FY16 PROJECT PROPOSAL SUMMARY PAGE Continuing, Multi-Year Projects

Project Title: PWS Herring Research and Monitoring: Herring Disease Program (HDP)

Project Period: February 1, 2016 – January 31, 2017

Primary Investigator(s): Paul Hershberger (USGS – Marrowstone Marine Field Station)

Study Location: Wild herring will be collected from NE PWS, laboratory studies will be performed at the

Marrowstone Marine Field Station

Project Website: http://pwssc.org/research/fish/pacific-herring/

Abstract*:

The *Herring Disease Program (HDP)* is part of a larger integrated effort, Prince William Sound Research and Monitoring (outlined in a separated proposal by Dr. Scott Pegau). Within this integrated effort, the *HDP* is intended to evaluate the impact of infectious and parasitic diseases on the failed recovery of the PWS herring population. The framework for the 2012 – 2016 *HDP* involves a combination of field surveillance efforts, field-based disease process studies, and laboratory-based controlled studies. Field surveillance efforts will provide continued and expanded infection and disease prevalence data for herring populations in Prince William Sound (PWS), Sitka Sound, and Puget Sound. During FY 2016 we will continue the health assessments of adult herring from Prince William Sound and Sitka Sound, we will continue to rear colonies of specific-pathogen-free Pacific herring for controlled studies in the laboratory, we will compare the relative sensitivities or four newly-developed diagnostic assays that are capable of identifying prior exposure to VHS virus in Pacific herring. Additionally, by employing the qPCR and chromogenic in situ hybridization tools that were developed as products of the HDP, we will begin searching for intermediate invertebrate hosts for *Ichthyophonus*.

Estimated Budget:

EVOSTC Funding Requested* (must include 9% GA):

FY12	FY13	FY14	FY15	FY16	TOTAL
		\$281.9K	\$291.9K	\$298.0K	\$871.8K

Non-EVOSTC Funds to be used:

FY12	FY13	FY14	FY15	FY16	TOTAL
				\$42.1K	

^{*}If the amount requested here does not match the amount on the budget form, the request on the budget form will considered to be correct.

Date: August 14, 2015

I. EXECUTIVE SUMMARY

Primary efforts completed during 2015 included submission of a review manuscript describing approximately 10 years of *Ichthyophonus* surveys in Pacific herring and a synthesis manuscript describing host and environmental characteristics that govern the epizootiology of VHS in Pacific herring. The latter manuscript is expected to provide the foundation for a mathematical model capable of forecasting potential VHS impacts in wild herring populations. Additional work included reporting an expansion of *Ichthyophonus* surveillance efforts in marine and freshwater fishes and a new description of the phylogeny this parasite, which indicates that multiple parasite species exist. Additionally, we have become successful at infecting Pacific herring by feeding with infected tissues, with transmission efficacy increasing through the use of multiple feedings. Finally, we have demonstrated that external signs of ichthyophoniasis on juvenile Pacific herring (i.e. pigmented ulcers on the flank) persist for extended periods and are not necessarily precursors of mortality. During 2016, we expect to finish the optimization of an antibody test capable of assessing prior exposure to VHSV, begin screening wild and laboratory-spiked zooplankton for *Ichthyophonus* using qPCR, and perform a controlled study intended to determine the reason(s) why *Ichthyophonus* can be easily transferred fish-to-fish in rainbow trout, but not in herring; for example, are these differences the result of water type (fresh / salt water), parasite genotype, or host species susceptibility. Fish health surveillances will continue in PWS and Sitka Sound, and will be expanded to include herring and other forage fishes in Puget Sound, WA.

2015 Publications:

- Hershberger, P.K., K.A. Garver, J.R. Winton. *Submitted*. Ecological Principles of viral hemorrhagic septicemia in wild marine fishes. Ecological Monographs.
- Purcell, M.K., S. Pearman-Gillman, R.L. Thompson, J.R. Winton, J.L. Gregg, L.M. Hart, E.J. Emmenegger, P.K. Hershberger. *In Review*. Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of a real-time PCR assay for quantification of ENV DNA. Journal of Veterinary Diagnostic Investigation.
- Gregg, J.L., R.L. Thompson, M.K. Purcell, C.S. Friedman, P.K. Hershberger. *In Review*. Phylogeny of parasites in the genus *Ichthyophonus*, and their prevalence in several fish hosts. Diseases of Aquatic Organisms.
- Hershberger, P.K., L.M Hart, A.M. MackKenzie, M.L. Yanney, C. Conway, D. Elliott. *Accepted*. Infecting Pacific herring with *Ichthyophonus sp*. in the laboratory. Journal of Aquatic Animal Health.
- Hart, L.M., C.M. Conway, D.G. Elliott, P.K. Hershberger. *Accepted*. Persistence of external signs in Pacific herring *Clupea pallasii* with ichthyophoniasis. Journal of Fish Diseases.
- Hershberger, P.K., J.L. Gregg, L.M. Hart, S. Moffitt, R. Brenner, K. Stick, E. Coonradt, T. Otis, J. J. Vollenweider, K. A. Garver, J. Lovy, T.R. Meyers. *In Press*. The parasite *Ichthyophonus* sp. in Pacific herring. Journal of Fish Diseases.
- Conway, C.M., M.K. Purcell, D.G. Elliott, P.K. Hershberger. 2015. Detection of *Ichthyophonus* by chromogenic *in situ* hybridization. Journal of Fish Diseases 38: 853-857.
- 4 additional manuscripts are currently in preparation; submission is anticipated in 2015

II. COORDINATION AND COLLABORATION

A. Within a EVOTC-Funded Program

- 1) Samples of wild herring are being collected in collaboration with both ADF&G spring herring assessment surveys and with surveillance efforts from the PWSSC.
- 2) Zooplankton samples are being provided by Dr. Rob Campbell in an effort to screen for intermediate hosts of *Ichthyophonus*.
- 3) Dr.'s Kristin Gorman and Scott Pegau (PWSSC) are providing monthly samples of juvenile herring from Cordova Harbor for heightened *Ichthyophonus* surveillance

4) Juvenile herring samples from PWS were provided by Yumi Arimitsu of Gulf Watch Alaska for additional *Ichthyophonus* sampling.

B. With Other EVOSTC-funded Projects

None

C. With Trustee or Management Agencies

- 1) Field samples are being processed by the ADF&G Fish Pathology Laboratory in Juneau
- 2) Samples of pre-spawn adult herring are provided by the very generous support of ADF&G herring biologists in Cordova and Sitka

III. PROJECT DESIGN – PLAN FOR FY15

A. Objectives for FY15

- Provision of disease prevalence data necessary for the ASA herring model
- Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS
- Collection of novel disease forecasting data
- Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships
- Compare sensitivity of recently-developed assays capable of identifying prior herring exposure to VHSV
- Collect and screen zooplankton for *Ichthyophonus*

B. Changes to Project Design

No changes in project design.

IV. SCHEDULE

A. Project Milestones for FY 16

Objective 1. Provision of disease prevalence data necessary for the ASA herring model *To be met by June 2016*

Objective 2. Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS

To be met by December 2016

Objective 3. Collection of novel disease forecasting data

To be met by June 2016

Objective 4. Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships *To be metamorphosed by August 2016*

Objective 5. Compare sensitivity of recently-developed assays capable of identifying prior herring exposure to VHSV

To be met by May 2016

Objective 6. Collect and screen zooplankton for *Ichthyophonus*

Collections will continue to occur throughout the year, processing by qPCR will start in the summer of 2016; any qPCR-positive samples will be confirmed by CISH by May 2016.

B. Measurable Project Tasks for FY 16

FY 16, 1st quarter (February 1, 2016 - April 31, 2016)

Field sampling, egg collections, laboratory experiments, manuscript preparation

FY 16, 2nd quarter (May 1, 2016-July 30, 2016)

Larval rearing, laboratory experiments, manuscript preparation

FY 16, 3rd quarter (August 1, 2016 – October 31, 2016)

Juvenile rearing, laboratory experiments, manuscript preparation

FY 16, 4th quarter (November 1, 2016- January 31, 2017)

Juvenile rearing, laboratory experiments, manuscript preparation

V. PROJECT PERSONNEL – CHANGES AND UPDATES

N/A

VI. BUDGET

A. Budget Forms

USGS

Budget Category:	Proposed	Proposed	Proposed	Proposed	Proposed	TOTAL	ACTUAL
	FY 12	FY 13	FY 14	FY 15	FY 16	PROPOSED	CUMULATIVE
Personnel	\$0.0	\$0.0	\$170,400.0	\$186,600.0	\$190,800.0	\$547,800.0	
Travel	\$0.0	\$0.0	\$17,000.0	\$17,000.0	\$18,400.0	\$52,400.0	
Contractual	\$0.0	\$0.0	\$12,000.0	\$12,000.0	\$12,000.0	\$36,000.0	
Commodities	\$0.0	\$0.0	\$46,000.0	\$39,000.0	\$39,000.0	\$124,000.0	
Equipment	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	
SUBTOTAL	\$0.0	\$0.0	\$245,400.0	\$254,600.0	\$260,200.0	\$760,200.0	
General Administration (9% of	\$0.0	\$0.0	\$22,086.0	\$22,914.0	\$23,418.0	\$68,418.0	N/A
General Administration (5% of	Ψ0.0	ψ0.0	Ψ22,000.0	Ψ22,914.0	Ψ23,410.0	ψου, 410.0	IN/A
PROJECT TOTAL	\$0.0	\$0.0	\$267,486.0	\$277,514.0	\$283,618.0	\$828,618.0	
Other Resources (Cost Share Funds)	\$0.0	\$0.0	\$0.0	\$0.0	\$42,100.0	\$0.0	

ADF&G

Budget Category:	Proposed	Proposed	Proposed	Proposed	Proposed	TOTAL	ACTUAL
	FY 12	FY 13	FY 14	FY 15	FY 16	PROPOSED	CUMULATIVE
Personnel	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	
Travel	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	
Contractual	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	
Commodities	\$0.0	\$0.0	\$13,200.0	\$13,200.0	\$13,200.0	\$39,600.0	
Equipment	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	
SUBTOTAL	\$0.0	\$0.0	\$13,200.0	\$13,200.0	\$13,200.0	\$39,600.0	
General Administration (9% of	\$0.0	\$0.0	\$1,188.0	\$1,188.0	\$1,188.0	\$3,564.0	N/A
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PROJECT TOTAL	\$0.0	\$0.0	\$14,388.0	\$14,388.0	\$14,388.0	\$43,164.0	
Other Resources (Cost Share Funds)	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	

B. Changes from Original Proposal

No Changes proposed

C. Sources of Additional Funding

\$42,100 In kind contribution from USGS includes salary and benefit contributions (20%) for P. Hershberger (\$26,400) and J. Gregg (\$15,700)