EVOSTC ANNUAL PROJECT REPORT

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'07: October 1, 2006 - September 1, 2007

Date of Report: ..... September 6, 2007

Report prepared by: ..... Paul Hershberger

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

Work Performed:

For the fourth consecutive year, we were successful at rearing specific pathogen-free (SPF), immunologically naïve Pacific herring in the laboratory at the Marrowstone Marine Field. Naturally deposited herring eggs were collected from two herring spawning stocks in Puget Sound (Quilcene Bay and Cherry Point). We are currently maintaining approximately 5,000 newly-metamorphosed herring juveniles from Quilcene Bay, and approximately 25,000 pre-metamorphosed larvae from Cherry Point. We will cull these fish to reasonable numbers (~1,000 Quilcene fish and ~2,000 Cherry Point fish) shortly to avoid overcrowding and food-limitation issues prior to over-wintering. We now maintain 4 age classes of SPF herring at the Marrowstone Marine Station (age 0-3 yr), and they continue to be utilized as test animals for empirical studies and development of forecasting disease tools.

Pacific herring were susceptible to waterborne challenge with viral hemorrhagic septicemia virus (VHSV) throughout their early life history stages, with significantly greater ($p < 0.001$) cumulative mortalities occurring among VHSV-exposed groups of age 9 d, 44 d, 54 d, and 76 d larvae than among respective control groups. Similarly, among age 89 d and 1+ yr post-metamorphosed juveniles, cumulative mortality was significantly greater ($p < 0.0001$) in VHSV - challenged groups than in respective control groups. Larval exposure to VHSV conferred partial protection to the survivors after their metamorphosis to juveniles because cumulative mortalities were significantly less ($p < 0.025$) among juvenile groups that survived a VHS epidemic as larvae than among groups that were previously naïve to VHSV. Magnitude of the protection, measured as relative percent survival, was a direct function of larval age at first exposure and was likely a reflection of gradual developmental onset of immunocompetence. These results indicate the potential for easily overlooked VHS epizootics among wild larvae in regions where the virus is endemic and emphasize the importance of early life history stages of marine fishes in influencing ecological disease processes.

Development of a molecular diagnostic tool to detect erythrocytic necrosis virus is underway. Briefly, blood was collected from several groups of VEN-positive (wild) and negative (SPF) herring, DNA was extracted and amplification of viral nucleic acid was attempted using four sets of conserved iridovirus primers. Molecular tool development is currently proceeding by attempted cloning of the bands.

Swimming performance studies are well under way to determine whether natural infections with *Ichthyophonus*, VENV, and VHSV result in decrease fitness among wild herring. Two cohoirts of wild, age 1+ yr Pacific herring were collected from Puget Sound (7/1/07 and 7/26/06) and introduced to a fabricated swim chamber during two swimming trials. Water velocity in the chamber was maintained at 1.0-1.4 ft / sec, and herring swam in the chamber for 4 hours (trial 1) and 72 hours (trial 2). Fish were euthanized from the chamber as they fatigued; at the termination of each swim trial, all un-fatigued fish were euthanized. Infection / disease data were collected from all sampled fish , including Ichthyophonus prevalence (explant tissue cultures) and intensity (histology), VEN prevalence and intensity (Giemsa-stained blood films), and VHSV prevalence and intensity (plaque assay of kidney

/ spleen homogenates). Preliminary analyses indicate that *Ichthyophonus* prevalence was similar among fatigued and un-fatigued groups; however, intensity of the infections (based on histological evaluation) is currently being evaluated. Similarly, blood films for VENV and plaque assays for VHSV are currently being processed. This experiment will be repeated once more (in September, 2007), using wild, age 0 herring.

To develop a standardized challenge model for erythrocytic necrosis virus (ENV) and determine its virulence to Pacific herring, a series of challenge studies are currently underway. Previous studies demonstrated that wild herring routinely undergo a VEN epizootic after confinement to laboratory tanks (Figure 1). We utilized this repeatable disease phenomenon as a source of VEN-positive material to conduct disease challenges using SPF herring. Wild, age 1+ herring were collected from a local herring bait fisher on 7/26/07 and transported to a flow-through tank in the laboratory. As a source of VEN-positive material for disease challenges, 20 herring VEN-positive herring were euthanized after 32d in the laboratory confinement. Kidney / spleen pools from the positive fish were homogenized, diluted 1:10, centrifuged, and the supernatant passed through a 0.45 µm filter; 0.05mL of the virus-positive filtrate was then injected into each of 30, age 1yr SPF herring. The remainder of the filtrate was added to tanks, containing 30 age 0 yr SPF herring. Thirty additional age 1 yr SPF herring were then cohabitated with the confined wild herring that were undergoing the VEN epizootic. The injection, waterborne, and cohabitation challenges are currently ongoing (day 8 post-challenge occurred on September 5, 2007), but preliminary scans of blood films sub-sampled from herring 5 d post-challenge indicated that VEN inclusions were present in herring exposed to infected tissue homogenates by both waterborne and injection challenge. Cumulative mortality, VEN progression, and mean hematocrits will be monitored from these exposed fish for 45d.

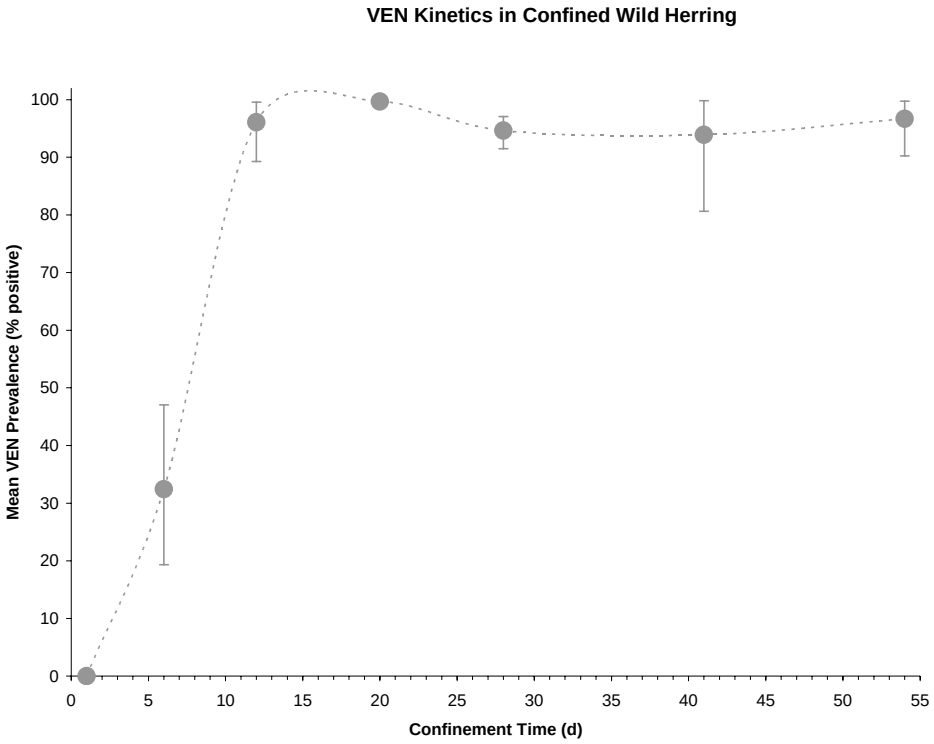

Figure 1. VEN prevalence in confined, age 0 Pacific herring held in tanks at the USGS Marrowstone Marine Field Station (from Hershberger et al. 2006).

Ongoing efforts are underway to develop and optimize a serum neutralization test to detect neutralizing antibodies for VHSV. Briefly, SPF herring and Atlantic salmon were exposed to VHSV, and the survivors were euthanized 29-34d post-challenge. Heparinized whole blood was separated by centrifugation, and the plasma portion was shipped to Dr. LaPatra for the first attempt at detecting neutralizing antibodies. All samples were assayed against two different VHSV isolates (99-001 from Pacific herring and 99-292 from Atlantic salmon, both from VHSV-Genogroup IV, the North American strain). All negative controls demonstrated a negative antibody titer (no virus neutralization at dilutions <1:20). However, few of the sera from the VHSV survivors were positive for neutralizing antibodies. Production of neutralizing antibodies to IHNV, a closely-related rhabdovirus, in rainbow trout is

temperature dependent. After further evaluation of our initial attempt to detect VHSV-neutralizing antibodies in herring, we hypothesize that antibody production proceeds slower than we initially anticipated. Therefore, we are currently repeating the study and we will euthanize a portion of the survivors 60 d post-challenge (Septemper 5, 2007 represents 49d into the challenge). The remainder of the surviving herring will be re-exposed to VHSV to attempt to detect an anamnestic antibody response. Heparinized whole blood will be centrifuged, and the plasma portion will be shipped to Dr. LaPatra in October, 2007 for a second attempt at detection of neutralizing antibodies.

As a pilot to studies planned in future years, the ability of naïve Pacific herring to develop innate resistance to VHSV with developmental ontogeny was tested by exposing three different age classes to VHSV. Briefly, cumulative mortality in all age classes (age 0, 1, and 2 yr) exposed to VHSV were similar (Figure 2), indicating that partial in protection to older wild age classes of Pacific herring likely results from components of the adaptive immune system. These age susceptibility studies will be repeater with older herring age classes in future years, but these preliminary data indicate that VHS-forecasting tools should target the adaptive arm of the immune system.

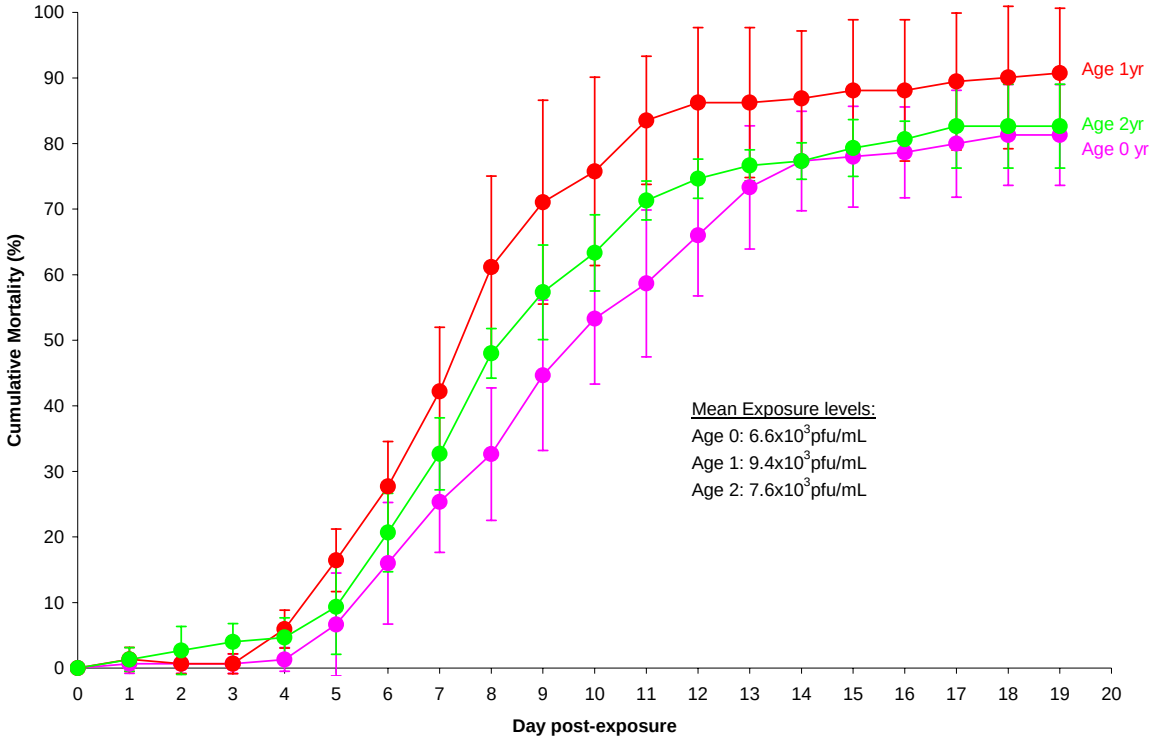

Figure 2. Susceptibility of 3 age classes of SPF herring to VHS. Data points indicate the back-transformed means of arc-sin transformed proportions for 5 replicate tanks in each age class. Replicate tanks each contained 30 herring at the beginning of the study. Mean cumulative mortality in each negative control age class was 5.6% after 20d.

As a no-cost addition to the '07 work plan, chlorine and iodine solutions were determined to be effective at inactivating *Ichthyophonus* spores in vitro. Inactivation in seawater increased directly with halogen concentration and exposure duration, with significant differences ($p < 0.05$) from controls occurring at all chlorine concentrations and exposure durations tested (1.5 - 13.3 ppm for 1-60 min) and at most iodine concentrations and exposure durations tested (1.2 ppm for 60 min and 5.9 - 10.7 ppm for 1-60 min). However, 10-fold reductions in spore viability occurred only after exposure to halogen solutions at higher concentrations and / or longer durations (13 ppm total chlorine for 1-60 min, 5.9 ppm total iodine for 60 min, and 10.7 ppm total iodine for 1-60 min). Inactivation efficacy was greater when halogen solutions were prepared in freshwater, presumably because of combined effects of halogen-induced inactivation and general spore instability in freshwater. The results have

practical implications for disinfection and biocontainment in research laboratories and other facilities that handle live *Ichthyophonus* cultures and / or infected fish.

As a no-cost addition to the '07 work plan, preliminary studies were conducted, using specific pathogen-free herring exposed to VHSV to identify herring immune response genes that may be useful in developing predictive tools that forecast disease epizootics. First, Expressed Sequence Tag (ESTs) database was initiated from Pacific herring, representing genes that are differentially regulated during VHSV infections. These candidate genes will later serve as sentinels of the immune response in herring for examining host factors that govern disease resistance and susceptibility. Second, we assessed the transcriptional regulation of these ESTs during VHSV infection in controlled laboratory challenges. The proteasome subunit beta type 9 protein gene sequence was submitted to, and is currently listed in GenBank (Accession #EF607279).

Surveys of wild herring were preformed in PWS, Sitka Sound, and Puget Sound during FY'07. Sixty adult herring were sampled from a spawning aggregation in Sitka Sound (south side of Cannon Island) on April 19, 2007. Prevalence of *Ichthyophonus* was 28.3% (17/60), with 18% (11/60) demonstrating visible signs of ichthyophoniasis. VEN was not detected (0/60), and kidney / spleen pools for VHSV assay are currently archived for future processing. Wild herring were collected from four temporally and spatially distinct herring pre-spawn aggregations in Puget Sound during in 2007 (including Johnson Point, Yukon Harbor, Skagit Bay, and Cherry Point); *Ichthyophonus* prevalence ranged from ranged from 7-35%. Kidney / spleen pools from the Cherry Point stock are archived for later VHSV assays. We partnered with monthly sampling excursions to Skagit Bay, to monitor the seasonal progression of VEN in juvenile herring. Samples are still being processed, but preliminary scans indicate a recurring, prolonged VEN epizootic among juvenile herring in Skagit Bay. Two samples of herring here obtained from Prince William Sound during 2007, one sample contained 60 adult, pre-spawn herring, and the other contained 60 juvenile herring. These fish were processed by the ADF&G pathology laboratory in Juneau, and the results are reported in the following laboratory reports:

ACCESSION NO: 07-0540

ALASKA DEPARTMENT OF FISH AND GAME
JUNEAU FISH PATHOLOGY LABORATORY, CFM&D DIVISION
3333 Old Glacier Highway - PO Box 25526, Juneau, AK 99802-5526
Phone: (907) 465-3577

REPORT OF LABORATORY EXAMINATION

LOT (YEAR, STOCK, SPECIES): Prince William Sound (St. Matthews Bay) Pacific herring *Clupea pallasi*

FACILITY: Western Fisheries Research Center, USGS

CONTACT PERSON/ADDRESS: Dr. Paul Hershberger, WFRC, USGS, 6505 NE 65th Street, Seattle, WA 98115-5016

SAMPLE DATE: 4/5/07 **DATE SAMPLE RECEIVED:** 4/8, 4/12/07

SPECIMEN TYPE: Kidney/spleen pools **LIFE STAGE:** Adult 3+ years **STATE:** Unfrozen/on ice
Blood smears Dried smears
Heart explant cultures Refrigerated explants

NUMBER IN SAMPLE: 60 kidney/spleen pools **WILD:** Yes
60 blood smears
60 explant cultures

HISTORY/SIGNS: Since the crash of this herring stock in 1993, there have been ongoing investigations regarding the recovery process and diseases in the population, the latter of which have focused on VHSV *Ichthyophonus hoferi* and VENV.

REASON FOR SUBMISSION: A recently funded project includes annual surveillance for the presence of VHSV, *Ichthyophonus* and VENV in Pacific herring from Prince William

Submit this report via e-mail to mandy.migura@alaska.gov . Thank you!

Sound, Alaska.

FINAL REPORT DATE: 5/18/07

CLINICAL FINDINGS:

VIROLOGY: Fish tissues processed without freezing; herring appeared normal at capture

0/60 kidney/spleen pools positive for virus on EPC cells after 14 days at 14.7 °C with a blind passage for another 14 days. The minimum level of detection was 50 infectious particles per gm of tissue sample.

ICHTHYOPHONUS:

25/60 (42%) heart explants with growth typical of *Ichthyophonus hoferi* after 14 days @ 14.7 °C

VENV:

0/60 peripheral blood smears with erythrocytic cytoplasmic inclusion bodies typical of VENV

COMMENTS/RECOMMENDATIONS:

No VHSV or VENV were detected in the samples submitted. *Ichthyophonus* was present at a moderate prevalence in the heart explants.

FISH HEALTH INVESTIGATOR(s): T.R. Meyers

TECHNICAL ASSISTANCE: I. Conte

COPIES TO: FY07, Herring, T. Meyers, S. Moffitt (Cordova)

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ACCESSION NO: 07-0543

ALASKA DEPARTMENT OF FISH AND GAME
JUNEAU FISH PATHOLOGY LABORATORY, CFM&D DIVISION
3333 Old Glacier Highway - PO Box 25526, Juneau, AK 99802-5526
Phone: (907) 465-3577

REPORT OF LABORATORY EXAMINATION

LOT (YEAR, STOCK, SPECIES): Prince William Sound (Simpson Bay) Pacific herring *Clupea pallasi*

FACILITY: Western Fisheries Research Center, USGS

CONTACT PERSON/ADDRESS: Dr. Paul Hershberger, WFRC, USGS, 6505 NE 65th Street, Seattle, WA 98115-5016

SAMPLE DATE: 4/19/07 **DATE SAMPLE RECEIVED:** 4/21/07

SPECIMEN TYPE: Kidney/spleen pools **LIFE STAGE:** Juvenile- 1 year **STATE:** Unfrozen/on ice
Blood smears Dried smears
Heart explant cultures Refrigerated explants

NUMBER IN SAMPLE: 60 kidney/spleen pools **WILD:** Yes

Submit this report via e-mail to mandy.migura@alaska.gov . Thank you!

60 blood smears
60 explant cultures

HISTORY/SIGNS: Since the crash of this herring stock in 1993, there have been ongoing investigations regarding the recovery process and diseases in the population, the latter of which have focused on VHSV *Ichthyophonus hoferi* and VENV.

REASON FOR SUBMISSION: A recently funded project includes annual surveillance for the presence of VHSV, *Ichthyophonus* and VENV in Pacific herring from Prince William Sound, Alaska.

FINAL REPORT DATE: 6/14/07

CLINICAL FINDINGS:

VIROLOGY: Fish tissues processed without freezing; juvenile herring appeared normal at capture

0/60 kidney/spleen pools positive for virus on EPC cells after 14 days at 14.7 °C with a blind passage for another 14 days. The minimum level of detection was 50 infectious particles per gm of tissue sample. Due to the small amount of tissue, individual pools had to be diluted 1:12 to 1:18 instead of the standard 1:10 (w/v)

ICHTHYOPHONUS:

9/60 (15%) heart explants with growth typical of *Ichthyophonus hoferi* after 14 days @ 14.7 °C

VENV:

10/60 (16.7%) peripheral blood smears with erythrocytic cytoplasmic inclusion bodies typical of VENV

2/10 light = < 20% cells infected/field- inclusions mostly in mature RBCs
7/10 moderate = 20-50% cells infected/field – large inclusions in mature and immature cells
1/10 severe = 60-80% cells infected/field – some cytopathology, many infected immature cells

COMMENTS/RECOMMENDATIONS:

No viral CPE (presumptive VHSV) was detected in the samples collected. *Ichthyophonus* was present at a low prevalence in the heart explants. Typical VENV erythrocytic inclusions were observed in nearly 17% of the juvenile fish. This is an interesting observation in that VENV inclusion bodies have been relatively rare in adult herring from PWS.

FISH HEALTH INVESTIGATOR(s): T.R. Meyers

TECHNICAL ASSISTANCE: I. Conte

COPIES TO: FY07, Herring, T. Meyers, S. Moffitt (Cordova)

Future Work:

No significant changes are proposed to the FY'08 study plan. Briefly, FY'08 objectives outlined in the study plan for The PWS Herring Disease Program include continuation of the herring disease index in PWS and Sitka Sound, paired with laboratory validation. Empirical studies include development of a standardized *Ichthyophonus* challenge model for Pacific herring, herring swimming performance studies with infected and uninfected groups, and continued development and validation of a VHSV serum neutralization test and ENV molecular diagnostic tool. No-cost additions to the study plan include:

1) Monitoring the infection / disease status of herring in Sitka Sound throughout the winter to begin to address the hypothesis that decreased over-wintering condition predisposes herring to disease-related mortality. These samples will be collected in collaboration with JJ Vollenweider, and no additional funds are requested.
2) Attempting to adapt a novel VHSV cultivation technique as a predictive tool to forecast wild herring susceptibility to future VHS epizootics. Briefly, herring fin explants from VHSV naïve individuals and survivors of a VHSV epizootic will be and will be incubated with live virus in vitro to determine whether less replication occurs in tissues of VHSV-resistant individuals. If decreased virus replication occurs in previously exposed, and if these individuals are less susceptible to VHS, then a screening tool to forecast potential for future VHSV epizootics can be developed. Potential for this technique to be used as a screening tool will be tested at the USGS-Marrowstone Marine Field Station, and no additional funds are requested.
3) Validating the sensitivity of a quantitative pcr for VHSV. Largely in response to the emergence of VHS epizootics in Great lakes fishes, Dr. Kyle Garver at DFO in Nanaimo recently developed a qPCR for VHSV. We will partner with Dr. Garver to compare the sensitivity of this novel assay to that of virus cultivation on cell culture, the current gold standard. If the qPCR is more sensitive, we will use this technique to investigate whether a latent carrier phase of viral infection occurs in herring that survived prior exposure.

Coordination/Collaboration:

The field components of this project relied heavily on collaboration with local and state collaborations. Herring from PWS and Sitka were collected by staff from Alaska Department of Fish and Game (ADF&G) – Cordova and Sitka, respectively and virology / parasitology samples were processed by the ADF&G Fish Pathology Laboratory in Juneau. Herring from Puget Sound were collected in collaboration with staff from Washington Department of Fish and Wildlife. Partnerships for future herring collections were formed with Keith Cox at Sheldon Jackson College and researchers at the Ted Stevens Marine Science Center in Juneau, including JJ Vollenweider, Ron Heintz, and Jeep Rice. Techniques for larval herring rearing were shared with Drs. Tim Linley (MariCal, Inc.) and Mike Schwartz (Virginia PolyTech).

Community Involvement/TEK & Resource Management Applications:

A facility poster describing activities at the Marrowstone Marine Field Station is currently being prepared and will be disseminated to the Prince William Sound Science Center. Reprints of all published manuscripts will be similarly disseminated.

Information Transfer:

Publications:

Hershberger P.K., J Gregg, C Pacheco, J Winton, J Richard, G. Traxler. 2007. Larval Pacific herring, *Clupea pallasii* (Valenciennes), are highly susceptible to viral hemorrhagic septicemia and survivors are partially protected after their metamorphosis to juveniles. Journal of Fish Diseases 30: 445-458.

PK Hershberger, Pacheco CA, JL Gregg,. *In Review*. Inactivation of *Ichthyophonus* Spores Using Sodium Hypochlorite and Polyvinyl Pyrrolidone Iodine (PVPI). Journal of Fish Diseases.

PK Hershberger, CA Pacheco, JL Gregg, CA Grady, M Purcell, SE LaPatra. *In Preparation*. Differential survival of Ichthyophonus isolates indicates parasite adaptation to the host . Parasitology.

Conference and Workshop Attendance:

P. Hershberger attended the 2007 Alaska Marine Science Symposium

P. Hershberger attended and presented at the 2007 Puget Sound / Georgia Basin Research Conference:

Hershberger PK, JL Gregg, JR Winton. March 26-29, 2007. Platform. Impacts of Disease to Wild Fish Populations with Special Reference to the Salish Sea Region. Georgia Basin / Puget Sound Research Conference. Vancouver, B.C.

Gregg J, C Pacheco, M Myers, P Hershberger. March 26-29, 2007. Platform. Prevalence of *Ichthyophonus* in copper rockfich (*Sebastes cayurinus*) from Puget Sound, WA. Georgia Basin / Puget Sound Research Conference. Vancouver B.C.

Other:

Deposition of the first herring immune response gene in Genbank (Accession Number EF607279) www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=156617987

Budget:

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

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