ATTACHMENT C EVOSTC Annual Project Report Form

Form Rev. 8.30.18

1. Program Number:

18120111-E

2. Project Title:

Herring Disease Program

3. Principal Investigator(s) Names:

Paul Hershberger, Maureen Purcell

4. Time Period Covered by the Report:

February 1, 2018-January 31, 2019

5. Date of Report:

April 2019

6. Project Website (if applicable):

http://pwssc.org/herring-research-and-monitoring/

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 10-13, 2018 to test for viral hemorrhagic septicemia virus (VHSV), *Ichthyophonus*, and viral erythrocytic necrosis (VEN) prevalence (Fig. 1):

Location	**Date**	**VHSV Prevalence**	***Ichthyophonus* Prevalence**	**VEN prevalence**
Hells Hole	April 10-11	0% (n=60)	10% (6/59)	0% (n=60)
Cedar Bay	April 12	0% (n=60)	22% (13/59)	0% (n=60)
Rocky Bay	April 13	0% (n=60)	8.3% (5/60)	0% (n=60)

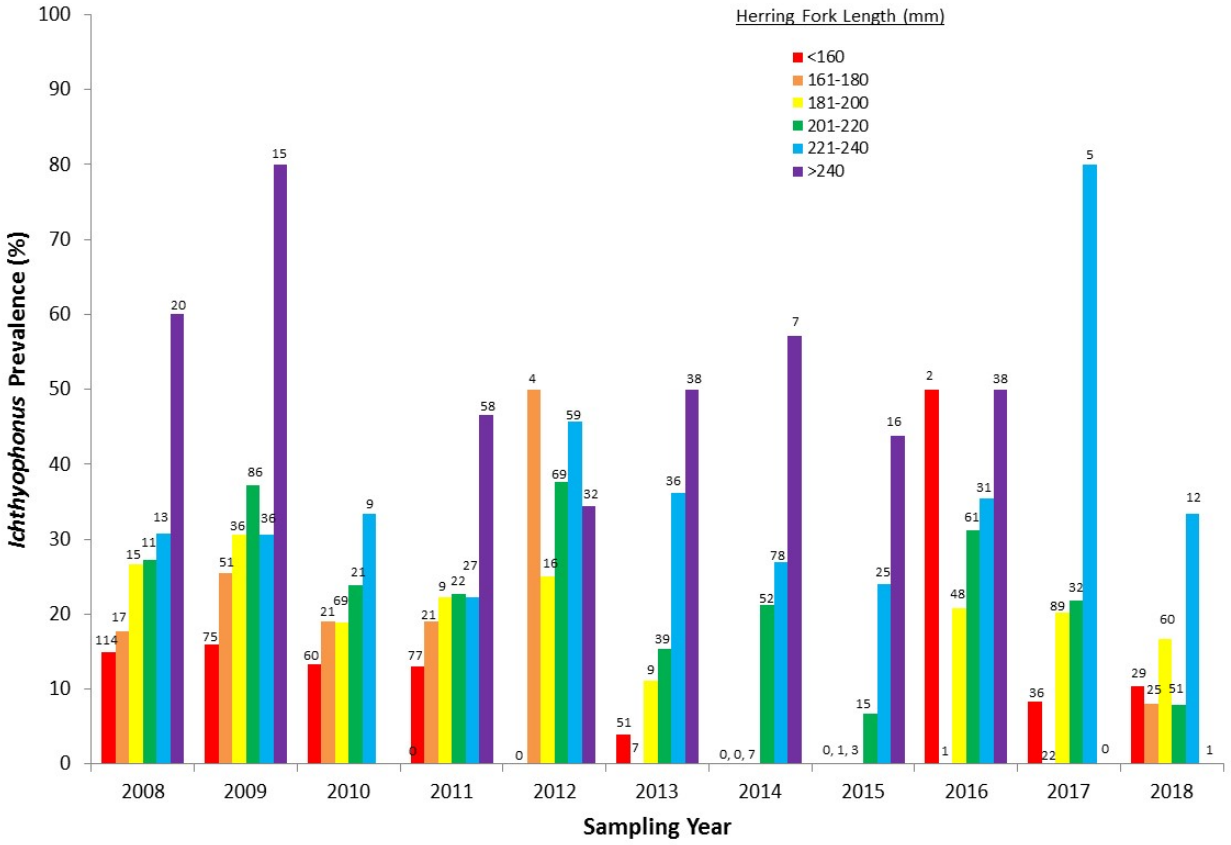

Figure 1. Temporal trend in *Ichthyophonus* infection prevalence in each size class of Prince William Sound herring. Numerals above each bar indicate 'n.'

B. Three samples of adult Pacific herring were collected from Sitka Sound during the spring pre-spawn period from March 22-23, 2018 to test for VHSV, *Ichthyophonus*, and VEN prevalence (Fig. 2):

Location	**Date**	**VHSV Prevalence**	***Ichthyophonus* Prevalence**	**VEN prevalence**
Guide Island	March 22	0% (n=56)	21% (12/56)	0% (n=55)
Unknown	March 23	0% (n=49)	18% (9/49)	0% (n=49)
Kruzof Island	March 23	0% (n=72)	24% (17/72)	0% (n=72)

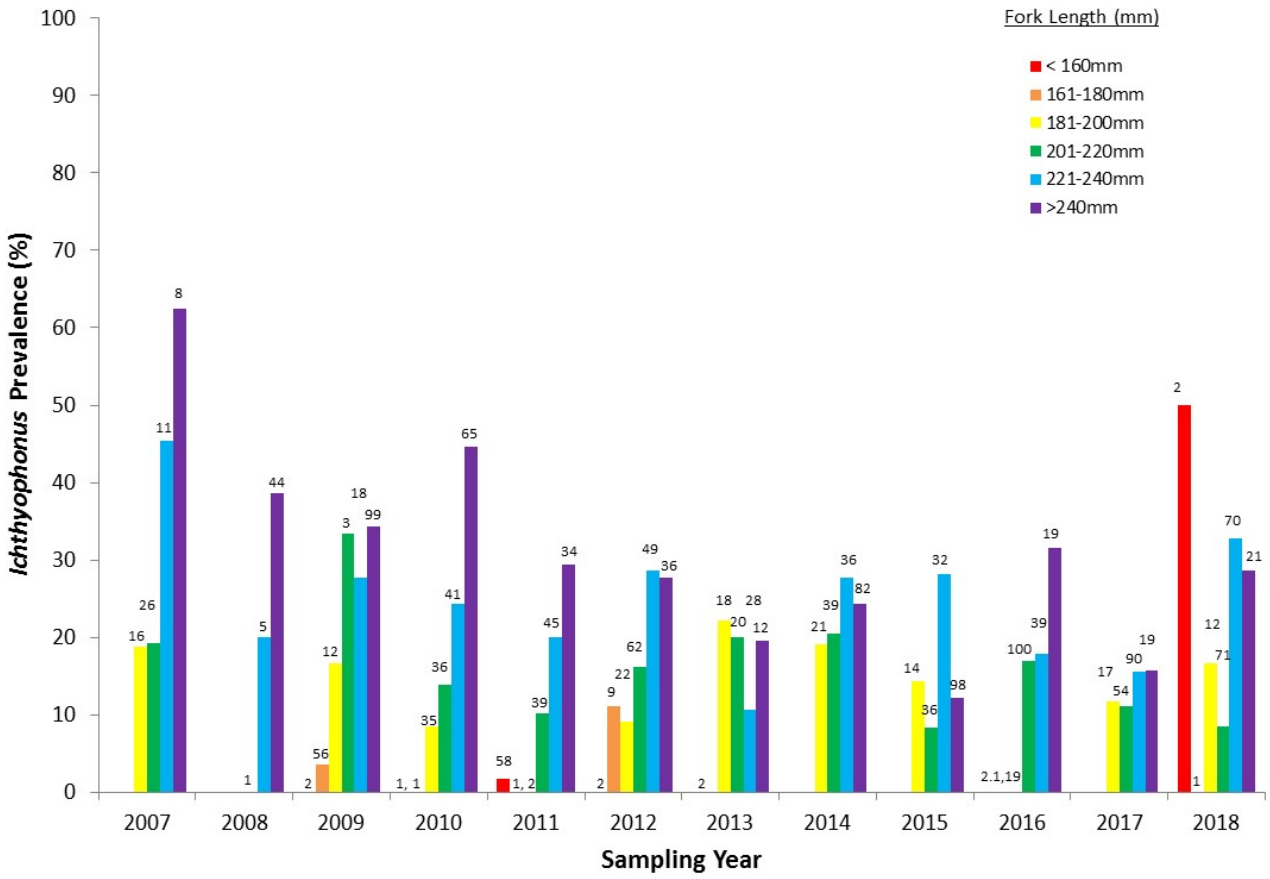

Figure 2. Temporal trend in *Ichthyophonus* infection prevalence in each size class of Sitka Sound herring.

C. *Ichthyophonus* infection prevalence was 3% (2/60) in a group of pre-spawn herring collected from south Puget Sound (Squaxin Pass) on February 8, 2018.

D. Plasma samples from Prince William Sound and Sitka Sound were processed by plaque neutralization test (PNT) to determine the presence and titer of neutralizing antibodies to VHSV (Fig. 3).

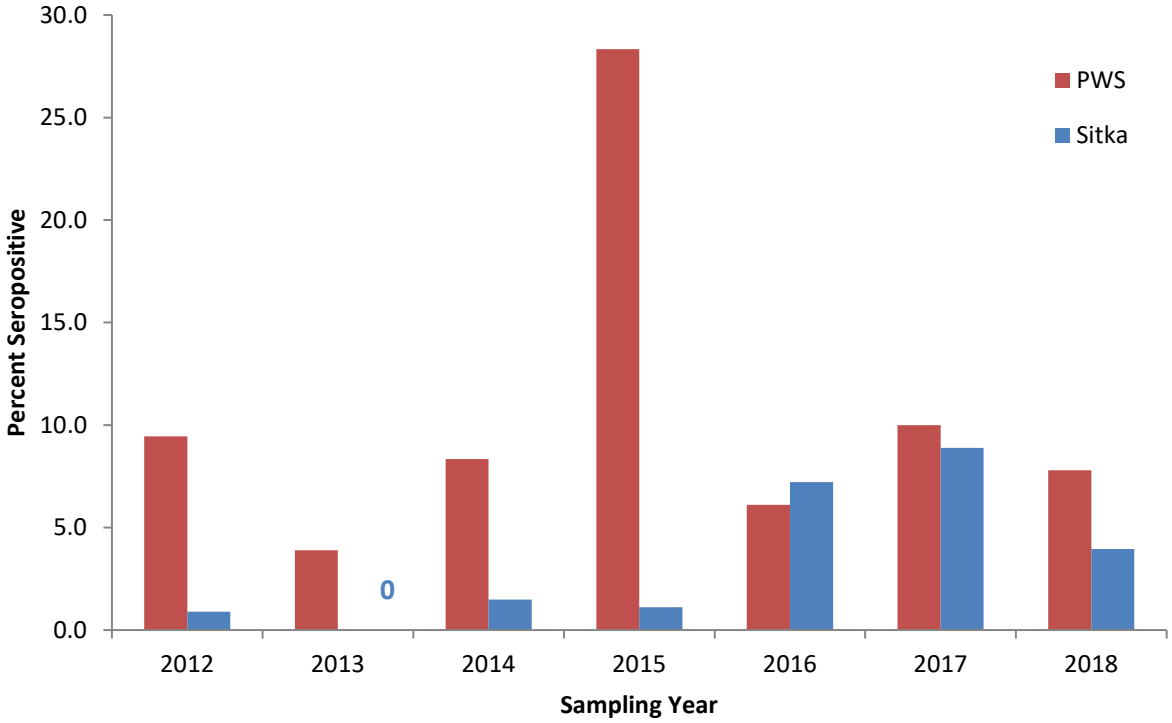

Figure 3. Percent of Prince William Sound and Sitka Sound herring that tested positive for VHSV neutralizing activity from 2012-2018.

Laboratory Studies

A large portion of our efforts throughout FY18 were directed towards validation of a method for understanding how neutralizing antibodies levels (e.g., Fig. 3) may be used to assign functional herd immunity to wild herring populations. A PNT was previously developed to detect VHSV neutralizing activity in the plasma of Pacific herring that survived prior exposure to the virus. The optimized PNT methods are highly sensitive and specific when applied to specific pathogen free herring with known exposure histories. However, we do not yet know the level of antibodies that are required to confer population-level herd immunity against VHS to wild populations.

Our approach involves assessing the levels of neutralizing antibodies present in herring populations that demonstrate solid herd immunity. This approach is made possible by a unique principle involving VHS and Pacific herring, whereby wild herring confined into net pens or laboratory tanks often (but not always) experience VHS epizootics. It can be deduced that any group of herring experiencing a VHS epizootic under these conditions did not possess herd immunity at the time of capture. In contrast, any group of herring failing to undergo a VHS epizootic under these confinement conditions most likely possessed herd immunity at the time of capture. Therefore, during 2018, we repeatedly collected groups of juvenile Pacific herring from various locations throughout Puget Sound, transported them alive to the laboratory, subsampled their VHSV antibody levels at the time of capture, then deduced their levels of herd immunity by confining them into laboratory tanks, observing whether a VHS epizootic ensued, and determining the levels of VHSV neutralizing antibodies in the population at time of capture and 21d post-confinement.

There are two possible scenarios to explain the absence of a VHS epizootic in confined herring:

- The population was not susceptible to VHS (i.e. demonstrated herd immunity); sufficient numbers of individuals survived prior exposure and possess protective antibodies.
- The population was susceptible to VHS; however, viral carriers were not present among the captured individuals, so exposure to VHSV did not occur in the tanks. The possibility of this scenario was explored by subsequently exposing survivors to known amounts of VHSV under controlled conditions. The re-exposure experiment was recently terminated (February 7, 2019) and complete results are not yet in; however, preliminary results indicate that all groups (F, H, and J) were not susceptible to VHS.

The figures below show the cumulative mortalities in 10 groups of juvenile Pacific herring (designated Groups A - J) collected from the Southern Salish Sea and transported alive to tanks at the Marrowstone Marine Field Station.

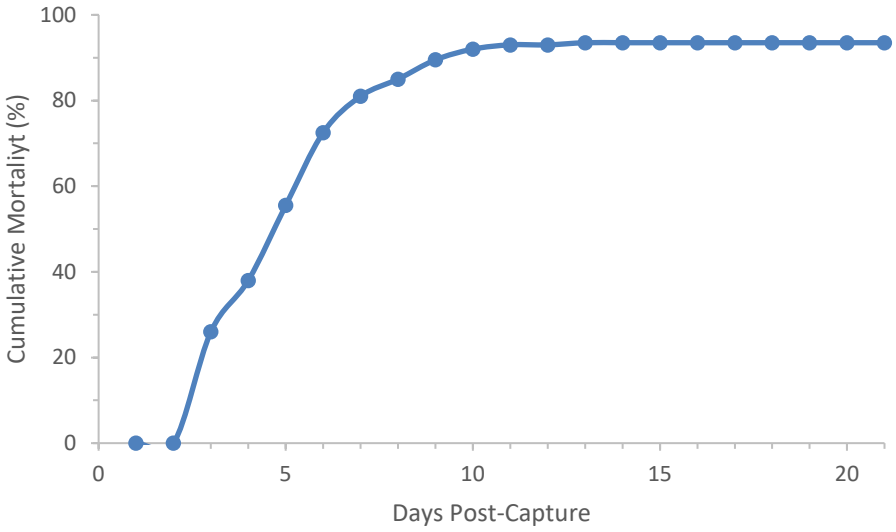

Figure 4 (Group A). Age 1 Pacific herring (N = 200) collected from Admiralty Inlet August 2, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	0% (n=41)	3% (1/29)
Day 21 survivors	69% (9/13)	77% (10/13)

Mortalities tested positive for VHSV from 4-9d post-capture

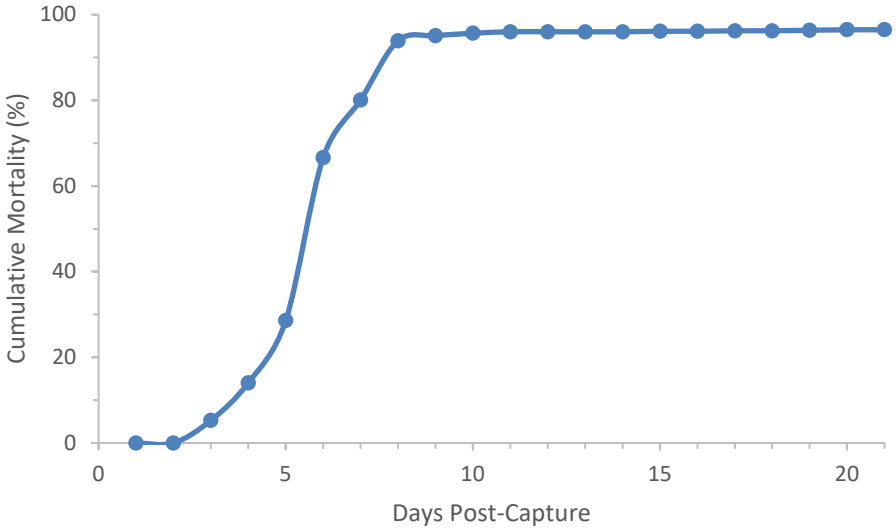

Figure 5 (Group B). Age 0 Pacific herring (N = 854) collected from Admiralty Inlet August 7, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	3% (1/30)	0% (0/30)
Day 21 survivors	69% (9/13)	50% (14/28)

Mortalities tested positive for VHSV from 1-18 d post-capture

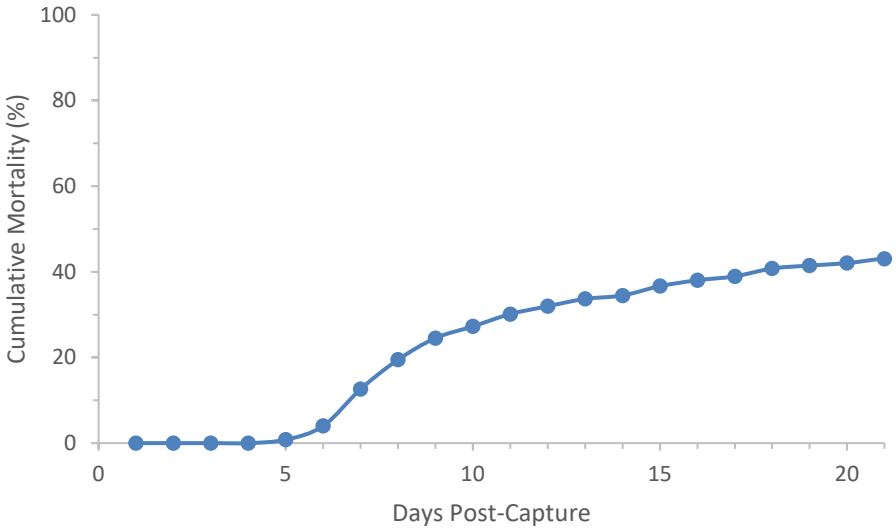

Figure 6 (Group C). Age 0 Pacific herring (N = 873) collected from Admiralty Inlet August 21, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	3% (1/30)	0% (0/30)
Day 21 survivors	0% (0/32)	67% (20/30)

Mortalities tested positive for VHSV from 4-20 d post-capture

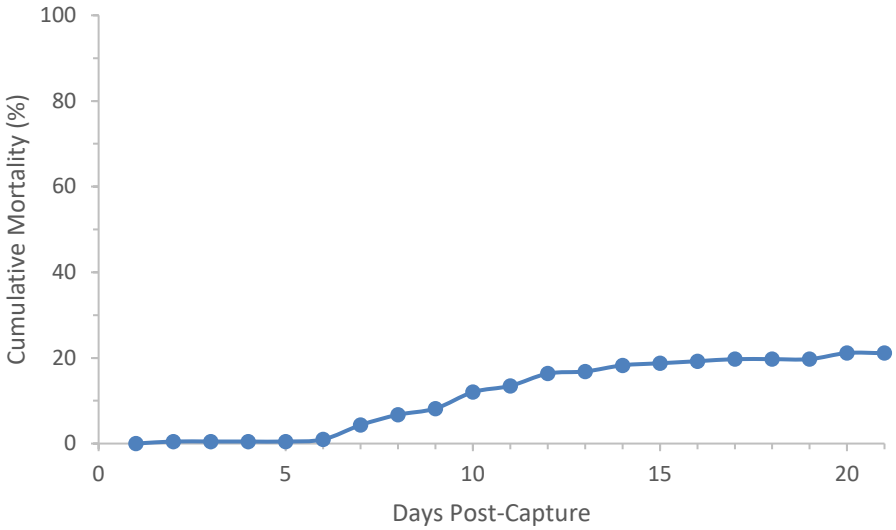

Figure 7 (Group D). Age 0 Pacific herring (N = 208) collected from Admiralty Inlet August 29, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	0% (0/30)	0% (0/30)
Day 21 survivors	3% (1/30)	63% (19/30)

Mortalities tested positive for VHSV from 5-19 d post-capture

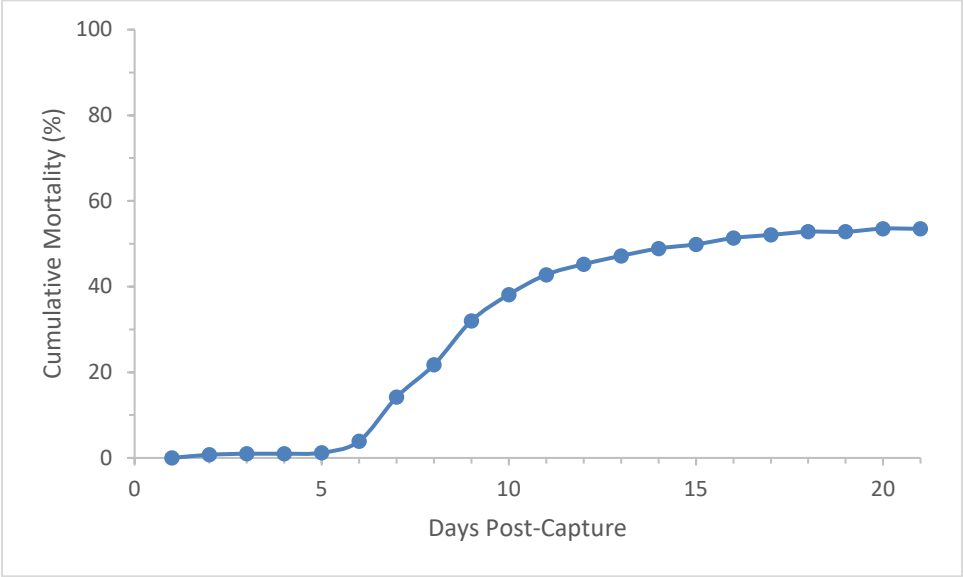

Figure 8 (Group E). Age 0 Pacific herring (N = 409) collected from Admiralty Inlet September 11, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	0% (0/30)	0% (0/30)
Day 21 survivors	3% (1/34)	80% (24/30)

Mortalities tested positive for VHSV from 4-15d post-capture

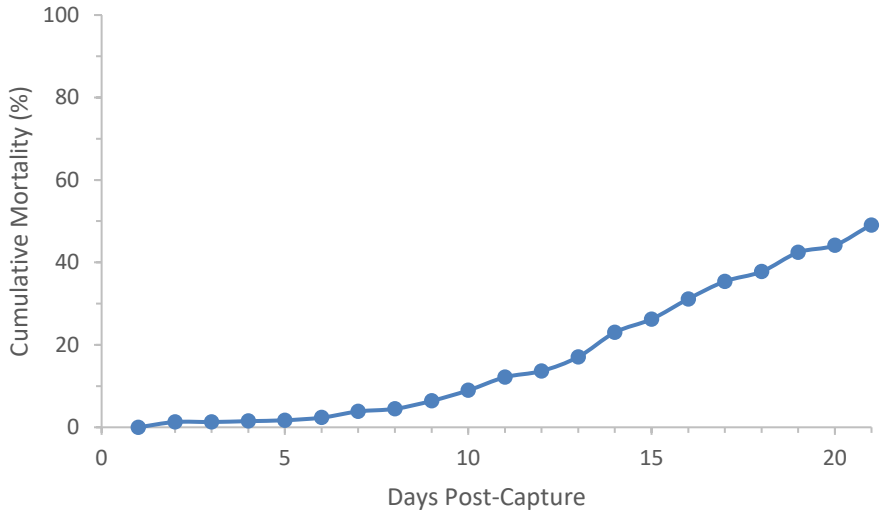

Figure 9 (Group F). Age 0 Pacific herring (N = 469) collected from Port Angeles Harbor September 18, 2018. These fish demonstrated herd immunity at the time of capture:

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	0% (0/30)	43% (13/30)
Mortalities	0% (0/146)	ND
Day 21 survivors	0% (0/41)	17% (5/30)

The atypical linear mortality curve in this group was most likely the result of VEN.

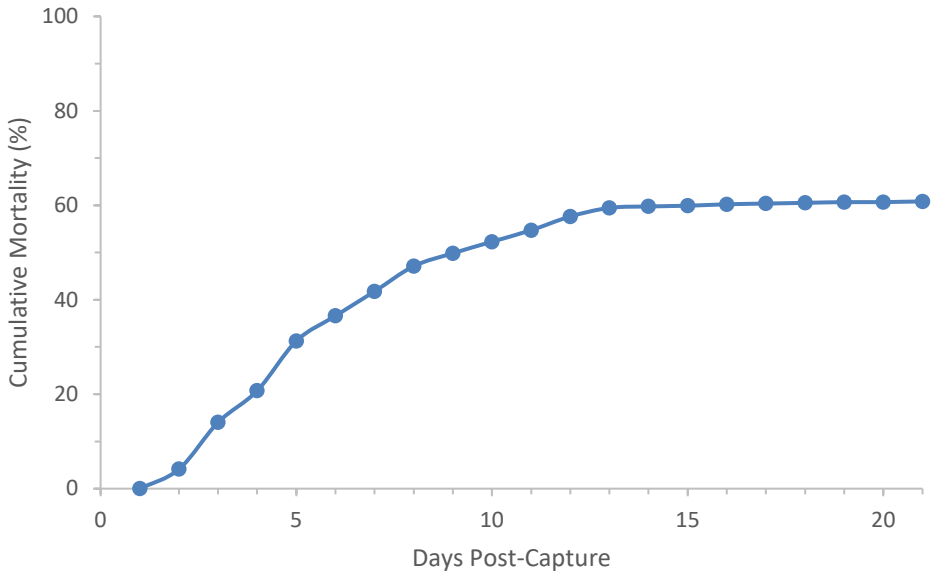

Figure 10 (Group G). Age 0 Pacific herring (N = 656) collected from the Strait of Juan de Fuca October 4, 2018. These fish did not demonstrate herd immunity when captured.

	VHSV Prevalence	VHSV Antibody Prevalence

Day 0 subsample	0% (0/30)	23% (7/30)
Day 21 survivors	0% (0/30)	33% (10/30)

Mortalities tested positive for VHSV from 4-16d post-capture

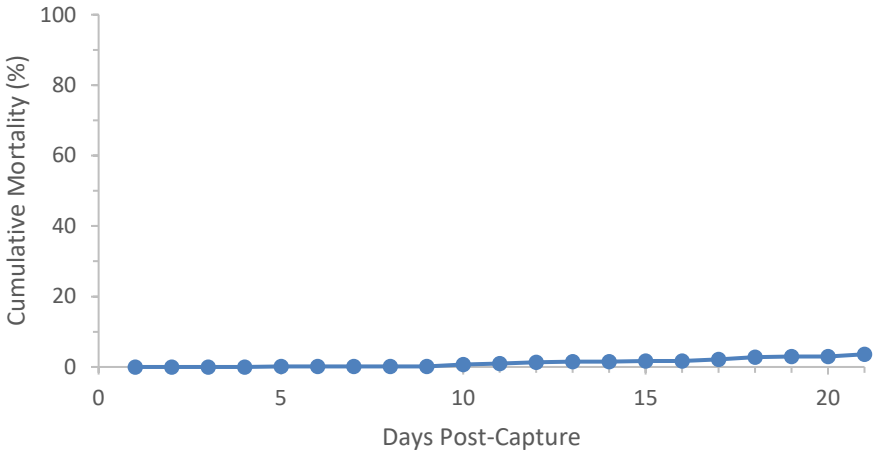

Figure 11 (Group H). Age 0 Pacific herring (N = 603) collected from Port Angeles Harbor October 9, 2018. These fish demonstrated herd immunity when captured:

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	7% (2/30)	0% (0/30)
Mortalities	0% (0/21)	ND
Day 21 survivors	0% (0/30)	13% (4/30)

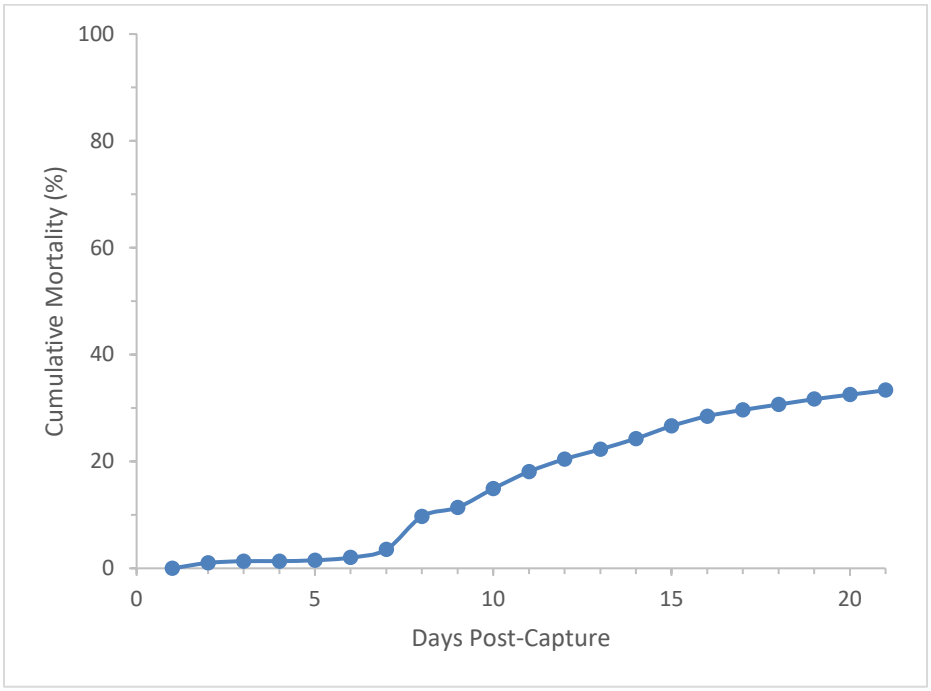

Figure 12 (Group I). Age 0 Pacific herring (N = 597) collected from the Strait of Juan de Fuca October 11, 2018. These fish did not demonstrate herd immunity when captured.

VHSV Prevalence VHSV Antibody Prevalence

Day 0 subsample	0% (0/30)	3% (1/30)
Day 21 survivors	0% (0/30)	33% (10/30)

Mortalities tested positive for VHSV from 5-20d post-capture

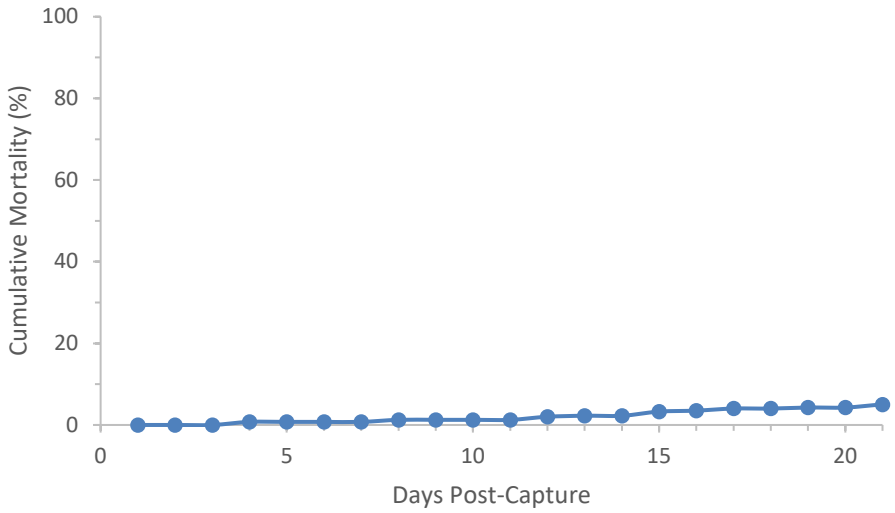

Figure 13 (Group J). Age 0 Pacific herring (N = 398) collected from Port Angeles Harbor November 5, 2018. These fish demonstrated herd immunity when captured:

	VHSV Prevalence	VHSV Antibody Prevalence
Day 0 subsample	7% (2/30)	13% (4/30)
Mortalities	0% (0/20)	ND
Day 21 survivors	0% (0/30)	10% (3/30)

Preliminary Summary

Results from Groups A-J should be considered preliminary, as we are continuing to process the data and interpret the results. For example, a distinct formula for assigning herd immunity is not available; rather, we are continuing to refine our analysis of epidemiological tools and interpretation of resulting datasets to provide a more quantitative assessment of herd immunity (details described below).

It was concluded that seven of these herring groups (A-E, G, and I) did not demonstrate herd immunity when they were collected because classic VHS epizootics (characterized by mortalities accompanied by high VHSV prevalence and tissue titers) ensued after their confinement into the tanks. The day 0 antibody profiles generally support this conclusion, as antibody prevalence at the time of capture was typically low ($\leq$3%). Group G was the exception to this pattern, where day 0 antibody prevalence was 21%; all samples from this group will be re-processed in 2019 to determine whether a laboratory error may have occurred.

It was concluded that three groups (F, H, and J) demonstrated herd immunity when they were collected because VHS epizootics did not ensue after their confinement into laboratory tanks[1]. Group F was a slight anomaly, as mortality did occur; however, it was not caused by VHS (the virus was not detected in any tissues from any of these fish, including mortalities). The mortalities in Group F were most likely cause by VEN, as the fish were severely anemic; follow up diagnostics are planned to determine whether these fish had VEN. The day 0 antibody profiles in groups F and J generally support this conclusion, with detectable levels of neutralizing antibodies occurring 13-43% when captured. Group H was the exception to this pattern, as

neutralizing antibodies were not detected in any fish at the time of capture; all samples from this group will be re-processed in 2019 to determine whether a laboratory error may have occurred.

[1]There are two possible scenarios to explain the absence of a VHS epizootic in confined herring:

- The population was not susceptible to VHS (i.e. demonstrated herd immunity); sufficient numbers of individuals survived prior exposure and possess protective antibodies.
- The population was susceptible to VHS; however, viral carriers were not present among the captured individuals, so exposure to VHSV did not occur in the tanks. The possibility of this scenario was explored by subsequently exposing survivors to known amounts of VHSV under controlled conditions. The re-exposure experiment was recently terminated (February 7, 2019) and complete results are not yet in; however, preliminary results indicate that all groups (F, H, and J) were not susceptible to VHS.

In addition to reprocessing plasma samples from Groups G and H, as mentioned above, we will be re-processing all plasma samples from all groups (A-J) using a slightly modified technique that dramatically increases the sensitivity of the PNT. The PNT is dependent on complement, a plasma protein that facilitates antibody neutralization, to provide accurate results. In the current methods, we assume that the plasma samples contain enough endogenous complement to satisfy the needs of the assay. Most PNT assays for other viruses employ a process of inactivating all endogenous complement, then adding back a known amount of exogenous complement so that each sample contains a known quantity of complement. During the development of the VHSV PNT, we recognized that the assay sensitivity could be increased dramatically after the addition of exogenous complement (Table 1); however, we had very strong justification for attempting to rely on endogenous complement. Based on these results from groups of herring demonstrating known levels of herd immunity, we now feel that additional assay sensitivity may be justified; therefore, all samples will be reprocessed using the more sensitive procedures. If the new results provide greater separation between the groups with various herd immunities, then all plasma samples from all wild herring collected from Prince William Sound and Sitka Sound (Fig. 3) will be re-processed using the more sensitive methods.

Table 1. Sensitivity of the PNT increases with the after addition of exogenous complement. Note the dramatic increase in detectable neutralization activity among VHS survivors (treatment) after the addition of exogenous complement (Heat-Inactivated + Complement column) compared to reliance on endogenous complement (Whole column). Table originally published in Hart et al. 2017.

Fish Treatment	Plasma Treatment		
	Whole Plasma (Endogenous Complement)	Heat-Inactivated (all Complement Removed)	Heat-Inactivated + Exogenous Complement
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	32
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	0
Neg. Control	0	0	0
Treatment	64	0	256
Treatment	128	0	2048
Treatment	16	0	16
Treatment	64	0	0
Treatment	256	0	2048
Treatment	256	0	2048
Treatment	256	0	1024
Treatment	64	0	0
Treatment	128	0	16
Treatment	0	0	256
Treatment	32	0	256
Treatment	0	0	0
Treatment	32	0	64
Treatment	256	0	128
Treatment	512	0	2048
Treatment	256	0	1024
Treatment	256	0	2048
Treatment	128	0	1024
Treatment	32	0	2048
Treatment	16	0	0

Published studies (2018)

- 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 ≤ 3% among the youngest cohorts (≤ 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.

- Harris, B.P., S.R. Webster, J.L. Gregg, P.K. Hershberger. 2018. *Ichthyophonus* in sport-caught groundfishes from southcentral Alaska. Diseases of Aquatic Organisms 128: 169-173.

This report of *Ichthyophonus* in common sport-caught fishes throughout the marine waters of south central Alaska represents the first documentation of natural *Ichthyophonus* infections in lingcod (*Ophiodon elongates*), yelloweye rockfish (*Sebastes ruberrimus*), and Pacific cod (*Gadus macrocephalus*). Additionally, the known geographic range of *Ichthyophonus* in black rockfish (*Sebastes melanops*) has been expanded northward to include southcentral Alaska. Among all species surveyed, the infection prevalence was highest (35%, n = 334) in Pacific halibut (*Hippoglossus stenolepis*). There were no gross indications of high-level infections or clinically diseased individuals. These results support the hypothesis that under typical conditions, *Ichthyophonus* can occur at high infection prevalence accompanied with low infection intensities among a variety of fishes throughout the eastern North Pacific Ocean, including southcentral Alaska.

- Lowe, V.C., P.K. Hershberger, C.S. Friedman. 2018. Analytical and diagnostic performance of a qPCR assay for *Ichthyophonus* spp. compared to the tissue explant culture 'gold standard'. Diseases of Aquatic Organisms 128: 215-224.

Due in part to the uneven distribution of *Ichthyophonus* throughout host tissues, the comparative sensitivity and accuracy of using molecular-based detection methods versus culture to estimate parasite prevalence in wild populations is under debate. We evaluated the analytical and diagnostic performance of an existing quantitative polymerase chain reaction (qPCR) assay in comparison to the 'gold standard' culture method using Pacific herring with known exposure history in a controlled environment. We determined that the assay is suitable for use in this host, and diagnostic specificity was consistently high (>98%) in both heart and liver tissues. Diagnostic sensitivity could not be fully assessed due to low infection rates in *Ichthyophonus*-inoculated fish, but our results suggest that qPCR is not as sensitive as culture under all circumstances. Diagnostic sensitivity of qPCR relative to culture is likely affected by the amount of sample processed. The prevalence values estimated by the two methods were not significantly different when sample amounts were equal (heart tissue), but when assayed sample amounts were unequal (liver tissue), the culture method detected a significantly higher prevalence of the parasite than qPCR. The culture method can accommodate a larger piece of tissue than the qPCR method, however, culture of liver also detected significantly more *Ichthyophonus* infections than culture of heart, suggesting that the density and distribution of parasites in tissues also plays a role in assay sensitivity. This sensitivity issue would be most problematic for fish with

light infections. Although qPCR does not detect the presence of a live organism, DNA-based pathogen detection methods provide the opportunity for alternate testing strategies when culture is not possible.

8. Coordination/Collaboration:

A. Projects Within a Trustee Council-funded program

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.

Processed water samples for Dr. Bishop's herring tagging project (Project 18160111-B) to test for VHSV; none was detected.

Preliminary serum neutralization results, to assess herd immunity by quantifying VHSV neutralizing titer, were shared with Dr. Trevor Branch (Project 18120111-C). He is currently waiting to incorporate these results into his model until we provide the re-processed results using the more sensitive PNT assay (with exogenous complement).

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.

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

1) Phase 1: This feeding study was completed using age 1 (2017 cohort) herring.
2) Phase 2: This feeding and injection study was completed using adult herring (2015 cohort)
3) Phase 3: This definitive injection and feeding study is currently being constructed (Feb 2019); anticipated completion date is June 2019.

Histological processing is currently underway for Phase 1 and 2:

1) VEN-positive tissues from wild herring were collected to provide a source of inoculum for future controlled exposure studies. Several VEN studies were initiated to investigate the effect of herring age on susceptibility to VEN.
2) Began processing archived histology samples from PWS 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.

2. Across Programs

a. Gulf Watch Alaska

Dr. Maya Groner participated in an information exchange outreach event in Port Graham on May 15, 2018 with Gulf Watch Alaska scientists.

b. Data Management

Survey data were provided with metadata to Axiom.

c. Lingering Oil

As in-kind contributions to Dr. Andrew Whitehead's project, we successfully fertilized herring eggs from three different stocks (Prince William Sound, Sitka Sound, and Puget Sound). Quadruplicate groups of

fertilized eggs from each stock were exposed to five different concentrations of Alaska North Slope Crude oil (plus additional negative control groups that were not exposed to oil); various metrics of embryonic and newly hatched larval health and survival were assessed between stocks and oil concentrations. From each stock, larvae exposed to two different oil concentrations (plus the unexposed controls) were reared through metamorphosis in duplicate tanks (Figs. 14 and 15).

Figure 14. Footprint of the 18 grow-out tanks for oil-exposed herring, including duplicate tanks for unexposed, medium-low oil, and medium-high oil exposures for each stock (Prince William Sound, Sitka Sound, and Puget Sound).

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Figure 15. Oil-exposed specific pathogen-free Pacific herring in one of the 18 tanks depicted in Figure 4 (Sitka Sound, medium-high oil exposure).

These groups of fish have been used as test animals in a series of trials to compare their relative susceptibilities to VHS:

Trial #1: Determine the effects of Temperature (13.1 vs 8.8 °C) on the susceptibility of Oiled vs Unoiled Survivors

Treatments consisted of triplicate tanks, each tank contained approximately 75 fish / tank. Fish were subsampled from the triplicate tanks on days 0, 2, 4, 7, 21 d post exposure (n = 4 / day) for gene expression (results pending; Fig. 16).

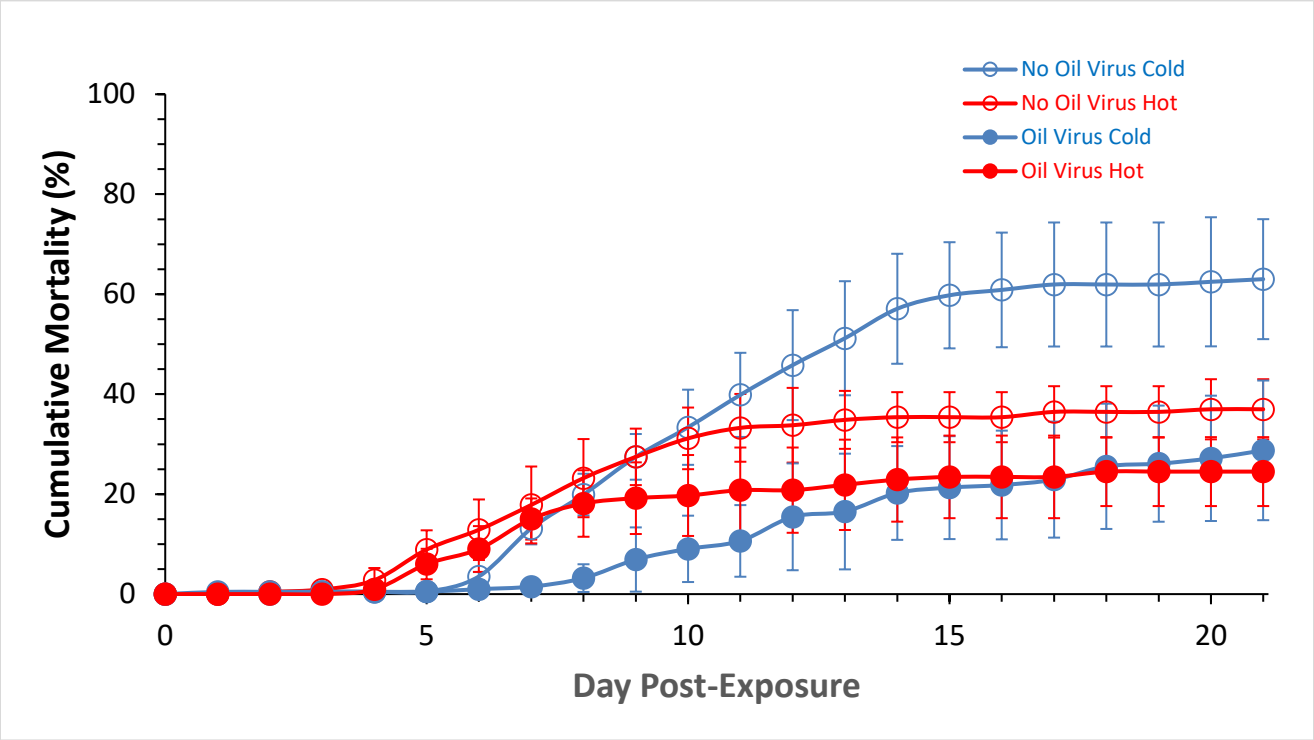


Figure 16. 1st Trial: Cumulative mortalities of herring that survived embryonic exposure to oil. The N's from each tank were adjusted for the subsamples that were removed on days 2, 4, 7, and 21 post exposure.

Results indicated lower VHS susceptibility among herring that survived early life stage exposure to oil. This effect was most pronounced at the colder temperature.

Trial #2: Repeat Trial #1 at cool temperature (8.5 ºC) and without subsampling.

This study was intended as a follow-up to the previous experiment, but we did not subsample during the experiment. One temperature (ambient), triplicate tanks / treatment, and N = 59 – 62 herring / tank (Fig. 17).

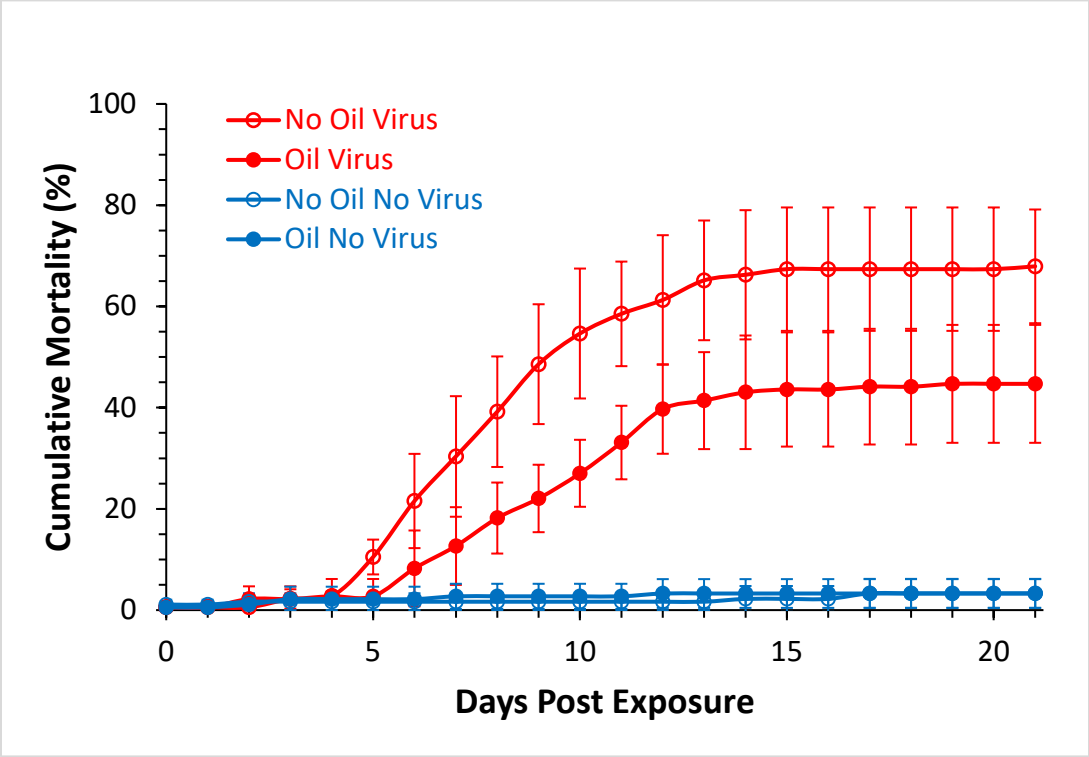


Figure 17. 2nd trial: Cumulative mortalities of herring that survived embryonic exposure to oil.

Results indicated lower VHS susceptibility among juvenile herring that survived early life stage exposure to oil.

Effects of multiple oil exposure levels on VHS susceptibility (7.6 ºC)

The previous two experiments provided evidence for decreased susceptibility to VHS after oil exposure. In both these previous experiments, we compared only two groups: unoiled controls vs survivors of a high oil treatment. However, in 2017, we reared 4 groups of herring, each with different oil exposure levels (no oil, low oil, medium oil, and high oil); the relative VHS susceptibilities in all 4 oil treatments were compared in triplicate during this study. Treatments consisted of triplicate tanks for each treatment, with each tank containing 58-62 herring (Fig. 18).

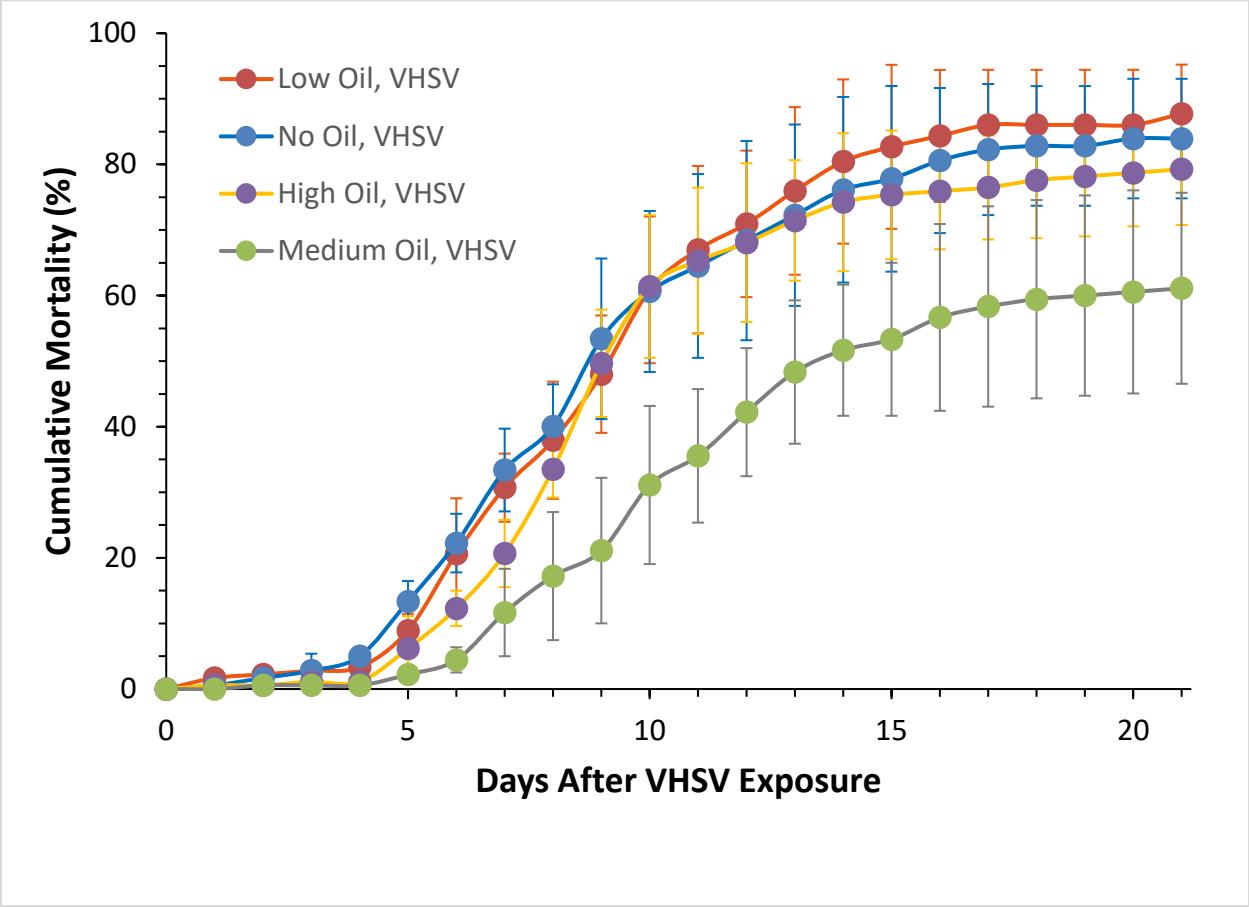


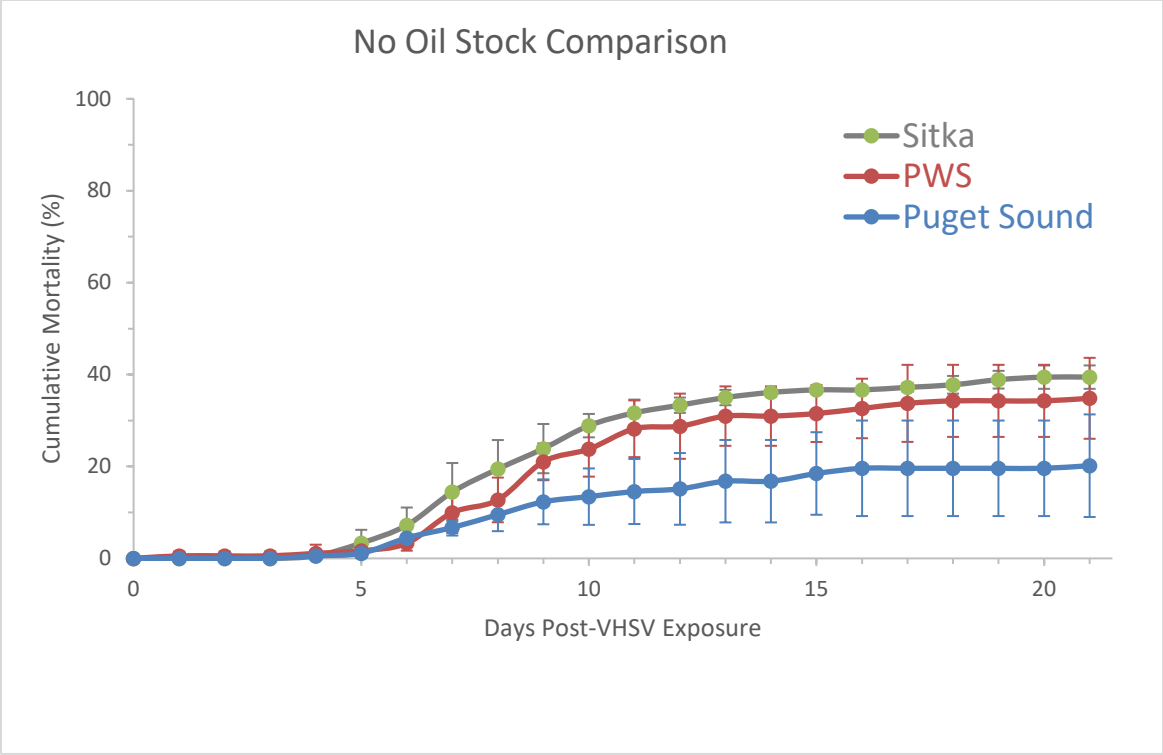
Figure 18. 3rd trial: Cumulative mortalities of herring that survived embryonic exposure to oil. Results from the negative controls, corresponding to each of the oil treatments but not exposed to VHSV, are not displayed (cumulative mortality in each negative control group was ≤ 6%).

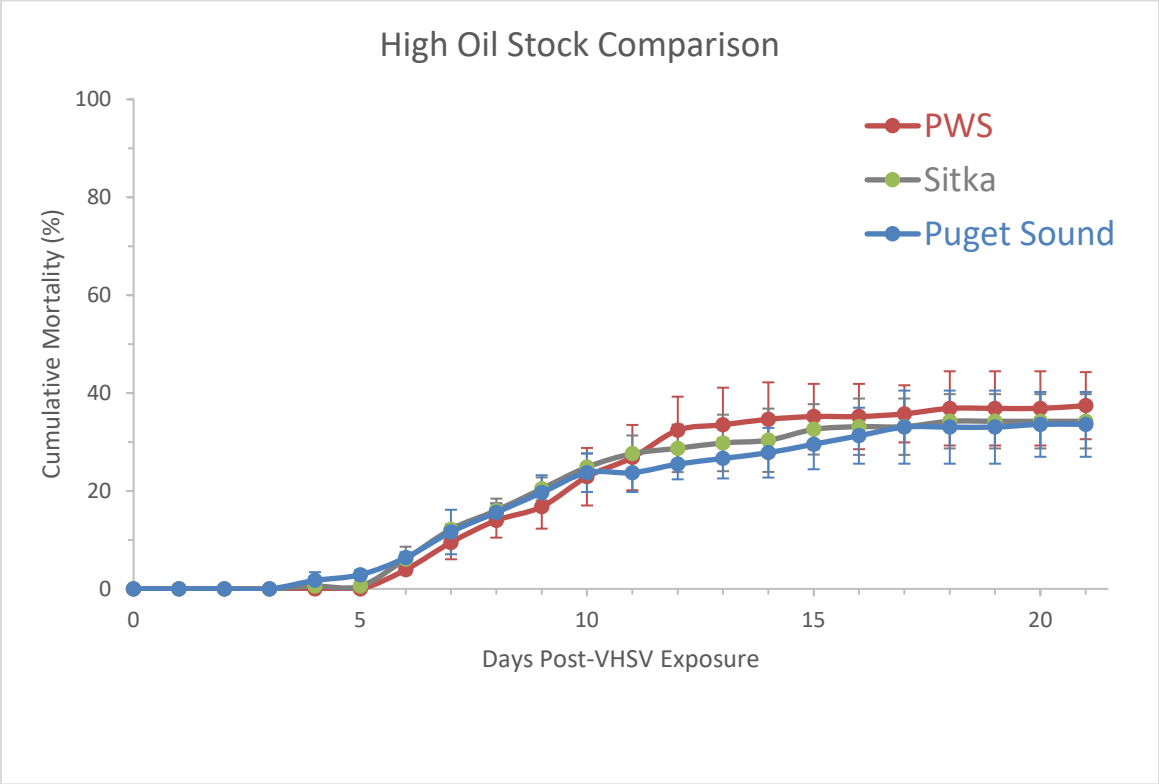
Results did not indicate a clear difference in VHS susceptibility between the oil treatment groups.

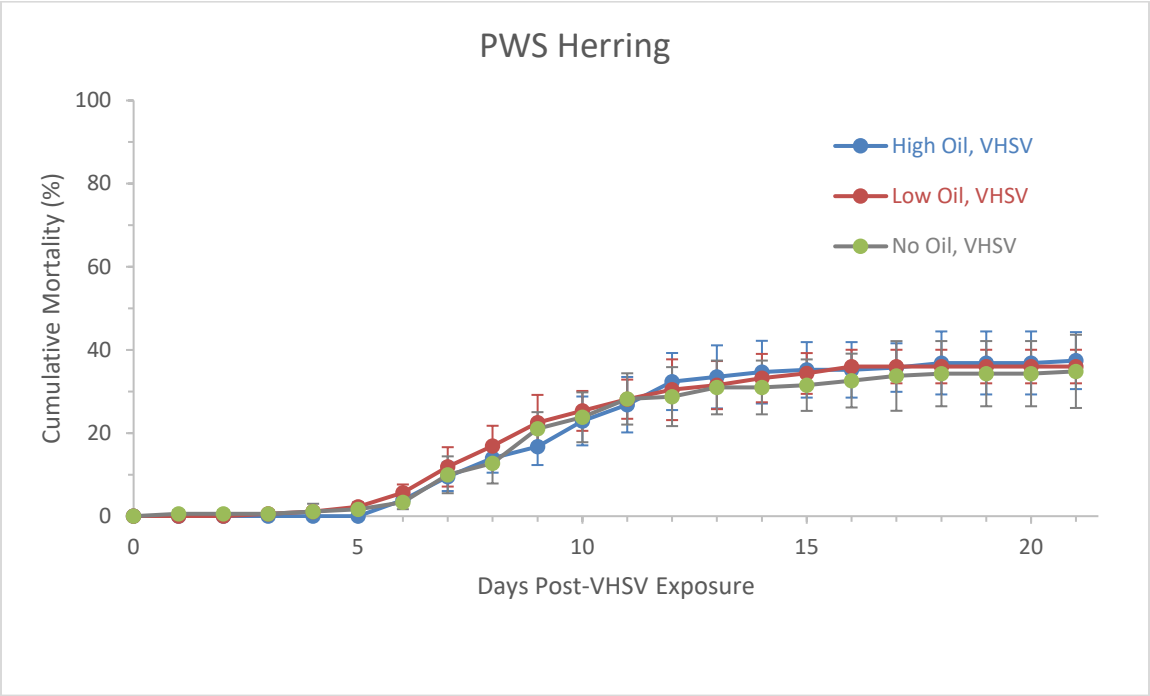
4th Trial: multiple stock / oil comparison at warm temperature (11.3 ^{0}C)

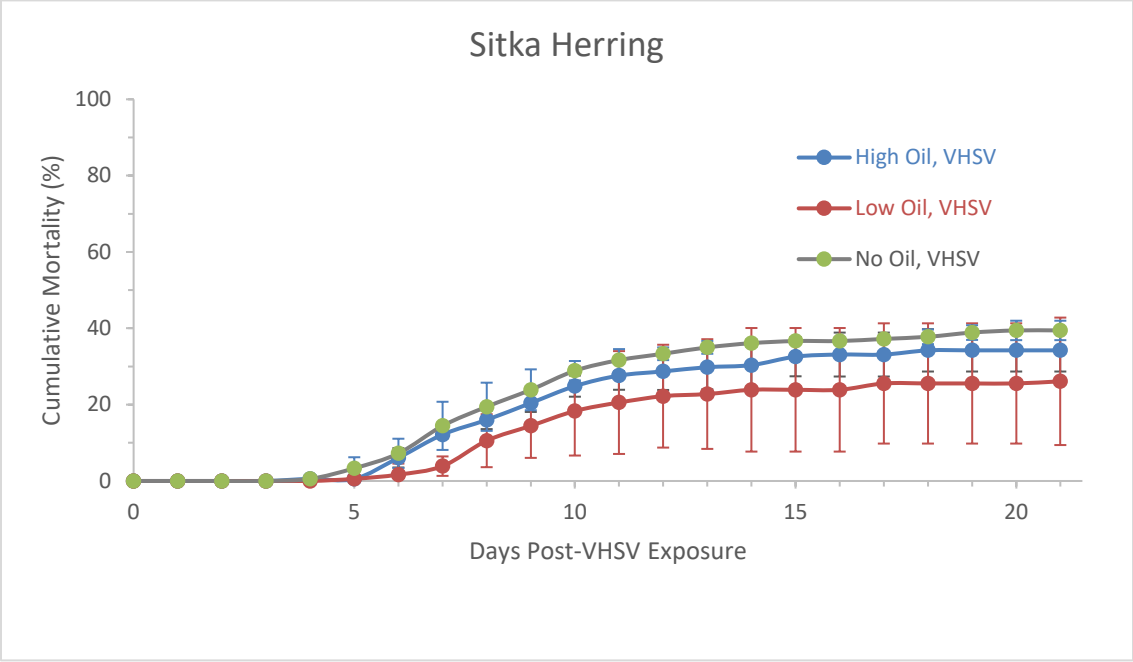
Trials #1-3 were performed using Puget Sound herring that survived embryonic exposure to oil (from oil-gravel columns) in 2017. In 2018, we expanded these studies to include multiple herring stocks (Prince William Sound, Sitka Sound, and Puget Sound) that survived embryonic exposure to oil (from the SINTEF generator) at each of 3 concentrations: (un-oiled controls - 0.02 ppm, medium-low oil - 0.24 ppm, and medium-high oil - 1.9 ppm). The relative susceptibilities of each of these treatments were evaluated in triplicate tanks, with each tank containing 60 herring. Tank layout is included below:

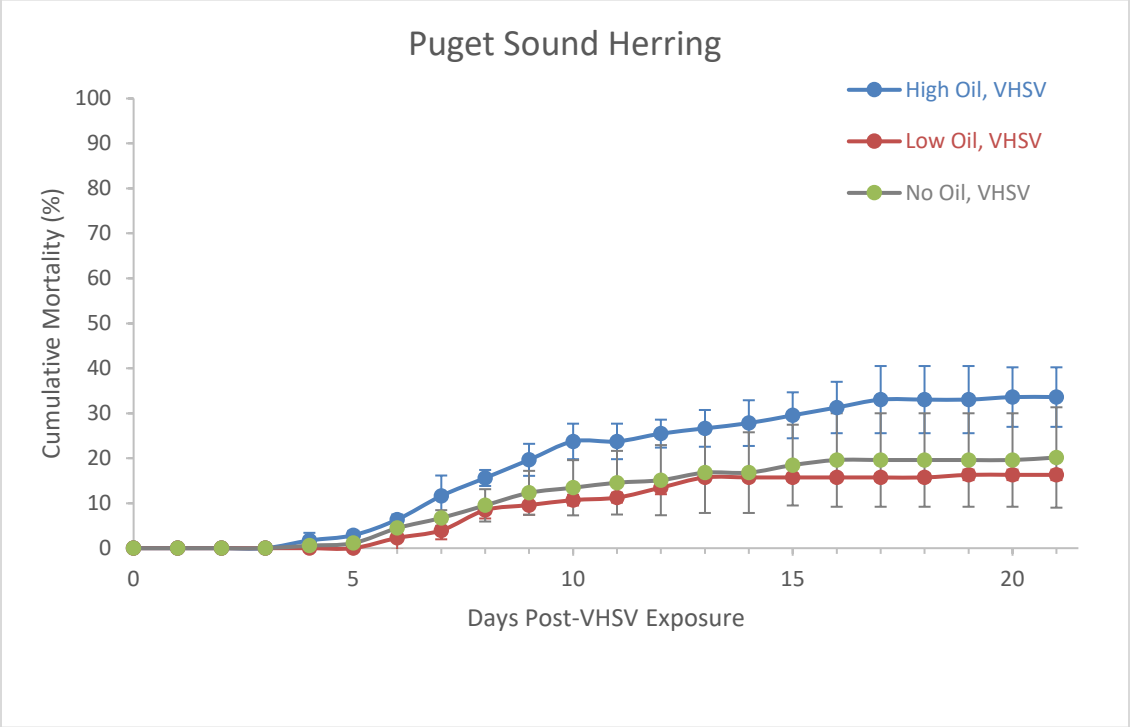
No Virus – Olympus Lab	Oil & Stock Treatment	Tank #
	Sitka Sound no-oil	301-303
	Sitka Sound medium-low oil	304-306
	Sitka Sound medium-high oil	307-309
	PWS no-oil	310-312
	PWS medium-low oil	313-315
	PWS medium-high oil	316-318
	Puget Sound no-oil	319-321
	Puget Sound medium-low oil	322-324
	Puget Sound medium-high oil	325-327
VHSV Exposure – Rainier Lab	Sitka Sound no-oil	207-209
	Sitka Sound medium-low oil	210-212
	Sitka Sound medium-high oil	213-215
	PWS no-oil	216-218
	PWS medium-low oil	219-221
	PWS medium-high oil	222-224
	Puget Sound no-oil	225-227
	Puget Sound medium-low oil	228-230
	Puget Sound medium-high oil	231-233




High Oil Stock Comparison
Cumulative Mortality (%)
Days Post-VHSV Exposure
PWS
Sitka
Puget Sound
0
20
40
60
80
100
0
5
10
15
20


PWS Herring
Cumulative Mortality (%)
Days Post-VHSV Exposure
High Oil, VHSV
Low Oil, VHSV
No Oil, VHSV
0
20
40
60
80
100
0
5
10
15
20





Results indicated no differences in VHS susceptibilities between various oil exposure concentrations, or between stocks. Note that these studies were performed at relatively warm temperature (11.4 ^{0}C). The results from Trial #1 (above) indicate that greater separation between groups may occur at cooler temperatures.

5th (definitive) Trial: multiple stock / oil comparison at cooler temperature

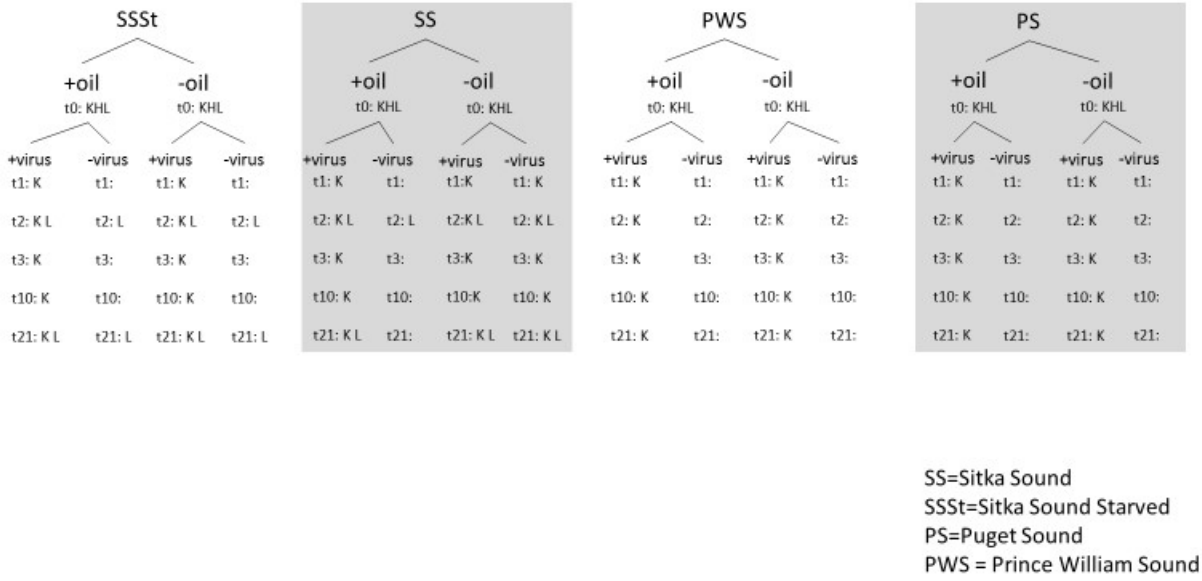
This will be a repeat of Trial #4 (above) but will be performed at cooler a temperature. Herring from each stock are the survivors of early life stage exposure to oil from the SINTEF generator at each of 3 concentrations: (un-oiled controls - 0.02 ppm, medium-low oil - 0.24 ppm, and medium-high oil - 1.9 ppm).

The relative susceptibilities of each of these treatments will be evaluated using triplicate tanks, with each tank containing 70-herring. Tank layout is depicted below:

No Virus – Olympus Lab	Oil & Stock Treatment	Tank #
	Sitka Sound no-oil	301-303
	Sitka Sound medium-low oil	304-306
	Sitka Sound medium-high oil	307-309
	PWS no-oil	310-312
	PWS medium-low oil	313-315
	PWS medium-high oil	316-318
	Puget Sound no-oil	319-321
	Puget Sound medium-low oil	322-324
	Puget Sound medium-high oil	325-327
VHSV Exposure – Rainier Lab	Sitka Sound no-oil	207-209
	Sitka Sound medium-low oil	210-212
	Sitka Sound medium-high oil	213-215
	PWS no-oil	216-218
	PWS medium-low oil	219-221
	PWS medium-high oil	222-224
	Puget Sound no-oil	225-227
	Puget Sound medium-low oil	228-230
	Puget Sound medium-high oil	231-233

VHSV exposure is projected to occur March 8, 2019. The current ambient water temperature at the Marrowstone Marine Field Station is 8.1 ^{0}C (as of Feb 14, 2018). The temperature is expected to warm slightly by the anticipated experimental start date.

Subsampling scheme:



SS=Sitka Sound
SSSt=Sitka Sound Starved
PS=Puget Sound
PWS = Prince William Sound

Day 0		
	OIL	No OIL
SSSt	K H L: 3 per tank (n=6)	K H L: 3 per tank (n=6)
SS	K H L: 3 per tank (n=6)	K H L: 3 per tank (n=6)
PWS	K H L: 3 per tank (n=6)	K H L: 3 per tank (n=6)
PS	K H L: 3 per tank (n=6)	K H L: 3 per tank (n=6)

kidney: virus challenge
heart: oil injury
liver: starvation challenge

Day 3				
	OIL		No OIL	
	Virus	No Virus	Virus	No Virus
SSSt	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
SS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	K: 2 per tank (n=6)
PWS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
PS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	

Day 1				
	OIL		No OIL	
	Virus	No Virus	Virus	No Virus
SSSt	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
SS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	K: 2 per tank (n=6)
PWS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
PS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	

Day 10				
	OIL		No OIL	
	Virus	No Virus	Virus	No Virus
SSSt	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
SS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	K: 2 per tank (n=6)
PWS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
PS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	

Day 2				
	OIL		No OIL	
	Virus	No Virus	Virus	No Virus
SSSt	K: 2 per tank (n=6)	L: 2 per tank (n=6)	K: 2 per tank (n=6)	L: 2 per tank (n=6)
SS	K: 2 per tank (n=6)	L: 2 per tank (n=6)	K: 2 per tank (n=6)	K L: 2 per tank (n=6)
PWS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
PS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	

Day 21				
	OIL		No OIL	
	Virus	No Virus	Virus	No Virus
SSSt	K: 2 per tank (n=6)	L: 2 per tank (n=6)	K: 2 per tank (n=6)	L: 2 per tank (n=6)
SS	K: 2 per tank (n=6)	L: 2 per tank (n=6)	K: 2 per tank (n=6)	K L: 2 per tank (n=6)
PWS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	
PS	K: 2 per tank (n=6)		K: 2 per tank (n=6)	

B. Projects not Within a Trustee Council-funded program

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 North Pacific Research Board (# 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 North Pacific Research Board 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.

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: *See*, Reporting Policy at III (C) (9).

A. Publications Produced During the Reporting Period

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.

Harris, B.P., S.R. Webster, J.L. Gregg, P.K. Hershberger. 2018. *Ichthyophonus* in sport-caught groundfishes from southcentral Alaska. Diseases of Aquatic Organisms 128: 169-173.

Lowe, V.C., P.K. Hershberger, C.S. Friedman. 2018. Analytical and diagnostic performance of a qPCR assay for *Ichthyophonus* spp. compared to the tissue explant culture 'gold standard'. Diseases of Aquatic Organisms 128: 215-224.

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

Scientific Presentations

Groner, M., E. Bravo, C. Conway, J. Gregg, P. Hershberger. January 28-31, 2019. A quantitative histological index to differentiate between endemic and epidemic ichtyhophoniasis in Pacific herring. Alaska Marine Science Symposium. Anchorage, AK.

Wendt, C., P. Hershberger, C. Wood. January 28-31, 2019. Patterns of *Ichthyophonus* sp. infection in age zero Pacific herring. Alaska Marine Science Symposium. Anchorage, AK.

Cypher, A.D., P. Hershberger, N. Scholz, J.P. Incardona. January 3-7, 2019. 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.

Bravo, E., C. Conway, P. Hershberger, J. Gregg, M. Groner. October 11-13, 2018. Poster. Do histological analyses of herring infected with *Ichthyophonus* sp. suggest a shift from endemic to epidemic disease? Society for the Advancement of Chicanos / Hispanics and Native Americans in Science. San Antonio, TX.

Invited Presentations

P. Hershberger. December 6, 2018. Causes of Pacific Herring Mortality: A Disease Perspective Prince William Sound Regional Citizens Advisory Council, Annual Science Night.

P. Hershberger. May 24, 2018. The Ecology of Disease in Marine Fishes: Insights from Pacific Herring. NOAA – Northwest Fisheries Science Center, Monster Seminar Jam

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

All field surveillance results have been provided to Axiom and are available on the GOA Data Portal.

10. Response to EVOSTC Review, Recommendations and Comments:

Science Panel Comments:

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 now post-doc and this project.

PI Response:

We have integrated Dr. Groner's work into the herring disease program. As we anticipated, her contributions have been beneficial to the herring disease program, the Herring Research Program as a whole, and to her scientific career.

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	$252.4
Travel	$20.1	$20.1	$20.1	$20.1	$20.1	$100.5	$20.9
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	$67.6
Equipment	$0.0	$0.0	$0.0	$0.0	$20.9	$20.9	$4
SUBTOTAL	$181.5	$210.0	$217.2	$223.2	$251.3	$1,083.2	$344.4
General Administration (9% of subtotal)	$16.3	$18.9	$19.5	$20.1	$22.6	$97.5	$35.2
PROJECT TOTAL	$197.8	$228.9	$236.7	$243.3	$273.9	$1,180.7	$379.6
Other Resources (Cost Share Funds)	$61.7	$63.6	$64.0	$65.2	$66.9	$321.4	

Literature Cited:

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.