1. Project Number:

20120111-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, 2010-January 31, 2021

5. Date of Report:

March 2021

6. Project Website (if applicable):

https://pwssc.org/herring/

7. Summary of Work Performed:

Field Sampling

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, scientific 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.

A. Prince William Sound Pre-spawn Adult Herring

Three samples of Pacific herring were collected from Prince William Sound (PWS) during the spring pre-spawn period from April 1-17, 2020, to test for viral hemorrhagic septicemia virus (VHSV) and *Ichthyophonus* prevalence (Table 1, Fig. 1). *Ichthyophonus* was detected in 17% (32/188) of heart cultures and 8.5% (7/82) of egg cultures from all sites combined. Herring egg and heart culture results did not correspond in 7 fish where *Ichthyophonus* was cultured from the hearts but not the eggs; the converse did not occur. Sample sizes differed between the two tissue types because contamination rendered many egg cultures unreadable. VHSV was not isolated from any samples, but neutralizing antibodies to VHSV were detected in 2.4% (7/294) of PWS herring in 2020 (Fig. 2). As in previous years, the prevalence of seropositives increased with herring age (Fig. 3).

Table 1. Infection prevalence results from Prince William Sound pre-spawn herring in 2020. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	*Ichthyophonus* Prevalence (Heart Cultures)	VEN prevalence
Canoe Pass	April 8	0% (n=130)	19% (25/130)	NC*
Double Bay	April 10	0% (n=70)	12% (7/58)	NC*

*NC: Samples for VEN were not collected in 2020 due to COVID-19 constraints.

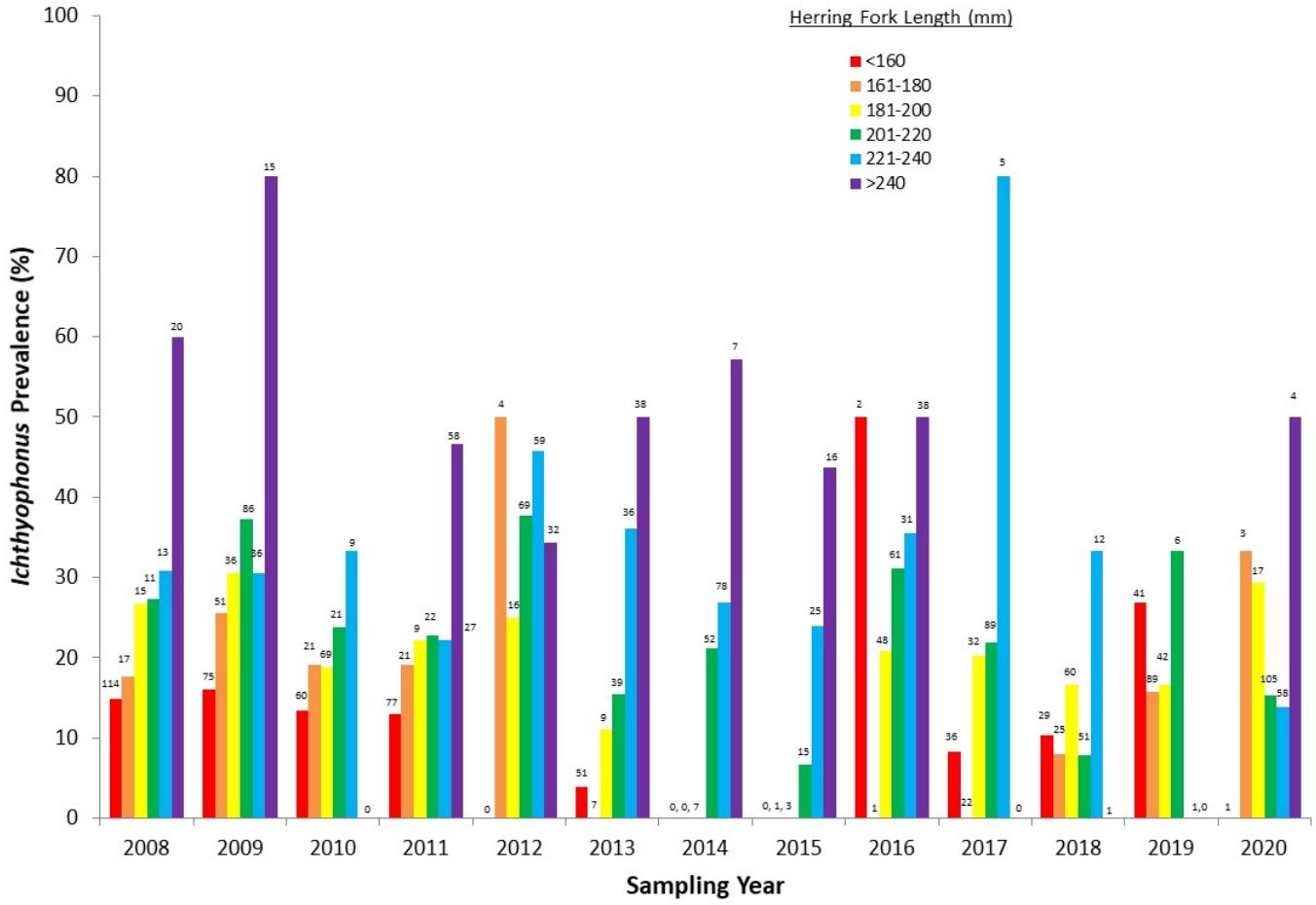

Figure 1. Temporal trend in Ichthyophonus infection prevalence in each size class of Prince William Sound herring. Numerals above each bar indicate sample size (n).

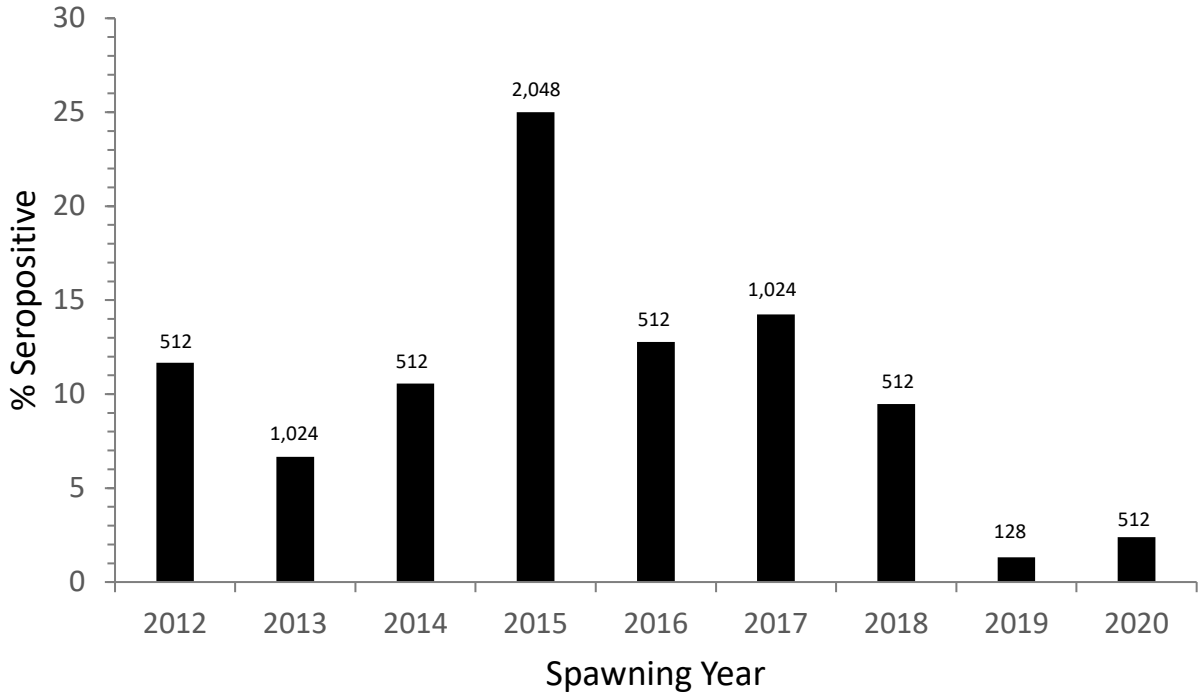

Figure 2. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Prince William Sound herring. Numerals above the bars indicate the median neutralizing titer in seropositives, reported as the reciprocal 50% inhibitory dilution – ID_{50} (titer range: 64 - 2,048).

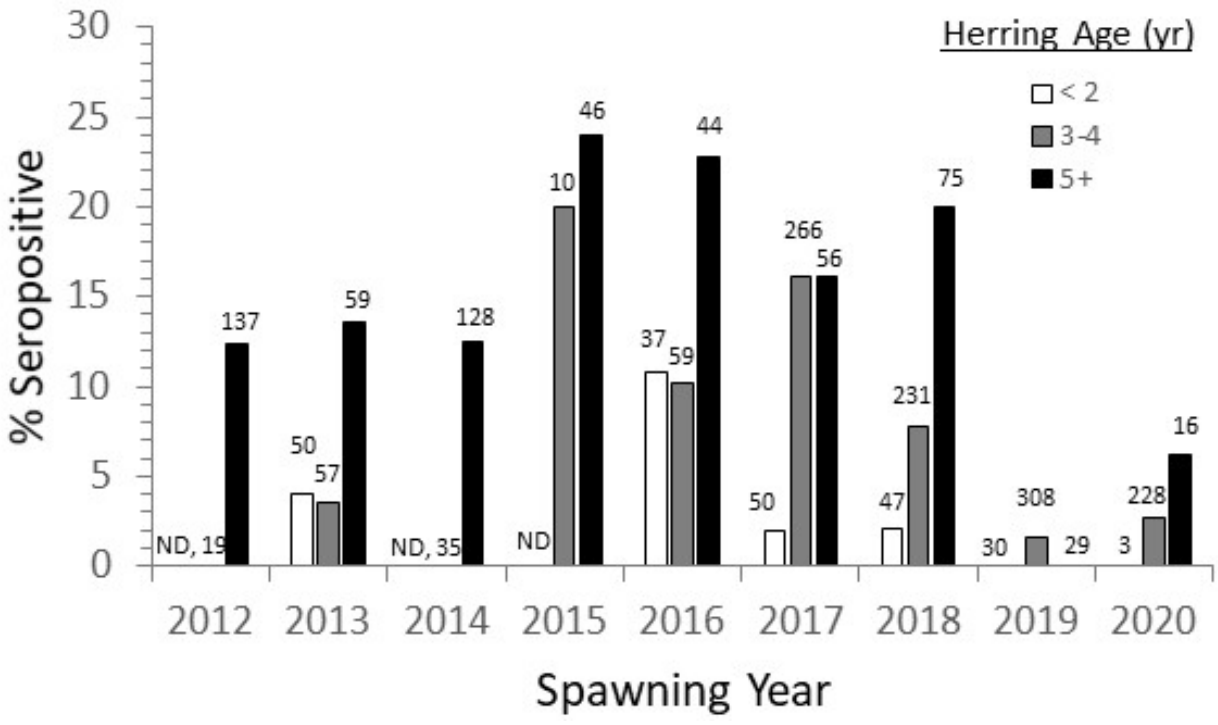

Figure 3. Inter-annual changes in prevalence of viral hemorrhagic septicemia virus neutralizing antibodies from Prince William Sound herring age classes (age determined by Alaska Department of Fish and Game from scales). Numerals above the bars indicate sample size (n).

B. Sitka Sound Pre-spawn Adult Herring

Three samples of adult Pacific herring were collected from Sitka Sound during the spring pre-spawn period from March 31-April 2, 2020, to test for VHSV and *Ichthyophonus* prevalence (Table 2, Fig. 4). *Ichthyophonus* was detected in 22% (39/180) of herring hearts; eggs were not cultured from Sitka Sound herring in 2020. VHSV was not detected in any samples. For the second consecutive year, neutralizing antibodies to VHSV were detected in only 1.7% (3/177) of herring plasma samples (Fig. 5).

Table 2. Infection prevalence results from Sitka Sound pre-spawn herring in 2020. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	**Date**	**VHSV Prevalence**	***Ichthyophonus* Prevalence (Heart Cultures)**	**VEN prevalence**
Kruzof	March 31	0% (n=60)	18% (11/60)	NC*
Low Island	April 1	0% (n=60)	18% (11/60)	NC*
Silver Bay	April 2	0% (n=60)	28% (17/60)	NC*

*NC: Samples for VEN not collected in 2020 due to COVID-19 constraints.

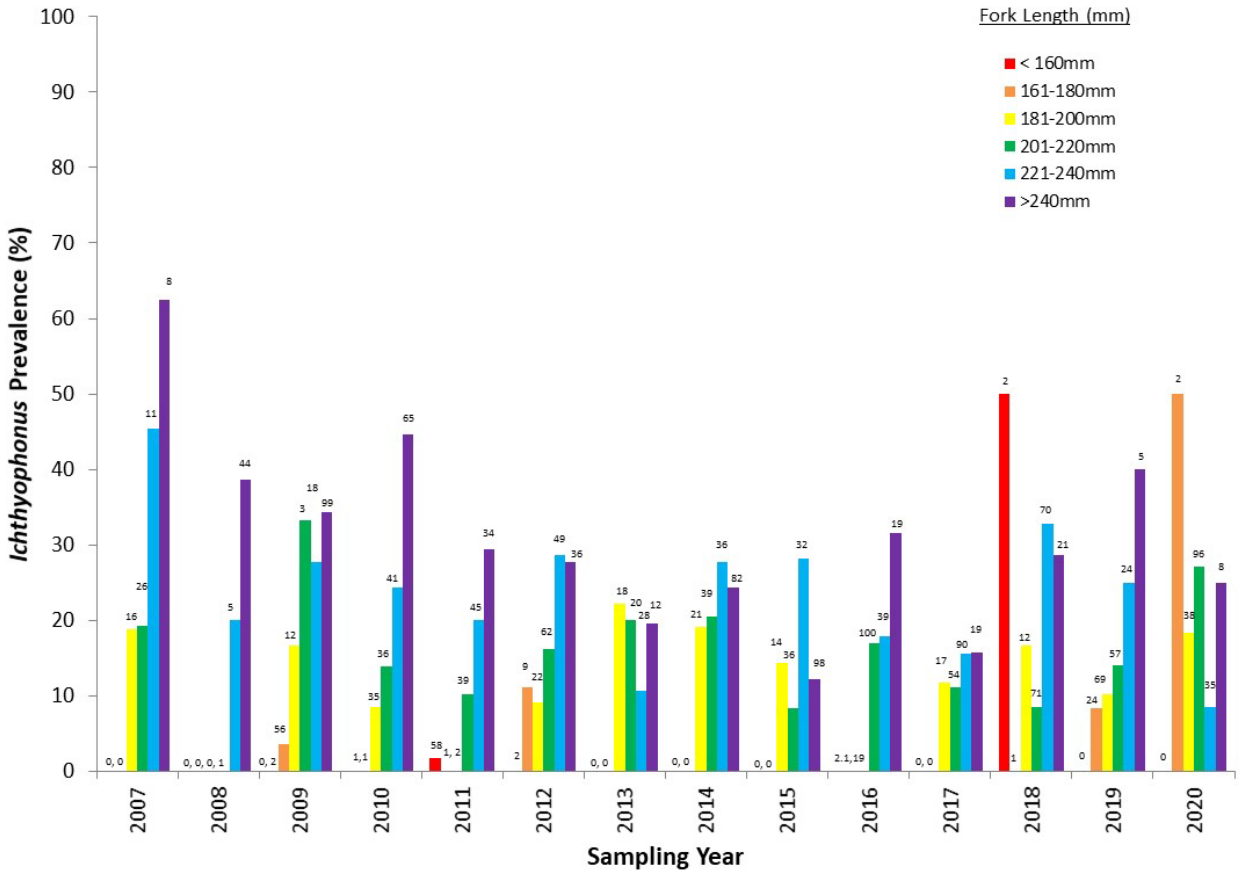

Figure 4. Temporal trend in Ichthyophonus infection prevalence in each size class of Sitka Sound herring. Numerals above each bar indicate (n).

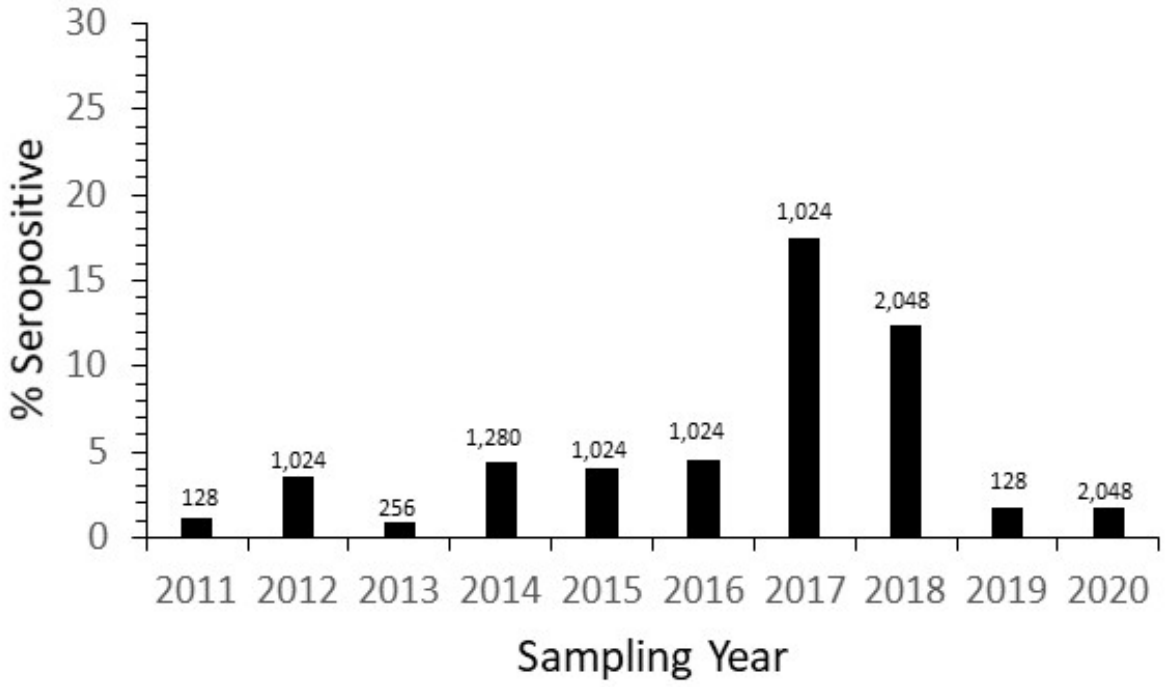

Figure 5. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Sitka Sound herring. Numerals above the bars indicate the median neutralizing titer among seropositives, reported as the reciprocal 50% inhibitory dilution – ID_{50} (titer range: 64 - 2,048).

C. Puget Sound Pre-spawn Adult Herring

Additional herring samples were collected from 3 sites in Puget Sound, WA. *Ichthyophonus* infection prevalence ranged from 0% in south Puget Sound (Squaxin Pass) to 17% in north Puget Sound (Semiahmoo Bay). Neither VEN nor VHSV were detected in herring from any location; additionally, neutralizing antibodies to VHSV were not detected in any samples (Table 3).

Table 3. Survey results from Puget Sound pre-spawn herring in 2020. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	Sample Size (n)	*Ichthyophonus* Prevalence (Heart Cultures)	VEN prevalence	VHSV Infection Prevalence	VHSV Antibody Prevalence
Squaxin Pass	Jan 27	26	0%	0%	0%	0%
Port Orchard	Jan 29	31	3%	0%	0%	0%
Semiahmoo Bay	Feb 18	30	17%	0%	0%	0%

D. Annual Recurrences of VHS Epizootics in Juvenile Herring

Continued surveillances of wild herring have documented several VHS epizootics in Age 0 herring, including some that recur annually in the same geographic locations (Table 4). For example, we have documented recurring VHS epizootics in Age 0 herring from Port Angeles Harbor in 2019 and 2020 and from Hot Springs Cove (west coast of Vancouver Island) in 2018 and 2019. Further, the progression of the VHS epizootic in Port Angeles Harbor was chronicled in 2020, when the virus was detected in 7% of juveniles at the time of larval metamorphosis (June 23); infection prevalence peaked at 63% from July 21 – Aug 4; and infections were no longer detected by September 22 (Fig. 6).

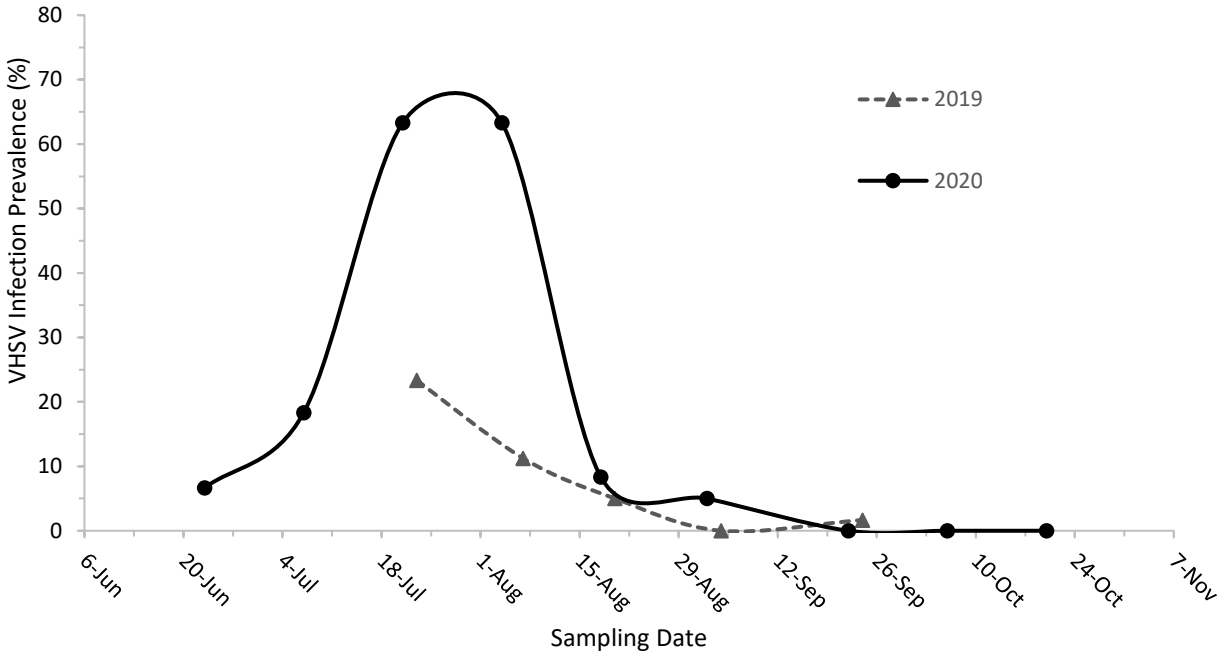

Figure 6. Progression of viral hemorrhagic septicemia virus (VHSV) epizootics in Age 0 Pacific herring from Port Angeles Harbor, Puget Sound, WA (2019-2020).

Table 4. Viral hemorrhagic septicemia (VHS) virus survey results from Pacific herring during atypical epizootic periods. "NA" indicates data "Not Available."

Region	Year	Stock	Collection Site	Collection Date	Mean fork le mm (SD)
AK, USA	2011	Sitka Sound	Bear Cove Bay	March 24	108 (11)
BC, Canada	2018	W. Vancouver Island	Hot Springs Cove	June 24	NA[A]
	2019	W. Vancouver Island	Hot Springs Cove	June 27	NA[B]
WA, USA	2014	Puget Sound	Lopez Island	Sept 11	NA[A]
		Puget Sound	Waldron Island	Sept 12	NA[A]
	2018	Puget Sound	Pt. Angeles Harbor	Sept 18 – Nov 5	67 (8.0) – 78 (
	2019	Puget Sound	Pt. Ludlow Harbor	July 25 – Sept 25	52 (2.4) - 78 (
		Puget Sound	Pt. Angeles Harbor	July 23 – Sept 24	64 (6.7) – 76 (
	2020	Puget Sound	Pt. Angeles Harbor	June 23 – Oct 20	37 (2.0) – 63 (

[A]Photographs suggest 50-60 mm length.

[B]Pacific herring were sampled from two locations in the north Puget Sound, WA region (San Juan Islands). External signs of VHS included hemorrhaging along the flank in a small proportion of individuals, increasing to approximately 10%of the population throughout the summer. Samples from 2014 were not randomly collected; rather, individuals demonstrating external signs of VHS were selected, frozen at -20°C, and submitted for laboratory diagnostics.

E. Walleye Pollock

Walleye pollock were collected from the Shelikof Strait, AK region from March 7-14, 2020, aboard a NOAA stock assessment cruise through the generous contribution of Dave McGowan. *Ichthyophonus* infection prevalence, based on tissue explant culture, was 42% (25/59) in livers and 52% (28/54) in eggs. These infection prevalence data support the hypothesis that herring predation on walleye pollock eggs reflects a plausible mechanism for *Ichthyophonus* transmission to Pacific herring; further these results provide the foundation for proposing additional studies with herring and pollock eggs in PWS.

Laboratory Studies

A. Long term detection of circulating antibodies to VHSV

A long-term study to document the kinetics of the VHSV antibody response was completed and preliminary results are back using a non-optimized plaque neutralization assay. The experiment was intended to assess how long circulating antibodies remain detectable after herring survive a single exposure to the virus. The experiment was terminated in July 2020, nearly three years after the herring were exposed to VHSV. Neutralizing antibodies were detected throughout the entire post-exposure period (Fig. 7). Peak seropositivity rate (100%) occurred 169 days post exposure (DPE) and dipped to 30% after 785 DPE. Median antibody titer among seropositives was highest (ID_{50} = 512) from 224 – 336 DPE. These samples will be re-processed using the optimized (heat inactivated plasma + exogenous complement) assay in FY 2021; final results will be presented next year.

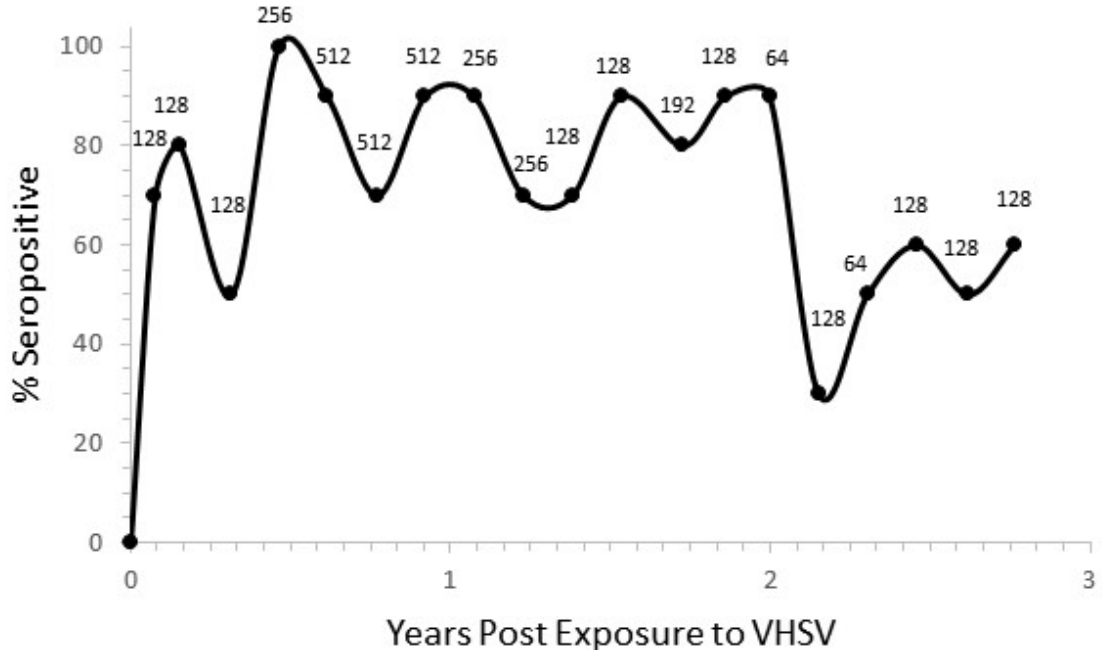

Figure 7. Preliminary results of neutralizing antibodies in herring that survived viral hemorrhagic septicemia virus (VHSV) exposure. Numerals above each data point indicate the median antibody titer among seropositive individuals, reported as the reciprocal 50% inhibitory dilution – ID50 (titer range: 64 - 2,048). Sample size (n) = 10 fish at each subsampling interval. None of the specific pathogen-free negative controls (n = 10 / sampling date) tested positive on any of the subsampling dates (data not shown). Note: these samples were processed by using the old (unoptimized) plaque neutralization methods; as such, they are considered preliminary results and are subject to change. Samples will be re-processed in 2021 using the optimized techniques and final (optimized) values will be reported next year.

B. Inability to demonstrate fish-to-fish transmission of *Ichthyophonus* in Pacific herring

Transmission of *Ichthyophonus* in clupeid hosts has been a subject of speculation since the earliest reported epizootics, but neither the mode of infection in herring nor the life cycle of *Ichthyophonus* spp. are adequately described.

Captive reared Specific Pathogen Free (SPF) sentinel herring (Age 1+) were cohabitated with *Ichthyophonus*-infected donor herring under four simulated environmental conditions: 1) ambient seawater, 2) ambient seawater with low salinity events, 3) chilled seawater, and 4) chilled seawater with low salinity events. Ambient seawater temperature ranged between 13.3 °C (24 Sept 2019) and 7.9 °C (16 Jan 2020) over the 5-month exposures, and chilled seawater temperatures ranged between 9.5 °C (6 Sept 2019) and 3.7 °C (15 Jan 2020) (Fig. 8). Mean temperature difference between treatments was 3.48 °C (1SD = 0.23 °C). Seawater salinity in all tanks remained in a narrow range, 27.9 to 32.3 ppt during the study, except during induced low salinity events which ranged from 11.7 to 13.5 ppt minimum concentration (Fig. 9).

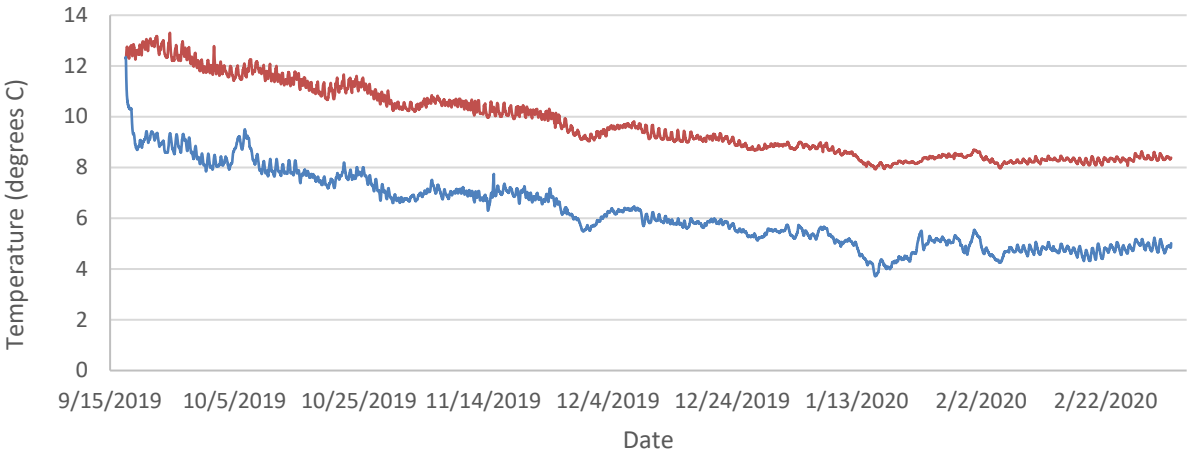

Figure 8. Seawater temperatures recorded from ambient (orange line) and chilled (blue line) treatments over the course of the Ichthyophonus transmission study. Data are means of three tanks per treatment collected at 15 min intervals.

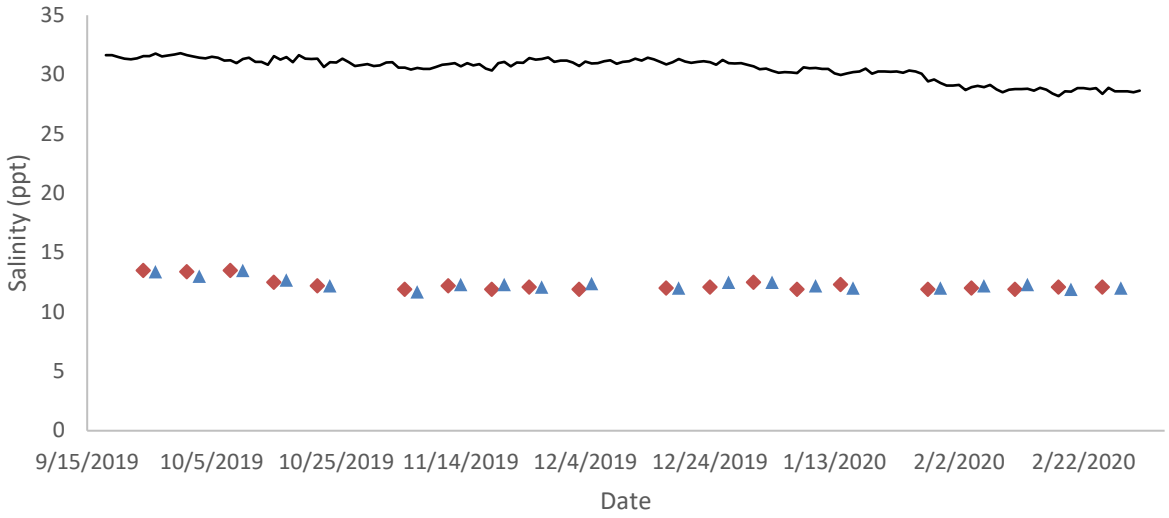

Figure 9. Mean seawater salinity readings (black line). Lowest salinity recorded during the ≈8 hr low salinity events is indicated by orange diamonds (ambient treatment) and blue triangles (chilled treatment).

Donor herring were injected with approximately 2,407 ± 1,574 (1SD) *Ichthyophonus* schizonts and large meronts plus thousands of smaller parasite cells in each 0.2 ml dose. These inoculations successfully established infections in 73% of donor herring, mean fork length (FL) = 148.9 ± 11 (1SD) mm and mean weight = 33.9 ± 8.9 (1SD) g. Infections progressed to disease in the donor herring and resulted in 20 to 40% mortality (Table 5). Mortalities began on first day of sentinel cohabitation (7 days after donor injections) and continued at a low rate throughout the duration of the study. No infections were detected in long-term or short-term sentinels in any treatment (Table 5).

The results of this study corroborate those of earlier studies in finding no support for fish-to-fish transmission of *Ichthyophonus* between Pacific herring. There are a wide range of conditions that herring experience in the wild that cannot be reproduced in the laboratory, and it is impossible to prove a negative. However, in this experiment and others, we have now cohabitated hundreds of herring under infection pressures (fish densities, infection intensities, etc.) that are much higher than those typically experienced in the wild, and we have been unable to confirm fish-to-fish transmission of *Ichthyophonus* in Pacific herring.

Future investigations into possible *Ichthyophonus* transmission routes, including possible transmission via predation on infected pollock eggs, may be helpful for elucidating other potential epizootic mechanisms in this system.

Table 5. Count of herring mortalities (dead) and survivors (live) from each treatment in this study. Donors were exposed to Ichthyophonus by IP injection, sentinels were Specific Pathogen Free herring. Number of Ichthyophonus-positive fish from each group indicated in parentheses. Overall percent infected for donors, long-term sentinels, and short-term sentinels indicated. No horizontal transmission was detected.

	Donors			Long-Term Sentinels[a]			Short-Term Sentinels[b]		
Treatment Group	Dead	Live	% infected	Dead	Live	% infected	Dead	Live	% infected
Control Ambient Seawater	0(0)	49[c] (0)	0%	0(0)	12(0)	0%	0(0) 0(0) 0(0) 0(0)	12(0) 12(0) 12(0) 12(0)	0%
Ichthyophonus Ambient Seawater	21(20)	29(19)	78%	0(0)	12(0)	0%	1(0) 0(0) 0(0) 0(0)	11(0) 12(0) 12(0) 12(0)	0%
Ichthyophonus Ambient Seawater with low salinity events	14(10)	36(23)	66%	1(0)	11(0)	0%	2(0) 0(0) 0(0) 0(0)	10(0) 12(0) 12(0) 12(0)	0%
Control Chilled Seawater	2(0)	48(0)	0%	1(0)	11(0)	0%	0(0) 0(0) 0(0) 0(0)	12(0) 12(0) 12(0) 12(0)	0%
Ichthyophonus Chilled Seawater	16(16)	34(22)	76%	1(0)	11(0)	0%	1(0) 0(0) 0(0) 0(0)	11(0) 12(0) 12(0) 12(0)	0%

	Donors			Long-Term Sentinels[a]			Short-Term Sentinels[b]		
Treatment Group	Dead	Live	% infected	Dead	Live	% infected	Dead	Live	% infected
Ichthyophonus Chilled Seawater with low salinity events	16(16)	34(20)	72%	0(0)	12(0)	0%	0(0) 0(0) 0(0) 0(0)	12(0) 12(0) 12(0) 12(0)	0%

C. Increased Herring Susceptibility to *Ichthyophonus* after embryonic exposure to PAHs

As a no-cost contribution to support the herring genetics project (PI Whitehead, project number 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 polycyclic aromatic hydrocarbons (PAHs) as embryos and raised under specific pathogen-free conditions through metamorphosis to juveniles at the U.S. Geological Survey (USGS) Marrowstone Marine Field Station. After metamorphosis, groups of previously oiled and unoiled SPF herring were injected with *Ichthyophonus*. After *Ichthyophonus* exposure, mortality was slightly higher among the group that survived oil exposure (Fig. 10); statistics on the mortality curves have not yet been performed.

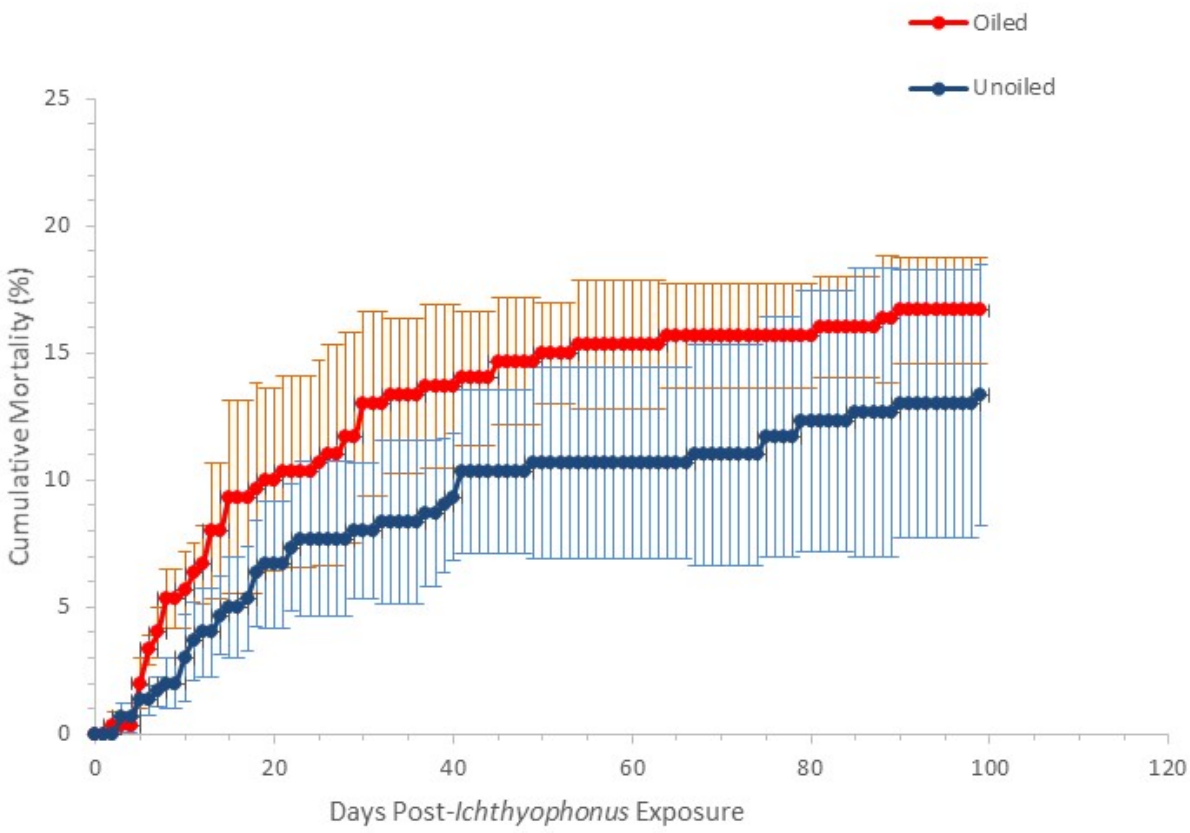

Figure 10. Cumulative mortality among two groups of Specific Pathogen Free herring (previously oiled and -unoiled) after exposure to Ichthyophonus by inter-coelomic injection. Mortality in both negative control groups (exposed to saline in lieu of Ichthyophonus) was negligible (data not shown). Each data point represents mean cumulative mortality from each of 3 tanks (n = 62 herring / tank), and error bars indicate 2 SD from the mean.

D. Decreased Herring susceptibility to VHS after surviving larval exposure to PAHs

As a no-cost contribution to support the herring genetics project (PI Whitehead, project number 20170115), a series of experiments were performed to evaluate the susceptibility of herring to VHSV after surviving larval exposure to oil.

First Test: Herring Larvae were Highly Susceptible to Direct Effects of Oil Exposure:

Herring embryos were collected from wild spawn in Puget Sound and transferred to the USGS 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 (results pending). A clear dose response occurred in larval survival to metamorphosis (Fig. 11) and fish mass at 85 d post-hatch (Fig. 12).

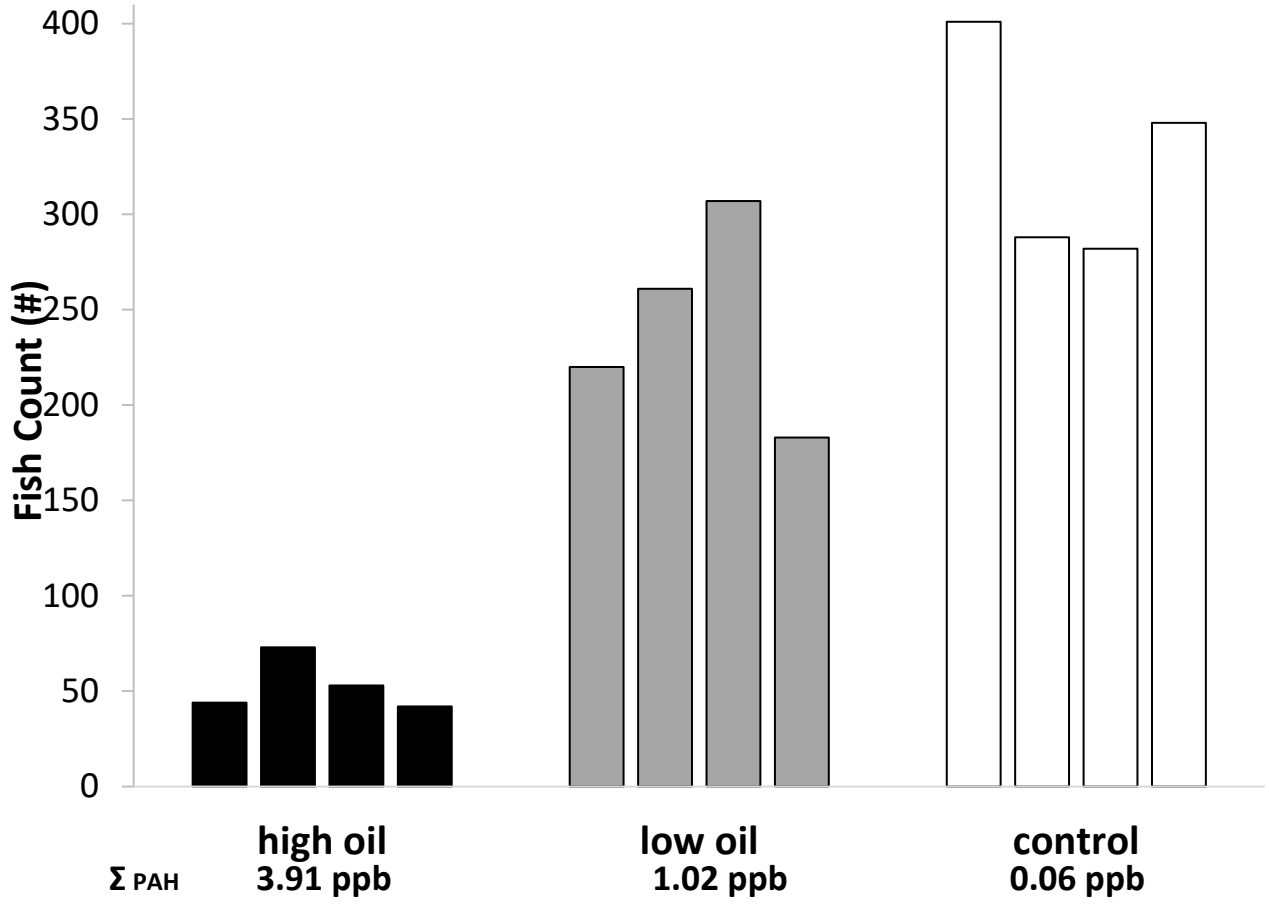

Figure 11. Larval survival after exposure to polycyclic aromatic hydrocarbons (PAHs). Each exposure was performed in 4 replicate tanks (indicated by the treatment bars), with each tank loaded with 1,000 newly-hatched larvae. Fish counts were made 85 d post hatch, after larvae were mostly

metamorphosed to juveniles. Note: development was retarded in the high PAH treatment group, where most individuals were not yet metamorphosed 85 d post-hatch.

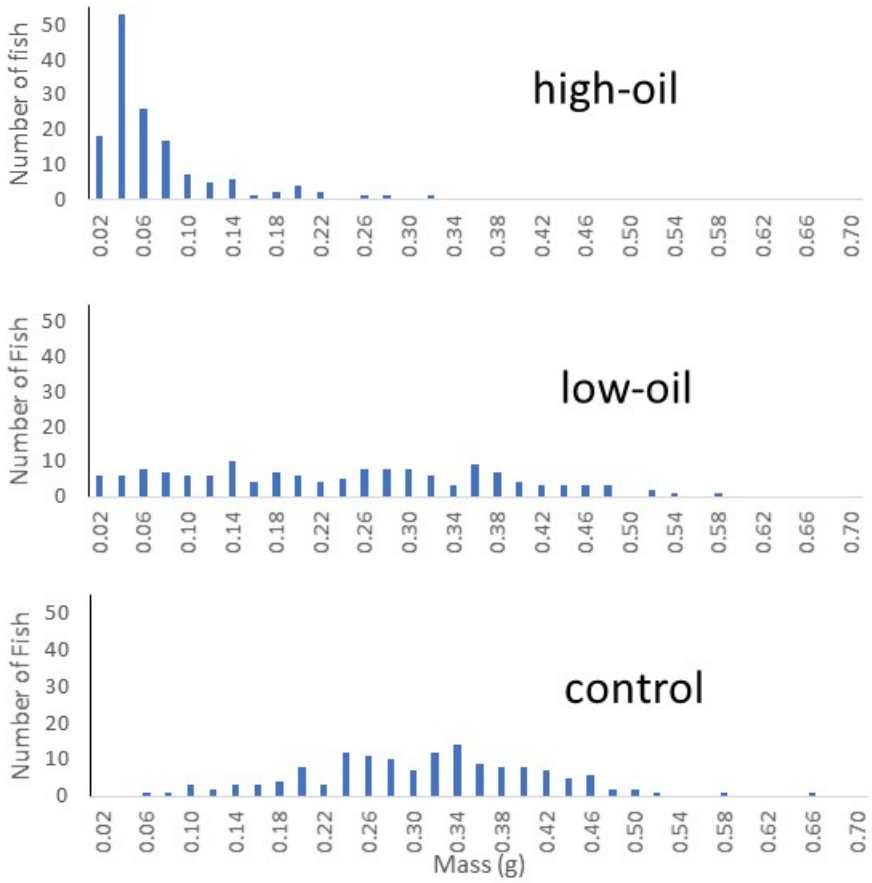

Figure 12. Frequency of wet weights among 85 d post-hatch juveniles in each of the polycyclic aromatic hydrocarbon (PAH) exposure treatments. Note: development was retarded in the high PAH treatment group, where most individuals were not yet metamorphosed 85 d post-hatch.

Second Test (Pilot Study): Decreased Susceptibility of Juveniles that Survived Larval PAH Exposure:

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 were used for an expanded VHSV study to assess VHS susceptibility. Additional fish from both groups (unoiled and oiled) remained and were used in a pilot study to assess whether larval exposure to PAHs impacts the susceptibility of juvenile herring to VHS. Pilot results indicated that the onset of VHS mortality was delayed among juvenile and 21d cumulative mortality was lower among herring that survived larval exposure to PAHs than unoiled herring (Fig. 13).

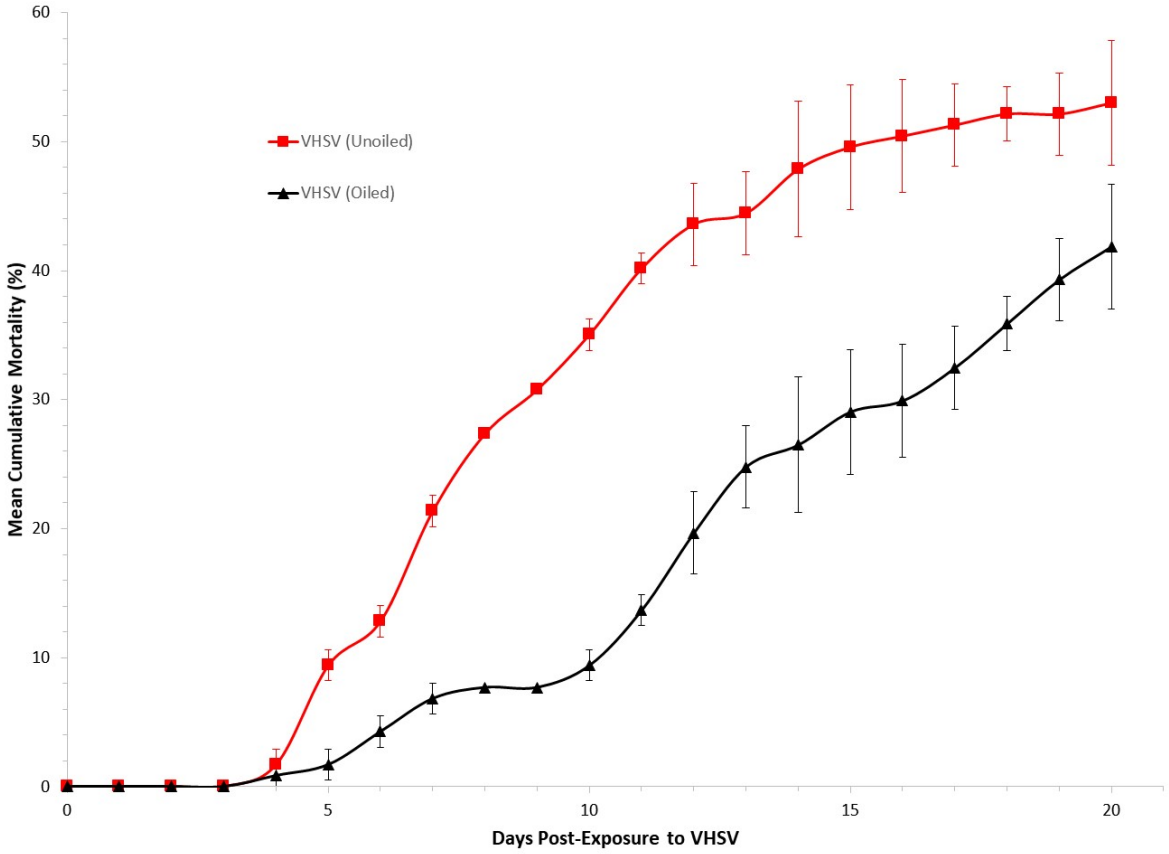

Figure 13. Pilot Study to examine the effects of larval polycyclic aromatic hydrocarbon exposure on the subsequent susceptibility of juvenile survivors to viral hemorrhagic septicemia virus (VHSV). Each data point represents mean cumulative mortality from each of 3 tanks (n = 39 herring / tank), and error bars indicate 2 SD. Mean cumulative mortalities in negative controls (exposed to saline in lieu of VHSV) for both treatments were ≤ 3.2% (results not shown).

Third Test (Expanded Study): Decreased Susceptibility of Juveniles that Survived Larval PAH Exposure:

An expanded study was performed using more robust sample sizes (n = 71-72 herring / tank x 3 replicate tanks / treatment), which permitted periodic subsampling for transcriptomics. Experimental animals were evenly represented by the original grow out tanks (e.g. Fig. 10). Fish were periodically sampled from each replicate to assess differences in the herring transcriptome between the various treatments (results pending). Additionally, genomic samples were collected to assess whether certain herring genotypes may be more susceptible to VHS (results pending). Mortality results were similar to those in the pilot study (Fig. 13) and were characterized by lower cumulative VHS mortalities among juveniles that survived larval exposure to PAHs than among unoiled cohorts (Fig. 14).

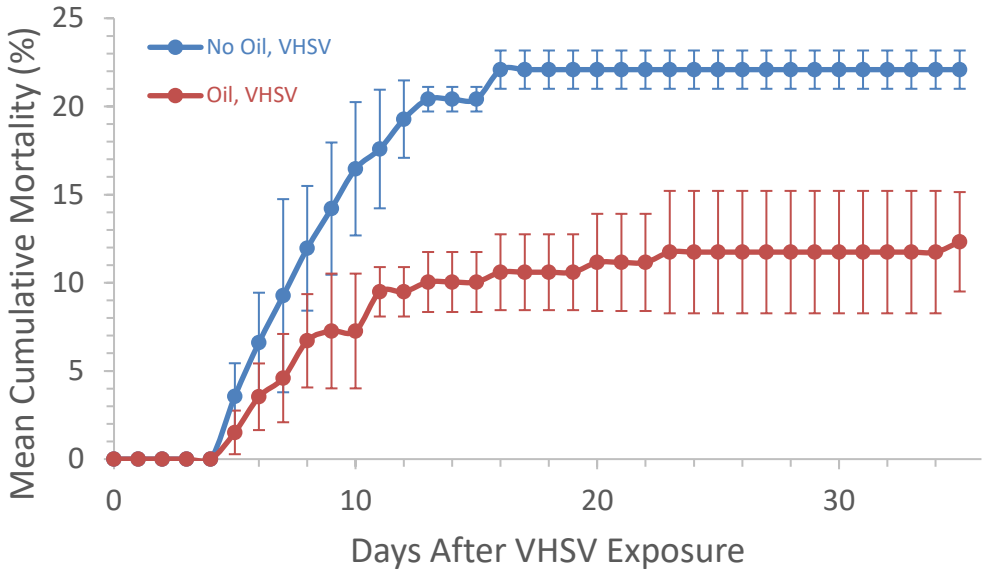

Figure 14. Expanded study to examine the effects of larval polycyclic aromatic hydrocarbon exposure on the subsequent susceptibility of juvenile survivors to viral hemorrhagic septicemia virus (VHSV). Each data point represents mean cumulative mortality from each of 3 tanks (n = 72 herring / tank), and error bars indicate 2 SD. Mean cumulative mortalities in negative controls (exposed to saline in lieu of VHSV) for both treatments were < 3.3% (results not shown).

Fourth Test: Ability of Oil-Exposed Survivors to Mount a protective Immune Response to VHSV:

In considering possible reasons for the VHS epizootic that occurred in PWS during the early 1990s, some have suggested that early life stage exposures of herring to PAHs compromised their ability to mount an adaptive (antibody) response to VHSV. If this were the case, then any prior exposures to VHS virus would not have resulted in the development of disease resistance in the form of herd immunity. As such, new recruits from 1989 year class would have all been susceptible to VHS in 1992 - 1993, regardless of their prior exposure histories to VHSV.

To test this hypothesis, survivors of the prior VHSV exposure (third test) were re-distributed to new replicates (n = 3 replicate tanks / treatment, with each tank containing 20-21 herring) and re-exposed to VHSV 59d after the initial virus exposure to assess whether larval exposure to PAHs impacted their ability to mount an adaptive immune response to VHSV. Cumulative mortality results provided no phenotypic indication that larval exposure to PAHs impacted the ability of juvenile survivors to mount a protective response after surviving VHSV exposure (Fig. 15); however, neutralizing antibody levels and transcriptomics will be examined to further explore this hypothesis (results will be presented next year).

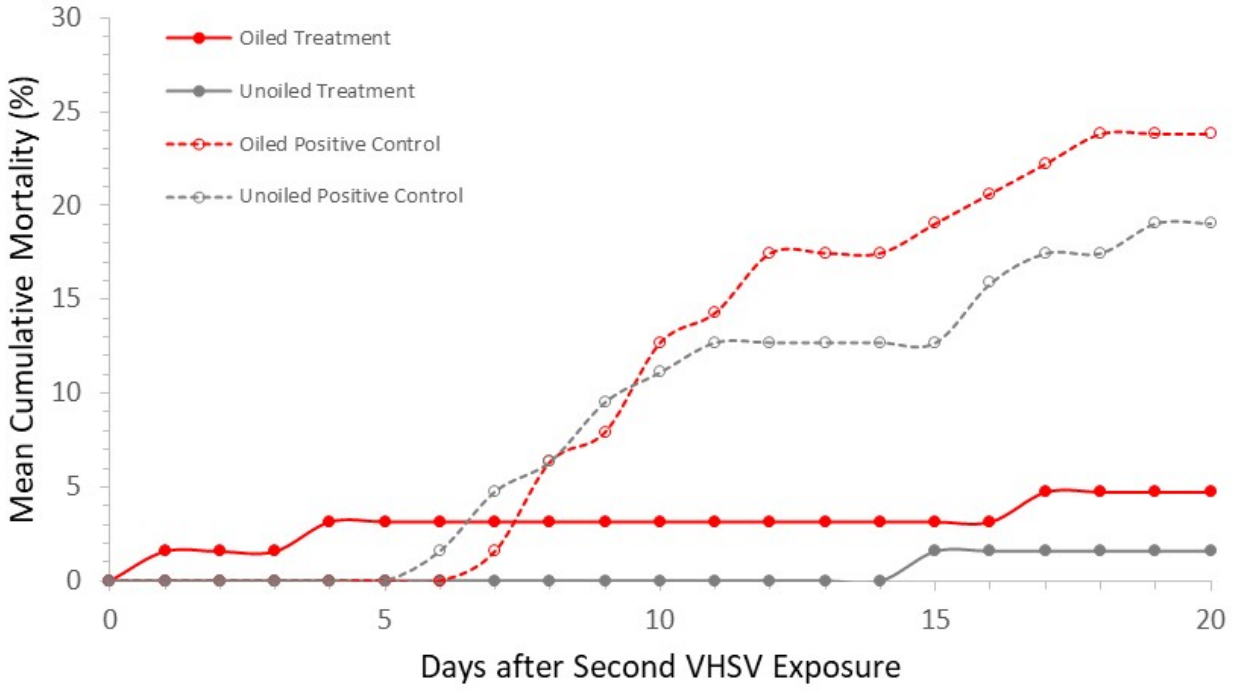

Figure 15. Ability of oiled survivors to mount a protective immune response against viral hemorrhagic septicemia virus (VHSV). Experimental fish were survivors from the previous experiment (Fig. 12). Treatment groups were exposed to VHSV twice (first exposure depicted in Fig. 12 and second exposure depicted here). Positive Controls were exposed to saline in the first experiment and VHSV in this second experiment. Two sets of negative controls were included: 1) Groups that survived VHSV exposure in the first experiment that were exposed to saline in this experiment and 2) Groups that were exposed to saline in both experiments (mean cumulative mortalities < 1.6% for both groups; data not shown).

E. *Ichthyophonus* Phyogenetics

The genetic relatedness of *Ichthyophonus* from Prince William Sound to isolates from other locations is being assessed. 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 (Fig. 16). Submission of a manuscript is expected in 2021.

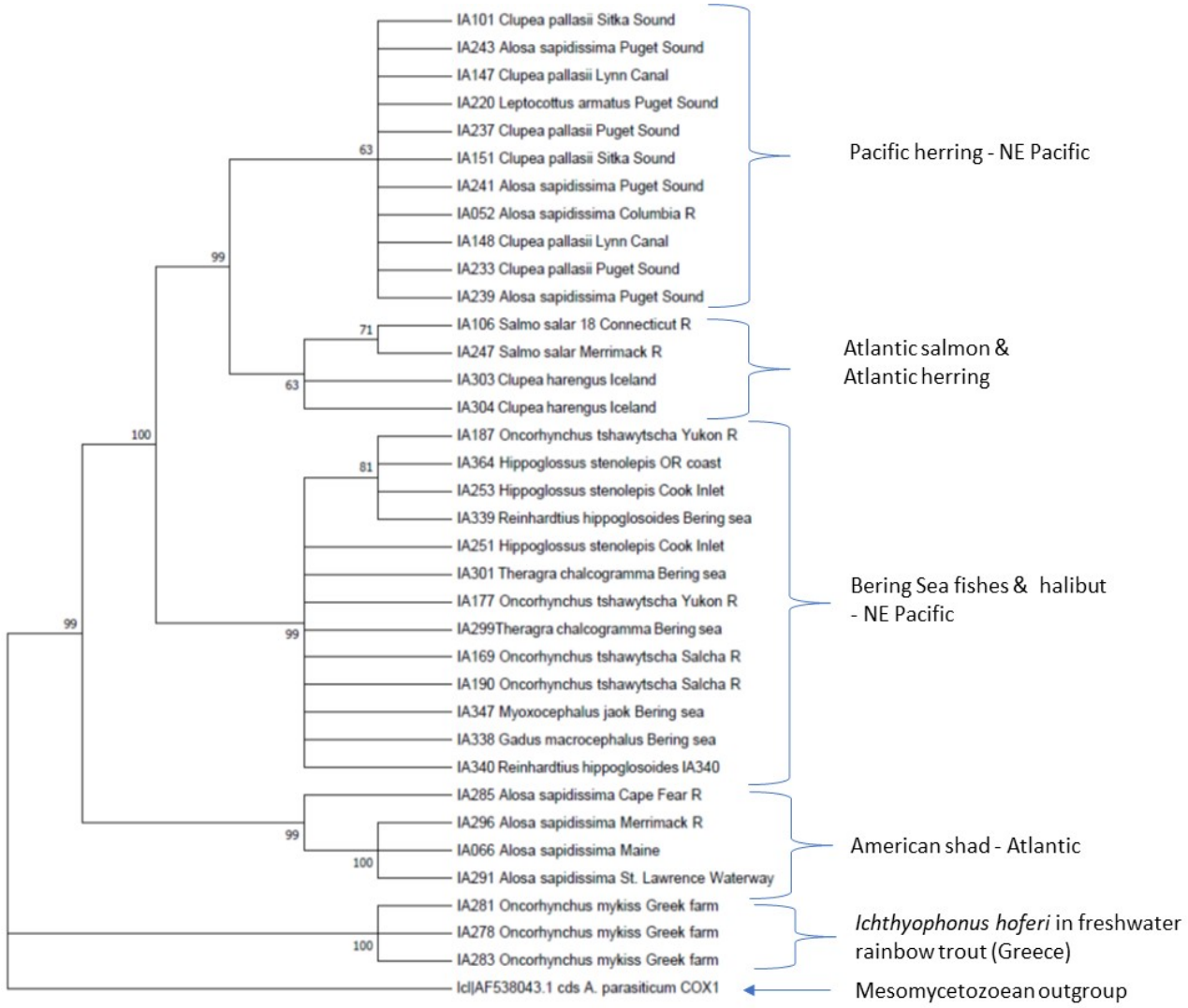

Figure 16. Ichthyophonus phylogenetics based on 1191 bases in the COX-1 gene. Values on the branches indicate percentages of bootstrap support, indicating the percentage of times the same branch occurred in repeated phylogenetic constructions.

F. *Ichthyophonus* in Pacific herring from 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 (Fig. 17). The cause of this increased prevalence was not determined, but may involve one of the following:

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.

C) Other disease co-factors that may occur in Cordova Harbor, including exposure to biofouling contaminants (e.g. TBT or copper-based paints), limited food availability, temperature differences, etc.

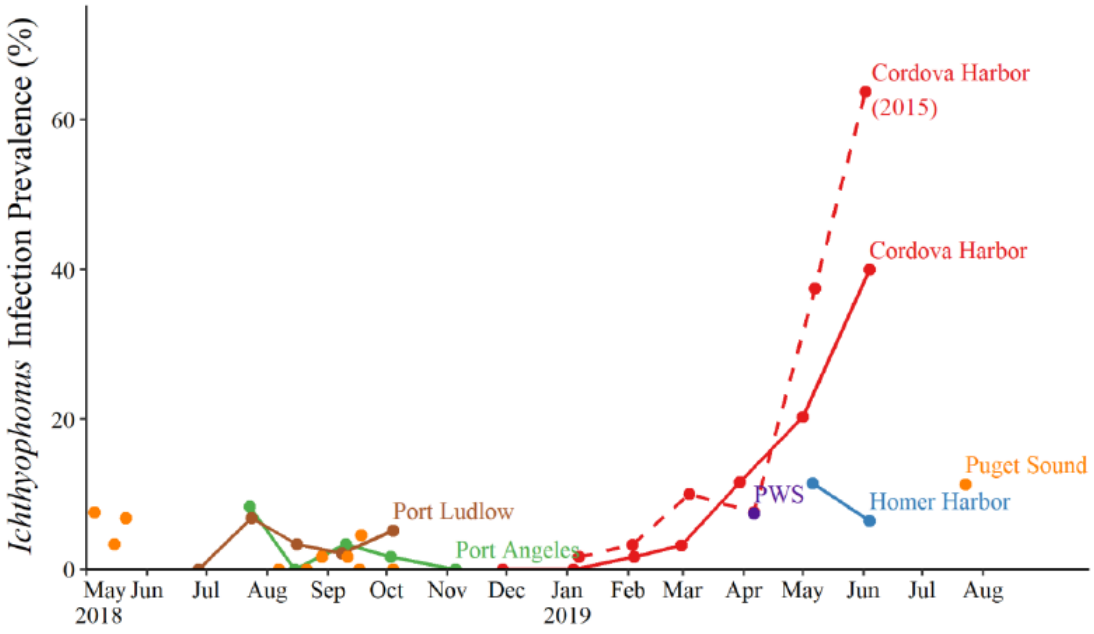

Figure 17. Ichthyophonus infection prevalence in Age 0 herring from various locations throughout the NE Pacific, including Cordova Harbor, throughout the year (May 2018 – Aug 2019).

G. Possible *Ichthyophonus* transmission through ovivory

Ovaries from walleye pollock and Pacific herring were transported to the USGS Marrowstone Marine Field Station and the eggs were fed to SPF herring to attempt *Ichthyophonus* transmission. *Ichthyophonus* was not recovered from any of the experimental 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* associated with these eggs 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 likelihood.

At the time of this writing (February 2021), we have obtained the first batch of 2021 herring eggs from Puget Sound and are performing new ovivory transmission experiments. The experimental challenge model is much improved since the 2020 feeding trials; results will be presented in the 2021 annual report.

8. Coordination/Collaboration:

All USGS field sampling and laboratory studies described in this report were approved by the USGS, Western Fisheries Research Center Institutional Animal Care and Use Committee (IACUC) Protocols #2008-51 and #2008-52.

A. Long-term Monitoring and Research Program Projects

1. Within the Program

- We worked closely with the Prince William Sound Science Center (project 20120111-A, Herring Research and Monitoring coordination) and Alaska Department of Fish and Game (ADF&G) (project 20160111-F, age-sex-length study and aerial milt survey, PI Haught) 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 (project 20120111-C). Additionally, revised antibody data for PWS were shared with Dr. Branch, for incorporation into a VHSV hindcasting model.

- As In-Kind contributions to Dr. Maya Groner's project (project 20120111-A, Herring Research and Monitoring Coordination), several experiments were initiated and are currently underway at the U.S. Geological Survey – Marrowstone Marine Field Station. Descriptions of the resulting *Ichthyophonus* models are included in the annual report from Dr. Pegau.

- We provided laboratory support, including larval oil exposures, herring rearing, pathogen exposure experiments, and coordination (including hosting meetings) for Dr. Andrew Whitehead's project (project 20170115, herring genetics).

2. Across Programs

a. Gulf Watch Alaska

None to report

b. Data Management

Survey data and metadata were entered onto the Workspace.

B. Individual Projects

We 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 North Pacific Research Board (NPRB; # 1807: *Ichthyophonus* in Pacific herring).

We partnered with Drs. John Incardona and Nat Sholtz (National Oceanic and Atmospheric Administration [NOAA] – 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 partnered with U.S. Geological Survey (Columbia River Research Laboratory and Washington Water Science Center) to determine the effects of polychlorinated biphenyl exposure on Pacific herring susceptibility to VHS. Results are currently being processed and will be reported in 2021. The availability of SPF herring from the EVOSTC-funded Herring Disease Program made this project possible.

C. With Trustee or Management Agencies

In addition to the above, we partnered with NOAA Fisheries (Dr. Dave McGowan - Alaska Fisheries Science Center) to collect walleye pollock samples from Shelikoff Strait. Samples were used to assess *Ichthyophonus* infection prevalence and attempt laboratory transmission studies through ovivory.

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

Burge, C.A., and 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.

Hershberger, P.K., M. Stinson, B. Hall, J.L. Gregg, A.M. MacKenzie, J.R. and Winton. 2020. Pacific herring are not susceptible to vibriosis under laboratory conditions. Journal of Fish Diseases 43:1607-1609.

LaDouceur, E.E.B., J. St Leger, A. Mena, A. MacKenzie, J. Gregg, M. Purcell, W. Batts, and P. Hershberger. 2020. *Ichthyophonus* Infection in Opaleye (*Girella nigricans*). Veterinary Pathology 57:316-320.

2. Reports

None to Report

3. Popular articles

None to Report

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, Jan 27-31.

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, Jan 27-31.

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

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

2. Public presentations

None to report

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

None to report

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:

Sept 2020: Science Panel Comment – FY21: Excellent progress has been made on multiple lines of investigation. The SP is very pleased to see the completion of MS student thesis as part of this research project. The SP has a few questions.

Hershberger response to Science Panel:

We would like to thank the Science Panel and Science Director for their thoughtful review of our proposal. Responses to specific questions are included below:

Sept 2020: Science Panel Comment – FY21: Figure 6 does not indicate that *Ichthyophonus* occurs in pink salmon. Please clarify whether pink salmon were not included in the analysis or whether they were included but not found to have *Ichthyophonus*.

Hershberger response to Science Panel:

Figure 6 and pink salmon susceptibility to *Ichthyophonus*: Sequencing data used to inform the figure were obtained from a combination of sources including an archive of *Ichthyophonus* isolates we maintain at USGS - Marrowstone and any isolates we have been able to obtain from global research partners. The figure is intended to reflect representative fish species from various regions; it is not a comprehensive list of all known susceptible species. However, in a previous publication (Gregg et al. 2016), we canvassed the scientific literature to identify all fish species known to be naturally infected with *Ichthyophonus*. Infections were confirmed in over 145 fish species, from the Barents Sea, AK, to the southern tip of Africa, in the Atlantic and Pacific Oceans, and in freshwater on 6 continents (list included below). Pink salmon were not included on this list; however, the amount of *Ichthyophonus* sampling effort in pink salmon remains unknown.

Sept 2020: Science Panel Comment – FY21: Also, in Figure 3 fish that are viral exposed and unoiled have greater mortality than exposed and oiled, but the text downplays this result. It seems to the SP that if the results were reversed (oiled + virus had LOWER survival) but of similar magnitude then the PIs might be making a bigger deal of this. Please discuss mechanisms for this result and elaborate on its significance or provide clearer reasons why the result is not biologically meaningful. Could it be that oil has an effect on the virus and offers some disease resistance?

Hershberger response to Science Panel:

Figure 3 and differences between treatments: Correct, this figure did appear to demonstrate reduced VHS susceptibility among groups that survived prior exposure to oil. We have performed some iteration of this experiment 6 times throughout the past several years and the results have been ambiguous (no difference in 3 of the trials, and slightly less susceptibility among the oil-exposed groups in 3 trials). Results depicted in Figure 3 were from a pilot experiment that was used to inform the design of our definitive experiment for 2020. Since submission of this proposal, the definitive experiment has been initiated, and the mortality results are analogous to those reported in Figure 3 (albeit even greater separation between the groups). The purpose of the definitive experiment is to determine the reasons for the differences between the treatment groups by performing full genome sequencing and RNA Seq. Although we have hypotheses that may account for these susceptibility differences, I am reluctant to speculate at this point because we should have scientifically justifiable explanations within the next several months.

Sept 2020: Science Panel Comment – FY21: What are the program and project contingency plans for FY21 in regard to accomplishing goals and field activities?

Hershberger response to Science Panel:

We don’t anticipate impacts to laboratory work. The USGS has given permission for people to continue working through the COVID-19 pandemic. We would work with researchers in Cordova to collect and process field samples, as was done in 2020.

Sept 2019: Science Panel Comment – FY20: The Science Panel appreciates the continued progress and willingness to adaptively manage the project to continue to produce novel results. The Panel

wondered what the long-term direction of the program will be. The Panel also recognizes the integrative effort to work our understanding of disease into the model with Branch.

Hershberger response to Science Panel:

We envision a several long-term goals for the Herring Disease Program. First, we are working towards complete validation and integration of the VHSV antibody assay into tools that can both hind-cast prior disease mortality events and forecast the potential for future disease epizootics. The hope is that we will be able to hand off a fully-vetted laboratory technique to the ADF&G pathology lab in Juneau, who will be able to work directly with ADF&G herring managers to incorporate near-real time disease metrics into their stock assessments. Second, during the next 5 years, we are interested in investigating potential interactions between pink salmon production in PWS and herring disease, as there are several diseases that cross over between the two species. Our hope is that the Herring Program will make pink salmon / herring interactions a theme and point of emphasis during the next 5-year project block (plans to discuss at the PI meeting in October 2019). Third, we are working towards understanding the basic transmission mechanisms for *Ichthyophonus*. It is our hope that elucidation of these processes will translate directly into tools that can forecast upcoming *Ichthyophonus* disease epizootics.

11. Budget:

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	$596.8
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	$32.7
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	$165.3
Equipment	$0.0	$0.0	$0.0	$0.0	$32.7	$32.7	
SUBTOTAL	$181.5	$210.0	$217.2	$223.2	$263.1	$1,095.0	$794.8
General Administration (9% of subtotal)	$16.3	$18.9	$19.5	$20.1	$23.7	$98.6	
PROJECT TOTAL	$197.8	$228.9	$236.7	$243.3	$286.8	$1,193.6	
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	$254.5