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: 070819

Project Title: PWS Herring Disease Program

PI Name: Paul Hershberger

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

Date of Report: ..... September 1, 2010

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.

Laboratory Rearing of Specific Pathogen-Free Herring:

For the seventh 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 (Squaxin Pass and Cherry Point). We currently maintain 4 age classes of SPF herring at the Marrowstone Marine Field Station, including age 0 (n ~ 30,000), age 1 (n = 120), age 3 (n = 505) and age 4 (n = 242); these fish continue to be utilized as test animals for empirical studies and for development of disease forecasting tools.

Laboratory Studies:

Procedures for a viral replication in excised fin tissue (VREFT) assay were adapted to Pacific herring (*Clupea pallasii)* and optimized to both reduce processing time and provide the greatest resolution between naïve herring and those previously exposed to viral hemorrhagic septicemia virus (VHSV), Genogroup IVa. The optimized procedures included removal of the left pectoral fin from a euthanized fish, inoculation of the fin with $>10^5$ plaque forming units (PFU) mL^{-1} VHSV for 1 h, rinsing the fin in fresh medium six times to remove unadsorbed virions, incubation of the fin in fresh medium for 4 d, and enumeration of the viral titer in a sample of the incubation medium by plaque assay. The optimized VREFT assay was effective at identifying the prior exposure history of laboratory-reared Pacific herring to VHSV. The geometric mean VREFT value was significantly greater ($P < 0.01$) among naïve herring (1.2×10^3 PFU mL^{-1}) than among groups that survived exposure to VHSV ($1.0\text{-}2.9 \times 10^2$ PFU

mL^{-1}); additionally, the proportion of cultures with no detectable virus was significantly greater ($P = 0.0002$) among fish that survived exposure to VHSV (39 - 47 %) than among naïve fish (3.3 %); Figure 1. The optimized VREFT assay demonstrates promise for identifying VHSV exposure history and forecasting disease potential in populations of wild Pacific herring.

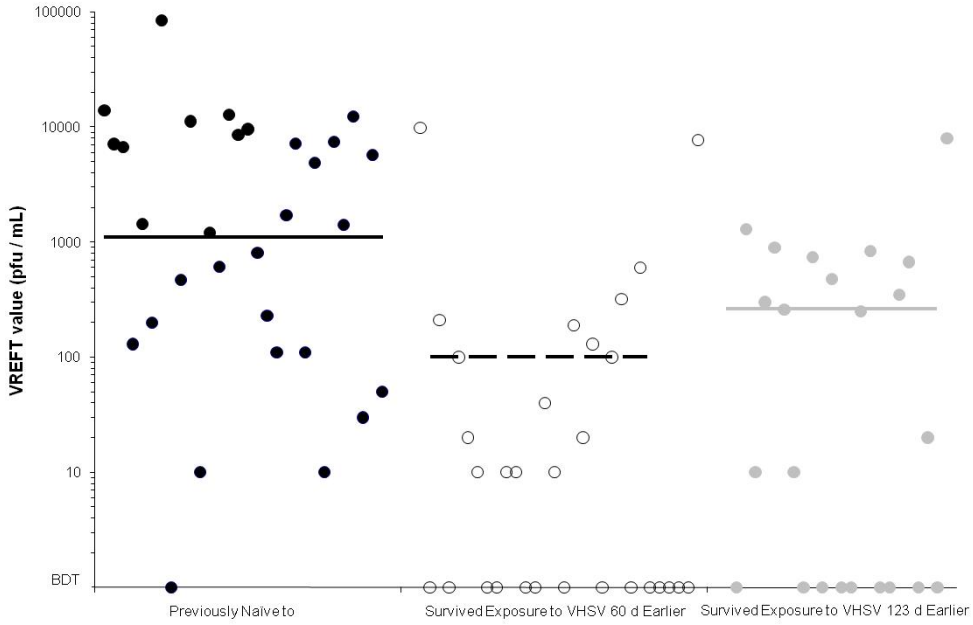

Figure 1. VREFT values from three groups of herring with different VHSV exposure histories. Data points indicate VREFT values for the individual replicates and horizontal lines (solid black, dashed black, and solid gray) indicate the geometric means for each of the three treatment groups. Samples with VREFT values below the detection threshold (10 PFU mL^{-1}) were assigned BDT.

A passive immunization assay was developed that is effective at determining the exposure history of Pacific herring to VHSV. When injected into the body cavity of highly susceptible Pacific herring, donor plasma from VHS survivors was highly effective at conferring protection against the disease. After VHSV exposure, survival in the group injected with plasma from VHS survivors (treatment; 50%) was significantly greater ($P < 0.025$) than that of herring injected with plasma from VHSV-naïve individuals (positive control; 6%; Figure 2). Additionally, the geometric mean viral tissue titer among VHSV-positive herring (mortalities and survivors), was significantly lower ($P < 0.001$; one-tailed t-test) in the treatment group (1.5×10^5 PFU g^{-1}) than in the positive control (3.7×10^6 PFU g^{-1}). Survival in the treatment group (50%) was not significantly different ($p = 0.10$) from that of the group exposed to saline in lieu of VHSV (negative control; 81%) and virus was not isolated from the tissues of any herring (mortalities or survivors) in the negative control group. Because it was effective at identifying the exposure history of herring to VHSV, *we* will be advancing the passive immunization technique in 2011 to determine whether it can forecast the potential for VHS epizootics in wild populations.

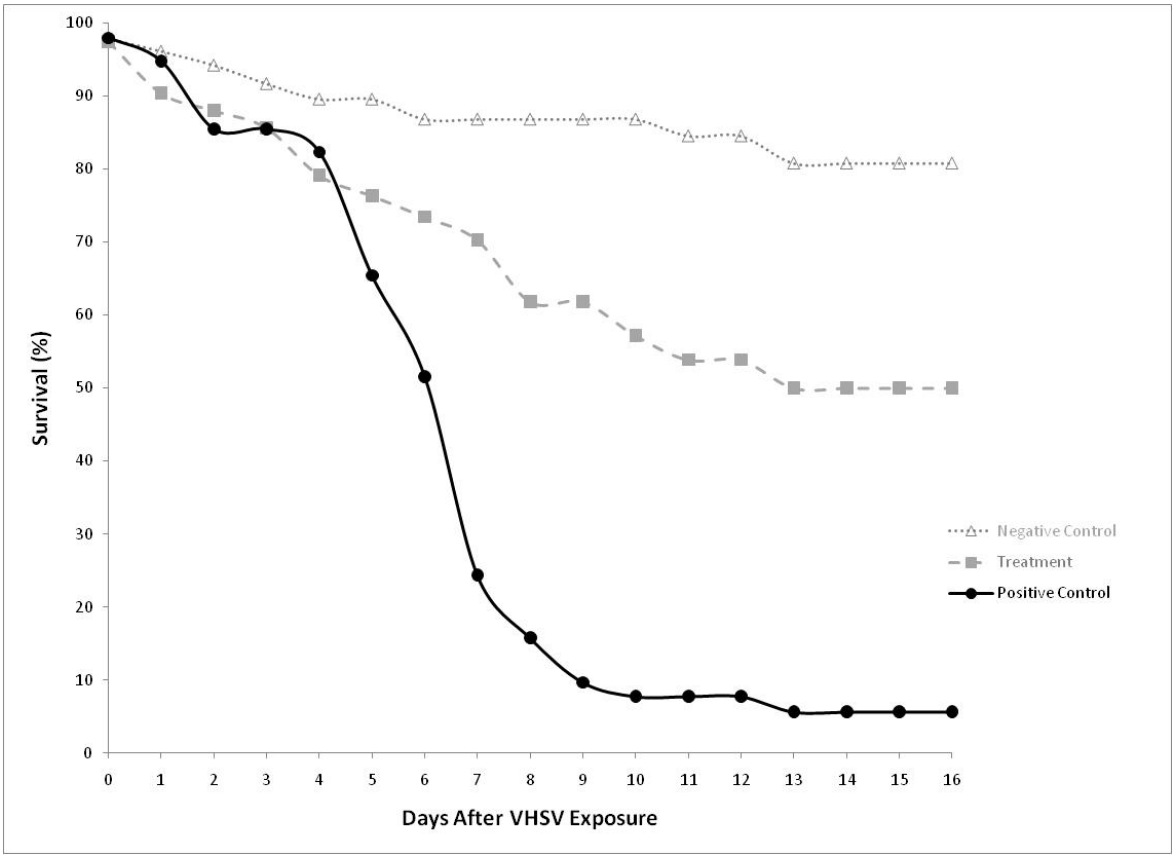

Figure 2. Survival after VHSV exposure to passively-immunized Pacific herring. Positive controls consisted of SPF Pacific herring that were passively immunized using serum from previously-naïve herring, then injected with VHSV. Treatments consisted of SPF Pacific herring that were passively immunized using serum from VHS survivors (approximately 28 months post-exposure), then injected with VHSV. Negative controls consisted of SPF Pacific herring that were passively immunized using serum from VHS survivors, then injected with saline. Data points represent back-transformed percentages corresponding to the means of arc sin transformed proportions from triplicate tanks (n = 10-13 herring / tank).

Chronic viral hemorrhagic septicemia virus (VHSV) infections were established in a laboratory stock of Pacific herring when held in a large-volume tank supplied with pathogen-free seawater at temperatures ranging from 6.8 to 11.6 °C. The infections were characterized by viral persistence for extended periods and near-background levels of host mortality (Figure 3). Infectious virus was recovered from mortalities occurring up to 167 d post-exposure and was detected in normal-appearing herring for as long as 224 d following initial challenge. Geometric mean viral titers were generally as high as or higher in brain tissues than in pools of kidney and spleen tissues with overall prevalence of infection being higher in the brain. Upon re-exposure to VHSV in a standard laboratory challenge, negligible mortality occurred among groups of herring that were either chronically infected or fully recovered, indicating that survival from chronic manifestations conferred protection against future disease. However, some survivors of chronic VHS infections were capable of replicating virus upon re-exposure. Demonstration of a chronic manifestation of VHSV infection among Pacific herring maintained at ambient seawater temperatures provides insights into the mechanisms by which the virus is maintained among populations of endemic hosts.

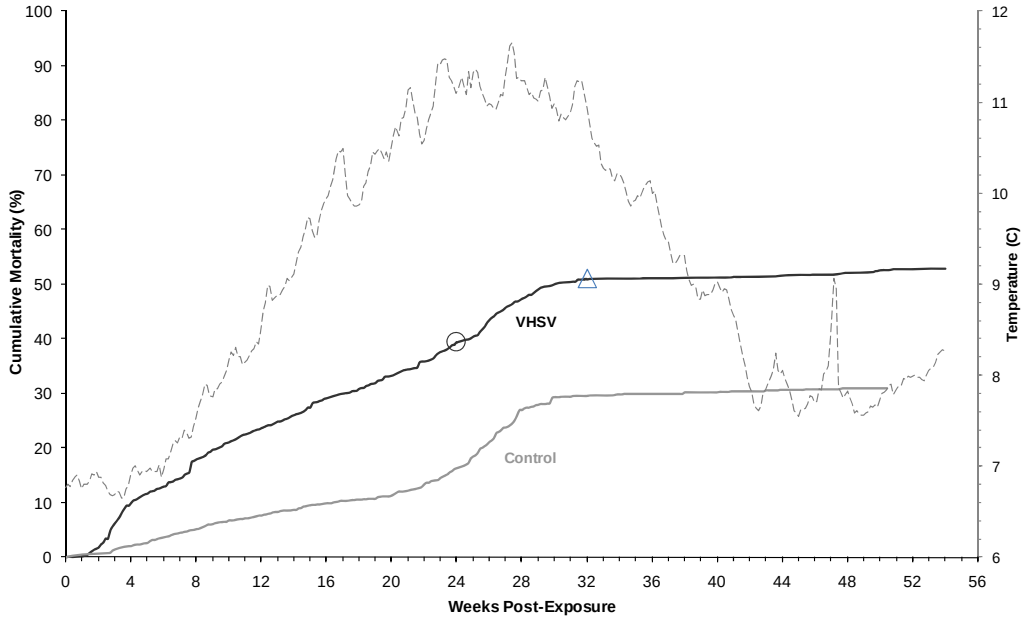

Figure 3. Chronic mortality following exposure of a colony of Pacific herring *Clupea pallasii* to VHSV in a large tank. Circle indicates the occurrence of the last VHSV-positive mortality (167d post-exposure). Triangle indicates the last day when positive survivors were known to exist in the exposed colony (224 d post-exposure). Dashed line indicates the daily ambient temperature in the tanks, reported as the daily mean of temperatures that were logged every 30 min.

Application of standard tissue clearing and histochemical staining techniques demonstrated that the distribution of *Ichthyophonus* schizonts in the skeletal muscle of infected fishes is not uniform; rather the parasite occurs more frequently in some sectors of the flank than others and often appears in non-randomly-distributed clusters, especially in the dark muscle (Figure 4). Non-random distribution of the parasite within infected host tissues likely accounts for differential sensitivities reported for different diagnostic techniques. For example, standard histological evaluation of 5 µm thick tissue sections detected only 47-59% of low-level and 84-92% of high-level *Ichthyophonus* infections in wild Chinook salmon. These results, when combined with similar false negatives reported using other diagnostic techniques, indicate that in vitro culture of tissue explants represents the most sensitive technique for detecting *Ichthyophonus* infections. As such, in vitro culture should be considered the preferred diagnostic technique for determining infection prevalence of *Ichthyophonus* in wild fish populations.

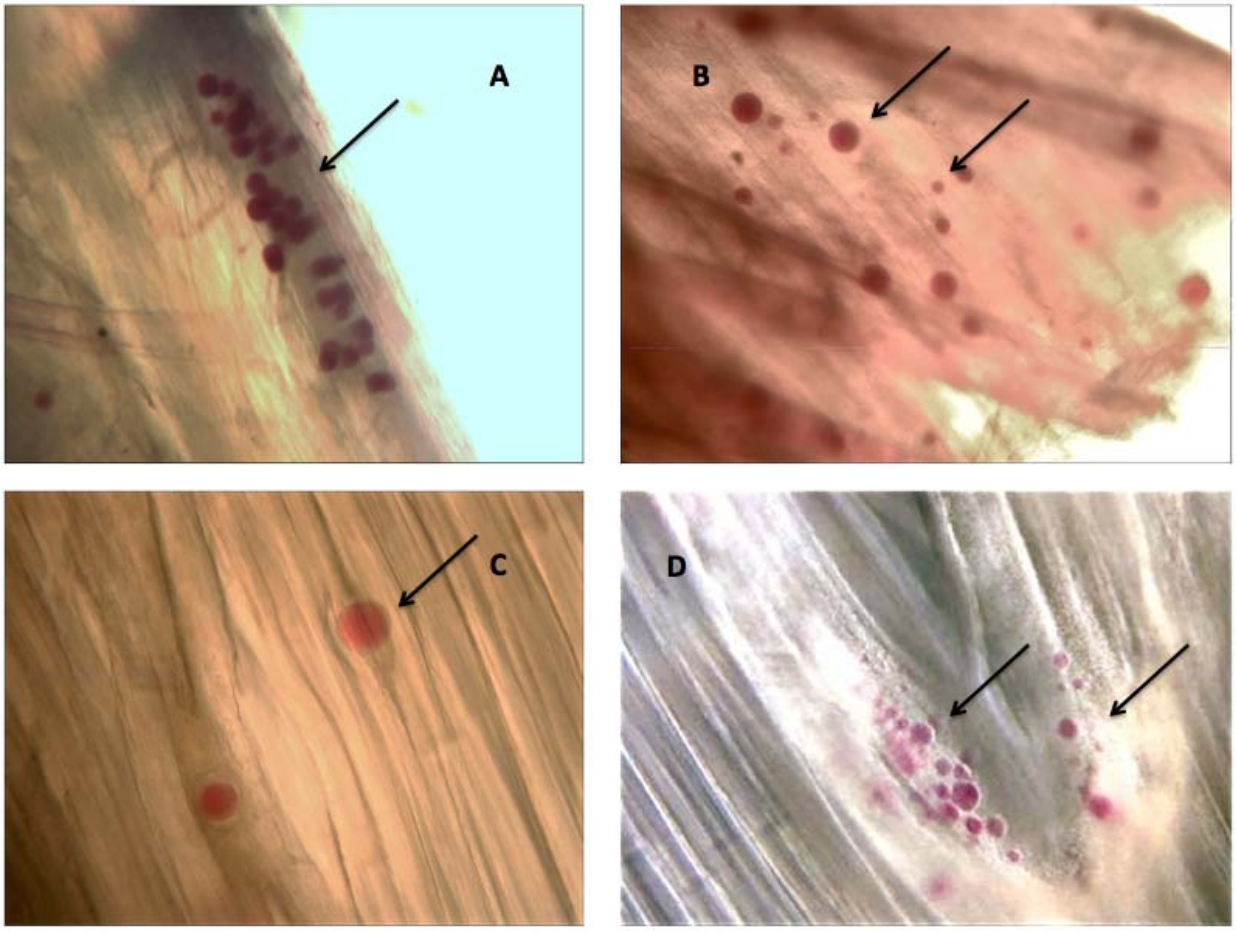

Figure 4. 3-dimentional view of *Ichthyophonus* schizonts in situ from different Pacific herring.
A- Clustering of similar size schizonts in the dark muscle (arrow). B- random distribution of various size schizonts throughout white muscle (arrows). C- Low-density of mature schizonts. D- Clustering of various sized schizonts in white muscle. Schizont diameter: 42 µm to 117 µm (stain – PAS following clearing).

Specific pathogen-free (SPF) larval and juvenile Pacific herring *Clupea pallasii* were not susceptible to infection by infectious hematopoietic necrosis virus (IHNV) by waterborne immersion in 10^{3-4} plaque forming units (pfu) of virus mL^{-1}. Cumulative mortalities among exposed groups were not significantly different from those of negative control groups (Figure 5). After waterborne exposure, IHNV was transiently recovered from the tissues of larvae but absent in tissues of juveniles. Additionally, no evidence of viral shedding was detected in the tank water containing exposed juveniles. After intraperitoneal (IP) injection of IHNV in juvenile herring with 10^3 pfu, IHNV was recovered from the tissues of sub-sampled individuals for only the first 5d post-exposure. The lack of susceptibility to overt disease and transient levels of IHNV in the tissues of exposed fish indicate that Pacific herring do not likely serve a major epizootiological role in perpetuation of IHNV among free-ranging sockeye salmon *Oncorhynchus nerka* and farmed Atlantic salmon *Salmo salar* in the NE Pacific.

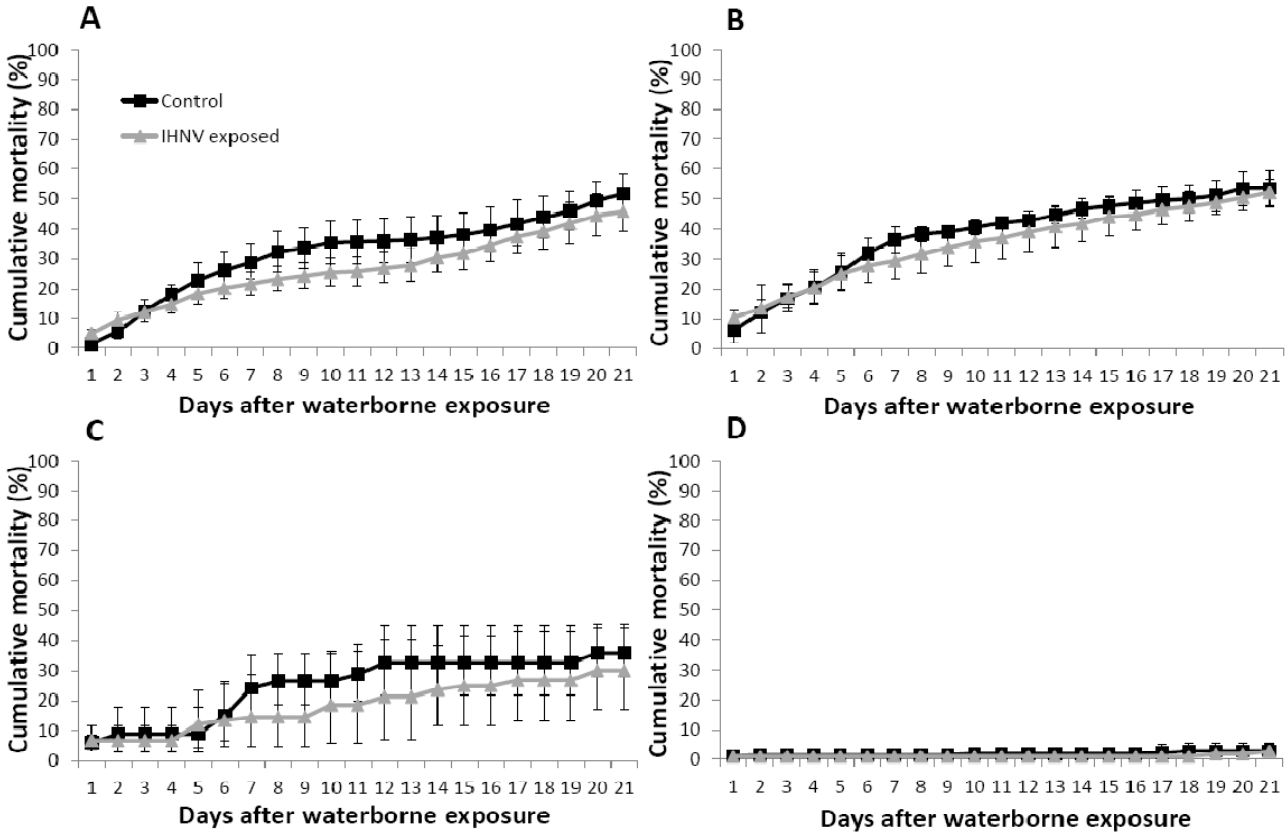

Figure 5. Susceptibility of Pacific herring to IHNV. A. Age 9d larvae. B. Age 56d larvae. C. Age 63d larvae. D. Age 1+ yr juveniles. Percent mortality represents the back-transformed mean of the transformed data from the five replicates for each group. Error bars represent one standard deviation from the mean.

A series of laboratory experiments was performed to determine the effects of *Ichthyophonus* infections on juvenile herring subjected to simulated over-wintering fast. These experiments were carried out in three temperature treatments, and in addition to defining input parameters for a herring bioenergetics model, they provided new insights into temperature effects on disease kinetics. Temperature manipulation had little effect on herring with established disease. In newly infected herring *Ichthyophonus* mortality was suppressed by both elevated and lowered temperatures. As initial parasite load was reduced an indirect relationship was detected between temperature and infection prevalence (Figure 6). These phenomena are likely the result of an interplay between temperature optima for parasite growth and host immune function.

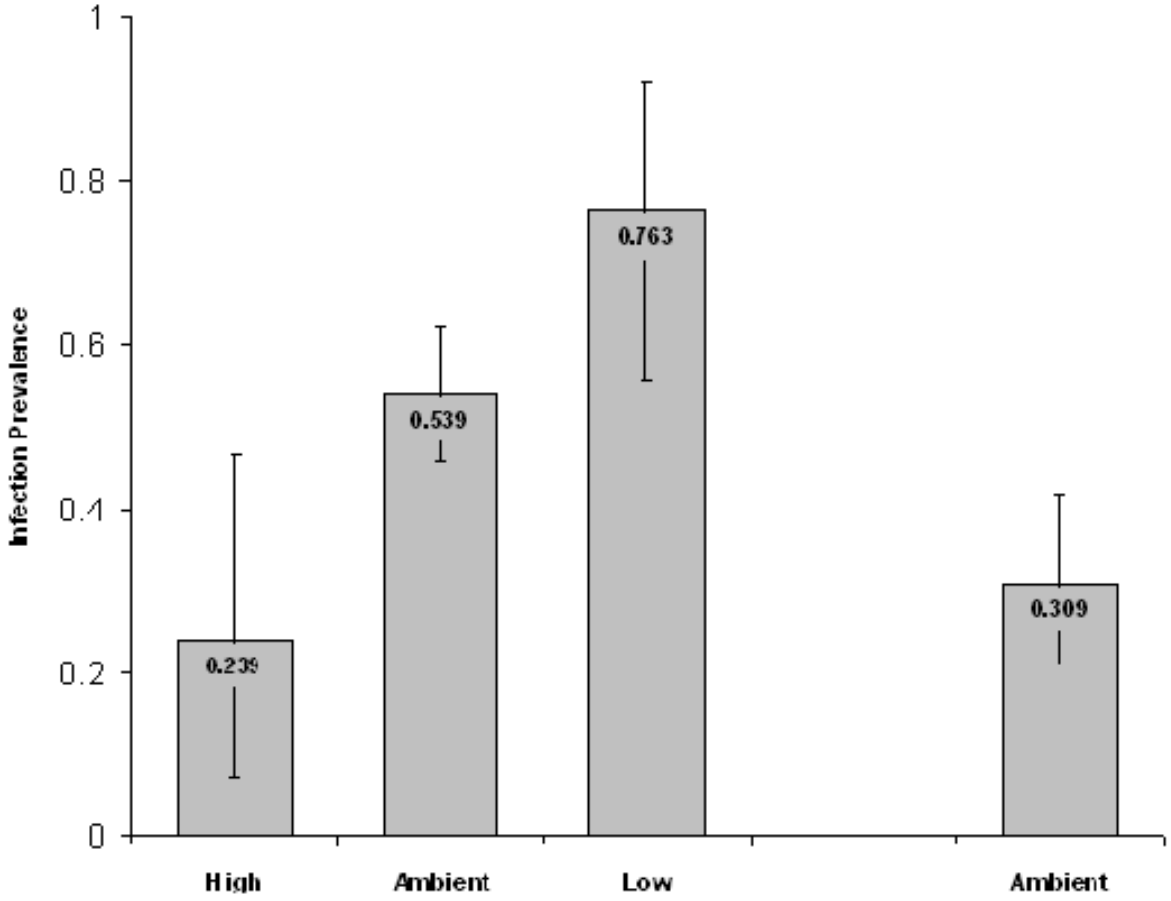

Figure 6: Infection prevalence in groups of Pacific herring held at 3 temperatures in Challenge-3. Data are means of three replicate tanks from each treatment. Mean temperatures were 9.3, 12.0, and 15.3°C for Low, Ambient, and High treatments respectively. Pre-challenge fast (Fast-Fed Treatments) lasted 56 days, after which fish were inoculated with *Ichthyophonus*, separated to treatments, and fed to satiation daily. Asterisk indicates Ambient Fed-Fed and High Fast-Fed treatments were significantly lower than Low Fast-Fed treatment.

Additional Empirical studies completed or initiated in 2010 include:
- Determined that temperature is extremely important in determining the disease kinetics after VHSV exposure of Pacific herring to the etiological agent. Briefly, at cooler temperatures (7°C), the virus is much more virulent and persists in the tissues of survivors for longer periods than at warmer temperatures (12°C). We consider this a major break-through in our understanding of VHS epizootiology; as such, these studies are being repeated and we will be concomitantly investigating the effects of temperature and VHSV exposure on the regulation of herring immune response genes.
- Determine the minimum lethal dose required to initiate VHS in herring (infections were established in 38% of herring injected with 0.07PFU / fish). This is an extremely low infection threshold (below the detection threshold of a standard plaque assay) and indicates that herring are highly susceptible to VHS.
- An *Ichthyophonus* review manuscript was initiated and is expected to be submitted during FY 2011. The primary objective of this manuscript is to clarify several misconceptions in *Ichthyophonus* life history, taxonomy, and terminology that commonly exist in the scientific literature.
- Determined the susceptibility of Pacific herring to the Great Lakes and European strains of VHSV
- Performed qualitative studies with erythrocytic necrosis virus, which indicate that the major viremia in infected herring occurs much earlier in the infection cycle than previously assumed. Briefly, the highest viremia occurs prior to the onset of complete inclusion body proliferation within the erythrocytes. The results have implications for developing a molecular diagnostic technique for the condition.
- Development of a molecular diagnostic tool to detect VEN from whole blood of Pacific herring
- Determine the effect of diet on herring susceptibility to VHSV

- Determine the susceptibility of Pacific herring embryos to VHSV
- Determine whether embryonic exposure to VHSV confers protection to the survivors after their metamorphosis to juveniles
- Determine a time series of histopathological damage resulting from herring exposure to VHSV (includes immunohistochemistry)

Field Surveillances of Infection and Disease Prevalences:
Surveys of the primary pathogens infecting wild adult herring were performed in Prince William Sound, Sitka Sound, and Lynn Canal, FY 2010. In fall samples (November 15-19, 2009) from Prince William Sound *Ichthyophonus* infection prevalence was 12% (7/60) in Port Gravina and 17% (10/60) in Elrington Pass. None of the adults tested positive for VHSV or VEN. In spring samples (March 16-20, 2010), *Ichthyophonus* infection prevalence was 18% (11/60) in Port Gravina, and 23% (14/60) in Port Fidalgo. None of the fish tested positive for VHSV, and VEN was detected in 2% (1/60) from Port Gravina and 3% (2/60) from Port Fidalgo; all VEN-positives were scored as light intensity.

In Sitka Sound (March 22-24, 2010), *Ichthyophonus* prevalence in pre-spawn adults was 27% (16/60) near the mouth of Indian River, 15% (9/60) between Boarder Rocks and Sitka Rocks, and 37% (22/60) at Mountain Point (Kruzof Island). None of the samples tested positive for VHSV and 3/180 were positive for VEN.

In Lynn Canal, *Ichthyophonus* infection prevalence in adult herring was 18% (11/60) on November 24, 2009, 10% (6/60) on December 7, 2009, 5% (3/56) on March 15 - 16, 2010, and 13% (5/40) on April 26, 2010. Virology was not performed on any of the samples from Lynn Canal.

In Puget Sound, annual WDFW herring trawl surveys were discontinued during 2010; therefore samples for *Ichthyophonus* prevalence were severely curtailed. However, we were able to collect herring samples from South Puget Sound (Squaxin Pass) on January 28, where the *Ichthyophonus* prevalence was 3% (2/60). Another sample of herring was collected from Holmes Harbor by gillnet on March 23, 2010, where the *Ichthyophonus* prevalence was 28% (17/60); it should be noted that the Holmes Harbor sample was likely biased because the largest fish were culled from this catch prior to collection of *Ichthyophonus* samples. Post-spawn herring were collected by trawl from Hood Canal on May 25, 2010, where the prevalence of *Ichthyophonus* was 45% (43/96). Among other hosts in Hood Canal, the prevalence of *Ichthyophonus* was 6% (1/17) in Pacific staghorn sculpin (May 25,2010) and 78% (28/36) in American shad (May 27, 2010).

Additionally, fish health surveys were preformed on juvenile herring during FY 2010; the results are presented in the Annual Report for Project #10100132-I.

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.*

The period of performance for this project (#070819) was scheduled to extend from FY 2007 – FY2010; as such, September 30, 2010 represents the projected end of this project. We will be requesting a no-cost extension to extend the period of performance into FY 2011. The extension will provide opportunity to draft a final project report, catch up on a back-log of virology samples from several laboratory studies, and finish submission of several additional manuscripts that resulted from this research.

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 from PWS were collected with the support of Tom Kline and Jennifer Todd (PWSSC) and Steve Moffitt and Dr. Rich Brenner (ADF&G – Cordova). Herring from Sitka Sound were collected in collaboration with Dr. Keith Cox (Sheldon Jackson College / NOAA-Fisheries), Eric Coonradt (ADF&G: Sitka), Heather Woody (Sitka Tribe), and JJ Vollenweider (NOAA – Fisheries). Herring from Lynn Canal were collected in collaboration with JJ Vollenweider, Ron Heintz, and Jeep Rice (Ted Stevens Marine Science Center in Juneau). Herring from Puget Sound were collected in collaboration with Wayne Palsson, Kurt Stick, Adam Lindquist, and Darcy Wildermuth (Washington

Department of Fish and Wildlife). Virology / parasitology samples for field surveillances were processed by the ADF&G Fish Pathology Laboratory in Juneau. Facility space, water, tanks, herring, and visiting scientist accommodations were made available at the Marrowstone Marine Field Station to accommodate the laboratory portion of the EVOSTC-funded project #090806.

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.

Plans are being made with the Prince William Sound Science Center to provide a herring disease seminar for residents of the City of Cordova. A smaller workshop is being planned to disseminate current research findings to ADF&G herring managers in Cordova and Sitka. A smaller workshop is being planned to disseminate current research findings to ADF&G herring managers in Cordova and Sitka. A guest lecture, entitled "What can we do about Diseases in Wild Marine Fishes?", was provided for students at the School of Aquatic and Fishery Sciences, University of Washington. A guest lecture, entitled "Influences of the Physical Environment on Diseases in Wild Fish Populations", was provided for students at Peninsula College. A seminar and workshop, entitled Herring Diseases in the NE Pacific; Implications for Fisheries Management", was provided for the Herring Conservation and research Society.

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.*

2010 Publications

Hershberger, P.K., J.L. Gregg, C.A. Grady, L. Taylor, S.E. Roon, J.R. Winton. *In Preparation*. Low doses of viral hemmorhagic septicemia virus are infectious for Pacific herring. Journal of Fish Diseases

Hershberger, PK, JL Gregg, CA Grady, JR Winton. *In Preparation*. Passive Immunization of Pacific herring *Clupea pallasii* against viral hemorrhagic septicemia. Fish and Shellfish Immunology.

Gregg J, J Vollenweider, C Grady, R Heintz, P Hershberger. *In Preparation*. Temperature modulated disease kinetics in juvenile Pacific herring (*Clupea pallasii*) infected with the parasite *Ichthyophonus*. MEPS

Hart L, GS Traxler, KA Garver, J Richard, JL Gregg, CA Grady, G Kurath, PK Hershberger. *Submitted*. Larval and juvenile Pacific herring *Clupea pallasii* are not susceptible to infectious hematopoietic necrosis. Diseases of Aquatic Organisms.

Cox MK, J Vollenweider, R Heintz, PK Hershberger, WJ Fournier. *Submitted*. Assessing the impacts of using Baltic herring (*Clupea harengus*) model parameters in bioenergetic modeling of Pacific herring (*C. pallasii*). Transactions of the American Fisheries Society

Kocan R, H Dolan, P Hershberger. *Submitted*. Diagnostic methodology is critical for accurately determining the prevalence of *Ichthyophonus* infections in wild fish populations. Journal of Parasitology.

Hershberger PK, JL Gregg, CA Grady, L Taylor, JR Winton. *Submitted*. Chronic and persistent viral hemorrhagic septicemia virus infections in Pacific herring. Diseases of Aquatic Organisms.

Grady, C.A., J.L. Gregg, R.M. Collins, P.K. Hershberger. *Accepted*. Viral Replication in Excised Fin Tissues (VREFT) corresponds with prior exposure of Pacific herring to *viral hemorrhagic septicemia virus* (VHSV). Journal of Fish Diseases.

Hershberger, P.K., B.K. van der Leeuw, J.L. Gregg, C.A. Grady, K. Lujan, S. Gutenberger, M. Purcell, J.C. Woodson, J.R. Winton, M. Parsley. *In Press.* Amplification and transport of an endemic disease by an invasive species. Biological Invasions.

Seitz, A.C., B.L. Norcross, J.C. Payne, A. Kagley, B. Meloy, J.L. Gregg, P.K. Hershberger. 2010. Feasibility of Surgically Implanting Acoustic Tags in Pacific Herring. Transactions of the American Fisheries Society 139: 1288-1291.

Kocan, R. M. , J. L. Gregg, P. K. Hershberger. 2010. Release of infectious cells from epidermal ulcers in *Ichthyophonus* sp. – infected Pacific herring (*Clupea pallasii*): evidence for multiple mechanisms of transmission. Journal of Parasitology 96: 348-352.

Hershberger PK, JL Gregg, CA Grady, RM Collins, JR Winton. 2010. Susceptibility of three stocks of Pacific herring to viral hemorrhagic septicemia. Journal of Aquatic Animal Health 22: 1-7.

Hershberger P, J Gregg, C Grady, R Collins, J Winton. 2010. Kinetics of viral shedding provide insights into the epidemiology of viral hemorrhagic septicemia in Pacific herring. Marine Ecology Progress Series 400: 187-193.

Rasmussen, C, M.K. Purcell, J.L. Gregg, S.E. LaPatra, J.R. Winton, P.K. Hershberger. 2010. Sequencing of the internal transcribed spacer (ITS) region reveals a novel clade of *Ichthyophonus* sp. from rainbow trout. Diseases of Aquatic Organisms 89: 179-183.

2010 Conferences and Workshops

Vollenweider, J.J., J. Gregg, R.A. Heintz, P.K. Hershberger. September 13-16, 2010. *Platform*. Impaired compensatory growth following disease exposure in fasting herring. American Fisheries Society 140th Annual Meeting. Pittsburgh, PA.

Hershberger, P.K., J.L. Gregg, M.K. Purcell, C.A. Grady, J.C. Woodson, J.R. Winton. September 5-9, 2010. Platform. Development of tools to forecast the potential for viral hemorrhagic septicemia epizootics in wild fish populations. Sixth International Symposium on Aquatic Animal Health. Tampa, Florida.

Gregg, J.L., J.J. Vollenweider C.A. Grady, R.A. Heintz, P.K. Hershberger. September 5-9, 2010. Effects of environmental temperature on the kinetics of ichthyophoniasis in juvenile Pacific herring (*Clupea pallasii*). Sixth International Symposium on Aquatic Animal Health. Tampa, Florida.

Emmenegger, E.J., J.A. Glenn, W.N. Batts, C.A. Grady, J.L. Gregg, S.E. Roon, J.R. Winton, P.K. Hershberger. September 5-9, 2010. Platform. Molecular characterization and infection kinetics of erythrocytic necrosis virus (ENV) in Pacific herring. Sixth International Symposium on Aquatic Animal Health. Tampa, Florida.

Hershberger, P.K., J.L. Gregg, C.A. Grady, L. Taylor, J.R. Winton. June 22-24, 2010. Platform. Chronic and persistent viral hemorrhagic septicemia virus infections in Pacific herring *Clupea pallasii*. Western Fish Disease Workshop. Corvallis, OR.

Hershberger, P.K., J.L. Gregg, C.A. Grady, L. Taylor, S.E. Roon, J.R. Winton. June 22-24, 2010. Platform. Low doses of viral hemorrhagic septicemia virus (VHSV) are infectious for Pacific herring *Clupea pallasii*. Western Fish Disease Workshop. Corvallis, OR.

VanderPol, J.A., J.L. Gregg, C.A. Grady, S. Roon, J.R. Winton, E.J. Emmenegger, P.K. Hershberger. June 22-24, 2010. Platform . Kinetics of viral load and erythrocytic inclusion body formation among Pacific herring with viral erythrocytic necrosis (VEN). Western Fish Disease Workshop. Corvallis, OR.

Hart, L.M., G.S. Traxler, K.A. Garver, J. Richard, J.L. Gregg, C.A. Grady, G. Kurath, P.K. Hershberger. June 22-24, 2010. Platform. Susceptibility of larval and juvenile Pacific herring *Clupea pallasii* to infectious hematopoietic necrosis virus. Western Fish Disease Workshop. Corvallis, OR.

Woodson, J.C., M.K. Purcell, S.M. Badil, C.A. Grady, J.L. Gregg, J.D. Hansen, E.S. Bromage, P.K. Hershberger. June 22-24, 2010. Platform. Development of immunological tools for the study of disease in Pacific herring *Clupea pallasii*. Western Fish Disease Workshop. Corvallis, OR.

Hershberger, P. K. C. Rasmussen, M.K. Purcell, J.L. Gregg, S.E. LaPatra, J.R. Winton. March, 2010. Poster. Genetic Characterization of the Internal Transcribed Spacer (ITS) Region Reveals Two Major Genetic Lineages of *Ichthyophonus hoferi*. USGS – Genomics and Genomics Symposium. Reston, VA.

Hershberger, P. K., C. Grady, J. Gregg, R. Collins, J.R. Winton. January 18-22, 2010. Poster. Herring fin explant cultures provide a reliable indication of host exposure history to viral hemorrhagic septicemia virus. Alaska Marine Science Symposium. Anchorage, AK. (Presented).

Cox, M. K., J. J. Vollenweider, R. A. Heintz, P.K. Hershberger. January 18-22, 2010. Poster. Bioenergetic models for Pacific herring; create or borrow. Alaska Marine Science Symposium. Anchorage, AK.

Vollenweider, J. J., J. Gregg, R. A. Heintz, P.K. Hershberger. January 18-22, 2010. Platform. The energetic toll of disease and starvation on overwintering juvenile herring. 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.