

**EVOSTC FY17-FY21 INVITATION FOR PROPOSALS  
FY19 CONTINUING PROJECT PROPOSAL SUMMARY PAGE**

*Proposals requesting FY19 funding are due to [shiwang.wang@alaska.gov](mailto:shiwang.wang@alaska.gov) and [elise.hsieh@alaska.gov](mailto:elise.hsieh@alaska.gov) by August 17, 2018. Please note that the information in your proposal and budget form will be used for funding review. Late proposals, revisions or corrections may not be accepted.*

**Project Number and Title**

19120111-E  
Herring Disease Program

**Primary Investigator(s) and Affiliation(s)**

Paul K. Hershberger, USGS – Marrowstone Marine Field Station  
Maureen K. Purcell, USGS – Western Fisheries Research Center

**Date Proposal Submitted**

August 17, 2018

**Project Abstract**

We will investigate fish health factors that may be contributing to the failed recovery of Pacific herring populations in Prince William Sound. Field samples will provide infection and disease prevalence data from Prince William Sound and Sitka Sound that will inform the ASA model, serological data that will indicate the prior exposure history and future susceptibility of herring to VHS, and diet information that will provide insights into the unusually high prevalence of *Ichthyophonus* that occurs in juvenile herring from Cordova Harbor. Laboratory studies will validate the newly-developed plaque neutralization assay as a quantifiable measure of herd immunity against VHS, provide further understanding of disease cofactors including temperature and salinity, investigate the possibility of an invertebrate host for *Ichthyophonus*, and assess the virulence of other endemic pathogens to Pacific herring. Information from the field and laboratory studies will be integrated into the current ASA model, a novel ASA-type model that is based on the immune status of herring age cohorts.

*\*The abstract should provide a brief overview of the overall goals and hypotheses of the project and provide sufficient information for a summary review as this is the text that will be used in the public work plan and may be relied upon by the PAC and other parties.*

EVOSTC Funding Requested* (must include 9% GA)					
FY17	FY18	FY19	FY20	FY21	TOTAL
Auth:\$197,800	Auth:\$228,900 <sup>a</sup>	\$236,700 <sup>a</sup>	\$243,300 <sup>a</sup>	\$251,100 <sup>a</sup>	\$1,157,900

Non-EVOSTC Funds to be used, please include source and amount per source:					
FY17	FY18	FY19	FY20	FY21	TOTAL
\$61,600	\$63,600	\$64,000	\$65,200	\$66,900	\$321,400

*\*If the amount requested here does not match the amount on the budget form, the request on the budget form will be considered to be correct. <sup>a</sup>Totals in FY '18-21 include additional annual requests of \$22,500 that will be used for processing additional herring plasma samples; results will be incorporated into a revised ASA model.*

## 1. PROJECT EXECUTIVE SUMMARY

Provide a summary of the program including key hypotheses and overall goals, as submitted in your original proposal. Please include a summary and highlights since your last annual report: preliminary results with figures and tables. If there are no preliminary results to present, please explain why (i.e., lab analysis is still in progress). List any publications that have been submitted and/or accepted since you submitted your last proposal and other products in *Section 7*. Prior annual reports will be appended to remind reviewers of progress in previous years.

A better understanding of the epidemiological principles governing herring diseases in PWS is necessary for the development of adaptive management strategies intended to mitigate the effects of diseases to wild herring populations. Early studies of known herring pathogens in PWS were conducted by Dr. Gary Marty, and provided valuable information on trends of infection prevalence and intensity since 1994. In an effort to document changes in pathogen prevalence and severity within the PWS herring population, these surveillance efforts were continued by Hershberger et al., in the form of the Herring Disease Program (HDP) from 2007 – present. The incorporation of laboratory-based manipulations and observations in the HDP has led to the realization that some of our prior assumptions of these diseases were incorrect. For example, in a typical herring population, the prevalence of VHSV generally falls below the realistic detection threshold obtained from 60-fish subsamples of a population. Even though the endemic prevalence is typically extremely low, an epizootic can occur very quickly as a result of changing host and environmental conditions. As such, the incorporation of VHSV prevalence data into the ASA model as a forecaster of future disease potential is inconsequential from an epidemiological perspective. For example, a prevalence of 0% (0/60) in a pre-spawn herring population provides no indication of whether the population previously experienced a VHS epizootic, or an epizootic is likely to occur in the future. For this reason, we have developed a serological assay (50% plaque neutralization assay - PNT) that we can use to determine whether herring have survived previous exposure to VHSV. Samples for this assay have been incorporated into the annual fish health assessments.

### Summary and Highlights since Feb. 2018

Results from the 2018 health assessments of pre-spawn herring were consistent with previous years. Overall *Ichthyophonus* infection prevalence in Prince William Sound was 13% (24/179); neither VHSV nor VEN was detected in any of the samples. Overall *Ichthyophonus* infection prevalence in Sitka Sound was 21% (38/177); neither VHSV nor VEN was detected in any of the samples. The prevalence of VHSV neutralizing antibodies was 8% in Prince William Sound and 4% in Sitka Sound (Figure 1).

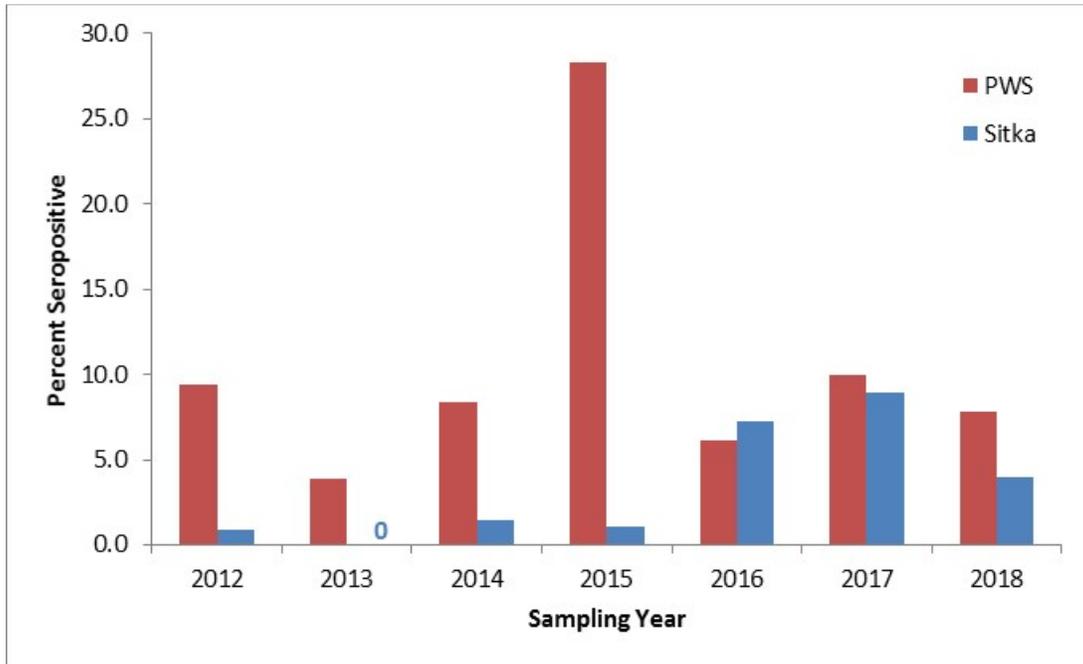


Figure 1. VHSV neutralizing antibody results from Prince William Sound and Sitka Sound herring. PWS data from 2017 and 2018 are represented from 360 samples (180 randomly-selected samples plus 60 fish selected from each of 3 size bins). These data will be separated into year classes and provided to Dr. Trevor Branch for incorporation into a revised ASA model structure.

We have initiated a long-term study to document the kinetics of this VHSV antibody response; particularly, we are assessing how long circulating antibodies remain detectable after herring survive a single exposure to the virus. We are currently 10 months into the experiment (post-virus exposure) and we continue to detect neutralizing antibodies in 70% of the previously exposed herring (Figure 2).

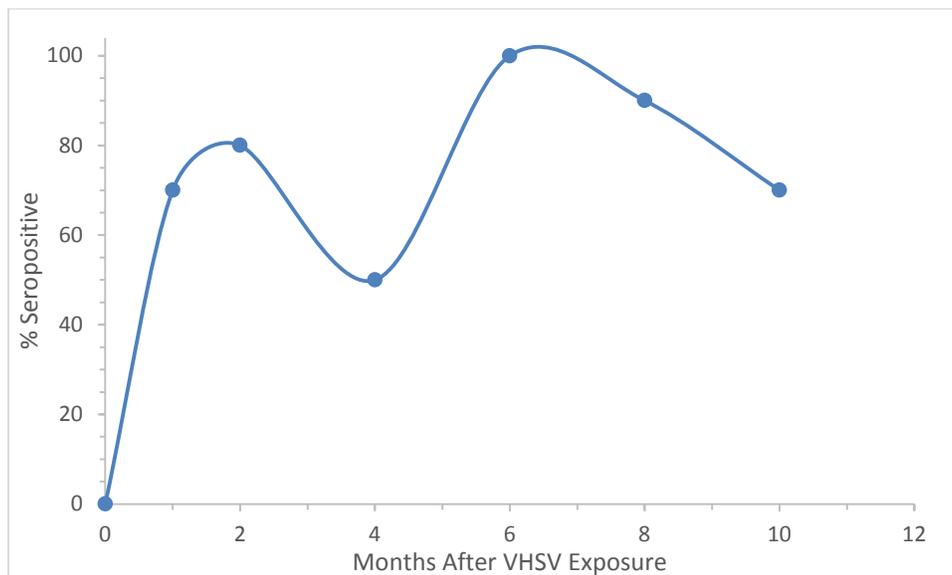


Figure 2. Monthly profile of neutralizing antibodies in Pacific herring that survived VHSV exposure. Each data point represents the percent seropositive from 10 subsampled herring. None of the specific pathogen-free negative controls (n = 10 / sampling date) tested positive for neutralizing antibodies. The experimental subsampling is ongoing (July 2018 is represented by month 10).

**2. PROJECT STATUS OF SCHEDULED ACCOMPLISHMENTS**

**A. Project Milestones and Tasks**

Milestones are annual steps to meet overall project objectives. For each milestone listed, specify the status (completed, not completed) when each was completed and if they are on schedule, as submitted in your most current proposal.

Tasks are annual steps to meet milestones. Specify, by each quarter of each fiscal year, when critical tasks (for example, sample collection, data analysis, manuscript submittal, etc.) were and will be completed.

Please identify any substantive changes and the reason for the changes. Reviewers will use this information in conjunction with annual program reports to assess whether the program is meeting its objectives and is suitable for continued funding.

**B. Explanation for not completing any planned milestones and tasks**

Please identify any substantive changes and the reason for the changes. If tasks were not completed as scheduled or delayed, please explain why and the anticipated completion date.

**C. Justification for new milestones and tasks**

Please identify any new milestones and tasks and the reason why they have been added.

**A. Project Milestones and Tasks**

Project milestone and task progress by fiscal year and quarter, beginning February 1, 2017. 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.

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
<b>Fieldwork</b>																				
Collect herring eggs for rearing SPF colonies	C				C				X				X				X			
Collect adult herring to assess annual infection and disease prevalence	C				C				X				X				X			
Collect zooplankton for investigation of possible <i>Ichthyophonus</i> intermediate host	C				C				X				X				X			
Collect herring from Cordova Harbor to assess <i>Ichthyophonus</i> -infected offal in the stomach bolus		C				X*														
<b>Captive Study</b>																				
Vibrio challenge experiments	C	C	C	C																
SPF herring metamorphosed to juveniles			C				C				X			X					X	
Assess effects of salinity on fish-to-fish transmission of <i>Ichthyophonus</i>					C	C	C	C												
Assess the effect of temperature on VHSV shedding									X	X	X	X								



### **3. PROJECT COORDINATION AND COLLABORATION**

#### **A. Within an EVOTC-Funded Program**

Provide a list and clearly describe the functional and operational relationships with any EVOTC-funded Program (Herring Research and Monitoring, Long-Term Research and Monitoring or Data Management Programs). This includes any coordination that has taken or will take place and what form the coordination will take (shared field sites or researchers, research platforms, sample collection, data management, equipment purchases, etc.).

#### **B. With Other EVOTC-funded Projects**

Indicate how your proposed project relates to, complements or includes collaborative efforts with other proposed or existing projects funded by the EVOTC that are not part of a EVOTC-funded program.

#### **C. With Trustee or Management Agencies**

Please discuss if there are any areas which may support EVOTC trust or other agency work or which have received EVOTC trust or other agency feedback or direction, including the contact name of the agency staff. Please include specific information as to how the subject area may assist EVOTC trust or other agency work. If the proposed project requires or includes collaboration with other agencies, organizations or scientists to accomplish the work, such arrangements should be fully explained and the names of agency or organization representatives involved in the project should be provided. If your proposal is in conflict with another project, note this and explain why.

#### **Within the HRM Program**

- We are working closely with the PWSSC and ADF&G to collect herring tissue and plasma samples during the spring herring cruises (shared research platform). Additionally, ADF&G continues to provide age data for the fish health samples.
- Serum neutralization results, to assess herd immunity by quantifying VHSV neutralizing titer, will be shared with Dr. Trevor Branch (U. Washington). These results will be used to create a novel age-structured assessment model that incorporates herd immunity by herring age class.
- As In-Kind contributions to Dr. Maya Groner's project, several experiments were initiated and are currently underway at the USGS - Marrowstone Marine Field Station.
  - 1) Pilot study to assess the histological threshold level of *Ichthyophonus* infection that is associated with herring mortality. This study was completed using age 1 (2017 cohort) herring. Results were used to inform experimental design for the definitive study using adult herring (*Ichthyophonus* exposures are anticipated to begin August 8, 2018).
  - 2) VEN-positive tissues from wild herring were collected to provide a source of inoculum for future controlled exposure studies.
  - 3) Began processing archived histology samples from PWS and Sitka Sound, dating to 2007. These samples will indicate whether the severity of *Ichthyophonus* infections have changed over time and whether these changes are associated with recent population changes in Sitka Sound.

#### **With Other EVOTC-Funded Programs and Projects**

As In-Kind contributions to Dr. Andrew Whitehead's project, we have successfully fertilized herring eggs from three different stocks (Prince William Sound, Sitka Sound, and Puget Sound). Quadruplicate groups of fertilized eggs from each stock were exposed to five different concentrations of Alaska North Slope Crude oil (plus additional negative control groups that were not exposed to oil); various metrics of embryonic and newly-hatched larval health and survival were assessed between stocks and oil concentrations. From each stock, larvae exposed to two different oil concentrations (plus the unexposed controls) were reared through metamorphosis in duplicate tanks (Figures 3 and 4). Additional metrics of health and survival are scheduled for these juveniles for Fall 2018, including the determination of their relative susceptibilities to VHSV and *Ichthyophonus*.



Figure 3. Footprint of the 18 grow-out tanks for oil-exposed herring, including duplicate tanks for unexposed, medium-low oil, and medium-high oil exposures for each stock (PWS, Sitka Sound, and Puget Sound).



Figure 4. Oil-exposed specific pathogen-free Pacific herring in one of the 18 tanks depicted in Figure 1 (Sitka Sound, medium-high oil exposure).

### **With Trustee or Management Agencies**

- We continue to partner with ADF&G – Cordova to collect herring infection and disease data onboard the shared ADF&G seining platform.
- We continue to partner with ADF&G – Sitka to collect herring infection and disease data from pre-spawn aggregations in Sitka Sound.
- We continue to partner with ADF&G – Juneau to provide consistent virologic methods between all EVOS-TC funded herring disease projects between 1994 – present.
- We have partnered with 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 EVOS-funded project were leveraged to obtain supplemental funding from the North Pacific Research Board (# 1807: *Ichthyophonus* in Pacific Herring).
- - We have partnered with Drs. John Incardona and Nat Sholtz (NOAA – Northwest Fisheries Science Science Center) to provide herring for their NPRB project investigating the long-term effects of embryonic oil exposure on herring cardiac morphology. Further, we are investigating the long-term impacts of these cardiac abnormalities on the health and survival of juvenile herring.

## **4. PROJECT DESIGN**

### **A. Overall Project Objectives**

Identify the overall project objectives for your project as submitted in your original proposal.

### **B. Changes to Project Design and Objectives**

If the project design and objectives have changed from your original proposal, please identify any substantive changes and the reason for the changes. Please include the revised objectives in this section. Include any information on problems encountered with the research or methods, if any. This may include logistic or weather challenges, budget problems, personnel issues, etc. Please also include information as to how any problem has been or will be resolved. This may also include new insights or hypotheses that develop and prompt adjustment to the project.

### **3A. Objectives for FY19**

- Provide pathogen and disease prevalence data to inform the ASA model
- Produce specific pathogen-free (SPF) Pacific herring for laboratory experiments
- Process new and archived herring plasma samples for indications of prior VHSV exposure
- Continue validating the plaque neutralization assay using wild herring
- Investigate the possibility of an invertebrate host for *Ichthyophonus*
- Determine the causes for abnormally high *Ichthyophonus* prevalence among juvenile Pacific herring that establish temporary residency in Cordova Harbor
- Determine the impacts of salinity on fish-to-fish transmission of *Ichthyophonus*

### **3B. Changes to Project Design**

As with the 2017 request, we would like to continue our expanded efforts to assess neutralizing VHSV neutralizing antibody levels in PWS herring. As a supplement to the typical 180 randomly selected samples (60 herring from each of 3 locations), we will select for 60 additional herring in each of 3 size classes. Using this expanded approach, we will achieve more robust sample numbers for each year class of herring (ages for all sampled herring will be provided by ADF&G). Requested supplemental funds will be used to process these expanded samples from 2017 onwards.

## **5. PROJECT PERSONNEL – CHANGES AND UPDATES**

If there are any staffing changes to Primary Investigators or other senior personnel please provide CV's for any new personnel and describe their role on the project.

Not Applicable

## 6. PROJECT BUDGET FOR FY19

### A. Budget Forms (Attached)

Provide completed budget forms.

### B. Changes from Original Proposal

If your FY19 funding request differs from your original proposal, provide a detailed list of the changes and discuss the reason for each change.

### C. Sources of Additional Funding

Identify non-EVOSTC funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

#### A. Budget Forms

#### B. Changes from Original Proposal

An additional \$24,500 / year is requested to enable the processing of additional herring plasma samples from PWS. This supplement would provide funds for additional plaque neutralization supplies (\$10,000), 2.5 months of support for a seasonal technician to assist with the processing of field samples (\$12,500), and 9% General Administration charges (\$2,000).

#### C. Sources of Additional Funding

- USGS provides matching funds for PI and support staff.
- We have partnered with Dr. Andrew Whitehead on his EVOS TC-funded project to evaluate possible delayed impacts of PAH exposure to Pacific herring.
- We have partnered with Drs. Nat Scholz and John Incardona (NOAA – Fisheries, Northwest Fisheries Science Center) on their NPRB-funded project to address similar objectives.
- The Herring Disease Program 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 Herring Disease Program was leveraged to support a M.S. student at Alaska Pacific University (Sioned Sitkiewicz) studying *Ichthyophonus* in Pacific halibut.
- The Herring Disease Program was leveraged to support a M.S. student at the University of Washington (Catrin Wendt) studying *Ichthyophonus* in Pacific herring

## 7. FY18 PUBLICATIONS AND PRODUCTS

Products include publications (include *in prep* and *in review*), published and updated datasets, presentations, and outreach during FY18.

### Publications

- Hershberger, P.K., J.L. Gregg, C. Dykstra. 2018. High-prevalence and low-intensity *Ichthyophonus* infections in Pacific Halibut (*Hippoglossus stenolepis*). *Journal of Aquatic Animal Health* 30:13-19.
- Harris, B.P., S.R. Webster, J.L. Gregg, P.K. Hershberger. 2018. *Ichthyophonus* in sport-caught groundfishes from southcentral Alaska. *Diseases of Aquatic Organisms* 128: 169-173.
- 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.

Sreenivasan, A., J. Vollenweider, J. Gregg, P. Hershberger. *In Review*. Utility of Nucleic Acid Ratios and Total-Body Lipid as Indices of Energy Allocation Strategies in Larval Pacific Herring (*Clupea pallasii*). Coastal and Marine Fisheries.

Sitkiewicz, S.E., N. Wolf, P.K. Hershberger, T.S. Smettz, S.R. Webster, B.P. Harris. *In Review*. Temporal changes in *Ichthyophonus* infection prevalence in Pacific halibut provide evidence for a stable host pathogen paradigm. Journal of Aquatic Animal Health.

*A previous publication was awarded the American Fisheries Society Publications Award for best paper of the year in Journal of Aquatic Animal Health:*

“Hart, L.M., M.K. Purcell, R. Powers, A. MacKenzie, P.K. Hershberger. 2017. Optimization of a plaque neutralization test to identify the exposure history of Pacific herring to viral hemorrhagic septicemia virus (VHSV). Journal of Aquatic Animal Health 29: 74-82.”

Other manuscripts in preparation

#### Presentations

Poster:

Sitkiewicz, S., P. Hershberger, N. Wolf. B. Harris. January 22-26, 2018. Poster. Effects of the parasite *Ichthyophonus* (spp.) on Pacific halibut (*Hippoglossus stenolepis*) growth and condition. Alaska Marine Science Symposium. Anchorage, AK.

Invited Seminar:

NOAA – Northwest Fisheries Science Center, Monster Seminar Jam (May 24, 2018)

“The Ecology of Disease in Marine Fishes: Insights from Pacific Herring.”