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'08: Oct. 1, 2007 – Sept. 30, 2008

Date of Report:..... Oct. 2, 2007

Report prepared by: ..... Paul Hershberger

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

Work Performed:

Laboratory Rearing of Specific Pathogen-Free Herring:

For the fifth 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 (Holmes Harbor and Cherry Point) and Prince William Sound, AK. We are currently maintaining approximately 10,000 newly-metamorphosed YOY juveniles from Cherry Point, 500 from Holmes Harbor, and 1,500 from PWS. We will cull the Cherry Point cohorts to reasonable numbers (~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 0-2 and 4 yr), and they continue to be utilized as test animals for empirical studies and development of forecasting disease tools.

Figure 1. SPF herring, representing 5 different hatch dates, reared at the USGS - Marrowstone Marine Field Station

Laboratory and Field Experiments:

During FY'08, we continued surveillances of viral erythrocytic necrosis in herring throughout the eastern North Pacific and documented the presence of VEN in juvenile herring from PWS. A manuscript describing epizootiology of this disease has been accepted for publication. Briefly, epizootics of VEN occurred among juvenile Pacific

herring in Skagit Bay, Puget Sound, WA during 2005 - 2007 and were characterized by high prevalences and intensities of cytoplasmic inclusion bodies within circulating erythrocytes. Prevalence of VEN peaked at 67% during the first epizootic in October, 2005, after which prevalence waned to 0% by August of 2006. A second VEN epizootic occurred throughout the summer of 2007, and was characterized by disease initiation and perpetuation in the 1+ yr, 2006 age class, followed by involvement of the 0+ yr, 2007 age class cohorts shortly after their larval metamorphosis to juveniles. The disease was detected in other populations of juvenile herring throughout Puget Sound and Prince William Sound, AK where prevalences and intensities typically did not correspond to those observed in Skagit Bay. The persistence and recurrence of VEN epizootics indicates that the disease is likely common among juvenile herring throughout the eastern North Pacific, and although population-level impacts likely occur, they are typically covert and not easily detected.

Table 1. Prevalence of VEN in Pacific herring from locations other than Skagit Bay.

Location	Collection Date	Mean length (SD) mm	n	VEN Prevalence %
Prince William Sound	4/5/07	224 (17)	60	0
Prince William Sound	4/19/07	86 (6)	60	17
Puget Sound: Cherry Point	4/30/07	184 (13)	60	0
Puget Sound: Skunk Bay	7/2/07	131 (4)	170	1.8
Puget Sound: Admiralty Inlet	8/1/07	129 (5)	60	0
Puget Sound: Port Townsend Bay	10/16/07	80 (6)	75	20

Table 2. Prevalence of VEN in each herring age cohort from Skagit Bay. All adult herring $\geq$ 2 years old were combined into the adult category. 'ND' indicates no data; none of the sampled herring were members of that particular age cohort. 'NA' indicates not applicable; the age cohort was either not yet born or larval metamorphosis to juveniles was not yet complete at the time of sampling.

	Age Cohort (Birth Year)									
	2004		2005		2006		2007		Age 2+ yr Adults	
Sampling Month	VEN Prevalence (n)	Length Bracket (mm)	VEN Prevalence (n)	Length Bracket (mm)	VEN Prevalence (n)	Length Bracket (mm)	VEN Prevalence (n)	Length Bracket (mm)	VEN Prevalence (n)	Length Bracket (mm)
2005 Sept.	0% (13)	115-150	48% (36)	85-110	NA		NA		36% (11)	155-180
Oct.	33% (3)	120-125	65% (49)	75-105	NA		NA		ND	
2006 May	Recruited to adults		33% (9)	85-140	NA		NA		7% (41)	155-200
June	Recruited to adults		28% (18)	125-145	NA		NA		30% (27)	150-205
July	Recruited to adults		43% (7)	115-150	4% (48)	45-100	NA		0% (5)	160-215
Aug.	Recruited to adults		ND		0% (60)	75-115	NA		ND	
Sept.	Recruited to adults		ND		0% (60)	75-120	NA		0% (60)	170-220
Oct.	Recruited to adults		ND		0% (60)	75-105	NA		ND	
2007 April	Recruited to adults		Recruited to adults		4% (56)	85-125	NA		0% (4)	195-210
May	Recruited to adults		Recruited to adults		35% (57)	90-120	NA		40% (5)	175-210
June	Recruited to adults		Recruited to adults		37% (60)	100-140	NA		50% (2)	185-200
July	Recruited to adults		Recruited to adults		43% (60)	100-145	0% (17)	40-85	100 (1)	185
Aug.	Recruited to adults		Recruited to adults		35% (48)	115-140	4.3% (23)	65-105	ND	
Sept.	Recruited to adults		Recruited to adults		35% (43)	115-150	33% (48)	70-110	100 (1)	180
Oct.	Recruited to adults		Recruited to adults		17% (6)	135-160	5.1% (59)	80-120	ND	

During FY'08 we performed studies demonstrating phenotypic differences in *Ichthyophonus* isolates from hosts sampled from different water types. A manuscript describing *Ichthyophonus* adaptation to its host environment is currently in press. Briefly, *in vitro* viability of *Ichthyophonus* spores in seawater and freshwater corresponded with the water type of the host from which the spores were isolated. Among *Ichthyophonus* spores from both marine and freshwater fish hosts (Pacific herring, *Clupea pallasii* and rainbow trout, *Oncorhynchus mykiss*; respectively), viability was significantly greater ($p < 0.05$) after incubation in seawater than in freshwater at all time points from 1-60 minutes post-immersion; however, magnitude of the spore tolerances to water-type differed with host origin.

Ichthyophonus adaptation to its host environment was indicated by greater seawater tolerance of spores from the marine host and greater freshwater tolerance of spores from the sympatric fish host. Prolonged aqueous survival of *Ichthyophonus* spores in the absence of a host provides insight into routes of transmission, particularly among planktivorous fishes, and should be taken into consideration when designing strategies to dispose of infected fish carcasses and tissues.

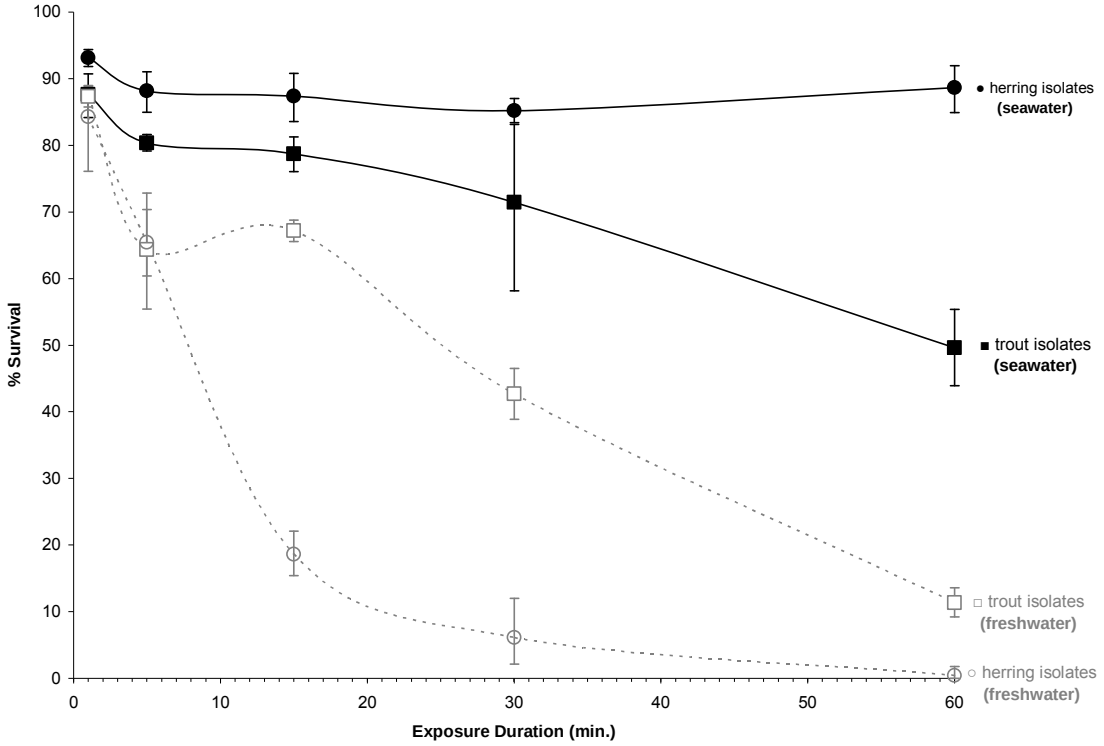

Figure 2. Survival of *Ichthyophonus* spores in seawater and freshwater. Data points indicate the percentages corresponding to the means of arc sin - transformed proportions for the replicates (n=3); 87 - 501 spores from each replicate were analyzed each sampling day. Error bars indicate 2 SD from the mean. Mean survival in duplicate control groups (not exposed to seawater or freshwater) was 87.6% (trout isolates) and 93.3% (herring isolates).

During FY'08, we investigated biocontainment procedures necessary to prevent release of *Ichthyophonus* from research laboratories. A manuscript describing appropriate halogen concentrations necessary to kill *Ichthyophonus* is currently in press. Briefly, chlorine and iodine solutions were 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.

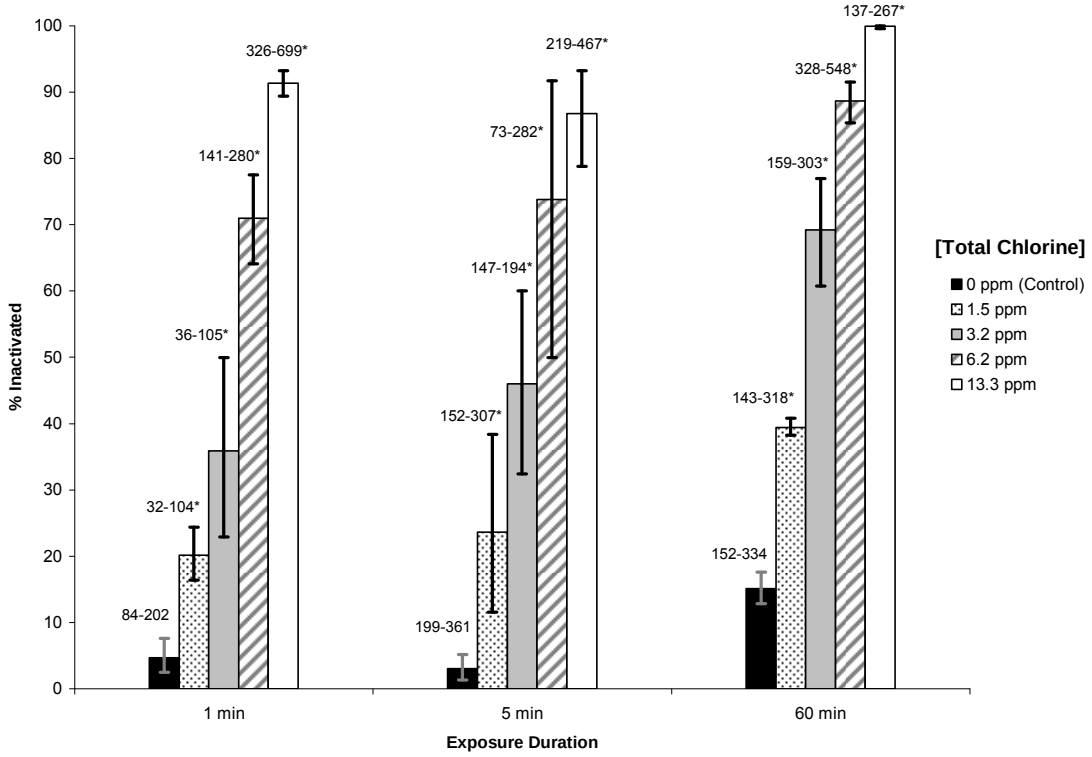

Figure 3. Inactivation of *Ichthyophonus* spores in chlorine / seawater (30‰) solutions. Bars indicate the percentages corresponding to the means of arc sine transformed proportions for the three replicates; error bars indicate 2 SD from the mean. Numerals above the bars indicate the number of spores per replicate (n = 3), and "*" indicates treatments where % inactivation was significantly greater ($p \leq 0.05$) than that in controls.

Other laboratory studies completed or initiated in 2008 include:
- investigations into the potential for cross-contamination of *Ichthyophonus* samples collected during field surveillances; cross contamination potential is low (LaPatra et al, In Press).
- investigations into the minimum waterborne exposure levels necessary to initiate VHS epizootics in Pacific herring (exposure levels as low as 50pfu / mL for 24 hrs were sufficient to initiate laboratory epizootics).
- investigations into viral shedding from Pacific herring after exposure to VHSV (herring shed up to 500 million pfu / day during the peak of the epizootic, approximately 6-10d post-exposure).
- challenge studies to compare the virulence of VHSV strains to Pacific herring, including Genogroup IVa (the strain endemic to the eastern North Pacific), Genogroup IVb (the strain currently emerging in the North American Great Lakes), and Genogroup I (the European strain).
- investigations into the susceptibility of copper and brown rockfish to *Ichthyophonus*
- investigations into the ability of euphasids and seal lice (*Lepeopthirius* sp.) to serve as intermediate hosts for *Ichthyophonus*
- initiated challenge studies and implemented subtractive hybridization techniques to develop a molecular diagnostic technique for VEN
- developed a molecular probe for the MHC-1b gene in Pacific herring and determined that both MHC-1b and PSMB-1 genes are both up-regulated during the early phases of VHS. It is anticipated that these and other genes may serve as proxy indicators of future susceptibility of herring populations to VHS.
- continued development of virus cultivation a system using *ex vivo* herring fin cultures to determine population susceptibility to VHS epizootics. The assay has been optimized, typical virus replication titers are approximately 1-2 logs lower in fins from herring that survived an epizootic than in fins from naïve herring.

Field Surveillances of Infection and Disease Prevalences:

Surveys of wild herring were preformed in Prince William Sound, Sitka Sound, Lynn Canal, and Puget Sound during FY'08. In Prince William Sound, 60 adult herring were sampled by hook-and-line from Sawmill Bay on November 30, 2007; prevalence of *Ichthyophonus* was 25% (15/60), with 12% (7/60) demonstrating visible signs of ichthyophoniasis on internal and / or external organs. Prevalences of VHSV and VEN were 0% (0/60). Additionally, 60 adult herring were captured by trawl from Simpson Bay on December 2, 2007; prevalence of *Ichthyophonus* was 37% (22/60), with 10% (6/60) demonstrating visible signs of ichthyophoniasis on internal and / or external organs. Pre-spawn samples of adult pacific herring were collected from PWS during March, 2008, and the complete ADF&G pathology report is included below:

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 (Fish and Whale Bays) 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: 3/17, 3/19, 3/24, 3/24/08 **DATE SAMPLE RECEIVED:** 3/29/08

SPECIMEN TYPE: Kidney/spleen pools **LIFE STAGE:** Adults/juveniles **STATE:** Refrigerated
Water samples — Dried smears
Blood smears — Fixed in formalin
½ Heart explant cultures
½ Hearts fixed in cassettes
Whole fish

NUMBER IN SAMPLE: 225 kidney/spleen pools **WILD:** Yes
180 blood smears
180 explant cultures
180 ½ fixed hearts in cassettes
15 whole fish
12 water samples

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: 5/8/08

CLINICAL FINDINGS:

VIROLOGY: Fish tissues processed without freezing –fish appeared normal in the field except for nematode infestations

0/225 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. The samples were highly contaminated when received requiring extensive re-plating after filtration (0.45 µ) - 127 filtered among 225 samples.

The 15 fish received whole were sampled 3/19 and in a state of decomposition when received. These samples were not processed.

0/12 filtered water samples positive for virus as processed above

ICHTHYOPHONUS: (3 numbered tubes contained no samples)

40/177 (22.6%) heart explants with growth typical of *Ichthyophonus hoferi* after 13 days @ 14.7 °C

Histological intensity – slides being processed and will be sent to the Seattle investigators

VENV: (1 smear too thick to read)

1/179 (0.55%) peripheral blood smears with erythrocytic cytoplasmic inclusion bodies typical of VENV
Moderate intensity

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

COMMENTS/RECOMMENDATIONS:

No viral CPE (presumptive VHSV) was detected in the VHSV samples collected. *Ichthyophonus* was present at a moderate prevalence in the heart explants. Typical VENV erythrocytic inclusions were observed in only one blood smear at a moderate intensity.

The integrity of the virus samples may have been somewhat compromised by the unnecessary handling to circumvent the excessive contamination due to wicking from the open sample bags received and the length of time (up to 15 days) before the tissues could be processed. Wicking of contaminating material from the whole fish sample bags may also have contributed to this problem.

Despite this issue, any presence of at least one log 10 of virus in a given sample should have been detectable. Recovery of lower virus titers requiring a blind passage for detection may have been less likely.

Suggestions for samples next year: Kidney/spleen tissues should be collected in 2 oz white-labeled Whirl-Pak bags and all whole fish should be processed in the field; many of the blood smears were too thick for optimum staining and interpretation.

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

TECHNICAL ASSISTANCE: I. Conte

COPIES TO: FY08, Herring, T. Meyers, S. Moffitt (Cordova)

In Puget Sound, samples of adult, pre-spawn herring were collected from Drayton Pass (Jan. 15, 2008), Yukon Harbor (Feb. 5, 2008), Skagit Bay (February 2, 2008), and Holmes Harbor (March 13, 2008); sampling was attempted from Cherry Point on two occasions, but the pre-spawn biomass in 2008 was too low to responsibly deploy a trawl. Prevalence of *Ichthyophonus* was 2% (1/60) in Drayton Pass (a subclinical case), 7% (4/60) in Yukon Harbor with 2% (1/60) demonstrating visible signs of ichthyophoniasis, 23% (14/60) in Skagit Bay with 10% (6/60) demonstrating visible signs of ichthyophoniasis, and 48% (29/60) in Holmes Harbor with 12% (7/60) demonstrating visible signs of ichthyophoniasis. Samples for virology were not collected from the Puget Sound samples; however, juvenile herring from Skagit Bay were screened for VEN during the summer of 2008, when 17% (4/23) were positive in April, 15% (8/53) were positive in June, 7% (8/111) were positive in July, and 0% (0/60) were positive in August.

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In Sitka Sound, adult herring were collected from a pre-spawn aggregation in North middle Island on March 26, 2008. ADF&G pathology report is included below:

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

ACCESSION NO: 08-0541

REPORT OF LABORATORY EXAMINATION

LOT (YEAR, STOCK, SPECIES): Sitka Sound (N. Middle Island) 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: 3/26/08 **DATE SAMPLE RECEIVED:** 3/28/08

SPECIMEN TYPE: Kidney/spleen pools **LIFE STAGE:** Adult **STATE:** Unfrozen/on ice
½ heart explant cultures
½ heart fixed

NUMBER IN SAMPLE: 60 kidney/spleen pools **WILD:** Yes
60 explant cultures
60 fixed hearts

HISTORY/SIGNS: Since the crash of the Prince William Sound (PWS) herring stock in 1993, there have been ongoing investigations regarding the recovery process and diseases in the population. Disease investigations have focused on VHSV, *Ichthyophonus hoferi* and VENV. Sitka Sound herring have been used as a control population.

REASON FOR SUBMISSION: A recently funded project includes annual surveillance for the presence of VHSV, *Ichthyophonus* and VENV in Pacific herring from PWS. Sitka Sound has been used as a control site.

FINAL REPORT DATE: 4/29/08

CLINICAL FINDINGS: VEN slides were taken back to Seattle for examination

VIROLOGY: Fish tissues processed without freezing; adult 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:

Heart Explant

17/60 (28.3%) heart explants with growth typical of *Ichthyophonus hoferi* after 11 days @ 14.7 °C

Histopathology- intensity – Processed slides will be sent to the Seattle investigators

COMMENTS/RECOMMENDATIONS:

No viral CPE (presumptive VHSV) was detected in the samples collected. *Ichthyophonus* was present at a moderate prevalence in the heart explants with varying intensities based on cursory histological examination..

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

TECHNICAL ASSISTANCE: I. Conte

COPIES TO: FY08, Herring, T. Meyers

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In Lynn Canal, prevalence of *Ichthyophonus* was monitored in the population of adult herring from November, 2007 – May, 2008 in coordination with Drs. Jeep Rice Ron Heintz, and J.J. Vollenweider under EVOS TC projects #090804 and #090806. ADF&G pathology report is included below:

ACCESSION NO: 08-0527

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): Lynn Canal (Juneau) 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: 11/10/07; 2/23, 4/12, 5/10/08 **DATE SAMPLE RECEIVED:** 11/16/07; 2/29, 4/15, 5/13/08

SPECIMEN TYPE: Excised hearts **LIFE STAGE:** Adult **STATE:** Unfrozen/on ice

NUMBER IN SAMPLES: 59-61 **WILD:** Yes

HISTORY/SIGNS: Since the crash of the Prince William Sound (PWS) herring stock in 1993, there have been ongoing investigations regarding the recovery process and diseases in the population. Disease investigations have focused on VHSV, *Ichthyophonus hoferi* and VENV. Because weights and lengths for Lynn Canal herring are accessible on a seasonal basis by NMFS, additional heart samples were collected to examine if there are seasonal changes in the prevalence of *Ichthyophonus* infections.

REASON FOR SUBMISSION: Determine prevalences of *Ichthyophonus* by explant culture and intensity of infection by histological examination. Each heart was cut in half longitudinally, ½ for culture and ½ fixed in 10% buffered formalin for histology. Only the hearts positive for *Ichthyophonus* in culture were processed for histology.

FINAL REPORT DATE: 6/3/08

CLINICAL FINDINGS:

Heart Explant Culture

First group-11/10/07

7/61 (11.4%) heart explants with growth typical of *Ichthyophonus hoferi* after 14 days @ 14.7 °C

Second group – 2/23/08

3/61 (4.9%) *Ichthyophonus* as above

Third group – 4/12/08

3/61 (4.9%) *Ichthyophonus* as above

Fourth group – 5/10/08

11/59 (18.6%) *Ichthyophonus* as above

Histopathology- intensity – Processed slides will be sent to the USGS Marrowstone Lab near Seattle for archiving and later examination

COMMENTS/RECOMMENDATIONS:

***Ichthyophonus* was present at low to moderate prevalences in the heart explants with varying intensities based on cursory histological examination.**

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

TECHNICAL ASSISTANCE: I. Conte, J. Schirmer

COPIES TO: FY08, Herring, T. Meyers

ACCESSION NO: 08-0538

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Future Work:

No significant changes are proposed to the FY'09 study plan. Briefly, FY'09 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.

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 Kurt Stick, Adam Lindquist, and Darcy Wildermuth (Washington Department of Fish and Wildlife). Herring from Sitka Sound were collected in collaboration with Dr. Keith Cox (Sheldon Jackson College / NOAA-Fisheries), and Eric Coonradt (ADF&G: Sitka). Herring from Lynn Canal were collected in collaboration with researchers at the Ted Stevens Marine Science Center in Juneau, including JJ Vollenweider, Ron Heintz, and Jeep Rice. Dr. Hershberger hosted tours of the Marrowstone Marine Field Station and the Western Fisheries Research Center for EVOSTC Executive Director, Drs. D. Hay, T. Linley, and Japanese researchers as part of the EVOS TC-funded project #090509. 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. Additionally, Hershberger spent four weeks during the summer of 2008 in Cordova to participate in the writing of the Prince William Sound Herring Restoration Plan.

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 will be hosted at the Marrowstone Marine Field Station Oct. 8-9, 2008.

Information Transfer:

Publications:

Hershberger, PK, NE Elder, CA Grady, JL Gregg, CA Pacheco, C Greene, C Rice, TR Meyers. *In Press.* Recurring viral erythrocytic necrosis (VEN) in juvenile Pacific herring from Puget Sound, WA, USA. Journal of Aquatic Animal Health.

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

Hershberger, PK, CA Pacheco, JL Gregg, M Purcell, SE LaPatra. *In Press*. Differential survival of *Ichthyophonus* isolates indicates parasite adaptation to its host environment. Journal of Parasitology.

LaPatra, S., R. Kocan, P. Hershberger. 2008. Potential for cross-contamination of *in vitro* explant cultures initiated from *Ichthyophonus* - infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 31: 317-320.

Conference and Workshop Attendance:

Hershberger, P.K. April 2, 2008. Invited seminar speaker at the Bodega Bay Marine Laboratories, University of California, Davis. "Ecology of Disease in Marine Fishes from the Eastern North Pacific."

Hershberger, P.K., J.L. Gregg, C.A. Grady, R.M. Collins. June 23 – 25, 2008. Platform. Virus Shedding from Pacific Herring after Exposure to Viral Hemorrhagic Septicemia Virus (VHSV). AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA. (Presented).

Hershberger, P.K., B.K. van der Leeuw, J.L. Gregg, C. A. Grady, K. Lujan, S. Gutenberger, J.H. Petersen, M.J. Parsley. June 23 – 25, 2008. Platform. Emergence of *Ichthyophonus hoferi* in the Columbia River by American Shad. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA. (Presented).

Hershberger, P.K. June 23-25, 2008. Platform. Recurring Viral Erythrocytic Necrosis Epizootics in Juvenile Pacific Herring from Puget Sound. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA. (Presented).

Garver, K., G. Traxler, P. Hershberger, S. LaPatra. June 23 – 25, 2008. Platform. VHSV in Farmed and Wild Fish in the Marine Waters of the Pacific Northwest. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA.

Kraus, D., P. Hershberger, C. Grady, J. Gregg, J. Winton, J. Hansen. June 23 – 25, 2008. Platform. Analysis of Immune Regulated Genes in Pacific Herring Challenged with Viral Hemorrhagic Septicemia Virus. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA.

Hershberger, P.K., C.A. Pacheco, JL. Gregg, M. K. Purcell, S.E. LaPatra. June 23-25, 2008. Poster. Differential Survival of *Ichthyophonus* Isolates Indicates Parasite Adaptation to its Host Environment. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA. (Presented).

Hershberger, P.K., C.A. Pacheco, J.L. Gregg. June 23-25, 2008. Poster. Inactivation of *Ichthyophonus* Spores using Sodium Hypochlorite and Polyvinyl Pyrrolidone Iodine. AFS - 49th Annual Western Fish Disease Workshop. Ocean Shores, WA. (Presented).

Hershberger, P.K. May 19-22, 2008. Platform. Emergence of *Ichthyophonus* in Fishes from the Eastern North Pacific. Native American fish and Wildlife Society National Meeting. Yakima, WA (Presented).

Hershberger, P.K., B.K. van der Leeuw, J.L. Gregg, C.A. Grady, K. Lujan, S. Gutenberger, J.H. Petersen, M.J. Parsley. May 5-8, 2008. Platform Emergence of *Ichthyophonus hoferi* in the Columbia River by American shad. Western Division, American Fisheries Society. Portland, OR. (Presented).

Hershberger, P.K., D. Fagergren. March 26, 2008. Forage Fish Health and Changes. South Sound Science Symposium. Tacoma, WA. Invited Speaker.

Hershberger, P.K., N.E. Elder, C.A. Grady, J.L. Gregg, C.A. Pacheco, C. Greene, C. Rice, T.R. Meyers. March 4-6, 2008. Platform. Recurring viral erythrocytic necrosis (VEN) epizootics in juvenile Pacific herring from Puget Sound. Fish Health / Disease Ecology Symposium Organizer and Session Chair. Annual Meeting: North Pacific International Chapter - American Fisheries Society. Bellingham, WA. (Presented).

Gregg, J, M Meyers, C Grady, J Word, P. Hershberger. March 4-6, 2008. *Ichthyophonus* in fishes from the northeastern Pacific Ocean. Annual Meeting: North Pacific International Chapter - American Fisheries Society. Bellingham, WA.

Hershberger P, J Gregg, C Pacheco, J Winton, J Richard, G Traxler. January 21-23, 2008. Platform. Larval Herring are highly susceptible to VHS and survivors are partially protected after their metamorphosis to juveniles. Alaska Marine Science Symposium. Anchorage, AK. (Presented).

Budget:

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