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

Form Rev. 9.14.17

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

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

17120111-E

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

Herring Disease Program – Herring Disease Program II

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

Paul Hershberger, Maureen Purcell

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

February 1, 2017 – January 31, 2018

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

February 2018

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

N/A

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 (PWS, n=60) during the spring pre-spawn period from April 7-10, 2017. VHSV = viral hemorrhagic septicemia virus, VEN = viral erythrocytic necrosis:

Location	Date	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Port Gravina	April 7	0% (n=60)	18% (11/60)	0% (n=60)
Rocky Bay	April 10	0% (n=60)	10% (6/60)	0% (n=60)
Port Fidalgo	April 10	0% (n=60)	23% (14/60)	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 24-25, 2017:

Location	Date	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Unknown	March 24	0% (n=60)	18% (11/60)	0% (n=60)
S. Magoun Isl.	March 25	0% (n=60)	15% (9/60)	0% (n=60)
Unknown	March 25	0% (n=60)	8% (5/60)	0% (n=60)

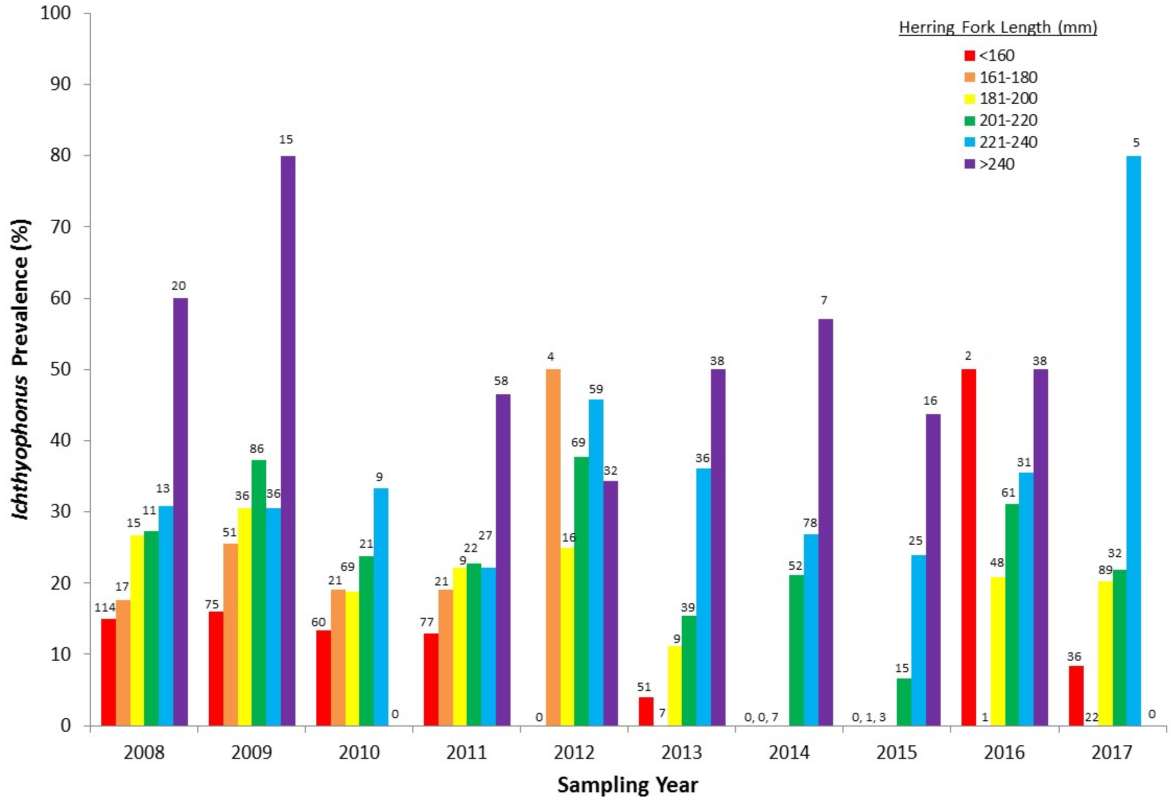

Figure 1. Temporal trend in *Ichthyophonus* infection prevalence in each size class of PWS herring. Numerals above each bar indicate 'n.'

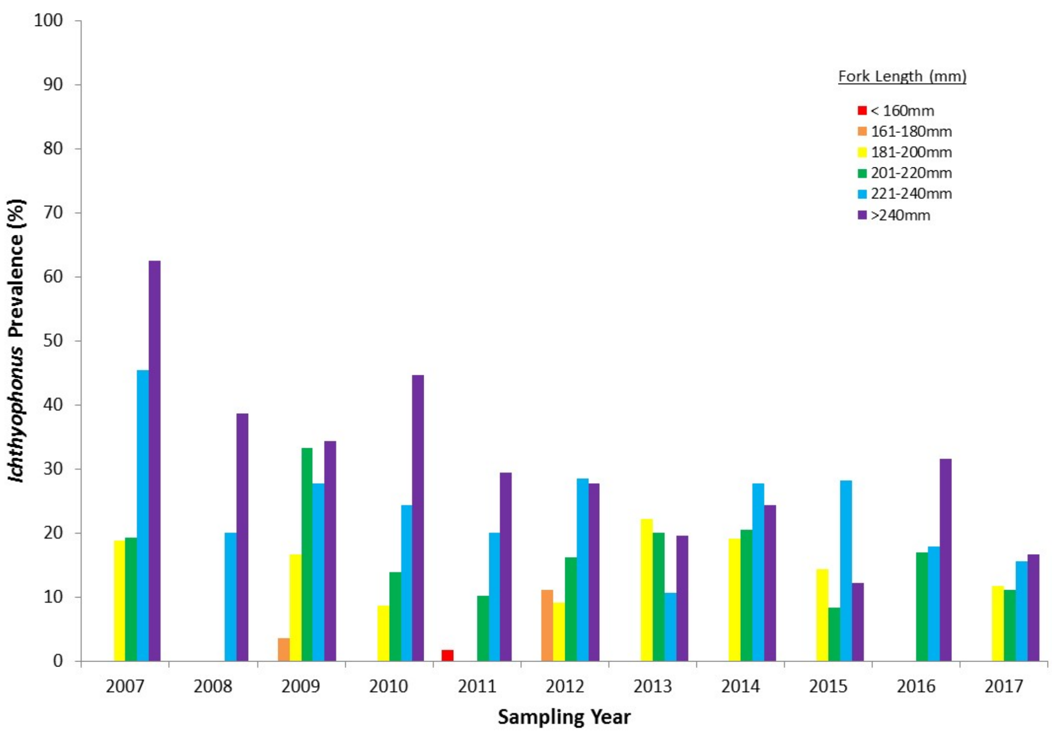

Figure 2. Temporal trend in *Ichthyophonus* infection prevalence in each size class of Sitka Sound herring.

C. Archived and current herring plasma samples from PWS and Sitka Sound were processed by plaque neutralization test (PNT) to determine the presence and titer of neutralizing antibodies to VHSV.

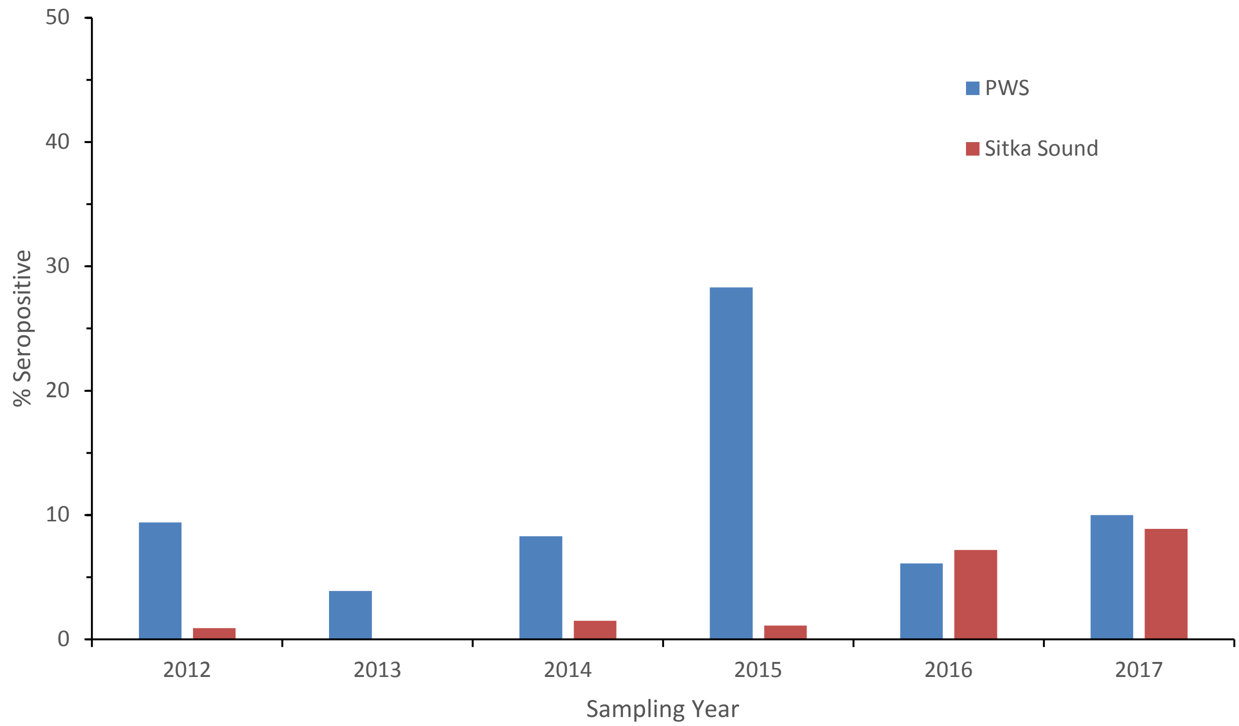

Figure 3. Percent of sampled herring that tested positive for VHSV neutralizing antibodies from 2012-2017.

Laboratory Findings:

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.

Methods for a plaque neutralization test (PNT) were optimized for the detection and quantification of viral hemorrhagic septicemia virus (VHSV) neutralizing activity in the plasma of Pacific herring *Clupea pallasii*. The PNT was complement-dependent, as neutralizing activity was attenuated by heat inactivation; further, neutralizing activity was mostly restored by the addition of exogenous complement from specific pathogen-free Pacific Herring. Optimal methods included the overnight incubation of VHSV aliquots in serial dilutions (starting at 1:16) of whole test plasma containing endogenous complement. The resulting viral titers were then enumerated using a viral plaque assay in 96 well micro plates. Serum neutralizing activity was virus-specific, as plasma from viral hemorrhagic septicemia (VHS) survivors demonstrated only negligible reactivity to infectious hematopoietic necrosis virus (IHNV), a closely-related rhabdovirus. Among Pacific herring that survived VHSV exposure, neutralizing activity was detected in the plasma as early as 37d post-exposure and peaked approximately 64 d post-exposure. The onset of neutralizing activity was slightly delayed at 6.0 ^{0}C relative to warmer temperatures (8.5 and 12.0 ^{0}C); however, neutralizing activity persisted for at least 345 d post exposure in all temperature treatments. It is anticipated that this novel ability to assess VHSV neutralizing activity will enable retrospective comparisons between a priori VHS exposures and year class recruitment failures. Additionally, the optimized PNT is expected to be employed as a forecasting tool capable of identifying the potential for future VHS epizootics in wild Pacific herring populations.

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.

Homologous and heterologous (genogroup Ia) DNA vaccines against viral hemorrhagic septicemia virus (VHSV – genogroup IVa) conferred partial protection in Pacific herring. Early protection at 2 wk post vaccination (PV) was low and occurred only at elevated temperature (12.6^{0}C, 189 degree days - DD), where the relative percent survival (RPS) following viral exposure was similar for the two vaccines (IVa and Ia, respectively) and higher than that of negative controls at the same temperature. Late protection at 10 wk PV was induced by both vaccines but was higher with the homologous vaccine at both 9.0^{0}C and 12.6°C. Virus neutralization titers were detected among 55% of all vaccinated fish at 10 wk PV. The results suggest that the immune response profile triggered by DNA vaccination of herring was similar to that reported for rainbow trout *Oncorhynchus mykiss* (Lorenzen and LaPatra 2005) where interferon responses occur in the early days PV and transition to adaptive response at later time points. However, the protective effect was far less prominent in herring, possibly reflecting different physiologies and or adaptations of the two fish species.

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.

Ichthyophonus occurred at high prevalence but low intensity in Pacific halibut (*Hippoglossus stenolepis*) throughout the west coast of North America, ranging from coastal Oregon to the Bering Sea. Infection prevalence in adults was variable on spatial and temporal scales, with the lowest prevalence typically occurring on the edges of the geographic range and highest prevalence consistently occurring inside Prince William Sound, AK (58-77%). Additionally, intra-annual differences occurred at Albatross - Portlock, AK (71% vs 32% within 2012) and inter-annual differences occurred along coastal Oregon (50% vs 12% from 2012 – 2015). The infection prevalence was influenced by host age, increasing from $\leq$ 3% among the youngest cohorts ($\leq$ age 6) to 39-54% among age 9-17 cohorts, then decreasing to 27% among the oldest (age 18+) cohorts. There was little indication of significant disease impacts to Pacific halibut, as the intensity of infection was uniformly low and length-at-age was similar between infected and uninfected cohorts. These results suggest that *Ichthyophonus* in Pacific halibut currently represents a stable parasite-host paradigm in the North Pacific.

Literature Cited:

Lorenzen, N. and S. E. LaPatra. 2005. DNA vaccines for aquacultured fish. Scientific and Technical Review of the Office International des Epizooties 24: 201-213.

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

A. Projects within a Trustee Council-Funded Program

- Collaboration with the Alaska Department of Fish & Game (ADF&G) – Cordova and Prince William Sound Science Center (PWSSC) to collect pre-spawn PWS herring to monitor infection prevalence and severity (shared research platform).

- 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. This work includes a new collaboration and graduate student (Catrin Wendt) mentoring at the University of Washington, School of Aquatic and Fisheries Sciences.

- Discussions with Trevor Branch regarding best approaches to integrate the VHSV antibody results into a revised ASA model.

- Plans to collect herring from Cordova Harbor to follow up on the elevated prevalence of *Ichthyophonus* infections.

- Molecular work PWSSC (Dr. Rob Campbell and Caitlin McKinistry) to identify a copepod parasite.

- Post doctoral mentoring for Dr. Maya Groner (PWSSC).

B. Projects not within a Trustee Council-Funded Program

- Rearing of SPF herring, embryonic oil exposures, and pathogen exposure studies for EVOST TC Project # 17170155, Lingering Oil – Immunological Compromise of Fish (Dr. Andrew Whitehead) All fish rearing, oil / virus exposures, and in vivo experiments are performed as an In Kind contribution from this project.

C. With Trustee or Management Agencies

- Collaboration with AFD&G – Sitka to collect pre-spawn herring samples to monitor infection prevalence and severity.

- Ongoing collaboration with ADF&G – Juneau to continue processing virology samples from spring herring collections.

- The interaction with Project # 17170155, Lingering Oil – Immunological Compromise of Fish has expanded to include investigators at the NOAA – Northwest Fisheries Science Center (Drs. Nat Schultz and John Incardona).

- Ongoing collaborations with Washington Department of Fish and Wildlife to assess the prevalence of *Ichthyophonus* in Puget Sound herring populations.

- Results represented in Figure 2 were integrated into a proposal submitted to the North Pacific Research Board. If funded, the EVOSTC-leveraged project (with strong support from ADF&G – Juneau) would identify temporal changes in the severity of *Ichthyophonus* infections, indicating whether recent demographic trends in Sitka Sound herring are associated with parasite-induced mortality.

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

In addition to the peer-reviewed manuscripts described in Section 1 (above), results were also presented at several scientific meetings and symposia:

Hershberger, P.K. Invited Talk. July 5-6, 2017. Washington State Disease Co-Managers Meeting. Long term shedding of VHS virus from Pacific herring: Demonstration of a marine reservoir host. Olympia, WA.

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.

Stinson, M.E., B.C. Hall, B.C. Stewart, P.K. Hershberger. June 20-22, 2017. Poster. Validation of improved *Listonella* (*Vibrio*) *anguillarum* vaccine in coho salmon. 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.

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

Science Panel Comments and Responses on Revised FY17-21 Proposal, September 2016

The PI adequately responded the questions the Panel raised about methodologies. The Panel fully supports the proposal by this PI. The brevity of this response should be seen as a tribute to the continued excellent work done in this project and the inter-projected cooperation and collaboration.

PI response: NA

Science Panel Comments and Responses on Revised FY18 Proposal, September 2017

The Panel is pleased with the results, supports the additional funding requested, and finds the request to be reasonable and justified. Would it be beneficial (and cost-effective) for the Post-Doc (Maya Groner) to help with this project without compromising her proposed research plan? If it can be managed, the Panel feels that this involvement would benefit both the new post-doc and this project.

PI Response (10/11/2017)
Thank you. We anticipate integrating Dr. Groner's work into the HDP, as we feel Dr. Groner's contributions will be beneficial the HDP, the Herring Research and Monitoring Program, and her scientific career. We foresee no conflicts and we are eager to start working with her.

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

Budget Category:	Proposed FY 17	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$122.4	$128.4	$135.6	$141.6	$148.8	$676.8	$ 116
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	$ 9
Contractual	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$ -
Commodities	$39.0	$39.0	$39.0	$39.0	$39.0	$195.0	$ 53
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$ -
Indirect Costs (*will vary by proposer*)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$ -
SUBTOTAL	$181.5	$187.5	$194.7	$200.7	$207.9	$972.3	N/A
General Administration (9% of subtotal)	$16.3	$16.9	$17.5	$18.1	$18.7	$87.5	$16.3
PROJECT TOTAL	$197.8	$204.4	$212.2	$218.8	$226.6	$1,059.8	$194.6
Other Resources (Cost Share Funds)	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	N/A

COMMENTS:
This summary page provides an five-year overview of proposed funding and actual cumulative spending. The column titled 'Actual Cumulative' must be updated each fiscal year as part of the annual reporting requirements. 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.