EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
FY21 (YEAR 10) CONTINUING PROJECT PROPOSAL SUMMARY PAGE

Project Number and Title

21120111-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 16, 2020

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, serological data will indicate the prior exposure history and future susceptibility of herring to viral hemorrhagic septicemia virus (VHSV), and diet information will provide insights into *Ichthyophonus* transmission mechanisms. Laboratory studies are intended to validate the newly developed plaque neutralization assay as a quantifiable measure of herd immunity against VHS and provide further understanding of disease cofactors including salinity and investigate possible routes of transmission. Information from the field and laboratory studies will be integrated into the current ASA model and inform a novel ASA-type model that is based on the immune status of herring age cohorts.

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

FY17	FY18	FY19	FY20	FY21	TOTAL
197,800	228,900[a]	236,700[a]	243,300[a]	251,100[a]	1,157,900[a]

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

FY17	FY18	FY19	FY20	FY21	TOTAL
61,600	63,600	64,000	65,200	66,900	321,400

[a]Totals in FY18-21 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

A better understanding of the epidemiological principles governing herring diseases in Prince William Sound (PWS) is necessary for the development of adaptive management strategies intended to account for and mitigate the effects of diseases to wild herring populations. The incorporation of laboratory-based experiments and field observations in the herring disease program (HDP) has led to the realization that some of our prior assumptions with respect to these diseases were incorrect. For example, in a typical herring population, the prevalence of viral hemorrhagic septicemia virus (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 because of changing host and environmental conditions. As such, the incorporation of VHSV prevalence data into the age structured assessment (ASA) model is inconsequential from population forecasting and epidemiological perspectives. For example, a prevalence of 0% (0/60) in a pre-spawn herring population provides no indication of whether the population previously experienced a viral hemorrhagic septicemia (VHS) epizootic, or whether an epizootic is likely to occur in the future. For this reason, we have developed a serological assay (50% plaque neutralization assay [PNT]) that can be used to identify whether herring have survived prior exposures to VHSV and whether they are refractory to future outbreaks. Once fully validated and vetted, these serological results will elucidate the prior VHSV exposure history and future disease potential; further, these serological results will replace the current VHSV infection prevalence inputs in the ASA model with biologically and ecologically meaningful values. Serological samples for this assay have been incorporated into annual herring health assessments for PWS and Sitka Sound.

Summary and Highlights since Feb. 2020

1) Travel restrictions due to the onset of the COVID-19 pandemic created logistical challenges for the herring pre-spawn disease surveillance sampling in 2020. Fortunately, our gracious research partners in Cordova (Prince William Sound Science Center and Alaska Department of Fish and Game) and Sitka (Sitka Sound Science Center and Alaska Department of Fish and Game) provided support to ensure that the normal herring disease samples were collected. Overall *Ichthyophonus* infection prevalence was 11% (32/188) in PWS and 22% (39/180) in Sitka Sound; VHSV was not detected in in any samples from PWS or Sitka Sound. Blood films for diagnosis of viral erythrocytic necrosis (VEN) were not collected from either stock in 2020 due to COVID-19 constraints.

   Additional herring samples were collected from 3 sites in Puget Sound, WA (Squaxin Island, Port Orchard, and Semiahoo Bay). Samples included tissues for *Ichthyophonus* infection prevalence, VEN infection prevalence and severity, and VHSV antibodies. Samples are still being processed; results will be presented in the annual report.

2) We just completed a long-term study to document the kinetics of the VHSV antibody response. The experiment was intended to assess how long circulating antibodies remain detectable after herring survive a single exposure to the virus. The experiment terminated in July 2020, three years after the herring were exposed to VHSV. Fish continued to demonstrate neutralizing antibodies, indicating immunity to VHS, throughout the entire post-exposure period (Fig. 1).

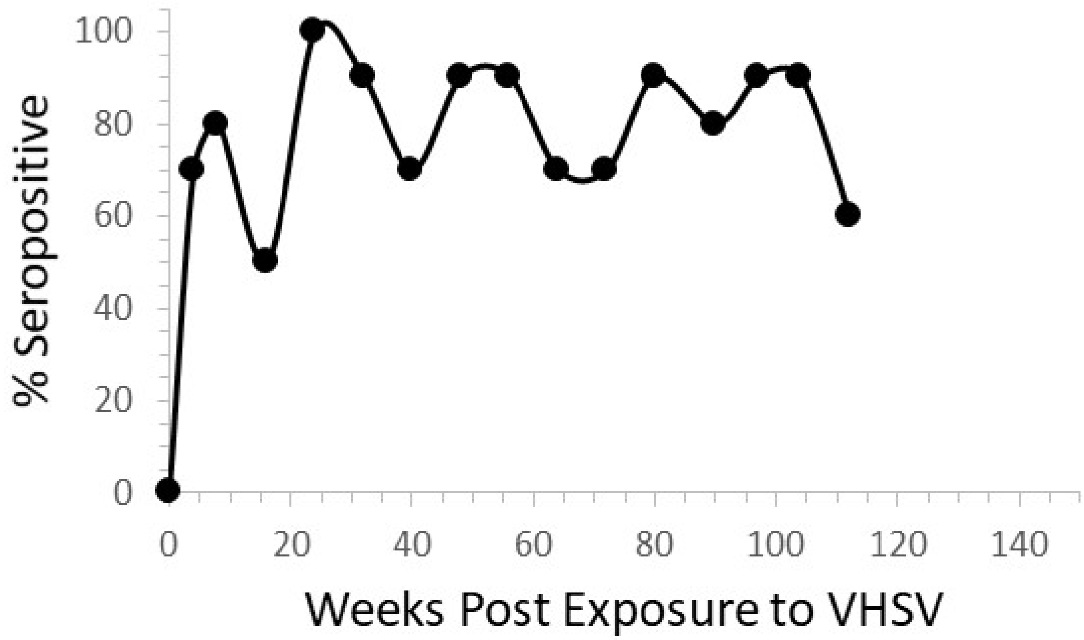

Figure 1. Monthly profile of neutralizing antibodies in Pacific herring that survived viral hemorrhagic septicemia virus exposure. Each data point represents the percent seropositive from 10 subsampled herring. None of the specific pathogen-free negative controls (n = 10 / sampling date) tested positive for neutralizing antibodies on any of the subsampling days (data not shown). The experiment was terminated in July 2020, nearly 3 years after exposure to VHSV. Samples from weeks 120, 128, 136, and 144 are currently being processed; results will be provided in the 2020 final report. Note: all these samples will be reprocessed using the updated assay methods that include the addition of exogenous complement; as a result, the final values are expected to change slightly.

3) As a no-cost contribution to the Whitehead project (#20170115), an exposure study was performed to evaluate the susceptibility of herring to *Ichthyophonus* after surviving embryonic exposure to oil. Briefly, groups of Pacific herring were exposed to PAH's as embryos and raised under specific pathogen-free conditions through metamorphosis to juveniles at the Marrowstone Marine Field Station. After metamorphosis, groups of previously oiled and -unoiled specific pathogen-free (SPF) herring were injected with *Ichthyophonus*. After *Ichthyophonus* exposure, mortality was slightly higher among the group that survived oil exposure (Figure 2); statistics on the mortality curves have not yet been performed.

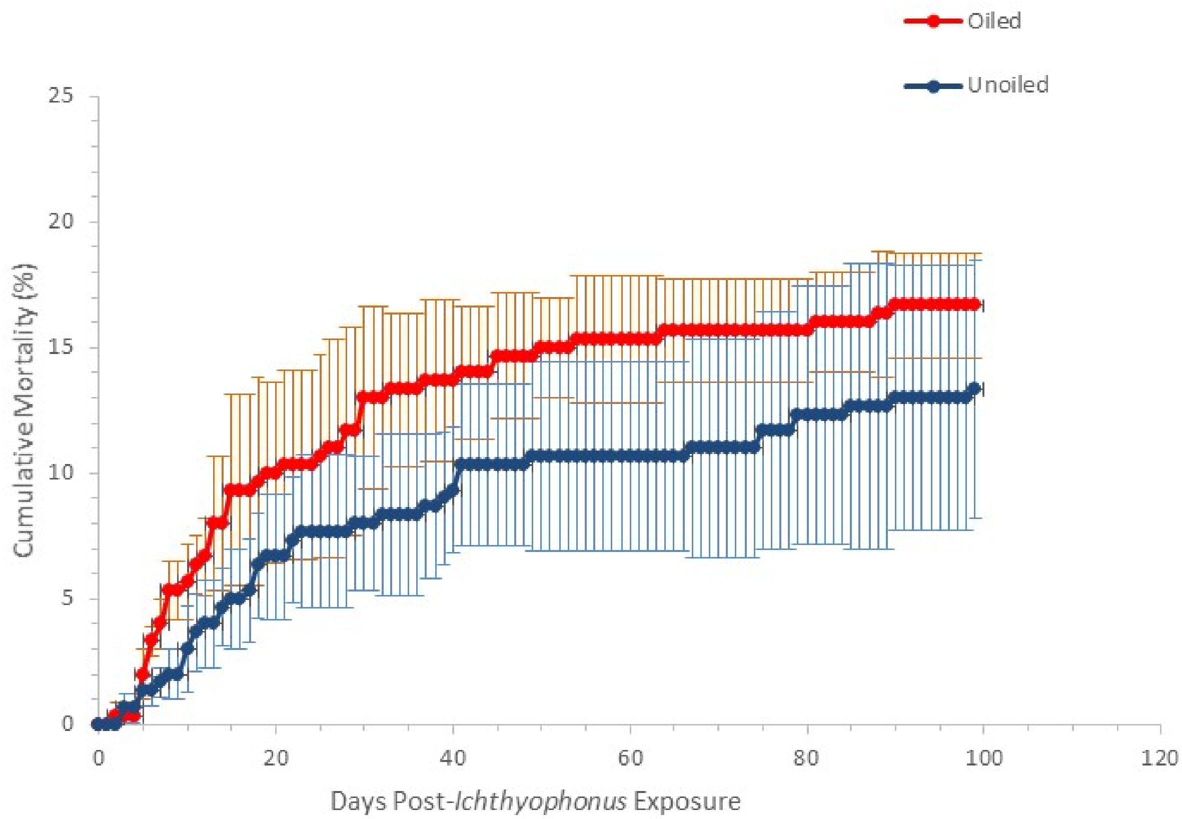

Figure 2. Cumulative mortality among two groups of SPF herring (previously oiled and -unoiled) after exposure to *Ichthyophonus* by inter-coelomic injection. Mortality in both negative control groups (exposed to saline in lieu of *Ichthyophonus*) was negligible (data not shown. Each data point represents mean cumulative mortality from each of 3 tanks (N = 62 herring / tank), and error bars indicate 2 SD from the mean.

4) As a significant contribution to the Whitehead project (#20170115), an exposure study was performed to evaluate the susceptibility of herring to VHSV after surviving larval exposure to oil. This was a pilot experiment, intended to set the sampling parameters for our definitive experiment that is scheduled to start in August 2020. Briefly, herring embryos were collected from wild spawn in Puget Sound and transferred to the Marrowstone Marine Field Station for grow-out. On the day of hatch, larvae were transferred to replicate tanks (N = 4 tanks / treatment; 1,000 larvae / tank) for each of 3 treatments (unoiled, exposure to low oil concentrations, and exposure to high oil concentrations). Groups were exposed to Alaska North Slope oil for 20 consecutive days, after which the oil generator was turned off and the larvae were raised through metamorphosis to juveniles. Total PAH concentrations were assessed from the exposure water and the herring tissues. Throughout this grow-out period, data were collected on total survival to metamorphosis and larval growth rates in each treatment (data not shown). After metamorphosis, the high oil group was terminated because too few fish survived through metamorphosis to justify further experimentation. Equal numbers of fish from the remaining treatments (unoiled and low oil) were transferred to larger tanks, where survival and growth assessments continued through the juvenile phase; these fish will be used for the definitive VHSV exposure study scheduled for August 2020. However,

additional fish from both groups (unoiled and oiled) remained and were used in this pilot study to assess whether surviving larval exposure to oil impacts the susceptibility of juvenile herring to VHS. Results indicated that the onset of VHS mortality was delayed among juvenile herring that survived larval exposure to PAH's (Figure 3).

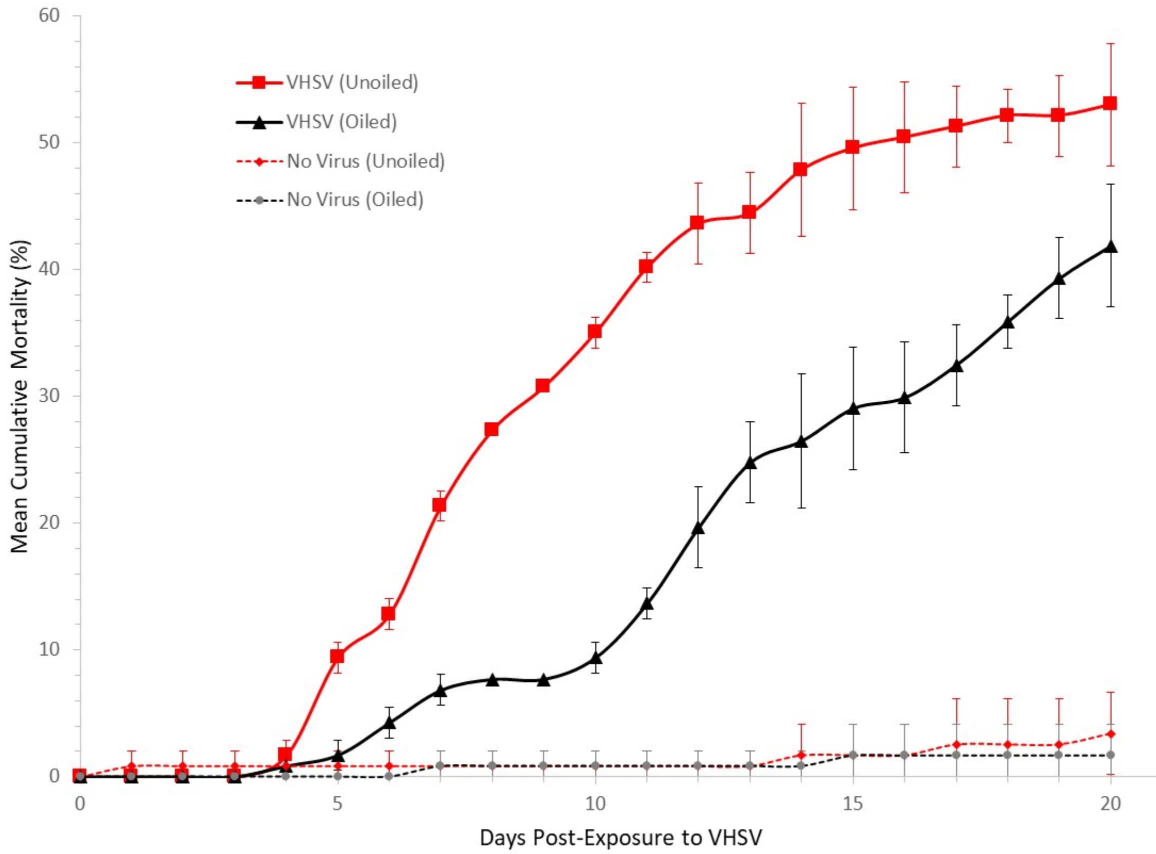

Figure 3. Effects of larval PAH exposure on the subsequent susceptibility of juvenile survivors to VHSV. Each data point represents mean cumulative mortality from each of 3 tanks (N = 39 herring / tank), and error bars indicate 2 SD from the mean

5) Surveillance of PWS herring for antibodies to VHSV.

A plaque neutralization assay was developed to detect antibodies to VHSV in Pacific herring. Last year the assay was optimized to increase detection sensitivity. Optimization included heat treatment of the plasma to inactivate interferons and other labile proteins that interfere with the assay; additionally, the optimized assay incorporates the addition of known amounts of exogenous complement back to the test plasma. All the historical plasma samples (2012 – present) will be reprocessed using the new methods to take advantage of the enhanced sensitivity, and all new samples going forward will be processed using the revised techniques.

Results between from the original and revised assay methods tracked each other and were generally slightly higher and mean antibody titers were much higher using the revised methods (Figure 4). Throughout the survey years 2012 – 2020, the prevalence of seropositive herring (those with detectable levels of neutralizing antibodies to VHSV) was highest in 2015 (25%; 15/60) and lowest in 2019 and 2020 (1.2 -1.3%; n = 380 & 165;

respectively). These results will be incorporated into an ASA-type model to assess whether the apparent lack of VHSV exposures in 2019 and 2020 resulted in population-level changes to herring year classes or recruitment. Within each year, the prevalence of seropositives generally increased with herring age (Figure 5). The prevalence of seropositives among the oldest cohorts ranged between 13-24% between 2012 – 2018 but declined to 0% in 2019 (age data for 2020 are not yet available). Archived plasma from Sitka Sound will be processed using the optimized assay methods next; it is anticipated that these results will be available by the end of FY20.

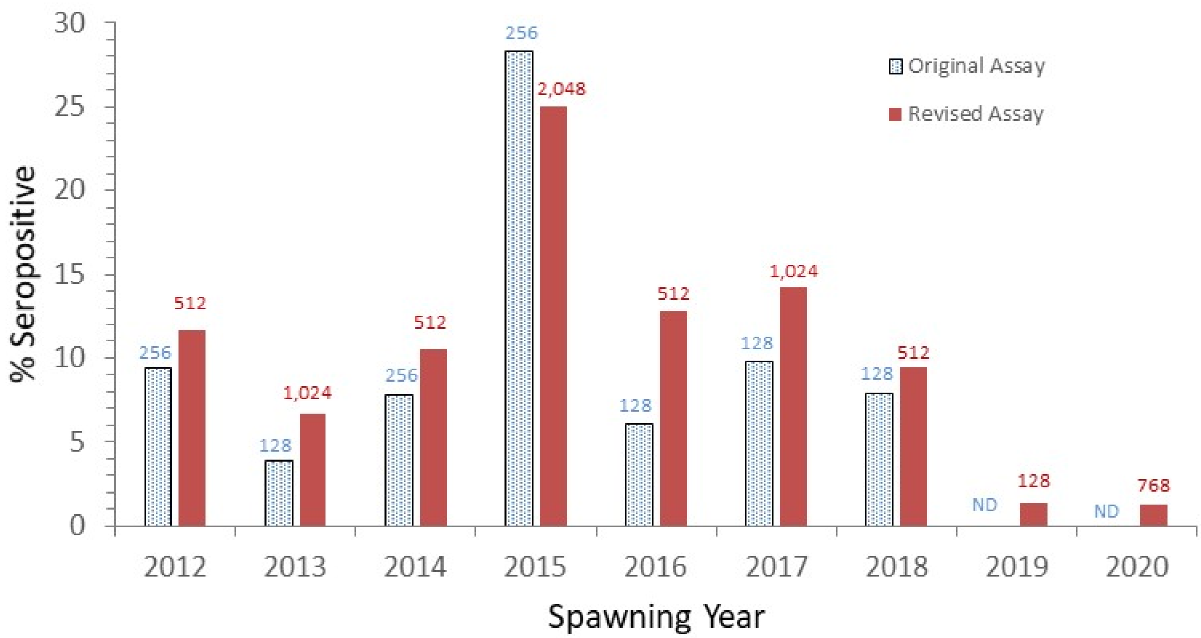

Figure 4. Comparison of VHS virus neutralizing antibody results in Prince William Sound herring using the original and revised (optimized) assay methods. ND = No Data; samples were not processed using the original assay methods in 2019 and 2020. Sample year 2020 includes samples from 165 fish; an additional 100 samples remain in Cordova and will be processed when they are received. Numerals above the bars represent the median antibody titer (reciprocal 50% inhibitory dilution – ID_{50}) in the samples. Detection limits for lower antibody titer = 64 and upper titer = 2,048.

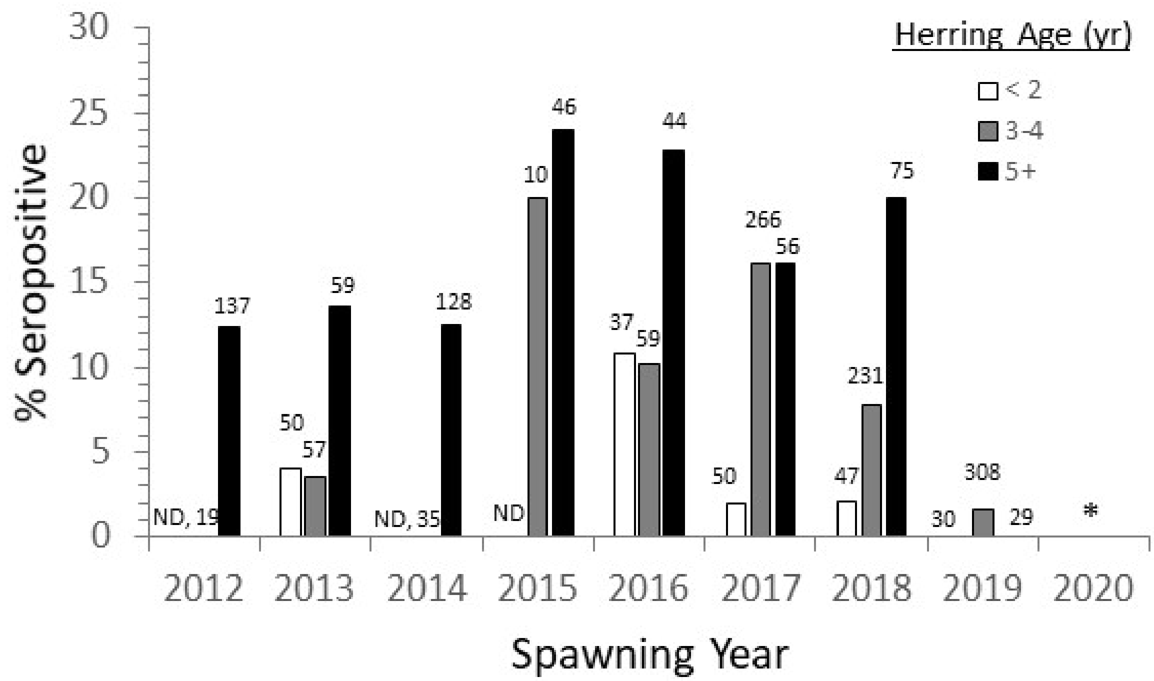

Figure 5. Prevalence of seropositives in each age bracket, including juveniles (<u><</u>Age 2), new recruits (Age 3-4), and older adults (Age 5+). Numerals above the bars indicate N. Age data from 2020 is currently pending.

6) *Ichthyophonus* Phylogenetics

As a no-cost addition to the project, we have been assessing the genetic relatedness of *Ichthyophonus* from Prince William Sound to isolates from throughout the world. The approach involves comparing the nucleotide sequences in 3 gene regions (COX-1, EF-1, and 18S). The analyses remain ongoing, but preliminary assessments indicate that more than one species of *Ichthyophonus* exists. The type species (*I. hoferi*) occurs most commonly in trout and other salmonids, primarily in freshwater aquaculture. Further, preliminary analyses suggest that enough genetic difference may occur to warrant a novel genus designation for the parasite that occurs in Prince William Sound and the North Pacific Ocean (Figure 6). A more complete analysis of the phylogenetic results will be available by the end of FY20.

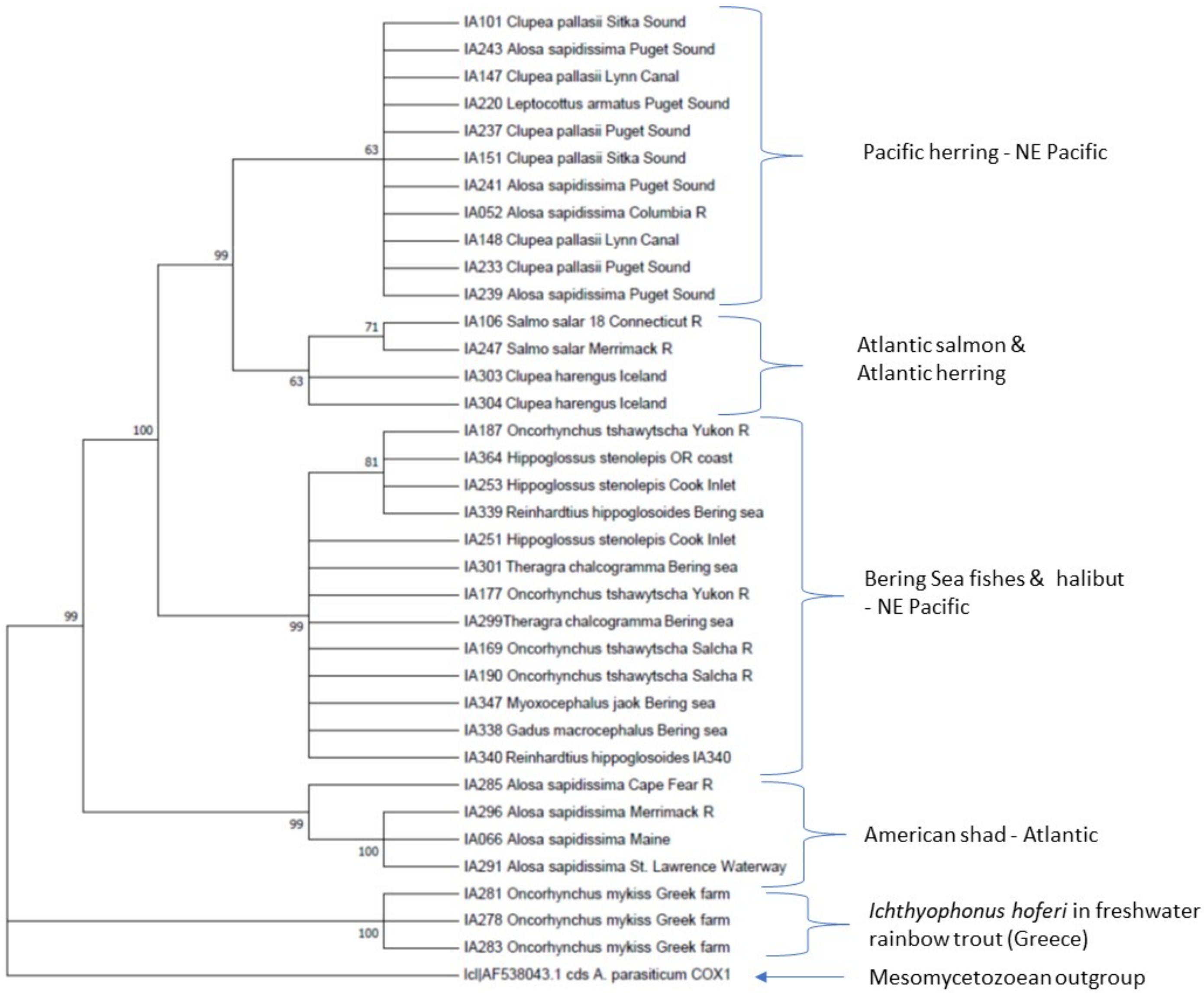

Figure 6. *Ichthyophonus* phylogenetics based on 1191 bases in the COX-1 gene. Values on the branches indicate percentages of bootstrap support, indicating the percentage of times the same branch occurred in repeated phylogenetic constructions.

7) *Ichthyophonus* in Cordova Harbor

Catrin Wendt defended her M.S. thesis at the University of Washington, School of Aquatic and Fishery Sciences, titled "*Ichthyophonus* in Pacific herring: Investigating a transmission hot spot". She determined that the prevalence of *Ichthyophonus* in age 0 herring rapidly increases in Cordova Harbor during the spring (Figure 7). The cause of this increased prevalence was not determined, but may involve:

A) An exodus of healthy herring from the harbor during the spring, leaving the infected herring behind if they are too sick to participate in the outmigration.

B) Increased infection pressures resulting from offal discharges when the fish processing plants become active in the spring.

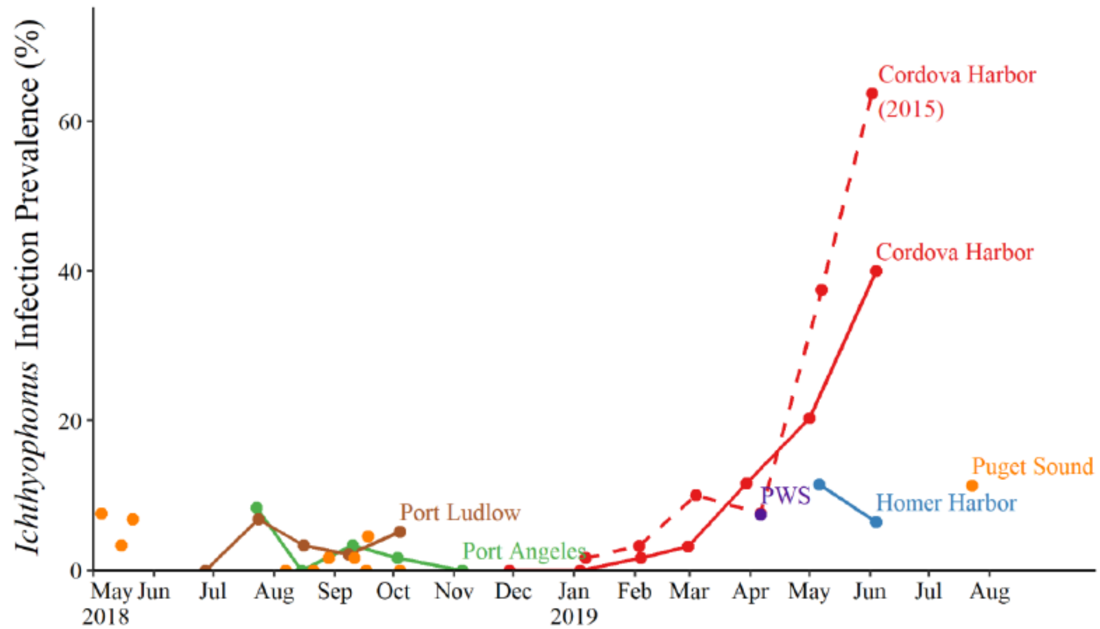

Figure 7. *Ichthyophonus* infection prevalence in Age 0 herring from various locations throughout the NE Pacific, including Cordova Harbor, throughout the year (May 2018 – Aug 2019).

8) *Ichthyophonus* associated with Pacific herring and walleye pollock eggs.

As a first step towards investigating the possibility of *Ichthyophonus* transmission to Pacific herring through ovivory, we examined fish eggs for the presence of *Ichthyophonus*. First, walleye pollock were collected from the Shelikof Strait region from March 7-14, 2020 aboard a NOAA stock assessment cruise through the generous contribution of Dave McGowan. Eggs were collected from female ovaries. *Ichthyophonus* infections prevalence, based on tissue explant culture, was 42% (25/59) in the liver and 52% (28/54) in the pollock eggs. Additionally, paired heart/egg samples were collected from PWS herring (April 8-17, 2020). *Ichthyophonus* prevalence was 11% (32/188) in hearts and 7% (6/82) in eggs. Ovaries from both sources (pollock and herring) were transported to the Marrowstone Marine Field Station and the eggs were repeatedly fed to SPF herring to attempt transmission. *Ichthyophonus* was not recovered from any of the herring fed with either egg type.

We were encouraged to detect *Ichthyophonus* in association with eggs from both hosts, as this was the primary objective of the 2020 work. It was not completely unexpected that consumption of eggs from the ovaries failed to transmit the parasite because the ovaries were 7+ days old by the time they were collected, shipped, received at the laboratory, and fed to the experimental fish. It is likely that any *Ichthyophonus* in these extracted ovaries was dead by the time the eggs were fed to our experimental fish. Experimental adjustments will be made in 2021 to try and account for this possibility. Additional studies in 2021 will be committed towards assessing the relationship between *Ichthyophonus* and the fish eggs (i.e. surface association or true infection).

2. PROJECT STATUS OF SCHEDULED ACCOMPLISHMENTS

A. Project Milestones and Tasks

Table 1. Project milestones and task progress by fiscal year and quarter, beginning February 1, 2017. Additional milestones and/or tasks have been added in red. C = completed, X = planned or not completed. 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
Milestone 1: Field Work																				
Task 1: Collect herring eggs for rearing SPF colonies	C				C				C				C				X			
Task 2: Collect adult herring to assess annual infection and disease prevalence	C				C				C				C				X			
Task 3: Collect zooplankton for investigation of possible *Ichthyophonus* intermediate host	C				C				X[1]				X[1]				X[1]			
Task 4: Collect herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus		C				X[2]		C	C	C										
Task 5: Determine whether *Ichthyophonus* is associated with fish eggs														C				X		
Milestone 2: Lab Experiments																				
Task 1: Vibrio challenge experiments	C	C	C	C																
Task 2: SPF herring metamorphosed to juveniles			C				C				C				C				X	
Task 3: Assess effects of salinity on fish-to-fish transmission of *Ichthyophonus*					C	C	C	C												
Task 4: Assess the effect of temperature on VHSV shedding									C	C	C	C								
Task 5: Assess challenge experiment to assess Ichthyophonus transmission through ovivory														C				X		
Task 6: Histology and immunochemistry to investigate *Ichthyophonus* association with fish eggs																			X	
Milestone 3: Lab Analyses																				
Task 1: Process spring adult herring to determine infection and disease prevalence		C				C				C				C				X		

	FY17				FY18				FY19				FY20				FY21			
Task 2: Validate the plaque neutralization assay using wild herring		C	C			C	C			C	C			C	C			X	X	
Task 3: Analysis of plasma samples			C				C				C								X	
Task 4: Analysis of archived plasma samples from 2012-16				C											C					
Task 5: Histological processing of herring from Cordova Harbor to assess *Ichthyophonus*-infected offal in the stomach bolus															C					
Task 6: PCR and CISH analyses of zooplankton samples for *Ichthyophonus*																			X[1]	
Milestone 4: Data																				
Task 1: Share 2012-16 plasma sample data with modelers				C												X				
Task 2: Upload data and metadata to Workspace				C				C				C								X
Milestone 5: Reporting																				
Task 1: Annual report					C				C				C				X			
Task 2: FY work plan (DPD)			C				C				C				C				X	
Task 3: FY17-21 Final Report																				X
Milestone 6: Meetings & Conferences																				
Task 1: Annual PI meeting				C				C				C								X
Milestone 7: Publications			C	C		C	C	C			C									X

B. Explanation for not completing any planned milestones and tasks

Nothing has changed since last year. The following changes were approved in the 2020 Work Plan

X[1]:2019 Zooplankton collections: Collections were discontinued: see explanation in section 'C' below.

X[2]: 2018 Cordova Harbor herring: Juvenile herring were not present in Cordova Harbor during 2018. Although sampling was attempted, no fish were collected in 2018. However, juvenile herring did occur in Cordova Harbor during 2019, and monthly samples (including fish, sediment, and water) were collected.

C. Justification for new milestones/tasks

Nothing has changed since last year. The following changes were approved in the 2020 Work Plan

To evaluate the possibility of an intermediate host in the *Ichthyophonus* life cycle, we processed numerous zooplankton samples using *Ichthyophonus*-specific qPCR primers. To date, these efforts have not returned consistent results, and we do not feel confident reporting any true positives. Additionally, to focus our surveillances on a copepod with a high likelihood of exposure to *Ichthyophonus*, we sampled sea lice from the flanks of herring demonstrating external ulcers from ichthyophoniasis; these lice were grazing on mucus and *Ichthyophonus* life stages that occurred on the external surfaces of heavily infected wild herring. These lice samples also returned inconclusive results using the *Ichthyophonus* primers. Therefore, until we have more convincing evidence that an intermediate host is involved with this parasite life history, we will be investigating possible alternative routes of *Ichthyophonus* transmission to Pacific herring (described in Section 4B).

3. PROJECT COORDINATION AND COLLABORATION

A. Within an EVOSTC-funded Program

Herring Research and Monitoring

- We are working closely with PWS Science Center (#20120111-G) and the Alaska Department of Fish and Game (20160111-F) to collect herring tissue and plasma samples during the spring herring cruises (shared research platforms). Additionally, ADF&G continues to provide age data for the fish health samples.
- As In-Kind contributions to Dr. Andrew Whitehead's project (#20170115), we provided samples for their reference genome, completed a series of experimental exposures of Specific Pathogen-Free (SPF) herring to VHSV, and initiated an experimental study to assess the relative susceptibility of oil-exposed herring to *Ichthyophonus*. Additionally, in 2020, we exposed SPF herring larvae to concentrations of Alaska North Slope Crude Oil and performed 2 VHSV challenge studies to determine the relative susceptibility of survivors to the disease.
- Serum neutralization results, to assess herd immunity by quantifying VHSV neutralizing titer were completed in July 2020; results will be shared with Dr. Trevor Branch (#20120111-C). 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 (#19120111-A), several experiments were initiated and are currently underway at the U.S. Geological Survey (USGS) - Marrowstone Marine Field Station.
    - An *in vivo* study was initiated to evaluate the histological infection threshold associated with herring mortality from ichthyophoniasis.
    - Archived histology samples were processed from PWS and Sitka Sound, dating to 2007. All culture-positive samples have now been processed and read.

Gulf Watch Alaska

Nothing to report

Data Management

Infection prevalence data from PWS and Sitka Sound are provided to the data management team annually.

B. With Other EVOSTC-funded Projects

Nothing to report

C. 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 *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) funded herring disease projects between 1994 – present.
- We have partnered with ADF&G – Sitka & Juneau 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 North Pacific Research Board (NPRB; # 1807: *Ichthyophonus* in Pacific Herring).
- We have partnered with Drs. John Incardona and Nat Sholtz (National Oceanic and Atmospheric Administration – Northwest Fisheries 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.
- We have partnered with WDFW to provide heath assessments of Puget Sound herring

4. PROJECT DESIGN

A. Overall Project Objectives

1. Provide pathogen and disease prevalence data to inform the ASA model
2. Produce specific pathogen-Free (SPF) Pacific herring for laboratory experiments
3. Process new and archived herring plasma samples for indications of prior VHSV exposure
4. Validate the novel plaque neutralization assay using wild herring
5. Investigate the possibility of *Ichthyophonus* transmission through the consumption of fish eggs containing the parasite
6. Determine the causes for abnormally high *Ichthyophonus* prevalence among juvenile Pacific herring that establish temporary residency in Cordova Harbor

B. Changes to Project Design and Objectives

Nothing changed since last year.

5. PROJECT PERSONNEL – CHANGES AND UPDATES

No changes to report since last year

6. PROJECT BUDGET

A. Budget Forms (See GWA FY20 Budget Workbook)

Please see project budget forms compiled for the program.

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	
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	
Contractual	$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	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
SUBTOTAL	$181.5	$210.0	$217.2	$223.2	$230.4	$1,062.3	
General Administration (9% of	$16.3	$18.9	$19.5	$20.1	$20.7	$95.6	
PROJECT TOTAL	$197.8	$228.9	$236.7	$243.3	$251.1	$1,157.9	
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	

B. Changes from Original Project Proposal

As approved in FY19, an additional $22,500 / year (FY19-21) is requested to enable the processing of additional herring plasma samples from PWS. This supplement will 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).

These expanded samples (in addition to the 180 random samples from the AWL collections) will be size-selected and are intended to provide more robust sample sizes for each available herring age class. The resulting reduction in variability around the age-specific immunity is needed by Dr. Branch for the revised ASA modelling.

C. Sources of Additional Project Funding

- USGS provides matching funds for PIs and support staff as part of their base appropriation.
- The HDP 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.

7. FY17-20 PROJECT PUBLICATIONS AND PRODUCTS

Publications

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.

Hart, L.M., N. Lorenzen, K. Einer-Jensen, M. Purcell, P.K. Hershberger. 2017. Influence of temperature on the efficacy of homologous and heterologous DNA vaccines against viral hemorrhagic septicemia (VHS) in Pacific herring. Journal of Aquatic Animal Health 29: 121-128.

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.

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, A. Lindquist, T. Sandell, M.L. Groner, D. Lowry. *Accepted*. A Geographic Hot Spot of *Ichthyophonus* infection in the Southern Salish Sea, USA. Diseases of Aquatic Organisms.

Gross, L., J. Richard, P. Hershberger, K. Garver. 2019. Low susceptibility of sockeye salmon *Oncorhynchus nerka* to viral hemorrhagic septicemia virus genotype IVa. Diseases of Aquatic Organisms 135: 201-209.Sitkiewicz, S.E., N. Wolf, P.K. Hershberger, T. Scott 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 Fish Diseases.

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

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

LaDouceur, E.E.B., J. St Leger, A. Mena, A. MacKenzie, J. Gregg, M. Purcell, W. Batts, P. Hershberger. *Accepted*. *Ichthyophonus* Infection in Opaleye (*Girella nigricans*). Veterinary Pathology 57: 316-320.

Hershberger, P.K., M. Stinson, B. Hall, J.L. Gregg, A.M. MacKenzie, J.R. Winton. *Accepted*. Pacific herring are not susceptible to vibriosis under laboratory conditions. Journal of Fish Diseases.

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

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

Published and updated datasets

Metadata and data describing infection prevalence results from herring health surveillance have been provided to Axiom annually.

Presentations

MacKenzie, A.H., J.L. Gregg, M.D. Wilmot, T. Sandell, D. Lowry, P.K. Hershberger. June 20-22, 2017. Poster. Temporal and spatial patterns of *Ichthyophonus* in Pacific herring throughout the southern Salish Sea. 58th Western Fish Disease Workshop. Suquamish, WA.

Sitkiewiz, S.E., B.P. Harris, P.K. Hershberger, N. Wolf. June 20-22, 2017. Poster. Effects of the parasite *Ichthyophonus* on groundfish growth and condition. 58th Western Fish Disease Workshop. Suquamish, WA.

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, M.D. Wilmot, R. Powers, M.K. Purcell. June 20-22, 2017. Platform. Long term shedding of viral hemorrhagic septicemia virus from Pacific herring. 58th Western Fish Disease Workshop. Suquamish, WA.

Sitkiewicz, S., B. Harris, P. Hershberger, N. Wolf. March 19-23, 2017. Poster. Impacts of the Parasite *Ichthyophonus* (sp.) on Groundfish Growth and Condition. Joint Meeting of the American Fisheries Society, Alaska Chapter American Water Resources Association, Alaska Section. Fairbanks, AK.

Hershberger, P.K., L. Hart, A. MacKenzie, R, Powers, M. Purcell. January 23-27, 2017. Poster. Quantifying the potential for disease impacts to Pacific Herring. Alaska Marine Science Symposium. Anchorage, AK.

Sitkiewicz, S., B. Harris, P. Hershberger, N. Wolf. January 23-27, 2017. Poster. Effects of the parasite *Ichthyophonus* (sp.) on groundfish growth and condition. Alaska Marine Science Symposium. Anchorage, AK.

Bravo, E., C. Conway, P. Hershberger, J. Gregg, M. Groner. October 11-13, 2018. Poster. Do histological analyses of herring infected with *Ichthyophonus* sp. suggest a shift from endemic to epidemic disease? Society for the Advancement of Chicanos / Hispanics and Native Americans in Science. San Antonio, TX.

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.

Hershberger, P.K., R.L. Powers, B.L. Besijn, J. Rankin, M. Wilson, B. Antipa, J. Bjelland, A.H. MacKenzie, J.L. Gregg, M.K. Purcell. June 17-20, 2019. Platform. Intra-annual variability in waterborne *Nanophyetus salmincola*. AFS Fish Health Section Annual Meeting and 60th Western Fish Disease Workshop. Ogden, UT.

Groner, M., E. Bravo, C. Conway, J. Gregg, P. Hershberger. January 28-31, 2019. Poster. A quantitative histological index to differentiate between endemic and epidemic ichtyhophoniasis in Pacific herring. Alaska Marine Science Symposium. Anchorage, AK.

Wendt, C., P. Hershberger, C. Wood. January 28-31, 2019. Poster. Patterns of *Ichthyophonus* sp. infection in age zero Pacific herring. Alaska Marine Science Symposium. Anchorage, AK.

Cypher, A.D., P. Hershberger, N. Scholz, J.P. Incardona. January 3-7, 2019. Larval cardiotoxicity and juvenile performance are likely contributors to the delayed fishery collapse of Pacific herring after the *Exxon Valdez* oil spill. Society for Integrative & Comparative Biology Annual Meeting. Tampa, FL.

Gill, J.A., P. Hershberger, J. Incardona, A. Whitehead. November 3-7, 2019. Poster. Interactions between oil exposure and immune function relevant for Pacific herring population collapse. Society of Environmental Toxicology and Chemistry. Toronto, Ontario, Canada.

Mena, A.J., J. St. Ledger, A. MacKenzie, J. Gregg, M. Purcell, W. Batts, P. Hershberger, E.E.B LaDouceur. May 16-20, 2020. Poster. *Ichthyophonus* sp. infection in opaleye (*Girella nigricans*). International Aquatic Animal Medicine Conference. Tampa, FL.

Pegau et al. Jan 27-31, 2020. Poster. Prince William Sound Herring Research and Monitoring Program. Anchorage, AK.

Cypher, A.D., P.K. Hershberger, J. Gregg, J. Incardona. Jan 27-31, 2020. Platform. Influence of embryonic crude oil exposure in overwinter fasting and disease susceptibility in juvenile Pacific herring (*Clupea pallasii*). Alaska Marine Science Symposium. Anchorage, AK.

Hershberger, P.K., A.H. MacKenzie, J.L. Gregg, R. Powers, M.K. Purcell. Jan 27-31, 2020. Poster. Long term shedding of viral hemorrhagic septicemia virus from Pacific herring. Alaska Marine Science Symposium. Anchorage, AK.

Outreach

Information about the herring disease program is included on the USGS - Marine Field Station (https://www.usgs.gov/centers/wfrc/science/marrowstone-marine-field-station-mmfs?qt-science_center_objects=0#qt-science_center_objects) and Prince William Sound Science Center (https://pwssc.org/herring/) web pages.

8. LITERATURE CITED

None