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).

PWS Herring Program #12120111-K

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

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, 2015 – January 31, 2016

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

February 2016

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. One sample of adult Pacific herring was collected from Prince William Sound (n=60) during the spring pre-spawn period on April 3, 2015:

	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Gravina Point	0% (n=60)	25% (15/60)	0% (n=60)

B. Three samples of adult Pacific herring were collected from Sitka Sound Sound (n=60 / collection) during the spring pre-spawn period from March 20 – 22, 2015:

	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Bieli Rock (May 20)	0%	10% (6/60)	0% (n=60)
Bieli Rock (May 22)	0%	13% (8/60)	0% (n=60)
Bieli Rock (May 22)	0%	20% (12/60)	0% (n=60)

C. One sample of adult Pacific herring was collected on April 27 by Ted Otis and Joe Loboy from Kamishak Bay; tissues were submitted to the ADF&G Anchorage Fish Pathology Laboratory for diagnostic testing (Courtesy J. Ferguson).

VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
0% (n=60)	2% (1/60)	0% (n=60)

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

	Date	Sample Size	VHSV Prevalence	VEN Prevalence	*Ichthyophonus* Prevalence
Simpson Bay*	Nov 6	46	0%	Results Pending	2%
Lower Herring	Nov 11	54	0%	Results Pending	2%
Whale Bay	Nov 12	60	0%	Results Pending	3%

*During the culturing of samples for VHSV, one sample from Simpson Bay produced questionable cytopathic effect (cell clumping) on EPC cells. The CPE was not indicative of VHSV or other viruses that typically cause plaques. The sample is currently under further investigation using molecular tools to determine whether a different agent was present and can be identified. Additionally, the sample was inoculated into SPF herring in an effort to perpetuate any live agent that may have caused the CPE.

E. Samples from the Craig herring fishery were submitted by Eric Coonradt (ADF&G – Sitka). A low proportion (approximately 1/100) of pre-spawn adult herring reportedly demonstrated external hemorrhages, possibly indicative of a pathogen, predator wounds, abrasion, or other wound. A random sample of 52 wild herring and a high-graded sample containing 8 affected herring (see image below) were frozen at -20C and shipped to the USGS – Marrowstone Marine Field Station for VHSV assessment using cell culture. VHSV was not detected in any of these fish (either the 8 high-graded samples or the 52 random samples). Although the samples were previously frozen at -20 °C prior to processing (not optimal for VHSV recovery), no CPE was detected in any of the samples, indicating that the lesions were not likely caused by high titers of VHSV. The lesions were possibly caused by predator- or net-induced injuries.

Laboratory Findings:

A. Hart, L.M., C.M. Conway, D.G. Elliott, P.K. Hershberger. *In Press*. Persistence of external signs in Pacific herring *Clupea pallasii* with ichthyophoniasis. Journal of Fish Diseases.

The progression of external signs of *Ichthyophonus* infection in Pacific herring *Clupea pallasii* Valenciennes was highly variable and asynchronous after intraperitoneal injection with pure parasite preparations; however, external signs generally persisted through the end of the study (429 d post-exposure). Observed signs included 'sandpaper skin,' open lesions, pigmented ulcers and / or bleeding ulcers. The prevalence of external signs plateaued 35 d post-exposure and persisted in 73-79% of exposed individuals through the end of the first experiment (147 d post-exposure). Among a second group of infected herring, external signs completely resolved in only 10% of the fish after 429 d. The onset of mortality preceded the appearance of external signs. Histological examination of infected skin and skeletal muscle tissues indicated an apparent affinity of the parasite for host red muscle. Host responses consisted primarily of granulomatous inflammation, fibrosis, and necrosis in the skeletal muscle and other tissues. The persistence and asynchrony of external signs and host response indicated that they were neither a precursor to host mortality nor did they provide reliable metrics for hind-casting the date of exposure. However, the long-term persistence of clinical signs in Pacific herring may be useful in ascertaining the population-level impacts of ichthyophoniasis in regularly observed populations.

B. Hershberger, P.K., J.L. Gregg, L.M. Hart, S. Moffitt, R. Brenner, K. Stick, E. Coonradt, T. Otis, J. J. Vollenweider,K. A. Garver, J. Lovy, T.R. Meyers. *In Press*. The parasite *Ichthyophonus* sp. in Pacific herring. Journal of Fish Diseases.

The protistan parasite *Ichthyophonus* occurred in populations of Pacific herring *Clupea pallasii* throughout coastal areas of the NE Pacific, ranging from Puget Sound, WA north to the Gulf of Alaska, AK. Infection prevalence in local Pacific herring stocks varied seasonally and annually, and a general pattern of increasing prevalence with host size and/or age persisted throughout the NE Pacific. An exception to this zoographic pattern occurred among a group of juvenile, age 1+ year Pacific herring from Cordova Harbor, AK in June 2010, which demonstrated an unusually high infection prevalence of 35%. Reasons for this anomaly were hypothesized to involve anthropogenic influences that resulted in locally elevated infection pressures. Inter-annual declines in infection prevalence from some populations (e.g. Lower Cook Inlet, AK; from 20-32% in 2007 to 0-3% during 2009-2013) or from the largest size cohorts of other populations (e.g. Sitka Sound, AK; from 62.5% in 2007 to 19.6% in 2013) was likely a reflection of selective mortality among the infected cohorts. All available information for *Ichthyophonus* in the NE Pacific, including broad geographic range, low host specificity, and presence in archived Pacific herring tissue samples dating to the 1980's, indicate a long-standing host-pathogen relationship.

C. Hershberger, P.K., L.M. Hart, A.H. MacKenzie, M.L. Yanney, C. Conway, D. Elliott. 2015. Infecting Pacific herring with *Ichthyophonus* sp. in the laboratory. Journal of Aquatic Animal Health 27: 217-221.

The protistan parasite *Ichthyophonus* sp. occurs in Pacific Herring *Clupea pallasii* populations in coastal areas throughout the northeast Pacific region, but the route(s) whereby these planktivorous fishes become infected is unknown. Several methods for establishing *Ichthyophonus* infections in laboratory challenges were examined. *Ichthyophonus* sp. infections were most effectively established after intraperitoneal injections with suspended parasite isolates from culture or after repeated feedings with infected fish tissues. Among groups that were offered infected fish tissues, infection prevalence was greater after multiple feedings (65%) than after a single feeding (5%). Additionally, among groups that were exposed to parasite suspensions prepared from culture isolates, infection prevalence was greater by intraperitoneal injection (74%) than by gastric intubation (12%); infections were not established in any experimental herring by flushing parasite suspensions over the gills. Although the consumption of infected fish tissues is not likely the primary route of *Ichthyophonus* sp. transmission in populations of wild Pacific Herring, this route may contribute to abnormally high infection prevalence in areas where juvenile herring have access to infected offal.

D. Conway, C.M., M.K. Purcell, D.G. Elliott, P.K. Hershberger. 2015. Detection of *Ichthyophonus* by chromogenic *in situ* hybridization. Journal of Fish Diseases 38: 853-857.

We developed a chromogenic in situ hybridization (CISH) assay capable of detecting *Ichthyophonus* nucleic acid in standard histology sections. The assay has utility for both diagnostic and research applications. The CISH assay can be used to confirm histological diagnosis of *Ichthyophonus* or be readily applied to archival material. There remain many unanswered questions regarding the *Ichthyophonus* life cycle and transmission routes. For example, although *Ichthyophonus* is typically observed as 50-250 µm diameter schizonts in tissues of live fishes, the parasitic life stages can be extremely pleomorphic in vitro and additional life stages have been reported in vivo, raising the possibility that small cryptic stages of the parasite have been overlooked when non-specific histological stains are used. If this is the case, the CISH assay may be useful for tracking sequential dissemination of *Ichthyophonus* throughout fish tissues following laboratory exposure. Additionally, the CISH assay may prove beneficial in ongoing studies intended to identify non-piscine intermediate hosts for the parasite.

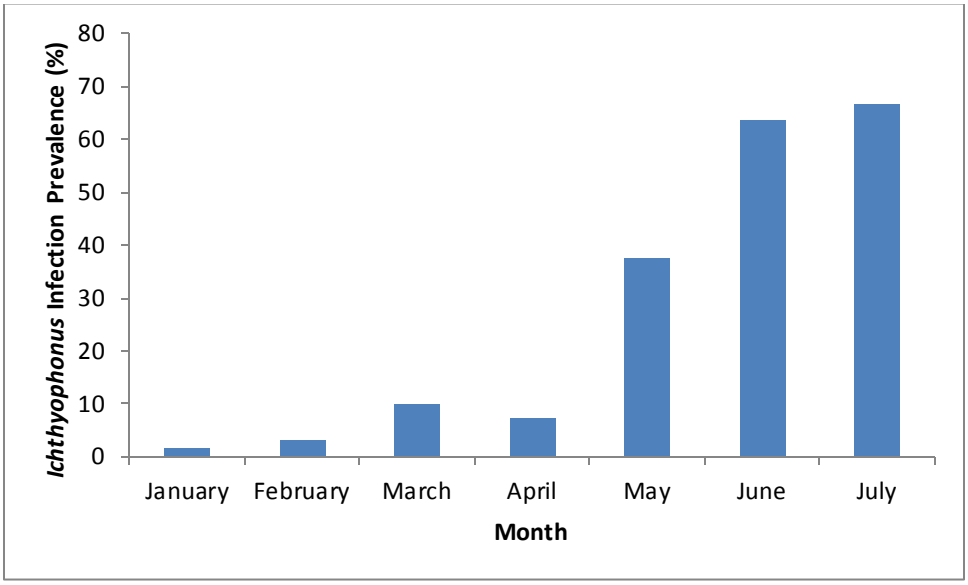
8. Coordination/Collaboration: *See*, Reporting Policy at III (C) (8).

Ongoing collaborations with partners within the PWS Herring Program include:

- Collection of shared zooplankton samples with Dr. Rob Campbell. These samples will be assessed for possible intermediate hosts for *Ichthyophonus*.

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

2015 Monthly Prevalence of *Ichthyophonus* in Juvenile Herring from Cordova Harbor



- Discussions and planned meetings with Trevor Branch regarding best approaches for the integration of herring infection and disease data into the ASA or other herring population models.

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:

Scientific Presentations

Gregg, J.L., R.L. Thompson, M.K. Purcell, C.S. Friedman, P.K. Hershberger. November 5-8, 2015. Phylogeny of *Ichthyophonus* parasites indicates majority of global impacts can be attributed to a single ubiquitous marine species. Western Society of Naturalists – 96th Annual Meeting. Sacramento, CA.

Elliott, D.G., C.L. McKibben, C.M. Conway, A. MacKenzie, P.K. Hershberger. September 7-11, 2015. Platform. Differential susceptibility of Yukon River and Salish Sea Chinook salmon (*Oncorhynchus tshawytscha*) stocks to *Ichthyophonus*. 17th International conference of Diseases of Fish and Shellfish. Las Palmas de Gran Canaria, Spain.

Hart, L.M., P.K. Hershberger. August 16-20, 2015. Platform. Integration of disease information into population assessments: the case of VHS and Pacific herring. American Fisheries Society 145th Annual Meeting. Portland, OR.

Chen, M., B. Stewart, P. Hershberger, K. Snekvik. May 27-29, 2015. Platform. *Nanophyetus salmincola* in outmigrating Puget Sound Steelhead. 2015 Salmon Recovery Conference. Vancouver, WA.

Hershberger, P.K., J.L. Gregg, A.H. MacKenzie, M.L. Yanney, C. Conway, D.Elliott. June 2-4, 2015. Poster. Infecting Pacific herring (*Clupea pallasii*) with *Ichthyophonus* in the laboratory. 56th Annual Western fish Disease Workshop. Steamboat Springs, CO.

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).

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
PROJECT BUDGET PROPOSAL AND REPORTING FORM

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	$ 258,069
Travel	$0.0	$0.0	$17.0	$17.0	$18.4	$52.4	$ 22,776
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	$ 79,096
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	$ 409,874
General Administration (9% of	$0.0	$0.0	$23.3	$24.1	$24.6	$72.0	$47,400.0
PROJECT TOTAL	$0.0	$0.0	$281.9	$291.9	$298.0	$871.8	$457,274.0
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. The column titled 'Actual Cumulative' should be updated each fiscal year to provide information on the total amount actually spent for all completed years of the project. On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation..

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