ATTACHMENT C ***EVOSTC Annual Project Report Form***

Form Rev. 10.3.14

*Please refer to the Reporting Policy for all reporting due dates and requirements.

1. Program Number: *See*, Reporting Policy at III (C) (1).

14120111-K

2. Project Title: *See*, Reporting Policy at III (C) (2).

PWS Herring Program - Herring Disease Program

3. Principal Investigator(s) Names: *See*, Reporting Policy at III (C) (3).

Paul K. Hershberger

4. Time Period Covered by the Report: *See*, Reporting Policy at III (C) (4).

Feb 1, 2014 – January 31, 2015

5. Date of Report: *See*, Reporting Policy at III (C) (5).

February 2015

6. Project Website (if applicable): *See*, Reporting Policy at III (C) (6).

http://pwssc.org/research/fish/pacific-herring/

7. Summary of Work Performed: *See*, Reporting Policy at III (C) (7).

Field Findings:

A. Three samples of Pacific herring were collected from Prince William Sound (n=60 / collection) during the spring pre-spawn period from March 26 – 29, 2014:

	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Gravina Point	2-8%*	25% (15/60)	0% (n=60)
Snug Corner Cove	0%	22% (13/60)	0% (n=60)
Red Head	0%	33% (20/60)	0% (n=60)

*Virology samples were processed in pools containing tissues from 5 fish. One pool of tissues produced cytopathic effect with very low titers after 2nd and 3rd passage. VHS virus was confirmed in the passed-material using a nested PCR and VHSV primers. Individual samples from these 5 fish were not processed, as the viral tissue titer in the pooled homogenate was $<10^1$ PFU / g.

B. Three samples of Pacific herring were collected from Sitka Sound Sound (n=60 / collection) during the spring pre-spawn period from March 26 – 28, 2014:

	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Causeway	0%	25% (15/60)	2% (1/60)
Middle Island	0%	22% (12/60)	0% (n=60)
Inner Point	0%	33% (16/60)	0% (n=60)

C. Juvenile herring were collected from PWS cruises in collaboration with the PWSSC surveys:

	Date	Sample Size	VHSV Prevalence	*Ichthyophonus* Prevalence
Simpson Bay	Nov 15	35	0%	0%
Bear Trap	Nov 16	61	0%	0%
Eaglek	Nov 19	61	0%	3%
Simpson	Nov 23	25	0%	4%

D. Juvenile herring were collected by beach seine from several locations in the Jan Juan Island region of Washington State from Sept 11-12, 2014. During the seining, it was observed that some individuals demonstrated external signs of VHS including hemorrhaging around the eyes and fin bases. A subsample of these lesioned individuals were selected and processed to confirm the presence of VHSV. Note: the samples were frozen at -20°C for several months prior to processing; this is a very unsuitable preservation method for the recovery of VHSV. From the first location (Sept 21) 27% (6/22) showed CPE indicative of VHSV, and from the second location (Sept 22) 12.5% (3/24) showed CPE indicative of VHSV. Tissue titers were as high as $5x10^3$ PFU / g. VHSV was confirmed by RT-PCR and viral sequencing.

Laboratory Findings:

A. Conway, C.M., M.K. Purcell, D.G. Elliott, P.K. Hershberger. In Press. Detection of Ichthyophonus by chromogenic in situ hybridization. Journal of Fish Diseases.

Ichthyophonus-like organisms have been reported in amphibians, reptiles, birds and invertebrates and may have been incorrectly classified under a single type species, *I. hoferi*. Although less sensitive than other detection techniques such as explant tissue culture, histopathological examination is effective for simultaneously evaluating host response and severity of *Ichthyophonus* infections. Histological sections showing positive periodic acid-Schiff (PAS) staining of multinucleate organisms 50-250 µm in diameter can be presumptive for *Ichthyophonus*, but lack of a definitive confirmatory test may lead to misdiagnosis, particularly when the organism is not cultured. We developed a chromogenic in situ hybridization (CISH) procedure that specifically detected *Ichthyophonus* ribosomal DNA in histological sections thereby complementing the histological diagnosis by providing highly specific molecular confirmation of the observed organism. A digoxigenin-labeled oligonucleotide probe was designed to target conserved portions of the 18S small subunit ribosomal gene of known *Ichthyophonus* species *I. hoferi* and *I. irregularis*. Formalin-fixed, paraffin-embedded tissues from naturally infected Chinook salmon (*Oncorhynchus tshawytscha*) and red-spotted newt (*Notophthalmus viridescens*), and experimentally infected Pacific herring (*Clupea pallasii*), rainbow trout (*O. mykiss*) and Pacific staghorn sculpin (*Leptocottus armatus*) were analyzed by CISH and PAS staining. Probe hybridization was indicated by dark purple precipitates and correlated with the distribution and morphology of parasites observed in PAS-positive tissues and also identified *Ichthyophonus* developmental stages in the presence of PAS-positive host cells. The CISH probe hybridized with PAS-positive, *Ichthyophonus*-like organisms in all host species except the red-spotted newt, supporting the hypothesis that the organism infecting amphibians is taxonomically distinct from fish-associated *Ichthyophonus*. The CISH has utility for both diagnostic and research applications.

B. Kocan, R., L. Hart, N. Lewandowski, P. Hershberger. 2014. Viability and infectivity of *Ichthyophonus sp.* in post-mortem Pacific herring, *Clupea pallasii*. Journal of Parasitology 100: 790-796.

Ichthyophonus-infected Pacific herring, *Clupea pallasii*, were allowed to decompose in flowing seawater, then serially sampled for 4 wk and examined for the presence of *Ichthyophonus* as determined by in vitro culture and single plane histology. The same tissues were fed to *Ichthyophonus*-free Pacific staghorn sculpins, *Leptocottus armatus*, to determine the duration of parasite infectivity. *Ichthyophonus* sp. was viable in decomposing herring viscera and muscle for at least 4 wk post-mortem and remained infectious for sculpins for up to 5 days post-mortem. Many of the morphologic changes observed were similar to those previously reported to occur during the first 24 hr following death of the host, but also included novel forms not previously described. The significance of extended survival and progressive morphologic transformation in the post-mortem host is unknown, but it could be inferred that it has survival value for the parasite.

C. Emmenegger, E.J., J.A. Glenn, J.R. Winton, W.N. Batts, J.L. Gregg, P.K. Hershberger. 2014. Molecular identification of erythrocytic necrosis virus (ENV) from the blood of Pacific herring (*Clupea pallasii*). Journal of Veterinary Microbiology 174: 16-26.

Viral erythrocytic necrosis (VEN) is a condition affecting the red blood cells of more than 20 species of marine and anadromous fishes in the North Atlantic and North Pacific Oceans. Presently, VEN is diagnosed by observation of typical cytoplasmic inclusion bodies in stained blood smears from infected fish. The causative agent, erythrocytic necrosis virus (ENV), is unculturable and a presumed iridovirus by electron microscopy. *In vivo* amplification of the virus in cultured Pacific herring and subsequent virus concentration, purification, DNA extraction, and high-throughput sequencing methodologies were applied to obtain genomic ENV sequences. Fragments with the highest sequence identity to the family *Iridoviridae* were used to design four sets of ENV-specific polymerase chain reaction (PCR) primers. Testing of blood and tissue samples from experimentally and wild infected Pacific herring as well as DNA extracted from other amphibian and piscine iridoviruses verified the four assays were specific to ENV. Sensitivity testing determined a limit of detection of 0.0003 ng. Preliminary phylogenetic analyses of a 1448 bp fragment of the putative DNA polymerase gene supported inclusion of ENV in a proposed sixth genus of the family *Iridoviridae* that contains other erythrocytic viruses from ectothermic hosts. This study provides the first molecular evidence of ENV's inclusion within the *Iridoviridae* family and offers a conventional PCR assay as a means of rapidly surveying the ENV-status of wild and propagated Pacific herring stocks.

D. Wilson, A. E., T. L. Goldberg, S. V. Marquenski, W. J. Olson, R. F. Goetz, P. K. Hershberger, K. L. Toohey-Kurth. 2014. Development and evaluation of a blocking enzyme-linked immunosorbent assay and virus neutralization assay to detect antibodies to viral hemorrhagic septicemia. Clinical and Vaccine Immunology 21: 435-442.

Currently, detection of VHSV relies on virus isolation, which is lethal to fish and only indicates current infection status. A serological method is required to ascertain prior exposure. Here, we report the development of two serologic tests for VHSV that are non-lethal, rapid, and species-independent: a virus neutralization assay (VN) and a blocking enzyme-linked immunosorbent assay (ELISA). Serum was collected from 34 uninfected fish (VHS negative group) and 28 fish that survived VHS virus infection (VHS positive group). The VN did not detect neutralizing antibodies in the serum of any of the 34 VHSV negative fish, demonstrating a test specificity of 100%. Neutralizing antibodies were detected in 12 of 28 VHS positive fish, indicating a test sensitivity of 42.9%. The anti-nucleocapsid blocking ELISA detected non-neutralizing VHSV antibodies in 4 of the 34 fish in the VHS negative group, indicating a specificity of 88.2%. Non-neutralizing antibodies were detected in 27 of 28 VHS positive fish, indicating a sensitivity of 96.4%. Used in parallel, the VN and ELISA correctly identified all survivors of VHSV infection and unexposed fish. Our VN and ELISA are valuable tools for assessing exposure to VHSV and should improve detection and surveillance efforts for both wild and commercial fish populations.

E. Burge, C. A., C. M. Eakin, C. S. Friedman, B. Froelich, P. K. Hershberger, E. E. Hofmann, L. E. Petes, K. C. Prager, E. Weil, B. L. Willis, S.E. Ford, C. D. Harvell. 2014. Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science 6: 249-277.

Infectious diseases are common in marine environments, but the effects of a changing climate on marine pathogens are not well understood. Here, we focused on reviewing current knowledge about how the climate drives host-pathogen interactions and infectious disease outbreaks. Climate-related impacts on marine diseases are being documented in corals, shellfish, finfish, and humans; these impacts are less clearly linked to other organisms. Oceans and people are inextricably linked, and marine diseases can both directly and indirectly affect human health, livelihoods, and well-being. We recommended an adaptive management approach to better increase the resilience of ocean systems vulnerable to marine diseases in a changing climate. Land-based management methods of quarantining, culling, and vaccinating are not successful in the ocean; therefore, forecasting of conditions that lead to outbreaks and designing tools/approaches to affect these conditions may be the best tool to manage marine disease.

8. Coordination/Collaboration: *See,* Reporting Policy at III (C) (8).

Ongoing collaborations with partners within the PWS Herring Program include:

- Collection of shared zooplankton samples with Dr. Rob Campbell

- Collection of samples from Cordova Harbor (monthly collections of juvenile herring, plankton collections, stomach samples, and bioenergetics samples) with Drs. Kristin Gorman and Scott Pegau.

- Collection of juvenile herring samples from PWS bays with the PWSSC.

Additionally, we discussed sampling contingency plans with partners (Yumi Armitsu and John Moran) from the Gulf Watch Alaska Program in the event that they encounter visibly diseased fish during their field efforts.

9. Information and Data Transfer: *See,* Reporting Policy at III (C) (9).

In addition to the manuscripts listed above (Section 7), the following presentations were delivered during the current reporting period:

Invited seminars

November 24, 2014: Cordova Weekly Seminar Series
Ecology of Disease in Pacific herring

August 16, 2014: National Science Foundation, Research Coordination Network
Invited Presentation - Pathogen Persistence and Perpetuation Strategies in Marine Fishes: Perspectives from Pacific Herring. Friday Harbor, WA.

May 30, 2014: School of Aquatic and Fishery Sciences, University of Washington: FISH 404.
Guest Lecture. "How Does Science Really Work? The Frustration of Dead Ends and the Satisfaction of Tiny Advancements."

May 21, 2014: Tribal Climate Change Webinar Series
Invited Webinar: Climate Change and Marine Issues
Shifting Ocean Currents and Infectious / Parasitic Diseases of Marine Fishes
Co-hosted by the Institute for Tribal Environmental Professionals – Northern Arizona University,– Pacific Northwest Tribal Climate Change Project - University of Oregon, and North Pacific Landscape Conservation Cooperative

Scientific Presentations

Conway, C.M, M.K. Purcell, D.G. Elliott, P.K. Hershberger. August 31 – September 4, 2014. Poster. Detection of *Ichthyophonus* by Chromogenic In Situ Hybridization. 7th International Symposium on Aquatic Animal Health. Portland, OR.

Garver, K.A., J. Lovy, P. K. Hershberger. August 31 – September 4, 2014. Platform. Trafficking of Viral Hemorrhagic Septicemia Virus from wild to farmed fish. 7th International Symposium on Aquatic Animal Health. Portland, OR.

Hart, L.M. C. Conway, D. Elliot, P.K. Hershberger. August 31 – September 4, 2014. Platform. A qualitative assessment of the progression of ichthyophoniasis related external signs and distribution of host response and parasite morphology in somatic tissues of Pacific herring *Clupea pallasii*. 7th International Symposium on Aquatic Animal Health. Portland, OR.

McKibben, C.L., P.K. Hershberger, M.K. Purcell, C.M. Conway, D.G. Elliott. August 31 – September 4, 2014. Poster. Influence of Temperature and Fish Stock on Progression of *Ichthyophonus* Infections in Chinook Salmon (*Oncorhynchus tshawytscha*). 7th International Symposium on Aquatic Animal Health. Portland, OR.

10. Response to EVOSTC Review, Recommendations and Comments: *See,* Reporting Policy at III (C) (10).

N/A

11. Budget: *See,* Reporting Policy at III (C) (11).

Budget Category:	Proposed FY 12	Proposed FY 13	Proposed FY 14	Proposed FY 15	Proposed FY 16	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$0.0	$0.0	$170.4	$186.6	$190.8	$547.8	$ 101,940
Travel	$0.0	$0.0	$17.0	$17.0	$18.4	$52.4	$ 10,468
Contractual	$0.0	$0.0	$12.0	$12.0	$12.0	$36.0	$ 49,933
Commodities	$0.0	$0.0	$59.2	$52.2	$52.2	$163.6	$ 44,653
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Indirect Costs (*will vary by proposer*)							
SUBTOTAL	$0.0	$0.0	$258.6	$267.8	$273.4	$799.8	$ 206,994
General Administration (9% of	$0.0	$0.0	$23.3	$24.1	$24.6	$72.0	$23,300.0
PROJECT TOTAL	$0.0	$0.0	$281.9	$291.9	$298.0	$871.8	
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	

COMMENTS:
This summary page provides an five-year overview of proposed funding and actual cumulative spending.
Some of the 'Personnel' charges appear under the 'Contractual' category because student interns were hired as contractors Travel and commodities costs are expected to be higher in FY'2015; as such we are planning to spend the remaining balance in the next fiscal year.

We appreciate your prompt submission and thank you for your participation.