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, Maureen Purcell, Jim Winton

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.

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). 2010 represented a single year of overlap between #070819 and this project; therefore the FY 2010 annual report for 070819 contains a more complete listing of all disease activities occurring in PWS herring during 2010. Here we describe progress on items identified solely in the 2010 study plan for this project (10100132-I).

Laboratory Studies:

Laboratory efforts during 2010 primarily involved development of an indirect enzyme-linked immunosorbent assay (ELISA) to detect specific antibodies in herring plasma that are directed against viral hemorrhagic septicemia virus (VHSV). Development of the ELISA is progressing as anticipated, and a fully-functional assay is anticipated within the next 2 months. Efficacy of the functional assay will then be tested using both laboratory-reared herring with known exposure histories and wild herring with unknown disease histories. Further, the ability of this ELISA to determine the susceptibility of populations to VHS (and forecast disease epizootics) will be tested by VHSV exposure to laboratory exposures to laboratory and field population demonstrating known ELISA values.

The general layering design of the indirect ELISA to detect herring antibodies to VHSV is depicted in Figure 1.
1) The bottom layer (VHSV) involves attaching target antigen to the surface of the plate. In this case, our test antigen is VHSV. Unfortunately, live or attenuated VHSV virions do not stick to the surface of the plastic ELISA plate. This problem was fully anticipated; therefore, we plan to first add a layer of lyophilized monoclonal antibody (MAb) against VHSV to the surface of the plate. This Mab will stick to the plastic plate, then the test antigen (VHSV) will be added and bind with the MAb. Depending upon the effectiveness of the assay, we may replace the test antigen (live VHSV) with a recombinant VHSV protein (glycoprotein), which we have determined to be the primary antigenic attachment utilized by herring antibodies for attachment to the virus. Optimization of this bottom layer represents the final phase of our ELISA development; all other layers have been optimized.
2) The next layer (herring serum, with anti-VHSV antibodies) represents the serum sample from the wild fish that we will be testing. During the optimization portion of this layer, we decided to use herring plasma instead of serum to facilitate ease of sampling in the field.
3) The next layer (Mouse anti-herring IgM) was is now fully complete. During the early developmental phases, we attempted to utilize a polyclonal antibody that we developed against herring IgM in rabbits. As expected, the polyclonal did not offer the specificity that we required in our assay, so we proceeded by developing a monoclonal antibody to herring IgM using mouse lymphocytes. Hundreds of potential hybridoma lines were developed and screened for specificity to herring IgM. After an extensive screening process involving Western Blots, ELISA's, and separation and selective binding to herring lymphocytes by flow cytometry (Figure 2), we are confident that we have developed and selected several MAb's that are specific to herring IgM.
4) The top layer (rabbit anti-mouse Ig) is a commercially available tag that fluoresces if all the constituent reagents successfully bind (ie, if herring antibodies to VHSV are present in the serum sample).

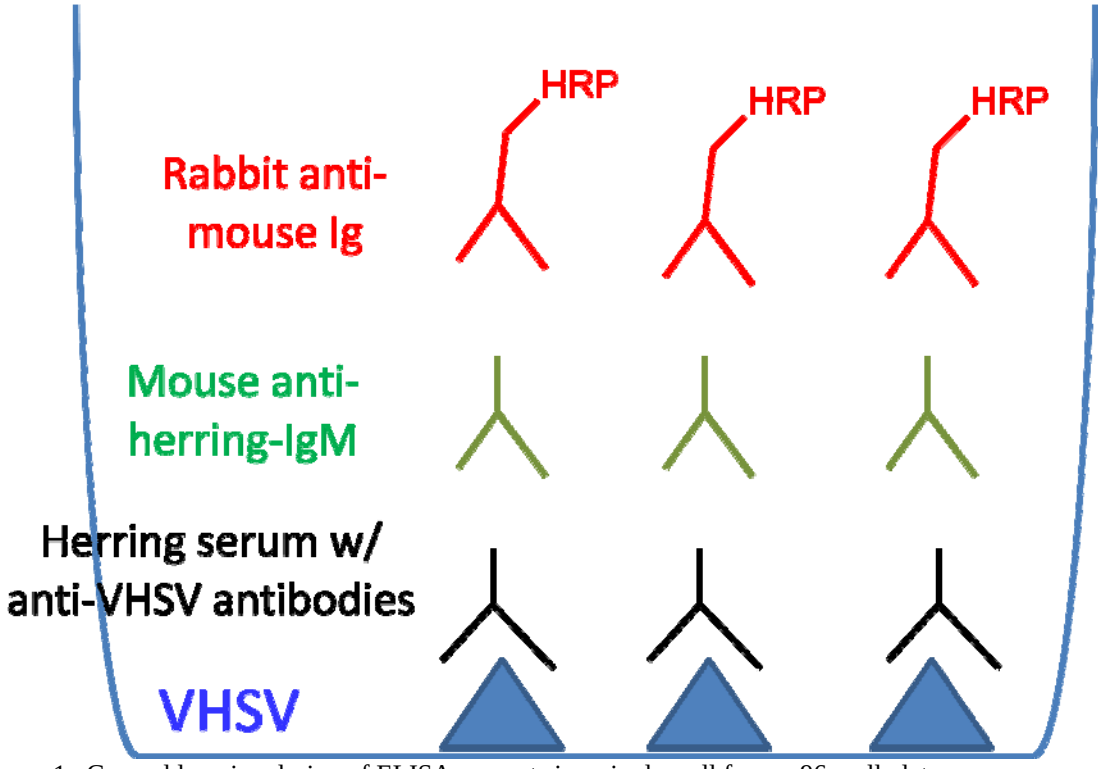

Figure 1. General layering design of ELISA reagents in a single well from a 96-well plate.

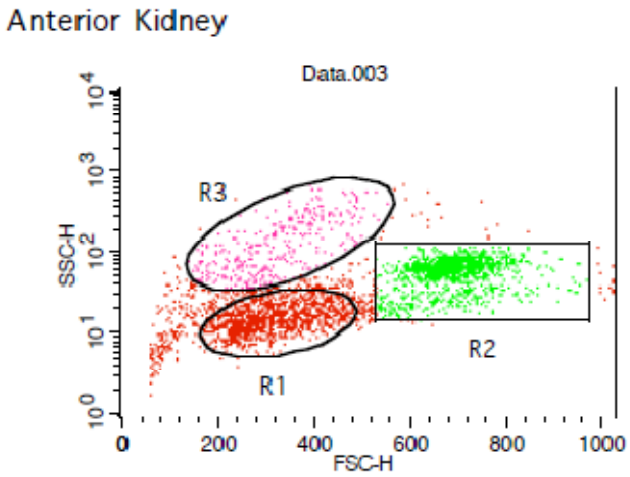

Figure 2. First-ever view of Pacific herring blood cells by flow-cytometry. The lymphocyte fraction (represented by the R1 gate) successfully bound to the newly developed herring MAb, indicating that our monoclonal recognizes herring IgM occurring on the surface of herring lymphocytes: SUCCESS!

Field Surveillances of Infection and Disease Prevalences:
The results of pathogen surveys in populations of adult herring are reported in the annual report for Project #070819; here we report the results from juvenile surveys only.

Prince William Sound juveniles
Among juvenile Pacific herring from Eaglik Bay, Lower Herring Bay, and Simpson Bay (November 11-19, 2009), *Ichthyophonus* prevalence was 3% (1/29), 0% (0/14), and 5% (1/20); respectively. VHSV was not isolated from any of the fish, and VEN prevalence was 16% (5/31), 21% (3/14), and 0% (0/33); respectively.

Among juvenile Pacific herring from Simpson Bay (March 20, 2010), the prevalence of *Ichthyophonus* was 13% (8/60); prevalence of VEN was 10% (6/60); and one tissue pool (from three fish) tested positive for VHSV. It should be noted that the VHSV positive sample occurred after blind passage, indicating that the intensity of the infection was extremely low.

Among another batch of juvenile Pacific herring from Eaglik and Simpson Bays (March 18-19, 2010), the prevalence of *Ichthyophonus* was 7% (4/62) and 3% (1/32); respectively. VHSV was not detected in the tissues of any of these fish; VEN samples were not processed because of water damage.

Disease Epizootics in Juvenile herring from Cordova Harbor
As documented by Dr. Tom Kline (PWSSC), juvenile herring appeared in Cordova Harbor during the summer of 2010. It was observed that the herring were in poor condition; therefore collections were made by Jennifer Todd and sent to the USGS Marrowstone Marine Field Station for processing. Laboratory diagnostics confirmed that the herring in Cordova Harbor were undergoing epizootics caused by several different etiological agents.

Mean Length:	84 mm
Prevalence of viral hemorrhagic septicemia virus (VHSV):	0% (0/49)
Prevalence of *Ichthyophonus*:	35% (17/49)
Prevalence of viral erythrocytic necrosis (VEN):	71% (34/48)

The prevalence of *Ichthyophonus* (35%) was higher than typical among juvenile Pacific herring in the NE Pacific. For example, the prevalence of *Ichthyohononus* among juvenile herring from Eaglik and Simpson Bays (PWS) during March, 2010 was 6.5% (4/62) and 3.1% (1/32); respectively.

The prevalence of VEN (71%) was the highest ever reported in PWS, and was similar to prevalences detected during VEN epizootics in other herring populations (Hershberger et al 2009, Journal of Aquatic Animal Health 21: 1-7). Among the VEN-positive herring, 92% of the infections were scored moderate or severe intensity.

As observed by underwater photography (courtesy of Dr. Tom Kline), sea lice infestations occurred on a high prevalence of the juvenile herring. An accurate prevalence of infestation was unobtainable because the lice often evacuated the host as the herring were sampled. Of the 49 herring sampled, sea lice remained on 18 at time of necropsy. The majority (16/18) were *Caligus clemensi*; however *Lepeopththerius* sp. (most likely *L. cuneifer*) also occurred on 2 sampled herring.

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 2011.

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.*

For a full list of herring health publications resulting from this project during 2010, please see the annual report for Project #070819. We anticipate the preparation of three additional manuscripts for publication from the work described above before the end of FY 2011, including:

- Development of a monoclonal antibody against herring immunoglobulin M.
- Development of an indirect enzyme linked immunosorbent assay to detect Pacific herring antibodies against VHSV.
- Identification of herring immune response genes that are up-regulated after VHSV exposure.

For a full list of conference and conference and workshop presentations resulting from this project during 2010, please see the annual report for Project #070819

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.