



Exxon Valdez Oil Spill Trustee Council
Proposal Amendment Form

Project Number: 23120111-E

Project Title: Herring Disease Program

Principal Investigator(s): Paul Hershberger & David Paez, U.S. Geological Survey

Submission date: 4/28/2023

Changes to the original scope or objectives of the project.

Project 22220203, *Assessment of Prince William Sound walleye pollock with investigations into walleye pollock-Pacific herring interactions*, was cancelled because a vessel was not available to perform the proposed stock assessment. However, the project was divided into separate ADF&G and USGS components. We propose proceeding with a greatly reduced scope of work that excludes the stock assessment, bioenergetics, and associated work; the truncated project would involve only the pollock ovivory components outlined in the original proposal.

This amendment will not change the original scope or objectives of the project. The objectives of the original proposal include quantifying impacts on disease on herring, including the investigating the association of *Ichthyophonus* with walleye pollock eggs and attempt to transmit the parasite to herring by feeding with wild pollock eggs.

- i. To quantify impacts of disease on herring**
 - a. Sea lice (*Caligus clemensii*) infestations cause deleterious impacts to the health of Pacific herring.
 - b. Viral erythrocytic necrosis (VEN) negatively impacts the health and survival of Pacific herring.
 - c. The swimming performance Pacific herring is affected by their infection status
 - d. *Ichthyophonus* can be transmitted to Pacific herring through ovivory on eggs of conspecifics and walleye pollock.**

Background:

It has long been recognized that walleye pollock and other piscivorous fishes become infected with *Ichthyophonus* through the consumption of infected prey items, including Pacific herring. However, it is unknown how the parasite cycles back from these predators to planktivorous fishes, like Pacific herring. Because of this uncertainty, the fate of *Ichthyophonus* in these predatory fishes is considered a life cycle dead end, whereby the parasite life history is presumed to be terminated and an alternative (unknown) cycle exists to transmit the parasite back to herring. This project will evaluate whether the parasite presence in these fish predators is a dead-end or if the parasite cycles back to Pacific herring through the consumption of pollock eggs containing the parasite. This hypothesis is supported by reports in the Sea of Okhotsk where



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herring consumed 11.4% of all pollock eggs spawned (Gorbatenko et al. 2012) and by the detection of *Ichthyophonus* on the eggs of > 50% of female pollock in Shelikof Strait (Hershberger unpublished data). Having investigated several other potential routes of *Ichthyophonus* transmission to herring without success, including fish-to-fish transmission and intermediate / paratenic hosts, the ovivory hypothesis remains the most well-supported and parsimonious transmission hypothesis.

Procedural and Scientific Methods:

Samples will be collected through project 23170111-F.

The presence of *Ichthyophonus* on pollock eggs will be assessed by tissue (liver and egg) explant culture. Pollock liver and egg tissue samples are proposed to be collected from gravid adult female pollock collected by project 23170111-F (n = 60 females / yr). See proposal amendment for project 23170111-F. The association of the parasite in / on the eggs will be assessed using chromogenic in situ hybridization on the eggs to assess whether the eggs are infected, or whether the parasite occurs in a loose association with the outside of the egg. Third, the ability of eggs to transmit the parasite to herring will be determined by feeding wild pollock eggs to laboratory colonies of specific pathogen-free herring under controlled conditions.

Detection of *Ichthyophonus* in pollock eggs and in herring fed pollock eggs will follow existing USGS Marrowstone Marine Laboratory protocols, procedures, and methods (Hershberger et al. 2016). Statistical comparisons will consist of standard parametric statistical tests for each objective.

Personnel changes.

No additional PIs or personnel changes are necessary.

Budget reallocation request.

Funding for the USGS component of project 22220203 was already received by USGS before the ADF&G component was terminated. We request that the funds from 22220203 USGS component be transferred to 22120111-E. These funds, totaling \$50,413, were originally intended to cover one year of *Ichthyophonus* ovivory work and one year of bioenergetics studies for project 22220203. We are proposing to carry these funds into FY24 and FY25 for project 22120111-E and proceed with two years of *Ichthyophonus* ovivory work in lieu of the bioenergetics studies. No additional funds are requested by USGS, beyond those already received in FY23 (\$50,413).



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Budget Category:		Proposed FY 22	Proposed FY 23	Proposed FY 24	Proposed FY 25	Proposed FY 26	5- YR TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel		\$0	\$46,250	\$0	\$0	\$0	\$46,250	
Travel		\$0	\$0	\$0	\$0	\$0	\$0	
Contractual		\$0	\$0	\$0	\$0	\$0	\$0	
Commodities		\$0	\$0	\$0	\$0	\$0	\$0	
Equipment		\$0	\$0	\$0	\$0	\$0	\$0	
Indirect Costs	Rate = 0%	\$0	\$0	\$0	\$0	\$0	\$0	
SUBTOTAL		\$0	\$46,250	\$0	\$0	\$0	\$46,250	
General Administration (9% of subtotal)		\$0	\$4,163	\$0	\$0	\$0	\$4,163	N/A
PROJECT TOTAL		\$0	\$50,413	\$0	\$0	\$0	\$50,413	
Other Resources (In-Kind Funds)							\$0	

Amendment to 23120111-E is a funds transfer from the USGS component of 22220203.

Milestone/task changes.

Associated amendments to the Milestones and Tasks project for the Herring Disease Program (23120111-E) are included in the table below. Additionally, other deliverables involving genetic and oil exposure studies were removed from the Table because they were originally offered as a no-cost extension for Dr. Whitehead’s proposed project (22170115 Genetic and physiological mechanisms of virus and oil interactions in Pacific herring). Dr. Whitehead’s project was not approved so those deliverables were removed.

Project milestones and tasks by fiscal year and quarter, beginning February 1, 2023. Fiscal Year Quarters: 1= Feb.1-April 30; 2= May 1-July 31; 3= Aug. 1-Oct. 31; 4= Nov. 1-Jan 31.

Highlight the line item to indicate an addition of a milestone/task. ~~Strikethrough~~ a line item to indicate the removal of a milestone/task.

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone 1: Produce SPF herring	X	X			X	X			X	X			X	X			X	X		
Milestone 2: Annual herring health Assessments																				
Task 1: Collect samples	X				X				X				X				X			
Task 2: Complete Lab diagnostics		X				X				X				X				X		
Task 3: Provide Data for ASA model				X				X				X				X				X
Milestone 3: Determine the spatial scale of herd immunity																				



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Milestone/Task	FY22				FY23				FY24				FY25				FY26				
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
Task 1: Collect plasma samples from disparate areas (including Kayak Island)					X				X				X	X						X	X
Task 2: Process plasma samples in the laboratory							X				X				X						
Milestone 4: Evaluate other Indicators of prior VHSV exposure																					
Task 1: Laboratory study evaluating RTqPCR to deduce VHSV exposure history																					
Task 2: Field studies validating RTqPCR as a proxy for prior exposure																					
Task 3: Process RTqPCR samples in the lab																					
Milestone 5: Develop epidemiological models for VHS																					
Task 1: Develop SEIR model to evaluate herd immunity, temperature, recruitment, etc.				X																	
Task 2: Develop state-space model to estimate mortality due to VHS infection using the VHS seroprevalence data collected yearly at Sitka and PWS.								X													
Milestone 6: Determine effects of sea lice on herring																					
Task 1: Quantify louse infestations on wild herring															X	X				X	X
Task 2: Lab exposures with sea lice							X	X			X	X									



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Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone 7: Determine Impacts of VEN to herring																				
Task 1: Determine the effects of temperature on VEN progression	X	X	X	X																
Task 2: Field assessment of VEN in herring and pink salmon			X	X			X	X			X	X			X	X			X	X
Task 3: Produce GLM																				
Milestone 8: Evaluate swimming performance																				
Task 1: Build flume	X	X	X	X																
Task 2: <i>Ichthyophonus</i> swimming performance studies										X	X			X	X					
Task 3: VHSV swimming performance studies																		X	X	
Task 4: VEN swimming performance studies																				
Milestone 9: <i>Ichthyophonus</i> Transmission																				
Task 1: Survey pollock eggs for <i>Ichthyophonus</i>						X				X				X				X		
Task 2: Determine the provenance of eggs in herring stomachs																				
Task 3: Use CISH to assess the relationship of <i>Ichthyophonus</i> on eggs																				
Task 4: Assess whether <i>Ichthyophonus</i> can be found in association with consumed eggs																				
Task 5: Perform controlled transmission studies involving herring or pollock eggs	X					X				X				X				X		
Milestone 10: Evaluate in vivo effects of oil / VHSV interactions																				



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Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Objective 1: Characterize TGIP and perturbation by oil																				
Task 1a: procure animals for pilot exposures	X				X															
Task 1b: adult exposure/spawn	X				X															
Task 1c: embryo/larval assessments		X	X	X		X	X	X												
Objective 2: Test for population differences in TGIP and perturbation by oil																				
Task 2a: procure gametes for PWS/SS exposures	X																			
Task 2b: Rear PWS and SS fish to adulthood	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Task 2c: adult exposure/spawn																				
Task 2d: embryo/larval assessments																				
Objective 3: Test for genotypic selection by oil and/or virus																				
Task 3a: procure gametes from PWS and SS fish for replicate 1					X															
Task 3b: Embryo oil, juvenile virus challenge for replicate 1						X	X	X												
Task 3c: procure gametes from PWS and SS fish for replicate 2									X											
Task 3d: Embryo oil, juvenile virus challenge for replicate 2										X	X	X								
Task 3e: procure gametes from PWS and SS fish for replicate 3													X							
Task 3f: Embryo oil, juvenile virus challenge for replicate 3														X	X	X				
Task 3g: procure gametes from PWS and SS fish for replicate 4																	X			
Task 3h: Embryo oil, juvenile virus challenge for replicate 4																		X	X	X
Reporting																				
Annual reports					X				X				X				X			
Final report																				
Deliverables																				



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Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Peer reviewed paper		X		X		X		X		X		X		X		X		X		X
Data posted online				X				X				X				X				X

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone: Fieldwork																				
Aerial surveys	X	X			X	X			X	X			X	X			X	X		
Acoustics and disease support survey	X				X				X				X				X			
Pollock egg histopathology, <i>Ichthyophonus</i> culture, and laboratory exposure studies										X	X	X		X	X	X				
Milestone: Lab Analysis																				
Herring ASL sample processing	X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	
Milestone: Data																				
Quality control ASL data		X				X				X				X				X		
Quality control and editing of aerial shape files		X				X				X				X				X		
Analysis of aerial survey data			X				X				X				X				X	
Combing aerial survey shape files into historical version			X				X				X				X				X	
Upload previous FY data/metadata to workspace		X				X				X				X				X		
Distribute ASL sample summary			X				X				X				X				X	
Milestone: Reporting																				
Annual reports	X				X				X				X				X			
Summary reports				X				X				X				X				X
Final report																				
Deliverables																				
Data posted online		X				X				X				X				X		

Internal use only:
 Fiscal Year of Request: FY23
 Approved by ED? No Yes
 Requires TC review? No Yes
 Copy sent to fiscal managing agency(yes)? No Yes