



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

Project Number: 23120111-E

Project Title: Herring Disease Program

Principal Investigator(s): Paul Hershberger and David Paez, U.S. Geological Survey, Western Fisheries Research Center, Marrowstone Marine Field Station

Reporting Period: February 1, 2023 – January 31, 2024

Submission Date: March 1, 2024

Project Website: <https://gulfwatchalaska.org/>

Please check all the boxes that apply to the current reporting period.

Project progress is on schedule.

Project progress is delayed.

Budget reallocation request.

Personnel changes.

This draft report is distributed solely for purposes of scientific peer review. Its content is deliberative and predecisional, so it must not be disclosed or released by reviewers. Because the report has not yet been approved for publication by the U.S. Geological Survey (USGS), it does not represent any official USGS finding or policy.

1. Summary of Work Performed:

Field Sampling

Prince William Sound Pre-spawn Adult Herring

Pacific herring (*Clupea pallasii*) were collected from three sites within Prince William Sound (PWS), Alaska and Kayak Island (Table 1) during the spring pre-spawn period from March 28-31, 2023, to test for viral hemorrhagic septicemia virus (VHSV), viral erythrocytic necrosis



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

(VEN), and *Ichthyophonus* prevalence (Table 1). *Ichthyophonus* was detected in 26% (46/177) of heart cultures from all sites combined and from 10% (6/60) of samples from Kayak Island. An inverted pattern of decreasing *Ichthyophonus* infection prevalence with age started around 2018 and continued through 2023 (Fig. 1). VHSV was isolated from three pooled samples from fish collected in Port Etches. Although the isolations were at very low viral titers, VHSV detections have been rare in most prior years. Neutralizing antibodies to VHSV were detected in 4% (15/377) of PWS herring in 2023 (Fig. 2). Erythrocytic inclusions indicative of VEN were not detected in any PWS herring (n=180) from 2023, but bacterial rods were noted in the blood films from three fish.

Table 1. Infection prevalence results from Prince William Sound pre-spawn herring in 2023. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	<i>Ichthyophonus</i> Prevalence (Heart Cultures)	VEN Prevalence ^B
Windy Bay	March 28	0% (n=60)	23% (14/60)	0% (0/60)
Port Gravina	March 29	0% (n=60)	18% (11/60)	0% (0/60)
Port Etches	March 31	3-25% (3/10 pools) ^A	37% (21/57)	0% (0/60)
Kayak Isl.	April 20	0% (0/60)	10% (6/60)	0% (0/60)

^AVHSV was detected at low levels in 3 pools of samples (4 or 5 fish pools). Pooled samples were positive for virus on *epithelioma papulosum cyprini* cells after 8-11 days at 14.7°C with a blind-passage for 14 days. In some cases, only ½ paired dilutions developed cytopathic effect (CPE) during the first passage requiring a second passage for CPE to develop in the second dilution. The minimum level of detection was 50 infectious particles /g of pooled tissue sample. Polyethylene glycol was used to increase cell culture sensitivity. Two isolates were selected and confirmed as VHSV by polymerase chain reaction.

^BBacterial rods were detected in 3 blood films.



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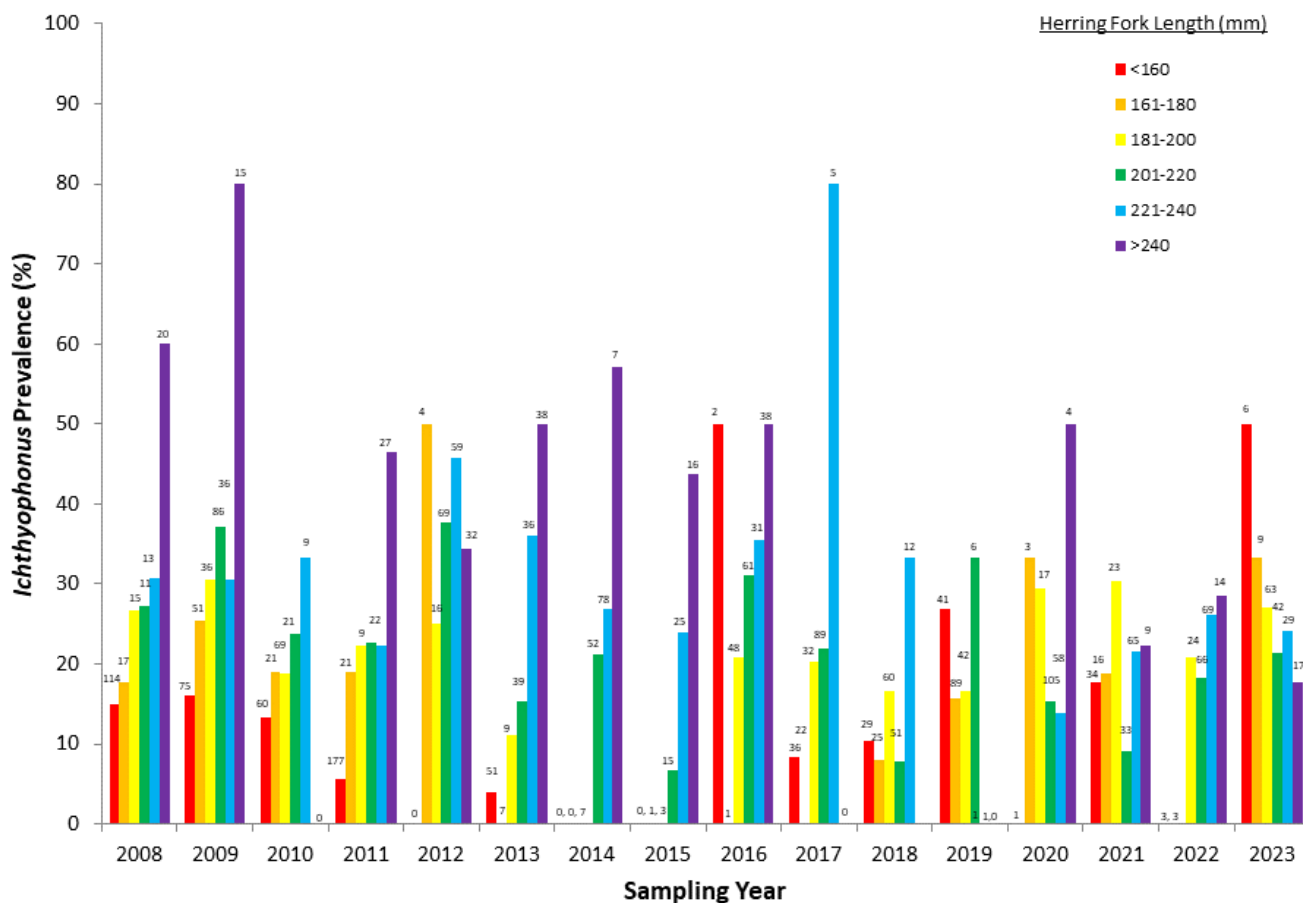


Figure 1. Temporal trend in *Ichthyophonus* infection prevalence in each size class of Prince William Sound herring. Numerals above each bar indicate sample size (n). Data do not include samples from Kayak Island.



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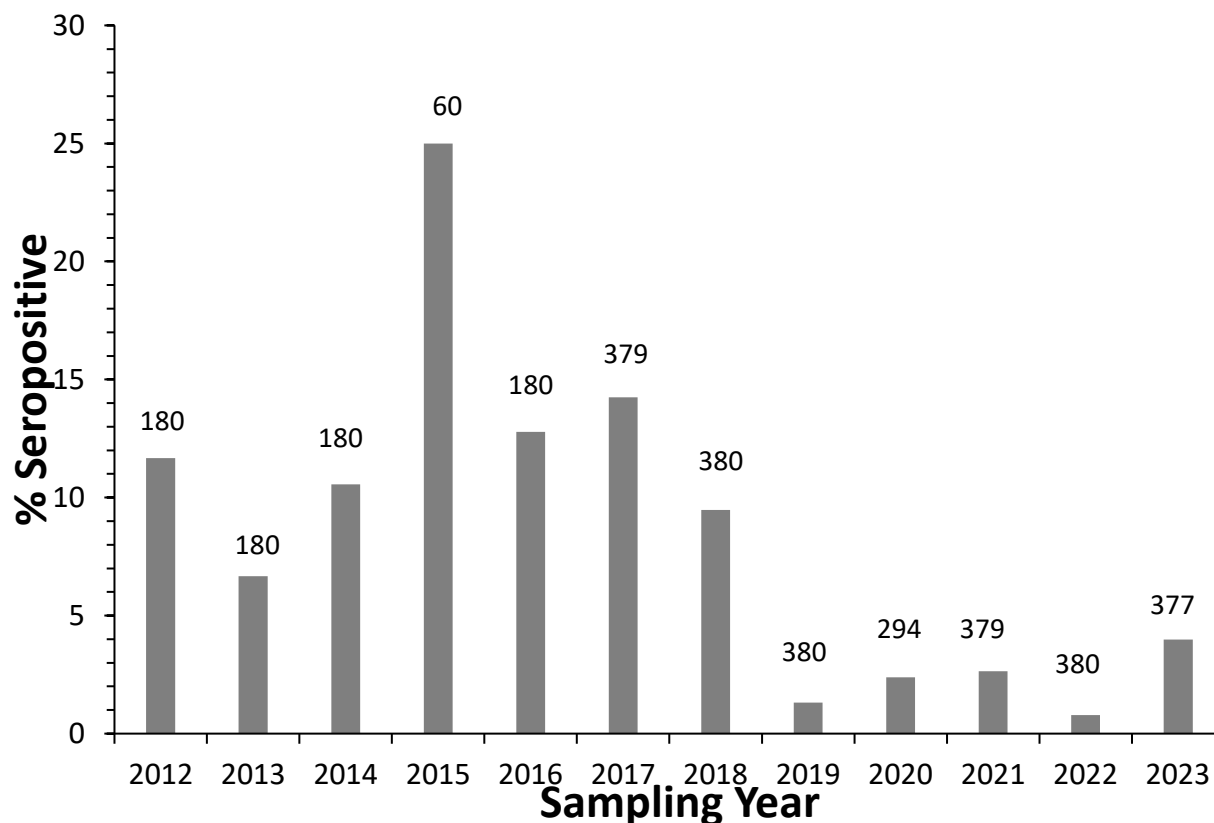


Figure 2. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Prince William Sound herring. Numerals above the bars indicate the sample size “n”. Age data were not yet available at the time of this report writing. Data from Kayak Island were not included, where 1/60 samples tested positive for neutralizing antibodies to viral hemorrhagic septicemia virus.

Sitka Sound Pre-spawn Adult Herring

Adult Pacific herring were collected from three sites in Sitka Sound, Alaska during the spring pre-spawn period from March 28-30, 2023, to test for VHSV, VEN, and *Ichthyophonus* prevalence (Table 2, Fig. 3). *Ichthyophonus* was detected in 27% (48/178) of herring hearts. Neither VHSV nor VEN were detected in any samples (n = 180 each). Neutralizing antibodies to VHSV were detected in only 1% (2/180) of herring plasma samples (Fig. 4). As with PWS, VHSV neutralizing antibody levels have been relatively low in Sitka Sound for the past five



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

years (2019-2023), inferring a paucity of VHSV exposures during this period and a current population of adult herring that remains susceptible to the resulting disease.

Table 2. Infection prevalence results from Sitka Sound pre-spawn herring in 2022. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	<i>Ichthyophonus</i> Prevalence (Heart Cultures)	VEN Prevalence
Deep Bay	March 28	0% (n=60)	30% (18/60)	0% (n=60)
Unknown	March 29	0% (n=60)	31% (18/59)	0% (n=60)
S. Frosty Reef	March 30	0% (n=60)	20% (12/59)	0% (n=60)



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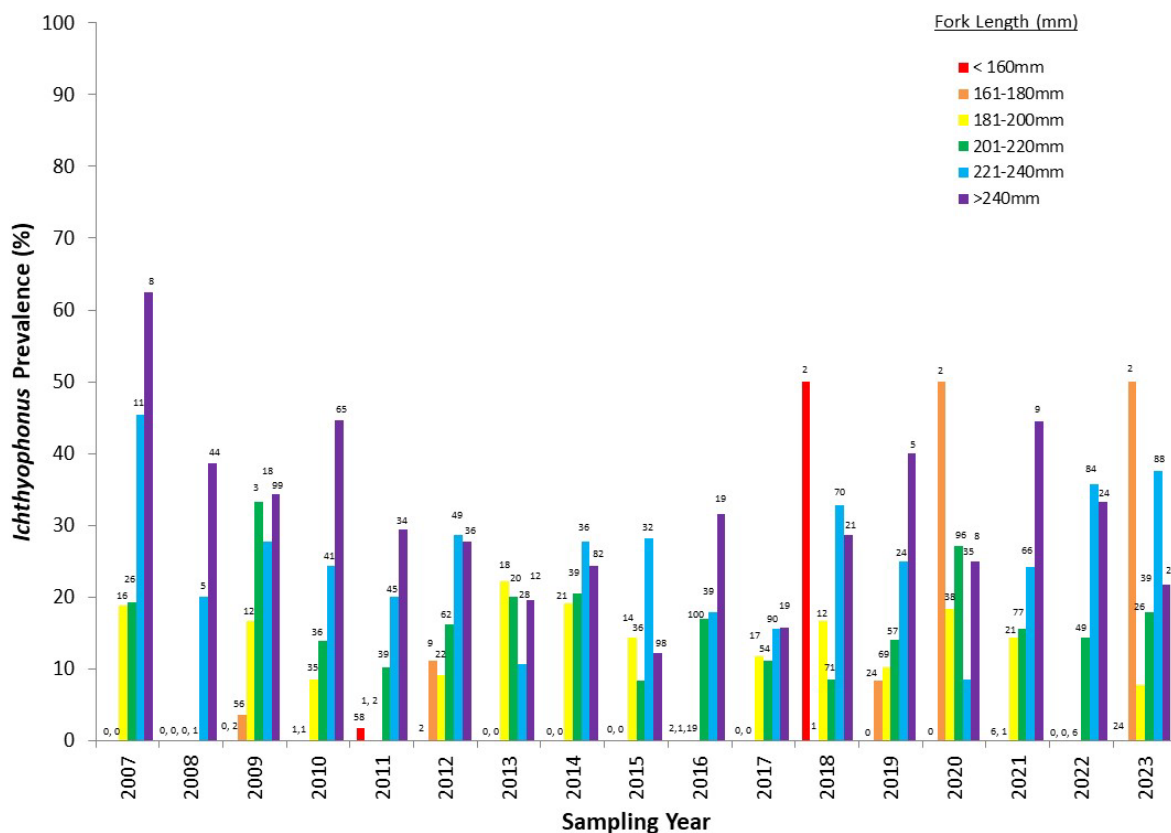


Figure 3. Temporal trend in *Ichthyophonus* infection prevalence in each size class of Sitka Sound herring. Numerals above each bar indicate 'n'.



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Annual Project Reporting Form

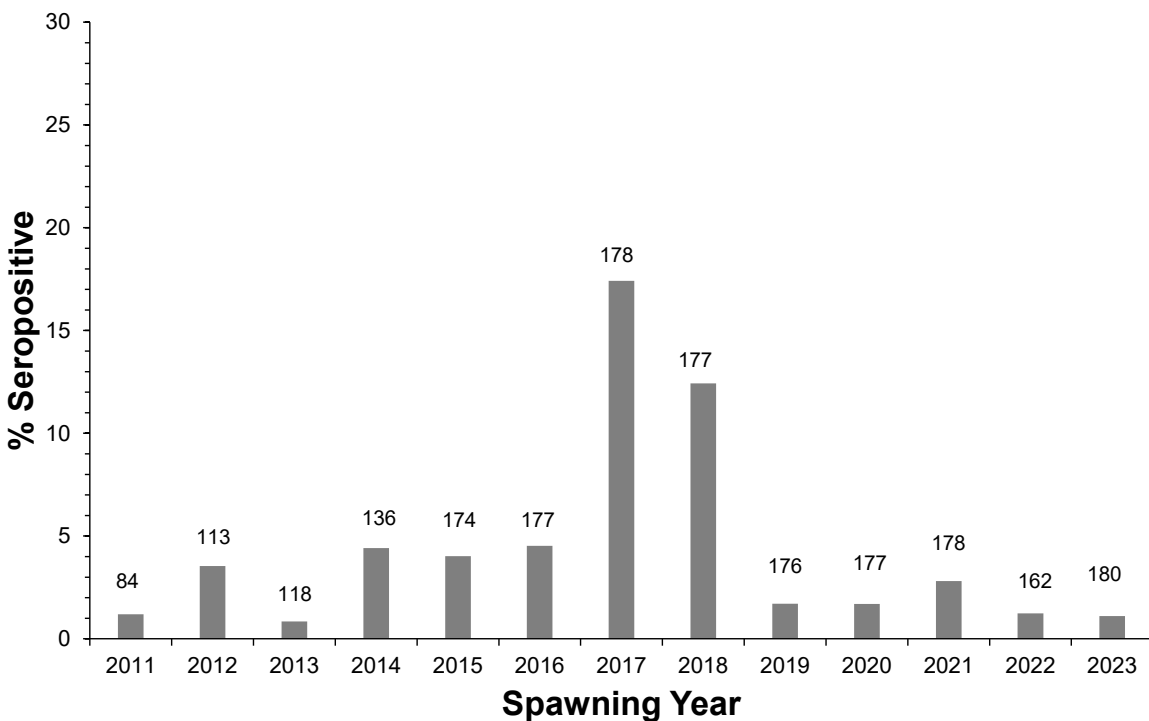


Figure 4. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Sitka Sound herring. Numerals above the bars indicate the sample size “n”.

American shad (Alosa sapidissima)

As an introduced member of the forage fish assemblage in the Northeast Pacific Ocean, American shad compete with Pacific herring for food and other resources. After their introduction to the west coast, shad have become a host for *Ichthyophonus*, and shad are known to traffic the parasite into previously naïve freshwater areas, including the Columbia River, where 33-72% of returning adults are infected. During 2023, we had an opportunity to sample age-1 juvenile shad (n=44) from Lake Washington, a watershed that feeds into Puget Sound. Typically, American shad out-migrate from freshwater at age 0, so there was some question whether these age-1 fish in Lake Washington were demonstrating a variant life history pattern involving freshwater holdover. Preliminary stable isotope analysis supported this hypothesis (data provisional and not presented). *Ichthyophonus* surveillance data further supported this hypothesis, because 0% (0/44) were positive for the parasite, suggesting that they had never



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

ventured into Puget Sound, where they would have become infected with this marine parasite. These negative results from the freshwater phenotype of age-1 shad in Lake Washington contrasts with the typical seawater phenotype of age 1 juvenile shad in Puget Sound, where *Ichthyophonus* was detected in 60% (6/10) during a 2004 survey.

Laboratory Studies

Swim Performance

Herring were exposed to VHSV and exercised in a swim flume to evaluate any long-term impacts of the infection on swimming performance. Preliminary analyses indicate that performance was negatively impacted for weeks after the fish recovered from the disease and the virus was longer be isolated from the survivors. We are finishing the histopathology for and anticipate manuscript complete analysis and manuscript submission by June 2024.

Further, herring were exposed to erythrocytic necrosis virus (ENV), the causative agent of VEN, in an analogous experiment to assess impacts of the resulting disease on swim performance. The experiment was terminated February 28, 2024, as this report was being finalized; results will be presented in the FY24 annual report.

Comparisons of temporal-, environmental-, and demographic- correlates of Ichthyophonus sp. infections in mature Pacific herring between PWS and Sitka Sound

Analysis of pathogen surveillance and population assessment datasets spanning 2007-2019 indicated that the age-based prevalence estimate of *Ichthyophonus* infection was, on average, 54% greater in PWS (a collapsed herring population), compared to a Sitka Sound (a relatively robust herring population). During the study years, the age-based infection prevalence ranged from 14-44% in PWS and 5-33% in Sitka Sound (Fig. 5). At both sites, the age-based infection prevalence declined over time, with an average decrease of 7% per year. Statistical analyses indicated that infection prevalence between the two populations was reduced by regional factors affecting both sites, and that these factors were independent of herring density. Infection prevalence in both populations was positively correlated with herring age and negatively correlated with the Pacific Decadal Oscillation.

As with other Pacific herring populations, there was a strong positive correlation between infection prevalence and host age in both study populations. Indeed, the slope of this relationship was remarkably similar across year classes, with a few notable exceptions, which may suggest similar prognosis for infected fish over time. For diseases such as ichthyophoniasis, where



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

recovery may not occur and herring can persist for long periods with infections, these positive correlations are typically attributed to conditions when transmission rates exceed disease mortality rates. Negative correlations between infection prevalence and host age may occur when recent natural mortality or disease mortality rates approach or exceed transmission rates. In Atlantic herring (*Clupea harengus*), negative correlations between infection prevalence and host age have occurred during ichthyophoniiasis epizootics associated with substantial host mortality. Interestingly, negative correlations between age-and infection prevalence occurred in the Prince William Sound stock in 2016-2019 for herring year classes 2011-2014 and flat age-prevalence relationships occurred in the Sitka Stocks between 2015 and 2017 for the 2010-2012 year classes (Fig. 6). Though the cause of these correlations cannot be conclusively demonstrated in this study, it is noteworthy that these herring would have experienced the extreme warm temperatures associated with the Pacific heatwave which arrived in the Gulf of Alaska in 2015 and 2016.



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

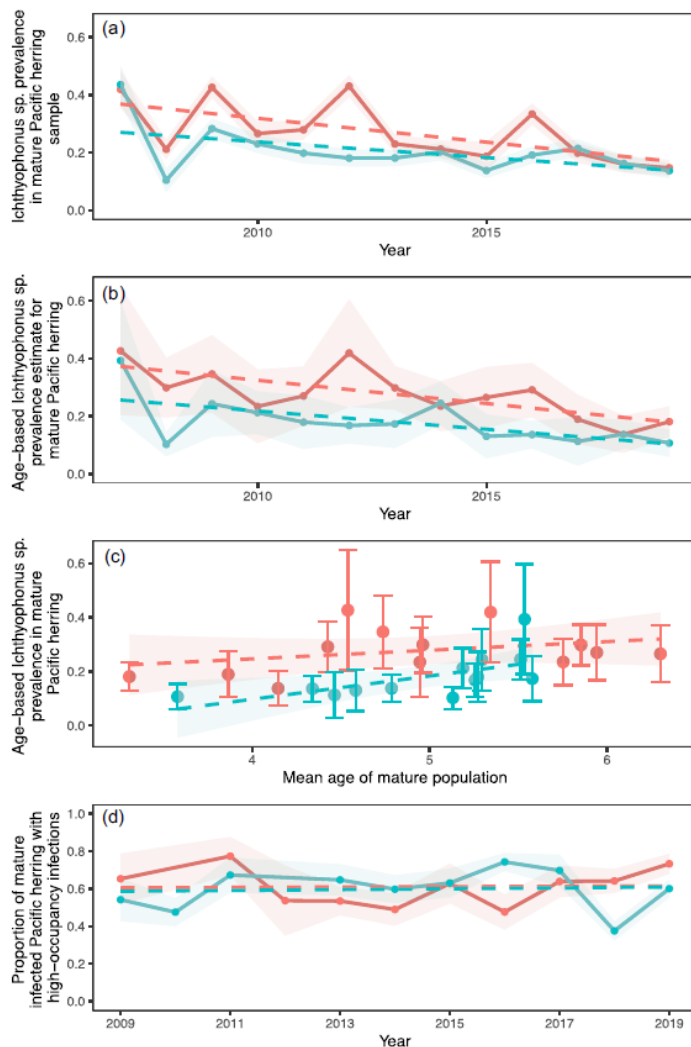


Figure 5. Age-based estimates of *Ichthyophonus sp.* infections in mature Pacific herring from Prince William Sound (pink) and Sitka Sound (blue). (A) Sample infection prevalence for the disease surveillances conducted from 2007-2019, (B) age-based estimate of yearly infection prevalence for the mature Pacific herring population from 2007-2019, (C) age-based infection prevalence as a function of mean age, and (D) the proportion of the infected population with high *Ichthyophonus sp.* parasite densities. Shading indicates the standard error. Straight dashed lines are linear models fit to the data.



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

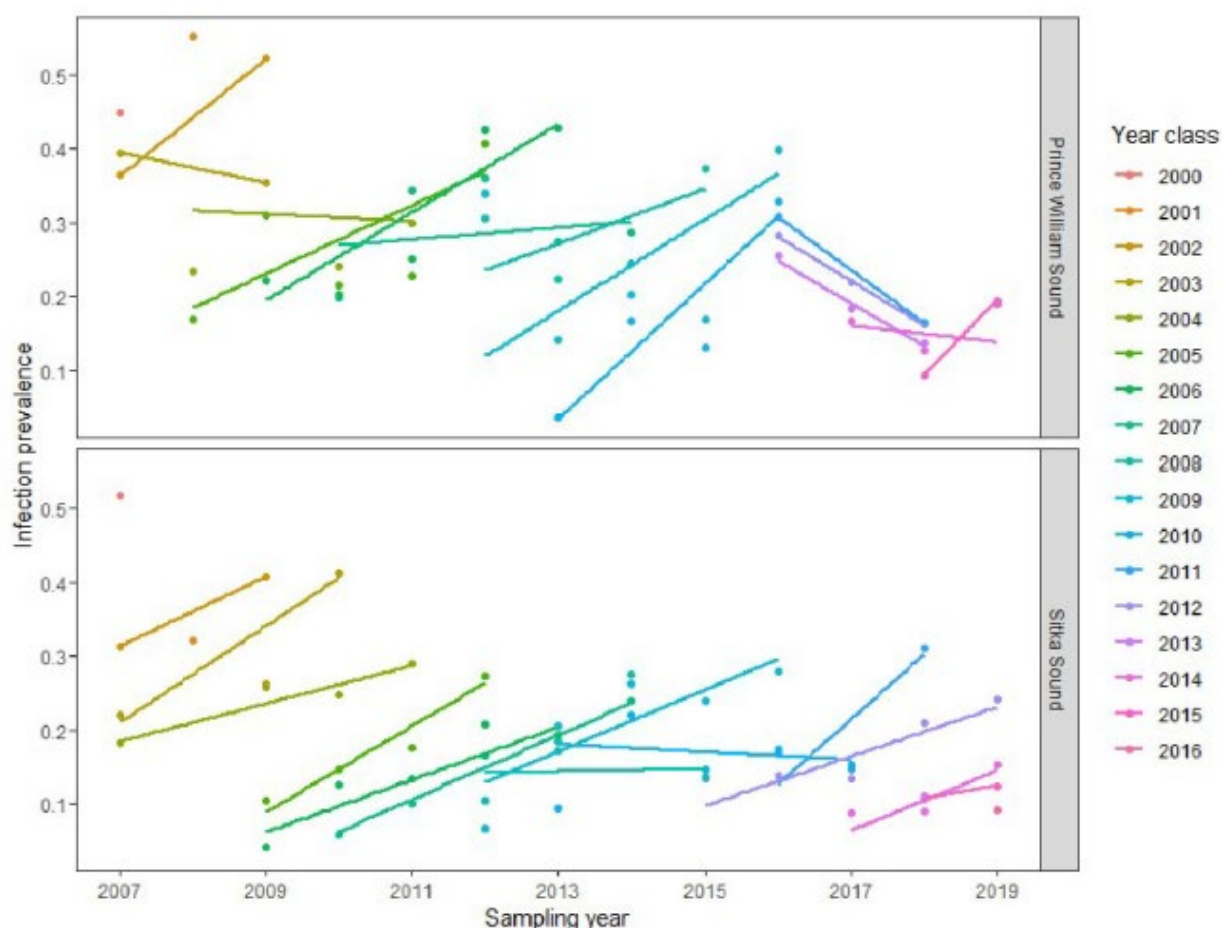


Figure 6. Patterns of *Ichthyophonus* sp. infection sample prevalence over time and age in Pacific Herring year-classes. Weighted linear regressions show trends in infection prevalence over time for each year-class, while dots represent the infection prevalence of a year class in a specific year (averaged across all sample sets in that year). Note that regressions could not be run for year-classes with data for less than three timepoints.

Characteristics of a sea louse (Caligus clemensi) epizootic in wild Pacific herring

We characterized a natural sea louse epizootic of *Caligus clemensi* and the effects of parasitism on Pacific herring *Clupea pallasii* in Port Angeles Harbor, Washington. Infestation started within



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

days after larval metamorphosis to juveniles and prevalence on newly metamorphosed age-0 Pacific herring reached 100% prevalence by mid-August (Figs. 7 and 8). At this time, the mean louse intensity was 4.6 lice / fish, and a positive correlation occurred between louse intensity and herring body length. The epizootic then waned, with infestation prevalence decreasing to less than 25% and the mean parasite intensity falling below 1 louse. While skin injuries were not detected, motile lice preferentially aggregated around head and anterior dorsal areas. However, louse tropism became evenly distributed over the body as the parasite intensity increased. Louse-induced mortality in herring was negligible in controlled experiments. These results indicate that *C. clemensi* epizootics reach high prevalence, but also fade from mid-summer to early fall. Due to the predominant presence of motile copepod stages, it is likely that the epizootic faded because lice completed their life cycle and dislodged from the host. Although the epizootic was documented only in a single harbor, we have observed similar outbreaks in herring from other location, including Cordova Harbor.

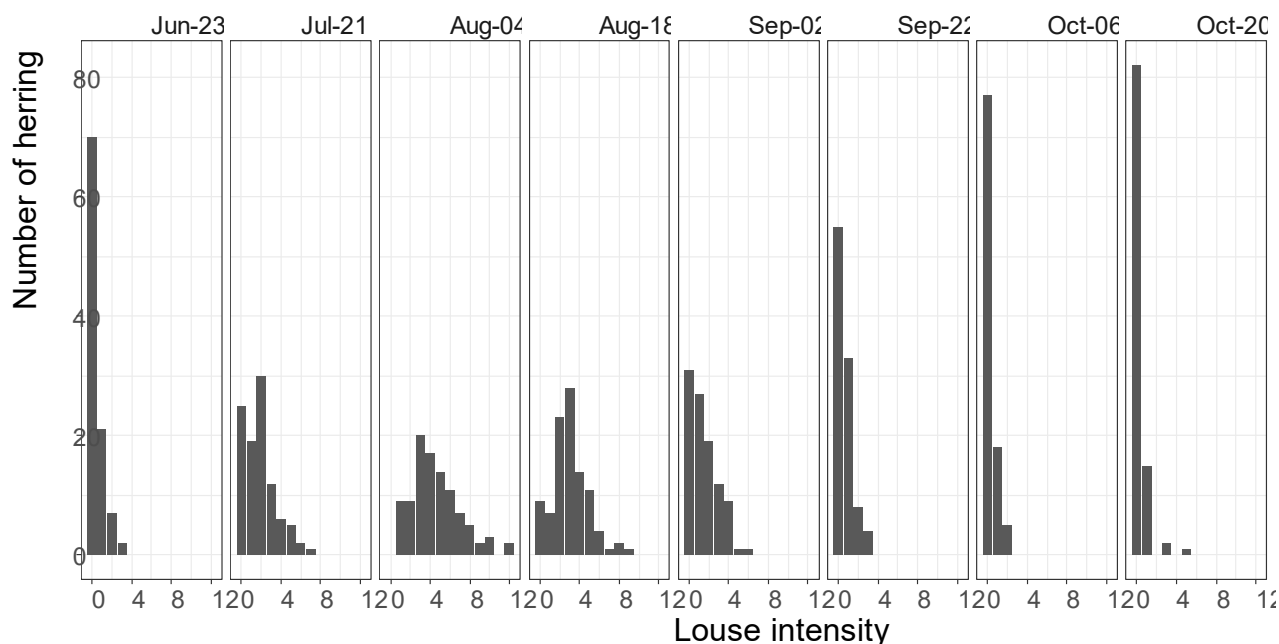


Figure 7. Variation in *C. clemensi* (louse) intensity on Pacific herring sampled from Port Angeles Harbor, Washington, over 8 time points. 100 herring were sampled at each timepoint.



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Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

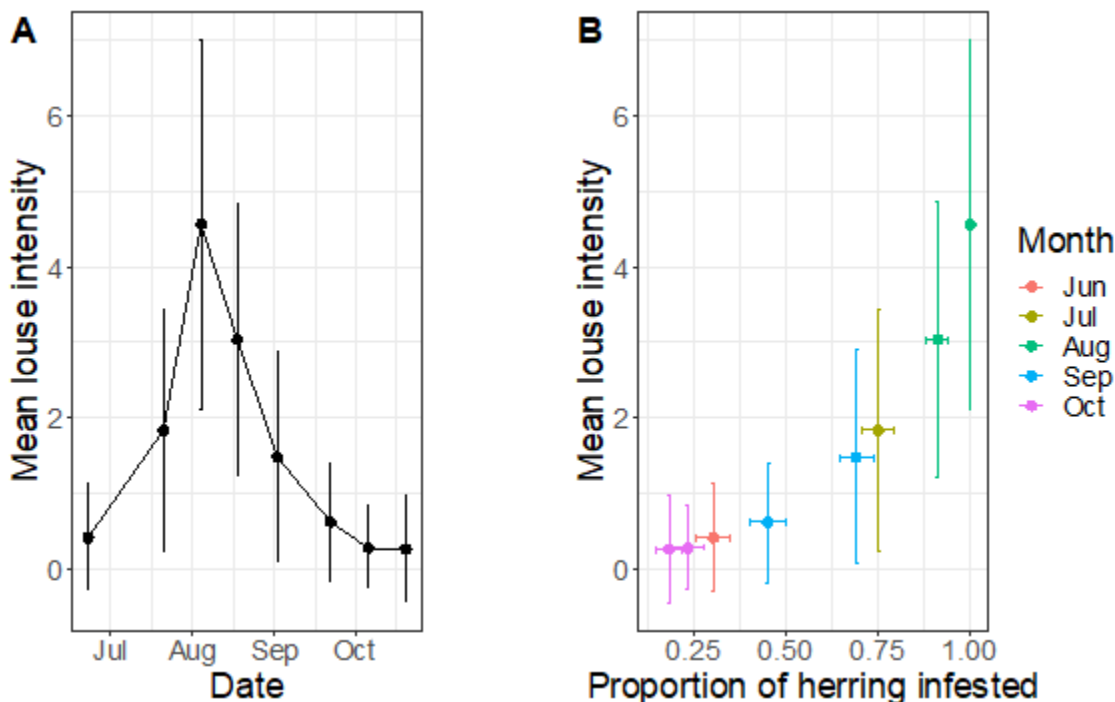


Figure 8. A) Mean *C. clemensi* (louse) intensity on Pacific herring as a function of sampling date. B) Louse intensity as a function of infestation prevalence at the different sampling time points. In both panels the dots are the mean intensity value whereas the error bars extend to plus and minus one standard deviation.

The developing heart of Pacific herring is responsive to crude oil at exposures below the limits of quantitation for PAHs in water and tissue

Herring embryos are highly susceptible to toxicity from oil spills and to chemicals leaching from oil stranded in intertidal and subtidal zones. The water-soluble components of crude oil trigger an adverse outcome pathway that involves disruptions to the physiological functions of cardiomyocytes in the embryonic herring heart. In previous studies, impaired ionoregulation (calcium and potassium cycling) in response to specific polycyclic aromatic hydrocarbons (PAHs) corresponds to lethal embryo-larval heart failure or subtle chamber malformations at the high and low ends of the PAH exposure range, respectively. Sublethal cardiotoxicity, which involves an abnormal outgrowth (ballooning) of the cardiac ventricular chamber soon after hatching, subsequently compromises juvenile heart structure and function, leading to



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Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

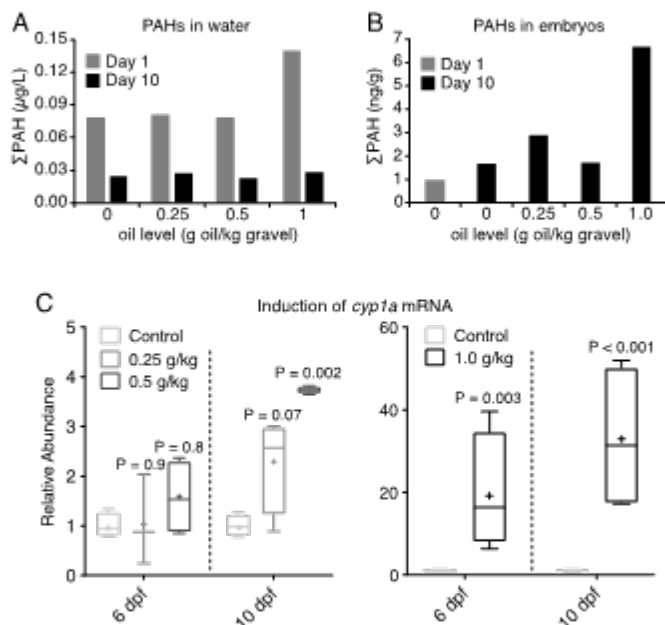


Figure 10. Σ Polyaromatic hydrocarbon (PAH) measures related to induction of *cyp1a* mRNA in Pacific herring embryos. (A) Σ PAH measures ($\mu\text{g/L}$) in effluent water at start of exposure (Day 1, grey bars) and at end exposure (Day 10, black bars). (B) Σ PAH measures (ng/g wet weight) in embryos at start of exposure (Day 1, grey bar) and at end exposure (Day 10, black bars). (C) Relative abundance (fold-change) of *cyp1a* mRNA measured by QPCR. Left plot shows values from lower dosing levels at 6 days post-fertilization (dpf) and 10 dpf (5 and 9 days of exposure, respectively), right plot shows values from the 1.0 g/kg dosing levels. Box and whiskers plots show the minimum and maximum values (whiskers), 25th to 75th percentile (box), median (line crossing box) and mean (plus sign) values.



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

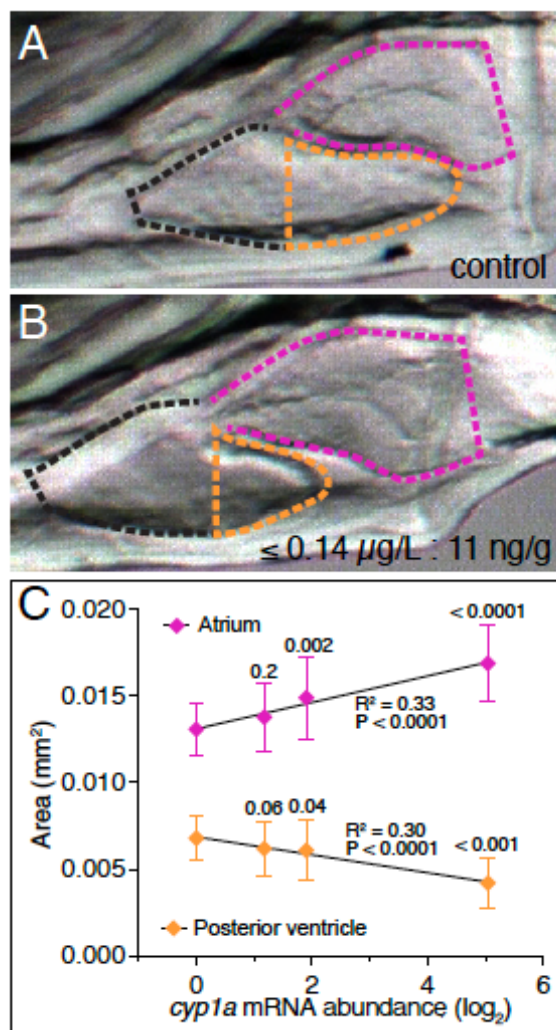


Figure 11. Relationship between cardiac chamber dimensions at hatch and *cyp1a* induction at 10 days post-fertilization (dpf). Representative lateral views of the heart in hatching stage larvae are shown for control (A) and the 1.0 g/kg oil (Σ polyaromatic hydrocarbon (PAH) 11 ng/g wet weight) dosing level (B). The periphery bounding the area measures are indicated by the dashed fuchsia line for the atrium and the dashed amber line for the posterior ventricle. The unmeasured anterior extent of the ventricle is indicated by the black dashed line. Still images represent video frames taken from the diastolic phase of the ventricle. (C) Linear regression plot of atrial (fuchsia points) and posterior ventricle (amber points) lateral area measures (mm²) against log₂ *cyp1a* mRNA relative abundance measured at 10 dpf (9 days post-exposure). Data represent mean \pm 1 S.D. for N = 40 measures.



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

Disease Modelling

To improve our understanding of the interactive roles of temperature and herd immunity on the dynamics of VHS we built a modified epidemiological model of VHS in Pacific herring. The specific type of model we used here is a Susceptible-Exposed-Infected-Carriers-Particle model, where the susceptible compartment represents fish that are naïve to the pathogen, exposed are the fish in the early stages of the disease, and not yet shedding virus, the infected are the symptomatic fish that are both shedding virus and vulnerable to disease mortality, and the carriers are the fish which survived the disease and are refractory to new infections. They can however shed the virus when exposed to cold temperatures. In contrast to most aquatic disease models, we explicitly model transmission via exposure to waterborne pathogen; the particle compartment tracks the waterborne virus (in quantities of plaque forming units) shed by infected and, at cold temperatures, carrier herring. Initial investigation shows that the model simulates experimental results with skill (Fig. 1). Using this model, we showed that VHS outbreaks are bigger and more lethal at cold temperatures because the virus persists in cold temperatures longer than it does in warmer temperatures, viral shedding rates are higher in cooler water, and carrier herring shed virus at cooler temperatures. Survivors of epizootics contribute to herd immunity, limiting the impact of viral exposure until new naïve fish arrive. Under warm temperatures (14 °C) the short persistence of the virus and the low shedding rate will quickly stop its spread. Under slightly cooler temperatures (11 °C) herd immunity requires 75% of herring to be completely refractory. However, in every simulation, cold temperatures (9 °C) will start viral shedding by carriers and start epizootics. Fisheries targeting year classes with high levels of susceptible fish will limit the loss of the protective effect of the carriers/refractory fish, while fisheries targeting year classes with high levels of carrier fish can result in a loss of herd immunity, contributing to future VHS outbreaks. The full manuscript describing these results is in preparation and will be included in the final report.

2. Products:

Peer-reviewed publications:

Groner, M. L., E. D. Bravo-Mendoza, A. H. McKenzie, J. L. Gregg, C. M. Conway, J. Trochta, and P. K. Hershberger. 2023. Temporal, environmental, and demographics correlates of



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

Ichthyophonus sp. infections in mature Pacific herring populations. ICES Journal of Marine Science 80:2342-2355. <https://doi.org/10.1093/icesjms/fsad147>.

Incardona, J. P., T. L. Linbo, J. Cameron, B. L. French, J. Bolton, J. L. Gregg, C. E. Donald, P. K. Hershberger, and N. L. Scholz. 2023. Biological responses of Pacific herring embryos to crude oil are quantifiable at exposure levels below conventional limits of quantification for PAHs in water and tissues. *Environmental Science and Technology* 57:19214-19222. <https://doi.org/10.1021/acs.est.3c04122>.

Páez, D. J., J. L. Gregg, A. H. MacKenzie, S. A. Hall, and P. K. Hershberger. 2023. Characteristics of a sea lice (*Caligus clemensi*) epizootic in wild Pacific herring (*Clupea pallasii*). *Canadian Journal of Fisheries and Aquatic Sciences* 80:1847-1856. <https://doi.org/10.1139/cjfas-2023-0070>.

Reports:

Hershberger, P. K. 2023. Herring Disease Program II. Long-Term Herring Research and Monitoring Project Final Report (Exxon Valdez Oil Spill Trustee Council Project 21120111-E). Exxon Valdez Oil Spill Trustee Council, Anchorage, Alaska.

Popular articles:

No new contributions for this reporting period.

Conferences and workshops:

Páez, D. J., J. L. Gregg, A. H. MacKenzie, S. A. Hall, and P. K. Hershberger. 2024. Characteristics of a sea louse (*Caligus clemensi*) epizootic in wild Pacific herring (*Clupea pallasii*). Poster presentation, Alaska Marine Science Symposium, Anchorage, Alaska, January.

Kroska, A. C., P. Hershberger, S. Sitkiewicz, T. S. Smeltz, C. L. Dykstra, J. Gregg, B. P. Harris, and N. Wolf. 2023. Exploring the relationship of *Ichthyophonus* exposure to infection prevalence and severity in wild-caught Pacific halibut. Platform presentation, PICES Annual Meeting. Seattle, Washington, October.

Harsha, M. L., Y. Salas-Ortiz, A. Cypher, E. Osborn, E. Turcios Valle, J. L. Gregg, P. K. Hershberger, P. Zito, M. A. Tarr, P. L. Tomco, and D. C. Podgorski. 2023. From molecules to morphology: A multidisciplinary investigation of the toxicity of



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

hydrocarbon oxidation products in Pacific herring embryos. Platform presentation, Society of Environmental Toxicology and Chemistry (SETAC) North America, November.

Lepeule, A., P. K. Hershberger, and M. Groner. 2023. A SICR models for VHS that informs management of Pacific herring fisheries. Platform presentation, Western Fish Disease Workshop. Parksville, British Columbia, June.

Public presentations:

No new contributions for this reporting period.

Data and/or information products developed during the reporting period:

No new contributions for this reporting period.

Data sets and associated metadata:

Hershberger, P. 2023. Herring: Herring disease program. Gulf of Alaska Data Portal. <https://gulf-of-alaska.portal.aaos.org/#metadata/61b4ec5a-f15c-4347-b0ba-8a25ad763675/project>.

Paez, D. J., J. L. Gregg, A. H. MacKenzie, S. A. Hall, and P. K. Hershberger. 2023. *Caligus Clemensi* prevalence and counts on *Clupea pallasii* from Port Angeles Harbor, WA and from a controlled laboratory experiment conducted at USGS Marrowstone Marine Field Station, WA. U.S. Geological Survey data release, <https://doi.org/10.5066/P9KRGX06>.

Additional Products not listed above:

No new contributions for this reporting period.

3. Coordination and Collaboration:

All U. S. Geological Survey (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. Additionally, this project was leveraged to obtain funding from the National Science Foundation (NSF) for a complementary herring disease study intended to evaluate the effects of climate change on



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

herring diseases. The NSF project partners with Alaska Pacific University and Northern Arizona University.

The Alaska SeaLife Center or Prince William Sound Science Center

This project coordinates closely with the Prince William Sound Science Center (PWSSC), as the lead coordinator for the Herring Research and Monitoring (HRM) component. PWSSC provides administrative support, serves as a logistical liaison coordinating PWS herring cruises, and assists with metadata transfer for the Herring Disease Program.

EVOSTC Long-Term Research and Monitoring Projects

The Herring Disease Program is within the HRM component of the Gulf Watch Alaska-Long-Term Research and Monitoring (GWA-LTRM) program. We work with other HRM component projects as the component as a whole works to understand the lack of recovery of PWS herring, particularly the following projects:

- We worked closely Alaska Department of Fish and Game (ADF&G; project 23170111-F: Herring surveys and age, sex, and size collection and processing) to collect herring tissue and plasma samples, using a shared platform for the spring herring cruises. 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 23120111-C: Modeling and stock assessment of PWS herring). Additionally, revised antibody data for PWS were shared with Dr. Branch, for incorporation into a VHSV hindcasting model.
- This project will be providing pathogen surveillance information for Drs. Rand and Gorman (project 23220111-I: Ecological interactions between Pacific herring and Pacific salmon in Prince William Sound). VEN surveillance results for pink salmon / herring interactions are included in their report.
- This project is partnering with the National Oceanic and Atmospheric Administration Resource Assessment and Conservation Engineering cruises to obtain samples near the pollock spawning locations in Shelikof Strait. Additional partnerships with ADF&G and the herring / pink salmon interaction project will provide pollock egg samples from PWS to investigate the possibility of *Ichthyophonus* transmission through ovivory.



Exxon Valdez Oil Spill Trustee Council

Long-Term Research and Monitoring, Mariculture, Education and Outreach

Annual Project Reporting Form

4. Response to EVOSTC Review, Recommendations and Comments:

No comments for FY23.

5. Budget:

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

Budget Category:	Proposed FY 22	Proposed FY 23	Proposed FY 24	Proposed FY 25	Proposed FY 26	5-YR TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	\$221,276	\$310,206	\$227,886	\$235,462	\$301,248	\$1,296,078	\$382,191
Travel	\$21,826	\$21,826	\$21,826	\$21,826	\$21,826	\$109,130	\$15,682
Contractual	\$0	\$0	\$0	\$0	\$0	\$0	\$0
Commodities	\$39,300	\$39,000	\$39,000	\$39,000	\$39,000	\$195,300	\$65,898
Equipment	\$15,000	\$0	\$0	\$0	\$0	\$15,000	\$10,583
Indirect Costs (varies by proposer)	\$18,424	\$18,424	\$0	\$0	\$0	\$36,849	\$18,634
SUBTOTAL	\$315,826	\$389,456	\$288,712	\$296,288	\$362,074	\$1,652,357	\$492,988
General Administration (9% of subtotal)	\$28,424	\$35,051	\$25,984	\$26,666	\$32,587	\$148,712	N/A
PROGRAM TOTAL	\$344,251	\$424,508	\$314,696	\$322,953	\$394,661	\$1,801,069	
Other Resources (In-Kind Funds)	\$124,245	\$127,724	\$131,396	\$135,129	\$138,910	\$657,404	

Above is the combined budget for PIs Hershberger and Paez (USGS) and Groner (Bigelow Labs). The project is behind in spending because of the delay in the release of funds that began in FY22. PI Groner's work was scheduled to phase out at the end of FY23; because of the delay in funding and the offset of the NOAA grant fiscal year from the EVOSTC fiscal year, her work will continue through FY24 to spend out authorized funds.