EVOSTC ANNUAL PROJECT REPORT

Recipients of funds from the *Exxon Valdez* Oil Spill Trustee Council must submit an annual project report in the following format by **Sept. 1 of each fiscal year** for which project funding is received (with the exception of the final funding year in which a final report must be submitted). **Please help ensure that continued support for your project will not be delayed by submitting your report by Sept. 1. Timely receipt of your report allows more time for court notice and transfer, report review and timely release of the following year's funds.**

Satisfactory review of the annual report is necessary for continuation of multi-year projects. Failure to submit an annual report by **Sept. 1** of each year, or unsatisfactory review of an annual report, will result in withholding of additional project funds and may result in cancellation of the project or denial of funding for future projects. **PLEASE NOTE:** Significant changes in a project's objectives, methods, schedule, or budget require submittal of a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.

Project Number: 10100132-I

Project Title: PWS Herring Survey: Herring Disease Program (HDP)

PI Name: Paul Hershberger

Time period covered: Oct. 1, 2010 – Sept. 1, 2011

Date of Report: ..... August 19, 2011

Report prepared by: ..... Paul Hershberger

Project website (if applicable): .....

Work Performed: Summarize work performed during the reporting period, including any results available to date and their relationship to the original project objectives. Explain deviations from the original project objectives, procedural or statistical methods, study area or schedule. Also describe any known problems or unusual developments, and whether and how they have been or can be overcome. Include any other significant information pertinent to the project.

This project represents an integration of PWS herring disease efforts with other herring efforts that are coordinated through the PWSSC. Additionally, this project is a logical extension and expansion of the previous project PWS herring Disease Program (#070819).

Field Surveillances of Infection and Disease Prevalence:
Adult and juvenile herring populations from Prince William Sound, Sitka Sound, Lynn Canal, and British Columbia were surveyed for the prevalence of primary pathogens during FY'11:

Prince William Sound pre-spawn adult herring

Location	Collection date	Length (mm)	n	*Ichthyophonus*	VEN	VHSV
St. Matthews Bay*	April 2, 2011	246	60	12%	0%	0%
Port Gravina	April 4 2011	219	60	27%	2%	0%
Hell's Hole	April 6, 2011	253	60	47%	2%	0%

*Samples from St. Matthews Bay were inadvertently frozen in transit by the airline; therefore, actual *Ichthyophonus* and VHSV prevalence may be higher than reported.

Prince William Sound juvenile herring

Location	Collection Date	Length (mm)	n	*Ichthyophonus*	VEN	VHSV
Simpson Bay	Nov 2, 2010	73	38	0%	6%	ND
Port Fidalgo	Nov 4, 2010	77	22	0%	5%	ND
Eaglik Bay	Nov 5, 2010	90	34	0%	26%	ND
Whale Bay	Nov 10-11, 2010	95	60	3.4%	18%	2%
Lower Herring	Mar 11, 2011	96	60	1.7%	23.3%	3%
Eaglik Bay	Mar 15, 2011	113	60	5%	1.7%	0%
Port Fidalgo	Mar 16, 2011	76	60	10.3%	13.3%	0%

Sitka Sound juvenile herring

Location	Collection Date	Length (mm)	n	*Ichthyophonus*	VEN	VHSV
Bear Cove	March 24, 2011	108	60	2%	7%	63%

Sitka Sound pre-spawn adult herring

Location	Collection Date	Length (mm)	n	*Ichthyophonus*	VEN	VHSV
Long Island	March 22, 2011	232	60	18%	0%	0%
Salsberry Isl.	April 6, 2011	228	60	20%	ND	ND

Lynn Canal adult herring

Collection Date	Length (mm)	n	*Ichthyophonus*
January 12, 2011	ND	60	2%
January 28, 2011	ND	60	10%
April 9, 2011	ND	60	18%
April 18 & June 4, 2011*	202	60	18%

* Herring sampled on April 18 and June 4 were cohabitated in a tank at the NOAA Ted Stevens Marine Science Laboratories prior euthanization and sampling for *Ichthyophonus*. Tank confinement likely had little impact on the reported prevalence because *Ichthyophonus* is not easily transferred between Pacific herring through cohabitation (Hershberger unpublished data).

British Columbia pre-spawn adult herring

Location	Collection Date	Mean length (mm)	n	*Ichthyophonus*
Strait of Georgia, Little Qualicum	March 1, 2011	180	60	23%
W. Coast Vancouver Island, Sydney Inlet	March 17, 2011	183	60	20%
Central Coast, Kwakshua Inlet	March 23, 2011	167	60	22%
Prince Rupert , Kitkatla area	March 24, 2011	194	60	27%
Queen Charlotte Isl's, Hail Gwaii	March 26, 2011	191	60	8.3%
Queen Charlotte Isl's, Haid Gwaii	March 30, 2011	190	60	5%

Laboratory Rearing of Specific Pathogen-Free Herring:
For the eighth consecutive year, we were successful at rearing specific pathogen-free (SPF), immunologically naïve Pacific herring in the laboratory at the USGS - Marrowstone Marine Field Station. Naturally deposited herring eggs were collected from adult herring spawning locations in Puget Sound, WA (Skagit Bay and Holmes Harbor). We currently maintain 4 age classes of SPF herring at the Marrowstone Marine Field Station, including age 0 (N ~ 30,000), age 1 (N = 4,839), age 4 (N = 204) and age 5 (N = 193); these fish continue to be utilized as test animals for empirical studies and for development of disease forecasting tools.

Laboratory Studies:

I. Results from laboratory-based empirical experiments provide insights into mechanisms that initiate epizootic cascades in populations of wild herring and have implications for the design of VHSV surveys in wild fish populations. Viral hemorrhagic septicemia virus, Genogroup IVa (VHSV) was highly infectious to Pacific herring (*Clupea pallasii* Valenciennes) even at exposure doses occurring below the threshold of sensitivity for a standard viral plaque assay; however, further progression of the disease to a population-level epizootic required viral amplification and effective fish-to-fish transmission. Among groups of herring injected with VHSV, the prevalence of infection was dose-dependent, ranging from 100%, 75%, and 38% after exposure to 19, 0.7, and 0.07 plaque-forming units (PFU) / fish, respectively. Among Pacific herring exposed to waterborne VHSV (140 PFU / mL), the prevalence of infection, geometric mean viral tissue titer, and cumulative mortality were greater among cohabitated herring than among cohorts that were held in individual aquaria where fish-to-fish transmission was prevented (Figure 1). Fish–to-fish transmission among cohabitated herring likely occurred via exposure to shed virus which peaked at 680 PFU/mL; shed virus was not detected in the tank water from any isolated individuals.

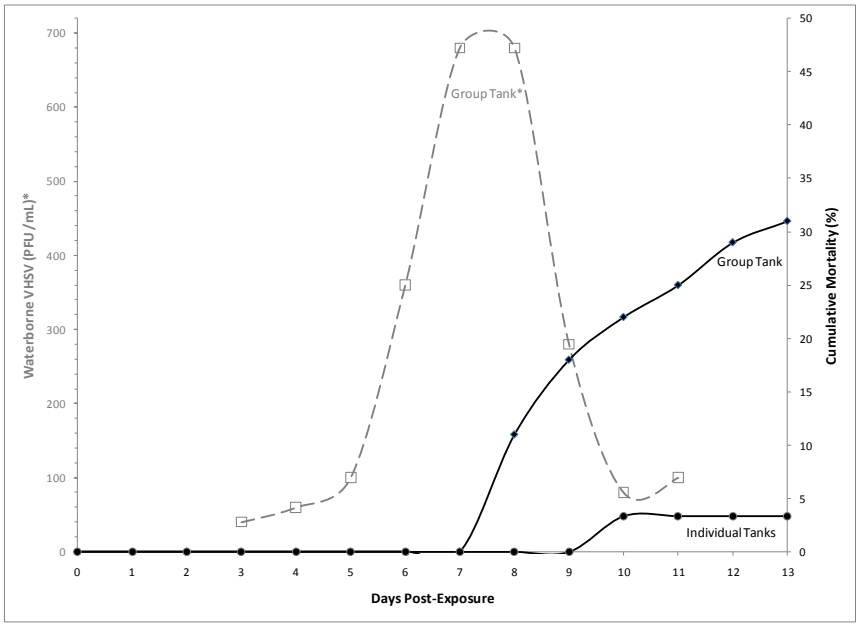

Figure 1. Cumulative mortality (solid lines) and waterborne virus titer (dashed line) in groups of VHSV-exposed Pacific herring either held together in a group tank (N=100) or in individual tanks (N=30). VHSV was isolated from the tissues of all mortalities in the group tank (n = 31) but not from the sole mortality in the individual tanks. * Waterborne VHSV was detected only in the group tank, not in any of the tanks containing individual herring.

II. Laboratory studies with viral erythrocytic necrosis (VEN) indicate that clinical signs of disease in herring do not necessarily correspond with viral load in the affected host. VEN is presumptively diagnosed by microscopic examination of blood films for the presence of a characteristic inclusion body within the cytoplasm of affected erythrocytes (Figure 2) or by observation of the causative iridovirus, erythrocytic necrosis virus (ENV; Figure 3), within erythrocytes using transmission electron microscopy. To better understand the kinetics of VEN, specific pathogen free Pacific herring *Clupea pallasii* were infected by intraperitoneal injection with ENV. At 1, 4, 7, 10, 14, 21 and 28 days post-infection, samples of blood, spleen and kidney tissues were collected and assessed for the number of cytoplasmic inclusion bodies in blood smears via light microscopy, and the number of virions within erythrocytes using transmission electron microscopy. The mean prevalence of cytoplasmic inclusion bodies in the blood cells increased from 0% at 0-4d post exposure to 94% at 28d. Viral load within circulating red blood cells peaked at 1 week post exposure, fell slightly, and then reached a plateau. However, blood cells observed within the kidney and spleen tissues reached high levels of ENV between days 14 and 28 (Table 1). The results indicated that virus load within erythrocytes did not correlate well with inclusion body prevalence and that the virus can persist in infected fish for more than 28 days.

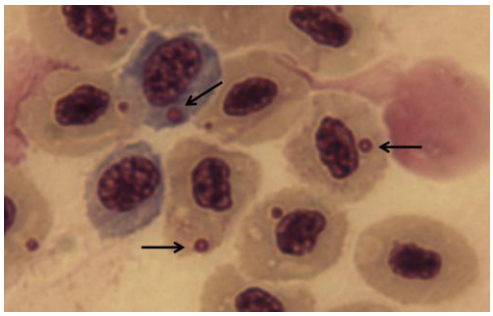

Figure 2. Geimsa stain of erythrocytic necrosis virus (ENV) infected erythrocytes and erythroblasts. Cytoplasmic inclusion bodies in some of the infected cells are indicated by the arrows. Uninfected cells are also present.

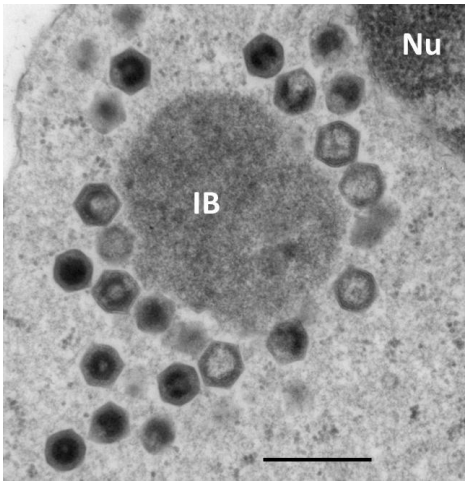

Figure 3. Electron micrograph of an ENV inclusion body (IB) surrounded by virions in the cytoplasm of a mature erythrocyte from a day 7 post-exposure blood sample. Virions are approximately 190nm in diameter. Nucleus (Nu) of the infected erythrocyte is present in the upper right corner. Bar represents 500nm.

Table 1.-Qualitative TEM assessment of virion intensity, determined by the average number of virions in virus positive cells per tissue type from 1 grid (containing 2-3 tissue sections per fish) was simultaneously measured by 3-4 investigators. No inclusion bodies or virions were found in the negative control fish tissue samples that were examined. Shaded regions indicated fish whose tissues (blood, kidney, and spleen) were examined via TEM. Each tissue was assigned to one of the following TEM virion intensity categories: -: no virus; +: 1-5 virions; ++: 6-15 virions; +++: 16-25 virions; ++++: >25 virions per grid.

Day Post Injection[a]	Prevalence of erythrocytes with inclusions	Blood	Kidney	Spleen
1	0			
1	0	-	-	-
1	0	-	-	-
1	0			
4	0			
4	1	-	-	-
4	0			
4	0	-	-	-
7	21	++++	-	++
7	6	++	-	-
7	15			
7	11			
10	28			
10	43			
10	16	++	-	-
10	46	+++	-	-
14	57	++	-	++
14	98			
14	98	++	++++	++++
14	60			
21	84	+++	++++	++++
21	100			
21	100	++	+	++
21	91			
28	95	++	++	++
28	96			
28	92			
28	92	+++	++	++++

III. Several Pacific herring genes associated with the innate immune response were identified, including the myxovirus resistance (Clpa-Mx), a major histocompatibility complex IB (named Clpa-UAA.001), the inducible immunoproteosome subunit 9 (Clpa-PSMB9) and the neutrophil chemotactic factor (Clpa-LECT2). Reverse transcriptase quantitative PCR (RT-qPCR) assays were developed based on these gene sequences to investigate the host immune response to acute viral hemorrhagic septicemia virus (VHSV) infection following both injection and immersion challenge. Virus levels were measured by both plaque assay and RT-qPCR and peaked at day 6 during the 10-day exposure period for both groups of fish. The interferon stimulated genes (Clpa-Mx, -UAA.001, and –PSMB9) were all up-regulated in response to VHSV infection at both 6 and 10 days post-infection in both spleen and fin (Figure 4). Results from these experiments indicate that Pacific herring mount a robust early antiviral response in both fin and spleen tissues. The immunological tools developed in this series of experiments will be useful for future studies to understand antiviral immunity in Pacific herring.

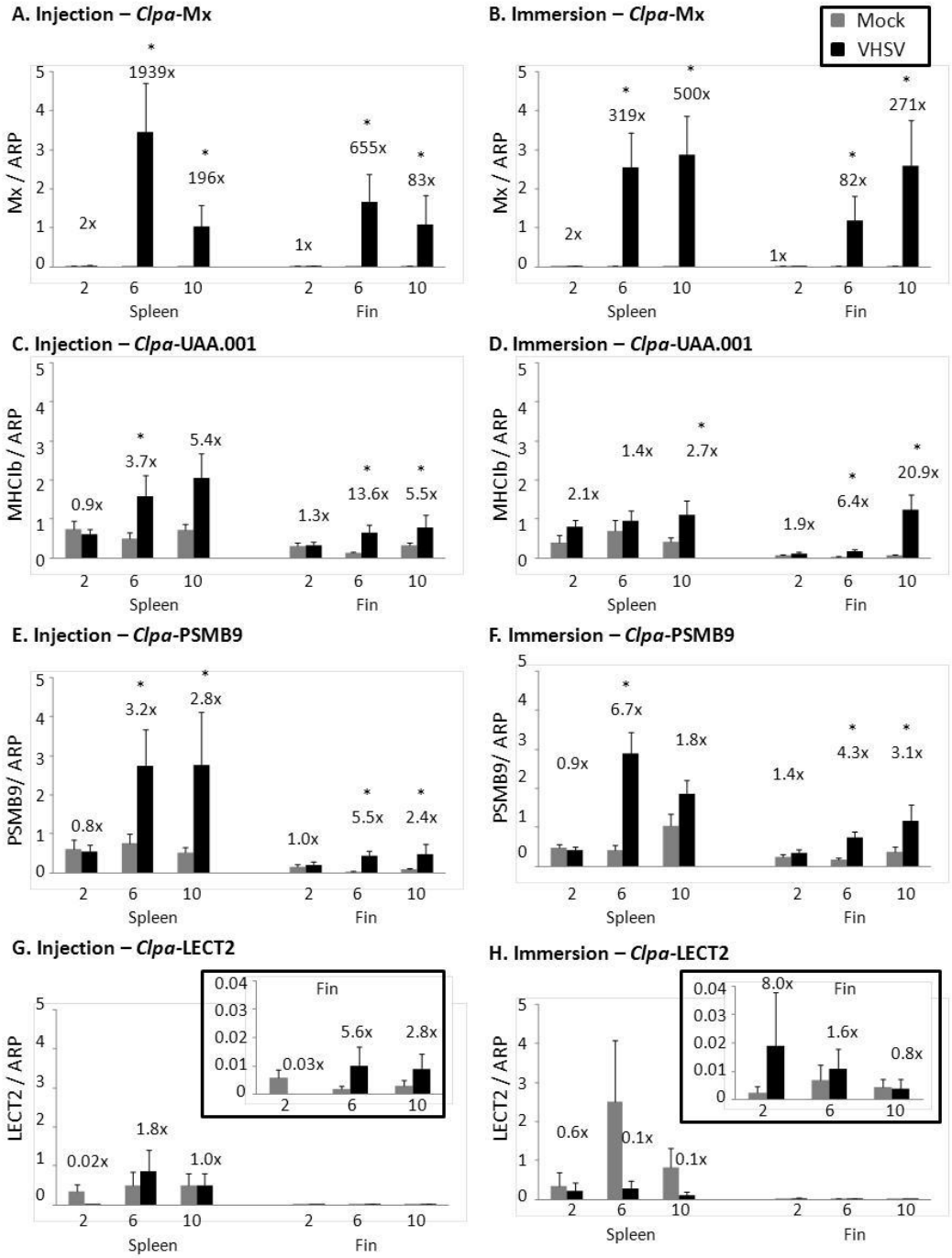

Figure 4. Normalized gene expression in Pacific herring given an intra-peritoneal injection VHSV or immersed in VHSV and sampled at 2, 6 and 10 days post-challenge. Grey columns indicate mock controls and black columns are VHSV infected. Fold change values (normalized VHSV expression / normalized mock control expression) are shown above the VHSV column. (A) *Clpa*-Mx expression following injection, (B) *Clpa*-Mx expression following immersion, (C) *Clpa*-UAA.001 expression following injection, (D) *Clpa*-UAA.001 expression following immersion, (E) *Clpa*-PSMB9 expression following injection, (F) *Clpa*-PSMB9 expression following immersion, (G) *Clpa*-LECT2 expression following injection, and (H) *Clpa*-LECT2 expression following immersion. Asterisks (*) indicate a significant difference ($P < 0.05$) between mock and VHSV groups.

IV. Additional laboratory studies indicate the influence of diet as a risk factor that influences the susceptibility of Pacific herring to VHS. Groups of specific pathogen-free (SPF) Pacific herring were highly susceptible to infection by *Viral hemorrhagic septicemia virus* (VHSV); however, the level of mortality was influenced by diet during the 40-70 d before, during, and after first exposure to the virus. Cumulative mortality was highest among herring maintained on an experimental soy-based pellet, intermediate among those maintained on a commercially available fish meal-based pellet, and lowest among those maintained on a second commercially available fish meal-based pellet containing β-glucans (Figure 5). Additionally, herring maintained on the experimental soy-based feed demonstrated retarded growth compared to those on the commercially-available feeds.

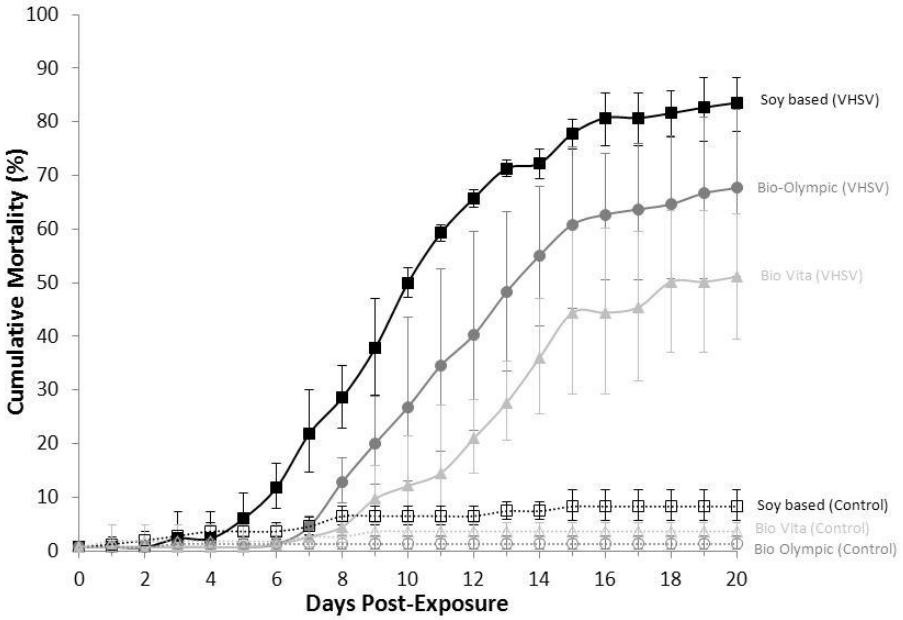

Figure 5. Experiment 2: Cumulative mortalities among groups fed with soy-based, Bio Vita, or Bio-Olympic diets 72d prior to VHSV exposure. Data points represent back-transformed percentages corresponding to the means of the arcsine-transformed proportions from the triplicate tanks. Error bars indicate 2 SD from the mean.

V. Neither dexamethasone nor temperature changes were successful at exacerbating chronic *Ichthyophonus* infections to overt disease and host mortality. Herring that survive the acute phase of ichthyophoniasis do not clear the infection; rather, they survive as carriers for extended periods. The fate of these carriers remains unknown. Here we tested the hypothesis that *Ichthyophonus* carriers, representing a large proportion of an adult herring population, may encounter suboptimal environmental conditions that result in exacerbation of this carrier state to active disease and mortality. This hypothesis was tested by developing a colony of herring that were *Ichthyophonus* carriers. Briefly, age 0 yr SPF herring were infected with *Ichthyophonus* by IP injection (~122 schizonts / fish); acute mortality occurred 20-80d post-exposure, afterwhich mortality plateaued and survivors were determined to be *Ichthyophonus* carriers. (Figure 6). After 139d, *Ichthyophonus* carriers and negative control groups were injected with either dexamethasone (an immunosuppressive drug) or saline and divided into 4 groups:

- *Ichthyophonus* carriers + dexamethasone
- *Ichthyophonus* carriers + saline
- Uninfected herring + dexamethasone
- Uninfected herring + saline.

Cumulative mortalities were not significantly different between any treatment groups (Figure 7), indicating that suppression of the cellular immune response by dexamethasone in *Ichthyophonus*-carriers did not result in increased host mortality.

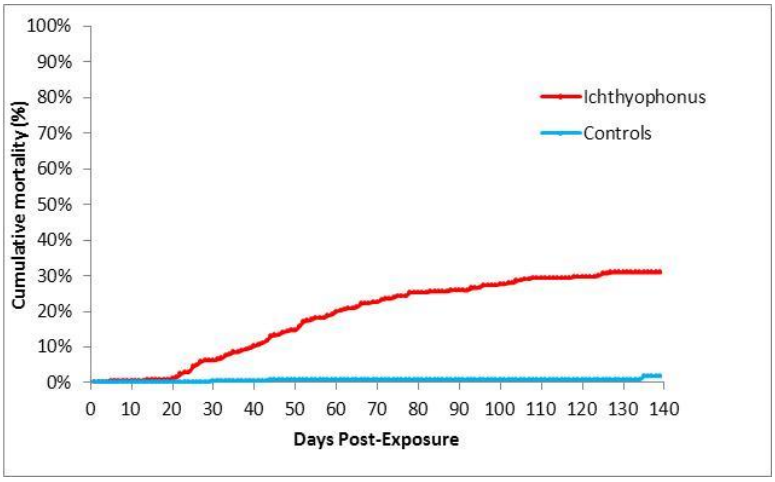

Figure 6. Mortality after exposure of Pacific herring to *Ichthyophonus*. All mortalities in the *Ichthyophonus*-exposed group (n = 105) cultured positive for *Ichthyophonus*; none of the mortalities (n =6) in the control group cultured positive.

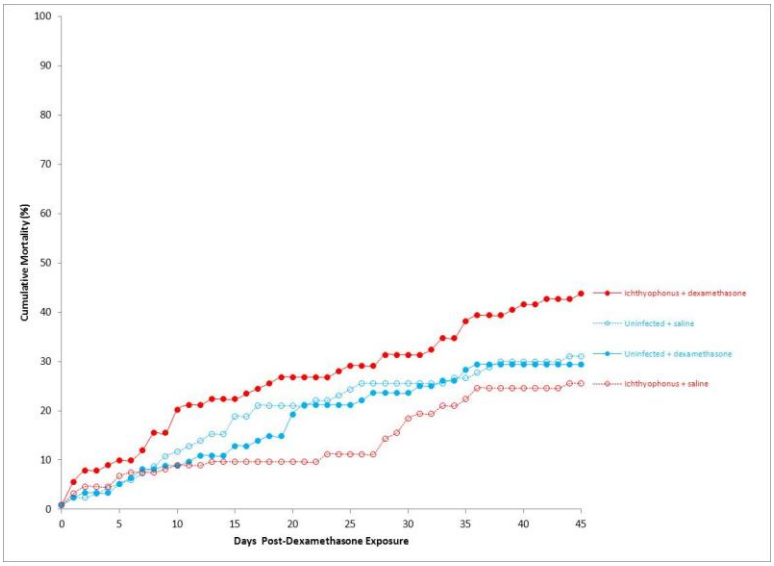

Figure 7. Mortality of *Ichthyophonus*-infected and – uninfected groups after exposure to dexamethasone or saline. Data points represent back-transformed means of arc sin transformed percentages from triplicate tanks per treatment; triplicate tanks each contained 29-30 herring.

To determine whether temperature adjustments result in exacerbation of chronic *Ichthyophonus* infections to acute disease and mortality, *Ichthyophonus* survivors from the dexamethasone experiment were transferred to different temperature treatments, including cold (~7°C), ambient (~10°C), and hot (~14°C). Differences in cumulative mortalities were not significant between any treatments (Figure 8), indicating that temperature manipulations did not exacerbate the progression of ichthyophoniasis from chronic host carriers.

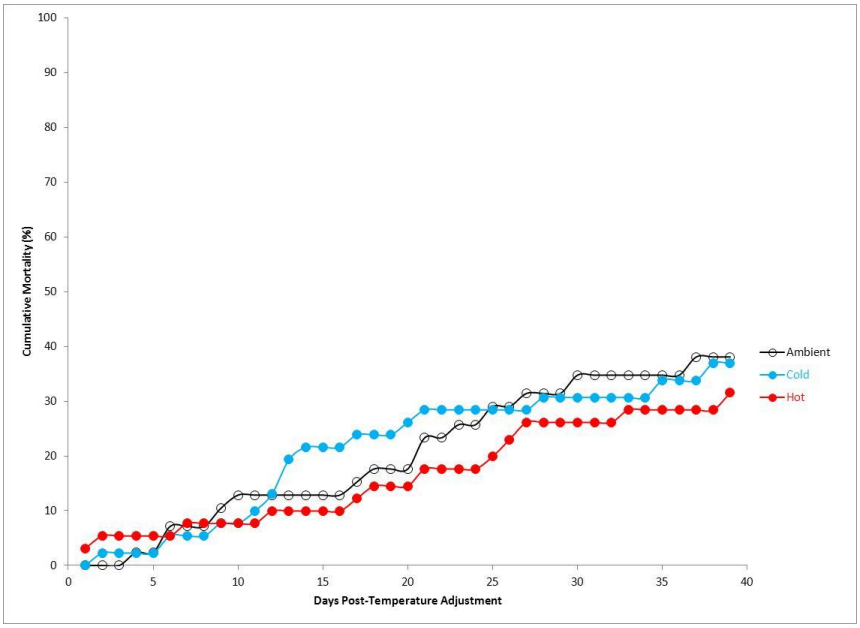

Figure 8. Effects of temperature manipulation on herring with chronic *Ichthyophonus* infections._Data points represent back-transformed means of arc sin transformed percentages from duplicate tanks per treatment; duplicate tanks each contained 15-22 herring. The experimental design also contained corresponding uninfected controls for each temperature (12-38% cumulative mortality, data not shown). Cumulative mortalities were not significantly different between any treatments.

Future Work: Summarize work to be performed during the upcoming year, if different from the original proposal. Describe any proposed changes in objectives, procedural or statistical methods, study area or schedule. ***NOTE****: Significant changes in a project's objectives, methods, schedule or budget require submittal of a new proposal subject to the standard process of proposal submittal, technical review and Trustee Council approval.*

No changes to the original work plan are anticipated during FY 2012.

Coordination/Collaboration: Describe efforts undertaken during the reporting period to achieve the coordination and collaboration provisions of the proposal, if applicable.

The field components of this project relied heavily on collaboration with local and state collaborations. Herring were collected by Jennifer Todd (PWSSC), Steve Moffitt Dr. Rich Brenner (ADF&G – Cordova), Eric Coonradt (ADF&G: Sitka), JJ Vollenweider, Ron Heintz, and Jeep Rice (Ted Stevens Marine Science Center in Juneau), Dr. Kyle Garver (DFO – Pacific Biological Station), Lorena Hamer and Dr. Jan Lovy (Herring Conservation and Research Society). Virology / parasitology samples for field surveillances were processed by the ADF&G Fish Pathology Laboratory in Juneau and the USGS, Marrowstone Marine Field Station.

Community Involvement/TEK & Resource Management Applications: Describe efforts undertaken during the reporting period to achieve the community involvement/TEK and resource management application provisions of the proposal, if applicable.

A herring disease seminar, hosted by the PWSSC was provided by Hershberger in Cordova and simulcast in Valdez. Five student interns and a post doc were partially supported by this project during FY'11.

Information Transfer: List (a) publications produced during the reporting period, (b) conference and workshop presentations and attendance during the reporting period, and (c) data and/or information products developed during the reporting period. ***NOTE:*** *Lack of compliance with the Trustee Council's data policy and/or the project's data management plan will result in withholding of additional project funds, cancellation of the project, or denial of funding for future projects.*

Publications

Hershberger. P.K.. *Submitted*. *Ichthyophonus* Disease (Ichthyophoniasis). In: American Fisheries Society, Fish Health Section Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens.

Beaulaurier, J., N. Bickford, J.L. Gregg, C.A. Grady, A. Gannam, J.R. Winton, P.K. Hershberger. *Submitted*. Susceptibility of Pacific herring *Clupea pallasii* to viral hemorrhagic septicemia (VHS) is influenced by diet. Journal of Aquatic Animal Health

Hansen, J.D., J.C. Woodson, P.K. Hershberger, C.A. Grady, M.K. Purcell. *Submitted*. Induction of anti-viral genes during acute infection with *Viral hemorrhagic septicemia virus* (VHSV) in Pacific herring *(Clupea pallasii*). Fish and Shellfish Immunology.

Glenn, J.A., E.J. Emmenegger, C. M. Conway, J. R. Winton, C.A. Grady, J.L. Gregg, S.E. Roon, P.K. Hershberger. *Submitted*. Kinetics of viral load and erythrocytic inclusion body formation in Pacific herring with viral erythrocytic necrosis (VEN). J. Fish Dis.

Hershberger, P.K., J.L. Gregg, C.A. Grady, L. Hart, S.E. Roon, J.R. Winton. *Accepted*. Factors controlling the early stages of viral hemorrhagic septicemia epizootics: low exposure levels, virus amplification, and fish-to-fish transmission. Journal of Fish Diseases.

Hershberger, PK, JL Gregg, CA Grady, SE LaPatra, JR Winton. *Accepted*. Passive immunization of Pacific herring *Clupea pallasii* against viral hemorrhagic septicemia. Journal of Aquatic Animal Health.

Kocan R, H Dolan, P Hershberger. 2011. Diagnostic methodology is critical for accurately determining the prevalence of *Ichthyophonus* infections in wild fish populations. Journal of Parasitology 97: 344-348.

Vollenweider, J.J., J. Gregg, R.A. Heintz, P.K. Hershberger. 2011. Energetic cost of *Ichthyophonus* infection in juvenile Pacific herring (*Clupea pallasii*). Journal of Parasitology Research.doi:1155/2011/926812, 10 pp.

Gregg J, J Vollenweider, C Grady, R Heintz, P Hershberger. 2011. Effects of environmental temperature on the dynamics of ichthyophoniasis in juvenile Pacific herring (*Clupea pallasii*). Journal of Parasitology Research. doi: 10.1155/2011/563412, 9pp.

Hart L, GS Traxler, KA Garver, J Richard, JL Gregg, CA Grady, G Kurath, PK Hershberger. 2011. Larval and juvenile Pacific herring *Clupea pallasii* are not susceptible to infectious hematopoietic necrosis under laboratory conditions. Dis, Aquat, Org. 93: 105-110.

Grady, C.A., J.L. Gregg, R.M. Collins, P.K. Hershberger. 2011. Viral Replication in Excised Fin Tissues (VREFT) corresponds with prior exposure of Pacific herring, *Clupea pallasii* (Valenciennes), to *viral haemorrhagic septicaemia virus* (VHSV). J. Fish Dis. 34: 34:-12.

Presentations

Kocan, R., P. Hershberger, L. Hart, S. LaPatra. September 26-30, 2011. Platform. Early Development of Ichthyophonus in two fish hosts; from circulating blood stage to fully mature tissue schizonts. 8th International Symp. on Fish Parasites. Viña del Mar, Chile

Hershberger, P.K., J.L. Gregg, C.A. Grady, L.M. Hart, S.R. Roon, K.A. Garver, J.R. Winton. June 14-16, 2011. Platform. Effects of temperature on the susceptibility of Pacific herring to viral hemorrhagic septicemia (VHS). 52nd Western Fish Disease Workshop & AFS Fish Health Section Meeting. Nanaimo, British Columbia, Canada. (Presented).

Hart, L.M., N. Lorenzen, S.E. LaPatra, C.A. Grady, S.E. Roon, J.L. Gregg, P.K. Hershberger. June 14-16, 2011. Platform. Efficacy of a DNA vaccine against VHS for Pacific herring. 52nd Joint Western Fish Disease Workshop & AFS Fish Health Section Meeting. Nanaimo, British Columbia, Canada.

Lovy,J., K.A. Garver, L. M. Hawley, L.M. Hart, P. K. Hershberger. June 14-16, 2011. Platform . Diseases of wild-captured and confined Pacific herring from British Columbia and their susceptibility to viral hemorrhagic septicemia. 52nd Joint Western Fish Disease Workshop & AFS Fish Health Section Meeting. Nanaimo, British Columbia, Canada.

Gregg, J.L., M.K. Purcell, P.K. Hershberger, C.S. Friedman. June 14-16, 2011. Platform . Analysis of rDNA internal transcribed spacer sequence from the parasite Ichthyophonus hoferi suggests closely related genotypes in several hosts from the northeast Pacific and northwest Atlantic. 52nd Joint Western Fish Disease Workshop & AFS Fish Health Section Meeting. Nanaimo, British Columbia, Canada.

Glenn, J.A., W.N. Batts, C.A. Grady, J.L Gregg, S.E. Roon, J.R. Winton, P.K. Hershberger, E.J. Emmenegger. June 14-16, 2011. Platform . Development of a molecular diagnostic assay to detect erythrocytic necrosis virus (ENV) in Pacific herring. 52nd Joint Western Fish Disease Workshop & AFS Fish Health Section Meeting. Nanaimo, British Columbia, Canada.

Kocan, R.M., P. Hershberger. June 1-3, 2011. Platform. Experimental evidence for the existence of a circulating blood stage of *Ichthyophonus* sp. in orally exposed Pacific staghorn sculpins (*Leptocottus armatus*). 86th Annual meeting of the American Society of Parasitologists. Anchorage, AK.

Zuray, S., R.M. Kocan, P. Hershberger. June 1-3, 2011. Poster. Long-term epidemiological trends in Ichthyophonus sp.-infected Chinook salmon (*Oncorhynchus tshawytscha*) in the Yukon River, Alaska; 1999 – 2010. 86th Annual meeting of the American Society of Parasitologists. Anchorage, AK.

Hershberger, P.K., J.R. Winton. February 13-18, 2011. Platform. An empirical approach to understanding the ecology of a viral disease affecting Pacific herring. American Society of Limnology and Oceanography. San Juan, Puerto Rico. (Presented). Also invited participant in a National Science Foundation-sponsored disease ecology workshop (February 11-12, 2011).

Hershberger, P., M. Purcell, J. Gregg, C. Grady, J. Winton. January 17-21, 2011. Platform. Development of tools to forecast the potential for viral hemorrhagic septicemia epizootics in Alaskan herring. Alaska Marine Science Symposium. Anchorage, AK. (Presented)

Gregg, J., C. Grady, P. Hershberger. January 17-21, 2011. Platform. Inability to demonstrate horizontal transmission of the highly pathogenic parasite *Ichthyophonus* from laboratory-infected Pacific herring (*Clupea pallasii*) to naïve conspecifics. Alaska Marine Science Symposium. Anchorage, AK.

Budget: Explain any differences and/or problems between actual and budgeted expenditures, including any substantial changes in the allocation of funds among line items on the budget form. Also provide any new information regarding matching funds or funds from non-EVOS sources for the project. ***NOTE:*** *Any request for an increased or supplemental budget must be submitted as a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.*

Budget expenditures are proceeding as per projections; no problems are anticipated.

We can accept your annual report as a digital file (Microsoft Word or WordPerfect), with all figures and tables embedded. Acrobat Portable Document Format (PDF) files (version 4.x or later) are also acceptable; please do not lock PDF files or include digital signatures.

Please submit reports electronically in ProjectView or by email to catherine.boerner@alaska.gov. Also, please be sure to post your annual report on your own website, if you have one.

We appreciate your prompt submission of your annual report and thank you for your participation.