EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
FY21 CONTINUING PROJECT PROPOSAL SUMMARY PAGE

Project Number and Title

21120111-A

Herring Research Program – Program Coordination

Primary Investigator(s) and Affiliation(s)

W. Scott Pegau, Prince William Sound Science Center

Date Proposal Submitted

August 14, 2020

Project Abstract

This proposal is to provide coordination of the Herring Research and Monitoring (HRM) program. In addition to the coordination efforts, it includes a postdoctoral researcher examining the relationships between herring diseases and physical and biological oceanographic conditions. Furthermore, it covers the community involvement and outreach activities of the program. The goal of the project is to provide coordination within the HRM program and with the Gulf Watch Alaska (GWA) and Data Management (DM) programs. The objectives of the project are:

1) Coordinate efforts among the HRM projects to achieve the program objectives, maximize shared resources, ensure timely reporting, and coordinate logistics.
2) Oversee a postdoctoral researcher.
3) Provide outreach and community involvement for the program.

Coordination is primarily through e-mail and teleconference. The management team of GWA and the lead of DM are included in the emails to HRM principal investigators to ensure they are aware of our activities. We also plan joint principal investigator meetings and community involvement activities.

The postdoctoral researcher, Dr. Maya Groner, was hired at the end of FY17 and is focusing her research on understanding the combined impacts of environmental conditions and disease on herring population dynamics using a field collected data, experiments and population models.

Outreach efforts are focused on providing up-to-date information on the projects and their findings. Community involvement includes regular communications with the Alaska Department of Fish and Game, to stay aware of their findings and observations. We also are participating with GWA in listening sessions in villages within the spill affected to seek additional local and traditional ecological knowledge.

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

FY17	FY18	FY19	FY20	FY21	TOTAL
$138,400	**$270,200**	**$302,500**	**$270,200**	**$159,600**	**$1,140,900**

Non-EVOSTC Funds to be used, please include source and amount per source: (see Section 6C for details)

FY17	FY18	FY19	FY20	FY21	TOTAL
$26,000	**$26,600**	**$90,000**	**$90,500**	**$28,300**	**$240,800**

1. PROJECT EXECUTIVE SUMMARY

The goal of this proposal is to provide the coordination necessary to continue integrating the research and monitoring projects to achieve the program goals. The objectives of the project are:

1) *Coordinate efforts among the Herring Research and Monitoring (HRM) projects to achieve the program objectives, maximize shared resources, ensure timely reporting, and coordinate logistics.*
2) *Oversee a postdoctoral researcher.*
3) *Provide outreach and community involvement for the program.*

Coordinate efforts among the HRM projects to achieve the program objectives, maximize shared resources, ensure timely reporting, and coordinate logistics

This project provides a program lead who works with the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) staff to ensure the EVOSTC's objectives and requirements are met. The coordinator facilitates the most cost-effective and scientifically supportive stream of funding, works with a program oversight panel to review proposals and reports and feedback on the program and individual projects, and other duties as identified in the invitation for proposals.

Coordination since the annual report has focused on the spring and summer sampling activities. These include the aerial and acoustic surveys of herring biomass, age-sex-length sampling of the herring biomass, and acoustic tagging of herring. The outbreak of COVID-19 resulted in restrictions in operations right at the beginning of the main sampling period for the HRM program. We were able to either gain permission to proceed with our sampling efforts or make alternative arrangements to collect the desired samples and data. We were able to complete all planned sampling through coordination among projects. For instance, the tagging project was able to bring back live herring for others in the program to process for the disease sampling project (20120111-E) because personnel from that project were unable to travel to sample themselves.

We participated in the Gulf Watch Alaska (GWA) teleconferences and with the Data Management (DM) program to make more data available through the Alaska Ocean Observing System (AOOS) Gulf of Alaska data portal. We worked with GWA to set the date of the fall principal investigator (PI) meeting. The HRM meeting will be November 17th in Cordova to allow PIs to collaborate with GWA investigators and DM team members. We are also considering the potential need for a video meeting if travel restrictions continue.

A synthesis of herring research was presented to the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) Public Advisory Committee and Science Panel. Five HRM investigators were asked to present findings from the synthesis. A general overview was provided by Dr. Pegau. Mr. Haught presented on sampling protocols used by the various monitoring projects, Dr. McGowan on the changes in spawn timing and location, Dr. Hershberger on the disease research, and Dr. Bishop on the herring tagging findings to date. While not all investigators presented their work, each of the chapters included information from multiple investigators so the presentations covered the breadth of the work underway. We received comments from the EVOSTC Science Panel on the written synthesis report and have been working to address those comments in the final draft of the synthesis document.

We were also funded by the Prince William Sound Regional Citizens' Advisory Council for aerial forage fish surveys that provide an indicator of the age-1 herring abundance. The data were used to support the GWA program's forage fish project (20120114-C). During June 2020, the count of age-1 herring schools was relatively low (Fig. 1). Validation efforts were conducted with vessels associated with the GWA forage fish project (project

20120114-C). There were issues finding fish or being able to sample schools this year that limited the number of validations completed.

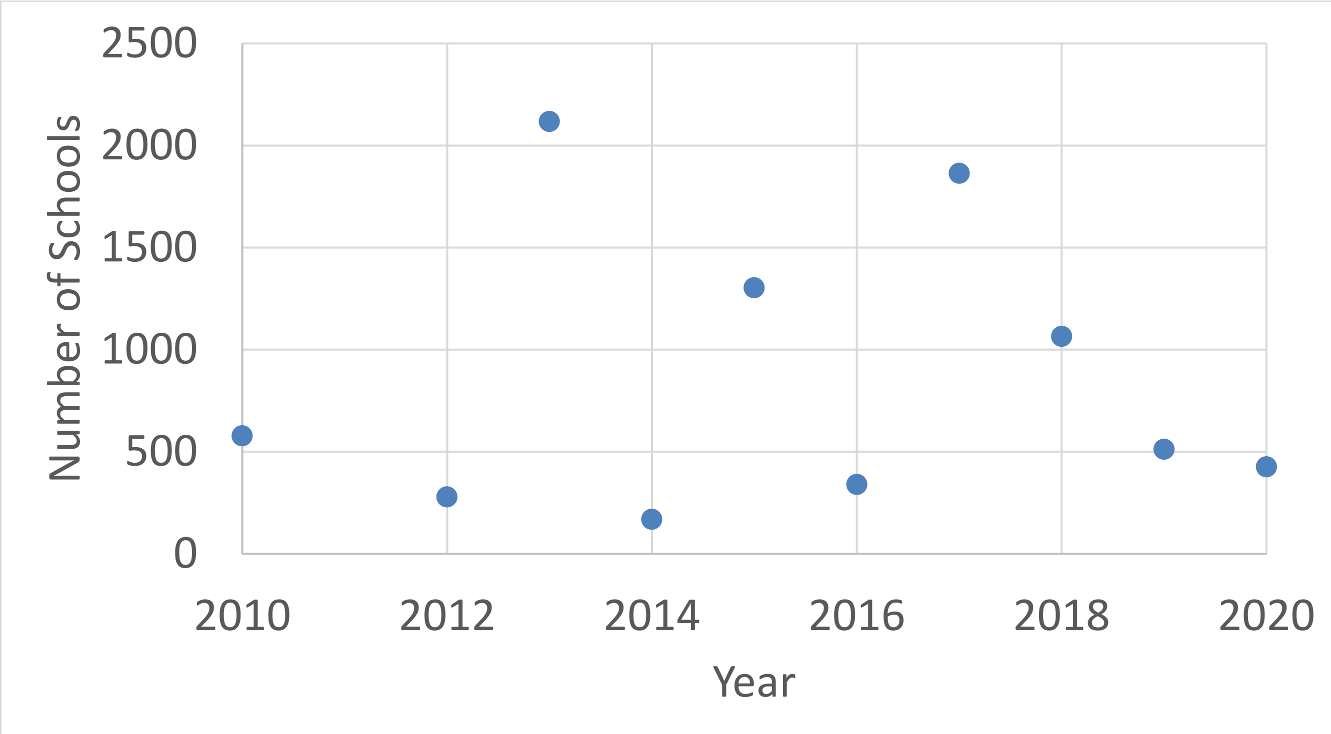

Figure 1. Number of schools of age-1 herring observed during aerial surveys of forage fish in Prince William Sound. The survey in 2011 was incomplete and results are not provided. Data are from projects 10100132-F (2010-2012) and 20120111-A.

Oversee a postdoctoral researcher

The project supported a post-doctoral researcher examining herring diseases. Dr. Maya Groner started at the beginning of the second year of the current 5-year funding cycle and is primarily collaborating with Dr. Hershberger in Washington. Research conducted to date has been focused on three topics: examining and modeling patterns of *Ichthyophonus* in herring, modeling the impact of viral hemorrhagic septicemia (VHS) on herring populations, and experimentally examining patterns of viral erythrocytic necrosis (VEN) infection across herring age classes.

The first goal of the herring diseases/environmental conditions post-doctoral study is to experimentally quantify the relationship between *Ichthyophonus* sp. pathogen load, host response, and mortality by using specific pathogen free herring at the U. S. Geological Survey (USGS) Marrowstone Laboratory. Replicate tanks (2 per treatment) of age-4 herring were exposed to *Ichthyophonus* via consumption of offal from ground up infected conspecifics or control offal from ground up uninfected conspecifics. Mortality, infection status, size, and cardiac tissue samples were collected from all tanks (Fig. 2). Pathogen load and host response has been quantified in dead, moribund, and subsamples of healthier fish through quantitative image analysis of histologically sectioned cardiac tissue (Fig. 3). These data demonstrate that herring tolerate higher parasite densities with increasing time since exposure. Increases in the host response to infection (e.g., by encapsulating *Ichthyophonus* schizonts with dense granulomas) suggest a mechanism for the change in tolerance (Fig. 4).

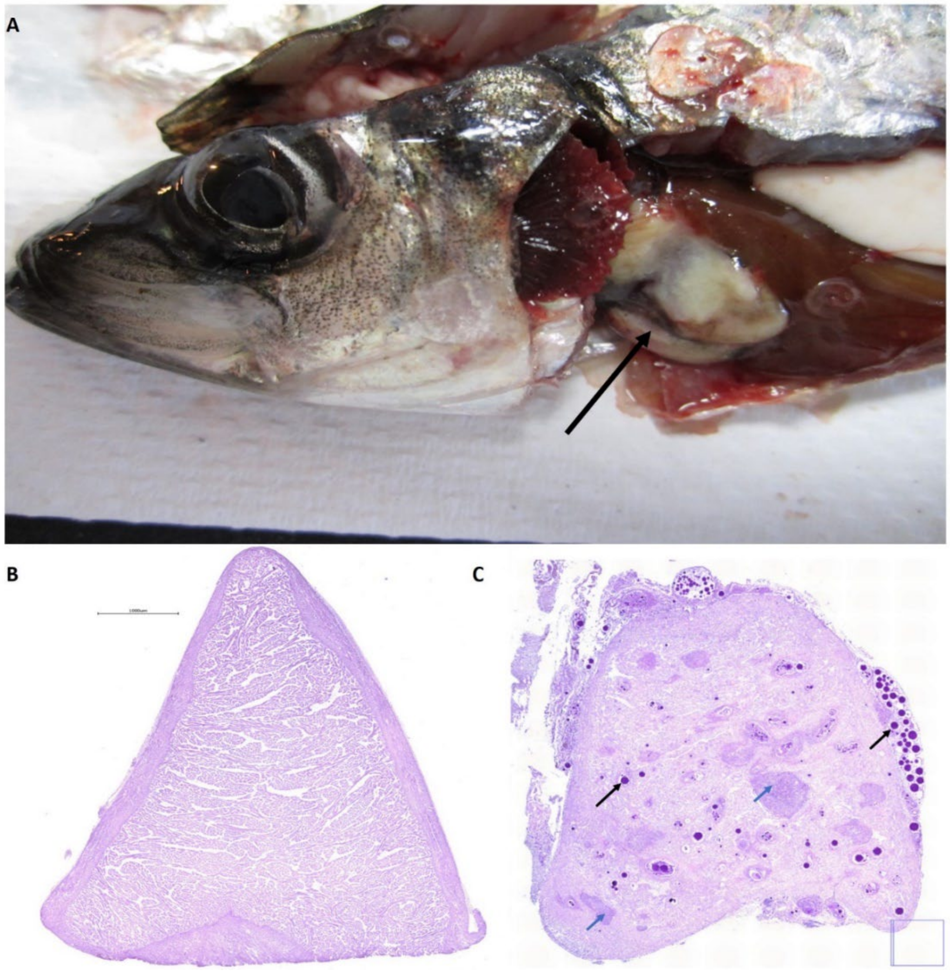

Figure 2. Hearts heavily infected with *Ichthyophonus* sp. can appear white instead of dark red (arrow) (A). Histological sections of a healthy herring ventricle (B) next to an infected ventricle (C). On the infected ventricle, the dark purple circles (black arrows) indicate *Ichthyophonus* sp. schizonts. Granulomas of darker staining tissue surround some of the schizonts (blue arrows).

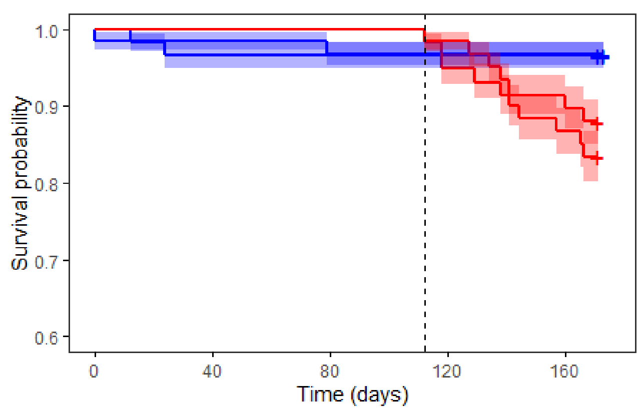

Figure 3. Survival of Pacific herring exposed to *Ichthyophonus* (red) or a control (blue). Initial exposures were done through consumption of ground up offal from diseased or healthy herring, however that did not cause mortality in exposed fish. On day 112 (dotted line) herring received intraperitoneal injections with *Ichthyophonus* or a control solution.

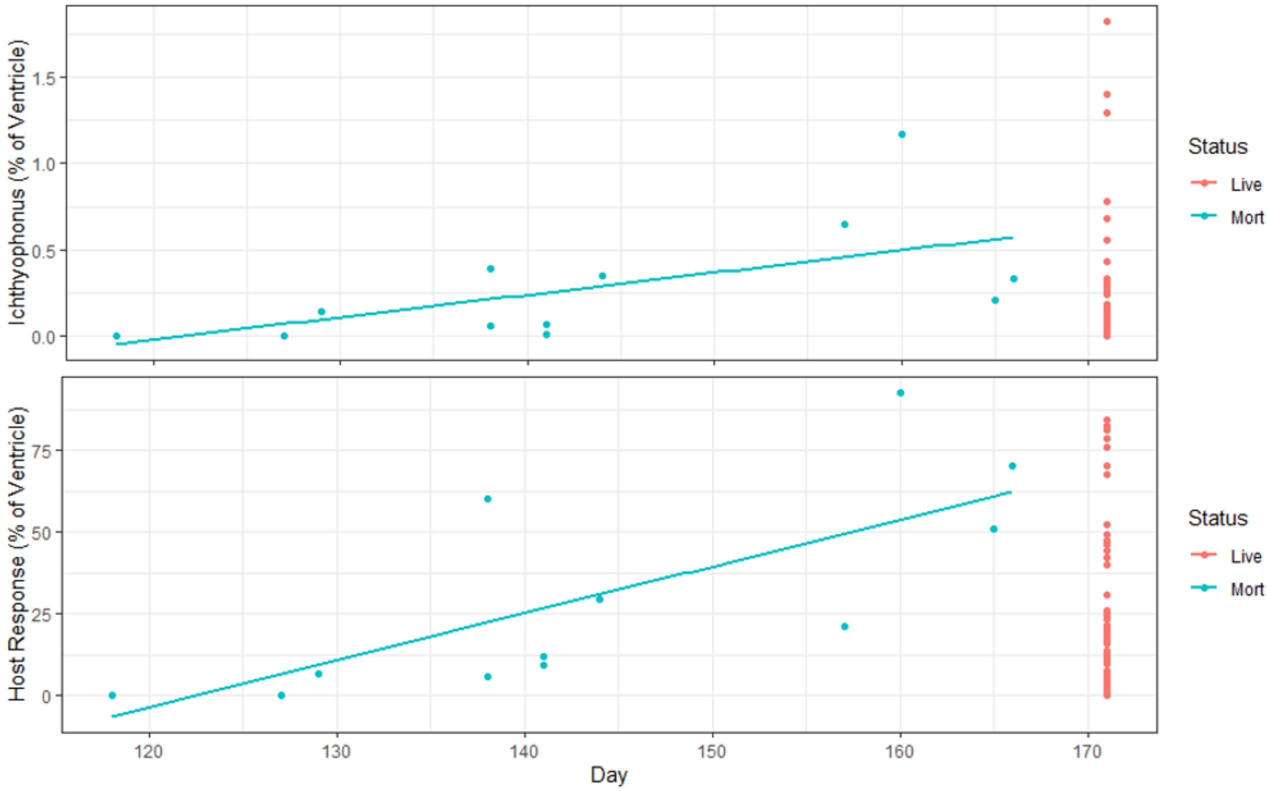

Figure 4. *Ichthyophonus* infection load and host response over time in age-4 herring that died during experiment (blue) and live herring sampled at the end of the experiment (pink). The lethal infection load increased with time since exposure. Exposure (via intraperitoneal injection) occurred on experiment day 112).

A second part of our work on *Ichthyophonus* has been to quantify infection loads in cardiac tissue across sizes classes Pacific herring collected at two sites, Sitka Sound and Prince William Sound (PWS), from 2009-2019 (180 hearts/ site/ year). This study expands on presentations of this data that examined pathogen presence/absence was reported across size classes (e.g., Hershberger et al. 2016). Examination of parasite density (# parasites/ area of ventricle observed) across age classes will allow us to determine if observed interannual differences in natural mortality can be attributed to *Ichthyophonus*. This work is motivated by shifting patterns in disease prevalence across fish size and an increase in observations of severely diseased herring particularly in Sitka Sound after 2013. We have finished processing, sectioning, staining, and imaging all hearts (>800). Moreover, all fish have been assigned an age based on a series of age-length keys that were calculated by using data from the Alaska Department of Fish and Game (ADF&G). The age-length distribution varied enough across years that unique age-length keys were calculated for each year and each site. These keys were weighted by the age structure of the populations as estimated by their respective age-structured assessments (Fig. 5). Generalized linear models are being run to identify potential demographic and environmental correlates of disease prevalence and severity (Fig. 6). This modelling work is in preliminary stages, however, early results show positive correlations between prevalence of *Ichthyophonus* in PWS and fish age, the estimated biomass of fish age-3 and older (from the age-structured assessment model), and the North Pacific Gyre Oscillation. Rob Campbell (GWA PI) is providing additional data on temperature and plankton community structure within PWS that will be included into these statistical models. Next steps on this project include publishing the results of the *Ichthyophonus* experiment and the statistical analyses of the historical dataset, and developing a mathematical model to estimate mortality of herring due to *Ichthyophonus* based on the age-structure and observed disease prevalence in these fish. This work has been partially facilitated by a North Pacific Research Board (NPRB) grant awarded to Groner and Hershberger.

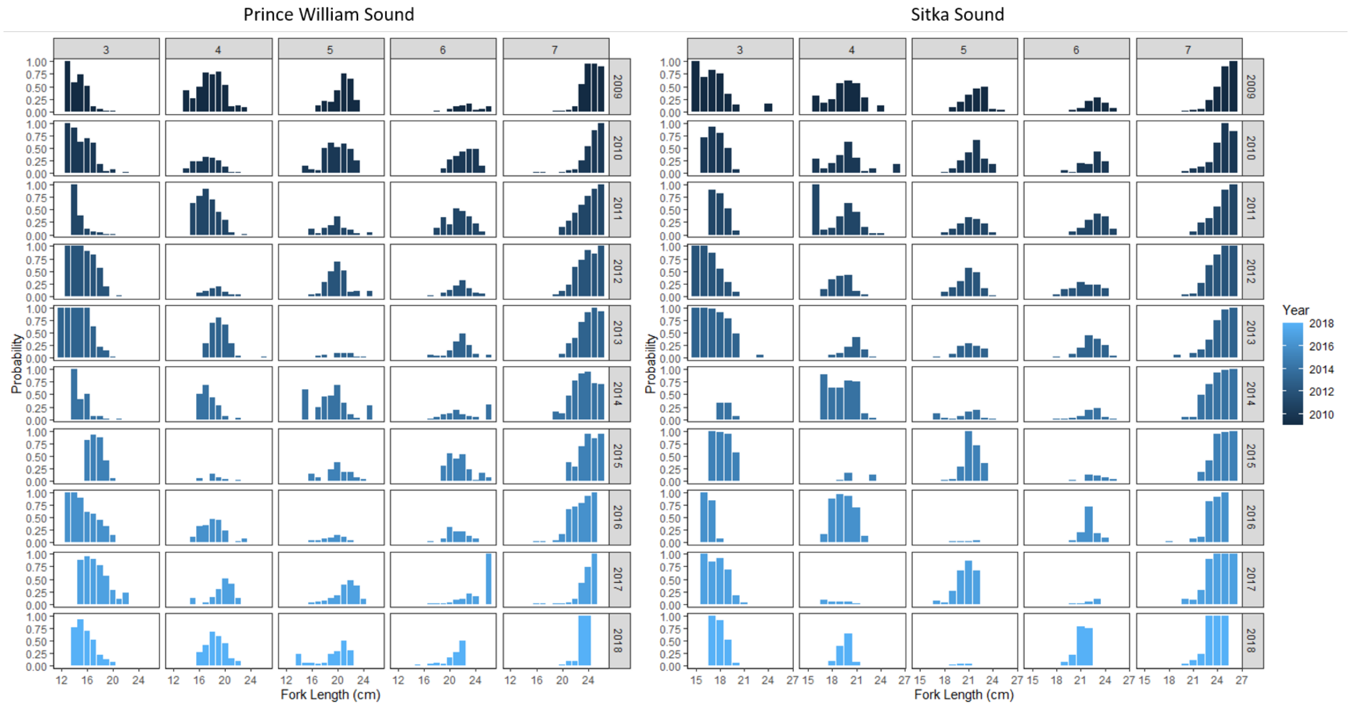

Figure 5. Weighted age-length keys for Pacific Herring in Prince William Sound and Sitka Sound. Ages (3-7) are shown in the top row. Standard length measurements provided by ADF&G were converted to fork length measurements prior to making this key. Herring sampled for the disease survey were measured for fork length.

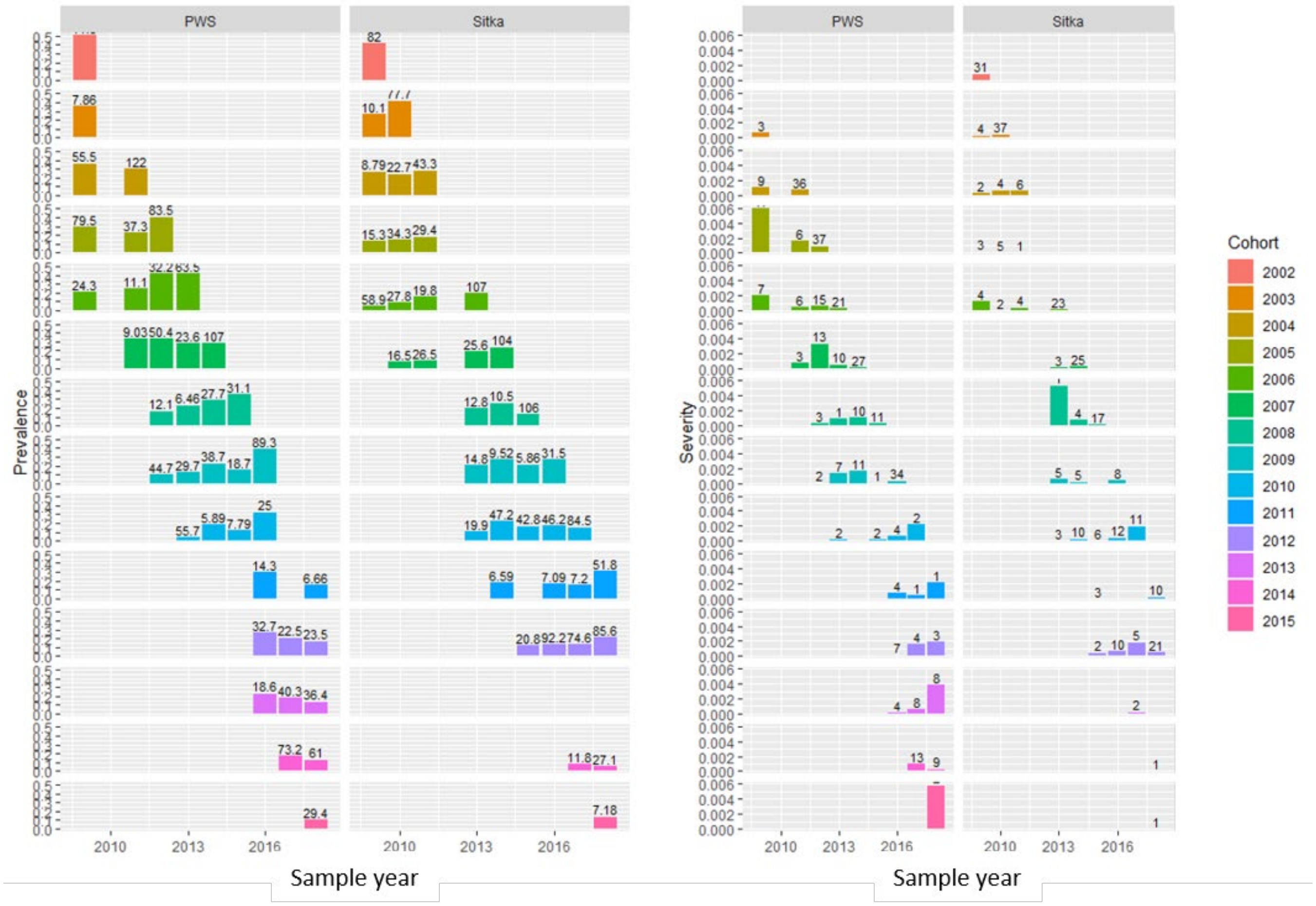

Figure 6. Prevalence and severity of *Ichthyophonus* infections in Prince William Sound for each cohort of Pacific herring observed between 2009 and 2018. Colors indicate unique cohorts of fish, while the years on the x-axis indicate the year the cohort was sampled. Estimated sample sizes for each age class are above the corresponding colored bar. The age estimations for most herring indicated that they had probabilities of being in several age classes. To account for this, prevalence values for each age class were weighted by their probability that a fish was in that age class. For the severity data, ages were assigned to each fish based on the probability distribution in the weighted age length key. Herring do not recover from *Ichthyophonus* infections, so the expectation is that prevalence will increase with increasing age within a cohort. Plateauing and/or decreasing prevalence across age within a cohort indicates that *Ichthyophonus* may be causing mortality. Decreases in severity across age within a cohort may indicate that the most severely infected individuals are dying and/or that new infections with low severity may be occurring. Mathematical models will be used to quantify mortality due to *Ichthyophonus* in FY 2021.

A second project pursued by Dr. Groner is to mathematically model VHS in herring populations. The model is designed to examine the impact that variable recruitment and fishing might have on dynamics of VHS across years. Herring immune function is suppressed at colder temperatures, which can result in increased mortality and more rapid spread of VHS. Fish that survive VHS infections have immunological memory; they produce antibodies to VHS infection that prevent them from getting infected again. Thus, fish that survive VHS outbreaks can dampen future disease dynamics by acting as dead ends in the transmission process. This is called herd immunity. This benefit can be compromised if fishing targets older fish (which are more likely to have immunity). Our model examines these interactions using a series of differential equations.

The framework is based on a classic SEIR model (Anderson and May 1991). This model is meant to imitate the dynamics of a directly transmitted parasitic pathogen in a population of herring, which is dependent on the prevalence of the VHS susceptible herring. The structure for our model is as follows:

$$dS/dt = (b - cN)N - (\mu + f + \lambda)S \quad (1)$$

$$dE/dt = \lambda S - (\mu + f + \gamma)E \quad (2)$$

$$dI/dt = \gamma E - (\mu + f + \alpha + \rho)I \quad (3)$$

$$dC/dt = \rho I - (\mu + f)C \quad (4)$$

where the state variables *S*, *E*, *I*, and *C* represent the numbers of susceptible, exposed (with latent infections), infected, and immune carriers, respectively. The size of the total host population (*N*) is given by $N = S + E + I + C$. Recruitment is indicated by b and c is the strength of density dependence. Terms μ, f, γ, λ, and ρ denote natural mortality, fishing catch, transmission rate, force of infection, and recovery rate, respectively. Here, newborns are susceptible to infection and survivors of the infection are considered immune for life. Model parameters have been estimated from experiments run by Paul Hershberger (Hershberger et al. 2010). A variety of time-dependent fishing (*f*) and recruitment rates (*b*) (ranging from a single pulse to a constant 'press') are used to examine the impact of timing of these events on disease dynamics.

The third ongoing project involves a series of experiments examining interactions between age and susceptibility of herring to VEN. In 2019, an experiment was conducted in which replicate tanks (3) of mixed aged herring (age 0, 1, 2, or 4) were exposed to erythrocytic necrosis virus (ENV, the causative agent of the disease VEN) or a control (sham) inoculum. Herring were tagged with elastomer tags to identify them to cohort. Mortality and water quality were monitored for two months and kidney samples were collected in fish after death and at the end of the experiment to determine infection status. For reasons that remain unknown, we did not observe substantial mortality or disease in this experiment. After this experiment ended, we ran two pilot trials to examine the virulence of archived VEN inocula. The inocula caused mortality in herring that were injected with the virus or exposed through waterborne virus. We then used this inoculum in a VEN experiment in 2020. The goal of the 2020 experiment was to quantify pathogen load, and immune responses (using gene expression) of herring to infection during different stages of the infection, including the incubation stage, the proliferation stage, and the viral shedding stage (Fig. 7). Similar to 2019, infection and mortality did not occur in this experiment. These results indicate that further work is required to standardize the VEN in vivo challenge model. At this point, we are going to hold off on repeating the experiments described above until we have a better challenge model.

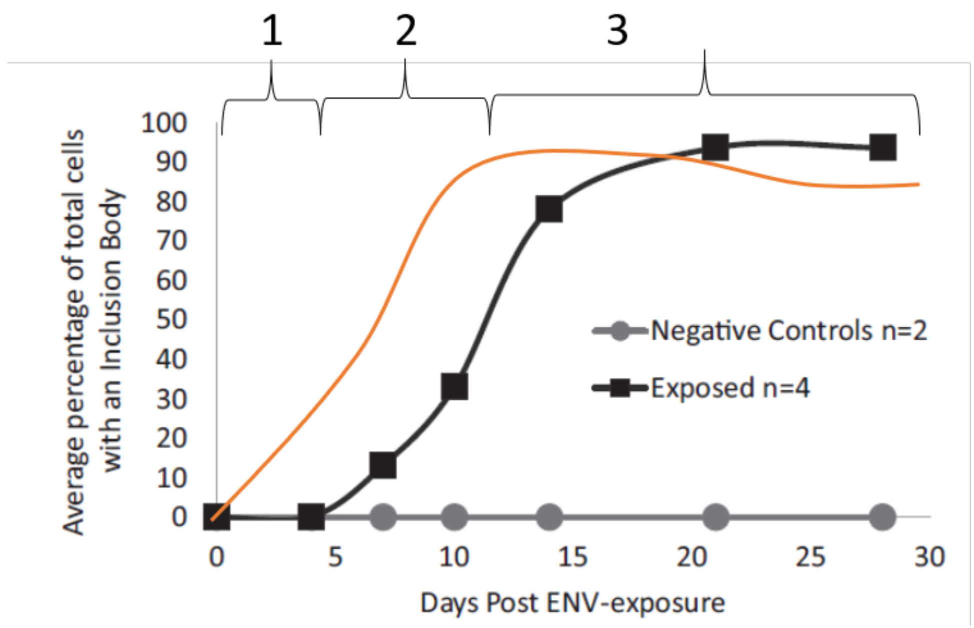

Figure 7. Typical time course of infection (from Glenn et al. 2012). Orange curve represents typical concentrations of ENV DNA in kidneys (determined from a reaction called quantitative PCR or qPCR) (based on Purcell et al. 2016), while the black curve includes real data on the percent of total erythrocytes (red blood cells) with an inclusion body (a visual indication that the cell is infected with ENV). Maximum qPCR load for ENV is about 10^7 copies per qPCR reaction. The stages of the infection include (1) incubation stage, (2) proliferation stage, and (3) period of illness and viral shedding.

In addition to her focal projects, over the past year, Dr. Groner has been working closely with John Trochta and Dr. Trevor Branch (project 20120111-C) on a series of models designed to evaluate how VHS antibody data can be used to make inference about previous outbreaks.

Dr. Groner has participated in several manuscripts that are tangentially related to this project including collaborations on (1) a book chapter on marine disease modeling (Ben-Horin et al. 2020), (2) a manuscript reviewing how management of marine resources (i.e., fisheries, marine protected areas, and rehabilitation) can indirectly alter host-pathogen interactions (in prep), (3) a methods paper on how to use coupled biophysical models to quantify the dispersal of marine pathogens (Cantrell et al. 2020) (4) a paper lead by Dr. Hershberger that evaluates patterns of *Ichthyophonus* infections in herring in Puget Sound, Washington (Hershberger et al. 2019), and (5) a paper led by Dr. Hershberger that compares immunological responses to *Ichthyophonus* infections in susceptible and resistant stocks of Chinook salmon (in prep). The book chapter was published in a textbook on marine diseases (Marine Disease Ecology) that was released earlier this year. Dr. Groner's contribution is focused on how to infer epidemiological processes by examining and modeling distributions of parasites within hosts. The theory in this chapter underlies her current work being applied to quantifying and interpreting *Ichthyophonus* intensities in herring hearts. The review paper (2) and methods paper (3) use examples of Pacific herring diseases to illustrate how fishing practices may alter disease processes, including size-selective harvests that may inadvertently target herring infected with *Ichthyophonus* and the spawn-on-kelp fishery which has been associated with shedding of VHS. Dr. Groner analyzed data and created figures for (4) and (5). These studies yield insight into patterns and processes associated with *Ichthyophonus* and VHS infections within and among individuals.

Provide outreach and community involvement for the program

Work within the project also includes support for outreach activities. We focused on updating the website to ensure current findings are being presented. Five short research articles were written for the 2020-2021 edition of Delta Sound Connections (https://pwssc.org/wp-content/uploads/2020/07/DSC-2020-web.pdf). We were in communications with people in Tatitlek to find an appropriate time to meet with them for a listening session for us to learn from their knowledge. That discussion ended at the start of the COVID-19 outbreak. We look forward to re-establishing that discussion and finding a date to visit.

2. PROJECT STATUS OF SCHEDULED ACCOMPLISHMENTS

A. Project Milestones and Tasks

Table 1. Project milestone and task progress by fiscal year and quarter, beginning February 1, 2017. Additional milestones and/or tasks have been added in red. Yellow highlight indicates proposed fiscal year Work Plan. Additional milestones and tasks may be added. C = completed, X = not completed or planned. Fiscal Year Quarters: 1= Feb. 1-April 30; 2= May 1-July 31; 3= Aug. 1-Oct. 31; 4= Nov. 1-Jan 31.

	FY17				FY18				FY19				FY20				FY21			
Milestone/Task	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Postdoctoral Researcher																				
Request proposals for postdoctoral researchers	C																			
Select project to include in FY18 proposal submission		C																		
Hire postdocs				C																
HRM Website																				
Annual update				C				C				C				X				X
Management																				
Ensure previous FY project data submission to workspace/AOOS		C				C					C				C				X	
Ensure project annual reports submitted					C				C				C				X			
Reporting																				
NOAA semi-annual report			C		C		C		C		C		C		C		X		X	
Annual reports					C				C				C				X			
FY work plan (DPD)			C				C				C				C				X	
EVOSTC Joint Science Workshop Report												C								
Draft FY17-21 Final Report																				X
Meetings & Conferences																				
Annual PI meeting				C				C				C				X				X
EVOSTC Joint Science Workshop													C							
Postdoc Milestones																				
(1) Wild herring disease assessments in Sitka and PWS						C				C				C				X		
(2) Experimentally investigate effects of VEN on different age classes							X	X	X											

Milestone/Task	**FY17**				**FY18**				**FY19**				**FY20**				**FY21**			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
(3) Experimentally investigate effects of temperature on susceptibility to ENV and mortality associated with infection								X	X	X										
(4) Publish manuscript on age-dependency and seawater temperature dependency of VEN in Pacific herring											X									
(5) Experimentally quantify Ichthyophonus mortality as a function of infection intensity						C	C	C												
(6) Quantify Ichthyophonus infection intensity in histological samples of cardiac tissue from PWS and Sitka 2009-2018							C	C	C	C										
(7) Publish manuscript on Ichthyophonus severity in PWS and Sitka from 2009-2018, comparing results to experimental infections																x				
(8) Model population-level effects of Ichthyophonus																	x	x	x	
(9) Publish Ichthyophonus model																			x	
(10) Examine relationships between food quality and disease susceptibility (bioenergetic-disease hypothesis) for Ichthyophonus, VHS and VEN												X	X							
(11) Publish on bioenergetics disease hypothesis														X	X					
(12) If data is sufficient, analyze relationships between environmental factors and prevalence of Ichthyophonus and VHS in PWS and SItka													C	C	C	x	x	x	x	
(13) Publish on environmental correlates of disease analysis																			x	
(14) *Pending funding:* Estimation of VHS and *Ichthyophonus* transmission rates																	x	x	x	x

B. Explanation for not completing any planned milestones and tasks

Two experiments have been conducted to investigate impacts of VEN on Pacific Herring. The first examined the impacts of VEN on herring of different age classes and the second was designed to examine effects of VEN infection on gene expression in herring. Gene expression (transcriptomics) analyses allow for investigation of numerous processes including stress response and immune function. We conducted these experiments using two different ENV inocula. While we detected low levels of ENV in our experimental tanks by qPCR, we did not see many signs of disease (more detail provided in the executive summary.) We are continuing to evaluate new and archived ENV inocula before we continue with this work.

A combination of both the time spent troubleshooting the challenges with the VEN inoculation experiments, and subsequent limitations in lab accessibility due to the COVID-19 pandemic have set us back in our efforts in the laboratory. We were not able to conduct experiments related to bioenergetics and disease susceptibility in herring (goals 11 and 12). Research efforts have instead been focused on developing a VHS model and analyzing the *Ichthyophonus* historical data set (described above). Continuation of disease modeling and finishing manuscripts will be the focus for FY21. If, during our evaluation of ENV inocula, we are able to develop an improved *in vivo* challenge model for ENV, we may also repeat the VEN/ ENV experiments described above.

C. Justification for new milestones and tasks

The new milestone is associated with a request for additional funding. Details of that request are provided in section 4B.

3. PROJECT COORDINATION AND COLLABORATION

A. Within an EVOSTC-Funded Program

Herring Research and Monitoring

This project provides the overall coordination between all projects within the HRM program, therefore is directly linked to each project. Dr. Pegau is the program team leader responsible for ensuring a coordinated and focused research program that leverages other assets whenever possible. Within program coordination is primarily through e-mail and phone communications. In-person meetings of participants occur in the fall of each year for exchange of information and to encourage collaboration between projects. Teleconference meeting are scheduled as needed.

Coordination occurs each spring when the main sampling period is being conducted to ensure each group is providing information to others about where they expect to be and what types of sampling is being done. This year we had to identify ways to collect data for projects unable to make it to the field due to COVID-19 concerns. This included age-sex-length (ASL) samples and samples for the disease prevalence measurements. We were able to work with a local fisherman and the other research cruises to collect samples for the ASL samples. They were also able to bring live fish to Cordova to sample for disease prevalence. Personnel from ADF&G and Prince William Sound Science Center (PWSSC) processed the live herring and sent the samples to the researchers from the United States Geological Survey. Further coordination occurred in ensuring aerial support for collection of herring and identification of schools for validation by the GWA forage fish project (project 20120114-C).

The projects within the HRM program worked together to develop and present a synthesis that described work by all except the lingering oil project. It was decided that results of the lingering oil project were too preliminary to justify including in the synthesis.

Gulf Watch Alaska & Data Management

This project provides the primary link between the HRM, GWA, and Data Management programs. Dr. Pegau participates in the GWA quarterly PI meetings and program management meetings. Mandy Lindeberg, the

GWA lead, and Dr. Carol Janzen, the Data Management lead, are included on the HRM email list so they are aware of what is going on in the HRM program. Administratively, the annual work plans and reports will continue to be developed together. We continue to plan joint PI meetings to encourage individuals to work with people in the other programs. We work together to design topics for analysis and development of joint scientific manuscripts. We work with the Data Management program to ensure timely submission of data and metadata.

B. With Other EVOSTC-funded Projects

We did not work with projects outside of the EVOSTC-funded programs.

C. With Trustee or Management Agencies

ADF&G is the primary trustee and management agency that the HRM program aims to serve. The success of the program is highly dependent on the information collected by ADF&G, so it is imperative that we work with the agency. We continue to have an ADF&G employee (Dr. Sherri Dressel) on our scientific oversight group. She remains our primary connection to the statewide herring program.

Drs. Groner and Hershberger have partnered with Sherri Dressel at ADF&G – Sitka to assess whether temporal changes in the severity of *Ichthyophonus* infections may be responsible for recent declines in the spawning herring biomass and age structure. Data and archived samples from the past 10 years of this EVOSTC-funded project were leveraged to obtain supplemental funding from the NPRB (# 1807: *Ichthyophonus* in Pacific Herring), for which ADF&G provided a letter of support. Stormy Haught (ADF&G – PWS) has also provided data relevant to this project.

4. PROJECT DESIGN

A. Overall Project Objectives

The original goals and objectives of the project are as follows. The **goal of this proposal is to provide the coordination necessary to continue integrating the research and monitoring projects** to achieve the program goals. The objectives of the project are:

1) *Coordinate efforts among the HRM projects to achieve the program objectives, maximize shared resources, ensure timely reporting, and coordinate logistics.*
2) *Oversee a postdoctoral researcher.*
3) *Provide outreach and community involvement for the program.*

The objectives have been modified to include the following that represent the work of the postdoctoral researcher that was selected.

1) *To quantify the role of VEN, VHS and Ichthyophonous in low recruitment and survival of Pacific herring*
2) *To evaluate the contribution of environmental (e.g. temperature) and biotic drivers of disease, particularly as they relate to decreased condition factor and disease susceptibility of juvenile herring*
3) *To provide estimates of the environmentally-dependent estimates of the effects of disease on Pacific herring recruitment to inform the Bayesian ASA model for Pacific herring assessment in PWS (Muradian et al. 2017)*
4) *Parameterize an age-structured disease model for Ichthyophonus sp. in Pacific herring.*

B. Changes to Project Design and Objectives.

Due to continuing challenges associated with transmitting VEN in the lab we have paused this work and experiments associated with disease and bioenergetics. We plan to pick up this work once we have an improved *in vivo* challenge model for VEN.

Both the VHS model development and *Ichthyophonus* characterization in Sitka and PWS have progressed on schedule. We are requesting additional funding to use existing data on these two diseases to quantify transmission processes in wild populations. See the project outlined below.

Request for additional funding for Maya Groner, PWSSC

Scope of work

Objective: Estimate transmission (force-of-infection) due to *Ichthyophonus* infections and VHS in Prince William Sound

Deliverables: One publication focused on age- and environmentally-dependent force of infection estimates for two diseases of Pacific Herring, viral hemorrhagic septicemia and *Ichtyophoniasis*

While recent research suggests that disease can have population impacts on herring populations, quantifying those impacts remains a challenge (Patterson 1996, Marty et al. 2003, Marty et al. 2010, Hershberger et al. 2016, Muradian et al. 2017). Both *Ichthyophoniasis* and VHS have been associated with episodic acute mortality events in Pacific herring. Caused by an RNA virus, VHS causes acute mortality events in herring, while the parasite *Ichthyophonus hoferi* causes chronic disease and mortality. Disease events are hard to observe in wild populations, particularly in remote locations. As a result, inferring epidemiological processes related to these diseases has been a challenge. Some disease processes can be quantified experimentally, such as mortality due to infection, and rates of disease progression or disease clearance; however, processes related to transmission, which depends on host susceptibility to infection, contact rates between susceptible and infected individuals, and population structure, are best estimated in the wild, because laboratory conditions have such a strong impact on these variables. Transmission rates, or the rate that infections spread through a population, are critical for identifying the conditions when outbreaks may occur.

Recent advances in our data collection and analysis have created an opportunity to estimate transmission processes for both VHS and *Ichthyophonus* using data from herring collected in the wild. The parameter that will be estimated is called the force-of-infection (FOI) and describes the rate that susceptible individuals become infected in a population. The first advance is that, as part of the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC)-funded HRM project, a novel plaque neutralization method has been developed to infer if herring have previously been exposed to VHS. Paul Hershberger (project 21120111-E) and his research team have optimized and validated this technique and now have data on the VHS seroprevalence for herring collected in PWS (since 2012) and are in the process of developing a similar dataset for herring from Sitka Sound (since 2011). The second advance is that all fish collected for health assessment (including VHS and *Ichthyophonus*) from Sitka Sound and PWS have been assigned probable ages based on age-length keys developed for each year of study in each of these regions (see executive summary above for details). Collectively, data on disease prevalence or antibody seroprevalence can be combined with data on host age to quantify FOI using a mathematical method developed by Heisey et al. (2006). This method has been successfully applied to quantify FOI for other diseases of marine fish including mycobacteriosis in striped bass, and the nematode *Anguillicoloides crassus* in eels (Gauthier et al. 2008, Warshafsky et al. 2019).

The FOI model employs a three-state irreversible disease model, which is modified from an age-dependent force-of-infection (FOI) model (Heisey et al. 2006) (Fig. 8). A variety of covariates can be applied to the model to determine if FOI is environmentally- and/or age-dependent. Model selection using Akaike Information Criteria can be used to select the best model.

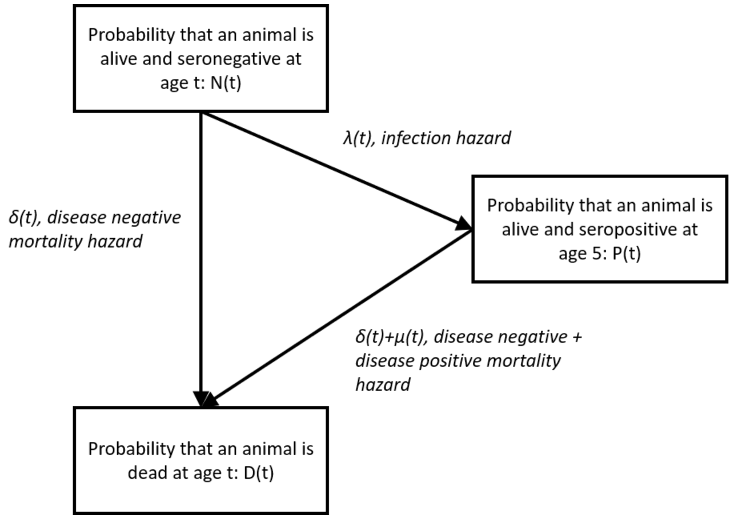

Figure 8. Conceptual model for inferring viral hemorrhagic septicemia force-of-infection and mortality. To change this model so that it applies to *Ichthyophonus*, seroprevalence data will be replaced with *Ichthyophonus* prevalence data. Key for the FOI model to be calculated in both of these systems is the fact that once seropositive (for VHS), or infected with *Ichthyophonus* (for Ichthyophoniasis), a fish will never revert to a seronegative or disease negative state.

Justification

The proposed work aligns with requests from the EVOSTC to synthesize research within the HRM program. These estimates will be valuable for more realistic incorporation of disease in the herring age-structured-assessment model and for creating disease models that can explore a variety of different environmental and/or demographic scenarios. For example, the mathematical models of VHS that are in development (both by Maya Groner and by John Trochta) do not have validated estimates of transmission because none are available. While transmission rates are not directly incorporated into the ASA, environmental factors that may alter transmission rates could potentially be included in the ASA if they have a large enough impact on population-level outcomes of infection. Lab experiments suggest that for VHS and *Ichthyophonus,* key epidemiological processes such as transmission and survival are temperature-dependent (Hershberger et al. 2010, Hershberger et al. 2016, Vollenwelder et al. 2011).

The proposed work overlaps with EVOSTC- and NPRB-funded research that Groner is currently writing up and that focuses on quantifying mortality due to a Mesomycetozoean parasite, *Ichthyophonus* sp., on herring populations in Sitka Sound and PWS. Both studies use the same dataset of wild sampled herring from 2009-2019, both studies will use the same demographically weighted age-length key developed by Groner to assign ages to these samples. Quantifying transmission rates is a valuable step towards inclusion of Ichthyophonus related mortality in the PWS age-structure assessment.

This work also compliments EVOSTC-funded research conducted by Trevor Branch and John Trochta (project 21120111-C) aimed at incorporating seroprevalence data into the PWS age-structured assessment of herring. Agreement between the results of the methods proposed by Branch and Trochta and the threestaged model proposed here will be valuable for validation of the model approach. The benefit of the approach described here is that independent yearly estimates of both force of infection and mortality can be regressed against environmental variables (including temperature and productivity metrics provided by GWA collaborators such as Rob Campbell) to identify possible drivers of disease for both the Sitka Sound and PWS herring stocks. Such environmental variables could potentially be included in the stock assessment model and/or incorporated in independent epidemiological models exploring how factors such as climate change may impact disease transmission and mortality.

Requested Budget

Item	Cost/unit	Units	Total
Salary and Benefits	$9600	4 months	$38,400
Travel	$100/ trip to Marrowstone Lab	3 trips	$300
Total Direct			$38,700
Overhead	35%		$13,500
Total			**$52,200**

Funding is requested to cover 4 months of salary for Groner and three trips to the USGS Marrowstone Lab (WA State). Paul Hershberger is located at the Marrowstone Lab, two hours away from where Groner is located at the USGS Western Fisheries Research Center in Seattle. Visits to the Marrowstone Lab will be made to discuss interpretation of results and facilitate writing of a manuscript.

5. PROJECT PERSONNEL – CHANGES AND UPDATES

There are no new personnel.

6. PROJECT BUDGET FOR FY20

A. Budget Forms

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$57.0	$153.3	$177.4	$171.9	$100.1	$659.5	
Travel	$6.4	$9.9	$6.4	$6.4	$6.7	$35.8	
Contractual	$24.7	$26.0	$26.2	$11.0	$4.4	$92.3	
Commodities	$3.8	$1.5	$3.5	$1.4	$1.5	$11.7	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Indirect Costs (*will vary by proposer*)	$35.1	$57.20	$64.0	$57.2	$33.8	$247.3	
SUBTOTAL	$127.0	$247.8	$277.5	$247.9	$146.5	$1,046.6	
General Administration (9% of subtotal)	$11.4	$22.3	$25.0	$22.3	$13.2	$94.2	N/A
PROJECT TOTAL	$138.4	$270.2	$302.5	$270.2	$159.6	$1,140.8	
Other Resources (Cost Share Funds)	$26.0	$26.6	$69.7	$90.5	$28.0	$240.8	

B. Changes from Original Project Proposal

We are requesting an additional $10,000 in salaries in FY21 to hire Donna Aderhold to provide an additional level of review of documents that the HRM program is submitting to the EVOSTC. Donna will coordinate primarily with the HRM program lead, W. Scott Pegau, and with individual project PIs as needed to complete document editorial reviews. For FY21, editorial support will include FY20 annual reports and final reports.

As described in Section 4, we are also requesting an additional $52,200 for Maya Groner and John Trochta to estimate FOI due to *Ichthyophonus* infections and VHS in PWS. These estimates will be valuable for more realistic incorporation of disease in the herring age-structured-assessment model and for creating disease models that can explore a variety of different environmental and/or demographic scenarios. The mathematical models of VHS that are in development (both by Maya Groner and by John Trochta) do not have validated estimates of transmission.

C. Sources of Additional Project Funding

The Oil Spill Recovery Institute provides up to two months of salary for Dr. Pegau to complete his coordination activities. The Prince William Sound Regional Citizens' Advisory Council is supporting aerial surveys of forage fish that provides information on the number of age-1 herring schools.

The Herring Disease Program (project 21120111-E) was leveraged to obtain NPRB funding (Groner and Hershberger) to evaluate the possible involvement of Ichthyophonus in the recent herring population trends occurring in Sitka Sound. The funding claimed here is only for the portion associated with Dr. Groner's work. The portion associated with Dr. Hershberger's work is shown in his proposal.

PRINCE WILLIAM SOUND
OIL SPILL RECOVERY INSTITUTE

To Whom it may Concern:

This letter is to confirm that the Oil Spill Recovery Institute (OSRI) has agreed to provide salary for up to two months in FY 20 and 21 for Dr. Pegau to support the Herring Research and Monitoring (HRM) program. Dr. Pegau's works for OSRI to identify efforts that help us understand recovery from oil spills and participation in the HRM program aligns with that mission. The value of Dr. Pegau's salary, benefits, and overhead is expected to be 18,500 per month in FY20 and 19,000 in FY 21.

Sincerely,

Katrina Hoffman
Executive Director
Oil Spill Recovery Institute

Mail address: P.O. Box 705 – Cordova, AK 99574 – Phone: (907) 424-5800 x 222; fax 424-5820
Physical address: 300 Breakwater Avenue, Cordova, AK 99574
www.pws-osri.org or www.pwssc.org – wspegau@pwssc.org

Regional Citizens' Advisory Council / "Citizens promoting environmentally safe operation of the Alyeska terminal and associated tankers."

In Anchorage: 3709 Spenard Road / Suite 100 / Anchorage, Alaska 99503 / (907) 277-7222 / FAX (907) 277-4523
In Valdez: P.O. Box 3089 / 130 South Meals / Suite 202 / Valdez, Alaska 99686 / (907) 834-5000 / FAX (907) 835-5926

July 14, 2020

To whom it may concern,

I am writing this letter in regards to forage fish survey work that the Prince William Sound Regional Citizens' Advisory Council (PWSRCAC) is currently funding. In May 2020, PWSRCAC contracted with the Prince William Sound Science Center, in an amount of $43,600, to conduct aerial surveys of juvenile forage fish (e.g. herring, capelin, sand lance) in Prince William Sound. These surveys took place in June 2020, and we anticipate receiving a report with the findings this fall. PWSRCAC has budgeted an additional $45,100 to conduct the same surveys in June 2021. This work uses the protocols of previous efforts funded by the *Exxon Valdez* Oil Spill Trustee Council, and the data will be combined with those prior year surveys. PWSRCAC is working with Dr. Scott Pegau at the Prince William Sound Science Center on this multi-year project.

The PWSRCAC Board of Directors believes that monitoring the long-term changes in forage fish distribution, and relative abundance in Prince William Sound, provides valuable information, especially since the Prince William Sound herring population has crashed to a low level. Understanding where herring and other forage fish are, and where the important nursery grounds exist, is important for prioritizing their protection during an oil spill response or identifying suitable places of refuge for oil tankers, or other vessels in distress.

As you are aware, the PWSRCAC is an independent non-profit corporation whose mission is to promote environmentally safe operation of the Valdez Marine Terminal and associated tankers. Our work is guided by the Oil Pollution Act of 1990, and our contract with Alyeska Pipeline Service Company. PWSRCAC's 18 member organizations are communities in the region affected by the 1989 Exxon Valdez oil spill, as well as commercial fishing, aquaculture, Native, recreation, tourism and environmental groups.

Please feel free to contact me if you have any questions or would like more information on PWSRCAC's work and interest in monitoring herring and other forage fish in Prince William Sound.

Sincerely

Donna Schantz

Donna Schantz
Executive Director

900.105.200714.ForageFish

7. FY17-20 PUBLICATIONS AND PRODUCTS

Publications

Aderhold, D.G.R., M.R. Lindeberg, K. Holderied, and W.S. Pegau. 2019. Spatial and temporal ecological variability in the northern Gulf of Alaska: What have we learned since the *Exxon Valdez* oil spill? Deep Sea Research II. 147:3-8. DOI 10.1016/j.dsr2.2017.11.015

Ben-Horin, T., G. Bidegain, G. de Leo, M.L. Groner, E. Hofmann, H. McCallum, and E. Powell. 2020. Modelling marine diseases. Marine Disease Ecology p. 233.

Cantrell, D.L., M.L. Groner, T. Ben-Horin, J. Grant, and C.W.Revie. 2020. Modeling pathogen dispersal in marine fish and shellfish. Trends in Parasitology *36*:239-249.

Elliott, D.G., C.M. Conway, C.L. McKibben, A.H. MacKenzie, L.M. Hart, M.K. Purcell, J.L. Gregg, M.L. Groner, and P.K. Hershberger. In prep*.* Differential susceptibility of Yukon River-and Salish Sea-origin Chinook salmon *Oncorhynchus tshawytscha* to ichthyophoniasis.

Groner, M.L., E. Bravo-Mendosa, C.M. Conway, D.G. Elliot, and P.K. Hershberger. In prep*.* Host response and infection load mediate lethal infectious dose of *Ichthyophonus* in Pacific herring.

Groner, M.L., E. Bravo-Mendosa, C.M. Conway, D.G. Elliot, A.H. McKenzie, J.L. Gregg, S. Dressel, and P.K. Hershberger. In prep. Environmental and demographic correlates of *Ichthyophonus* severity and prevalence in Pacific herring from 2009-2019.

Gorman, K.B., T.C. Kline, M.E. Roberts, F.F. Sewall, R.A. Heintz, and W.S. Pegau. 2019. Spatio-temporal variation in stable carbon and nitrogen isotope signatures and condition of juvenile herring (*Clupea pallasii*) in Prince William Sound, Alaska: teleconnections with the Gulf of Alaska. Deep Sea Research II 147:116-126. DOI 10.1016/j.dsr2.2017.10.010

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, A. Lindquist, T. Sandell, M.L. Groner, and D. Lowry. 2019. A geographic hot spot of Ichthyophonus infection in the southern Salish Sea, USA. Diseases of Aquatic Organisms 136:157-162.

Shore-Maggio, A., M.L. Groner, C.A. Burge, R. Carnegie, and P. Hershberger. In prep. Disease transmission in managed marine systems.

Published and updated datasets

The forage fish data available from the AOOS data portal was updated with the aerial survey data collected in 2020.

Presentations

Bishop M.A., T. Branch, K. Gorman, M. Groner, S. Haught, P. Hershberger, S. Pegau, P. Rand, J. Trochta, and A. Whitehead. 2020. PWS Herring Research. Poster presentation, Alaska Marine Science Symposium, January, Anchorage, AK.

Outreach

Groner, M. 2019. 'Ich-Y' diseases in Pacific herring. Delta Sound Connections. Prince William Sound Science Center.

Hoover, H. 2017, 2018, 2019, 2020. The need for Herring Research and Monitoring. Delta Sound Connections. Prince William Sound Science Center.

Hoover, H. and S. Pegau. 2020. Herring population estimates. Delta Sound Connections. Prince William Sound Science Center.

Pegau, S. 2019. Changes in forage fish. Delta Sound Connections. Prince William Sound Science Center.

We completed updates to the website (http://pwssc.org/research/) for all of the ongoing projects within the HRM program and continue updating past projects to include more findings.

Dr. Groner participated in the listening session held at Port Graham in 2018 with along with GWA researchers.

8. LITERATURE CITED

Ben-Horin, T., G. Bidegain, G. de Leo, M.L. Groner, E. Hofmann, H. McCallum, and E. Powell. 2020. Modelling marine diseases. Marine Disease Ecology p. 233.

Cantrell, D.L., M.L. Groner, T. Ben-Horin, J. Grant, and C.W. Revie. 2020. Modeling pathogen dispersal in marine fish and shellfish. Trends in Parasitology *36*:239-249.

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Hershberger, P.K., K.A. Garver, and J.R. Winton. 2016. Principles underlying the epizootiology of viral hemorrhagic septicemia in Pacific herring and other fishes throughout the North Pacific Ocean. Canadian journal of fisheries and aquatic sciences 73:853-859.

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, A. Lindquist, T. Sandell, M.L. Groner, and D. Lowry. 2019. A geographic hot spot of *Ichthyophonus* infection in the southern Salish Sea, USA. Diseases of Aquatic Organisms 136:157-162.

Marty, G.D., T.J.Q. Ii, G. Carpenter, T.R. Meyers, and N.H. Willits. 2003. Role of disease in abundance of a Pacific herring (*Clupea pallasi*) population. Canadian Journal of Fisheries and Aquatic Sciences 60:1258-1265.

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Patterson, K.R. 1996. Modelling the impact of disease-induced mortality in an exploited population: the outbreak of the fungal parasite (*Ichthyophonus hoferi*) in the North Sea herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences 53:2870-2887.

Vollenweider, J.J., Gregg, J.L., Heintz, R.A. and Hershberger, P.K., 2011. Energetic cost of *Ichthyophonus* infection in juvenile Pacific herring (*Clupea pallasii*). Journal of parasitology research, 2011:926812.

Warshafsky, Z.T., T.D.Tuckey, W.K. Vogelbein, R.J. Latour, and A.R. Wargo. 2019. Temporal, spatial, and biological variation of nematode epidemiology in American eels. Canadian Journal of Fisheries and Aquatic Sciences, 7:1808-1818.