FY 22-31 *PROJECT* PROPOSAL
LONG-TERM RESEARCH AND MONITORING PROGRAM

Does this proposal contain confidential information? ☒No ☐Yes

Project Number and Title

Gulf Watch Alaska Long-Term Research and Monitoring Program: Herring Research & Monitoring Component

22120111-E Herring Disease Program

Primary Investigator(s) and Affiliation(s)

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Project Abstract (maximum 300 words)

The Herring Disease Program (HDP) involves a combination of field observations, controlled laboratory experiments, novel tool development, and mathematical models to better understand, forecast, and mitigate disease impacts to Prince William Sound (PWS) herring populations. Field surveillances will involve annual assessments of the primary herring pathogens occurring in PWS, including viral hemorrhagic septicemia virus (VHSV), *Ichthyophonus*, and erythrocytic necrosis virus (ENV). Additional field studies will investigate how other Gulf of Alaska and PWS fishes impact the ecology of these pathogens for Pacific herring. *In vivo* laboratory experiments will be based on the successful production of specific-pathogen-free (SPF) Pacific herring and will be directed towards understanding basic epizootiological principles of these diseases. A large laboratory focus will involve evaluating possible *Ichthyophonus* transmission routes to Pacific herring, including the possible involvement of egg consumption on transmission. Novel disease forecasting tools will be developed and further optimized, including the plaque neutralization test to detect VHSV neutralizing antibodies and the possible application of RTqPCR on gill tissues to assess VHSV exposure history in Pacific herring. Finally, disease models will be developed to evaluate the relative importance of disease cofactors and evaluate roles of VHSV antibodies and herd immunity in disease potential. The HDP is either fully integrated, or sharing sampling platforms, with other proposed *Exxon Valdez* Oil Spill Trustee Council projects including Genetic and physiological mechanisms of virus and oil interactions in Pacific herring (Whitehead), Herring / Pink Salmon interactions (Rand et al.), Modeling and stock assessment of Prince William Sound herring (Branch), PWS Herring Assessment (Morella), and Pacific Herring Connectivity Between PWS and Kayak Island (Cypher).

EVOSTC Funding Requested* (must include 9% GA)

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$344,250	$374,095	$314,696	$322,954	$394,661	$1,750,655
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
$406,945	$419,543	$432,420	$376,436	$387,092	$2,022,436
				FY22-31 Total	*$3,773,091*

**If the amount requested here does not match the amount on the budget form, the request on the budget form will considered to be correct.*

Non-EVOSTC Funds to be used, please include source and amount per source:

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$124,245	$127,724	$131,396	$135,129	$138,910	$657,404
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
$142,857	$146,917	$151,092	$155,387	$159,804	$756,057
				FY22-31 Total	*$1,413,461*

Matching U.S. Geological Survey funds include partial salary + benefits coverage for Paul Hershberger (20%), Maureen Purcell (10%), and Jacob Gregg (50%).

1. EXECUTIVE SUMMARY (maximum ~1500 words, not including figures and tables)

This proposal addresses how top-down forces, particularly infectious and parasitic diseases, and their environmental drivers, affect the failed recovery of Pacific herring, an injured resource in Prince William Sound (PWS). The biomass of adult herring PWS collapsed from 111,000-121,000 mt in 1988-1989 to 30,000 mt in the early 1990s; since then, the population has remained depressed. Consequently, the PWS herring population is currently classified as an "injured resource" that is "not recovering" (EVOSTC 2002) and commercial herring fisheries have remained closed for over 20 years. In addition to the human economic impacts of the population decline, the long-term ecological impacts were devastating. In marine systems such as PWS, forage fishes, including Pacific herring, represent the primary energy link in the biological community, exerting both top-down control over primary and secondary production (phytoplankton and zooplankton) and bottom-up control over higher order predators (Rice 1995 and Currey et al 2000). The critical ecological position occupied by Pacific herring is equally important for bridging the flow of inorganic nutrients (mobilized by primary and secondary production) and organic nutrients (utilized by higher trophic level predators). As such, they represent a critical link in the food web and biomass fluctuations cause cascading impacts to upper and lower trophic levels.

Definitive cause(s) of the herring population decline and failed recovery in PWS remain uncertain; however, a leading hypothesis involves chronic and epizootic mortality resulting from infectious and / or parasitic diseases (Marty et al. 1998, 2003). In 1993 only 20% of the anticipated adult herring biomass appeared in the known spawning areas. Returning fish were lethargic and demonstrated external hemorrhages consistent with viral hemorrhagic septicemia (VHS). The etiological agent, VHS virus (VHSV), was later isolated from moribund individuals. Subsequently, other suspected pathogens were identified in the PWS herring population, including *Ichthyophonus hoferi*, Anisakid worms, lymphocystis virus, *Goussia* sp (an intestinal parasite), *G. clupearum* (a liver parasite), a testicular coccidian, a myxosporean in the gall bladder, *Ortholinea orientalis*, *Ceratomyxa auerbachi*, *Gyrodactylus spp* (monogenean trematodes), branchial ciliated protozoans, a renal myxosporean, *Epitheliocystis*, gastric trematodes, intestinal trematodes, intestinal cestodes (Marty et al. 1998), and erythrocytic necrosis virus (ENV; Hershberger et al. 2009). Among the pathogens occurring in PWS herring, VHSV, *Ichthyophonus*, and ENV are considered the primary pathogens of concern because they have been

associated with epizootics in populations of wild herring, pilchards, and other forage species (Meyers et al. 1986, Hershberger et al. 2009, Garver et al. 2013, Burge et al. 2014). Alternative and complementary hypotheses accounting for the herring population dynamics include competition with pink salmon for limited resources (Deriso et al. 2008) and predation on herring populations by humpback whales and other predators.

The overarching goal of all studies within the herring disease program (HDP) is to advance an understanding of the basic ecology of the primary diseases and use this information to develop adaptive disease management strategies that are capable of forecasting and mitigating disease impacts to PWS herring. This broad goal would be unachievable using traditional scientific approaches that include focused research objectives that are often siloed within their traditional scientific fields. However, by incorporating our expansive network of fish health professionals at the U.S. Geological Survey (USGS) Western Fisheries Research Center, the robust team of herring experts within the Herring Research and Monitoring (HRM) component, and the broader base of fisheries professionals within the newly amalgamated Long-Term Research and Monitoring (LTRM) program, this network presents a truly unique opportunity within the field of disease ecology. Within this broad team, we have brought together experts in virology, parasitology, microbiology, molecular biology, immunology, herring biology, food webs, predator ecology, stock assessment, and ecological modelling. This breadth of expertise combined with the 10-year project horizon positions the HDP to make significant advancements in both basic disease ecology and the ecology of diseases in Pacific herring.

Our approach is multi-tiered, encompassing a combination of field surveillances and observations, controlled laboratory studies, novel disease forecasting tools, and ecological modelling. A detailed list of objectives and hypotheses is included in the next section. However, it is important to highlight two primary objectives because of their potential ecological and management impacts, 1) to evaluate epidemiological consequences of herd immunity to VHSV and 2) to identify *Ichthyophonus* transmission mechanisms.

Herd immunity refers to the proportion of immune animals in a population that is sufficient to provide population-level protection against a particular disease. This protection results if the critical transmission pathways required to maintain the infection become interrupted. This concept is particularly important in the case of VHSV in Pacific herring because we have previously demonstrated that survivors of a single exposure develop life-long immunity against future outbreaks of the disease. This academic concept has practical applications because knowledge of the prior exposure history of herring to VHSV informs the potential for future disease outbreaks. For example, a population that survived prior exposure and demonstrates herd immunity is refractory to the disease, and disease mortality can be removed from future population assessment models. Conversely, a naïve population without herd immunity retains the potential for future disease impacts. During previous HDPs we developed, optimized, and validated a tool (plaque neutralization test) that can detect herring neutralizing antibodies to VHSV. Further work confirmed that herd immunity is achieved when antibodies are detectable in $\geq$27% of individuals in a herring population (Hershberger and Purcell 2020). This method requires further refinement to determine how large the herd is, and how broad a geographic area must be sampled to assign herd immunity in the metapopulation. Additionally, although herd immunity is achieved when antibodies are detected in $\geq$27% of the population, we know through experimentation that the remaining 73% without detectable levels of circulating antibodies are also immune. To capture a greater proportion of the refractory individuals in a population, we propose to explore alternatives to the plaque neutralization test for deducing the exposure history of wild Pacific herring.

An understanding of *Ichthyophonus* transmission mechanisms in Pacific herring is critical if we are to develop an early warning system to forecast interannual changes in disease mortality. *Ichthyophonus* disease is periodically responsible for large fish kills and declines in marine clupeid populations throughout the northern hemisphere; however, a basic understanding of conditions that preface these epizootics remains unknown. Hypotheses accounting for the sudden appearance of epizootics in herring populations have evolved throughout the past several years of the HDP. For example, we initially hypothesized that disease sometimes occurred through the exacerbation of chronic *Ichthyophonus* infections to overt disease, presumably after exposure to a host or environmental disease cofactor. However, laboratory experiments involving several disease cofactors have repeatedly failed to exacerbate chronic *Ichthyophonus* infections, presumably because the established infections are well-encapsulated by a robust host cellular immune response. Therefore, a new hypothesis has been advanced, whereby disease occurs after periodic exposures to high levels of the parasite that can overwhelm the host cellular response. This hypothesis is supported by laboratory studies which repeatedly indicate that disease progression and host fate are a reflection of exposure level, whereby exposure to high parasite levels results in disease and mortality, but exposure to low parasite levels results in chronic infections and host survival. Unfortunately, the natural mechanism(s) of *Ichthyophonus* exposure and transmission to Pacific herring remain enigmatic and currently represent a major impediment to advancing our understanding of this hypothesis. Here, we propose to evaluate whether *Ichthyophonus* transmission occurs via ovivory on eggs of walleye pollock and herring conspecifics. The hypothesis is supported by two lines of evidence:

- survey results in 2021 detected *Ichthyophonus* in association with the eggs of both walleye pollock and Pacific herring
- eggs of both walleye pollock and Pacific herring are often consumed by Pacific herring in copious amounts (Norcross et al. 2001, Gorbatenko et al. 2012, personal observation).

If this hypothesis is supported, then we may be able to utilize this ovivory transmission mechanism to develop an early warning system that forecasts *Ichthyophonus* impacts. Such a system may conceivably involve some combination of *Ichthyophonus* infection prevalence in walleye pollock with the spatial overlap of herring feeding on spawned pollock eggs. During years when these disease cofactors align, we may expect to have higher herring mortality from *Ichthyophonus*. If so, then management actions could take advantage of this knowledge to open herring fisheries and harvest product before it is removed from the ecosystem by disease mortality. Additionally, it may be possible to manage the disease in herring by adjusting harvest levels of walleye to curb *Ichthyophonus* transmission to herring.

2. RELEVANCE TO THE INVITATION (maximum 300 words)

As part of the Gulf Watch Alaska (GWA)-LTRM program, this proposal is one of several collective projects that address the Herring Research and Monitoring Component of the Invitation. This proposal specifically addresses "*Top-down forces that may limit herring recovery*", with particular emphasis on topic #8 "*Continued study of the role of disease in herring recovery and the potential for developing tools to aid management agencies in the detection and management of disease outbreaks, including research of other vectors of disease transmission such as egg predation (ovivory).*" It is well integrated with other proposed *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) funded projects that are nested within and outside the HRM component.

3. PROJECT HISTORY (maximum 400 words)

The HDP is a continuation of project 21120111-G. Precursors to the HDP began with herring disease projects that started within the Sound Ecosystem Assessment (SEA) Program and were led by Drs. Richard Kocan (laboratory experiments) and Gary Marty (field assessments) from 1994 to 2003 (project numbers 94320-S, 95320-S, 96062, 96162, 97162 (including Supp), 98162, 981626, 99162-A, 99238, 99462, and 00462). This early herring disease work was continued by Hershberger when the HDP was launched in 2007. Since its inception the HPD was a part of the evolving EVOSTC programmatic structure, including the Integrated Herring Restoration Program from 2007 to 2013 (project numbers 070819 and 10100132-I) and the HRM program from 2012 to present (project numbers 12120111-K, 13120111-K, 14120111-K, 15120111-K, 16120111-K, 17120111-E, 18120111-E, 19120111-E, 20120111-E, and 21120111-E). With the current amalgamation of the HRM program and GWA program into the LTRM program, this broader scientific effort offers much greater opportunity to understand the ecological principles that influence the primary disease of Pacific herring. During the most recent four year period (2017 – date), the HDP has delivered 17 presentations at scientific meetings and 11 publications in the peer reviewed scientific literature (not including two additional publications that are currently in review and 4 that are anticipated for submission in 2021).

4. PROJECT DESIGN

A. Objectives and Hypotheses

i. To produce laboratory-reared specific pathogen-free Pacific herring for controlled laboratory studies.

ii. To continue annual herring health assessments in PWS and reference locations, including Sitka Sound, Puget Sound, and other opportunistic locations.

   a. Infection and disease prevalence provide higher resolution of natural mortality in herring age-structured-assessment model
   b. Environmental and demographic factors influence *Ichthyophonus* infection prevalence and VHS seroprevalence

iii. To evaluate herd Immunity to VHS

   a. Herd immunity against VHS occurs at varying spatial scales in Pacific herring.
   b. Evidence of prior exposure to VHSV can be deduced using novel tools that are cheaper, faster, and more universal than the current plaque neutralization test.
   c. Herd immunity can be conceptualized and incorporated into management decisions using an epidemiological model.

iv. To quantify impacts of disease on herring

   a. Sea lice (*Caligus clemensii*) infestations cause deleterious impacts to the health of Pacific herring.
   b. Viral erythrocytic necrosis (VEN) negatively impacts the health and survival of Pacific herring.
   c. The swimming performance Pacific herring is affected by their infection status
   d. *Ichthyophonus* can be transmitted to Pacific herring through ovivory on eggs of conspecifics and walleye pollock.

v. To determine whether exposures of herring to oil and / or VHSV can have cross-generational effects

B. Procedural and Scientific Methods

i. To produce laboratory-reared specific pathogen-free Pacific herring for controlled laboratory studies.

A critical component of both the field surveillance efforts and the empirical disease process studies involves the availability of laboratory host animals with known exposure and disease histories. We have developed techniques to rear specific pathogen-free (SPF) herring and we currently maintain several thousand SPF herring in each of four-year classes (2015 and 2018 – 2020) for use as experimental animals. Additional colonies will be reared to satisfy the needs described in this proposal. These experimental animals will be reared and manipulated under highly controlled laboratory conditions to address specific questions involving pathogenicity, pathogen transmission, direct and indirect effects of disease, etc. Additionally, a source of SPF herring is needed as a source of complement from Pacific herring that are known to be naïve to VHS virus. This complement is a critical ingredient of the plaque neutralization test to assess antibodies to VHS virus (Fig. 1).

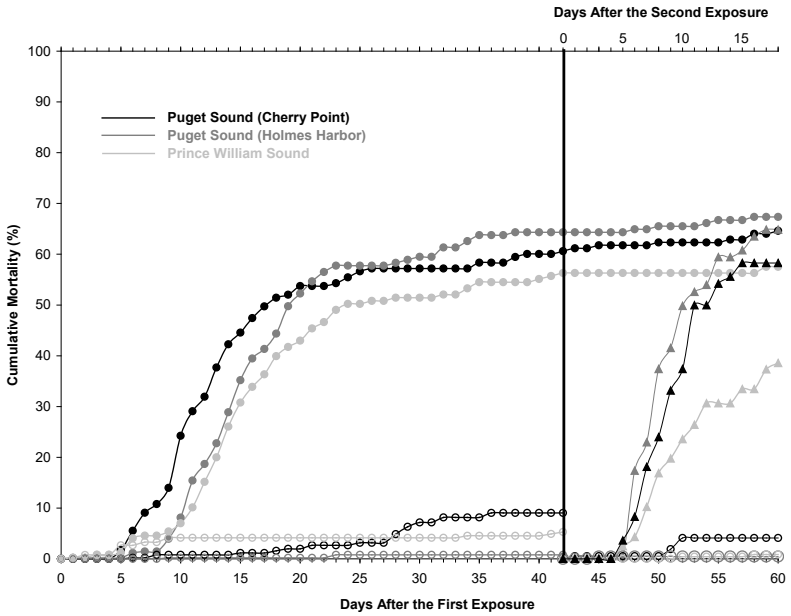

Figure 1. Relative susceptibilities of Pacific herring from three different stocks (Holmes Harbor, Cherry Point, and Prince William Sound) to viral hemorrhagic septicemia (VHS). Closed circles indicate treatment groups exposed to VHS virus (VHSV) and open circles indicate negative control groups (exposed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

VHS virus pathogenicity and protective host response are consistent across different populations of Pacific herring; therefore, eggs can be sourced from Puget Sound, WA for most of these studies. However, SPF herring from additional stocks, including PWS, will be reared when the need arises in other EVOSTC projects, including the project proposed by Dr. Whitehead (project 2217015, *Genetic and physiological mechanisms of virus and oil interactions in Pacific herring*).

ii. To continue annual herring health assessments in PWS and reference locations, including Sitka Sound, Puget Sound, and other opportunistic locations.

a. Infection and disease prevalence provide higher resolution of natural mortality in herring age-structured-assessment model.

Disease is a component in the Age-Structure-Analysis (ASA) model for PWS; however, it is not part of the Alaska Department of Fish and Game (ADF&G) sponsored surveys. We will provide the disease information for the ASA model by determining annual prevalence and intensity data for the most virulent pathogens occurring in the PWS herring populations, including VHSV, ENV, and *Ichthyophonus*. Monitoring efforts will consist of the annual collection and processing of sixty adult herring per site from each of three sites in PWS and Sitka Sound to test for infection and disease prevalence. Diagnostic techniques for these pathogens will follow standard procedures described in the "Blue Book: Standard procedures for the detection and identification of select fish and shellfish pathogens (American Fisheries Society)." Additionally, plasma samples will be collected from sampled fish in PWS and Sitka; samples will be processed using a novel plaque neutralization assay that can determine prior VHSV exposure history. In an effort to increase age class resolution of the serology data from PWS herring, the sample size will be increased to n = 360, including 180 randomly sampled individuals, and 60 fish from each of three size bins (small, medium, and large). Ages (from scales) will be obtained for all 360 PWS herring. Similar pathogen and serological sampling will occur from Pacific herring populations in Puget Sound, WA, and other locations throughout the NE Pacific as sampling opportunities become available. When combined, these survey results will inform the ASA model and serological results will inform a novel model intended to assess the contribution of VHS to annual natural mortality rates.

iii. To evaluate herd immunity to VHS.

Throughout the past several years, a major contribution of the Herring Disease Program involved the development, optimization, validation, and application of an antibody assessment technique (plaque neutralization test) to assess herd immunity in Pacific herring. The assay has been a resounding success, as application of this tool to wild herring populations can now deduce the VHSV exposure history and inform the future potential for VHS epizootics. Experiments are proposed here to further refine the details of this herd immunity concept so the results can be applied to adaptive management strategies.

a. Herd immunity against VHS occurs at varying spatial scales in Pacific herring.

Field surveillances of wild Pacific herring have determined that herd immunity occurs when $\geq$27% of the individuals are seropositive for neutralizing antibodies. However, questions remain regarding the variability of herd immunity on different geographic scales. We propose to compare VHSV neutralizing antibody levels within the same metapopulation from groups of herring that are collected from geographically disparate sites throughout Prince William Sound (e.g. Port Fidalgo, Port Gravina, Montague Island, and Kayak Island) and Puget Sound (e.g. south, central, and north Sound). Age of herring sampled from PWS / GOA will be determined from scales. By covering this geographic scale, we will be able to assess the geographic extent of herd immunity within a metapopulation and determine the sampling resolution required to inform population assessment

models. This objective relies on contributions from the project proposed by Dr. Cypher "Pacific Herring Connectivity Between PWS and Kayak Island" for samples from Kayak Island.

b. *Evidence of prior exposure to VHSV can be deduced using novel tools that are cheaper, faster, and more universal than the current plaque neutralization test.*

The newly developed and validated antibody assay provides a reliable indication of prior exposure history, indicating that a seroprevalence of ≥27% corresponds with herd immunity in herring populations. A common misinterpretation of these results is that only 27% of the population are resistant and the remainder (73%) remain susceptible. This interpretation is incorrect because all herring (including those with no detectable antibodies) are refractory to VHS after controlled re-exposure to the virus. As we would expect antibodies to occur in >90% of individuals after herd immunity has been achieved, it is apparent that some further refinement may be useful. Reasons for the apparent incongruities lie in the detailed immunological mechanisms that confer resistance. For example, animals produce many different types of neutralizing and non-neutralizing antibodies in response to a foreign agent (including VHSV). It is very likely that the neutralizing antibodies we are reporting with the plaque neutralization test (PNT) can be protective at circulating titers that fall well-below the minimum detection threshold of the assay. Additionally, other non-neutralizing antibodies are certainly present and not detected by our PNT. Further, it is energetically costly for animals to produce antibodies for extended periods after the antigen (VHSV) is no longer present. In these cases, the levels of circulating antibodies are expected to fall to sub-detectable levels. However, the activated lymphocytes responsible for producing these antibodies can mobilize very quickly after another exposure; thereby providing a mechanism for rapid onset of adaptive immunity without the need for the continuous production of circulating neutralizing antibodies.

Work on the antibody assay will continue; however, we will also evaluate alternative techniques for assessing herd immunity. For example, a direct measure of adaptive immunity (neutralizing antibodies) may not be necessary for assigning herd immunity status to a population. Rather, our previous work has repeatedly demonstrated that herring develop resistance to VHS after surviving only a single exposure to the virus. Therefore, quantification of herd immunity may be achievable by indirectly deducing the exposure history of individuals to VHSV, without the need to detect neutralizing antibodies. We recently determined that gills of 90% of herring that survived prior VHSV exposure in the laboratory continue to test qPCR positive for extended periods after full convalescence from the disease. If the virus remains associated with the gills of the survivors for extended periods and this viral signature can be detected by reverse transcriptase quantitative polymerase chain reaction (RTqPCR), then a molecular assay may offer a much quicker and cheaper indication of prior exposures and resulting herd immunity. We will test this hypothesis using a series of long-term laboratory exposures, accompanied with field validations in wild herring. Combined, this approach will determine whether RT-qPCR assessment of gill tissues may provide a deducible measure of herd immunity against VHSV in Pacific herring.

c. *Herd immunity can be conceptualized and incorporated into management decisions using epidemiological models.*

Due to the intensive data collection efforts in past years, it is now possible to parameterize epidemiological models of VHS using data from lab experiments (e.g., Hershberger et al. 2013). This opens numerous opportunities to evaluate the impact of management scenarios on the epidemiology of this disease. We propose to use a VHS disease model developed and parameterized during the current funding cycle to do the following:

1) Quantify the interactive roles of herd immunity, temperature, recruitment, and fishing on VHS outbreak size, mortality, and duration using a classic SEIR (susceptible, exposed, infected, recovered) disease model.

2) Estimate mortality due to VHS infection using the VHS seroprevalence data collected yearly at Sitka and PWS. This model will utilize a modified version of the partially observed Markov process model structure that was recently developed during the 2017-2021 HDP. These mortality rates could be used to estimate the contribution of VHS to mortality of 3+ year old herring in PWS; mortality estimates could then be fed into the age-structured assessment model.

iv. Quantify impacts of disease on herring

a. *Sea lice infestations cause deleterious impacts to the health of Pacific herring.*

Sea lice are common ectoparasites of numerous fish species including Pacific herring (e.g., Beamish et al. 2009). The life cycle of *C. clemensi* involves both planktonic and parasitic stages. Eggs (attached to an adult female) hatch into planktonic nauplii which develop into infective copepodids. If these copepodids find a suitable host, they will feed on their mucus and epidermis while they develop through four chalimus stages followed by motile pre-adult and adult stages; the motile stages are often the most damaging to hosts. While generally not considered lethal to adult fish, the impacts of sea lice on juvenile fish are not trivial; infestations of a related sea lice species (*Lepeophtheirus salmonis*) have been associated with 39% declines in recruitment of adult Atlantic salmon (Krkošek et al. 2013). Mortality is frequently associated with loss of osmoregulation, secondary infestations, increased vulnerability to predation and/or a reduction in feeding success (Godwin et al. 2015). For example, juvenile sockeye infected with *C. clemensi* have reduced ability to compete for limited resources, reduced stomach fullness, and reduced growth (Godwin et al. 2015, 2017, 2018). Robust investigations have not been conducted to quantify the prevalence and intensity of sea louse infestations and their associated fitness costs to Pacific herring. However, preliminary surveys suggest that infestation loads can be substantial and may be increasing. In a survey of *C. clemensi* infestations in the Gulf Islands in 2008, the average infestation was 5.0 sea lice on juvenile herring (n=12) and 4.9 on spawning adult herring (n=50) (Beamish et al. 2009). During biweekly sampling in 2019 our research team observed *C. clemensi* infestations on Pacific herring (Fig. 2), particularly juveniles sampled along the Strait of Juan de Fuca and in Cordova Harbor. Sea lice infestations are highly temperature-dependent; generation time decreases and the reproductive ratio (R_0) of sea lice increases with temperature (Groner et al. 2014). While numerous additional factors affect sea lice

abundance, thermal conditions will be conducive to louse populations increases in the future. The impact of these infestations on juvenile Pacific herring has not been explored.

Figure 2. Pacific herring demonstrating heavy infestation with *Caligus clemensi*. The top fish has an unusually high parasite load.

Aim 1: To quantify sea louse infestations in wild juvenile herring

Sampling will occur at nearshore sites by purse seine or beach. After deployment, the seine will be closed, but fish will remain in the water to reduce exodus of lice which commonly occurs when *C. clemensi* are pulled out of the water. 60 fish per sampling event/ location will be randomly sampled for sea lice; data will include, length, weight, stomach fullness (measured by regressing stomach weight on length), and hematocrit. Upon returning to the lab, sea lice from all samples will be enumerated and staged (as adult-female, adult male, or nonmotile stages (i.e., pre-adult or chalimus). Fish dry weight/ wet weight ratio will be recoded on half of the fish as a proxy for energy density. A calibration curve for dry weight/ wet weight will be made by correlating paired samples of herring tissue for energy density measured using bomb calorimetry and the dry weight/ wet weight method

Aim 2: To experimentally determine impacts of sea lice to herring

Controlled experiments using SPF herring will be performed to quantify impacts of sea louse infestations on juvenile herring survival and fitness. Herring in each of 4 replicate tanks will be experimentally exposed to *C. clemensi* negative controls will consist of a sham treatment.

Individuals will be subsampled periodically after exposure to evaluate metrics of herring health, including of gene expression, hematocrit, energy density, and histological changes to the skin. Effects will be evaluated using a nested design where tank is treated as a random effect and motile and non-motile sea louse infestation intensities are used as a covariate.

b. VEN negatively impacts the health and survival of Pacific herring.

VEN, a disease characterized by anemia and the presence of cytoplasmic inclusion bodies in erythrocytes of affected fishes, has been reported in more than 20 species of wild marine and anadromous fish worldwide (MacMillan and Mulcahy 1979). Along the Pacific coast of North America, natural hosts include Pacific herring, pink salmon, and chum salmon, which appear to be highly susceptible. Epizootics, sometimes accompanied with mass mortalities, periodically occur among Pacific herring populations in near shore areas of the NE Pacific (MacMillan and Mulcahy 1979, Meyers et. al. 1986, Hershberger et al. 2009). Affected herring often demonstrate severe blood dyscrasias, which culminate in anemia, lethargy, and the presence of pale gills. The disease is diagnosed by the detection of characteristic cytoplasmic inclusion bodies in affected erythrocytes of Giemsa-stained blood films.

Aim 1: Assess impacts of temperature on VEN progression

The effect of temperature on VEN progression (including prevalence of infection, infection density, histopathological changes, and gene expression) will be assessed in Pacific herring using triplicate 760 L tanks (n = 45 herring / tank) at each of three temperatures (ambient, cool, and warm). Treatment groups will be exposed to ENV by waterborne immersion and controls will be exposed to MEM in lieu of virus. Experimental animals will consist of age 1 SPF Pacific. Temperature treatments will consist of ambient (approximately 8.5 °C), chilled (approximately 6.5 °C), and warm (approximately 14 °C) for treatment and negative control groups.

Subsampling will occur on at prescribed intervals post-exposure. Samples will include hematocrits, fork length, mass, blood films (to quantify the presence of erythrocytic inclusions), kidneys (to quantify viral load by qPCR), and livers (to quantify gene expression) from all fish. The study will tentatively be terminated after approximately 30 days if VEN disease progresses in at least one of the temperature treatments. Upon experimental termination, all the surviving fish will be euthanized and processed to assess differences in disease progression between the three temperature treatments.

Aim 2: Assess the effects of juvenile pink salmon and Pacific herring sympatry on VEN

As part of the pink salmon / herring interaction projects proposed by Rand et al. and Heintz and Gorman (projects 22220111-I and 22220111-L, respectively), we will investigate the prevalence and infection intensity of erythrocytic necrosis virus (the causative agent of VEN), on juvenile pink salmon at various timepoints throughout their PWS outmigration. Analogous VEN observations will be made on sympatric juvenile herring collected during these cruises. Coherence between VEN infection prevalence and load in both species will be evaluated.

c. The swimming performance Pacific herring is affected by their infection status

In addition to direct impacts of diseases on the survival of affected herring, many hosts often survive with chronic infections that predispose them to indirect mortality via decreased performance, predator avoidance, immune capacity, etc. Here, we propose to evaluate some of these sublethal impacts by determine the effects of the major herring pathogens on host swimming performance.

The effects of VHSV, ENV, and *Ichthyophonus* on the swimming performance of herring will be evaluated under controlled conditions in the laboratory. The effects of these pathogens on the host swimming performance will be evaluated at different stages of infection, the timing of which will be unique to each pathogen. Although *Ichthyophonus* has been demonstrated to cause decreased swimming performance in rainbow trout, these sublethal effects have never been evaluated in Pacific herring. Additionally, we have recently learned that the parasite evaluated in rainbow trout is markedly distinct, and likely a different species (possibly even a different genus) than that occurring in Pacific herring. The impact of the other pathogens on swimming performance in Clupeids has not been evaluated either.

A lack of appropriate equipment prevented us from testing these performance metrics in previous HDP efforts. Although we were in possession of a respirometer that was capable of evaluating swimming capacity, Pacific herring displayed markedly abnormal swimming behavior in the device; as a result, we were concerned that any attempted comparisons between infected and uninfected groups would be based on spurious results that were simply a reflection of the abnormal environment. We propose to alleviate these concerns by building an experimental swim flume to elicit more normal swimming behavior from Pacific herring. This newly constructed flume will be employed as the experimental devise in controlled experiments to compare swimming performance between infected and uninfected cohorts.

d. Ichthyophonus can be transmitted to Pacific herring through ovivory on eggs of conspecifics and walleye pollock.

In developing techniques to forecast disease impacts, the most important data gap for marine fishes often involves a basic understanding of epidemiological principles governing transmission. For example, all available data indicate that the apparent stochasticity of large, recurring fish kills from ichthyophoniasis likely result from punctuated exposures to high levels of the parasite.

Although *Ichthyophonus* has been described for over a hundred years and is one of the most ecologically and economically important pathogens of marine fishes in the world, some basic tenets of its epizootiology remain unresolved. The most pressing knowledge gap involves mechanisms by which Pacific herring and other planktivorous fishes become exposed-to and infected-with the parasite. Our laboratory has been investigating possible transmission routes and has been unsuccessful at demonstrating *Ichthyophonus* transmission to herring via cohabitation or immersion in high concentrations of parasite isolates. Additionally, we have been unsuccessful at detecting the parasite in wild zooplankton that serve as typical food items for Pacific herring. Repeated feedings with infected tissues have been minimally successful, with approximately 7% of individuals becoming infected after consuming infected offal; however, consumption of infected offal cannot explain the global distribution of this parasite in clupeids.

We recently learned that overwintering Pacific herring in the Gulf of Alaska are often found with fish eggs (presumably walleye pollock eggs) in their stomachs. Additionally, during the herring spawn, we have observed Pacific herring stomachs gorged with newly spawned eggs from conspecifics. Because both fishes (walleye pollock and Pacific herring) are known hosts for *Ichthyophonus*, and the parasite tends to occur in multiple tissues within an infected host, we hypothesize that herring ovivory may represent a natural transmission route for *Ichthyophonus*. As a first step towards investigating this hypothesis, we documented that *Ichthyophonus* occurs in association with both herring and pollock eggs in 2020. For example, *Ichthyophonus* was recovered from 8.5% of herring egg cultures from PWS (17% of paired heart cultures) and 52% of walleye pollock egg cultures from Shelikof Strait (42% of paired liver cultures).

These preliminary results provide proof-of-concept for the *Ichthyophonus* ovivory transmission hypothesis and provide the most promising support for any *Ichthyophonus* transmission mechanism for clupeids to date. Extensive investigations are necessary to successfully demonstrate this transmission route. To test the ovivory hypothesis, we propose to address the following objectives:

1) Repeat surveillance of *Ichthyophonus* in pollock and herring eggs.
2) Determine the provenance of eggs in the stomachs of herring eggs using DNA barcoding to confirm they are from walleye pollock and Pacific herring.
3) Use chromogenic in situ hybridization (CISH) to assess of the location and life stages of *Ichthyophonus* occurring on herring and / or pollock eggs.
4) Assess whether *Ichthyophonus* can be found in association with consumed eggs (pollock and/or herring) in the stomach of herring consumers.
5) Attempt *Ichthyophonus* transmission studies by feeding pollock and / or herring eggs to laboratory reared specific pathogen-free herring under controlled conditions.

It should be emphasized that the ability to successfully test these objectives relies heavily on synergisms with other proposed EVOSTC projects, including '*Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions'* (Fournier et al., new proposed project outside of the GWA-LTRM program) and '*Surveys and age, sex, and size collection and processing'* (Haught, project 22170111-F).

If true, this hypothesis would represent a major knowledge advancement at several levels. First, we are unaware of any other parasite in any other host that relies on ovivory as a primary mechanism for transmission. As such, this mechanism would represent a major contribution to basic parasite ecology by describing a new transmission mechanism. Second, if transmission through ovivory does occur, then it could be employed as an early warning system for future *Ichthyophonus* disease impacts. For example, the combination of pollock spawning biomass, *Ichthyophonus* infection prevalence in adult pollock, and spatial overlap of spawning pollock with foraging herring schools may be used to deduce inter-annual changes in infection pressures (based on transmission risk) and resulting disease impacts to herring populations. This knowledge could easily be incorporated into adaptive disease management practices to mitigate the impacts of ichthyophoniasis, such as culling pollock populations in certain situations to reduce ovivorous transmission to herring.

v. To determine whether exposures of herring to oil and/or VHSV can have cross-generational effects.

Our overarching hypothesis is that oil exposure affects the ability of adult Pacific herring to immunologically prime their offspring, thereby increasing susceptibility to viral disease during development. Animals are especially susceptible to disease during early life because their immune systems are not yet mature. One line of protection from infectious disease for early life (embryo/larval stages) is through parental priming of immune factors; that is, parental exposure to pathogens up-regulates immune factors that can then be passed to offspring for example through maternal loading of those immune factors into eggs. This is a phenomenon called trans-generational immune priming (TGIP), and is found across vertebrates (e.g., fish, birds, mammals) and invertebrates. Like many modes of non-genetic inheritance, TGIP may be perturbed by environmental exposures including to toxicants. We have two key questions: 1) does oil exposure perturb TGIP, and 2) does TGIP and the influence of oil vary between PWS and other populations?

Detailed methods and timelines required to address these overarching hypotheses are included in the project proposed by Dr. Whitehead (project 22170115, *Genetic and physiological mechanisms of virus and oil interactions in Pacific herring*). All in vivo experimental work involving Pacific herring will be performed at the USGS Marrowstone Marine Field Station as an in-kind contribution of the Herring Disease Program, including polycyclic aromatic hydrocarbon exposures, VHSV exposures, herring egg fertilizations, fish rearing, live feed production, VHSV antibody diagnostics, etc.

C. Data Analysis and Statistical Methods

Standard statistical comparisons for pathogen virulence studies will be employed in all experiments. For example, percent cumulative mortalities in replicate tanks / aquaria will be arc sin transformed and transformed means from all groups will be statistically compared using Student's T-test (1-tailed) or analysis of variance followed by the Tukey test for multiple comparisons. In non-replicated tanks, percent mortality in control and treatment groups will be statistically compared using the Chi Square statistic (χ^2). Statistical significance will be assigned to all comparisons with $p \leq 0.05$. Prevalence of infection and disease in wild populations from PWS, Sitka Sound, and Puget Sound will be based on minimum sample sizes of 60 fish, sufficient to detect 5% prevalence in the population with 95% confidence.

To ensure continuity in VHSV surveillance results dating to the early 1990s, VHSV surveillances from PWS and Sitka Sound will be processed by ADF&G – Juneau Fish Pathology Laboratory as an in-kind contribution to this project.

D. Description of Study Area

The study area includes locations throughout PWS and Sitka Sound where pre-spawn herring aggregate. PWS disease surveillances will be performed within and near the Spill Affected Area.

Laboratory studies described in this proposal will be conducted at the USGS-Marrowstone Marine Field Station, and USGS-Western Fisheries Research Center (WFRC) where facilities ideally designed to conduct experiments using endemic fish pathogens safely and responsibly. The Marrowstone Marine Field Station represents the sole seawater-based biological research facility for the USGS. Facilities include three large wet laboratory buildings with approximately 10,000 sf of wet laboratory space, replicated with approximately 60,000-L tank capacity, and supplied with 400 gpm of high quality filtered and ultraviolet 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 WFRC is recognized as an international leader in fish health research. The WFRC maintains fish health laboratory facilities which are among the newest and best in the nation. The facility operates a state-of-the-art fresh water wet laboratory that is completely climate controlled and automated for disease challenges and studies in physiology and pathology. The nation's only Biosafety Level III disease containment wet laboratory for fish is also part of this facility. Additionally, the Center maintains fully equipped laboratories for molecular biology, virology, bacteriology, immunology, and histopathology.

5. COORDINATION AND COLLABORATION

A. With the Alaska SeaLife Center or Prince William Sound Science Center

The HDP is closely aligned with multiple principal investigators (PIs) and projects at the Prince William Sound Science Center (PWSSC; see below). Additionally, one of the PIs on this HDP (Groner) is a current employee at the PWSSC.

B. Within the EVOSTC LTRM Program

Environmental Drivers Component

While this project currently does not collaborate with Environmental Drivers Component projects, oceanographic information, particularly within PWS from the oceanographic conditions in PWS (project 22120114-G) and Seward Line (project 22120114-L) projects could provide interesting insights into future work.

Pelagic Monitoring Component

This project currently does not collaborate with pelagic monitoring component projects. However, future coordination with the forage fish project (22120114-C) may be beneficial.

Nearshore Monitoring Component

This project currently does not collaborate with the nearshore monitoring component. We look forward to increasing coordination with the PIs of the nearshore project (22120114-H) in future years.

Lingering Oil Monitoring Component

Because lingering oil data are collected once in a 5-year period and the oil is not currently bioavailable, we do not anticipate incorporating these data into our project. We look forward to status reports from the Lingering Oil Component.

Herring Research and Monitoring component

This project coordinates with multiple HRM component projects, as follows:

- *20120111-B Annual Herring Migration Cycle: Movement between Kayak Island and Prince William Sound*
  This project will provide plasma samples from Kayak Island to address the geographic scope of herd immunity in Pacific herring.

- *22170111-F Surveys and age, sex, and size collection and processing*
  This project has will provide a sampling platform and technical assistance for performing annual pathogen prevalence surveillances in PWS.

- *22170115 Genetic and physiological mechanisms of virus and oil interactions in Pacific herring*
  This project is completely integrated with the HDP. We will perform all in vivo experiments for this project including fish crosses, fish rearing, and all in vivo manipulations including oil and PAH exposures.

- *22220111-L Ecological interactions between juvenile salmon and Pacific herring in southwestern Prince William Sound*
  This project has agreed to provide field samples for sea lice and VEN from pink salmon and sympatric herring.

Synthesis and Modeling Component

The HPD will provide annual infection and disease surveillance results for the ASA herring modelling project (22120111-C: *Modeling and stock assessment of Prince William Sound herring*) and will directly or indirectly be used in other Synthesis and Modeling Component analyses (2222LTRM-A&B).

Data Management Project

The HDP will provide annual pathogen surveillance results and associated metadata to the data management project within required timeframes.

C. With Other EVOSTC-funded Projects (not within the LTRM Focus Area)

Current EVOSTC-funded projects not within the LTRM focus area have not intersected with this project so far. As the EVOSTC funds future projects outside the GWA-LTRM program we will evaluate their applicability to our project and coordinate as appropriate. Of particular interest to this project is the project proposed by Fournier et al. “Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions.” If funded, our project will provide bi-annual samples of walleye pollock for the HDP and serve as a source of pollock samples (including eggs) to address the ovivory hypothesis outlined in the HDP.

D. With Proposed EVOSTC Mariculture Focus Area Projects

We look forward to working with the EVOSTC’s Mariculture Program and projects they embark on. We anticipate they will be interested in GWA-LTRM datasets and we expect there will be opportunities for coordination and collaboration.

E. With Proposed EVOSTC Education and Outreach Focus Area Projects

The GWA-LTRM program will develop an outreach plan that includes coordination and collaboration with the Trustee’s Education and Outreach Program and projects. We look forward to participating in education and outreach opportunities where our project findings can contribute to a better understanding of the Gulf of Alaska ecosystem by the general public.

F. With Trustee or Management Agencies

Two of the PIs for this project are employees of USGS, a Department of Interior agency. The HDP will continue to work closely with ADF&G herring managers in Cordova and Sitka to collect annual pathogen prevalence

information. Additionally, we will work closely with ADF&G – Homer to collect walleye pollock samples and associated *Ichthyophonus* information. Finally, we will continue to work closely with ADF&G – Juneau, who will continue to process the virology samples for the annual pathogen assessments in PWS and Sitka Sound.

G. With Native and Local Communities

The GWA-LTRM program and this project are committed to involvement with local and Alaska Native communities. Our vision for this involvement will include active engagement with the Education and Outreach Focus Area (see above), program-directed engagement through the Program Management project (2222LTRM-A&B), and project-level engagement. During the first year of the funding cycle (FY22), the GWA program will reach out to local communities and Alaska Native organizations in the spill affected area to ask what engagement they would like from us and develop an approach that invites involvement of PIs from each project, including this one. Our intent as a program is to provide effective and meaningful community involvement that complements the work of the Education and Outreach Focus Area and allows communities to engage directly with scientists based on local interests.

6. DELIVERABLES

As with previous HDP projects, we anticipate producing at least two manuscripts per year for the scientific literature (previous HDP projects averaged four per year). Among these, if results provide support for the herd immunity and ovivory hypotheses, two of these manuscripts will be submitted to the highest tier scientific journals. Annual reports will be provided to the EVOSTC. Additionally, we will prepare at least one presentation / year for the Alaska Marine Science Symposium. Additional presentations will be presented at the prominent fish health symposia, including the Western Fish Disease Workshop. Pathogen surveillance data and accompanying metadata will be posted annually.

7. PROJECT STATUS OF SCHEDULED ACCOMPLISHMENTS

Project milestones and tasks by fiscal year and quarter, beginning February 1, 2022. Fiscal Year Quarters: 1= Feb. 1-April 30; 2= May 1-July 31; 3= Aug. 1-Oct. 31; 4= Nov. 1-Jan 31.

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone 1: Produce SPF herring	X	X			X	X			X	X			X	X			X	X		
Milestone 2: Annual herring health Assessments																				
Task 1: Collect samples	X				X				X				X				X			
Task 2: Complete Lab diagnostics		X				X				X				X				X		
Task 3: Provide Data for ASA model				X				X				X				X				X
Milestone 3: Determine the spatial scale of herd immunity																				
Task 1: Collect plasma samples from disparate areas (including Kayak Island)					X				X				X	X					X	X
Task 2: Process plasma samples in the laboratory							X				X				X					

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone 4: Evaluate other Indicators of prior VHSV exposure																				
Task 1: Laboratory study evaluating RTqPCR to deduce VHSV exposure history																				
Task 2: Field studies validating RTqPCR as a proxy for prior exposure																				
Task 3: Process RTqPCR samples in the lab																				
Milestone 5: Develop epidemiological models for VHS																				
Task 1: Develop SEIR model to evaluate herd immunity, temperature, recruitment, etc.				X																
Task 2: Develop state-space model to estimate mortality due to VHS infection using the VHS seroprevalence data collected yearly at Sitka and PWS.								X												
Milestone 6: Determine effects of sea lice on herring																				
Task 1: Quantify louse infestations on wild herring															X	X			X	X
Task 2: Lab exposures with sea lice							X	X			X	X								
Milestone 7: Determine Impacts of VEN to herring																				
Task 1: Determine the effects of temperature on VEN progression	X	X	X	X																
Task 2: Field assessment of VEN in herring and pink salmon			X	X			X	X			X	X			X	X			X	X
Task 3: Produce GLM																				
Milestone 8: Evaluate swimming performance																				
Task 1: Build flume	X	X	X	X																
Task 2: *Ichthyophonus* swimming performance studies										X	X			X	X					
Task 3: VHSV swimming performance studies																		X	X	
Task 4: VEN swimming performance studies																				
Milestone 9: *Ichthyophonus* Transmission																				
Task 1: Survey pollock eggs for *Ichthyophonus*					X				X								X			

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Task 2: Determine the provenance of eggs in herring stomachs																				
Task 3: Use CISH to assess the relationship of *Ichthyophonus* on eggs																				
Task 4: Assess whether *Ichthyophonus* can be found in association with consumed eggs																				
Task 5: Perform controlled transmission studies involving herring or pollock eggs	X				X				X				X				X			
Milestone 10: Evaluate in vivo effects of oil / VHSV interactions																				
Objective 1: Characterize TGIP and perturbation by oil																				
Task 1a: procure animals for pilot exposures	X				X															
Task 1b: adult exposure/spawn	X				X															
Task 1c: embryo/larval assessments		X	X	X		X	X	X												
Objective 2: Test for population differences in TGIP and perturbation by oil																				
Task 2a: procure gametes for PWS/SS exposures	X																			
Task 2b: Rear PWS and SS fish to adulthood	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Task 2c: adult exposure/spawn																				
Task 2d: embryo/larval assessments																				
Objective 3: Test for genotypic selection by oil and/or virus																				
Task 3a: procure gametes from PWS and SS fish for replicate 1					X															
Task 3b: Embryo oil, juvenile virus challenge for replicate 1						X	X	X												
Task 3c: procure gametes from PWS and SS fish for replicate 2									X											
Task 3d: Embryo oil, juvenile virus challenge for replicate 2										X	X	X								
Task 3e: procure gametes from PWS and SS fish for replicate 3													X							
Task 3f: Embryo oil, juvenile virus challenge for replicate 3														X	X	X				
Task 3g: procure gametes from PWS and SS fish for replicate 4																	X			
Task 3h: Embryo oil, juvenile virus challenge for replicate 4																		X	X	X

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Reporting																				
Annual reports					X				X				X				X			
Final report																				
Deliverables																				
Peer reviewed paper		X		X		X		X		X		X		X		X		X		X
Data posted online				X				X				X				X				X

Milestone/Task	FY27				FY28				FY29				FY30				FY31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone 1: Produce SPF herring	X	X			X	X			X	X			X	X			X	X		
Milestone 2: Annual herring health Assessments																				
Task 1: Collect samples	X				X				X				X				X			
Task 2: Complete Lab diagnostics		X				X				X				X				X		
Task 3: Provide Data for ASA model				X				X				X				X				X
Milestone 3: Determine the spatial scale of herd immunity																				
Task 1: Collect plasma samples from disparate areas (including Kayak Island) **Need to include timing for Puget Sound samples**																				
Task 2: Process plasma samples in the laboratory	X																			
Milestone 4: Evaluate other Indicators of prior VHSV exposure																				
Task 1: Laboratory study evaluating RTqPCR to deduce VHSV exposure history	X	X	X	X	X	X	X	X	X											
Task 2: Field studies validating RTqPCR as a proxy for prior exposure			X	X			X	X			X	X								
Task 3: Process RTqPCR samples in the lab													X	X						
Milestone 5: Develop epidemiological models for VHS																				
Task 2: Develop state-space model to estimate mortality due to VHS infection using the VHS seroprevalence data collected yearly at Sitka and PWS.																				
Milestone 6: Determine effects of sea lice on herring																				
Task 1: Quantify louse infestations on wild herring																				

Milestone/Task	FY27				FY28				FY29				FY30				FY31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Task 2: Lab exposures with sea lice																				
Milestone 7: Determine Impacts of VEN to herring																				
Task 1: Determine the effects of temperature on VEN progression																				
Task 2: Field assessment of VEN in herring and pink salmon		X	X																	
Task 3: Produce GLM					X															
Milestone 8: Evaluate swimming performance																				
Task 1: Build flume																				
Task 2: *Ichthyophonus* swimming performance studies																				
Task 3: VHSV swimming performance studies		X	X																	
Task 4: VEN swimming Performance studies						X	X			X	X									
Milestone 9: *Ichthyophonus* Transmission																				
Task 1: Survey pollock eggs for *Ichthyophonus*	X								X				X							
Task 2: Determine the provenance of eggs in herring stomachs																	X	X		
Task 3: Use CISH to assess the relationship of *Ichthyophonus* on eggs													X	X	X	X				
Task 4: Assess whether *Ichthyophonus* can be found in association with consumed eggs									X	X	X	X								
Task 5: Perform controlled transmission studies involving herring or pollock eggs	X				X				X				X				X			
Milestone 10: Evaluate in vivo effects of oil / VHSV interactions																				
Objective 1: Characterize TGIP and perturbation by oil																				
Task 1a: procure animals for pilot exposures																				
Task 1b: adult exposure/spawn																				
Task 1c: embryo/larval assessments																				
Objective 2: Test for population differences in TGIP and perturbation by oil																				
Task 2a: procure gametes for PWS/SS exposures																				

Milestone/Task	FY27				FY28				FY29				FY30				FY31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Task 2b: Rear PWS and SS fish to adulthood																				
Task 2c: adult exposure/spawn	X																			
Task 2d: embryo/larval assessments		X	X	X																
Objective 3: Test for genotypic selection by oil and/or virus																				
Task 3a: procure gametes from PWS and SS fish for replicate 1																				
Task 3b: Embryo oil, juvenile virus challenge for replicate 1																				
Task 3c: procure gametes from PWS and SS fish for replicate 2																				
Task 3d: Embryo oil, juvenile virus challenge for replicate 2																				
Task 3e: procure gametes from PWS and SS fish for replicate 3																				
Task 3f: Embryo oil, juvenile virus challenge for replicate 3																				
Task 3g: procure gametes from PWS and SS fish for replicate 4																				
Task 3h: Embryo oil, juvenile virus challenge for replicate 4																				
Reporting																				
Annual reports	X				X				X				X				X			
Final report																				X
Deliverables																				
Peer reviewed paper		X		X		X		X		X		X		X		X		X		X
Data posted online				X				X				X				X				X

8. BUDGET

A. Budget Forms (Attach)

Please see Gulf Watch Alaska - Long-Term Research and Monitoring workbook.

This project includes two years (FY22 and FY23) of funding to PWSSC for a contract with Maya Groner at Biglow Labs to complete work she has been performing for the disease program during the FY17-21 funding period.

Budget Category:	Proposed FY 22	Proposed FY 23	Proposed FY 24	Proposed FY 25	Proposed FY 26	5-YR TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$193,776	$236,456	$227,886	$235,462	$301,248	$1,194,828	
Travel	$21,826	$21,826	$21,826	$21,826	$21,826	$109,130	
Contractual	$45,924	$45,924	$0	$0	$0	$91,848	
Commodities	$39,300	$39,000	$39,000	$39,000	$39,000	$195,300	
Equipment	$15,000	$0	$0	$0	$0	$15,000	
Indirect Costs (varies by proposer)	$0	$0	$0	$0	$0	$0	
SUBTOTAL	$315,826	$343,206	$288,712	$296,288	$362,074	$1,606,106	
General Administration (9% of subtotal)	$28,424	$30,889	$25,984	$26,666	$32,587	$144,550	N/A
PROGRAM TOTAL	**$344,250**	**$374,095**	**$314,696**	**$322,953**	**$394,661**	**$1,750,655**	
Other Resources (In-Kind Funds)	**$124,245**	**$127,724**	**$131,396**	**$135,129**	**$138,910**	**$657,404**	

Budget Category:	Proposed FY 27	Proposed FY 28	Proposed FY 29	Proposed FY 30	Proposed FY 31	5-YR TOTAL PROPOSED	ACTUAL CUMULATIVE	TEN YEAR TOTAL
Personnel	$312,518	$324,076	$335,890	$284,528	$294,304	$1,551,316		$2,746,143
Travel	$21,826	$21,826	$21,826	$21,826	$21,826	$109,130		$218,260
Contractual	$0	$0	$0	$0	$0	$0		$91,848
Commodities	$39,000	$39,000	$39,000	$39,000	$39,000	$195,000		$390,300
Equipment	$0	$0	$0	$0	$0	$0		$15,000
Indirect Costs (report rate here)	$0	$0	$0	$0	$0	$0		$0
SUBTOTAL	$373,344	$384,902	$396,716	$345,354	$355,130	$1,855,446		$3,461,551
General Administration (9% of subtotal)	$33,601	$34,641	$35,704	$31,082	$31,962	$166,990	N/A	$311,540
PROGRAM TOTAL	**$406,945**	**$419,543**	**$432,420**	**$376,436**	**$387,092**	**$2,022,436**		**$3,773,091**
Other Resources (In-Kind Funds)	**$142,857**	**$146,917**	**$151,092**	**$155,387**	**$159,804**	**$756,057**		**$1,413,461**

B. Sources of Additional Funding

Non-EVOSTC Funds to be used, please include source and amount per source:

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$124,245	$127,724	$131,396	$135,129	$138,910	$657,404
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
$142,857	$146,917	$151,092	$155,387	$159,804	$756,057
				FY22-31 Total	*$1,413,461*

Matching USGS funds include partial salary + benefits coverage for Paul Hershberger (20%), Maureen Purcell (10%), and Jacob Gregg (50%). Details are provided in Table 1.

Table 1. Proposed in-kind salary and benefit contributions to this project by the U.S. Geological Survey.

Personnel	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031
Hershberger	$38,580	$39,582	$40,639	$41,696	$42,723	$43,834	$44,974	$46,143	$43,343	$48,574
Purcell	$21,944	$22,549	$23,188	$23,837	$24,504	$25,190	$25,895	$26,620	$27,365	$28,131
Gregg	$63,721	$65,593	$65,569	$69,596	$71,683	$73,833	$76,048	$78,329	$80,679	$83,099
Total	**$124,245**	**$127,724**	**$131,396**	**$135,129**	**$138,910**	**$142,857**	**$146,917**	**$151,092**	**$155,387**	**$159,804**

9. LITERATURE CITED

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10. PROJECT PERSONNEL

Paul K. Hershberger, Ph.D.
U.S. Geological Survey - Marrowstone Marine Field Station
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Education:

Ph.D. Fisheries, University of Washington: 1998
M.S. Fisheries, University of Washington: 1995
B.S. Chemistry & Biology, Northland College (Manga Cum Laude): 1993

Recent Positions

2003 - Present: Station Leader & Research Fishery Biologist – USGS, Marrowstone Marine Station
2016 - Present: Affiliate Professor: School of Aquatic and Fishery Sciences, University of Washington.
2013 – 2014: President, American Fisheries Society, Fish Health Section

Ten Recent Publications Relevant to this Proposal:

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, M.D. Wilmot, R.L. Powers, M.K. Purcell. *Accepted*. Long-term shedding and asymptomatic carriers indicate that Pacific herring are a marine reservoir for viral hemorrhagic septicemia virus. Diseases of Aquatic Organisms.

Elliott, D.G., C.M. Conway, C.L. McKibben, A.H. MacKenzie, L.M. Hart, M.K. Purcell, J.L, Gregg. *In Press*. Differential susceptibility of Yukon River – and Salish Sea-origin Chinook Salmon *Oncorhynchus tshawytscha* to ichthyophoniasis. Diseases of Aquatic Organisms.

Hershberger, P.K., M. Stinson, B. Hall, J.L. Gregg, A.M. MacKenzie, J.R. Winton. 2020. Pacific herring are not susceptible to vibriosis under laboratory conditions. Journal of Fish Diseases 43: 1607-1609.

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Burge, C.A., P.K. Hershberger. 2020. Chapter 5: Climate change can drive marine diseases. pp. 83-94 *In:* Marine Disease Ecology. and Donald C. Behringer, Brian R. Silliman, and Kevin D. Lafferty, (Eds.) Oxford University Press. New York.

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Lowe, V.C., P.K. Hershberger, C.S. Friedman. 2018. Analytical and diagnostic performance of a qPCR assay for *Ichthyophonus* spp. compared to the tissue explant culture 'gold standard'. Diseases of Aquatic Organisms 128: 215-224.

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Five Additional Publications

Travis, B.A., W.N. Batts, M.L. Groner, P.K. Hershberger, S. Fradkin, C.M. Conway, L. Park, M.K. Purcell. *Accepted*. A novel diagnostic tool for the putative agent of bacterial gill disease in razor clams (*Siliqua patula*). Journal of Invertebrate Pathology.

Losee, J.P., W.N. Batts, S.R.M. Jones, C.A.E. McKinstry, P.K. Hershberger. 2020. Anadromous coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) as a host for *Argulis pugettensis* (Crustacea, Branchiura): parasite prevalence, intensity, and distribution. Northwest Science 94: 111-117.

Hershberger, P.K., R.L. Powers, B.L. Besijn, J. Rankin, M. Wilson, B. Antipa, J. Bjelland, A.H. MacKenzie, J.L. Gregg, M.K. Purcell. 2019. Intra-Annual Changes in Waterborne *Nanophyetus salmincola*. Journal of Aquatic Animal Health 31: 259-265.

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

B. Antipa, B. Berejikian (NOAA), R. Brenner (ADFG), C. Burge (U. Maryland), C. Closek (Penn State U.), C. Dykstra (IPHC), D. Elliott (USGS), K. Einer-Jensen (Danish National Veterinary Institute), D. Elliott (USGS), E. Emmenegger (USGS), B. Harris (APU), L. Hart (NWSC), C. Friedman (U. Washington), L. Fuess (U. Texas – Arlington), S. Fradkin (NPS), K. Garver (DFO), J. Hansen (USGS), C.D. Harvell (Cornell U.), S. Jones (DFO), R. Kocan (UW-SAFS), G. Kurath (USGS), E. LaDouceur (Sea World), N. Lorenzen (Danish National Veterinary Institute), L. Lossee (WDFW), J. Lovy (New Jersey F&W), D. Lowry (WDFW), T. Meyers (ADF&G), K. Miller (DFO), S. O'Neil (WDFW), T. Otis (ADFG), L. Park (NOAA), L. Rhodes (NOAA), T. Sandell (WDFW), M. Schmidt (LLTK), M. Wilson (WDFW), J. Winton (USGS), N. Wolf (APU).

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EDUCATION

University of Washington	Seattle, WA	Aquatic and Fisheries Sciences	Ph.D. 2005
University of Maine	Orono, ME	Zoology	M.S. 1997
Washington State University	Pullman, WA	Zoology	B.S. 1993

EMPLOYMENT

Apr 2017 to present: Chief, Fish Health Section Chief / Supervisory Research Microbiologist; US Geological Survey – Western Fisheries Research Center (USGS WFRC), Seattle, WA

2013 to present: Affiliate Associate Professor; School of Aquatic and Fisheries Sciences (SAFS), University of Washington, Seattle, WA

Jan 2007 to Mar 2017: Research Microbiologist; USGS WFRC, Seattle, WA

Dec 2005 to Dec 2007: Microbiologist; USGS WFRC, Seattle, WA

Aug 2000 to Nov 2005: Graduate Student, SAFS, University of Washington, Seattle, WA.

PUBLICATIONS RELATED TO PROPOSAL

Elliott D.G., C.M. Conway, C.L. McKibben, A.H. MacKenzie, L.M. Hart, M.L. Groner, **M.K. Purcell**, J.L. Gregg, P.K. Hershberger. 2021. Differential susceptibility of Yukon River and Salish Sea stocks of Chinook salmon *Oncorhynchus tshawytscha* to ichthyophoniasis. Dis. Aquat. Org. *in press.*

Hart, L.M., A. MacKenzie, **M.K. Purcell**, R.L. Powers, and P.K. Hershberger. 2017. Optimization of a plaque neutralization test (PNT) to identify the exposure history of Pacific Herring to viral hemorrhagic septicemia virus (VHSV). J. Aquat. Anim. Health 29(2): 74-82.

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Purcell, M.K., S. Pearman-Gillman, R.L. Thompson, J.L. Gregg, L.M. Hart, J.R. Winton, E.J. Emmenegger, and P.K. Hershberger. 2016. Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of quantitative real-time PCR assays for quantification of ENV DNA. J. Vet. Diagn. Invest. 28(4): 382-391.

Gregg, J.L., R.L. Powers, **M.K. Purcell**, C.S. Friedman, and P.K. Hershberger. 2016. *Ichthyophonus* parasite phylogeny based on ITS rDNA structure prediction and alignment identifies six clades, with a single dominant marine type. Dis. Aquat. Org. 120(2): 125-141.

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OTHER PUBLICATIONS

Purcell, M.K., R.L. Powers, J. Evered, J. Kerwin, T.R. Meyers, B. Stewart, and J.R. Winton. 2018. Molecular testing of adult Pacific salmon and trout (*Oncorhynchus* spp.) for several RNA viruses demonstrates widespread distribution of piscine orthoreovirus in Alaska and Washington. J. Fish Dis. 41(2): 347-355.

Breyta, R., I. Brito, P. Ferguson, G. Kurath, K.A. Naish, **M.K. Purcell**, A.R. Wargo, and S. LaDeau. 2017. Transmission routes maintaining a viral pathogen of steelhead trout within a complex multi-host assemblage. Ecol. Evol. 7(20): 8187-8200.

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Hutchins P.R., A.J. Supulveda, H. Hartikainen, K.D. Staigmiller, S.T. Opitz, R.M. Yamamoto, A. Huttinger, R. Cordes, T. Weiss, L. Hopper, **M.K. Purcell**, and B. Okamura. 2021. Exploration of the 2016 Yellowstone River fish kill and proliferative kidney disease in wild fish populations. Ecospheres. *in press.*

COLLABORATORS PAST 4 YEARS

Last Name	First Name	Affiliation	Last Name	First Name	Affiliation
Bader	Joel	USDA	LaDeau	Shannon	CARY INSTITUTE
Blair	Marlyn	USFWS	Martin	Barbara	USGS
Bowers	Jim	KING COUNTY	McKenney	Doug	USGS
Breyta	Rachel	U. WASHINGTON	Meyers	Ted	ADF&G
Burdick	Summer	USGS	Naish	Kerry	U. WASHINGTON
Chase	Dorothy	USGS	Okamura	Beth	UK
Cordes	Rick	USFWS	Opitz	Scott	MONTANA STATE
Creekmore	Lynn	USDA	Ostberg	Carl	USGS
Elliott	Diane	USGS	Paez	David	U. WASHINGTON
Evered	Joy	RETIRED	Palti	Yniv	USDA
Ferguson	Jayde	ADF&G	Park	LInda	NOAA
Fradkin	Stephen	NPS	Polinski	Mark	DFO
Garver	Kyle	DFO	Powers	Rachel	USGS
Goodwin	Andrew	USFWS	Rhodes	Linda	NOAA
Gregg	Jacob	USGS	Sfomo	Todd	NORTH SLOPE BOROUGH
Groner	Maya	PWSSC	Snekvik	Kevin	WASH STATE UNIV
Gustafson	Lori	USDA	Staigmiller	Ken	MONTANA STATE
Gutenberger	Susan	USFWS	Stewart	Bruce	NWIFC
Hansen	John	USGS	Supulveda	Adam	USGS
Hartikainen	Hanna	UK	Taksdal	Torunn	NORWAY
Hershberger	Paul	USGS	Travis	Brook	CORNELL
Hopper	Lacey	USFWS	Warg	Janet	USDA
Hutchins	Patrick	USGS	Wargo	Andrew	VIMS
Kerwin	John	RETIRED	Warheit	Ken	WDFW
Kurath	Gael	USGS	Whaley	Janet	NOAA
Kyle	David	TROUT UNLIMITED	Winton	Jim	USGS

United States Department of the Interior

U.S. GEOLOGICAL SURVEY

July 29, 2021

To: Mandy Lindeberg - NOAA, GWA-LTRM Program Lead
Shiway Wang, EVOSTC Executive Director

Re: Letter of Commitment

We are pleased to provide this letter of commitment for the proposed project "*Herring Disease Program, 22120111-E*" led by principal investigator (PI), Paul Hershberger. This proposal was drafted by the PI in response to the EVOSTC's FY22-31 Invitation for Proposals and subsequent request for final submission on August 13, 2021. The cost for this project over a ten-year period will be $3,461,091 (without EVOSTC GA). This includes some non-EVOSTC funds that are in-kind contributions we support totaling an estimated $1,413,461 for the life of the project (e.g., salaries of permanent staff, commodities, and equipment use).

This project proposal is part of the larger multi-agency Gulf Watch Alaska Long-Term Research and Monitoring (GWA-LTRM) program proposal package. This package represents a continued commitment of the successful long-term research and monitoring projects supported by the EVOSTC and various agencies and organizational investments, including the Herring Disease Program since 2007.

USGS funds included as in-kind or as contributions are included for planning purposes only and nothing contained in this proposal shall be construed as binding USGS to expend in any one fiscal year any sum in excess of its appropriations or funding in excess or what it has received for the collaborative work outlined in this proposal or involving the Federal government in any obligation to pay money before funds have been appropriated for that purpose unless otherwise allowed by law.

Sincerely,

Eric Janney

Eric Janney
Center Director (Acting)
U.S. Geological Survey - Western Fisheries Research Center
ecjanney@usgs.gov
Phone: (541) 273-8689, Ext 202