Exxon Valdez **Oil Spill Trustee Council**

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

Annual Project Reporting Form

Project Number: 22120111-E

Project Title: Herring Disease Program

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

Reporting Period: February 1, 2022 – January 31, 2023

Submission Date: March 8, 2023

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**

All field surveillances, diagnostic testing, and laboratory / field experiments in the Herring Disease Program are on schedule. There are two associated projects / deliverables that are slightly behind schedule due to funding delays.

- The susceptible, exposed, infected, recovered (SEIR) disease model development for VHSV is slightly behind schedule due to unexpected delays with funding arrival at Bigelow Labs. It is anticipated that this deliverable will be back on schedule by the end of FY23.
- Fish health contributions to Project #22220111-I (Ecological interactions between Pacific herring and Pacific salmon in Prince William Sound) were delayed with the first year of this project.
- Fish health contributions to Project #22220203 (Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions) were delayed with the first of this project.

☐ **Budget reallocation request.**

☒ **Personnel changes.**

Dr. Maureen Purcell has changed positions and will no longer serve as a co-Principal Investigator on this project. Dr. David Paez has been hired to work on this project and will assume the Co-PI responsibilities that were vacated by Dr. Purcell. This change in project leadership is cost-neutral and will not impact the budget.

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1. Summary of Work Performed:
Field Sampling

A. Prince William Sound Pre-spawn Adult Herring

Three samples of Pacific herring (*Clupea pallasii*) were collected from Prince William Sound (PWS) during the spring pre-spawn period from April 2-3, 2022, to test for viral hemorrhagic septicemia virus (VHSV) and *Ichthyophonus* prevalence (Table 1, Fig. 1). *Ichthyophonus* was detected in 22% (40/179) of heart cultures from all sites combined. VHSV was not isolated from any samples (n = 180), but neutralizing antibodies to VHSV were detected in 0.79% (3/380) of PWS herring in 2022 (Fig. 2). The prevalence of seropositives was generally low across all age classes from 2019-2022 (Fig. 3), inferring a paucity of VHSV exposures during this period and a current population of adult herring that remains susceptible to the resulting disease. Erythrocytic inclusions indicative of viral erythrocytic necrosis (VEN) were not detected in any PWS herring (n =180) from 2022.

Table 1. Infection prevalence results from Prince William Sound pre-spawn herring in 2022. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	VHSV Prevalence	*Ichthyophonus* Prevalence (Heart Cultures)	VEN prevalence
Redhead	April 2	0% (n=60)	23% (14/60)	0% (n=60)
Cedar Bay	April 3	0% (n=60)	20% (12/60)	0% (n=60)
Port Etches	April 3	0% (n=60)	24% (14/59)	0% (n=60)

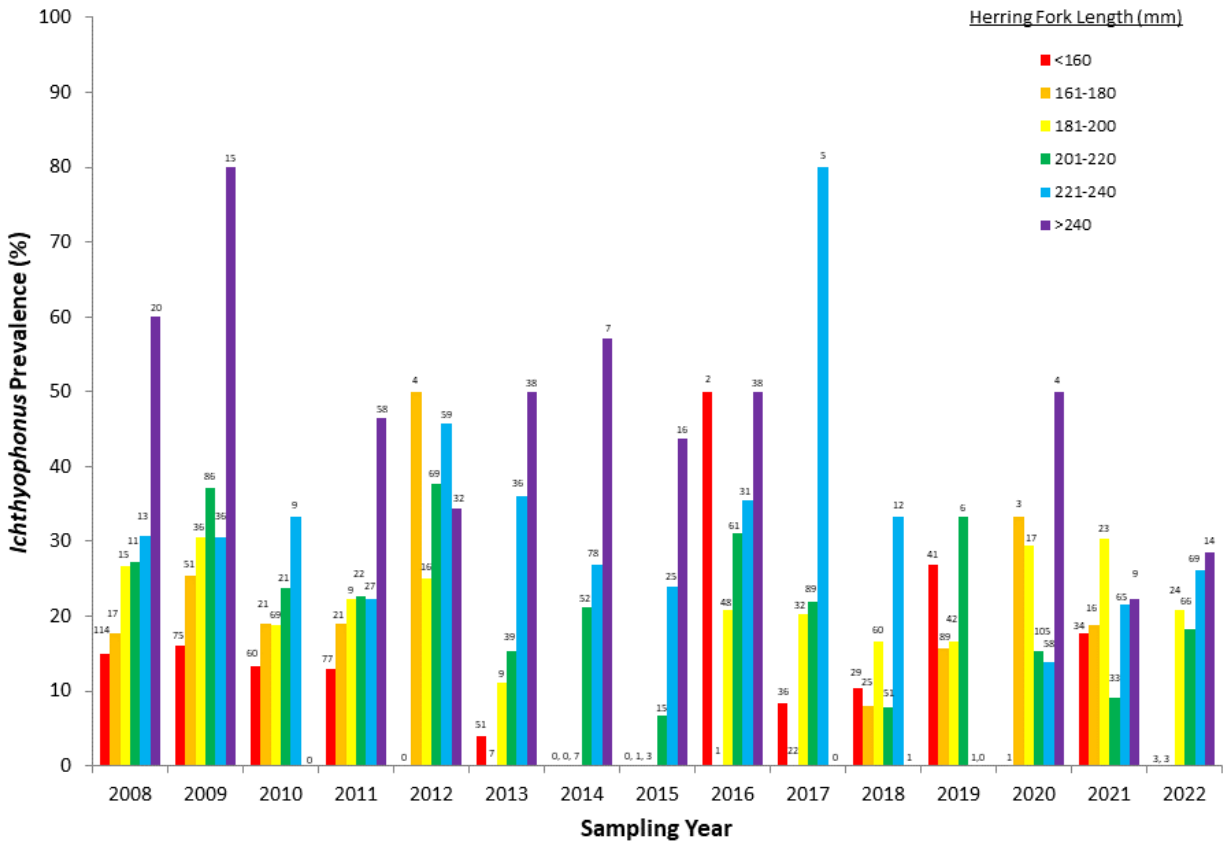

Figure 1. Temporal trend in Ichthyophonus infection prevalence in each size class of Prince William Sound herring. Numerals above each bar indicate sample size (n).

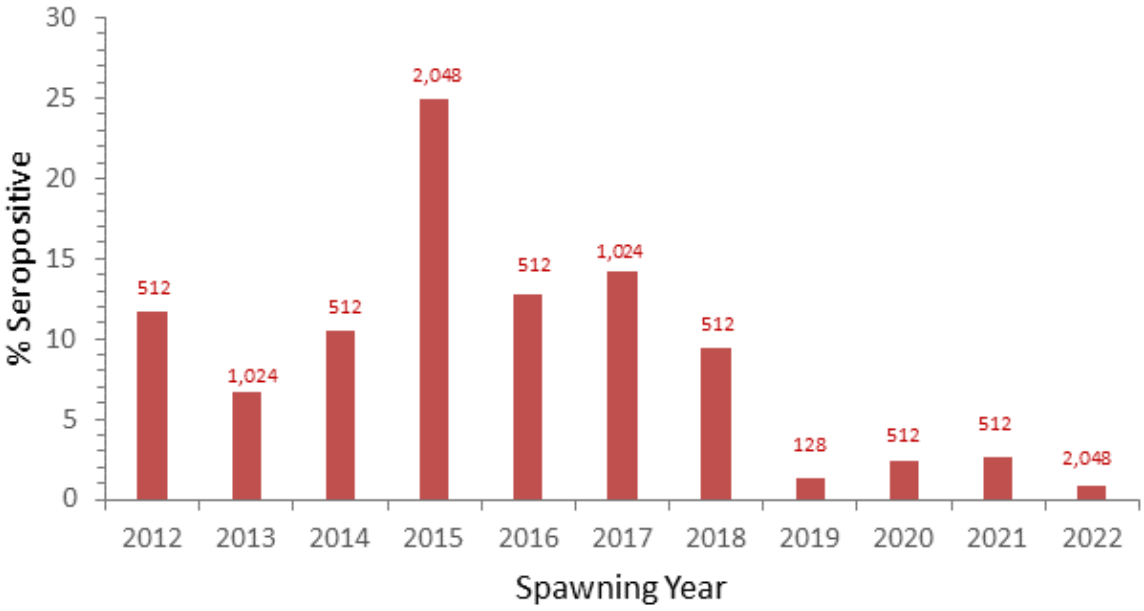

Figure 2. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Prince William Sound herring. Numerals above the bars indicate the median neutralizing titer in seropositives, reported as the reciprocal 50% inhibitory dilution – ID_{50} (titer range: 64 - 2,048).

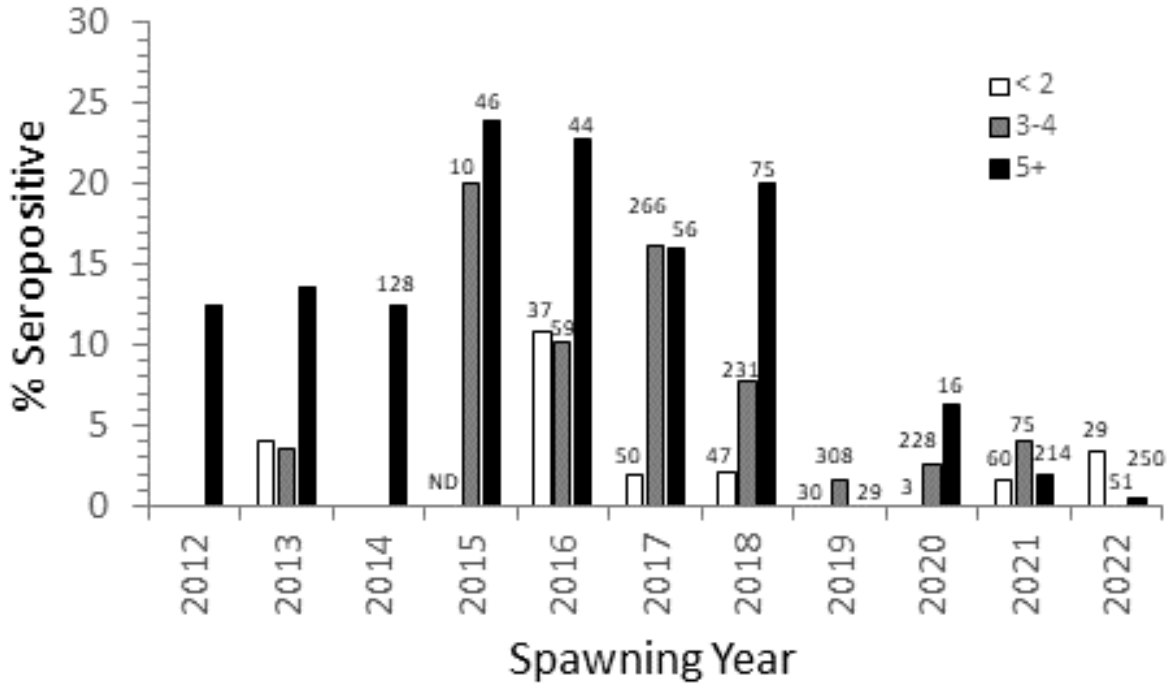

Figure 3. Inter-annual changes in prevalence of viral hemorrhagic septicemia virus neutralizing antibodies from Prince William Sound herring age classes (age determined by Alaska Department of Fish and Game from scales). Numerals above the bars indicate sample size (n).

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 28-31, 2022, to test for VHSV and *Ichthyophonus* prevalence (Table 2, Fig. 4). *Ichthyophonus* was detected in 27% (45/164) of herring hearts. Neither VHSV nor VEN were detected in any samples (n = 164 and 158; respectively). Neutralizing antibodies to VHSV were detected in only 1.2% (2/162) of herring plasma samples (Fig. 5). As with PWS, VHSV neutralizing antibody levels have been relatively low in Sitka Sound for the past 4 years (2019-2022), 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	*Ichthyophonus* Prevalence (Heart Cultures)	VEN prevalence
N. Inner Point	March 28	0% (n=60)	25% (15/60)	0% (n=60)
SW Middle Island	March 29	0% (n=60)	23% (14/60)	0% (n=60)
M. Middle Island	March 31	0% (n=60)	36% (16/44)	0% (n=60)

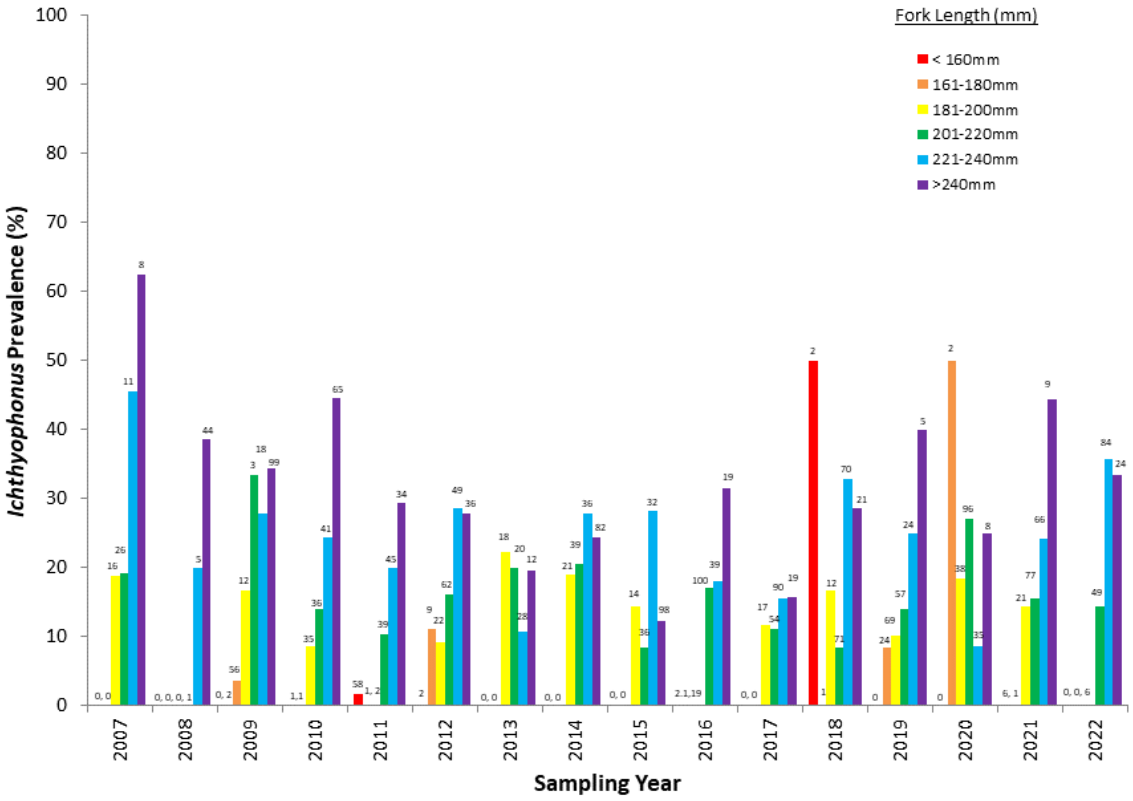

Figure 4. Temporal trend in Ichthyophonus infection prevalence in each size class of Sitka Sound herring. Numerals above each bar indicate 'n'.

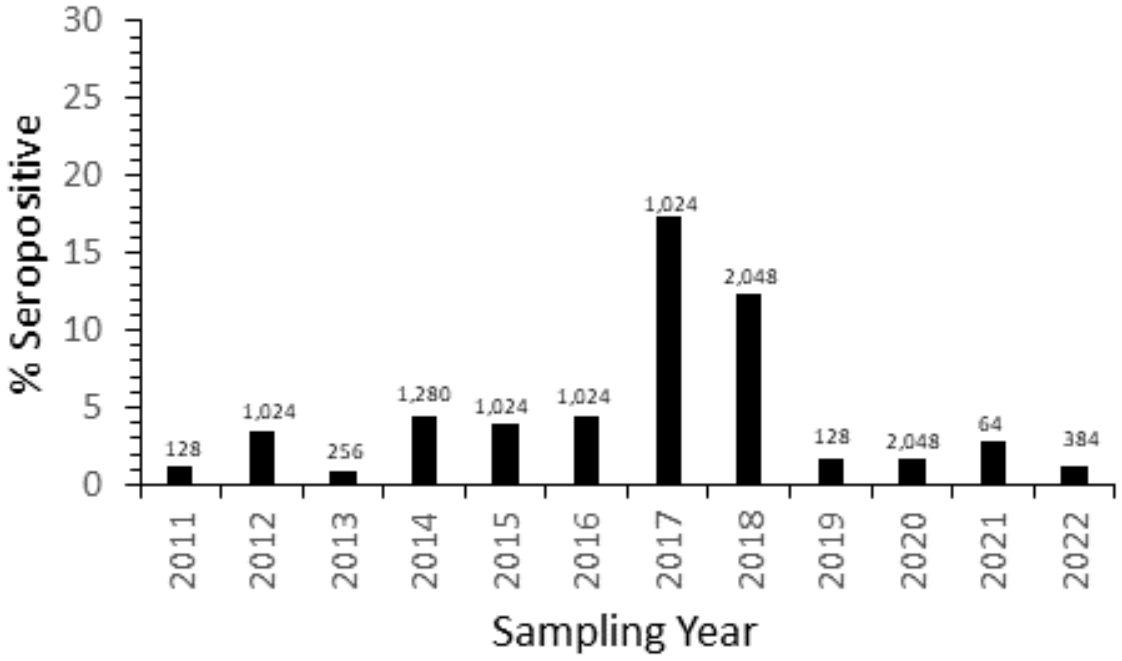

Figure 5. Annual prevalence of viral hemorrhagic septicemia virus neutralizing antibodies in Sitka Sound herring. Numerals above the bars indicate the median neutralizing titer among seropositives, reported as the reciprocal 50% inhibitory dilution – ID_{50} (titer range: 64 - 2,048).

C. Puget Sound Pre-spawn Adult Herring

Additional herring samples were collected from several sites in Puget Sound, WA during 2022. *Ichthyophonus* infection prevalence ranged from 3.3% Yukon Harbor and Pt. Madison to 66% (19/29) in Quilcene Bay. The prevalence of Ichthyophonus has been elevated in Quilcene By the past several years and is coincident with record high herring biomass in this stock. VEN was not detected from herring Semiahmoo Bay or Quilcene Bay. Samples were not collected for VHSV tissue titers from any location; however, neutralizing antibodies to VHSV were detected in 3.1% (2/65) of herring from Semiahmoo Bay and 0% (n = 30) from Quilcene Bay (Table 3).

Table 3. Survey results from Puget Sound pre-spawn herring in 2022. VHSV = viral hemorrhagic septicemia virus and VEN = viral erythrocytic necrosis.

Location	Date	*Ichthyophonus* Prevalence (Heart Cultures)	VEN prevalence	VHSV Infection Prevalence	VHSV Antibody Prevalence
Semiahmoo Bay	Feb 9	20% (6/30)	0% (0/30)	ND	6.7% (2/30)
Yukon Harbor	Feb 17	3.3% (2/60)	ND	ND	ND
Shilshole	Feb 18	5.0% (3/60)	ND	ND	ND
Quilcene Bay	Feb 22	66% (19/29)	0% (0/30)	ND	0% (n=30)
Pt. Madison	Mar 15	3.3% (2/60)	ND	ND	ND
Holmes Harbor	Mar 16	9.7% (4/41)	ND	ND	ND

Laboratory Studies

A. Swim Flume

A swim flume (Fig. 6) was constructed and is currently fully operational. The flume is intended to compare the relative swimming performance (critical swimming velocity) between groups of Pacific herring at different stages of infection with various pathogens, including VHSV, ENV, and *Ichthyophonus*. The first four-month-long experiment was recently completed with VHSV, and results are currently being analyzed (will presented in the FY23 annual report). Maximum water velocities in the flume are approximately 5-6 body length / sec for age 0 herring (80-110 mm). With aspirations of swimming larger fish, we are currently retrofitting the flume with redesigned venturi, turning veins, and acrylic lid to achieve faster current velocities.

Figure 6. Photograph of the newly-constructed swim flume, including the motor (shrouded in the background), propellor, shaft, venturi, turning veins, laminizer tubes, and swim box.

B. Different strains of *Ichthyophonus* exist sympatrically in the NE Pacific

In recent decades evidence has accumulated to suggest that the widespread and highly variable parasite *Ichthyophonus hoferi* is actually a species complex. Highly plastic morphology and a general lack of defining structures has contributed to the likely underestimate of biodiversity within this group. Molecular methods are a logical next step in the description of these parasites, but markers used to date have been too conserved to resolve species boundaries. Here we use mitochondrial encoded cytochrome-c oxidase (MTCO1) gene sequences and phylogenic analysis to compare *Ichthyophonus spp.* isolates from several marine and anadromous fish hosts. The resulting phylogeny displays lineage separation among isolates, and possible host/niche segregation not previously described (Fig. 7). The parasite type that infects Pacific herring, Atlantic herring (*Clupea harengus*), Atlantic salmon (*Salmo salar*), and Pacific staghorn sculpin (*Oligocottus maculosus*) (Clade A) is different from that which infects Chinook salmon (*Oncorhynchus tshawytscha*), walleye pollock (*Gadus chalcogrammus*), Greenland halibut (*Reinhardtius hippoglossoides*), and Pacific halibut (*Hippoglossus stenolepsis*) (Clade B). MTCO1 sequences confirmed the presence of a more divergent *Ichthyophonus* sp. isolated from American shad (*Alosa sapidissima*) in rivers of Eastern North America (Clade C), while American shad introduced to the Pacific Ocean are infected with the same parasite that infects Pacific herring (Clade A). Currently there are no consensus criteria for delimiting species within *Ichthyophonidae*, but MTCO1 sequences hold promise as a potential species identifying marker and useful epizootiological tool.

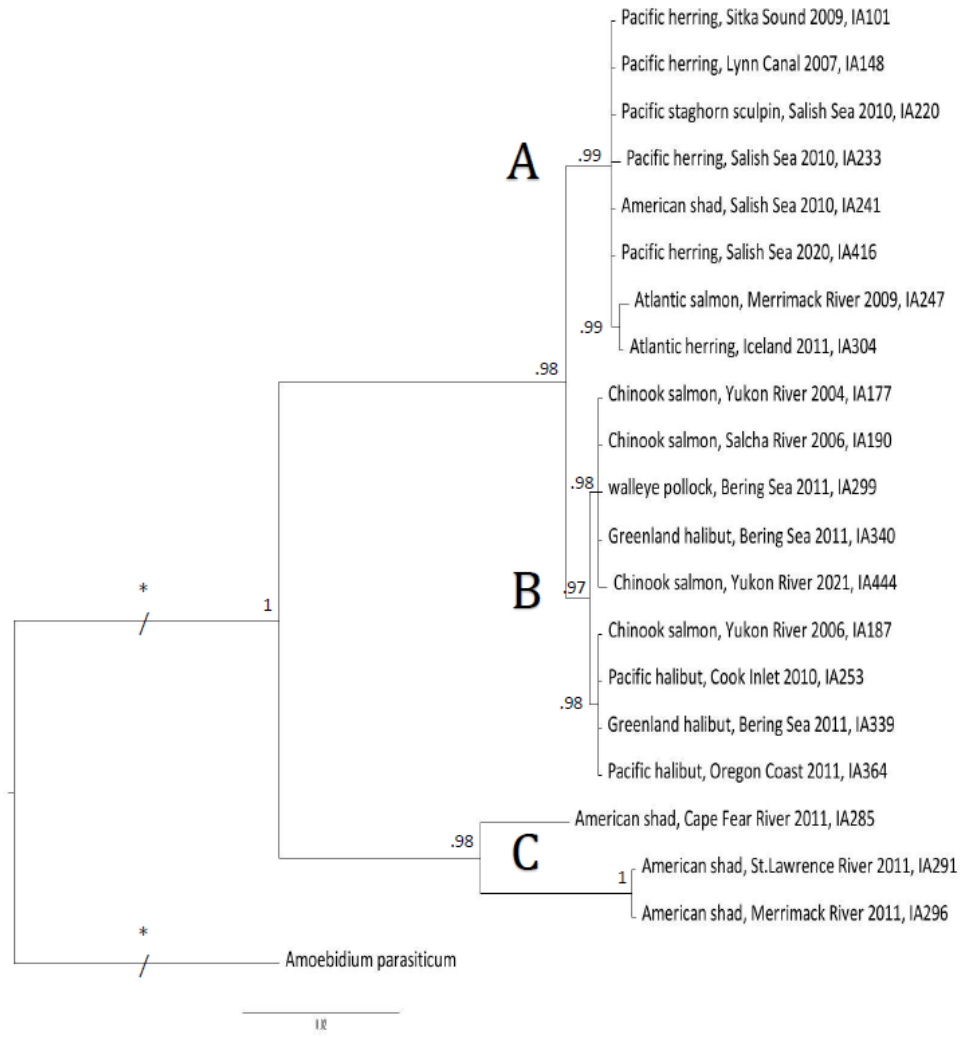

Figure 7. Phylogeny of Ichthyophonus hoferi isolates based on MTCO1 gene sequences. Tip labels indicate host, collection location, collection year, and isolate number. Node labels are posterior probabilities. MTCO1 sequences from Amoebidium parasiticum included as outgroup taxon. Displayed genetic distance to outgroup has been reduced by a factor of 10 to improve visibility of tree near tips, shortened branches indicated with asterisk. Phylogeny is the majority consensus from 1502 trees sampled during MCMC, see text for details. Clades arbitrarily labelled A through C for discussion purposes.

C. A novel approach for directly incorporating disease into fisheries stock assessment: the powerful potential of seroprevalence data

When estimating mortality from disease with fisheries population models, common disease surveillance data such as infection prevalence are not always informative, especially for fast-acting diseases that may go unobserved in infrequently sampled populations. In these cases, seroprevalence—the proportion of fish with measurable antibody levels in their blood—may be more informative. In cases of life-long immunity, seroprevalence data require less frequent sampling intervals than infection prevalence data and can reflect the cumulative exposure history of fish. We examined the usefulness of seroprevalence data in an age-structured fisheries stock assessment for a simulated fast-acting disease in a herring-like population. We developed a novel epidemiological model to simulate population dynamics and seroprevalence data and fitted to these data in an integrated catch-at-age model with equations that estimate age- and time-varying mortality from disease (Fig. 8). We found that simulated seroprevalence data can provide accurate estimates of infection history and disease-associated mortality. Importantly, even models that mis-specified nonstationary processes in background or disease-associated mortality but included seroprevalence data accurately estimated annual infection and population abundance.

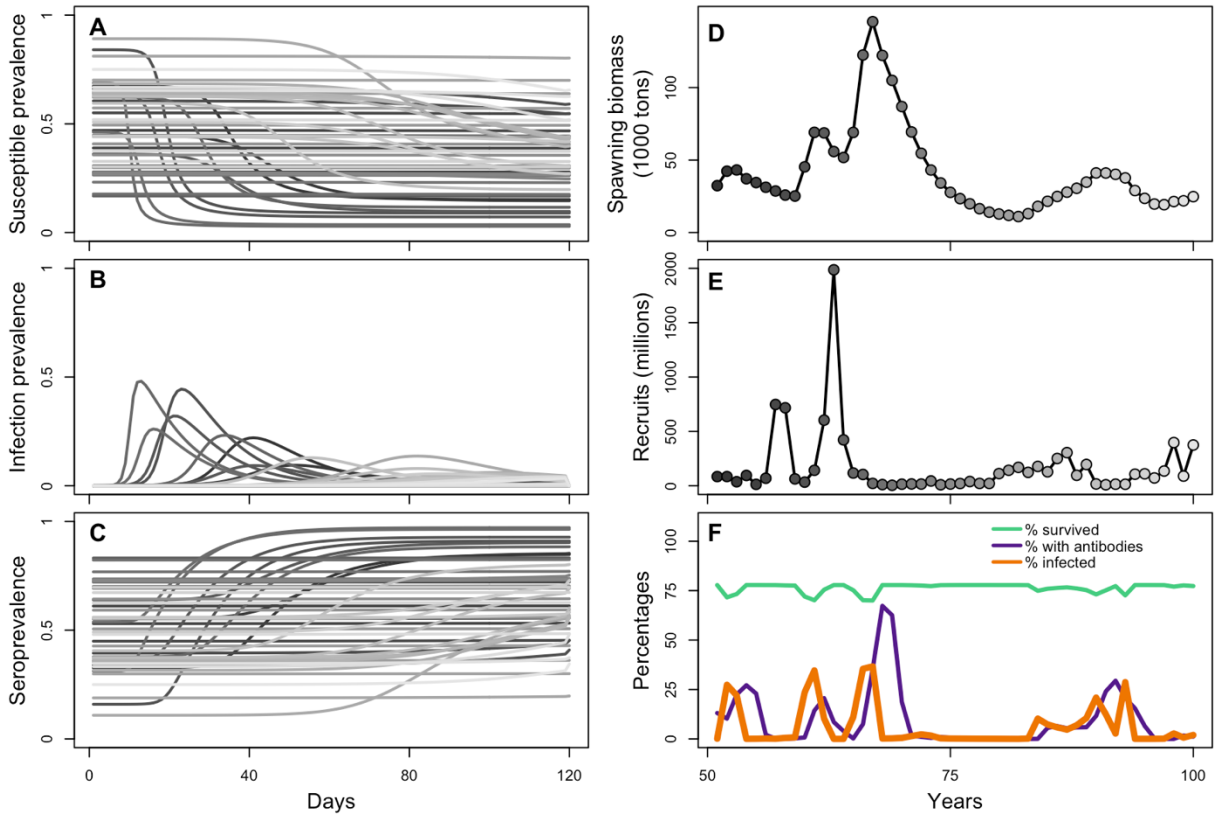

Figure 8. Population and epidemiological dynamics over 50 years from a single simulation of the operating model. Daily disease stage transitions are shown as the proportions of the overall population that are (A) susceptible, (B) infected, or (C) recovered and now carriers on each day of the fixed transmission period. Each line is a different year. Grey shading denotes the corresponding years between plot A-C and D-E. The annual population level dynamics are shown in the right column including (D) annual spawning biomass, (E) stochastic recruits as the number of age-0 fish, and (F) the realized population survival (the annual percentage that survive), seroprevalence (the annual percentage that are currently immune), and infection incidence (the percentage that becomes infected) of the population (excluding the plus age group) in each year.

D. Elevated temperature increases disease progression and host response of Pacific herring to erythrocytic necrosis virus

Controlled laboratory exposure studies provided evidence for a direct relationship between temperature and the progression of viral erythrocytic necrosis (VEN) in Pacific herring. Waterborne exposure of Pacific herring to kidney homogenates containing the causative iridovirus (erythrocytic necrosis virus - ENV) resulted in the establishment of infections, characterized by high infection prevalence (89%; 40/45) and mean viral loads (5.5 $\log_{10}$ gene copies / ug DNA) in kidney tissues at 44 d post exposure. Viral loads were higher in herring from the ambient (9.0 °C) and warm (13.5 °C) treatments (6.1 - 6.2 $\log_{10}$ gene copies / total DNA) than in the cool (6.9 °C) treatment (4.3 $\log_{10}$ gene copies / total DNA). Similarly, disease signs were temperature dependent ($P < 0.001$), with cytoplasmic inclusion bodies occurring in 20% of herring in the cool, 47% in the ambient, and 60% in the warm treatments. The mean disease load in each fish (enumerated as the percent of erythrocytes with cytoplasmic inclusions), increased with temperature from 13% in the cool, 47% in the ambient, and 32% in the warm treatments at 44 days post exposure. Transcriptional analysis indicated that the number of differentially expressed genes among ENV-exposed herring also increased with temperature, time post exposure, and viral load. Correlation network analysis of transcriptomic data showed robust activation of interferon and viral immune responses in hepatic tissue of infected individuals independent of other experimental variables (Fig. 9).

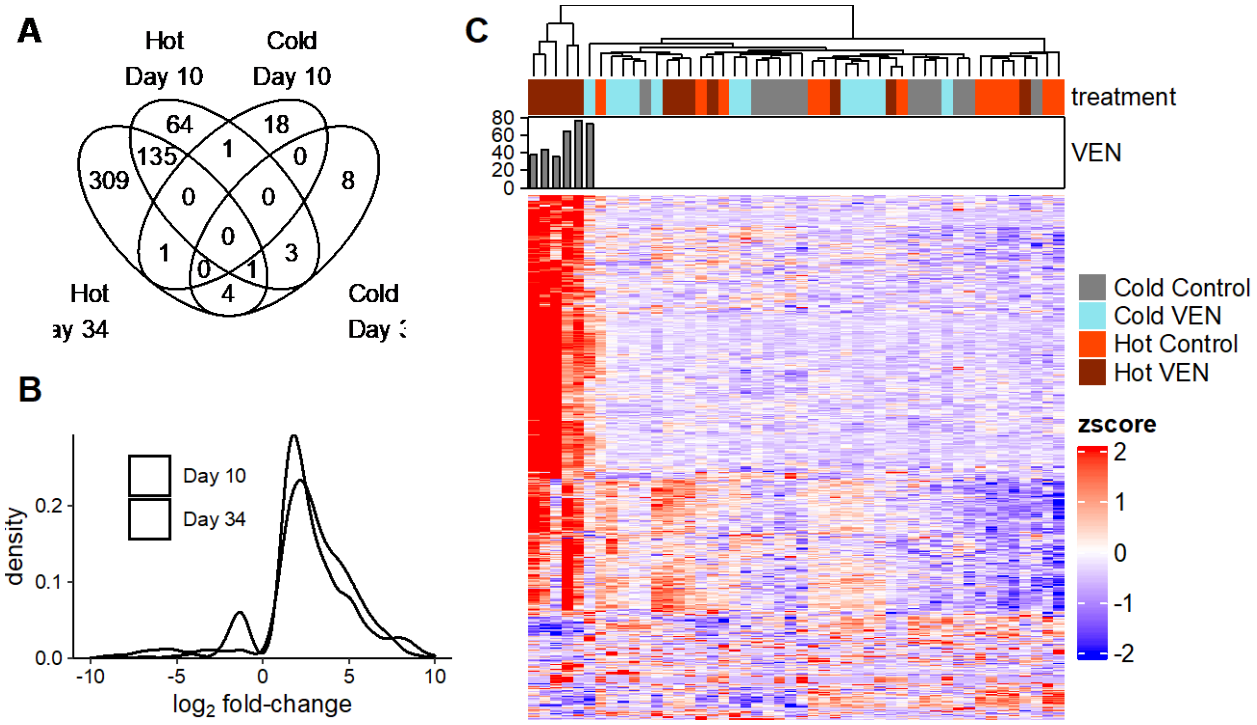

Figure 9. A) Venn diagram of overlapping Differentially Expressed Genes in the four viral erythrocytic necrosis (VEN) treatments compared to time and temperature matched controls. B) Density plot of differential expression in warm temperature treatments at days 10 and 34. C) Heatmap of genes differentially expressed in any treatment. Each column represents an individual, with each row depicting a different gene. Bar graphs at the top represent measured disease load for the sample (determined by blood film). Top dendrogram indicates sample relationships and showed a distinct expression profile for erythrocytic necrosis virus (ENV)-positive fish from the warm treatments.

2. Products:

Peer-reviewed publications:

Trochta, J., M. L. Groner, P. K. Hershberger, and T. A. Branch. 2022. A novel approach for directly incorporating disease into fisheries stock assessment: a case study with seroprevalence data. Canadian Journal of Fisheries and Aquatic Sciences 79:611-630.

Gregg, L. L., P. K. Hershberger, A. S. Neat, H. T. Jayasekera, J. A. Ferguson, R. L. Powers, and M. K. Purcell. 2022. A phylogeny based on cytochrome-c oxidase gene sequences identifies sympatric *Ichthyophonus* genotypes in the NE Pacific Ocean. Diseases of Aquatic Organisms 150: 61-67.

Reports:

Hershberger, P.K. Draft. Herring Disease Program II. *Exxon Valdez* Oil Spill Trustee Council Herring Research and Monitoring Project Final Report (Project #21120111-E). *Exxon Valdez* Oil Spill Trustee Council, Anchorage, AK.

Popular articles:

Salzer, J., M. Groner, and P. Hershberger. 2022-2023. Temperature-induced disease progression in Pacific herring. Delta Sound Connections 2022-2023. https://pwssc.org/wp-content/uploads/2022/06/DSC-2022-WEB.pdf.

Conferences and workshops:

Salzer, J. E., J. Greer, M. Groner, A. MacKenzie, J. Gregg, and P. Hershberger. 2023. Elevated temperature increases disease progression and host response of Pacific herring to erythrocytic necrosis virus. Poster presentation, Alaska Marine Science Symposium, Anchorage, AK, January.

Hershberger, P. K. 2022. Intersection of Species management and Disease Ecology. Virtual, USGS Pacific Coast Diadromous and Marine Fish Symposium, September.

Salzer, J. E., J. B. Greer, M. L. Groner, A. H. MacKenzie, J. L. Gregg, and P. K. Hershberger. 2022. Effects of temperature on viral erythrocytic necrosis (VEN) in Pacific herring. Western Fish Disease Workshop. Hood River, OR, May.

Spanjer, A., T. Liedtke, P. Hershberger, K. Conn, and K. Snekvik. 2022. Immunotoxic response and disease susceptibility in adult Pacific Herring, *Clupea pallasii*, fed a complex PCB congener mixture. 2022 Salish Sea Ecosystem Conference. Virtual, April.

Murray, C. S., J. Gregg, H. Jayasekera, W. Richards, A. Malloy, and P. Hershberger. 2022. Does ocean acidification affect the bioenergetics and susceptibility to viral disease in Pacific herring? Poster presentation, Salish Sea Ecosystem Conference. Virtual, April.

Groner, M. L., E. D. Bravo-Mendosa, A. H. McKenzie, J. L. Gregg, C. M. Conway, J. Trochta, and P. K. Hershberger. 2022. Reconstruction of infection history indicates consistently elevated transmission and prevalence of *Ichthyophonus* sp. in a collapsed population of Pacific herring. Ocean Sciences Meeting, Honolulu, HI, March.

Public presentations:

Hershberger, P.K. June 23, 2022. What's Happening Down at the Point? USGS - Marrowstone Marine Field Station. Invited Seminar: Friend of Fort Flagler. Nordland, WA.

Hershberger, P.K. August 3-4, 2022. Understanding and Mitigating the Impacts of Marine Disease. Invited Seminar and Laboratory Instructor: Friday Harbor Laboratories, University of Washington, Friday Harbor, WA.

Hershberger, P.K. September 29, 2022. The Ecology of Disease in Marine Fishes: Insights from Pacific Herring. Invited seminar: Memorial University, Newfoundland, Canada.

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

None

Data sets and associated metadata:

All herring surveillance metadata resulting from this project are housed on DataOne (https://search.dataone.org/view/10.24431%2Frw1k32b) and metadata from the laboratory studies are housed on ScienceBase Additional Products not listed above:

3. Coordination and Collaboration:

All US Geological Survey 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.

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 22170111-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 #22120111-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 Heintz (project # 22220111-I: Ecological interactions between Pacific herring and Pacific salmon in Prince William Sound); however, field work was delayed a year due to funding logistics, and active engagement between the projects is now scheduled to start in FY23.
- This project will be partnering with Wyatt Rhea-Fournier (project #22220203: Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions); however, field work was delayed a year due to funding logistics, and active engagement between the projects is now scheduled to start in FY23.

EVOSTC Mariculture Projects

The GWA-LTRM program is coordinating with the Mariculture ReCon project funded by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) on mariculture projects within the spill-affected region. We will participate in these collaborative efforts as appropriate.

EVOSTC Education and Outreach Projects

The GWA-LTRM program is coordinating with members of the CORaL network funded by the EVOSTC on collaborative outreach within the spill-affected region. We will participate in outreach efforts as appropriate.

Individual EVOSTC Projects

The Herring Disease Program works with the Data Management program to ensure our data are properly reviewed, have current metadata, and are posted to the Gulf of Alaska data portal within required timeframes. We will work with other individually funded EVOSTC projects if collaborative efforts make sense based on data collected.

Trustee or Management Agencies

We partner with ADF&G, a Trustee Agency on a variety of disease issues, specifically the following:

- We collect herring infection and disease data onboard the shared ADF&G seining platform in coordination with Cordova-based biologists.
- We collect herring infection and disease data from pre-spawn aggregations in Sitka Sound with Sitka-based biologists.
- We continue to partner with ADF&G Juneau Fish Pathology Laboratory who has provided consistency for processing all fish virology samples from PWS and Sitka Sound since the onset of herring health assessments in the early 1990's.

Native and Local Communities

None to report.

4. Response to EVOSTC Review, Recommendations and Comments:

May 2021 EVOSTC Science Panel Comment: This is a very productive research project that continues to make many contributions to the primary literature as well as provide essential information to regional managers and scientific colleagues. Noteworthy in this proposal is the expanded degree of interaction and collaboration with other PIs. This has been one of the most successful projects, and the new 10-year proposal is well thought out overall. This proposal provides some of the most comprehensive information about marine fish disease ecology worldwide. There are two primary objectives that relate to their potential ecological and management impacts: 1) to evaluate epidemiological consequences of herd immunity to VHSV and 2) to identify *Ichthyophonus* transmission mechanisms.

The focus on herd immunity of the PWS herring population is very interesting. The proposed approach of PCR analysis for VHSV should provide a much more sensitive assay for exposure/immunity than neutralizing antibody assays. Is it known in fish that previous exposure may not include circulating VHSV antibodies, but that T-cell responses can ramp up upon exposure? While this is established in mammals, is this a possible situation in herring? Some comparisons of populations from other locations may be telling, as could the rapidity of an antibody response in fish presumed to have had previous exposure to VHSV.

PI Response: Yes, an analogous process to what the Science Panel describes most likely occurs in the herring / VHSV model system. The Science Panel is correct that the presence of circulating antibodies to a particular agent are typically transient and often decline to undetectable levels after several months. The host typically remains protected after these antibody levels are no longer detectable because the lymphocytes responsible for producing these antibodies are primed and ready to start production as soon as re-exposure occurs. We recognize that something similar occurs in the herring / VHSV system because we have found fully protected groups of fish with only 27% of the individuals demonstrating detectable levels of neutralizing antibodies. Our task in this project is to identify and quantify an immune system marker, specific to VHSV, that is a reliable indicator of prior exposure. We are hopeful that RT qPCR detections on the gills will provide this deductive ability. If this technique is not effective, we will move onto other specific immune markers that may include identification of activated lymphocytes or immunoglobulin T responses in herring. As the reviewer suggests, we plan to compare these responses in controlled situations using laboratory herring with known exposure histories and using wild herring from different various locations.

May 2021 EVOSTC Science Panel Comment: The proposed focus on ovivory in herring and *Ichthyophonus* transmission is appropriate. It is unclear if the pollock egg consumption (winter?) is of unfertilized or fertilized eggs (embryos) at spawning of developing embryos in the water column, please clarify. What will be used in experiments and how does this compare to natural exposures in PWS? Do embryos or developing pollock larvae need to be consumed for transmission, or does *Ichthyophonus* exist in ovarian eggs prior to spawning? The proposed research in this proposal appears to be duplicated in proposal 22220203; this needs to be reconciled.

PI Response: We will start the experimentation by using eggs collected from female pollock and herring ovaries, as this represents the most available source of eggs. These eggs will be assessed for the presence of Ichthyophonus and they will be fed out to SPF laboratory herring to attempt parasite transmission. We will also attempt to collect some naturally spawned and fertilized eggs from pollock and herring; however, the collection logistics are much more difficult to solidify using the available sampling efforts and platforms. We are less interested in sampling larval herring for Ichthyophonus, as we have no indication that the parasite demonstrates true vertical

transmission (i.e., is present inside the egg and infected the F1 generation). Rather, it is more likely that the parasite occurs on the outside of the chorion and is transmitted to adult herring that consume the parasite occurring out the outside eggs surface. From this perspective, we feel justified starting our investigations using eggs collected from inside the ovaries.

The apparent redundancy with the walleye pollock proposal (22220203) is an administrative artifact because that proposal is not part of GWA. The pollock proposal will provide all the pollock samples from the field and the Herring Disease Program (22120111-E) will provide all the laboratory diagnostics and experimentation. The Herring Disease Program was limited to a certain budget within the GWA program; therefore, the expanded efforts needed to accommodate the pollock diagnostics and experimentation are reflected by a modest staffing request for laboratory support in the walleye pollock proposal (22220203). This budget item will be better defined in the revised pollock proposal.

May 2021 EVOSTC Science Panel Comment: We appreciated the focus on sublethal impacts of disease and oil on herring. It is likely that this impact will be much greater than simply studying mortalities. The cross-generational effects of oil and disease exposure is exciting, as is the continued use of pathogen-free herring established by the PI.

We also greatly appreciate the effort and productivity of the PIs. However, there was some concern that PI Hershberger may be over-committed with collaborations and other efforts on collaborating proposals. It is suggested that the PI Hershberger describe percent effort on herring disease research in terms of what is proposed across all collaborative projects.

PI Response: The Science Panel's recognition of our scope of work is much appreciated. Indeed, the project is very expansive and cross-disciplinary. As we have mentioned before, the cross-disciplinary nature of the EVOSTC programs, including GWA provides a unique opportunity in the field of disease ecology. The typical impediment to addressing these comprehensive studies in disease ecology has been cost and the unavailability of interdisciplinary teams involving specialists in disease ecology, population assessment, food webs, genetics, toxicology, and ecology. In this case, these and other specialists reflect the fundamental pillars of GWA and other EVOSTC programs. We consider this a generational opportunity to address real issues in disease ecology and we plan to take full advantage of the opportunity.

Other Changes to the proposal since the initial submission:

Since this proposal was initially submitted, Dr. Groner accepted a new position at Bigelow Laboratories in Maine. Owing to her geographic and career change, a new junior scientist will be hired to assume a portion of the laboratory and field tasks. With her new position, Dr. Groner's contributions to the project be greatly diminished, and these changes are reflected in the revised project administration and budget. Briefly, she is no longer listed as a Co-PI; rather,

she will contribute as a PWSSC contractor during FY22 and FY23, during which time she will complete the two modelling exercises described in the proposal. Her involvement in the project will sunset after FY23. As a result of these administrative changes, some budgeting details have changed since the original submission:

- *a new subcontract is requested for Bigelow Labs for Dr. Groner (administered through the PWSSC) to accomplish the disease modelling exercises outlined in the proposal,*
- *requested personnel funding for the USGS Marrowstone Marine Field Station was reduced to accommodate the Bigelow contract.*

The budgeting changes are cost-neutral and, although annual budget requests have changed slightly, the total EVOS TC funding request for the 10-year Herring Disease Program remains the same.

September 2021 EVOSTC Science Panel Comment: The PIs have addressed questions adequately. The percentage effort for the primary PI (Hershberger) is not completely clear. We request that the PIs include the time commitment of the PIs that are on multiple proposals in terms of person months rather than the broad percentage provided in the table that is simply percentage for the overall project, not PI-specific. Based on previous track record, there is minimal concern regarding them accomplishing what they have proposed, even though it is quite ambitious.

PI response: Across all EOVSTC projects , the anticipated Hershberger contribution is 35%, including Project # 22120111-E: Herring Disease Program (20%), Project #22120111-C: Modeling and stock assessment of PWS herring (5%), Project #22220111-I: Ecological interactions between Pacific herring and Pacific salmon in Prince William Sound (5%), Project #22220203: Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions walleye pollock / herring interactions (5%).

September 2021 EVOSTC Science Panel Comment: Regarding ovivory, we understand that assessing unfertilized eggs collected from sexually mature females would be logistically more feasible, but some data on presence of parasites in oocytes from the ovary is needed and probably should have been presented or mentioned. We would like to know why samples of eggs and embryos from different species cannot be screened using molecular techniques in order to determine *Ichthyophonus* presence. It seems like this would be a good approach to determine the stage (oocyte, egg/embryo, late embryo) and the potential for transmission through ovivory. This would also provide some quantification in terms of exposure through diet when fed to lab herring. Collection of herring spawn over the first days to week of deposition seems critical and certainly seems feasible. For pollock, it seems that at least some sampling of unfertilized oocytes (from females collected) and some fertilized eggs/embryos from the environment should be sampled via the commercial fishery or agency observers and analyzed for *Ichthyophonus*. While

it is understandable that this effort is not easy, it is critical to the hypothesis if pollock is to be included in this proposal. Are the herring and pollock "egg cultures" where *Ichthyophonus* was recovered from unfertilized or fertilized eggs known?

PI response: *This is a great suggestion, and much-appreciated. We will make every effort to collect naturally fertilized eggs from the environment as well as eggs from the ovaries of the donor fish. We have a few concerns about the naturally fertilized eggs that we will have to be cognizant of. First, we do not know how long the parasite would survive on the released / fertilized eggs. If the parasite is highly labile outside the ovary, then we may not be able to detect it on eggs that have been outside the ovary for extended periods of time. From a transmission perspective, this lability is of minimal concern because the herring likely consume the eggs within minutes after they are released; however, lability would certainly influence our ability to detect the parasite on eggs that have been in the environment for extended periods. Second, we would like to steer clear of screening the eggs using molecular techniques because interpretation of a qPCR- positive sample would certainly be challenged in the peer reviewed literature. A qPCR positive would only determine whether a portion of the parasite genome is present; reviewers would justifiably argue that we detected nucleic acid from a dead parasite that was no longer infectious. This leaves parasite culture and histopathology as our available diagnostic tools. Culture is by-far the most sensitive diagnostic test (even more sensitive than qPCR); however, we will have to deal with overgrowth of environmental contaminants (esp. yeast and mold) in our broth media. Histopathology is by far the least sensitive diagnostic technique; so much so, that it is not worth exploring for this research question. However, we will work with our histopathologist to determine whether there may be a way to use chromogenic in situ hybridization on large quantities of whole eggs to screen for the parasite on the chorion of formalin-fixed eggs without having to embed and section.*

September 2021 EVOSTC Science Panel Comment: Finally, a component of this project relies on sample collection and analyses described in proposal 22220203. If the proposal 22220203 is not funded, the PIs will need to consider how they will include a pollock component in this proposal.

PI response: *Fortunately, the pollock proposal was funded.*

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	$193,776	$236,456	$227,886	$235,462	$301,248	$1,194,828	$100,528
Travel	$21,826	$21,826	$21,826	$21,826	$21,826	$109,130	$2,435
Contractual	$45,924	$45,924	$0	$0	$0	$91,848	$8,149
Commodities	$39,300	$39,000	$39,000	$39,000	$39,000	$195,300	$28,927
Equipment	$15,000	$0	$0	$0	$0	$15,000	$10,583
Indirect Costs (varies by proposer)	$0	$0	$0	$0	$0	$0	$0
SUBTOTAL	$315,826	$343,206	$288,712	$296,288	$362,074	$1,606,106	$150,622
General Administration (9% of subtotal)	$28,424	$30,889	$25,984	$26,666	$32,587	$144,550	N/A
PROGRAM TOTAL	**$344,250**	**$374,095**	**$314,696**	**$322,953**	**$394,661**	**$1,750,655**	
Other Resources (In-Kind Funds)	**$124,245**	**$127,724**	**$131,396**	**$135,129**	**$138,910**	**$657,404**	

COMMENTS:
This is the combined budget for PIs Hershberger and Paez at USGS and collaborator Groner at Bigelow Lab. The contract for collaborator Groner will run through PWSSC's NOAA grant. Please see attached budgets for details.

FY22-26	Project Number: 22120111-E Project Title: Herring Disease PI(s): Hershberger & Purcell (USGS) Collaborator: Groner (Bigelow)	SUMMARY TABLE

Spending is behind schedule because of delays in receiving the funds, staffing turnover at the Marrowstone Marine Field Station, and subsequent delays in hiring federal staff because of COVID-19. We are currently in the final stages of back-filling staff, and personnel spending is expected to catch up in FY23. Additional logistical delays occurred with funding transfers and hiring for the Bigelow contract; that work is expected to catch up in FY23 also.

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Education:

Ph.D. Fisheries, Université Laval, Québec, Canada: 2011
B.S. Monash University, Melbourne, Australia: 2004

Recent Positions

2022 – Present: Fish Biologist, U.S. Geological Survey, Western Fisheries Research Center, Marrowstone Marine Field Station

2018 – 2022: Research Associate, School of Aquatic and Fishery Sciences, University of Washington

2017 – 2018: Post Doc, University of Alabama, Department of Biological Sciences

2015 – 2017: Post Doc, Montana State University, Department of Biological Sciences

2011 – 2015: Post Doc, University of Chicago, Department of Ecology and Evolution

Ten Recent Publications Relevant to this Proposal:

Mihaljevic, J. R. & **Páez, D. J.** Systematic shifts in the variation among host individuals must be considered in climate-disease theory. *bioRxiv* (2021).

Páez, D. J., Powers, R. L., Jia, P., Ballesteros, N., Kurath, G., Naish, K. A. & Purcell, M. K. Temperature variation and host immunity regulate viral persistence in a salmonid Host. *Pathogens* **10,** 855 (2021).

Páez, D. J., LaDeau, S. L., Breyta, R., Kurath, G., Naish, K. A. & Ferguson, P. F. Infectious hematopoietic necrosis virus specialization in a multihost salmonid system. *Evolutionary applications* **13,** 1841–1853 (2020).

Páez, D. J. & Fleming-Davies, A. E. Understanding the evolutionary ecology of host–pathogen interactions provides insights into the outcomes of insect pest biocontrol. *Viruses* **12,** 141 (2020).

Páez, D. J. & Dodson, J. J. Environment-specific heritabilities and maternal effects for body size, morphology and survival in juvenile Atlantic salmon (*Salmo salar*): evidence from a field experiment. *Environmental biology of fishes* **100,** 209–221 (2017).

Páez, D. J., Dukic, V., Dushoff, J., Fleming-Davies, A. & Dwyer, G. Eco-evolutionary theory and insect outbreaks. *The American Naturalist* **189,** 616–629 (2017).

Mazé-Guilmo, E., Loot, G., **Páez, D. J.**, Lefèvre, T. & Blanchet, S. Heritable variation in host tolerance and resistance inferred from a wild host–parasite system. *Proceedings of the Royal Society B: Biological Sciences* **281,** 20132567 (2014).

Dodson, J. J., Aubin-Horth, N., Thériault, V. & **Páez, D. J.** The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews* **88,** 602–625 (2013).

Páez, D. J., Brisson-Bonenfant, C., Rossignol, O., Guderley, H., Bernatchez, L. & Dodson, J. Alternative developmental pathways and the propensity to migrate: a case study in the Atlantic salmon. *Journal of Evolutionary Biology* **24,** 245–255 (2011).

Roberge, C., **Páez, D. J.**, Rossignol, O., Guderley, H., Dodson, J. & Bernatchez, L. Genome-wide survey of the gene expression response to saprolegniasis in Atlantic salmon. *Molecular immunology* **44,** 1374–1383 (2007).

Five Additional Publications

Páez, D. J. & Fleming-Davies, A. E. Understanding the evolutionary ecology of host–pathogen interactions provides insights into the outcomes of insect pest biocontrol. *Viruses* **12,** 141 (2020).

Páez, D. J., Restif, O., Eby, P. & Plowright, R. K. Optimal foraging in seasonal environments: implications for residency of Australian flying foxes in food-subsidized urban landscapes. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373,** 20170097 (2018).

Páez, D. J., Dukic, V., Dushoff, J., Fleming-Davies, A. & Dwyer, G. Eco-evolutionary theory and insect outbreaks. *The American Naturalist* **189,** 616–629 (2017).

Páez, D. J., Giles, J., McCallum, H., Field, H., Jordan, D., Peel, A. J. & Plowright, R. K. Conditions affecting the timing and magnitude of Hendra virus shedding across pteropodid bat populations in Australia. *Epidemiology & Infection* **145,** 3143–3153 (2017).

Plowright, R. K., Manlove, K. R., Besser, T. E., **Páez, D. J.**, Andrews, K. R., Matthews, P. E., Waits, L. P., Hudson, P. J. & Cassirer, E. F. Age-specific infectious period shapes dynamics of pneumonia in bighorn sheep. *Ecology letters* **20,** 1325–1336 (2017).