EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
FY18 CONTINUING PROJECT PROPOSAL SUMMARY PAGE

Proposals requesting FY18 funding are due to shiway.wang@alaska.gov and elise.hsieh@alaska.gov by August 23, 2017. 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

#18120111-E EVOS 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

July 26, 2017

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, 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
$197,800	$228,900*	$236,700*	$243,400*	$259,600*	$1,070,100

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

**Totals in FY '18-21 include additional annual requests of $24,500 that will be used for processing additional herring plasma samples; results will be incorporated into a revised ASA model.*

1. EXECUTIVE SUMMARY

Please provide a summary of the project including key hypotheses and overall goals, as submitted in your original proposal. If there are highlights that you would like to include from your FY17 work, please include them here. Also, please list any publications that have been submitted and/or accepted since you submitted your last proposal.

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 is capable of determining whether herring have survived previous exposure to VHSV. This knowledge is extremely important from a disease forecasting perspective because survivors of prior VHSV exposure remain refractory to the disease for a very long time; presumably for life.

A major advancement in our knowledge of VHS disease ecology was realized in FY 2017 with the development and optimization of a laboratory test (plaque neutralization assay) that is capable of enumerating neutralizing antibody levels for viral hemorrhagic septicemia virus (VHSV). This novel tool provides the ability to deduce the prior exposure history of Pacific herring to VHSV. Preliminary application of this tool using archived plasma samples indicated a consistently higher prevalence of antibodies (i.e. evidence for elevated VHSV exposures) in herring from Prince William than from Sitka Sound (Figure 1). Further, the highest levels of neutralizing antibodies occurred in PWS during 2015, a year when field assessments indicated an unanticipated paucity of new recruits and a drop in adult spawning stock biomass. Because of the potential implications of these results for the failed recovery of the PWS herring population, we propose to double future herring sampling numbers and begin tracking VHSV antibody levels within specific herring year classes. In addition to our standard sampling of 60 random herring from each of 3 separate sets, we propose to selectively sample 60 PWS herring from each of three sizes classes. Herring age (from scales) will later be assigned to all 360 sampled fish in an effort to achieve statistically relevant sampling numbers for multiple year classes. By continuing this sampling over consecutive years, we will be able to follow the antibody levels in specific year classes and deduce when VHSV exposures occurred. This expanded sampling protocol was prophylactically implemented in FY 2017 after we recognized the dramatic differences depicted in Figure 1. Supplemental funds of $25,000 / year in FY 18-21 are requested to enable the processing of these additional field samples. We have spoken with Dr. Trevor Branch (University of Washington), who is eager to incorporate these age-specific antibody levels into a revised Age Structured Assessment (ASA) model.

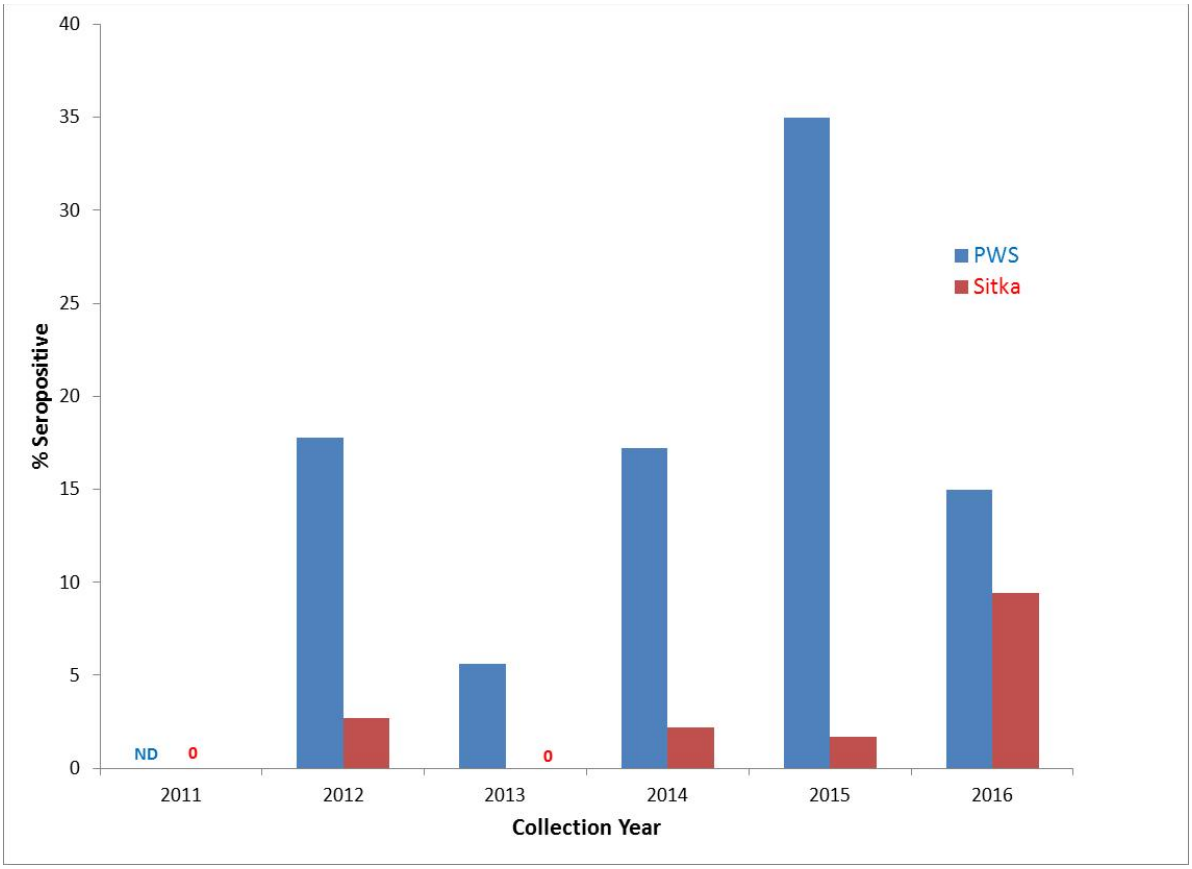

Figure 1. Prevalence of Pacific herring with detectable levels of neutralizing antibodies to VHSV (% seropositive) from Prince William Sound (PWS) and Sitka Sound. ND = No Data from PSW in 2011; '0' indicates 0% seropositive from Sitka Sound in 2011 and 2013.

Although *Ichthyophonus* is one of the most significant parasites of wild marine fishes, causing recurring population-level impacts during the past century (reviewed in Burge et al. 2014), very little is known about its natural life cycle. From a disease forecasting perspective, the most important information gap involves unresolved routes of exposure and transmission to planktivorous fishes. Laboratory studies indicate that the parasite is not readily transmitted from herring-to-herring via direct contact or through the water (Gregg et al. 2012). Recently, we have successfully established infections in herring by habituating them to the consumption of large quantities of infected fish tissues (Hershberger et al 2015); however, the relevance of this exposure route to wild populations of Pacific herring remains questionable, as herring are generally considered planktivores. These and other results have resulted in the elevation of a hypothesis that an invertebrate, intermediate host may be involved in completing the *Ichthyophonus* life cycle. However, until recently, appropriate scientific tools did not exist for examining the possibility of an *Ichthyophonus* intermediate host. Recent work performed in the Herring Disease Program was successful in developing novel tools (quantitative PCR and chromogenic in situ hybridization) that will be useful for assessing wild zooplankters as intermediate hosts for *Ichthyophonus*. Here we will continue to assess possible natural route(s) of *Ichthyophonus* transmission by expanding laboratory studies to assess horizontal transmission, examining a particular location (Cordova Harbor) where *Ichthyophonus* infection prevalence is unusually high, and by screening common herring food items as possible intermediate hosts for the parasite.

Finally, we will continue to advance our understanding of basic epizootiological principles that govern the primary diseases of herring by continuing to employ specific pathogen-free (SPF) laboratory animals in controlled laboratory experiments. For example, we will evaluate the effects of salinity on horizontal transmission of *Ichthyophonus* between herring.

2. COORDINATION AND COLLABORATION

A. Within an EVOTC-Funded Program

Provide a list and clearly describe the functional and operational relationships with any EVOSTC-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 EVOSTC-funded Projects

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

C. With Trustee or Management Agencies

Please discuss if there are any areas which may support EVOSTC trust or other agency work or which have received EVOSTC 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 EVOSTC 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

- Herring collections from Cordova Harbor will be provided by Drs Kristen Gorman and Scott Pegau (PWSSC). Stomachs from these fish will be assessed for indications that *Ichthyophonus*-infected offal may contribute to the unusually high infection prevalence detected among juvenile herring in Cordova Harbor.
- 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.

With Other EVOSTC-Funded Programs and Projects

- Long Term Monitoring: Yumi Arimitsu (USGS – Alaska Science Center) and John Moran (NOAA Fisheries - Auke Bay Labs) continue to send samples of suspect sick herring and other forage fish to us for diagnosis.
- Long Term Monitoring: Zooplankton collections from throughout Prince William Sound will be provided by Dr. Rob Campbell (PWSSC). Subsamples from these collections will be assessed by qPCR and CISH to look for an *Ichthyophonus* invertebrate host.
- Lingering Oil: We have partnered with Dr. Andrew Whitehead on his proposal to investigate the effects of PAH exposure to genetic pathways that directly and indirectly influence immune-competence. In kind laboratory space and SPF herring for Dr. Whitehead's projects will be provided at the USGS - Marrowstone Marine Field Station.

With Trustee or Management Agencies

- We will continue to partner with ADF&G – Cordova to collect herring infection and disease data onboard the ADF&G seining platform used to assess pre-spawn herring biomass in PWS.
- We will continue to partner with ADF&G – Sitka to collect herring infection and disease data from pre-spawn aggregations in Sitka Sound.

3. PROJECT DESIGN – PLAN FOR FY18

A. Objectives for FY18

Identify the primary objectives for your project for FY18 as submitted in your original proposal.

B. Changes to Project Design

If the project design has changed from your original proposal, please identify any substantive changes and the reason for the changes. 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 FY18

i. Continue collecting infection and disease prevalence data to inform the ASA model
ii. Produce specific pathogen-Free (SPF) Pacific herring for laboratory experiments
iii. Process new herring plasma samples for VHSV neutralizing antibodies
iv. Validate the novel plaque neutralization assay using wild herring
v. Determine the impacts of salinity on fish-to-fish transmission of *Ichthyophonus*

3B. Changes to Project Design

Based on novel serological results (Figure 1) which indicate that antibodies to VHSv consistently occur among higher proportions of herring in PWS than in Sitka Sound, we feel it is necessary to expand the PWS field sampling efforts to more fully understand the demographics of these observations. The data in Figure 1 represent the percent positive from all samples that were collected each year ($\leq$ 180 fish / year). If these annual serological data were separated into age classes, then we would be able to follow the antibody profiles in each particular year class and hind-cast at which herring life stage the VHSv exposures occurred. These data could then be dovetailed with population assessments to determine whether interannual changes in herring abundance (or year class strength) were associated with recent VHSv exposures. Unfortunately, with $\leq$ 180 samples / year, any attempt to compare age or year classes would be met with statistical power issues involving low sample sizes. Here we propose to double the number of herring plasma samples we collect from PWS by supplementing the random samples of 180 herring (n=60 from each of 3 sets) with 180 additional herring that are selected from each of three size bins (n = 60 / size bin). Herring age (from scales) will be determined from all 360 samples. Using this approach, we should be able to achieve n > 30 / age class in each forthcoming sampling year and provide enough statistical power for robust comparisons between age classes. In anticipation of this proposed expansion in sample size, we took the opportunity to collect the full complement of 360 plasma samples during the 2017 spring herring assessments. Requested supplemental funds would be used to process these expanded samples from 2017 onwards.

4. SCHEDULE

A. Program Milestones for FY18

For each project objective listed, specify when critical project tasks will be completed, as submitted in your original proposal. Please identify any substantive changes and the reason for the changes.

B. Measurable Project Tasks for FY18

Specify, by each quarter of each fiscal year, when critical project tasks (for example, sample collection, data analysis, manuscript submittal, etc.) will be completed, as submitted in your original proposal. Please identify any substantive changes and the reason for the changes.

A. Program Milestones for FY18

For each project objective listed, specify when critical project tasks will be completed, as submitted in your original proposal. Please identify any substantive changes and the reason for the changes.

i. Provide pathogen and disease prevalence data to inform the ASA model
Laboratory diagnostics for pre-spawn herring aggregations in PWS and Sitka Sound will be completed by June of each year.

ii. Rear Specific Pathogen-Free (SPF) Pacific herring
Annual rearing of SPF herring to juveniles will be completed by August.
iii. Data Archive
Data and metadata will be submitted to the Ocean Workspace by Dec 31.
iv. Process herring plasma samples for the detection of neutralizing antibodies to VHSV. Samples collected in 2018 will be processed by August of the same year.
v. Determine the impacts of salinity on fish-to-fish transmission of *Ichthyophonus*
December 2018

B. Measurable Project Tasks for FY18

1st Quarter (Feb. 1 – Apr. 30)

- Project funding approved by TC
- Collect herring eggs for rearing SPF colonies
- Collect adult herring to assess annual infection and disease prevalence
- Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host

2nd Quarter (May 1 – Jul. 31)

- Finish processing spring adult herring to determine infection and disease prevalence.
- Continue studies to validate the plaque neutralization assay using wild herring
- Collect herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus

3rd Quarter (Aug. 1 - Oct. 31)

- Brood Year 2018 SPF herring metamorphosed to juveniles
- Complete analysis of 2018 plasma samples
- Continue studies to validate the plaque neutralization assay using wild herring

4th Quarter (Nov. 1 - Jan. 31)

- Annual PI meeting
- Complete experiments intended to assess the effects of salinity on fish-to-fish transmission of *Ichthyophonus*

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.

No Changes

6. Budget

A. Budget Forms (Attached)

Provide completed budget forms.

B. Changes from Original Proposal

If your FY18 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 Attached

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$122.4	$140.9	$148.1	$154.1	$161.3	$726.8	$56.7
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	$7.8
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Commodities	$39.0	$49.0	$49.0	$49.0	$49.0	$235.0	$18.6
Equipment	$0.0	$0.0	$0.0	$0.0	$7.8	$7.8	$0.0
SUBTOTAL	$181.5	$210.0	$217.2	$223.2	$238.2	$1,070.1	$83.1
General Administration (9% of subtotal)	$16.3	$18.9	$19.5	$20.1	$21.4	$96.3	$16.3
PROJECT TOTAL	$197.8	$228.9	$236.7	$243.3	$259.6	$1,166.4	$99.4
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	$30.9

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.

Additionally, 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 also partnered with Drs. Nat Shutlz and John Incardona (NOAA – Fisheries, Northwest Fisheries Science Center) on their NPRB-funded project to address similar objectives.