV) is periodically associated with epizootics in wild marine species where it can be highly virulent. Monospecific VHS epidemics 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). Additionally, VHS is proposed to have a great effect on unexpected population change in the PWS herring population (Marty et al 2003). Epidemics 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 epidemics 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 epidemics among the confined populations (Hershberger et al 1999, Kocan et al 2001, Hedrick et al 2003). In Pacific herring prevalence and severity of VHS decreases with age (Kocan et al 1997, Hershberger et al 1999, Marty et al 2003). Wild juvenile herring in Puget Sound are exposed to VHSV as early as 3 months post-hatch, shortly after their metamorphosis from larvae (Kocan et al 2001). As juveniles, Pacific herring are highly susceptible to VHS, with laboratory exposures resulting in 66-100% mortality. 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, or the onset of innate resistance with adult age.



Mortality occurring after waterborne exposure of specific pathogen-free Pacific herring juveniles to VHSV in the laboratory (Hershberger et al 2006).

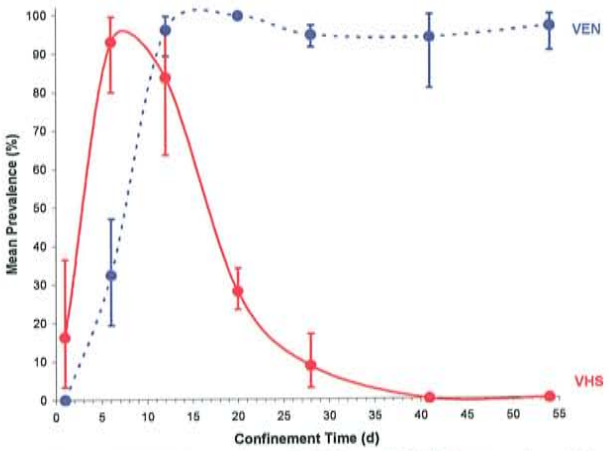
Ichthyophonus hoferi is a member of the Mesomycetozoea, a monophyletic class of protozoans that includes several other pathogenic organisms (Ragan et al 1996, Herr et al 1999, reviewed in Mendoza et al 2002). Currently *I. hoferi* (reviewed in McVicar 1999) and *I. irregularis* (Rand et al 2000) are 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). Additional molecular phylogenetic studies are necessary to better understand the relatedness of *I. hoferi* isolates (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 epidemics were described in Atlantic herring (*Clupea harengus*) from the Western North Atlantic (Sindermann 1990, McVicar 1999). More recently, a massive *Ichthyophonus*-related epidemic 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).

Wild herring demonstrating gross signs of ichthyophoniasis. Note the presence of black ulcers on the flank.

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 associated with an 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 an epizootic of wild herring in Alaska (Meyers et al 1986).

Populations of wild herring are often infected with, or susceptible to, multiple infections with VHSV, VEN, and *Ichthyophonus*, and capture and confinement into laboratory tanks results in initiation of the respective diseases. Timing and progression of the three resulting diseases differ. The VHS epidemic 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 generally followed by a VEN epidemic that, within 12 d of confinement, progresses from undetectable levels to 100 % infection prevalence with >90 % of erythrocytes demonstrating inclusions. The VEN epidemic 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 multiple stressors associated with capture, transport, confinement, and progression of the viral diseases. These results illustrate the differences in disease ecology and possible synergistic effects of 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).



Progressive epidemics of VHS and VEN occur in wild Pacific herring after their confinement in laboratory tanks (Hershberger et al 2006).

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. A multi-year survey of known herring pathogens in PWS has been conducted since 1994, providing valuable information on trends in infection prevalence and intensity. It is recommended that these surveys be continued in an effort to document changes in pathogen prevalence and severity within the PWS herring population; further, these surveys provide insight into the efficacy of adaptive management strategies intended to mitigate detrimental effects of disease. However, by lacking the capacity for empirical manipulation, field surveys are limited in their ability to demonstrate cause-and-effect relationships between the host, pathogen, and environment. To address these relationships and provide quantifiable metrics useful for an adaptive management approach, we propose to expand the ongoing PWS herring disease surveys into a herring disease program (HDP) that incorporates laboratory validation of the ongoing disease index, experimental studies intended to demonstrate cause-and-effect relationships, and predictive immunological and molecular tools that can be used to forecast disease epidemics. Traditional experimental approaches to addressing disease ecology issues in marine fish populations were historically limited by the lack of specific pathogen-free hosts with known disease and exposure histories. However, researchers at the Marrowstone Marine Station have overcome this significant impediment by developing colonies of laboratory-reared, specific pathogen–free Pacific herring. These colonies, consisting of thousands of individuals in each of 3 age cohorts (age 0, 1, and 2 yr) are offered as an USGS in-kind contribution towards these proposed laboratory studies. Using these fish as laboratory host models, we can empirically manipulate single variables of concern and demonstrate cause-and-effect relationships that can later be incorporated into predictive population models and adaptive management techniques intended to mitigate the population-level effects of disease in PWS herring.

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 2007.

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 herring disease 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 invitation for proposals, a specific solicitation for herring proposals is categorized in Appendix A, and disease is included a primary topic for funding consideration. This proposal seeks to determine the impact of infectious and parasitic diseases on the early life stage survival and population growth of Pacific herring, an area indicated by the Council as a critical information gap. Information from these studies will provide cause-and effect epidemiological relationships that offer a scientifically sound basis for developing adaptive management methods intended to mitigate the effects of disease in PWS herring populations. Further, when these empirical disease studies are combined with continued disease prevalence surveys in PWS (also identified as a herring disease research need in the invitation for proposals), the effectiveness of these adaptive management strategies can be tested.

PROJECT DESIGN

Empirical studies described in this multiple year proposal, combined with ongoing herring disease monitoring surveys conducted by Alaska Department of Fish and Game (ADF&G), provide the framework for a Herring Disease Program (HDP) intended to develop and validate an adaptive disease management plan for PWS. The empirical study components of the HDP will provide:

1) Cause-and-effect relationships that will be incorporated into predictive herring population models.
2) Predictive metrics useful in forecasting disease epidemics in PWS herring populations.
3) Adaptive disease management strategies designed to mitigate the effects of infectious and parasitic diseases on populations of Pacific herring in PWS.

A critical component of this empirical component involves the availability of laboratory host models with a known exposure and disease history. Researchers at the USGS - Marrowstone Marine Field Station developed the techniques to rear specific pathogen-free (SPF) herring and we currently maintain thousands of SPF herring in each of 3 age classes (age 0, 1, and 2 yr) for use as experimental animals. These laboratory animals are the only SPF herring known to exist and are offered as an USGS in kind contribution to initiate the HDP. Cause-and effect relationships, determined by empirical studies using these SPF host, will be used to forecast potential for future herring disease epidemics and will be used to develop adaptive management strategies intended to mitigate the effects of disease. Within the HDP, effectiveness of these management strategies will be validated by the ongoing herring disease monitoring surveys conducted by ADF&G.

The initial phase of the HDP, described in this proposal, consists of a four-year plan that is heavily weighted towards understanding epidemiological details associated with VHS for two reasons. First, VHSV is currently presumed to be the primary disease factor in PWS because, compared to other known herring pathogens in PWS "VHSV has more of an effect on unexpected population change" (Marty et al 2003). Second, a standardized VHS laboratory challenge model has been developed in our laboratory and we currently have all the tools available to start investigating individual cause-and-effect relationships with this known pathogen and its herring host. During the initial phase of the HDP, we will begin developing standardized laboratory challenge models for other pathogens of concern to PWS herring, including *Ichthyophonus* and ENV, and preliminary empirical studies will be conducted with these pathogens. If it is determined that these, or other pathogens are population-limiting factors in PWS, then we will expand future proposals to include empirical studies with these pathogens.

Objectives

I. Provide diagnostic confirmation of infection and disease prevalence in PWS herring populations.

Clinical signs of disease are rarely pathognomonic in fish, and diagnosis of disease signs based solely on gross signs can easily result in misdiagnoses. Additionally, low level infections, possibly prefacing the onset of an epidemic, are undetectable by gross observation. Therefore, laboratory diagnostic studies are proposed to confirm disease prevalences reported during ADF&G stock assessment surveys and to determine the overall prevalence of infection, including low level infections.

II. Determine the susceptibility of PWS herring to known pathogens.

Mortality rates within a population can be estimated or incorporated into predictive recruitment models when morbidity and mortality rates due to specific pathogens are known. Epidemiological details associated with individual pathogens will likely have to be incorporated into these models singularly to account for differences in disease kinetics associated with each pathogen.

III. Determine susceptibility of larval (pre-metamorphosis) Pacific herring following exposure to VHSV and determine whether early life stage exposure confers protection to survivors that later metamorphose to juveniles.

If exposure of larval herring confers protection against subsequent VHSV exposure after survivors undergo metamorphosis to juveniles, then the resulting juvenile population will be resistant to VHSV. If this occurs, then susceptibility of larval and juvenile herring to VHSV can be used as a predictive metric to forecast the potential for future VHS epidemics in PWS herring populations.

IV. Determine the relationships between age of juvenile / subadult Pacific herring and susceptibility to VHSV.

There is evidence for both acquired and innate immunity to VHS in Pacific herring. If herring develop innate resistance to VHS as they age, then their previous exposure history to VHSV (eg. acquired immunity) as juveniles and larvae is inconsequential to adult survival after exposure. Answers to this question of innate vs acquired immunity will address the larger question of whether the herring die-off following the PWS oil spill was attributed to VHS. For example, if adult herring develop innate resistance to VHS and they are completely protected regardless of their prior exposure history, then etiology of the 1993 herring crash in PWS may have been caused by something other than VHS.

V. Determine whether Ichthyophonus infections in herring result in decreased swimming performance.

Perhaps low level (subclinical) infections that are below the detection capabilities of standard histological techniques fail to immediately result in direct, parasite-induced mortality; rather, they may decrease host swimming performance to the point where they are selectively targeted by predators. If this is the case, direct and indirect *Ichthyophonus*-related mortality may be much higher than originally estimated and indirect disease mortality should be accounted for in population forecasting models.

VI. Develop immunological and molecular tools and techniques useful in predicting the risk of disease epizootics in herring populations.

Knowledge of a population's innate or acquired resistance to a specific pathogen provides the first step in developing predictive tools that can be used to forecast the potential for future disease epidemics.

A. *Development of a neutralizing antibody test that quantifies humoral resistance to VHS and forecasts potential for future epidemics in wild herring populations..*
B. *Identification of herring immune response genes that predict VHS susceptibility and the potential for future epidemics in wild herring.*
C. *Development of a diagnostic tool to identify viral erythrocytic necrosis.*

Procedural and Scientific Methods

I. Provide diagnostic confirmation of infection and disease prevalence in PWS herring populations.

Disease prevalence surveys conducted by Dr. Gary Marty effectively documented the serious disease problems in PWS herring and demonstrated the need for long term disease monitoring programs. Consequently, Alaska Department of Fish and Game integrated disease monitoring studies into their

herring stock assessment surveys in PWS. Biologists, trained by Dr. Marty, record the prevalence of disease signs, including nodular lesions of the heart and internal organs (indicative of ichthyophoniasis) and focal / petechial skin reddening (indicative of VHS), during their stock assessment surveys. This method of disease surveillance is the least sensitive and does not identify subclinical infections. Consequently, we propose to enhance this ongoing disease monitoring program through the annual addition of laboratory confirmations of clinical and subclinical infection and disease prevalences. Herring from PWS (30 fish / site x 4 sites), will be collected by purse seine or gill net and screened for disease signs. Kidney and spleen from all screened fish will be aseptically removed and analyzed for VHSV by plaque assay. Heart tissue will be cultured in Eagles Minimum Essential Medium and screened for the presence of *Ichthyophonus*. Blood films from all screened fish will be air dried, fixed in methanol, stained with Giemsa, and screened for VEN. Samples from fish with any additional lesions that occur in high prevalences will be fixed in 10% Formalin for later histological analysis. This sampling design will not only confirm the presumed disease diagnoses conducted in the field, but it will also indicate the total prevalence of infection (clinical and subclinical) in PWS herring populations.

II. Determine the susceptibility of PWS herring to known pathogens.

Determination of the cause of the PWS herring decline and failed recovery is confounded by the isolation and identification of multiple potential pathogens after the population crash in 1993. Among the major identified pathogens, VHSV has been shown to be highly virulent (Kocan et al 1997), and injection of *Ichthyophonus* spores results in high pathogenicity and mortality (Kocan et al 1999). However, details of the *Ichthyophonus* challenge model are not yet resolved, and disease kinetics resulting from a natural route of infection remain undetermined. Additionally, difficulties associated with isolation and cultivation of other pathogens, including erythrocytic necrosis virus (ENV), have limited our understanding of their impacts to PWS herring populations. A reductionist approach will be employed to determine morbidity and mortality rates for individual pathogens of concern to PWS herring. For example, we propose to minimize potential confounding variables and determine the pathogenicity and virulence of the major PWS herring pathogens, individually. Then, when disease kinetics for individual pathogens are understood, we will investigate confounding effects of multiple infections and stressors herring health and survival.

During FY 07, we propose to determine the virulence of ENV to Pacific herring by developing a standard laboratory challenge model. Past attempts to conduct laboratory challenge studies with ENV were limited to unnatural routes of infection, particularly injection of transplanted blood from infected individuals, because the causative agent is refractory to established cell lines. We propose to circumvent this problem by utilizing wild herring as a source of ENV. For example, capture of wild, juvenile herring and confinement in laboratory tanks results in progressive outbreaks of VHS and VEN (see detailed description, pg. 3). Therefore, wild herring will be captured, confined to laboratory tanks, and allowed to experience the full VHS epidemic. After the VHS epidemic is complete and during the peak of the VEN epidemic, laboratory-reared SPF herring will be exposed to ENV by cohabitation with the wild fish. As a positive control, blood from wild herring that are heavily diseased with VEN will be injected into SPF herring. Disease kinetics will be quantified by recording daily mortalities in the exposed SPF herring. ENV-exposed individuals will be subsampled from the tanks weekly and infection intensity will be quantified by enumerating VEN inclusions in Giemsa-stained blood films; pathological damage associated with VEN will be determined by examining histological sections (Hematoxylin and Eosin stain) of spleen and kidney tissues. To insure any pathological damage and mortality resulted from VEN and not VHS, dead and moribund fish will be assayed for VHSV by plaque assay. These studies set the groundwork for future studies that will be designed to determine immunological components involved in protection from VEN (ie. passive immunization studies) and the effects of multiple pathogens and stressors to the health and survival of Pacific herring.

During FY 08, we propose to determine the virulence of *Ichthyophonus* to Pacific herring by developing a standard laboratory challenge model. Although carnivorous fishes can become infected with *Ichthyophonus* through the consumption of infected tissue (Kocan et al 1999), Pacific herring are planktivorous and direct consumption of infected fish tissues does not represent a plausible route of natural infection. Therefore, herring likely become infected either by direct consumption of neutrally buoyant ~200μm spores in the water column, or by consumption of an intermediate or parentetic host that carries an infectious stage of the parasite. Past attempts to infect SPF herring with *Ichthyophonus* per os have met with limited success, possibly as a result of temperature requirements necessary to establish infections, or by failure to utilize the infectious stage of the polymorphic parasite during the exposure. Development of a standard infection model will provide basic information on survival (mortality risk) and time-to-death after exposure.

During FY '09-'10, we will utilize the previously developed infection model for VHSV and the newly developed infection models for ENV and *Ichthyophonus* to investigate the confounding effects of multiple infections and stressors on Pacific herring. Because *Ichthyophonus* prevalence increases with herring age and older age cohorts are experiencing dramatic unexplained mortalities in certain parts of the eastern North Pacific, we will use the newly developed *Ichthyophonus* infection model to determine the effects SPF herring age to progression of ichthyophoniasis. Additionally, we will determine the effects of multiple infections with VHSV, ENV, and *Ichthyophonus* to SPF Pacific herring, incorporating different environmental and physiological variables including temperature, nutritional status, and hormonal levels into standard challenge studies.

III. Determine susceptibility of larval (pre-metamorphosis) Pacific herring following exposure to VHSV and determine whether early life stage exposure confers protection to survivors that later metamorphose to juveniles.

Because a standard laboratory challenge model currently exists in our laboratory for VHSV in Pacific herring, we have the tools in hand to investigate the effects of environmental and physiological variables on the ecology of disease with VHS. Newly metamorphosed Pacific herring juveniles are highly susceptible to VHSV, and exposure typically results in 60-100% cumulative mortality. However the susceptibility of premetamorphosed larvae to VHSV remains uninvestigated. If pre-metamorphosed larvae are susceptible to VHSV and they are exposed to exogenous virus in the wild, then the resulting VHS epidemics in wild larvae would be inconspicuous and would likely go unnoticed because of the small size, transparency, and rapid post mortem decomposition of dead larvae. Therefore, the scale of VHSV-induced mortality in wild herring would be much greater than originally estimated. Further, if exposure of herring to VHSV as larvae confers protection to the surviving cohorts that metamorphose to juveniles, then the larval stages of herring likely are very important components contributing to the ecology of VHS disease in wild populations.

During FY 07, we propose to determine the susceptibility of larval herring to VHS by conducting concomitant exposures of SPF age 2, 6, 7, and 9 wk larvae and age 3 and 12 mo juveniles to VHSV. Herring will be exposed to the North American isolate of VHSV (Genogroup IV). All age groups will be exposed to VHSV (~10^4 PFU mL^{-1}) or Hanks Buffered Saline Solution (HBSS; negative controls) by waterborne immersion for 1 hr. Exposed herring will then be distributed to flow-through 760 L tanks supplied with processed seawater for grow-out. Dead and moribund larvae and juveniles from each treatment will be pooled daily and archived at -80 °C for later quantification of VHSV prevalence and / or tissue titers.

To determine whether larval exposure to VHSV confers protection to subsequent exposure after survivors metamorphose to juveniles, a second round of challenges will be conducted, re-exposing survivors to VHSV after they metamorphosed to juveniles. Concomitant exposures will be performed in triplicate for each of three treatment groups (negative control, positive control, and VHSV treatment). Each replicate will consist of a flow-through 280 L tank containing 30-60 juvenile herring. Processed

seawater to the tanks will be turned off during the 1 hr exposure period when groups will be exposed to VHSV (~10^4 pfu mL^{-1}) or HBSS by waterborne immersion. Negative control groups will be exposed to HBSS during the first and second exposures. Positive control groups will be exposed to HBSS during the first exposure and VHSV during the second (i.e. naïve to VHSV until the second challenge). VHSV treatment groups will be exposed to live virus during both the first and second exposures. Dead and moribund individuals from each replicate tank will be collected daily and survivors will be euthanized after 17 d with an overdose of neutral buffered tricaine methane sulfonate (MS-222). All samples will be archived at -80 °C for later quantification of VHSV tissue titers.

Presence and / or titer of VHSV in herring tissues and exposure water will be determined by plaque assay. Duplicate samples of exposure water will be diluted in tris-buffered Eagles Minimum Essential Medium (MEM) supplemented with 100 IU / ml penicillin, 100 µg/ ml streptomycin, 100 µg/ ml gentamycin, and 2.5 µg / ml amphotericin B; exposure level will be reported as mean titer in the duplicate samples. Pooled whole larvae and individual juvenile herring samples (minus head and tail) will be diluted and homogenized in MEM by mortal and pestle or Stomacher-80™. Serial 10-fold dilutions of water samples and tissue homogenates will be plated onto polyethylene glycol pretreated monolayers (Batts and Winton 1989) of *epithelioma papulosum cyprini* (EPC) cells (Fijan et al. 1983). Cell cultures will be overlayed with methylcellulose, incubated at 15°C for 7 d, then fixed and stained with crystal violet - Formalin solution prior to plaque enumeration. Tissue titers of VHSV will be reported as plaque forming units (PFU) g^{-1}, and mean waterborne exposure levels will be reported as PFU mL^{-1}.

IV. Determine the relationship s between age of juvenile / sub-adult Pacific herring and susceptibility to VHSV.

Preliminary laboratory studies indicate that susceptibility of Pacific herring to VHS decreases with host age (Kocan et al 1997); however, confounding variables inherent to these studies require a more comprehensive study. For example, the range of herring ages examined for susceptibility spanned only 8 months (age 5, 9, and 13 mo cohorts). Additionally, failure to perform the exposures concomitantly resulted in subtle differences in water temperature and exposure level that can have dramatic impacts on the disease process.

Confirmative studies are proposed during FY 08, whereby SPF herring age cohorts, encompassing a more expanded age range from newly metamorphosed juveniles through sexually mature adults, will be exposed concomitantly to VHSV Genogroup IV in the laboratory.

Groups of age 0, 1, 2, and 3, and 4 yr SPF herring will be concomitantly exposed to the same isolate of VHSV, Genogroup IV in a waterborne exposure studies (5 replicate tanks / age class x 4 age classes = 20 experimental tanks + 20 control tanks). Rearing of SPF herring in the laboratory will continue through FY 07 and 08 and the full complement of SPF herring age classes (age 0-4 yr) will be available for experimental studies in FY 08. During the exposure studies, each replicate will consist of a 280L tank, containing 30 herring from a single age class. Herring in each experimental replicate will be exposed to ~10^3 PFU mL^{-1} VHSV for 1 hr; herring in the respective control tanks will be concomitantly exposed to similar volumes of HBSS. Dead and moribund individuals will be sampled daily and frozen at -80 °C for later enumeration of virus tissue titers by plaque assay, as described previously.

V. Determine whether Ichthyophonus infections cause decreased swimming performance in herring.

Ichthyophonus infections can be highly pathogenic to Pacific herring and result in direct mortality; however, infections are more often chronic and hosts are often able to survive for extended periods. Swimming stamina in rainbow trout, measured as time-to-fatigue, is approximately 60% less in *Ichthyophonus*-infected individuals than in pathogen-free controls (Kocan et al. *In press.*); however, analogous studies on herring swimming performance have not been performed. If similar performance reductions occur in *Ichthyophonus*-infected herring, then infected individuals would be less able to mount an effective predator avoidance response and selective predation on infected cohorts would likely occur. Considering the prevalence of

Ichthyophonus in Pacific herring ranges from 11-55% (Hershberger et al 2002, Jones and Dawe 2002), the summation of direct and indirect mortality resulting from *Ichthyophonus* infections may constitute a major population-limiting factor.

During FY 07, naturally infected, wild herring will be captured by purse seine and transferred alive to laboratory tanks, where swimming stamina studies will be performed. Wild adult herring, which generally have *Ichthyophonus* infection prevalences of 30-60% (Hershberger et al 2002), will be acclimated to laboratory tanks for 2 weeks, then swam to exhaustion in a calibrated swim chamber at a predetermined flow velocity. Time to exhaustion will be recorded for each fish. Exhausted herring will be euthanized and their infection status will be determined by in vitro explant culture as described previously. *Ichthyophonus* infection intensity will be quantitatively enumerated histologically based on parasite load and cellular inflammatory response surrounding the parasite (Sanders et al in preparation). Other naturally occurring parasites will also be recorded, quantified and correlated with swimming performance. Observer bias will be eliminated because the infection status of each fish will remain unknown until each exertion test is terminated and explant cultures are examined.

To eliminate the confounding effects of multiple pathogens and unknown history inherent to utilizing wild herring as experimental animals, we propose to expand upon the wild herring swimming stamina studies in FY 08 using experimentally infected SPF herring. SPF herring will be infected with *Ichthyophonus* spores suspended in Hanks Buffered Saline Solution (HBSS); control groups will be treated similarly but not exposed to the pathogen. To determine the effect of infection intensity on swimming performance, subsamples of infected and control groups will be subjected to swimming exertion tests 2, 6, and 8 wk post-infection. During each exertion test, infected and uninfected fish (twenty each) will be pooled into a swim chamber at a predetermined flow velocity. Time to exhaustion will be recorded for each fish. Exhausted herring will be euthanized and their infection status and intensity will be determined by in vitro explant culture and histology, as described previously. Observer bias will be eliminated because the infection status of each fish will remain unknown until each exertion test is terminated and explant cultures are examined. The extent of tissue damage caused by the parasite will be determined histologically.

During FY 09, we will determine whether predators selectively target *Ichthyophonus*-infected individuals within a population. Experimentally-infected and uninfected SPF herring will be introduced to a community tank containing predatory fish (likely to include coho and / or Chinook salmon). Predators will be allowed to forage on herring for a predetermined period, after which all fish in the tank will be euthanized by an overdose of MS-222. Predator selection for, or against *Ichthyophonus*-infected herring will be determined by comparing the prevalence of *Ichthyophonus* in unconsumed herring with that of cohorts that were successfully captured and eaten by the predators. *Ichthyophonus* prevalence will be determined as described previously.

VI. Develop immunological and molecular tools and techniques useful in predicting the risk of disease epizootics in herring populations.

If, as suggested by Marty et al (2003), VHSV is the primary pathogen responsible for "unexpected population change" in PWS herring populations, then techniques to quantify the susceptibility of herring populations to VHS can be used as predictive tools to forecast the potential for future disease epidemics. For example, if it can be demonstrated that a herring population, or cohorts of a population, are resistant to VHS, then the potential for an unexpected epidemic within the population or cohort can be considered minimal. Conversely, if a tool or technique demonstrates that a population or cohort of a population is highly susceptible to VHS, then the potential for an epidemic can be considered high, especially if the susceptible fish are exposed to exogenous virus. This type of predictive tool can then be incorporated into herring management schemes in PWS by adapting annual management strategies with the changing disease potentials.

A. Development of a neutralizing antibody test that quantifies humoral resistance to VHS and forecasts potential for future epidemics in wild herring populations.

Serum neutralization tests can be used to quantify the level of humoral protection that an individual demonstrates for a chosen pathogen. By plating serial dilutions of serum containing neutralizing antibodies in wells containing virus and susceptible cells, a quantifiable metric of humoral protection against a specific virus is achieved. Plaque neutralization tests are routinely performed in virology laboratories to identify unknown viruses using known levels of neutralizing antibodies specific to the suspected virus. However, an adaptation of the plaque neutralization technique can be employed to screen wild herring and quantify their currently levels of humoral protection against a chosen pathogen. Previous attempts to develop a neutralizing antibody test for VHSV in wild herring met with limited success (Kocan unpublished data), likely because of details specific to the fish immune system response to rhabdoviral infections. For example, to optimize sensitivity of the plaque neutralization test for VHSV and infectious hematopoietic necrosis virus (IHNV) in rainbow trout serum, exogenous complement from naive individuals is routinely incorporated into the plaque neutralization procedure (Dorson and Torchy 1979, LaPatra et al. 1993, LaPatra 1996). We propose to adapt these standard complement supplementation techniques and optimize a plaque neutralization test for VHSV from Pacific herring serum. As a source of supplemented complement, serum from SPF rainbow trout and Pacific herring will be tested. The VHSV plaque neutralization test will be optimized for Pacific herring in FY 07; studies using serum from wild PWS fish will verify the technique in FY 08, and studies in future years will be directed towards application of this tool as a predictive metric to forecast the potential for a VHSV epidemic in PWS herring.

B. Identification of herring immune response genes that predict VHS susceptibility and the potential for future epidemics in wild herring.

In addition to detecting neutralizing antibodies to VHSV in serum of wild herring, molecular immunological tools will be developed as epidemiological biomarkers capable of forecasting the susceptibility of herring populations to VHS. During FY07 we will identify molecular sentinels of the immune response to VHSV infection using a tow-fold approach. The first approach is a candidate gene approach based upon alignments of known vertebrate immune response genes (including those from teleost species) to identify conserved stretches of amino acids for the development of degenerate primer sets. The degenerate primer sets will be used to amplify portions of specific target genes by RT-PCR using cDNAs derived from herring immune tissues as the templates for amplification. This is standard methodology in our laboratory. The remaining portions of the candidate genes will be cloned from herring full-length cDNA libraries or from herring RACE (rapidly amplified cDNA ends-5' and 3' portions of genes) cDNA libraries both of which represent additional products for this proposal. The four genes that have been chosen (Mx, interferon-A, MHC 1A and immunoglobulin) represent innate and adaptive immune response genes that are differentially regulated in other teleosts.

The second approach represents a global approach in which the majority of genes (cDNAs) that are either up or down regulated during infection will be identified, regardless of whether they are currently known or unknown. We will use a subtractive approach to directly clone and characterize genes that are differentially regulated in infected herring relative to controls. There are several different strategies for subtraction-based cloning, but for the "best value" in both monetary measure and time spent, suppression-based subtractive hybridization (SSH) is the most efficient and easiest method for cloning a suite of differentially regulated genes. Clonetech introduced the first SSH kit in 2000 and now offers the third generation kit that greatly reduces false positives (i.e. genes that are not subtracted from one another). In simplest terms, two pools (naïve and infected) of cDNA are subtracted from one another and converted into double stranded cDNA and cut with a restriction enzyme into smaller fragments (200-600 bp) that are more easily hybridized with one another. Adaptors containing specific primer sites are then ligated onto the ends of one of the pools of cDNAs. cDNA pools are then mixed, heated to facilitate denaturation and genes common to both pools will hybridize (re-anneal). An excess of the pool lacking the primer site (i.e. cDNA pool that was not ligated to the adaptor) is used in the reaction to ensure that

most if not all of the genes common to both pools are hybridized. The remaining, non-hybridized cDNAs are amplified using primers specific for the adaptors. The reaction can be driven in both directions resulting in the amplification of up and down regulated genes. This is a robust method for cloning differentially regulated genes that are representative of the innate and adaptive immune responses.

There are several ways that the subtraction process can be analyzed. We plan to use the most straightforward approach, as our goal for FY07 is to identify 4-5 molecular sentinels of the immune response. After performing the PCR suppression reactions in both directions and with several tissue sources, aliquots of the reactions will be electrorphoresed and stained with EtBr for visualization of bands. If the reaction yields a single, distinct band then the amplicon can be cloned using the pTOPO cloning kit (Invitrogen). Ligated plasmids will then be transformed into *E. coli*, screened by blue/white selection, purified using standard plasmid procedures and sequenced using our in-house ABI 3130 automated sequencer to reveal the identity of the cloned cDNAs. Sequences will then be compared against GenBank using BlastX homology searches (NCBI, www.ncbi.nlm.nih.gov). Typically though, a single band generated using this kit actually contains several cDNAs (genes) that share the same size range for the amplicon. Thus after the amplification step, 20 randomly selected colonies from the transformation reaction will be analyzed by EcoRI restriction digests whereupon several different sized inserts would then be sequenced. All cDNA sequences that are derived from this study will be deposited into GenBank regardless of whether they encode immune related genes as these sequences will likely be useful to other researchers studying herring performance traits. Real time qPCR assays will be developed using genes obtained by these two approaches in FY 07 that will lay the groundwork for proposed studies in future years designed to profile the immune responses of herring after infection with VHSV. The Fish Health Lab at WFRC is well adept with real-time RTPCR assays (Purcell et al 2004, 2006). Our goal is to incorporate up and down regulation of these immune system genes into epidemiological biomarkers that can be used as predictive tool to forecast future disease epidemics in PWS herring populations.

During FY 08 and 09, we will utilize the real time qPCR developed in FY07 to map the immune response of SPF herring after infection with VHSV and vaccination using a newly constructed DNA vaccine derived from VHSV Genogroup IV. This will profile the timing and magnitude of the immune response to VHSV after survival from an induced epidemic and after immunization. Studies in future years will be directed towards application of these molecular immune response genes into predictive metrics to forecast the potential for a VHSV epidemic in PWS herring

C. Development of a diagnostic tool to identify viral erythrocytic necrosis.

In addition to VHS, other diseases of herring should be considered population-limiting factors, including viral erythrocytic necrosis (VEN). Although VEN is a known disease of Pacific herring and has been associated with epidemics in wild Pacific herring from Alaska (Meyers et al 1986), the limiting effects of VEN on herring populations remain largely uninvestigated. Research with VEN has been impeded by the unavailability of standard virology tools necessary to characterize and cultivate the causative agent. The primary impediment precluding development of these tools is that the causative agent does not replicate in any known cell line (Nicholson and Reno 1981). VEN is currently diagnosed by observation of erythrocytic inclusion bodies in stained blood films, and 'confirmation' is by detection of iridovirus-like particles in transmission electron micrographs. Although useful, these methods do not confirm the identity of a single virus species.

We propose to circumvent these problems with virus isolation by developing molecular diagnosis tools based on the polymerase chain reaction (PCR), to confirm the etiology of the presumed causative agent from whole herring blood with intracytoplasmic inclusion bodies. Because VEN epidemics occur with great predictability in confined, juvenile herring (Hershberger et al 2006), we plan to obtain VEN-infected herring blood by confining wild herring in laboratory tanks until the epidemic peaks. Anemic individuals will be euthanized and whole blood from heavily diseased individuals will be collected in

sodium citrate coated capillary tubes. Giemsa-stained blood films from each euthanized fish will confirm VEN infections. DNA will be extracted from herring whole blood and amplified in a thermocycler using multiple degenerate iridovirus primer pairs. Amplicons of the correct size will be identified by gel electrophoresis, excised, cloned, and sequenced. The generated sequences will be compared to database sequences by a BlastX homology search to determine VEN's relatedness to iridoviruses. To confirm the viral origin of the amplicons, blood from laboratory-reared SPF herring will be utilized as negative controls. If the degenerate iridovirus primers fail to amplify the extracted DNA from VEN-infected blood alternative strategies include subtractive hybridization as described previously and purification of the presumed iridovirus by a cesium gradient column, amplification by random primers, and cloning. Ultimately, the generated VEN sequence products will be used to design higher specificity primers for a diagnostic PCR.

Development of a molecular tool to detect ENV represents a logical first step in a larger VEN work plan intended to determine epidemiological mechanisms including virus transmission and ecological mechanisms of infection and disease. Understanding these basic epidemiological mechanisms will lead to development of the ability to forecast VEN epidemics and offer management options intended to mitigate the population-level effects of disease. A molecular diagnosis tool will be developed in FY '07 and further epidemiological studies, that utilize this new tool, will be described and proposed in future years.

Data Analysis and Statistical Methods

Standard statistical comparisons for pathogen virulence studies will be employed in all experiments. For example, cumulative mortalities (percents) 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 comparisons in the swimming performance studies will be performed using the Student's t-test. Statistical significance will be assigned to all comparisons with $P \leq 0.05$.

Description of the 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. Proposed field studies in PWS are partnered with ADF&G stock assessment surveys and are intended to provide laboratory confirmation of infection and disease prevalences.

Laboratory studies described in this proposal will be conducted at the USGS-Marrowstone Marine Field Station, USGS-Western Fisheries Research Center, and Clear Springs Foods, Inc. Facilities at the Marrowstone Marine Station and Western Fisheries Research Center are 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 and the Department of Interior. 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, histopathology, and stress biochemistry. Clear Springs, Inc. is the world's largest producer of rainbow trout, producing over 20 million pounds / year. The fish health program at Clear Springs has European complement-mediated plaque neutralization techniques into standard operating procedures to determine the susceptibility of their rainbow trout to IHNV; further these techniques are used to forecast the potential for future disease epidemics. These methods will be adapted to VHSV, the closely related rhabdovirus infection, in Pacific herring.

Coordination and Collaboration with Other Efforts

This HDP represents a large collaborative effort between private, state, and federal entities. These studies dovetail with Alaska Department of Fish and Game herring population assessments and disease surveys by providing laboratory diagnostic confirmation using paired samples. Laboratory confirmation of the ongoing disease severity index will be accomplished via subcontract to the Alaska Department of Fish and Game, Juneau Fish Pathology Laboratory. Empirical studies outlined in this proposal are designed to demonstrate cause-and-effect epidemiological relationships. These relationships will then be incorporated into predictive metrics that become integrated into a herring population assessment model (to be proposed in FY '10). Additionally, results from the empirical studies will be used to offer adaptive management options intended to mitigate the effects of disease in wild herring populations. The effectiveness of these adaptive management plans will be evaluated by continued confirmation of the ADF&G disease severity index. Our proposed herring disease approach is supported by Alaska Department of Fish and Game PWS herring managers and by the Prince William Sound Fisheries Research Application and Planning (PWSFRAP), a group dedicated to resolving the disconnect between science and its application (see attached letter of support from Ross Mullins).

SCHEDULE

Project Milestones

Objective I. Provide diagnostic confirmation of infection and disease prevalence in PWS herring populations.

Laboratory analyses of wild fish disease surveys: completed by September 1 for each spawning year.

Objective II: Identify, isolate, and determine the virulence of known PWS herring pathogens.

Virulence of VEN to SPF herring: completed by September 1, 2007.

A standard infection model for *Ichthyophonus*: developed by September 1, 2008.

Effects of multiple infections: September 1, 2010.

Rearing of SPF herring: continue through 2010, when 6 year classes of laboratory-reared herring will be available for experimental purposes.

The effect of herring age on ichthyophoniasis: September 1, 2010.

Objective III. Determine susceptibility of larval (pre-metamorphosis) Pacific herring following exposure to VHSV and determine whether early life stage exposure confers protection to survivors that later metamorphose to juveniles: September 1, 2007.

Objective IV. Determine the relationships between age of juvenile / subadult Pacific herring and susceptibility to VHSV: September 1, 2008.

Objective V. Determine whether Ichthyophonus infections in herring result in decreased swimming performance.

Relative swimming performance of wild herring: September 1, 2007

Relative swimming performance of SPF herring experimentally infected with *Ichthyophonus*: September 1, 2008.

Objective VI. Develop immunological and molecular tools and techniques useful in predicting the risk of disease epizootics in herring populations.

A. Development of a serum neutralization test to predict the potential for future VHSV epidemics in wild herring.

Techniques optimized: September 1, 2007.

Validation of the techniques using wild PWS herring: September 1, 2008.
Application of this tool as a predictive metric to forecast VHS epidemics: September 1, 2009.

B. Identification of herring immune response genes that predict VHS susceptibility and the potential for future epidemics in wild herring.
Candidate genes identified and qPCR optimized: September 1, 2007.
Expression profiling after exposure to live virus and DNA vaccine: September 1, 2008 and 2009, respectively.
Application of this tool as a predictive metric to forecast VHS epidemics: September 1, 2009.

C. Development of a diagnostic tool to identify viral erythrocytic necrosis.
Technique developed and optimized: September 1, 2007
Validation of the technique, using PWS and Puget Sound herring: September 1, 2008.
Application of this tool as a predictive metric to forecast VEN epidemics: September 1, 2009.

Measurable Project Tasks (FY 07)*

FY07, 1st quarter (October 1, 2006-December 31, 2006)

Oct.	- Project funding approved by Trustee Council
	- Capture juvenile herring as a source of VEN-positive blood for the development of a molecular diagnostic tool
Nov. 1	- Initiate VHSV exposure studies as a source of serum for plaque neutralization test and as a source of VHSV-positive tissue for the identification of immune response genes.

FY07, 2nd quarter (January 1, 2007-March 31, 2007)

Jan. 23-27	Annual Marine Science Symposium (attend and present preliminary results)
Feb. 1 -	- Collect herring eggs for continuation of SPF herring rearing
May 31	- ADF&G collects herring from stock assessment surveys, estimates disease prevalence, and sends tissues to diagnostic laboratory for confirmation
	- Collect adult herring for us as experimental animals to determine effects of natural *Ichthyophonus* infections on swimming performance.

FY07, 3rd quarter (April 1, 2007-June 30, 2007)

June 1 - 21	Initiate VHSV exposure studies with larval herring
June 30	- Initiate swimming performance studies using wild herring
	- Complete laboratory confirmation of infection and disease prevalences in wild PWS herring populations.

FY07, 4th quarter (July, 2007-September 30, 2007)

July 15	Collect wild, juvenile herring as a source of ENV for virulence studies
Aug. 1	Re-expose survivors of the larval VHSV exposure
Aug. 7	Cohabitate wild, VEN-diseased herring with SPF herring in the laboratory and initiate virulence study
Aug. 31	Complete laboratory assays for larval herring exposure studies
	Complete development of serum neutralization test to quantify the level of protection against VHS
	Complete development of the VEN molecular diagnostic tool
	Identify VHSV immune response genes

*Measurable project tasks for studies beyond FY'07 will be included in future proposals.

RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

Community Involvement and Traditional Ecological Knowledge (TEK)

This Herring Disease Plan proposes to integrate local Alaskan expertise in herring biology and stock assessment techniques with empirical disease resources that are available only at the Marrowstone Marine Station, including pathogen disinfection / containment facilities and availability of SPF Pacific herring colonies. Local Alaskans will be involved with PWS sample collection, disease screening procedures

(ADF&G - Cordova), and laboratory diagnostic confirmation of infection / disease prevalences (ADF&G – Juneau Fish Pathology Laboratory). Copies of all presentations will disseminated to the public via the Prince William Sound Science Center.

Resource Management Applications

The proposed epidemiological approach in this Herring Disease Program (HDP) is similar to that employed by the World Health Organization (WHO) and Centers for Disease Control (CDC) for emerging diseases in human populations, and consists of disease surveillance and empirical epidemiological studies. Combined, these data are useful in developing metrics and tools necessary to predict future disease epidemics and develop disease mitigation strategies. Our proposed approach utilizes the natural host (SPF Pacific herring) in empirical manipulation studies and represents a distinct advantage over the WHO and CDC approach that is limited to experimental manipulation using surrogate species. Therefore, this proposed HDP represents an integrated and cutting-edge approach to advancing our understanding of disease ecology in wild marine fish populations and streamlines the existing approaches utilized by the world's leading human health epidemiologists.

We have a demonstrated publication record of working with resource managers to develop disease mitigation strategies in populations of wild marine fishes (Hershberger et al 2001, Kocan et al 2005), and the PWS herring manager supports our proposed approach (See attached letter of support from Steve Moffitt). Prior to conducting the empirical studies, we can only speculate at management applications; however, basic fishery management techniques may be used to encourage or curtail harvest during years of high or low disease prevalence. Additionally, if managers are capable of forecasting the severity of diseases each year, then effort can be shifted to alternative herring gear types. For example, if herring age and water temperature are inversely correlated with VHS prevalence, then herring fisheries during cool spawning years, predominated by newly-recruited cohorts, should be shifted away from spawn-on-kelp fisheries because impoundment would result in an epidemic. During such a year, an effective management strategy may be to direct fishery effort towards sac roe fisheries. Alternatively, if herring age is directly related to ichthyophoniasis, a much more chronic disease, then perhaps SOK fisheries should be encouraged during years predominated by older recruits. Negative effects of this management strategy may be weighed against the possible effects of elevated temperature at exacerbating *Ichthyophonus* infections. These epidemiological relationships, and their management implications, will remain purely conjectural until controlled empirical studies are performed and adaptive management strategies are verified by long term disease surveillance studies.

PUBLICATIONS AND REPORTS

We will make every effort to publish the results from these laboratory studies in peer reviewed scientific journals. During the first two years of the HDP we anticipate submitting manuscripts on the following subjects:

1) Susceptibility of naïve Pacific herring larval and previously exposed juveniles to VHS
2) Differential susceptibility of different age classes of Pacific herring to VHS
3) Virulence of ENV to Pacific herring
4) Effect of *Ichthyophonus* on the swimming performance of Pacific herring
5) A plaque neutralization test to detect neutralizing antibodies to VHSV in Pacific herring
6) Regulation of immune system genes in Pacific herring after exposure to VHSV
7) A molecular diagnosis tool to identify ENV from whole blood

Additionally, results from these studies will be presented at national and international professional meetings including the Ecological Society of America, American Fisheries Society National meeting, and AFS Fish Health Section Annual Meeting.

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Rahimian H, J Thulin. 1996. Epizootiology of *Ichthyophonus hoferi* in herring populations off the Swedish west coast. Diseases of Aquatic Organisms 27:187-195.

Rand TG, K White, JJ Cannone, RR Gutell, CA Murphy, MA Ragan. 2000. *Ichthyophonus irregularis* sp. nov. from the yellowtail flounder *Limanda ferruginea* from the Nova Scotia shelf. Diseases of Aquatic Organisms 41: 31-36.

Rice, J. 1995. Food web theory, marine food webs, and what climate change may do to northern marine fish populations. In Climate Change and Northern Fish Populations, pp. 516-568. Ed. By R.J. Beamish. Canadian Special Publication of Fisheries and Aquatic Sciences, 121.

Sanders, GS, C Conway, PK Hershberger, R Kocan, and JR Winton. *In Preparation*. Comparison of standard *in vivo* culture technique to serial histopathological sectioning for the identification of *Ichthyophonus*-infected Yukon River Chinook salmon. Journal of Fish Diseases.

Sindermann C. 1990. Fungi. Pp 57-78 *In:* Principal diseases of marine fish and shellfish, Second Edition, Volume 1: Diseases of Marine Fish. Academic Press, New York

Traxler GS, D Kieser. 1994. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) from herring (*Clupea harengus pallasi*) in British Columbia. Fish Health Section American Fisheries Society Newsletter 22(1): 8.

ABBREVIATED 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)
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: 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:

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, 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.

Hershberger, P.K., N.E. Elder, G.D. Marty, J. Johnson, and R.M. Kocan. 2001. Management of Pacific herring closed pound spawn-on-kelp fisheries to optimize fish health and product quality. North American Journal of Fisheries Management 21: 550-555.

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.

Kocan, R., P. Hershberger, T. Mehl, N. Elder, M. Bradley, D. Wildermuth, and K. Stick. 1999. Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring (*Clupea pallasi*) and its early appearance in wild Puget Sound herring. Diseases of Aquatic Organisms 35: 23-29.

Five Additional Selected Publications

Kocan, R., S. LaPatra, J. Gregg, J. Winton, P. Hershberger. *In Press*. *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in rainbow trout. Journal of Fish Diseases.

Kocan, R.M., P.K. Hershberger. *In Press*. Differences in *Ichthyophonus* prevalence and infection severity between upper Yukon and Tanana River Chinook salmon stocks. Journal of Fish Diseases.

Hershberger, P.K., N.E. Elder, J. Wittouck, K. Stick, and R.M. Kocan. 2005. Abnormalities in Larvae from the Once-Largest Pacific Herring Population in Washington State Result Primarily from Factors Independent of the Spawning Location. Transactions of the American Fisheries Society 134: 326-337.

Lefebvre, K.A., N.E. Elder, P.K. Hershberger, V.L. Trainer, C.M. Stehr, N.L. Sholtz. 2005. Dissolved saxitoxin exposure causes transient inhibition of sensorimotor function in larval herring *Clupea pallasi*. Marine Biology 147: 1393-1402.

Van der Straaten, N, A. Jacobson, D. Halos, P. Hershberger, A. Kocan, R. Kocan. 2005. Prevalence and spatial distribution of intraerythrocytic parasite(s) in Puget Sound rockfish (*Sebastes emphaeus*) from the San Juan Archipelago, Washington (USA). Journal of Parasitology 91:980-982.

Recent Collaborators and Co-Authors:

W. Batts (USGS-WFRC), B. Bui (UW-FHL), N. Elder (USGS), C. Carroll (UW-FHL), B. Fall (UW-FHL), J. Gregg (USGS), D. Halos (UW-FHL), C. Halpenny (U. Montreal), A. Hart (UW), H. Hsu, A. Jacobson (UW-FHL), A. Kocan (Oklahoma State U.), R. Kocan (UW-SAFS), S. LaPatra (Clear Springs Foods), K. Lefebvre (NMFS - NW Center), C. Mork (Boston College), J. Perry (Colorado State U.), J. Richard (DFO), N. Sholtz (NMFS – NW Center), C. Stehr (NMFS – NW Center), K. Stick (WDFW), E. Sweeney (UW-FHL), V. Trainer (NMFS – NW Center), G. Traxler (DFO), N. Van der Straaten (UW-FHL), J. Winton (USGS-WFRC), J. Wittouck (UW-SAFS)

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Education:

1985	School of Fisheries, University of Washington, WA	Ph.D
1976	School of Fisheries, University of Washington, WA	M.S.
1971	College of Fisheries, University of Washington, WA	B.S.

Employment:

1986-present	Research Microbiologist, Project Leader, Bacteriology and Histology Sections, Western Fisheries Research Center, USGS, Seattle WA
1995-present	Affiliate Associate Professor, University of Washington, Seattle, WA
1986-1995	Affiliate Assistant Professor, University of Washington, Seattle, WA
1984-1986	Research Fishery Biologist, National Fisheries Research Center, USFWS, Seattle, WA
1979-1981	Research Fishery Biologist, Northwest and Alaska Fisheries Center, NMFS/NOAA, Seattle, WA,
1976-1979	Research Fish Health Biologist, Tavolek Inc., Redmond, WA,
1974-1976	Fish Pathologist/Disease Inspector, Biometrics, Inc., Tacoma, WA

Professional Organizations and Certification

American Society for Microbiology
American Fisheries Society (Certified Fish Pathologist, FHS/AFS)
European Society of Fish Pathologists (U.S. Branch Officer)
New York Academy of Sciences
Sigma Xi

Selected Publications:

Chase, D.M., D.G. Elliott, and R.J. Pascho. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction. Journal of Veterinary Diagnostic Investigation (in press).

Coady, A.M., A.L. Murray, D.G. Elliott, and L.D. Rhodes. 2006. Both *msa* genes in *Renibacterium salmoninarum* are needed for full virulence in bacterial kidney disease. Applied and Environmental Microbiology 72:2672-2678.

Bruno D.W., B. Nowak, and D.G. Elliott. 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. Diseases of Aquatic Organisms 70:1-36.

Kurath, G., K.A. Garver, S. Corbeil, D.G. Elliott, E.D. Anderson, and S. E. LaPatra. 2006. Protective immunity and lack of histopathological damage two years after DNA vaccination against infectious hematopoietic necrosis virus in trout. Vaccine 24:345-354.

Garver, K.A., C.M. Conway, D.G. Elliott, and G. Kurath. 2005. Analysis of DNA vaccinated fish reveals viral antigen in muscle, kidney and thymus, and transient histopathological changes. Marine Biotechnology 7:540-553.

Yasutake, W.T., and D.G. Elliott. 2003. Epizootiology and histopathology of *Parvicapsula* sp. in coho salmon *Oncorhynchus kisutch*. Diseases of Aquatic Organisms 56:215-221.

Pascho, R.J., D.G. Elliott, and D.M. Chase. 2002. Comparison of traditional and molecular methods for detection of *Renibacterium salmoninarum*. Pages 157-209 *in* Cunningham, C.O. (ed.) Molecular Diagnosis of Salmonid Diseases. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Elliott, D.G. and R.J. Pascho. 2001. Evidence that coded-wire-tagging procedures can enhance transmission of *Renibacterium salmonminarum* in chinook salmon. J. Aquat. Anim. Health 13:181-193.

O'Farrell, C.L., D.G. Elliott, and M.L. Landolt. 2000. Mortality and kidney histopathology of chinook salmon *Oncorhynchus tshawytscha* exposed to virulent and attenuated *Renibacterium salmoninarum* strains. Dis. Aquat. Org. 43:199-209.

Elliott, D.G. 2000. Integumentary system: microscopic functional anatomy. Pages 271-306 *in* G.K. Ostrander, editor. The Laboratory Fish. Academic Press, London.

Recent Collaborators and Co-Authors

Anderson ED (Maine BioTek Inc.), Applegate LM (USGS-WFRC), Bruno DW (FRS Marine Laboratory, Scotland), Campton DE (USFWS), Carper M (NOAA), Chase DM (USGS-WFRC), Coady AM (NOAA), Conway CM (USGS-WFRC), Corbeil S (CSIRO Animal Health Laboratory, Australia), Garver KA (DFO Canada), Gilbreath L (NOAA), Hard JJ (NOAA), Kurath G (USGS-WFRC), LaPatra SE (Clear Springs Foods Inc.), Maule A (USGS-CRRL), McComas RL (NOAA), McKibben C (USGS-WFRC), McMichael GA (Battelle), Mesa M (USGS-CRRL), Murray AM (USGS-WFRC), Nowak B (University of Tasmania), Park LK (NOAA), Pascho RJ (USGS-WFRC), Purcell M (USGS-WFRC), Rhodes LD (NOAA), Ryan B (NOAA), Smith S (NOAA), Vucelick JA (Battelle), Winton J (USGS-WFRC), Yasutake WT (USGS-WFRC)

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EDUCATION

H.D.	1982	West High School, Alaska	
B.S.	1987	Oregon State University	Fisheries
B.S.	1987	Oregon State University	Microbiology
M.S.	1994	University of Washington	Fisheries

PROFESSIONAL EXPERIENCE

Research Microbiologist, USGS, Biological Resources Division,
Western Fisheries Research Center, Seattle, Washington
October 1994 - present

Fisheries Biologist, US Fish and Wildlife Service,
National Fisheries Research Center, Seattle, Washington
June 1992 - October 1994

Microbiologist, Alaska Department of Fish and Game,
Fish Pathology Section, Anchorage, Alaska
August 1990 - September 1991

Adjunct Lecturer, University of Alaska,
Biology Department, Anchorage, Alaska
September 1988 - June 1991

HONORS

1995 On the Spot Award, National Biological Service
1994 Quality Performance Award, National Biological Service
1993 University of Washington School of Fisheries Graduate Scholarship
1987 OSU Department of Fisheries and Wildlife "Senior of the Year"
1987 Graduated with honors in Microbiology (OSU)
1987 Graduated with honors in Fisheries (OSU)
1987 Academic All-American
1986 Oregon Western Rod and Reel Scholarship recipient
1986 OSU Department of Fisheries and Wildlife "Undergraduate of the Year"
1986 Elected Vice President of the OSU College of Science Council
1984 Elected as the Associated Students of OSU science senator
1983 Elected to Phi Kappa Phi honor society, Oregon State University

SELECTED PUBLICATIONS:

Kurath, G., K.A. Garver, W.N Batts, and E.J. Emmenegger. 2005. Genetic typing of infectious hematopoietic necrosis virus. Aquaculture, R.C. Cipriano, I.S. Shchelkunov, and M. Failal, eds. In press.

Emmenegger, E.J., R.M. Troyer, and G. Kurath. 2003. Characterization of the mutant spectra of a fish RNA virus within individual hosts during natural infections. Virus Research 96:15-25.

Kurath, G., K.A. Garver,. R.M. Troyer, E. J. Emmenegger, K. Einer-Jensen, and E.D. Anderson. 2003 Phylogeography of infectious hematopoietic necrosis virus in North America. Journal of General Virology 84:803-814.

Emmenegger, E.J., and G. Kurath. 2002 Genetic characterization of infectious hematopoietic necrosis virus of coastal salmonid stocks in Washington State Journal of Aquatic Animal Health 14:25-34.

Emmenegger, E.J., T.R. Meyers, T.O. Burton, and G. Kurath. 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Diseases of Aquatic Organisms 40: 173-176.

Anderson, E.D., H. M. Engelking, E.J. Emmenegger, and G. Kurath. 2000. Molecular epidemiology reveals emergence of a virulent infectious hematopoietic necrosis virus strain in wild salmon and its transmission to hatchery fish. Journal of Aquatic Animal Health 12: 85-99.

Kurath, G, R.M. Troyer, E.D. Anderson, and E. J. Emmenegger. 2000. Genetic diversity patterns and evolution of an aquatic rhabdovirus. Proceedings of the Noble Foundation Virus Evolution Workshop, Oct. 1999, Ardmore, Oklahoma. Available by internet at http://www.noble.org/virusevolution/abstracts/KurathProc.htm.

Emmenegger, E., M. Landolt, S. LaPatra, and J. Winton. 1997. Immunogenicity of synthetic peptides representing antigenic determinants on the infectious hematopoietic necrosis virus glycoprotein. Diseases of Aquatic Organisms 28: 175-184.

RECENT COLLABORATORS

G. Kurath (USGS), J. Winton (USGS), Bill Batts (USGS), M. Purcell (USGS), J. Ranson (USGS), G. Sanders (U. Washington), S. LaPatra (Clear Springs Food), K. Einer-Jensen (Danish Veterinary Lab.), T. Meyers (ADF&G), T. Burton (ADF&G), G. Traxler (DFO), K. Garver (DFO), E. Anderson (Maine Bio-Tek), and R. Troyer (Colorado State)

John D. Hansen, Ph.D.

Current Position

Research Immunologist, Immunology Project Leader in the Fish Health Section at the Western Fisheries Research Center, USGS-Biological Resources Division in Seattle, WA. 6505 NE 65th Street, Seattle, WA, 98115. 206-526-6588(O) 6654 (F). jhansen@usgs.gov

Education

B.S. Zoology and Molecular Biology, University of Wisconsin at Eau Claire 1988
Ph.D. Genetics, Oregon State University (Department of Microbiology) 1995

Additional Professional Experience

2004-present: Affiliate Associate Professor with graduate faculty status in the Department of Pathobiology, University of Washington.
2001-2004 Assistant Professor, Center of Marine Biotechnology, University of MD
2001-present Adjunct Assoc. Professor, Molecular Medicine, Univ. of MD School of Medicine
1995-2001 Member of the Basel Institute for Immunology, Basel Switzerland

Major interests

Innate and adaptive immunity in teleost fish. Host-pathogen interactions. Development of cellular and molecular reagents for teleost leukocytes.

Professional Memberships

International Society of Developmental and Comparative Immunology (1993) and the American Association of Immunologists (1997).

Editorial Boards

Developmental and Comparative Immunology. Immunogenetics.

Selected Publications (in chronological order, 10 of 40 excluding reviews/chapters)

1. Hansen, J.D., and Kaattari, S.L. The recombination activating gene-1 (*RAG1*) of rainbow trout (*Oncorhynchus mykiss*): cloning, expression and phylogenetic analysis. Immunogenetics 42: 188-195, 1995.

2. Hansen, J.D. Characterization of terminal deoxynucleotidyl transferase in rainbow trout. TdT and RAG1 co-expression define the primary lymphoid tissues. Immunogenetics 46: 367-375, 1997.

3. Hansen, J.D., Strassburger, P., Thorgaard, G.H., Young, W.P., and Du Pasquier, L. Expression, linkage and polymorphism of MHC related genes in rainbow trout, *Oncorhynchus mykiss*. J Immunol 163: 774-786, 1999.

4. Hansen, J.D., and Strassburger, P. Description of an ectothermic TCR co-receptor, CD8□from rainbow trout. J Immunol164: 3132-3139, 2000.

5. Hansen, J. D., and La Patra, S. Induction of the rainbow trout MHC class I pathway during acute IHNV infection. Immunogenetics 54: 654-661, 2002.

6. Phillips, R., Zimmerman, A., Noakes, M., Palti, Y., Morasch, M., Ristow, S., Thorgaard, G.H. and Hansen, J.D. Physical and genetic mapping of the rainbow trout major histocompatibility regions: evidence for duplication of the class I region. Immunogenetics 55:561-569. 2003.

7. Ohta, Y., Boulay,T., Phillips, R., Flajnik, M.F. and Hansen, J.D. Orthologs of CD83: isolation and characterization of a putative dendritic cell marker from elasmobranch and teleost fish. J Immunol. 173:4553-60. 2004

8. Hansen JD, Landis ED, Phillips RB. Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: Implications for a distinctive B cell developmental pathway in teleost fish. Proc. Natl. Acad. Sci (PNAS). U S A. 2005 102:6919-24. 2005.

9. Landis, E.L., Palti, Y., DeKoning, J., Drew, R, Phillips, R. and Hansen, J.D. Identification and regulatory analysis of rainbow trout tapasin and tapasin-related genes. Immunogenetics-58: 56-69. 2006.

10. Laing, K., Zhou, J., Purcell, M. Secombes., C.J. and Hansen, J.D. Evolution of the CD4 family: teleost fish possess two divergent forms of CD4 in addition to LAG-3. J Immunology-in press (2006)

Recent Collaborators and Coauthors: Srassburger, P (Switzerland), LaPatra (Clear Spings), Thorgaard, G (WSU), Bailey, G (OSU), Williams, D (WSU), Kaattari, S (VIMS), Ristow, S (WSU), Winton, J (WFRC), Bartholomew, J (OSU), Walsh, P (USDA), Nagler, J (UI), Buhler, D (OSU), Overturf, K (USDA), Young, W (UI), Rexroad, C (USDA), Palti, Y (USDA), Hershburger, P (USGS), Zimmerman, A (USC), Noakes, M (WSU), Phillips, R (WSU), Cupit, P (UNM), Cunningham, C (UNM), McCarty, Boudinot, P (France), A (UCSF), Smale, S (UCSF), Landis, E (UMD), Sunyer, O (UPENN), Bashra, H (UPENN), Haliniewski, D (UMD), Lorenzen, N (Denmark), Kurath, G (USGS), Ohta, Y (UMD), Flajnik, M (UMD), Boulay, T (Switzerland), Robison, B (UI) Leong, J-A (UH), Köllner, B (Germany), Kim, C (UM), Lambris, J (UPENN), Fisher, U (Germany), DuPasquier, L (Switzerland), Rombout, J (Netherlands), Secombes, C (Scotland), Laing, K (FHCRC-Seattle), Purcell, M (USGS), Zou, J (Scotland), Drew, R (UI), DeKoning, J (WSU), Boulay, T (France), Moracsch, M (WSU), Jun, L (UPENN), Chioda, M (Norway), Spada, F (Norway), Maltapudi, A (UPENN), Tort, L (UPENN), Bernard, D (France), Benmansour, A (France), Riteau, B (France), LeFranc (France), Nichols, K (Purdue), Park, L (NMFS), Dijkstra, J (Japan), Shiina, T (Japan), Aoki, T (Japan), Wiens, G (USDA), Anderson, E (Maine-Biotek), Clouthier, S (Maine-Biotek).

RICHARD M. KOCAN, PH.D.

Education:

Hiram College Hiram, Ohio	B.A. (Biology/General Science)	1963
Michigan State University East Lansing, Michigan	M.S. (Microbiology & Pub. Health) Topic: Avian malaria	1965
Michigan State University East Lansing, Michigan	Ph.D.(Microbiology & Pub. Health) Topic: Autoimmune response to malaria	1967

Honors and Awards:

Hiram College Alumni Achievement Award	1984
Sigma Xi	
National Science Foundation Fellow	1962 -1964
Most Significant Research paper for 1999, Amer. Fisheries Soc.	2000
Most Significant Publication for 2001, Amer. Fisheries Soc.	2002

Professional Experience:

School of Aquatic & Fisheries Science; Univ. of Washington, Seattle, Wa

2001 – present	Professor Emeritus,
1981-2000	Professor
1984-1989	Adjunct Res. Prof.; Pathology

Department of Pathology, School of Medicine; University of Washington, Seattle, WA

1978-1981	Research Assistant Professor

U.S. Fish & Wildlife Service

1967-1976	Fish & Wildlife Health Lab., Madison, Wisc. '75-'76 Eastern Fish Disease Lab., Leetown, W. Va. '73-'75 Patuxent Wildlife Res. Center, Laurel, Md. '67-'73
1966-1967	Kellogg Gull Lake Laboratories Mich. State Univ. Richland, Michigan
1963-1966	Teaching/Research Assistant Veterinary Science Michigan State Univ.
1962-1963	Teaching Assistant - Parasitology Kellogg Gull Lake Labs Michigan State Univ.

Five Selected Publications Relevant to this Proposal

Kocan, R. & P. Hershberger (2006) Differences in *Ichthyophonus* prevalence and infection severity between upper Yukon River and Tanana River Chinook salmon stocks. J. Fish Diseases 29: (In press).

Kocan, R, S. LaPatra, J. Gregg, J. Winton, P. Hershberger (2006) *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids. J. Fish Diseases, 29: (In Press)

Kocan, RM, PK Hershberger, NE Elder, JW Winton (2001) Epidemiology of viral hemorrhagic septicemia (VHS) among juvenile Pacific herring and Pacific sandlance in Puget Sound, Washington J. Aquatic Anim. Health. 13: (2) 77-85

Kocan,-R.M.; Hershberger,-P.; Mehl,-T.; Elder,-N.; Bradley,-M.;Wildermuth,-D.; Stick,-K. (1999) Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring *Clupea pallasi* and its early appearance in wild Puget Sound herring. Dis-Aquat-Org.35: 23-29

Kocan, RM, M Bradley, N Elder, T Meyers, W Batts, J Winton (1997) The North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring (*Clupea pallasi*). J. Aquatic Animal Health. 9:279-290.

Five Other Selected Publications

Noelle van der Straaten, Anthony Jacobson, Daniel Halos, Paul Hershberger, A. Alan Kocan, and **Richard Kocan** (2005). Prevalence and Spatial Distribution of Intraerythrocytic Parasite(s) in Puget Sound Rockfish (*Sebastes emphaeus*) from the San Juan Archipelago, Washington (USA) Journal of Parasitology: Vol. 91 (4): 980–982.

Halos, D. S. Alexandra Hart, H. Hsu, P. Hershberger, **R. Kocan** (2005). *Ichthyophonus* in Puget Sound Rockfish, *Sebastes emphaeus*, from the San Juan Archipelago and Puget Sound, Washington, USA J. Aquat. Animal Health 17 (3): 222-227.

Kocan, R.M., P.K. Hershberger, J.M. Winton. 2004. Ichthyophoniasis: An Emerging Disease of Chinook Salmon, *Oncorhynchus tshawytscha* in the Yukon River J. Aquat. Animal Health. 16:58-72

Clewley, A., **R.M. Kocan**, A.A. Kocan. 2003. An intraerythrocytic parasite from the spiny dogfish, *Squalus acanthias* L., from the Pacific Northwest. J. Fish Dis. 25: 693-696.

Kocan, RM and JE Hose. (1997) Correspondence between laboratory and field observations of sublethal damage in marine fish larvae: Lessons from the effects of the *Exxon Valdez* oil spill on Prince William Sound Herring. pp 167-176. In: "Chemically Induced Alterations in Functional Development and Reproduction of Fishes." Rolland RM, Gilbertson M, Peterson RE, eds. SETAC Press, Pensacola FL.

Recent Collaborations

Bering Sea Fishermen's Association (Alaska), Yukon River Drainage Fisheries Association (Alaska), U.S. Fish & Wildlife Service – Office of Subsistence Management and Restoration and Enhancement (Alaska), Virgil Umphenor (Interior Alaska Processors, Fairbanks), Bill Fliris & Pat Moore (Tanana, AK fishermen), P. Hershberger (USGS-Marrowstone Island Research Station, Wash), J. Winton (USGS –BRD, Seattle, Wash), M. Reichard (Vet Medicine, Oklahoma State Univ., Stillwater, OK), S. LaPatra (Clear Springs Foods), D. Willows (Friday Harbor Marine Labs, Wash).

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Current Position

Molecular biology project leader, Fish Health Section, U.S.G.S. Western Fisheries Research Center, 1992-present; and Affiliate Associate Professor, Department of Pathobiology, University of Washington (1994-present).

Professional Interests

Virology, fish health, RNA virus evolution, molecular epidemiology, virulence and host specificity, fish vaccines

Membership in Professional Organizations

American Society for Virology, member since 1981
American Fisheries Society, Fish Health Section, member since 1993

Education:

Ph.D. Oregon State University, 1985, Microbiology;
M.S. Oregon State University, 1980, Marine Microbiology.
B.A. Miami University, Oxford, Ohio, 1978

Selected Publications: *from a total of 58 papers in peer-reviewed journals*

Kurath, G., and Winton, R. Fish rhabdoviruses (Rhabdoviridae). In, Encyclopedia of Virology, B. W. J. Mahy and M. H. V. van Regenmortel, eds. In press.

Garver, K. A., Batts, W. N., and Kurath, G. Virulence comparisons of infectious hematopoietic necrosis virus (IHNV) U and M genogroups in sockeye salmon (Oncorhynchus nerka) and rainbow trout (O. mykiss). J. Aquat. Anim. Health, in press.

Purcell, M. K., Nichols, K. M., Winton, J. R., Kurath, G., Thorgaard, G. H., Wheeler, P., Herwig, R. P., and Park, L. K. (2006) Comprehensive gene expression profiling following DNA vaccination of rainbow trout against infectious hematopoietic necrosis virus. Molecular Immunology 43:2089-2106.

Kurath, G., Garver, K.A., Corbeil, S., Elliott, D.G., Anderson, E.D., and LaPatra, S.E. (2006) Protective immunity and lack of histopathological damage two years after DNA vaccination against infectious hematopoietic necrosis virus in trout. Vaccine 24:345-354.

Kurath, G. 2005. Overview of recent DNA vaccine development for fish. *In*, Progress in Fish Vaccinology, P.J. Midtlyng ed., Developments in Biologicals Vol 121:201-213, Karger, Basel.

Enzmann, P.J., Kurath, G., Fichtner, D., and Bergmann, S.M. (2005) Infectious hematopoietic necrosis virus: Monophyletic origin of European IHNV isolates from North-American genogroup M. Dis. Aquat. Org. 66:187-195.

Purcell, M, Kurath, G., Garver, K.A., Herwig, R., and Winton, J.R. (2004) Quantitative expression profiling of immune response genes in rainbow trout during IHNV infection or following vaccination. Fish and Shellfish Immunology 17:447-462.

Emmenegger, E.J., Troyer, R.M., and Kurath, G. (2003) Characterization of the mutant spectra of a fish RNA virus within individual hosts during natural infections. Virus Res. 96:15-25.

Kurath, G., Garver, K.A., Troyer, R.M., Emmenegger, E. J., Einer-Jensen, K., and Anderson, E.D. (2003) Phylogeography of infectious hematopoietic necrosis virus in North America. J. Gen. Virol. 84:803-814.

Troyer, R. M., and Kurath, G. (2003) Molecular epidemiology of infectious hematopoietic necrosis virus reveals complex virus traffic and evolution within southern Idaho aquaculture. Dis. Aquat. Org. 55:175-185.

Garver, K. A., Troyer, R. M., and Kurath, G. (2003) Two distinct phylogenetic clades of infectious hematopoietic necrosis virus overlap within the Columbia River basin. Dis. Aquat. Org. 55:187-203.

Emmenegger, E.J., and Kurath, G. (2002) Genetic characterization of infectious hematopoietic necrosis virus of coastal salmonid stocks in Washington State. J. Aquat. Anim. Health 14:25-34.

Troyer, R.M., LaPatra, S., and Kurath, G. (2000) Genetic analyses reveal unusually high diversity of infectious hematopoietic necrosis virus in rainbow trout aquaculture. J. Gen. Virol. 81:2823-2832.

Anderson, E.D., Engelking, H.M., Emmenegger, E.J., and Kurath, G. (2000) Molecular epidemiology reveals emergence of a virulent IHN virus strain in wild salmon and transmission to hatchery fish. J. Aquat. Anim. Health 12:85-99. (invited feature article).

Emmenegger, E.J., Meyer, T.R., Burton, T.O., and Kurath, G. (2000) Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Dis. Aquat. Org. 40:163-176.

Recent Collaborators and Co-Authors:

W. Ahne (U. Munich), E.D. Anderson (Maine Biotek), W.N. Batts (USGS), H. V. Bjorklund (Orion Pharma), S. Corbeil (CSIRO), E.J. Emmenegger (USGS), K. A. Garver (DFO), LaPatra, Scott E. (Clear Springs Foods.,Inc., M. Purcell (U.Washington), S. St. Hilaire (Idaho Sate U), G. Traxler (DFO), R. M.Troyer (Colorado State U.), J.R. Winton (USGS)

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Current Position

Director of Research and Development
Associate Professor (Affiliate Faculty), University of Idaho, Department of Fisheries and Wildlife Resources
Associate Professor (Affiliate Faculty), Idaho State University, Department of Biology

Professional Interests

Infectious diseases of fish, integrated fish health management, fish cell and tissue culture, vaccinology, seroepidemiology and epidemiology of fish pathogens.

Membership in Professional Organizations

American Society for Microbiology, American Society for Virology, American Fisheries Society, American Institute of Fishery Research Biologists, European Association of Fish Pathologists, Japanese Society of Fish Pathologists,
International Society of Developmental and Comparative Immunology, Nordic Immunology Society.

Education

1979, B.S., Oregon State University, Corvallis, OR .
1989, Ph.D., Oregon State University, Corvallis, OR.

Recent Honors and Professional Recognition

2000 Re-certified as Fish Pathologist, Fish Health Section, American Fisheries Society.
2000 American Fisheries Society Fish Health Section Special Achievement Award.
2002 Editorial Board, Journal of Fish Diseases .
2001 Special Award from Seattle Mayor's Office for work on Cedar River Hatchery Design Committee.
2002 Tibbett's Award, Small Business Innovation Research, Small Business Administration.
2003 Re-certified American Fisheries Society, Fisheries Scientist and Fish Health Inspector.
2004 Re-certified as United States Titel 50 Inspector and Canadian Fish Health Official.
2006 Palouse Unit Award for Excellence in Student Mentoring, American Fisheries Society

Five Selected Publications Relevant to this Proposal

LaPatra, S.E., T. Turner, K.A. Lauda, G.R. Jones, and S. Walker. 1993. Characterization of the humoral response of rainbow trout to infectious hematopoietic necrosis virus. Journal of Aquatic Animal Health 5:165-171.

LaPatra, S.E. 1996. The use of serological techniques for virus surveillance and certification of finfish. Annual Review of Fish Diseases 6:15-28.

Traxler, G.S., J.R. Roome, K.A. Lauda, and S.E. LaPatra. 1997. The appearance of infectious hematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockeye salmon (Oncorhynchus nerka) during their migration and maturation period. Diseases of Aquatic Organisms 28:31-38.

LaPatra, S.E. 1998. Factors affecting pathogenicity of infectious hematopoietic necrosis virus for salmonid fish. Journal of Aquatic Animal Health 10:121-131.

LaPatra, S.E., S. Ireland, J.M. Groff, K. Clemens, and J. Siple. 1999. Adaptive disease management strategies for the endangered population of Kootenai River white sturgeon *Acipenser transmontanus*. Fisheries 24(5):6-14.

Five Other Selected Publications

Lorenzen, N., and S.E. LaPatra. 1999. Humoral immune response to rhabdovirus antigens in salmonid fish. Fish and Shellfish Immunology 9:345-360.

LaPatra, S.E., W.N. Batts, K. Overturf, G.R. Jones, W.D. Shewmaker, and J.R. Winton. 2001. Negligible risk associated with the movement of processed rainbow trout from an infectious hematopoietic necrosis virus (IHNV) endemic area. Journal of Fish Diseases 24: 399-408.

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LaFrentz, B.R., S.E. LaPatra, G.R.Jones, J.L. Congelton, B. Sun, and K.D. Cain. 2002. Characterization of serum and mucosal antibody responses of rainbow trout (*Oncorhynchus mykiss*) to *Flavobacterium psychrophilum*. Journal of Fish Diseases 25:703-713.

Overturf, K., M. Casten, S. LaPatra, C. Rexroad, and R.W. Hardy. 2003. Comparison of growth performance, immunological response, and genetic diversity of five different strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 217:93-106.

Recent Collaborators and Co-Authors

E. Anderson (Maine BioTech), W. Batts (USGS), M. Benko (Hungry), V. Blazer (USGS), K. Cain (UI), D. Call (WSU), S. Clouthier (Maine BioTek), J. Congleton (UI), S. Corbeil (CSIRO), J. Drennan (Schering-Plough), K. Einer-Jensen (Denmark), D. Elliott (USGS), E. Emmenegger (USGS), H. Ferguson (Scotland), K. Garver (DFO), A. Goodwin (UAPB), J. Hansen (USGS), L. Hanson (MSU), R. Hardy (UI), R. Hedrick (UCD), P. Hershberger (USGS), M. House (NWIFC), J. Jones (ODFW), S. Kaattari (VIMS), M. Kent (OSU), C. Kim (UM), R. Kocan (UW), T. Kono (Japan), G. Kurath (USGS), B. LaFrentz (UI), M. Le Haneff (France), J-A. Leong (UH), Y. Lu (UH), N. Lorenzen (Denmark), B. May (UCD), P. Millard (UM), H. Ogut (Turkey), K. Overturf (USDA), M. Purcell (USGS), C. Rexroad (USDA), P. Reno (OSU), J. Richard (DFO), S. Ristow (WSU), J. Rolland (USDA), M. Sakai (Japan), W. Shewmaker (Clear Springs), L. Skinner (UBC),C. Smith (USFWS), O. Sunyer (UP), G. Thorgaard (WSU), G. Traxler (DFO), A. Weighall (Clear Springs), T. Welch (USDA), P. Wheeler (WSU), G. Wiens (USDA), M. Yoshimizu (Japan).

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Current Position

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

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.

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 Honors and Professional Recognition

2000 Re-certified as Fish Pathologist, Fish Health Section, American Fisheries Society.
2000 American Fisheries Society Fish Health Section S. F. Snieszko Distinguished Service Award.
2000 Editorial Board, Journal of Fish Diseases (continuing term).
2001 Special Award from Seattle Mayor's Office for work on Cedar River Hatchery Design Committee.
2002 Award for most significant paper of 2001 in Journal of Aquatic Animal Health.
2002 Certificate of Appreciation from USDA for technical assistance on Infectious Salmon Anemia.
2004 Designated Expert and Office of International Epizooties Reference Laboratory for BKD & IHNV.
2006 U.S. Department of Interior Distinguished Service Award.

Five Selected Publications Relevant to this Proposal

Kocan, R., M. Bradley, N. Elder, T. Meyers, W. Batts, and J. Winton. 1997. The North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring (*Clupea pallasi*). Journal of Aquatic Animal Health 9:279-290.

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

Hershberger, P.K., K. Stick, B. Bui, C. Carroll, B. Fall, C. Mork, J.A. Perry, E. Sweeney, J. Whittouck, J. Winton and R.K. Kocan. 2002. Incidence of *Ichthyophonus hoferi* in Puget Sound fishes and its increase with age of Pacific herring. Journal of Aquatic Animal Health 14:50-56.

Hedrick, R.P., W.N. Batts, S. Yun, G.S. Traxler, J. Kaufman and J.R. Winton. 2003. Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. Diseases of Aquatic Organisms 55:211-220.

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

Five Other Selected Publications

Winton, J.R. and K. Einer-Jensen. 2002. Chapter 3 - Molecular diagnosis of infectious hematopoietic necrosis and viral hemorrhagic septicemia. Pp 49-79. *In:* Cunningham, C. (ed.) Molecular Diagnosis of Salmonid Diseases. Kluwer, Dordrecht.

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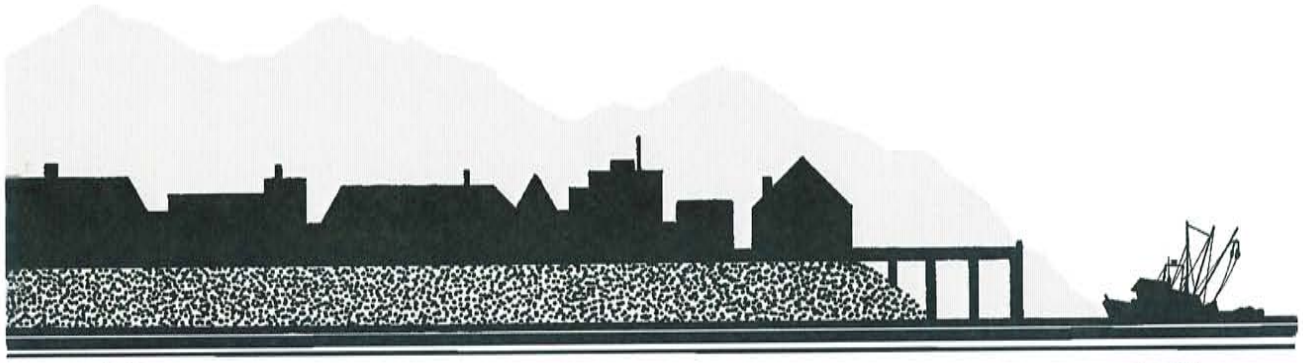
Einer-Jensen, K., J. Winton and N. Lorenzen. 2005. Genotyping of the fish rhabdovirus, viral haemorrhagic septicaemia virus, by restriction fragment length polymorphisms. Veterinary Microbiology 106: 167-178.

Purcell, M.K., K.D. Smith, A. Aderem, 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.

Purcell, M.K., K.M. Nichols, J.R. Winton, G. Kurath, G.H. Thorgaard, P. Wheeler, J.D. Hansen, R.P. Herwig and L.K. Park. 2006. Comprehensive gene expression profiling following DNA vaccination of rainbow trout against infectious hematopoietic necrosis virus. Molecular Immunology 43: 2089-2106.

Recent Collaborators and Co-Authors

W. Ahne (U. Munich), E. Anderson (Maine BioTech), G. Bailey (OSU), I. Bandin (Spain), J. Bartholomew (OSU), W. Batts (USGS), D. Beauchamp (UW), K. Beauchamp (USGS), H. Bjorklund (Finland), V. Blazer (USGS), D. Bouchard (Micro Technol.), R. Brunson (USFWS), D. Buhler (OSU), B. Bui (UW), D. Campton (USFWS), C. Carroll (UW), M-S. Chien (Taiwan), S. Chilmonczyk (France), J. Coll (USFWS), A. Colwell (NPS), J. Congleton (UI), S. Corbeil (CSIRO), R. Devlin (DFO), C. Dopazo (Spain), K. Einer-Jensen (Denmark), N. Elder (USGS), D. Elliott (USGS), E. Emmenegger (USGS), S. Essbauer (U. Munich), J. Evered (USFWS), M. Faisal (MSU), B. Fall (UW), N. Fijan (Croatia), T. Flagg (NMFS), J. Franke (France), 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), P. Hershberger (USGS), R. Herwig (UW), B. Hill (CEFAS), R. Hilborn (UW), L. Hood (ISB), M. House (NWIFC), C. Huang (Taiwan), A. Jacobson (UW), S. Kaattari (VIMS), J. Kaufman (ODFW), M. Kent (OSU), B. Kerrans (MSU), C. Kim (UM), A. Kocan (OSU), R. Kocan (UW), G. Kurath (USGS), Scott LaPatra (Clear Springs Foods), J-A. Leong (UH), J. Levin (NCS), C-M. Liao (Taiwan), N. Lorenzen (Denmark), E. MacConnell (USFWS), C. Mahnken (NMFS), S. McDiarmid (New Zealand), T. Meyers (ADFG), K. Miller (DFO), T-J. Ming (DFO), C. Mork (Boston University), S. Mumford (USFWS), J. Nagler (UI), K. Naish (UW), K. Nichols (NMFS), 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), R. Pascho (Seattle), C. Patterson (USFWS), J. Perry (CSU), S. Pipoppinyo (Thailand), 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), I. Romero-Brey (Spain), M. Rust (NMFS), G. Sanders (UW/USGS), A. Schulze (DFO), 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).



PRINCE WILLIAM SOUND
FISHERIES RESEARCH APPLICATIONS AND PLANNING

July 28,2006
Box 1848
Cordova, Ak. 99574

Dear Dr. Hershberger,

I am writing this letter in support of your multi-year "Herring Disease Project" for Prince William Sound herring restoration that you are submitting to the EVOSTC for funding. Thank you for sharing your draft. PWSFRAP group looks forward to collaborating with you in any aspect of your work where we can provide assistance.

PWSFRAP is anticipating organizing and managing the intervention/egg translocation and chemical marking efforts utilizing local commercial fishermen and other equipment designed for that special purpose. If we are able to successfully obtain TC funding for our intervention plans then we will be in a position to help provide necessary animal samples at various stages of growth. We anticipate raising some herring from hatch through metamorphosis as one part of our intervention plan. We would like to create a lab on board our working vessels for the purpose of preparing samples in forms that will be needed by project investigators.

We trust that the Council will see the wisdom of structuring the herring recovery/restoration program as a multi-disciplinary, multi-year plan, in which the PI's themselves are encouraged to develop a consensus on objectives and milestones, through a workshop process. I appreciate your comments in this regard.

Sincerely,

Ross Mullins Ken Adams Vince Patrick

Data Management and 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.

Budget Justification – FY 2007 – FY 2010 = $1,035.0

Personnel Costs = $444.0K

FY07 ($103.2K)
A new GS-7 Biological Fisheries technician ($46.8K) will be hired at the USGS-Marrowstone Marine Field Station to perform standard cell culture duties associate with the empirical studies that comprise the HDP. The GS-7 technician will also be involved in experimental set up, laboratory sampling, and assistance with the field surveys. This position will be under the direct supervision of Dr. Hershberger (P.I.). A new GS-9 molecular / histopathologist ($56.4K) will be hired at the WFRC to assist with identification of immune genes that are regulated after VHSV exposure. This biologist will also assist with the histopathological screening of tissues from the empirical studies that will be conducted at the Marrowstone Marine Station. This position will be under the direct supervision of Drs. Hansen and Elliott (PI's).

FY08 ($109.2K)
Continuation of GS-7 Fisheries Technician ($49.2K) and GS-9 Molecular Biologist / Histopathologist ($60.0K) positions is requested for processing tissues from empirical laboratory studies and profiling VHSV immune response genes.

FY09 ($112.8K)
Continuation of GS-7 Fisheries Technician ($50.4K) and GS-9 Molecular Biologist / Histopathologist ($62.4K) positions is requested for processing tissues from empirical laboratory studies and application of VHSV immune response genes.

FY10 ($118.8K)
Continuation of GS-7 Fisheries Technician ($52.8K) and GS-9 Molecular Biologist / Histopathologist ($66.0K) positions is requested for processing tissues from empirical laboratory studies and application of VHSV immune response genes.

Travel Costs = $21.0K

FY07 ($5.4K)
Round trip travel costs from Seattle, WA are requested to assist with field sampling and exchange scientific information with the local community in Cordova ($2.0K), attend the annual Marine Science Symposium ($2.0K), and project-related Western Fish Disease Workshop in June ($1.4K).

FY08 ($4.0K)
Round trip travel costs from Seattle, WA are requested to assist with field sampling and exchange scientific information with the local community in Cordova ($2.0K), attend the annual Marine Science Symposium ($2.0K).

FY09 ($5.8K)
Round trip travel costs from Seattle, WA are requested to assist with field sampling and exchange scientific information with the local community in Cordova ($2.0K), attend the annual Marine Science Symposium ($2.0K), and assist with the Sitka field sampling ($1.8K).

FY10 ($5.8K)
Round trip travel costs from Seattle, WA are requested to assist with field sampling and exchange scientific information with the local community in Cordova ($2.0K), attend the annual Marine Science Symposium ($2.0K), and assist with the Sitka field sampling ($1.8K).

Contractual Costs = $236.6K

FY07 ($54.0K)
A subcontract ($12.0K), administered through WFRC, will be set up to include two months salary for Dr. Richard Kocan. A subcontract ($7.4K), administered through the WFRC, will be set up for the ADF&G, Juneau Fish Pathology Laboratory to cover sampling costs incurred during the laboratory validation of PWS herring infection and disease prevalence. Student contractors will be hired at the WFRC ($19.5K) and Marrowstone Marine Field Station ($15.1K) to assist with development of a molecular diagnosis tool for ENV and rearing of SPF herring (Supervised by Emmenegger and Hershberger, PI's).

FY08 ($59.4K)
A subcontract ($12.0K), administered through WFRC, will be set up to include two months salary for Dr. Richard Kocan. A subcontract ($11.4K), administered through the WFRC, will be set up for the ADF&G, Juneau Fish Pathology Laboratory to cover sampling costs incurred during the laboratory validation of PWS herring infection and disease prevalence. Student contractors will be hired at the WFRC ($20.3K) and Marrowstone Marine Field Station ($15.7K) to assist with validation of a molecular diagnosis tool for ENV and rearing of SPF herring (Supervised by Emmenegger and Hershberger, PI's).

FY09 ($60.8K)
A subcontract ($12.0K), administered through WFRC, will be set up to include two months salary for Dr. Richard Kocan. A subcontract ($11.4K), administered through the WFRC, will be set up for the ADF&G, Juneau Fish Pathology Laboratory to cover sampling costs incurred during the laboratory validation of PWS herring infection and disease prevalence. Student contractors will be hired at the WFRC ($21.1KK) and Marrowstone Marine Field Station ($16.3KK) to assist with development of a molecular diagnosis tool for ENV and rearing of SPF herring (Supervised by Emmenegger and Hershberger, PI's).

FY10 ($62.4K)
A subcontract ($12.0K), administered through WFRC, will be set up to include two months salary for Dr. Richard Kocan. A subcontract ($11.4K), administered through the WFRC, will be set up for the ADF&G, Juneau Fish Pathology Laboratory to cover sampling costs incurred during the laboratory validation of PWS herring infection and disease prevalence. Student contractors will be hired at the WFRC ($22.0K) and Marrowstone Marine Field Station ($17.0K) to assist with development of a molecular diagnosis tool for ENV and rearing of SPF herring (Supervised by Emmenegger and Hershberger, PI's).

Annual Commodities = $251.4K

FY 07, FY 08 & FY 10 = $64.2K; FY 09 = $58.8K
Annual commodities include laboratory supplies for the Marrowstone Marine Station ($15.0K for fish food, $10.0K for dry lab supplies), WFRC ($8.8K for molecular supplies for ENV tool component), and Clear Springs Foods ($15.0K for VHSV neutralizing antibody component), and standard tank / bench fees at the Marrowstone Marine Station ($10.0K) cover costs associated with seawater, plumbing, and tank maintenance; and WFRC histology supplies for empirical studies ($5.4K).

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 reor requested for this project.

Matching funds: $272.3K - $294.3K/yr

Partial annual salaries and benefits for PI's and technicians are offered as in kind matching funds. Rearing and feeding costs associated with existing SPF herring ($133.0K) are included as an additional match.

Data Management and QA/QC Statement:
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. All laboratories involved in this HDP conform to Title 21 Code of Federal Regulations: Good Laboratory Practice for Nonclinical Laboratory Studies.

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:	Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	TOTAL	USGS $993.4	ADF&G $41.6	TOTAL $1,035.0
Personnel	$103.2	$109.2	$112.8	$118.8	$444.0			
Travel	$5.4	$4.0	$5.8	$5.8	$21.0			
Contractual	$53.4	$58.5	$59.9	$61.5	$233.3			
Commodities	$64.2	$64.2	$58.8	$64.2	$251.4			
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0			
Subtotal	$226.2	$235.9	$237.3	$250.3	$949.7			
General Administration	$20.3	$21.2	$21.3	$22.5	$85.3			
Project Total	$246.5	$257.1	$258.6	$272.8	$1,035.0			
Full-time Equivalents (FTE)		2.0	2.0	2.0	2.0			
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$272.3	$279.3	$286.6	$294.3			

Comments:

USGS: FY 07 $239.1
FY 08 $245.7
FY 09 $247.2
FY 10 $261.4

ADFG: FY 07 $7.4
FY 08 $11.4
FY 09 $11.4
FY 10 $11.4

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Lead Agency: USGS

FORM 2A
MULTI-TRUSTEE
AGENCY
SUMMARY

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:	Authorized FY 2006	Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	Total Project
Personnel		$103.2	$109.2	$112.8	$118.8	$444.0
Travel		$5.4	$4.0	$5.8	$5.8	$21.0
Contractual		$46.6	$48.0	$49.4	$51.0	$195.0
Commodities		$64.2	$64.2	$58.8	$64.2	$251.4
Equipment		$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$0.0	$219.4	$225.4	$226.8	$239.8	$911.4
General Administration		$19.7	$20.3	$20.4	$21.6	$82.0
Project Total	$0.0	$239.1	$245.7	$247.2	$261.4	$993.4
Full-time Equivalents (FTE)		2.0	2.0	2.0	2.0	
	Dollar amounts are shown in thousands of dollars.					
Other Resources		$272.3	$279.3	$286.6	$294.3	

Comments:
Matching Funds: FY 07, 08, 09, 10
Hershberger salary + benefits (PI) : 20% = $17,481, $18,355, $19,273, $20,236
Gregg salary + benefits (Fisheries Biologist, Marrowstone): 20% = $11,730, $12,317, $12,932, $13,579
Elliott salary + benefits (PI): 20% = $23,431, $24,603, $25,833, $27,124
Conway salary + benefits (Histology Technician, WFRC): 20% = $13,205, $13,865, $14,559, $15,286
Emmenegger salary + benefits (PI) : 20% = $18,384, $19,303, $20,269, $21,282
Hanson salary + benefits (PI): 20% = $20,537, $21,564, $22,642, $23,774
LaPatra salary + benefits (PI): 10% = $12,015, $12,616, $13,247, $13,909
Clear Springs technical Support: 20% = $7,862, $8,255, $8,668, $9,101
Winton salary + benefits (PI): 10% = $14,695, $15,430, $16,201, $17,011
Annual contribution of existing SPF herring [labor ($29,325/yr) and feed ($15,000/yr) for 3 year classes] = $132,975

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2007
Name	Position Description					
New Hire (Marrowstone)	Biological Fisheries Technician	GS-7, Step 1	12.0	3.9		46.8
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 1	12.0	4.7		56.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	8.6	0.0	
					Personnel Total	$103.2

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2007
Description					
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2007)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2007) and exchange scientific information	0.6	1	7	0.2	2.0
Seattle - Jackson, WY: Western Fish Disease Wrkshp (June 2007)	0.3	2	4	0.2	1.4
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5.4

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2007
Description	
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool development $15.25 / hr x 1280 hrs	19.5
Marrowstone Student Contractor (fish care): $14.91 / hr x 1040 hrs	15.1
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$46.6

Commodities Costs:	Proposed FY 2007
Description	
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
WFRC histology supplies for empirical studies:	5.4
ENV challenge: 10 fish / group /week x 9 weeks = 270 fish (1 parafin block + 1 H&E @$11ea) = 2970	
Ichthyophonus wild fish stamina studies: 60 fish @$11 ea (H&E) + $10.50 (recut + PAS)+ $9.50 ea (recut + Giemsa) = $1860	
General histology supplies for 240 fish (fixative, jars, cassettes) =$567	
Clear Springs supplies for development of VHSVneutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
Commodities Total	$64.2

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2007
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2008
Name	Position Description					
New Hire (Marrowstone)	Biological Fisheries Tech	GS-7, Step 2	12.0	4.1		49.2
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 2	12.0	5.0		60.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	9.1	0.0	
					Personnel Total	$109.2

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2008
Description					
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2007)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2007) and exchange scientific information	0.6	1	7	0.2	2.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$4.0

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2008
Description	
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool verification $15.86 / hr x 1280 hrs	20.3
Marrowstone Student Contractor (fish care): $15.10 / hr x 1040 hrs	15.7
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$48.0

Commodities Costs:	Proposed FY 2008
Description	
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
WFRC histology supplies for empirical studies:	5.4
Clear Springs supplies for verification of VHSV neutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$64.2

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2008
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2009
New Hire (Marrowstone)	Biological Fisheries Tech	GS-7, Step 3	12.0	4.2		50.4
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 3	12.0	5.2		62.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	9.4	0.0	
					Personnel Total	$112.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2009
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2008)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2008) and exchange scientific information	0.6	1	7	0.2	2.0
Seattle - Sitka: assist with field sampling 2008	0.4	1	7	0.2	1.8
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5.8

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2009
Description	
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool application $16.49 / hr x 1280 hrs	21.1
Marrowstone Student Contractor (fish care): $15.70 / hr x 1040 hrs	16.3
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$49.4

Commodities Costs:	Proposed FY 2009
Description	
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
Clear Springs supplies for application of VHSV neutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$58.8

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:	Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	TOTAL	USGS $993.4	ADF&G $41.6	TOTAL $1,035.0
Personnel	$103.2	$109.2	$112.8	$118.8	$444.0			
Travel	$5.4	$4.0	$5.8	$5.8	$21.0			
Contractual	$53.4	$58.5	$59.9	$61.5	$233.3			
Commodities	$64.2	$64.2	$58.8	$64.2	$251.4			
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0			
Subtotal	$226.2	$235.9	$237.3	$250.3	$949.7			
General Administration	$20.3	$21.2	$21.3	$22.5	$85.3			
Project Total	$246.5	$257.1	$258.6	$272.8	$1,035.0			
Full-time Equivalents (FTE)		2.0	2.0	2.0	2.0			
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$272.3	$279.3	$286.6	$294.3			

Comments:

USGS: FY 07 $239.1
FY 08 $245.7
FY 09 $247.2
FY 10 $261.4

ADFG: FY 07 $7.4
FY 08 $11.4
FY 09 $11.4
FY 10 $11.4

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Lead Agency: USGS

FORM 2A
MULTI-TRUSTEE
AGENCY
SUMMARY

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:	Authorized FY 2006	Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	Total Project
Personnel		$103.2	$109.2	$112.8	$118.8	$444.0
Travel		$5.4	$4.0	$5.8	$5.8	$21.0
Contractual		$46.6	$48.0	$49.4	$51.0	$195.0
Commodities		$64.2	$64.2	$58.8	$64.2	$251.4
Equipment		$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$0.0	$219.4	$225.4	$226.8	$239.8	$911.4
General Administration		$19.7	$20.3	$20.4	$21.6	$82.0
Project Total	$0.0	$239.1	$245.7	$247.2	$261.4	$993.4
Full-time Equivalents (FTE)		2.0	2.0	2.0	2.0	
	Dollar amounts are shown in thousands of dollars.					
Other Resources		$272.3	$279.3	$286.6	$294.3	

Comments:
Matching Funds: FY 07, 08, 09, 10
Hershberger salary + benefits (PI) : 20% = $17,481, $18,355, $19,273, $20,236
Gregg salary + benefits (Fisheries Biologist, Marrowstone): 20% = $11,730, $12,317, $12,932, $13,579
Elliott salary + benefits (PI): 20% = $23,431, $24,603, $25,833, $27,124
Conway salary + benefits (Histology Technician, WFRC): 20% = $13,205, $13,865, $14,559, $15,286
Emmenegger salary + benefits (PI) : 20% = $18,384, $19,303, $20,269, $21,282
Hanson salary + benefits (PI): 20% = $20,537, $21,564, $22,642, $23,774
LaPatra salary + benefits (PI): 10% = $12,015, $12,616, $13,247, $13,909
Clear Springs technical Support: 20% = $7,862, $8,255, $8,668, $9,101
Winton salary + benefits (PI): 10% = $14,695, $15,430, $16,201, $17,011
Annual contribution of existing SPF herring [labor ($29,325/yr) and feed ($15,000/yr) for 3 year classes] = $132,975

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2007
Name	Position Description					
New Hire (Marrowstone)	Biological Fisheries Technician	GS-7, Step 1	12.0	3.9		46.8
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 1	12.0	4.7		56.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	8.6	0.0	
					Personnel Total	$103.2

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2007
Description					
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2007)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2007) and exchange scientific information	0.6	1	7	0.2	2.0
Seattle - Jackson, WY: Western Fish Disease Wrkshp (June 2007)	0.3	2	4	0.2	1.4
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5.4

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2007
Description	
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool development $15.25 / hr x 1280 hrs	19.5
Marrowstone Student Contractor (fish care): $14.91 / hr x 1040 hrs	15.1
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$46.6

Commodities Costs:	Proposed FY 2007
Description	
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
WFRC histology supplies for empirical studies:	5.4
ENV challenge: 10 fish / group /week x 9 weeks = 270 fish (1 parafin block + 1 H&E @$11ea) = 2970	
Ichthyophonus wild fish stamina studies: 60 fish @$11 ea (H&E) + $10.50 (recut + PAS)+ $9.50 ea (recut + Giemsa) = $1860	
General histology supplies for 240 fish (fixative, jars, cassettes) =$567	
Clear Springs supplies for development of VHSVneutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
Commodities Total	$64.2

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2007
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2008
Name	Position Description					
New Hire (Marrowstone)	Biological Fisheries Tech	GS-7, Step 2	12.0	4.1		49.2
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 2	12.0	5.0		60.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	9.1	0.0	
					Personnel Total	$109.2

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2008
Description					
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2007)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2007) and exchange scientific information	0.6	1	7	0.2	2.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$4.0

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2009
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed
Description	FY 2010
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool application $17.15 / hr x 1280 hrs	22.0
Marrowstone Student Contractor (fish care): $16.33 / hr x 1040 hrs	17.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$51.0

Commodities Costs:	Proposed
Description	FY 2010
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
WFRC histology supplies for empirical studies:	5.4
Clear Springs supplies for develoment of VHSVneutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$64.2

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:		Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	
Personnel		$0.0	$0.0	$0.0	$0.0	
Travel		$0.0	$0.0	$0.0	$0.0	
Contractual		$6.8	$10.5	$10.5	$10.5	
Commodities		$0.0	$0.0	$0.0	$0.0	
Equipment		$0.0	$0.0	$0.0	$0.0	
Subtotal		$6.8	$10.5	$10.5	$10.5	Total Project
General Administration		$0.6	$0.9	$0.9	$0.9	
Project Total		$7.4	$11.4	$11.4	$11.4	$41.6
Full-time Equivalents (FTE)		0.0	0.0	0.0	0.0	
	Dollar amounts are shown in thousands of dollars.					
Other Resources						

Comments:

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2009
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2010
New Hire (Marrowstone)	Biological Fisheries Tech	GS-7, Step 4	12.0	4.4		52.8
New Hire (WFRC)	Molecular Biologist / Histopathologist	GS-9, Step 4	12.0	5.5		66.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	9.9	0.0	
					Personnel Total	$118.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2010
Seattle-Anchorage: Annual Marine Science Symposiun (Jan 2009)	0.5	2	5	0.2	2.0
Seattle - Cordova: Assist with field sampling (May 2009) and exchange scientific information	0.6	1	7	0.2	2.0
Seattle - Sitka: assist with field sampling 2009	0.4	1	7	0.2	1.8
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5.8

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed
Description	FY 2010
Subcontract: RM Kocan: Assistance with laboratory challenge studies at the Marrowstone Marine Station	12.0
WFRC Student Contractor: assistance with ENV molecular tool application $17.15 / hr x 1280 hrs	22.0
Marrowstone Student Contractor (fish care): $16.33 / hr x 1040 hrs	17.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$51.0

Commodities Costs:	Proposed
Description	FY 2010
Fish Food, enrichments, and live feed for production of SPF herring	15.0
ENV molecular tool development: Invitrogen TPO cloning kit, Ambion Ribo-Pure Blot kit, Qiagen DNAeasy, Promega Enzymes for amplification, IDT nucleotide primers, ABI Big Dye Sequencing Reagent, and other standard molecular biological supplies	8.8
Marrowstone Marine Station Laboratory supplies (cell culture, molecular biology, parasitology, virology, etc.)	10.0
WFRC histology supplies for empirical studies:	5.4
Clear Springs supplies for develoment of VHSVneutralizing antibody test (complement from SPF rainbow trout, plasticware, pipettes, fish food, etc.	15.0
Marrowstone tank fees ($5 / day x 100 days x 20 tanks)	10.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$64.2

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2010
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Equipment
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Budget Category:		Proposed FY 2007	Proposed FY 2008	Proposed FY 2009	Proposed FY 2010	
Personnel		$0.0	$0.0	$0.0	$0.0	
Travel		$0.0	$0.0	$0.0	$0.0	
Contractual		$6.8	$10.5	$10.5	$10.5	
Commodities		$0.0	$0.0	$0.0	$0.0	
Equipment		$0.0	$0.0	$0.0	$0.0	
Subtotal		$6.8	$10.5	$10.5	$10.5	Total Project
General Administration		$0.6	$0.9	$0.9	$0.9	
Project Total		$7.4	$11.4	$11.4	$11.4	$41.6
Full-time Equivalents (FTE)		0.0	0.0	0.0	0.0	
	Dollar amounts are shown in thousands of dollars.					
Other Resources						

Comments:

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2007
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2007
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2007
Description	
Subcontract: ADF&G Juneau Fish Pathology Lab (Dr. Ted Meyers): laboratory confirmation of infection and disease prevalence in wild PWS herring ($31.35 / sample (includes VEN blood films, Ichthyophonus cultures, and VHSV plaque assays) x 120 samples + $2000 (histopathology) + $1,000 (travel)	6.8
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$6.8

Commodities Costs:	Proposed FY 2007
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$0.0

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2007
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY07

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Equipment
DETAIL

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2008
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2008
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: DOI - USGS - WFRC - Marrowstone Marine Field Station

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2008
Description	
Subcontract: ADF&G Juneau Fish Pathology Lab (Dr. Ted Meyers): laboratory confirmation of infection and disease prevalence in wild PWS herring ($31.35 / sample (includes VEN blood films, Ichthyophonus cultures, and VHSV plaque assays) x 240 samples + $2000 (histopathology) + $1,000 (travel) (includes PWS and Sitka)	10.5
	0.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$10.5

Commodities Costs:	Proposed FY 2008
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$0.0

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2008
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY08

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Equipment
DETAIL

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2009
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2009
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2009
Description	
Subcontract: ADF&G Juneau Fish Pathology Lab (Dr. Ted Meyers): laboratory confirmation of infection and disease prevalence in wild PWS herring ($31.35 / sample (includes VEN blood films, Ichthyophonus cultures, and VHSV plaque assays) x 240 samples + $2000 (histopathology) + $1,000 (travel) (includes PWS and Sitka)	10.5
	0.0
	0.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$10.5

Commodities Costs:	Proposed FY 2009
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$0.0

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2009
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY09

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2010
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2010
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Contractual Costs:	Proposed FY 2010
Description	
Subcontract: ADF&G Juneau Fish Pathology Lab (Dr. Ted Meyers): laboratory confirmation of infection and disease prevalence in wild PWS herring ($31.35 / sample (includes VEN blood films, Ichthyophonus cultures, and VHSV plaque assays) x 240 samples + $2000 (histopathology) + $1,000 (travel) (includes PWS and Sitka)	10.5
	0.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$10.5

Commodities Costs:	Proposed FY 2010
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$0.0

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

Prepared:

2007 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2006 - September 30, 2007

Those purchases associated with replacement equipment should be indicated by placement of an R.

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2010
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY10

Project Number: 070819
Project Title: PWS Herring Disease Program
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared: