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: ..... Prince William Sound Herring Disease Program

PI Name:..... Paul Hershberger, Richard Kocan, John Hansen, Diane Elliott, Eveline Emmenegger, Geal Kurath, Scott LaPatra, Jim Winton

Time period covered: ..... FY'08: Oct. 1, 2008 – Sept. 30, 2009

Date of Report:..... August 28, 2009

Report prepared by: ..... Paul Hershberger

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

Work Performed:

Laboratory Rearing of Specific Pathogen-Free Herring:

For the sixth 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 (Quilcene Bay and Cherry Point). We are currently maintaining approximately 29,000 newly-metamorphosed YOY juveniles for FY 2010 experiments. We now maintain 4 age classes of SPF herring at the Marrowstone Marine Field Station (age 0, 2, 3 and 5 yr), and they continue to be utilized as test animals for empirical studies and development of forecasting disease tools.

Laboratory and Field Experiments:

Losses from infectious disease are an important component of natural mortality among marine fish species, but factors controlling the ecology of these diseases and their potential response to anthropogenic changes are poorly understood. We used viral hemorrhagic septicemia virus (VHSV) and a laboratory stock of Pacific herring to investigate the kinetics of viral shedding and its effect on disease transmission and mortality. Outbreaks of acute disease, accompanied by mortality and viral shedding, were initiated after waterborne exposure of herring to concentrations of VHSV as low as 10^1 plaque-forming units (pfu) mL^{-1}. Shed virus in flow-through tanks was first detected 4-5 days post-exposure, peaked after 6-10 days, and was no longer detected after 16 days. Shedding rates, calculated from density, flow, and waterborne virus titer reached 1.8-5.0 $x10^8$ pfu $fish^{-1}$ day^{-1}. Onset of viral shedding was dose-dependent and preceded initial mortality by 2 days. At 21 days, cumulative mortality in treatment groups ranged from 81-100% and was dependent not on challenge dose, but on the kinetics and level of viral shedding by infected fish in the tank. When extrapolated to the population scale, these results provide insights into virus transmission, infection pressure and resulting disease among free-ranging populations of Pacific herring.

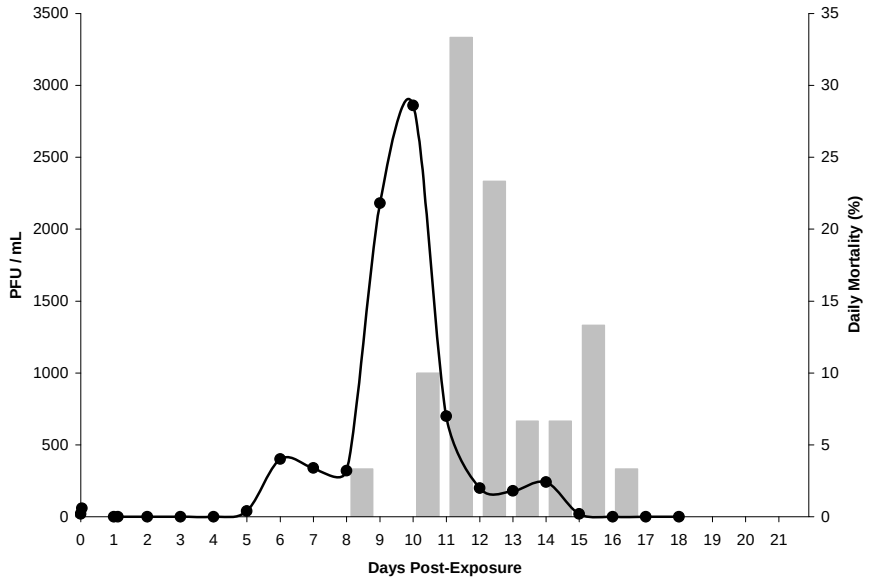

Figure 1. Waterborne VHSV titer (line) and daily mortality (bars) in a flow-through tank of Pacific herring (n=30) exposed to 23-27 pfu mL^{-1} for 24 hr.

Laboratory challenges of specific-pathogen-free Pacific herring obtained from three distinct populations revealed that stock origin had no effect on the susceptibility of herring to viral hemorrhagic septicemia virus (VHSV, Genogroup IVa) or on the ability of survivors to mount an adaptive immune response. Cumulative mortalities in all stocks were significantly greater ($p < 0.002$) in groups exposed to VHSV (56.3-64.3%) than in groups of unexposed controls (0.8-9.0%), indicating that all populations were highly susceptible to the virus upon initial exposure. Inter-stock differences were not significant ($p = 0.79$). Virus load in the tissues of mortalities was 10^1 to 10^4 times higher during the acute phase of the epizootics (day 13 post-exposure) than during the recovery phase (day 30-42 post-exposure). Solid immunity to VHSV was conferred to survivors of the laboratory challenges, with re-exposures resulting in significantly less ($p < 0.00026$) mortality (1.2-4.0%) than among groups of positive controls (38.1-64.4%). Inter-stock differences in the strength of the adaptive immune response were not significant ($p = 0.58$). The results indicate that data from experiments designed to understand the ecology of VHSV in a given stock of Pacific herring are likely applicable to herring stocks throughout the NE Pacific.

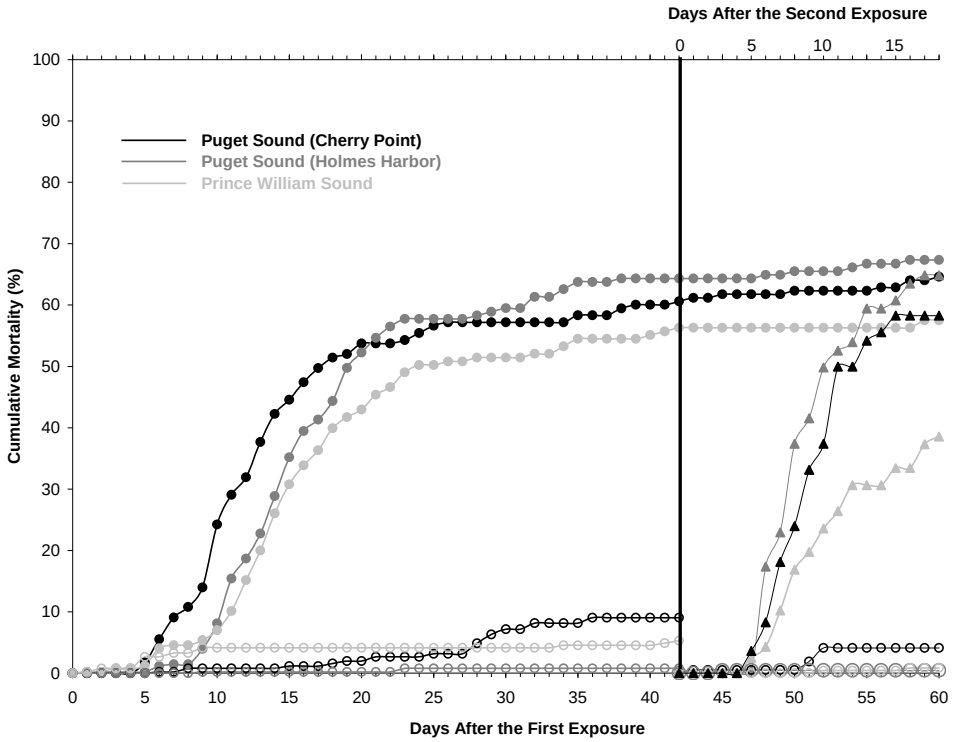

Figure 2. Relative susceptibilities of Pacific herring from three different stocks (Holmes Harbor, Cherry Point, and Prince William Sound) to VHS. Closed circles indicate treatment groups exposed to VHSV and open circles indicate negative control groups (exposed to saline). All survivors in the VHSV treatment groups from the first exposure were re-exposed to VHSV in the same tanks 42d later. All survivors in the negative control groups after 42 days were split into two groups (positive controls and negative controls) for the second exposure. Positive controls for the second exposure (closed triangles starting on day 42) were exposed to VHSV for the first time on day 42. Negative controls for the second exposure (open circles starting on day 42) were re-exposed to saline on day 42. All data points represent back-transformed percentages corresponding to the means of arc sin transformed proportions from triplicate tanks.

Disease selective predation in juvenile Pacific herring (*Clupea pallasii*) was studied in predation trials conducted in indoor mesocosms. Pairs of rainbow trout (*Oncorhynchus mykiss*) and lingcod (*Ophiodon elongatus*) were allowed to prey on comingled schools of juvenile herring that contained both *Ichthyophonus*-infected and uninfected individuals. Behavioural observations were conducted to determine pursuit rate, strike rate and attack success by the two predator species. Selection index was calculated from composition of infected vs non-infected prey before and after predation had reach 30-50 %, while size selective mortality was analyse by cumulative size distributions. Three trials with 5 replicates for each predator were performed during a period of 3 weeks to determine how disease progression may affect selectivity. We demonstrate selective predation on *I. hoferi*-infected herring, starting 38 days post-infection. Furthermore, predator species did not differ in their selection patterns even though both pursuit rate and strike rate differed. Attack success was not different between predators, suggesting that mechanisms late in the predation cycle were of importance to selection. We also demonstrate a disease specific size selection where predators consumed smaller infected fish more readily. We discuss our results in light of ecological consequences of selection and population dynamics.

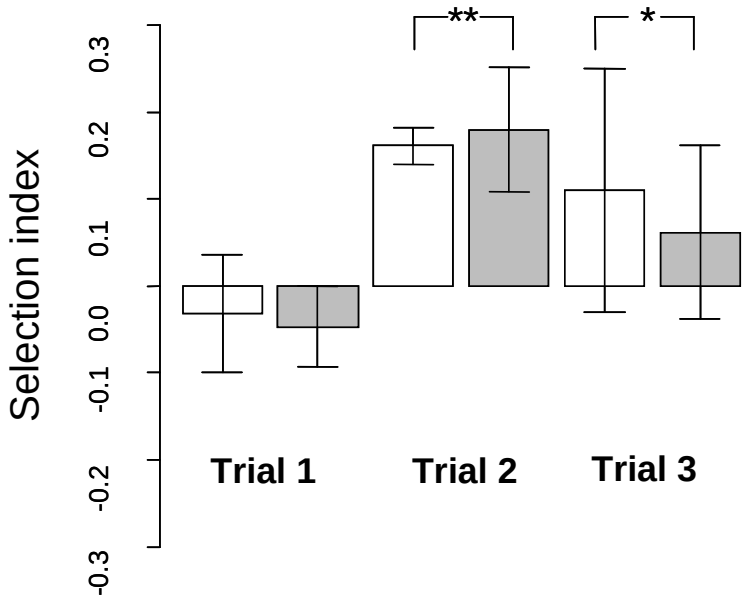

Figure 3. Selection index for the three trials. White bars indicate lingcod while grey bars indicate trout pooled for the five day trials. Bars indicate 95 % confidence intervals. Significant selection is indicated for pooled data within each trial (* $p<0.05$, ** $p<0.01$).

A common clinical sign of ichthyophoniasis in herring and trout is “sandpaper” skin, a roughening of the epidermis characterized by the appearance of small papules followed by ulceration and sloughing of the epithelium. Early investigators hypothesized that these ulcers might be a means of transmitting the parasite, *Ichthyophonus*, without the necessity of ingesting an infected host. We examined the cells associated with the epidermal lesions and confirmed they were viable *Ichthyophonus* cells that were readily released from the skin into the mucous layer and ultimately into the aquatic environment. The released cells were infectious when injected into the body cavity of specific pathogen-free herring, supporting our hypothesis that different mechanisms for transmission occur in carnivorous and planktivorous hosts.

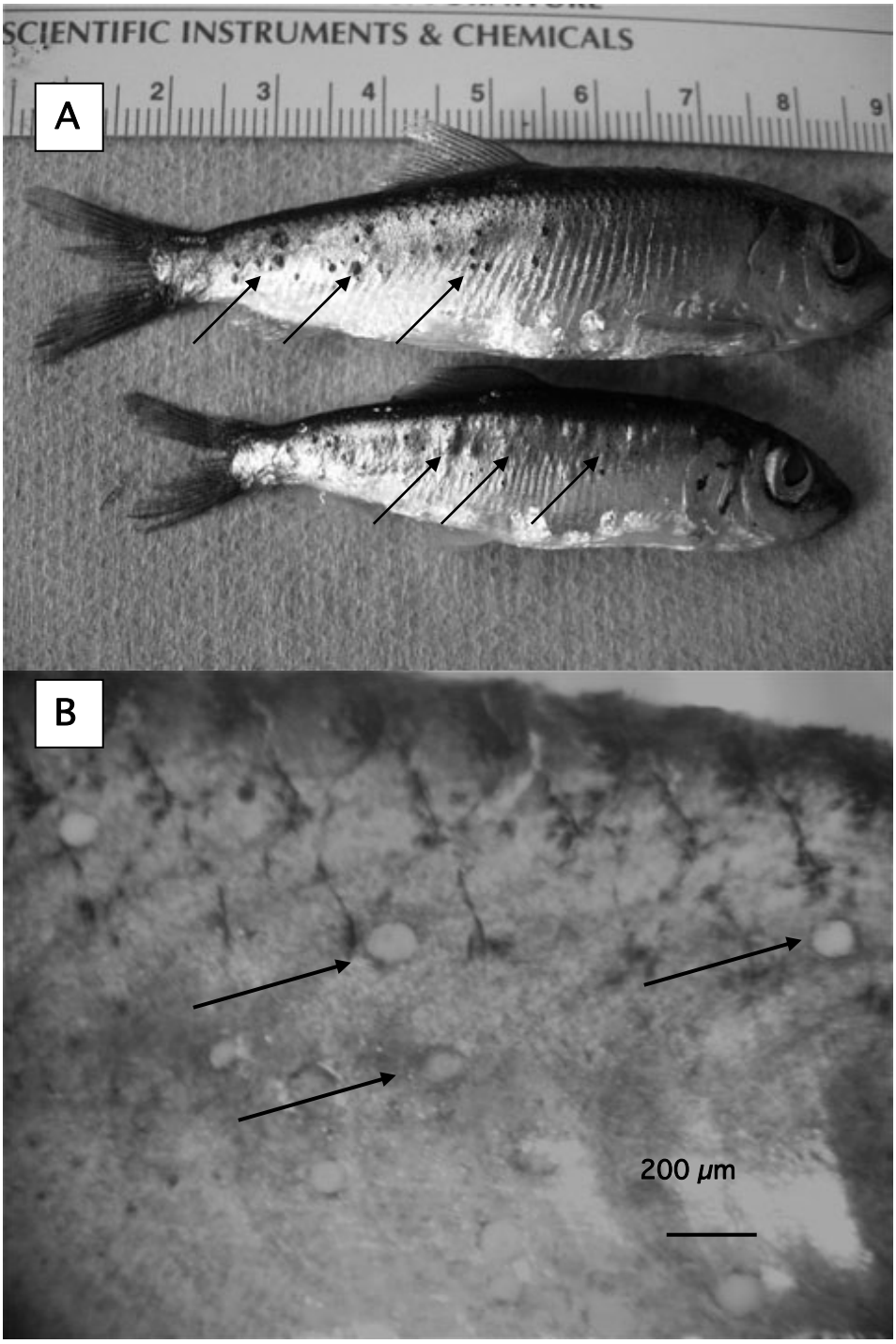

Figure 4. A. Epidermal ulcers (upper fish) and papules (lower fish) in Pacific herring caused by *Ichthyophonus*. B. Magnified view (5X) of ulcers showing whitish parasite cell(s) protruding through ulcerated skin.

Ichthyophonus is most commonly associated with marine fish hosts but the parasite is also endemic in the freshwater rainbow trout (*Oncorhynchus mykiss*) aquaculture industry. It is not certain how the parasite was introduced into rainbow trout culture but it may be associated with the historical practice of feeding raw, ground common carp (*Cyprinus carpio*) caught by commercial fisherman. We detected a major genetic division between west coast freshwater and marine isolates of the *Ichthyophonus hoferi*. Sequence differences were not detected in two regions of the highly conserved small subunit (18s) rDNA gene; however, nucleotide variation was seen in internal transcribed spacer loci (ITS1 and ITS2), both within and among the isolates. Intra-isolate variation ranged from 2.4 to 7.6 nucleotides over a region consisting of approximately 740 bp. Consensus sequences from marine / anadromous hosts differed in only 0-3 nucleotides (99.6-100% nucleotide identity) while those derived from freshwater rainbow trout were 100% identical. However, the consensus sequences from freshwater trout differed from those of marine / anadromous hosts by 13-16 nucleotides (97.8 - 98.2% nucleotide identity).

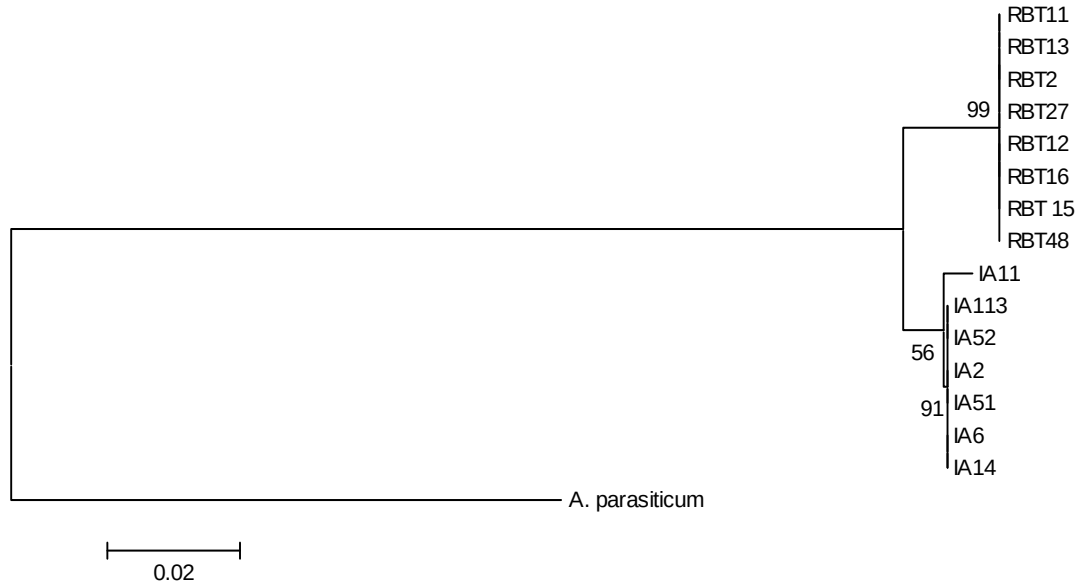

Figure 5. Evolutionary relationships of *Ichthyophonus* isolates based on the consensus sequence of the ITS region (ITS1-5.8s-ITS2) inferred using the neighbor-joining method (1000 bootstrap iterations). Similar tree topology was obtained using the maximum parsimony method. Isolates are derived from rainbow trout (RBT-11, 13, 2, 27, 12 16, 15 and 48), Pacific herring (IA2, IA113 and IA51), American shad (IA6, IA52 and IA14), and Copper rockfish (IA11). *Amoebidium parasiticum* (AY388646) was used as an outgroup.

Rainbow trout (*Oncorhynchus mykiss*) were infected with *Ichthyophonus sp*. and held at 10 ^{o}C, 15 ^{o}C and 20 ^{o}C for 28 days to monitor mortality and disease progression. Infected fish demonstrated more rapid onset of disease, higher parasite load, more severe host tissue reaction and reduced mean-day-to-death at higher temperature. In a second experiment, *Ichthyophonus*-infected fish were reared at 15 ^{o}C for 16 weeks then subjected to forced swimming at 10 ^{o}C, 15 ^{o}C and 20 ^{o}C. Stamina improved significantly with increased temperature in uninfected fish; however, this was not observed for infected fish. The difference in performance between infected and uninfected fish became significant at 15 ^{o}C (P = 0.02) and highly significant at 20 ^{o}C (P = 0.005). Infected fish were smaller at the time of testing, but body size did not explain the differences among treatments. These results have implications for changes in the ecology of fish diseases in the face of global warming and demonstrate the effects of higher temperature on the progression and severity of ichthyophoniasis as well as on swimming stamina, a critical fitness trait of salmonids. This study helps explain field observations showing the recent emergence of clinical ichthyophoniasis in Yukon River Chinook salmon later in their spawning migration when water temperatures were high, as well as the apparent failure of a substantial percentage of infected fish to successfully reach their natal spawning areas.

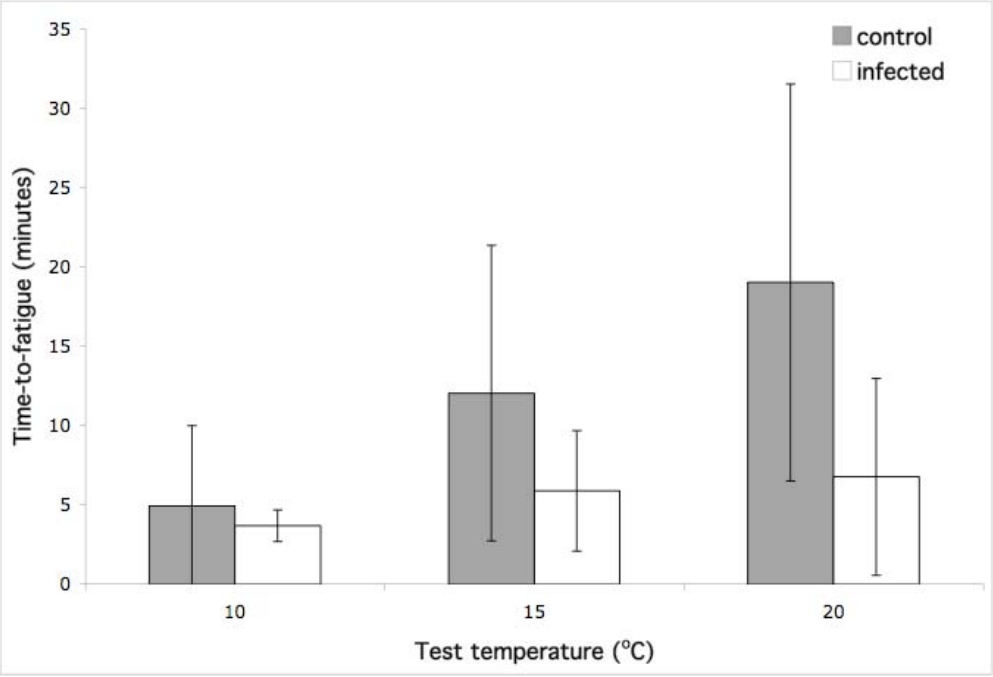

Figure 6. Effect of temperature on the swimming performance (time-to-fatigue) of *Ichthyophonus*-infected and uninfected rainbow trout. Increase in stamina significant (AOVA; P = 0.007) for controls but not for infected fish (P = 0.26). Bars = 1 SD above and below the mean, * = significant difference in performance between infected and control fish (paired t-Test).

Acoustic tags are a research tool that enables one to gather time-space movement information from marine animals. We examined the feasibility of implanting acoustic tags in adult Pacific herring as a method of monitoring their movements. Two sizes of dummy tags were surgically implanted (9 mm diam x 21 mm length, 1.6 g, n=50) and (7 mm diam x 18 mm length, 0.7 g, n=50) in adult Pacific herring ranging from 165 to 215 mm FL and 41.6 to 142.6 g. After 135 days, the fish in this study experienced relatively low mortality (4%) and tag shedding (4%) rates. Furthermore, their growth was not significantly different than those in two control groups. Pacific herring appear to be amenable to acoustic tag implantation, given careful handling and surgical procedures.

Treatment	Sample Size	Mortalities	Extrusions
Anesthesia	25	0	na
Incision and anesthesia	24	0	na
Vemco V7-1L dummy tag	50	2	1
Vemco V9-6L dummy tag	50	2	3

Table 1. Mortality and acoustic tag shedding events by Pacific herring in the acoustic transmitter implantation experiment. Vemco V7-1L dummy tag mortalities occurred 10 and 25 days post-surgery while the extruded tag was found on the bottom of the holding tank 39 days post-surgery. Vemco V9-6L dummy tag mortalities occurred 9 and 15 days post-surgery while extruded tags were found on the bottom of the holding tank 51 (n=2) and 53 days post-surgery.

Additional empirical studies completed or underway in 2009 include:
- Development of a passive immunization test as a reliable indicator of herring exposure history to VHSV
- Development of herring fin explants as a reliable indicator of exposure history to VHSV
- Development of a molecular diagnostic tool to detect VEN from whole blood of Pacific herring
- Determine the minimum lethal dose required to initiate VHS in 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)
- Breakthrough empirical studies indicating that VHS can manifest as a chronic disease in Pacific herring, and subsist in a population for extended periods.

Field Surveillances of Infection and Disease Prevalences:

Surveys of the primary pathogens infecting wild herring were preformed in Prince William Sound, Sitka Sound, Lynn Canal, and Puget Sound during FY'09. In fall samples (November 8-12, 2008) from Prince William Sound *Ichthyophonus* infection prevalence was 24% (19/80) in adults from Port Gravina and 0% (0/78) in juveniles from Simpson Bay. None of the adults tested positive for VHSV, and the juvenile samples were not plated onto cell cultures. VEN was not detected in any adults, but was detected in 1.4% of juveniles. In spring samples (March 20-22, 2009), *Ichthyophonus* infection prevalence was 43% (26/60) in pre-spawn adults from Port Gravina, 25% (15/60) in juveniles from the head of Port Gravina, and 13% (8/60) in smaller juveniles from Simpson Bay. None of the fish tested positive for VHSV, and VEN was only detected in 5% (3/60) of the juveniles from Simpson Bay (VEN was not detected in any adult or juvenile herring from Port Gravina). In a second spring sample of adults (April 4-9, 2009) *Ichthyophonus* prevalence was 45% (27/60); virology was not performed on these fish. In a final collection pre-spawn adults in PWS, collected in Snug Corner Cove on April 13, 2009, *Ichthyophonus* prevalence was 26% (16/62); virology was not performed on these fish.

In Sitka Sound, *Ichthyophonus* prevalence was 40% in pre-spawn herring collected near Guide Island on February 15-16, 2009; tissues from these fish were not processed for virology. A second collection occurred in Sitka Sound from March 24-27, 2009; *Ichthyophonus* prevalence was 45% (20/44) in prespawn adults provided by the Sitka Tribe (specific collection location unknown), 31% (21/67) in pre-spawn adults from St. John Babtist Bay, and 4% (3/69) in juveniles. None of the samples tested positive for VHSV or ENV.

In Lynn Canal, *Ichthyophonus* infection prevalence was 7% (3/44) among adults collected from February 11-12, 2009 and 13% (8/60) among adults collected from March 18-19, 2009. Virology was not performed on any of the samples from Lynn Canal.

In Puget Sound, *Ichthyophonus* prevalence was 3% (2/60) among adults collected from Yukon Harbor (Port Orchard / Port Madison) on February 2, 18% (11/60) among adults collected from Skagit Bay on February 2, 27% (16/60) among adults collected from Port Gamble on February 12, and 22% (13/60) among adults collected from Holmes Harbor on March 18, 2009. Virology was not performed on the Puget Sound samples. However, VEN prevalence among juvenile herring from Skagit Bay was 55% (33/60) in June, 32% (19/60) in July, and 4% (2/54) in August, 2009.

Additionally, *Ichthyophonus* was not detected in Pacific herring from San Francisco Bay, including 81 fish collected from Pt. Chauncey on February 11 and 60 fish collected from the same location on February 25, 2009.

Future Work:

No significant changes are proposed to the FY'10 study plan. Briefly, FY'10 objectives outlined in the study plan for The PWS Herring Disease Program include continuation of the herring disease index in PWS and Sitka Sound, and Puget Sound. Empirical studies will include investigations into the effects of multiple pathogens to SPF herring, effects of temperature on the virulence of VHSV, and continued development of VHS forecasting tools. Additionally, a comprehensive project report, integrating and summarizing the results of the 4 year HDP will be prepared and submitted to EVOS TC for peer review.

Coordination/Collaboration:

The field components of this project relied heavily on collaboration with local and state collaborations. Herring from PWS were collected with the support of 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 Kurt Stick, Adam Lindquist, and Darcy Wildermuth (Washington Department of Fish and Wildlife). Herring from San Francisco Bay were collected in collaboration with Kathy Hieb and Aaron Ngo (California Department of Fish and Game). 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 and for Pacific Ocean Shelf Tracking (POST) and UAF researchers who assisted with the herring acoustic tagging studies.

Community Involvement/TEK & Resource Management Applications:

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 VHSV min-summit, that included all the leading VHS in the NE Pacific region, was hosted at the USGS - Marrowstone Marine Field Station Oct. 8-9, 2008.

Information Transfer:

Publications:

Vollset, K.W., P.K. Hershberger, J.L. Gregg, A. Folkvord. *In Review*. Selective predation on *Ichthyophonus*-infected young of the year herring. Marine Ecology Progress Series.

Seitz, A.C., B.L. Norcross, J.C. Payne, A. Kagley, B. Meloy, J.L. Gregg, P.K. Hershberger. *In Review*. Feasibility of Surgically Implanting Acoustic Tags in Pacific Herring. Transactions of the American Fisheries Society.

Kocan, R. M. , J. L. Gregg, P. K. Hershberger. *In Review*. Release of infectious cells from the skin of Pacific herring (*Clupea pallasii*) with ichthyophoniasis. Journal of Parasitology.

Rasmussen, C, M.K. Purcell, J.L. Gregg, S.E. LaPatra, J.R. Winton, P.K. Hershberger. *In Review*. Sequencing of the internal transcribed spacer (ITS) regions indicate genetic heterogeneity between freshwater and marine *Ichthyophonus* Sp. Isolates. Diseases of Aquatic Organisms.

Hershberger, P.K., J.L. Gregg, C.A. Grady, R.M. Collins, J. R. Winton. *In Review*. Relative susceptibilities of Pacific herring stocks to viral hemorrhagic septicemia. Journal of Aquatic Animal Health

Hershberger, PK, JL Gregg, CA Grady, RM Collins, JR Winton. *In Review*. Viral shedding is a key driver in the ecology of an acute infectious disease of marine fish. Marine Ecology Progress Series.

Kocan, R., P. Hershberger, G. Sanders, J. Winton. *In Press*. Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout (*Oncorhynchus mykiss*) infected with *Ichthyophonus* sp. Journal of Fish Diseases.

Conference and Workshop Attendance:

Hershberger, P.K. May 21, 2009. Invited seminar speaker at the School of Aquatic and Fishery Sciences, University of Washington "Ecology of Diseases in Wild Fish Populations."

Grady, C. A., J. L. Gregg, R. M. Collins, P. K. Hershberger. June 7-10, 2009. Platform. Optimization of an in vitro technique to replicate viral hemorrhagic septicemia virus (VHSV) in herring fin explant cultures. 50th Western Fish Disease Workshop and AFS Fish Health Section Annual Meeting . Park City, UT.

Rasmussen, C., M. K. Purcell, J.L. Gregg, S. E. LaPatra, J. R. Winton, P. K. Hershberger. June 7-10, 2009. Poster. Sequencing of the internal transcribed spacer (ITS) regions indicate genetic heterogeneity between freshwater and marine Ichthyophonus sp. isolates. 50th Western Fish Disease Workshop and AFS Fish Health Section Annual Meeting . Park City, UT.

Seitz, A., J. Gregg, P. Hershberger, A. Kagley, B. Meloy, J. Payne. April 20-23, 2009. Platform. A Pilot study of mortality and tag loss in captive Puget Sound herring surgically implanted with acoustic tags. 2009 Annual General Meeting of the Washington - British Columbia Chapter of American Fisheries Society. Shelton, WA.

Hershberger, P.K.. January 29-30, 2009. Platform. Impacts of diseases to wild fish populations and the exacerbating effects of temperature. USGS – Climate Change, Natural Resources, and Coastal Management: A Workshop on the Coastal Ecosystems of California, Oregon, and Washington. San Francisco, CA, (Presented).

Hershberger, P.K., J.L. Gregg, C.A. Grady, R.M. Collins. January 19-22, 2009. Platform. Virus shedding after exposure to viral hemorrhagic septicemia virus (VHSV). Alaska Marine Science Symposium. Anchorage, AK. (Presented).

Winton, J., D. Elliott, P. Hershberger, G. Kurath, M. Purcell. Nov. 5-7, 2008. Platform. The Ecology and population effects of disease – bacterial kidney disease and viral hemorrhagic septicemia as examples from the Great Lakes and Pacific Northwest. Great Lakes Fishery Commission: Understanding the relationships between ecosystem dysfunction and fish health in the Great Lakes. Ann Arbor, MI.

Hershberger, P.K. October 15-17, 2008. Platform. Disease impacts on populations of wild marine fishes. USGS Interdisciplinary Microbiology Workshop. Estes Park, CO. (Presented).

Budget:

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

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