1. Project Number:

19120111-E

2. Project Title:

Herring Disease Program

3. Principal Investigator(s) Names:

Paul Hershberger, Maureen Purcell, U.S. Geological Survey – Marrowstone Marine Field Station

4. Time Period Covered by the Report:

February 1, 2019-January 31, 2020

5. Date of Report:

March 2020

6. Project Website (if applicable):

https://pwssc.org/herring/

7. Summary of Work Performed:

Field Sampling

A. Three samples of Pacific herring were collected from Prince William Sound during the spring pre-spawn period from April 5-6, 2019 to test for viral hemorrhagic septicemia virus (VHSV), *Ichthyophonus*, and viral erythrocytic necrosis (VEN) prevalence (Table 1, Fig. 1). An apparent increase in *Ichthyophonus* infection prevalence occurred among the smallest (<160mm) size cohort in 2019 (Fig. 1).

Table 1. Infection prevalence results from Prince William Sound pre-spawn herring in 2019. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	*Ichthyophonus* Prevalence	VEN prevalence
Hawkins / Double Bay	April 5	0% (n=60)	18% (11/60)	
Canoe Pass	April 6	0% (n=60)	18% (11/60)	
Windy Bay / Whiskey Pete	April 6	0% (n=59)	20% (12/60)	Total = 2% (3/176)*

*All 3 VEN-positive samples had low densities of intraerythrocytic inclusions (<10% of red cells demonstrated viral inclusion bodies).

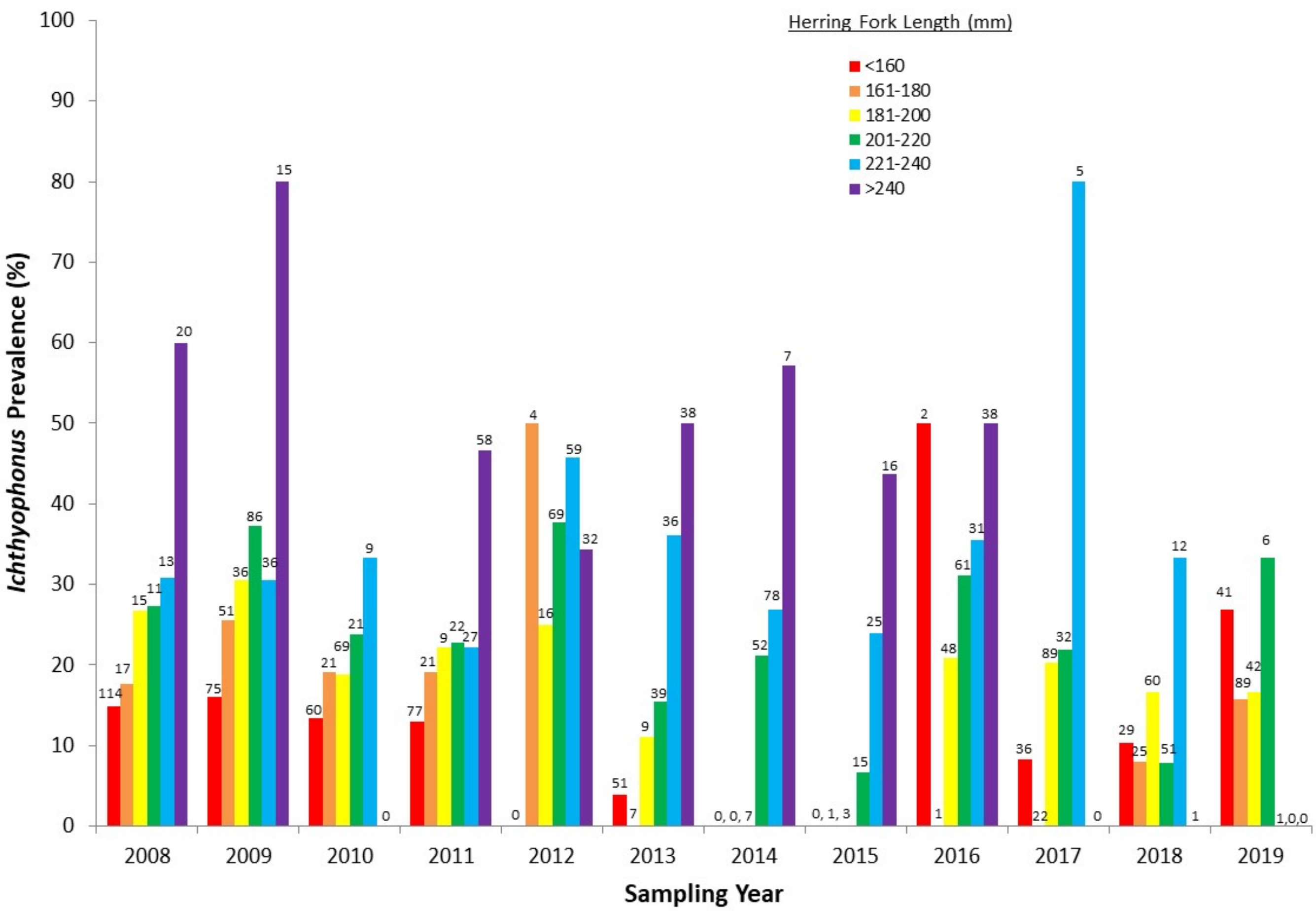

Figure 1. Temporal trend in Ichthyophonus *infection prevalence in each size class of Prince William Sound herring. Numerals above each bar indicate 'n'.*

B. Three samples of adult Pacific herring were collected from Sitka Sound during the spring pre-spawn period from March 25-27, 2018 to test for VHSV, *Ichthyophonus*, and VEN prevalence (Table 2, Fig. 2). After a decrease in *Ichthyophonus* infection prevalence in the largest (> 240mm) size cohort from 2007-2015, the infection prevalence in this size cohort is currently increasing to more typical levels (Fig. 2).

Table 2. Infection prevalence results from Sitka Sound pre-spawn herring in 2019. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	***Ichthyophonus*** **Prevalence**	VEN prevalence
Kristoff	March 25	0% (n=60)	8% (5/60)	0% (n=60)
Kristoff	March 26	0% (n=60)	18% (11/60)	0% (n=60)
Whitestone Narrows	March 27	0% (n=60)	15% (9/60)	0% (n=60)

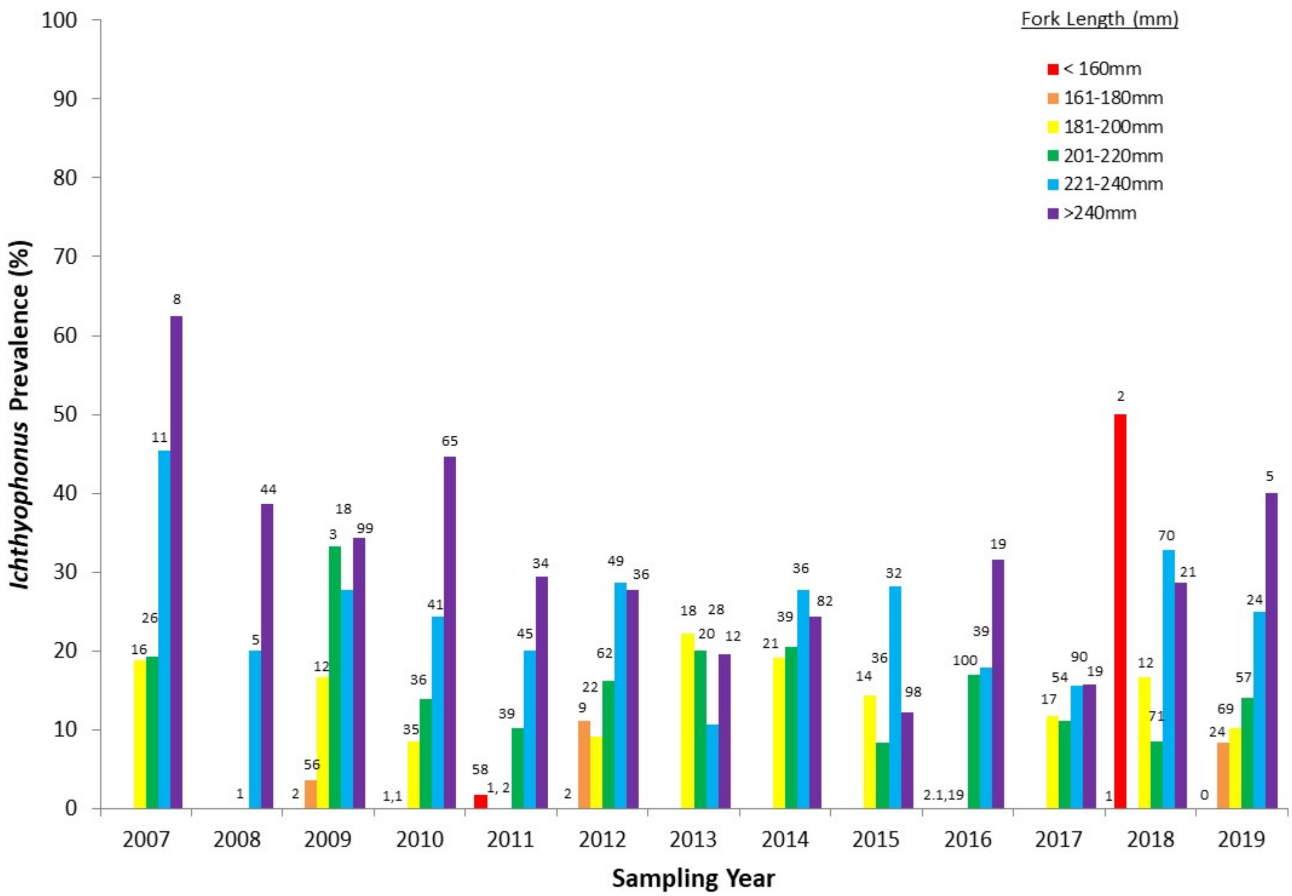

Figure 2. Temporal trend in Ichthyophonus *infection prevalence in each size class of Sitka Sound herring. Numerals above each bar indicate 'n'. Note the restructuring of a more typical direct relationship between infection prevalence and herring size in recent years.*

C. *Ichthyophonus* infection prevalence was 0% (0/30) and VHSV infection prevalence was 0% (0/60) in a group of herring (2018 year class) collected from south Puget Sound (Vashon Island) during a fish kill event on Jan 24 -25, 2019.

D. *Ichthyophonus* was detected in heavily diseased opaleye and sheepshead wrasse from display tanks at Sea World that were experiencing mortality. Cause of mortality was acute ichthyophoniasis. The manuscript describing the results has been submitted and accepted for publication.

E. A thorough quality assurance process was performed to validate and optimize the methods for the VHSV antibody assay. The revised methods, involving heat inactivation of the plasma sample followed by addition of exogenous complement, were applied to wild herring with known VHSV exposure histories to validate the antibody results in the context of herd immunity (discussed later). We now feel comfortable with the renewed methods and we are in the process of re-analyzing all archived plasma samples from Sitka Sound and Prince William Sound (2012 – present) with the revised methods. To date, 2019 samples from Sitka Sound have been processed with the revised methods; 2% (3/172) with detectable neutralizing antibodies; neutralizing titers = 64, 218, and 512. Anticipated completion date for all archived samples is August 2020.

Laboratory Studies

- A large portion of our laboratory efforts in 2019 involved the provision of technical assistance for Andrew Whitehead's project investigating the impacts of early life stage PAH exposure to subsequent disease susceptibility in Pacific herring (project 19170115). We performed the oil exposures, reared the exposed herring through metamorphosis, performed the pathogen exposures, and provided logistics support for the research teams of Dr. Andrew Whitehead and John Incardona (related North Pacific Research Board [NPRB] project). Results are summarized in Dr. Whitehead's report.
- We provided technical assistance and mentoring for Dr. Maya Groner's studies including assessment of *Ichthyophonus* infection intensities in Sitka Sound herring, VHSV modelling, controlled VEN exposures, etc. (results are summarized in Dr. Pegau's report 19120111-A).
- We continue making progress documenting an *Ichthyophonus* hot spot among age-0 herring in Cordova Harbor. This is part of a graduate student thesis (Catrin Wendt, University of Washington); anticipated thesis completion is 2020. Briefly, a molecular technique to detect *Ichthyophonus* eDNA was optimized for water samples, and samples from Cordova Harbor were processed using this technique. The first round of quantitative polymerase chain reactions (qPCRs) are completed, and suspect positive samples are currently being sequenced for confirmation. Additionally, a rapid increase in *Ichthyophonus* infection prevalence was demonstrated in Cordova Harbor again during spring 2019; herring stomach samples collected during this outbreak are currently being processed.
- A localized VHS epizootic in age-0 herring was documented in newly metamorphosed juvenile herring from Port Angeles Harbor, WA (Fig. 3). The herring were VHSV-positive, with high tissue titers at the first sampling event in July 2019, days after they metamorphosed from larvae, and the epizootic persisted through the end of August. A similar, but truncated

epizootic occurred among newly metamorphosed herring in Port Ludlow Harbor, WA. Samples for VEN were also collected from both locations; results are pending.

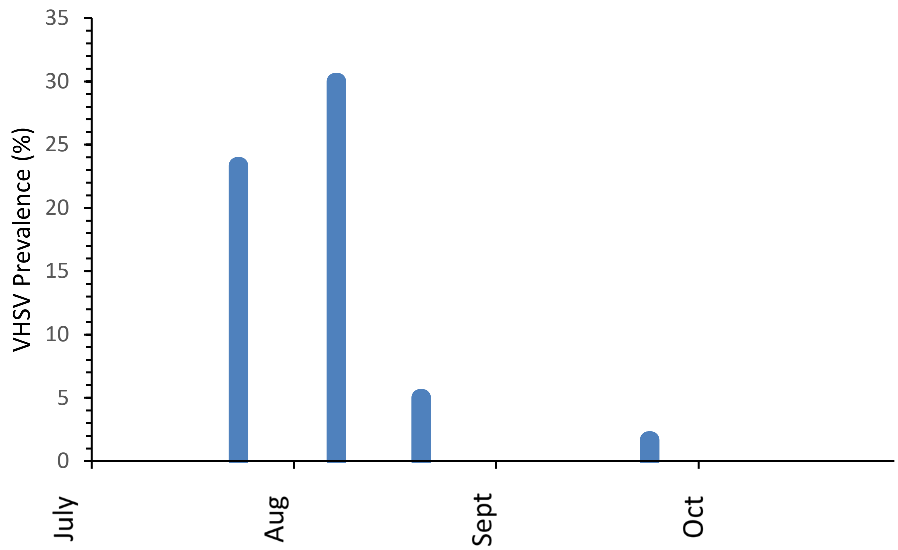

Figure 3. Viral hemorrhagic septicemia virus prevalence in kidney / spleen homogenates among age-0 (2019 year class) herring from Port Angeles Harbor during 2019 (n = 60 fish / sampling event). The mid-July sampling event occurred shortly after larvae metamorphosed to juveniles.

- An experiment is underway to determine whether *Ichthyophonus* can be transmitted from herring-to-herring via cohabitation under ideal environmental conditions (i.e., low temperatures and low salinities). We are approximately 6 months into this 8-month *in vivo* experiment; a complete summary is anticipated by the end of FY20.
- New phylogenetics work is demonstrating major differences in *Ichthypohonus* types, based on nucleic acid sequences in multiple genes. Sequence comparisons from *Ichthyophonus* isolates collected throughout the world, including hundreds of isolates from Alaska, indicate that *Ichthyophonus* is not panmictic, as previously thought. Rather, sequence variants indicate that several parasite species likely exist, including at least two species in the marine waters of Alaska. Preliminary analysis indicates that parasite speciation appears to be structured geographically. A complete synopsis is anticipated by the end of FY20.
- During FY19, the sensitivity and specificity of the VHSV neutralizing antibody assay was significantly improved. The new technique employs heat to inactivate endogenous complement and any non-specific virus neutralization factors (including interferons) that may return false positive results. Exogenous complement from naïve herring is then added back to the heat-inactivated plasma samples. The improved technique correctly identified 6 groups of wild herring that were naïve to VHS and 3 groups that survived prior exposure to VHSV (Table 3). The latter included a group from Port Angeles Harbor (October 9, 2018) that survived prior exposure; the antibodies were undetectable using the original (pre-optimized) methods. Additionally, the median antibody titers were dramatically increased using the improved methods. These results indicate that the improved methods are much more informative for assessing exposure history and herd immunity; as a result, we are currently

re-processing all archived herring plasma from Prince William Sound and Sitka Sound using the improved techniques.

Table 3. Comparison of viral hemorrhagic septicemia virus (VHSV) neutralizing antibody results between the original and improved methods in the Plaque Neutralization Test.

Collection Location	Collection Date	VHSV exposure history[1]	Original Methods		Improved Methods	
			% with antibodies	Mean antibody titer[2]	% with antibodies	Mean antibody titer[2]
Admiralty Inlet	Aug 2, 2018	Naive	3% (1/30)	128	0% (0/29)	NA
Admiralty Inlet	Aug 7, 2018	Naive	0% (0/30)	NA	0% (0/29)	NA
Admiralty Inlet	Aug 21, 2018	Naive	0% (0/30)	NA	0% (0/24)	NA
Admiralty Inlet	Aug 29, 2018	Naive	0% (0/30)	NA	0% (0/30)	NA
Admiralty Inlet	Sept 11, 2019	Nai`ve	0% (0/30)	NA	0% (0/30)	NA
Pt. Angeles Harbor	Sept 18, 2018	Prior Exposure	43% (13/30)	162	31% (8/26)	608
Protection Isl.	Oct 4, 2018	Ambiguous[3]	13% (4/30)	80	27% (8/30)	676
Pt. Angeles Harbor	Oct 9, 2018	Prior Exposure	0% (0/30)	NA	26% (7/27)	421
St of Juan de Fuca	Oct 11, 2018	Naive	3% (1/30)	64	3% (1/30)	128
Pt Angeles Harbor	Nov 5, 2018	Prior Exposure	13% (4/30)	80	33% (10/30)	1,331

[1]VHSV exposure history was deduced by observing whether the sampled herring school experienced a VHS epizootic after transport and confinement into laboratory tanks. Those that experienced an epizootic were assigned “Previously Naïve” status, as they were susceptible to the disease at time of capture and antibody levels were generally low / undetectable. Those that did not experience an epizootic were deduced to have evidence of “Prior Exposure” to VHSV, as they were not susceptible to the disease, and neutralizing antibodies were detected.

[2]Antibody titer is reported as the inverse serum dilution required to neutralize 50% of VHSV in the antibody neutralization test. A larger numeral indicates a higher titer of neutralizing antibodies.

[3]The exposure history of this sample is ambiguous, as antibodies were detected at the time of collection (indicating prior exposure), yet some of the fish died from VHS in the laboratory (indicating they were naïve). This ambiguity may reflect a mixed school demonstrating differing exposure histories.

- The prevalence of *Ichthyophonus* infection in Pacific herring was spatially heterogeneous in the Southern Salish Sea, WA. Over the course of 13 months, 2,232 Pacific herring were sampled from 38 midwater trawls throughout the region. Fork length was positively correlated with *Ichthyophonus* infection at all sites. After controlling for the positive relationship between host size and *Ichthyophonus* infection, the probability of infection was approximately 6X higher in North Hood Canal than in Puget Sound and the northern Straits (12% versus 2% predicted probability for a 100 mm fish and 30% versus 7% predicted probability for a 180 mm fish). Temporal changes in *Ichthyophonus* infection probability were explained by seasonal differences in fish length, owing to Pacific herring life history and movement patterns. Reasons for the spatial heterogeneity remain uncertain but may be associated with density dependent factors inherent to the boom-bust cycles that commonly occur in Clupeid populations.

- The susceptibility of Atlantic salmon and sockeye salmon to VHSV-IVa was evaluated using exposure routes including injection, static immersion, and cohabitation with diseased Pacific herring. Exposed fish were monitored for mortality and external pathology, mortalities were tested by cell culture, and live fish were regularly sampled and screened for infection. Among injected sockeye, VHSV was detected in one mortality (n = 195) and two sub-sampled fish (n = 30), whereas sockeye exposed by immersion and cohabitation did not experience mortality nor was systemic infection indicated by tissue screening. Injection and cohabitation exposure routes confirmed the susceptibility of Atlantic salmon to VHSV. Neither sockeye nor Atlantic salmon surviving the cohabitation served as a reservoir of VHSV, but Pacific herring did. The results suggest that VHSV-IVa poses low risk to sockeye salmon under natural routes of exposure.

8. Coordination/Collaboration:

A. Long-term Monitoring and Research Program Projects

1. Within the Program

- We worked closely with the Prince William Sound Science Center and Alaska Department of Fish and Game (ADF&G) to collect herring tissue and plasma samples during the spring herring cruises (shared research platform). Additionally, ADF&G provided age data for the fish health samples.

- Pathogen survey data are shared with Dr. Trevor Branch for incorporation into the age structured analysis model; revised antibody data will be shared as soon as the results are processed and QA'ed.

- As In-Kind contributions to Dr. Maya Groner's project, several experiments were initiated and are currently underway at the U.S. Geological Survey – Marrowstone Marine Field Station.

    1. Studies to assess the histological threshold level of *Ichthyophonus* infection that is associated with herring mortality.

2. Finished processing archived histology samples from Prince William Sound and Sitka Sound, dating to 2007. These samples will indicate whether the severity of *Ichthyophonus* infections have changed over time and whether these changes are associated with recent population changes in Sitka Sound.
3. Initiated experiments to investigate the pathogenicity and virulence of VEN.

- Provided laboratory support, including embryonic oil exposures, herring rearing, pathogen exposure experiments, and coordination (including hosting meetings) for Dr. Andrew Whitehead's project (project 19170115).

2. Across Programs

a. Gulf Watch Alaska

Scientists from GWA provided Alaskan razor clam samples to develop a novel diagnostic technique for nuclear inclusion X disease.

b. Data Management

Survey data and metadata were entered onto the Research Workspace.

B. Individual Projects

We have partnered with ADF&G – Sitka 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 *Exxon Valdez* oil spill Trustee Council (EVOSTC)-funded project were leveraged to obtain supplemental funding from the 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 provided technical support to Sea World with the identification and characterization of *Ichthyophonus* in their display fish.

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 EVOSTC funded herring disease projects between 1994 and present.

9. Information and Data Transfer:

A. Publications Produced During the Reporting Period

1. Peer-reviewed Publications

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.

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.

2. Reports

Hershberger, P., and M. Groner. 2019. Chapter 5 herring disease synthesis. In Pegau, W.S., and D.R. Aderhold, editors. Herring Research and Monitoring Science Synthesis. Herring Research and Monitoring Synthesis Report, (*Exxon Valdez* Oil Spill Trustee Council Program 20120111). *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Hershberger, P., and M. Purcell. 2019. Herring disease program. FY18 annual report to the *Exxon Valdez* Oil Spill Trustee Council, project 18120111-E. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, AK.

3. Popular articles

None.

B. Dates and Locations of any Conference or Workshop Presentations where EVOSTC-funded Work was Presented

1. Conferences and Workshops

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

Cypher, A.D., P. Hershberger, N. Scholz, and J.P. Incardona. 2019. Poster. 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, January.

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.

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

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

Pegau, W.S., T. Branch, D. McGowan, J. Trochta, A. Whitehead, P. Hershberger, M. Groner, P. Rand, K. Gorman, M.A. Bishop, and S. Haught. 2020. 2020. Prince William Sound Herring Research and Monitoring Program. Poster, Alaska Marine Science Symposium. Anchorage, AK, January.

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

2. Public presentations

Hershberger, P. 2019. "Principles of Viral Hemorrhagic Septicemia Virus." Guest Lecture in FHL 568: Ecology of Infectious Marine Disease. University of Washington, Friday Harbor Laboratories, June.

C. Data and/or Information Products Developed During the Reporting Period, if Applicable

None.

D. Data Sets and Associated Metadata that have been Uploaded to the Program's Data Portal

Pathogen survey data from Prince William Sound and Sitka Sound.

10. Response to EVOSTC Review, Recommendations and Comments:

EVOSTC Science Panel Comment. The Science Panel remains impressed with the level of productivity of the PI and the project. At what age are Abs first present in serum? Is there a difference between Sitka and PWS fish in this regard? In the comparison, were fish of the same age between the sites were they all just pooled? It is not clear in the figure. If younger fish are most impacted by VHSV and survivors are the ones with serum Abs, then it is an interesting question and related to the Whitehead studies on when herring mount an Ab response and if this differs between populations. Some clarifications would be appreciated. We would like to see more detail regarding this topic so we can better understand the intriguing data presented.

PI Response. We would like to thank the Science Panel and Science Coordinator for their constructive feedback on the proposed FY19 work in the Herring Disease Program. We are also very excited about the VHSV antibody results from the fish health surveys in Prince William Sound and Sitka Sound. At this point, we are reluctant to overanalyze these observational data until we have more experimental data to facilitate their interpretation. Specifically, we have spent most of the summer of 2018 assessing the levels of antibodies in additional groups of wild herring and determining how these antibody levels correspond to population herd immunity against VHSV. We hope to have these results summarized for the final report of the FY'18 project. Additionally, we suspect that the antibody data presented in Figure 1 may be more meaningful when analyzed by herring year class in Dr. Branch's revised age structured analysis model. We will be working with Dr. Branch to facilitate this integration during the fall of 2018.

EVOSTC Science Panel Comment. Also, does warmer water enhance disease prevalence?

PI Response. The question of temperature and disease is rather complex, and Hershberger is currently working to address this issue in more detail by co-authoring a chapter in a Disease Ecology Textbook, describing the impacts of global climate change on disease. In short, the proximate effects of temperature are disease-specific. However, temperature can also influence host, pathogen, and plankton (intermediate host) assemblages that indirectly influence certain diseases.

11. Budget:

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

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	$432.8
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	$31.4
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	$115.5
Equipment	$0.0	$0.0	$0.0	$0.0	$31.4	$31.4	
SUBTOTAL	$181.5	$210.0	$217.2	$223.2	$261.8	$1,093.7	$579.7
General Administration (9% of subtotal)	$16.3	$18.9	$19.5	$20.1	$23.6	$98.4	
PROJECT TOTAL	$197.8	$228.9	$236.7	$243.3	$285.4	$1,192.1	
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	