Exxon Valdez Oil Spill
Long-Term Herring Research and Monitoring Program Final Report

Herring Disease Program

Exxon Valdez Oil Spill Trustee Council Project 16120111-K
Final Report

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Study History: The biomass of Pacific herring (*Clupea pallasii*) in Prince William Sound, Alaska decreased from 120,000 metric tons to less than 30,000 tons following the *Exxon Valdez* Oil Spill in 1989. Cause(s) of this population decline remain unresolved; leading hypotheses include combinations of direct and indirect mortality from oil exposure, predation, competition for limited resources, and disease-related mortality. The *Exxon Valdez* Oil Spill Trustee Council launched early efforts to investigate the possible involvement of infectious and parasitic diseases in the initial population decline. These early efforts from 1994-2003 (Restoration projects 94320S, 95320S, 96162, 97162, 98162, 99328, 99462, 00462, 01462, 02462, 03462) consisted primarily of fish health surveillances lead by Dr. Gary Marty (formerly University of California Davis, currently British Columbia Ministry of Agriculture and Lands). However, larger impacts of the spill occurred in the ensuing decades since 1989, as the Prince William Sound herring population failed to recover. As a result, restoration goals shifted towards understanding the factors (including mortality from infectious and parasitic diseases) that may be contributing to the ongoing failed population recovery. Beginning in 2007, the Herring Disease Program expanded upon the early work by Dr. Marty by continuing the annual disease surveillances and providing disease data for the age-structured assessment model used by the Alaska Department of Fish and Game; however, a significant portion of the Herring Disease Program also includes controlled experimental studies intended to determine cause-and-effect disease relationships. The first phase of the Herring Disease Program (Project 070819) was initiated as a 4 year project from FY 2007-2010; a one year no cost extension was granted for FY 2011. The Herring Disease Program was continued as a follow-up study (Project 10100132-I) from 2010-2013, as the Herring Disease Program became an integrated project within the Prince William Sound Herring Survey Program. The integration of the Herring Disease Program within the Prince William Sound Herring Survey Program continues with this most recent project. Funding was provided during 2012-2014; as such, this report only covers the primary Herring Disease Program results from those years. Results from previous Herring Disease Program projects are provided in the earlier final reports.

Abstract: This study includes annual field surveys of *Ichthyophonus*, viral hemorrhagic septicemia virus, and erythrocytic necrosis virus in adult and juvenile Pacific herring (*Clupea pallasii*) in Prince William Sound, Alaska and several reference populations in Alaska, British Columbia, and Washington. Results from controlled experimental studies with *Ichthyophonus* indicated that:

- Pacific herring could become infected after repeated feedings on simulated infected offal,
- -Gross external signs of ichthyophoniasis can persist for extended periods without causing direct host mortality,
- A novel tool (chromogenic in situ hybridization) was developed to confirm the presence of *Ichthyophonus* in histological sections,
- A circulating stage of *Ichthyophonus* was detected in the blood of infected hosts,
- *Ichthyophonus* remains viable in a fish carcass for up to 4 weeks and remains infectious for at least 5 days post mortem,
- Six distinct genetic types of *Ichthyophonus* were identified in different hosts throughout the world,
- Tissue explant culture was confirmed to be more sensitive than qPCR for detecting low - intensity *Ichthyophonus* infections directly from fish tissues.

Results from controlled experimental studies with viral hemorrhagic septicemia virus indicated that:

- Cooler temperatures are more conducive to viral hemorrhagic septicemia epizootics in Pacific herring,
- A blocking ELISA was developed to detect fish antibodies to viral hemorrhagic septicemia virus,
- A more sensitive plaque neutralization test was optimized to detect herring neutralizing antibodies to viral hemorrhagic septicemia virus,
- The relative susceptibility of Pacific herring to other viral hemorrhagic septicemia virus genotypes was assessed, experimental spill-over, amplification, and spill-back was demonstrated between Atlantic salmon (*Salmo salar*) and Pacific herring,
- The efficacy of homologous and heterologous DNA vaccines against viral hemorrhagic septicemia virus was demonstrated in Pacific herring.

Controlled experimental studies with erythrocytic necrosis virus resulted in the development of a conventional PCR technique that is capable of the virus in the blood and the development of a quantitative PCR technique that is capable of detecting the virus in any herring tissues.

Key Words: Pacific herring, disease, VHS, viral hemorrhagic septicemia, *Ichthyophonus*, ichthyophoniasis, viral erythrocytic necrosis, VEN, erythrocytic necrosis virus, ENV

Project Data: *Description of data* – data were collected from field surveys and controlled experiments performed at the USGS – Marrowstone Marine Field Station. Field samples were processed at the USGS – Marrowstone Marine Field Station and the ADF&G Juneau Fish Pathology Laboratory (Case record numbers are reported in Table 1). Results from controlled laboratory experiments are maintained in notebooks at the USGS – Marrowstone Marine Field Station.

All of the data are publically available on the Alaska Ocean Observing System (AOOS) data portal (http://portal.aoos.org/gulf-of-alaska.php#metadata/fc5b0956-ef7c-49df-b261-c8e2713887fc/project).

The AOOS contact is Carol Janzen, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org, http://portal.aoos.org/gulf-of-alaska.php.

There are no limitations on the use of the data, however, it is requested that the authors be cited for any subsequent publications that reference this dataset. It is strongly recommended that careful attention be paid to the contents of the metadata file associated with these data to evaluate data set limitations or intended use.

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TABLE OF CONTENTS

Executive Summary 8
List of Tables 10
List of Figures 11
Chapter 1: Surveillance of Pathogens and Diseases in Wild Herring Populations 22
Chapter 2: Experimental Studies Involving *Ichthyophonus* spp. 22
2.1 The Parasite *Ichthyophonus* in Pacific Herring. 22
2.2 Infecting Pacific Herring with *Ichthyophonus* in the Laboratory 22
2.3 Persistence of External Signs in Pacific Herring *Clupea pallasii* with Ichthyophoniasis. 23
2.4 Detection of *Ichthyophonus* by Chromogenic in Situ Hybridization. 24
2.5 Identification of the Infectious Stage of *Ichthyophonus* sp. and Description of a Circulating Blood Stage 25
2.6 Viability and Infectivity of *Ichthyophonus sp.* in Post-Mortem Pacific Herring 26
2.7 *Ichthyophonus* Phylogeny Based on ITS rDNA Structure Prediction and Alignment Identifies Six Clades, with a Single Dominant Marine Type. 27
2.8 Analytical and Diagnostic Performance of a qPCR Assay for *Ichthyophonus* spp. Compared to the Tissue Explant Culture 'Gold Standard'. 29
Chapter 3: Experimental Studies involving Viral Hemorrhagic Septicemia Virus (VHSV) 31
3.1 Principles Underlying the Epizootiology of Viral Hemorrhagic Septicemia in Pacific Herring throughout the North Pacific Ocean. 31
3.2 Influence of Temperature on Viral Hemorrhagic Septicemia (Genogroup IVa) in Pacific Herring, *Clupea pallasii*. 32
3.3. Development of a Blocking Enzyme-Linked Immunosorbent Assay and Virus Neutralization Assay to Detect Antibodies to VHSV. 33
3.4 Optimization of a Plaque Neutralization Test to Identify the Exposure History of Pacific Herring to Viral Hemorrhagic Septicemia Virus. 33
3.5 Virulence of Viral Hemorrhagic Septicemia Virus (VHSV) Genotypes Ia, IVa, IVb, and IVc in Five Fish Species. 34
3.6. Demonstration of Atlantic Salmon as a Host and Reservoir of Viral Hemorrhagic Septicemia Virus Type IVa 35
3.7. Influence of Temperature on the Efficacy of DNA Vaccines Against Viral Hemorrhagic Septicemia in Pacific Herring. 36
Chapter 4: Experimental Studies Involving Viral Erythrocytic Necrosis (VEN). 38
4.1. Molecular identification of Erythrocytic Necrosis Virus (ENV) from the Blood of Pacific Herring. 38
4.2. Identification of the Major Capsid Protein of ENV and Development of Quantitative Real-time PCR Assays. 39
Chapter 5: Additional Studies 40
5.1 Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. 40
5.2 Infectious Diseases of Fishes in the Salish Sea. 42
5.3 Molecular Characterization of Hepatic Coccidiosis and a Morphological Report of a Novel Intestinal Coccidian. 42
List of Scientific Products and Literature Cited 44

Acknowledgments 48

Executive Summary

All available data continue to support the hypothesis that direct and indirect mortalities from infectious and parasitic diseases, including viral hemorrhagic septicemia (VHS), viral erythrocytic necrosis (VEN), and ichthyophoniasis contribute to population-level impacts on Pacific herring (*Clupea pallasii*) populations in Prince William Sound (PWS) and throughout the NE Pacific. Results from laboratory-based studies indicate that Pacific herring are highly susceptible to these diseases and exposure to the causative agents often results in host mortality.

Ichthyophonus occurred in populations of Pacific herring throughout the NE Pacific. Infection prevalence varied with geographic location, season, and population age structure, with prevalence in adult herring from Prince William Sound ranging from 24 - 47% during 2014 – 2016; infection prevalence in juvenile cohorts was typically much lower. A review paper describing the demographic patterns detected in *Ichthyophonus* surveys throughout the NE Pacific from 2003-2013 was published. One interesting anomaly to the demographic patterns occurred in Cordova Harbor, where infection prevalence was unusually high among juvenile cohorts; follow-up studies are planned to address this anomaly. Additionally, we investigated the appearance of external ichthyophoniasis signs on the flank of juvenile herring. The characteristic open ulcers can be quite persistent on infected cohorts, but the appearance of these signs does not necessarily precede host mortality. A very small stage of *Ichthyophonus* was detected in the blood of infected fish. This parasitic life stage is most certainly involved in dissemination throughout the host tissues, and it may represent the infectious stage. We also determined that *Ichthyophonus* life stages remain viable and infectious in a dead host for extended periods; this ability of the parasite to survive both saprophytically and facultatively likely provides the parasite with a persistence strategy in the wild. We also collected *Ichthyophonus* isolates from around the world and determined that at least six distinct genotypes exist. The predominant type in the NE Pacific is common among most hosts we examined, including Pacific herring. Interestingly, a different genotype is pervasive in freshwater rainbow trout aquaculture facilities throughout the world; in all likelihood, this is freshwater genotype represents the original type species *I. hoferi*. Follow-up studies are underway to evaluate the relative virulence of the different genotypes. Finally, we confirmed that tissue explant culture is the most sensitive diagnostic technique available for detecting low-intensity *Ichthyophonus* infections; it is even more sensitive than quantitative PCR.

During the study period 2012 – 2014, VHS virus (VHSV) was detected at low prevalence and low titer in random samples of adult herring from Sheep Bay (2014). This low prevalence is neither surprising nor uncommon, considering the prevalence of VHSV is generally extremely low during endemic periods (i.e. below the detection threshold of 5% prevalence with 95% confidence provided by a 60 fish sample size). However, VHS epizootics accompanied by high mortality can occur within days after these same fish become confined in high densities or subjected to limited water exchange. An apparent VHS epizootic was detected in juvenile herring from the nearshore regions of the San Juan Islands (north Puget Sound) during 2014. Field biologists recognized lethargic juvenile herring demonstrating characteristic hemorrhages during routine beach seining exercises. Hemorrhaged individuals were high-graded from the catches, frozen at -20°C, and submitted for VHSV screening. Even using these sub-optimal handling procedures, VHSV was detected in high tissue titers among 13-27% of the samples. It is very likely that this type of small-scale VHSV epizootic - occurring in the apparent absence of any

appreciable mortality event - is quite common, yet overlooked because of a paucity of sampling efforts in wild marine fish. Laboratory studies indicated that cooler water temperatures were more conducive to VHS epizootics, resulting in greater mortality, higher viral tissue titers, and longer viral persistence in the host tissues. This inverse relationship between temperature and VHS was likely mediated by an enhanced immune response at warmer temperatures, where a robust type I interferon response was indicated by rapid and significant upregulation of the herring Mx gene. An important advancement during this funding cycle included the development and optimization of a number of techniques (ELISA, virus neutralization, and plaque neutralization – PNT) that are capable of measuring VHSV antibodies in surviving fish. The PNT was by far the most sensitive technique in Pacific herring, and future work is focusing on this technique. Further development of the PNT as a forecasting tool is currently underway to determine whether it can be employed as both a hindcasting tool capable of identifying prior VHS epizootics and a forecasting tool capable of assessing the potential for future VHS epizootics. Other studies determined the susceptibility of Pacific herring to other VHSV genotypes and assessed the effects of temperature on the efficacy of DNA vaccines against VHSV in Pacific herring.

During the study period 2012 – 2014, a low prevalence of VEN was detected in herring populations throughout the NE Pacific, including Cook Inlet (2014), Sitka (2014), and Prince William Sound (2016). These were generally low-intensity infections, with the exception of the positive fish from Cook Inlet, which demonstrated a high proportion of circulating erythroblasts and erythrocytes demonstrating VEN inclusions. Additionally, the methods for two new VEN diagnostic techniques (conventional and quantitative PCR's) were published, thereby enabling confirmation of this condition from standard tissue samples. This important advancement will enable us to diagnose the condition in the absence of blood films, which are not typically included in standard necropsy procedures.

Additional products included two additional synthesis/review papers and a description of coccidian parasites in herring. Because ichthyophoniasis is the most ecologically and economically significant disease of wild marine fishes in the world, it was included in a review paper describing the impacts of climate change on marine diseases (Burge et al 2014). As part of this exercise, all the published reports of *Ichthyophonus* impacts on wild fishes were consolidated. Additionally, we published a review of the primary diseases impacting marine fishes in the Salish Sea, including bacterial kidney disease, infectious hematopoietic necrosis, VHS, VEN, and ichthyophoniasis). Finally, we described the occurrence of two coccidian parasites in herring and the apparent paucity of *Ichthyophonus* and VHSV in Atlantic herring populations.

Status of Project Objectives:

- Provision of disease prevalence data necessary for the age structured assessment herring model – **Completed (Chapter 1)**
- Provision of disease process studies intended to investigate the seasonality of herring diseases in PWS – **Completed (Chapter 1)**
- Collection of novel disease forecasting data – **Completed (Section 3.4)**
- Production of Specific Pathogen-Free Pacific herring intended as laboratory hosts for controlled experiments intended to determine cause-and-effect disease relationships – **Completed (used in Chapters 2-4)**
- Development of a novel diagnostic technique (fluorescent in situ hybridization) intended to provide confirmatory diagnosis of *Ichthyophonus* from histology sections. – **Completed (Section 2.4)**

Detailed descriptions of the studies designed to address each of these objectives are included in the following chapters.

List of Tables

Table 1	Results of pathogen prevalence surveys in Pacific herring	13
Table 2	Prevalence of *Ichthyophonus* sp. infection in Pacific herring after single and multiple feedings with infected tissues	22
Table 3	Diagnostic sensitivity (DSe) and specificity (DSp) of the *Ichthyophonus* qPCR test on experimentally inoculated *Ichthyophonus*-exposed (ICH) and control (PBS) *Clupea pallasii*.	30
Table 4	Relative percent survival (RPS) 15 d after exposure of Pacific herring to viral hemorrhagic septicemia virus.	37
Table 5	Reported ichthyophoniasis epizootics in wild fishes.	41

List of Figures

Figure 1	Representative external signs observed in Pacific herring after treatment with *Ichthyophonus* by i.p. injection.	23
Figure 2	*Ichthyophonus* identified in (a) Pacific herring epicardial connective tissue and (c) rainbow trout stomach tunica propria by hybridization with an oligonucleotide probe targeting an *Ichthyophonus* 18s rDNA sequence.	24
Figure 3	Transformation of *Ichthyophonus* schizonts in the stomach (bolus) of Pacific staghorn sculpins, *Leptocottus armatus,* following ingestion of infected herring tissue.	25
Figure 4	Periodic Acid-Schiff (PAS) stained histologic sections of post-mortem herring (*Clupea pallasii*) tissue.	26
Figure 5	Phylogeny of *Ichthyophonus* based on Bayesian MCMC analysis of ITS rDNA consensus sequences under a six-partition model.	28
Figure 6	VHS epizootic and associated fish kill involving wild herring in Vancouver Island, B.C, Canada.	31
Figure 7	Effects of temperature on the cumulative mortality of VHSV exposed herring.	32
Figure 8	Kinetics of virus neutralizing activity in the plasma of VHSV survivors.	34
Figure 9	Susceptibility of Pacific herring to VHSV genotypes.	35
Figure 10	Gross clinical signs of VHSV in Atlantic salmon.	36
Figure 11	Maximum likelihood (ML) phylogenetic tree for ENV.	38
Figure 12	Relationship of erythrocytic necrosis virus (ENV) to other iridoviruses based on partial sequencing of the major capsid protein (MCP).	39
Figure 13	*Goussia clupearum*	43

Chapter 1: Surveillance of Pathogens and Diseases in Wild Herring Populations

Annual infection and disease surveillance for VHSV, ENV, and *Ichthyophonus* continued during 2014-2016. The primary purpose of these efforts remains the provision of disease inputs to the PWS herring age structured assessment (ASA) model. As reference locations, herring populations outside PWS were sampled whenever opportunities arose. The three primary pathogens continue to occur throughout the NE Pacific, and the prevalence and intensity of VHSV and ENV remained low during typical endemic periods. However, indications of localized viral epizootics exist, including a likely VHS event among juvenile herring cohorts that occurred in north Puget Sound during 2014; this event was not accompanied with any observed mortality. Additionally, we investigated another case involving hemorrhaged adult herring near Craig, AK in 2015. VHSV was not detected among any of the Craig fish; rather, it is suspected that the observed lesions likely resulted from predator escapes. *Ichthyophonus* also remains endemic throughout the region; however, during typical endemic periods this parasite occurs in much higher infection prevalence than the viral agents. Unusually high *Ichthyophonus* infection prevalence and intensity were documented among juvenile herring from Cordova Harbor, and future studies are planned to investigate the causes for this anomaly.

As an attempted consolidation, survey results in the tables below indicate a summation of all results since the beginning of the Herring Disease Program in 2007. The results from 2010 – present are novel to this project.

Table 1. Results of pathogen prevalence surveys in Pacific herring. Results from 2007-2010 were reported in HDP final reports from previous years, but are also included here as a complete inventory. Results from 2011-2016 are novel to this final report. ND = no data.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2007	PWS	St. Matthews Bay	April 5	A	224 (17)	42% (25/60)	0% (0/60)	0% (0/60)	ADF&G #07-0540
		Simpson Bay	April 19	J	86 (6)	15% (9/60)	0% (0/60)	17% (10/60)	ADF&G #07-0543
		Sawmill Bay	Nov. 30	A	215 (21)	25% (15/60)	0% (0/60)	0% (0/60)	MMFS #PWS 07-2
		Simpson Bay	Dec. 2	A	187 (13)	37% (22/60)	0% (0/60)	0% (0/60)	MMFS #PWS 07-2
	Cook Inlet	Kamishak B	May 16	A	ND[1]	32% (19/60)	ND	ND	
		Kamishak B	May 27	A	ND[1]	20% (12/59)	ND	ND	
		Kamishak B	May 27	A	ND[1]	28% (17/60)	ND	ND	
	Sitka Sound	S. Cannon Island	April 19	A	215 (18)	28.3% (17/60)	0% (0/60)	0% (0/60)	MMFS #VHSV07-1 & ICH07-5
	Lynn Canal		Nov. 10	A	199	11% (7/61)	ND	ND	ADF&G #08-0527
	Puget Sound[2]	Johnson Point	Jan 18	A	181 (8)	7% (4/59)	ND	ND	MMFS #ICH 07-1
		Port Orchard (Yukon Harbor)	Feb 1	A	181 (11)	17% (10/60)	ND	ND	MMFS #ICH 07-1
		Skagit Bay	Feb 8	A	184 (11)	37% (22/60)	ND	ND	MMFS #ICH 07-1
		Cherry Point	April 30	A	184 (13)	25% (15/60)	ND	0% (0/60)	MMFS #ICH 07-1
		Skagit Bay	April 25-26	J	117 (25)	ND	ND	3% (2/60)	MMFS #VEN Surveys
		Skagit Bay	May 22-24	J	111 (25)	ND	ND	37% (22/60)	MMFS #VEN Surveys
		Skagit Bay	June 19-20	J	116 (17)	ND	ND	38% (23/60)	MMFS #VEN Surveys
		Skagit Bay	July 24-25	J	110 (25)	ND	ND	35% (27/78	MMFS #VEN Surveys
		Skagit Bay	Aug 21-22	J	112 (21)	ND	ND	25% (18/71)	MMFS #VEN Surveys
		Skagit Bay	Sept 18-20	J	109 (23)	ND	ND	36% (32/92)	MMFS #VEN Surveys
		Skagit Bay	Oct. 16	J	109 (14)	ND	ND	6% (4/65)	MMFS #VEN Surveys
		Skunk Bay	Jul 2	J	134 (4)	ND	ND	2% (3/170)	MMFS #VEN Surveys
		Admiralty Inlet	Aug 1	J	129 (5)	ND	ND	0% (0/60)	MMFS #VEN Surveys
		Port Townsend Bay	Oct 16	J	80 (6)	ND	ND	20% (15/75)	MMFS #VEN Surveys

[1]Herring lengths in Cook Inlet were recorded as standard length, not fork length.
[2]160 northern anchovies were also sampled from Puget Sound (Holmes Harbor) on March 11; neither VHSV nor *Ichthyophonus* was detected.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2008	PWS	Fish Bay	Mar 19	A	236 (27)	33% (19/58)	0% (0/45)	2% (1/60)	ADF&G #08-0541
		Evans Pt	Mar 24	A	208 (18)	ND	0% (0/60)	ND	ADF&G #08-0541
		?	Mar 17	J	141 (11)	20% (12/59)	0% (0/60)	0% (0/60)	ADF&G #08-0541
		Whale Bay	Mar 24	J	149 (22)	15% (9/60)	0% (0/60)	0% (0/59)	ADF&G #08-0541
		Port Gravina	Nov 8-12	A	197 (23)	24% (19/80)	0% (0/80)	0% (0/80)	ADF&G #09-0522
		Simpson Bay	Nov 8-12	J	65 (7)	0% (0/78)	ND	1% (1/69)	AFD&G #09-0522
	Sitka Sound	Beli Rock	Mar 5	A	262 (14)	30% (18/60)	ND	ND	MMFS #AK-081A
		N. Middle Island	March 26	A	249 (14)	28% (17/60)	ND	2% (1/60)	ADF&G #08-0538 & #AK08-1C
	Lynn Canal		Feb 23	A	ND	5% (3/61)	ND	ND	ADF&G #08-0527
			April 12	A	ND	5% (3/61)	ND	ND	ADF&G #08-0527
			May 10	A	ND	19% (11/59)	ND	ND	ADF&G #08-0527
	Puget Sound	Drayton Pass	Jan 15	A	144 (7)	2% (1/60)	ND	ND	MMFS #ICH 08-1
		Port Orchard[3]	Feb. 5	A	154 (16)	7% (4/60)	ND	ND	MMFS #ICH 08-1
		Skagit Bay	Feb 2	A	176 (17)	23% (14/60)	ND	ND	MMFS #ICH 08-1
		Holmes Harbor	Mar 13	A	193 (8)	48% (29/60)	ND	ND	MMFS #ICH 08-1
		Skagit Bay	May 29	J	148 (26)	ND	ND	17% (4/23)	MMFS #VEN FF08
		Skagit Bay	June 23-25	J	145 (24)	ND	ND	15% (8/53)	MMFS #VEN FF08
		Skagit Bay	July 22	J	109 (33)	ND	ND	7% (8/111)	MMFS #VEN FF08
		Skagit Bay	Aug 19	J	93 (9)	ND	ND	0% (0/60)	MMFS #VEN FF08
		Skagit Bay	Sept 17	J	89 (12)	ND	ND	2% (1/61)	MMFS #VEN FF08
		Skagit Bay	Oct 8	J	91 (9)	ND	ND	2% (1/60)	MMFS #VEN FF08

[3]Four Pacific Sardines were collected from Port Orchard on March 5; none tested positive for VHSV.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2009	PWS	Port Gravina	Mar 20	A	199 (15)	43% (26/60)	0% (0/60)	0% (0/60)	ADF&G #09-0543 & MMFS #AK 09-1
		Port Gravina	Mar 20	J	168 (11)	25% (15/60)	0% (0/60)	0% (0/60)	ADF&G #09-0543 & MMFS #AK 09-1
		Simpson Bay	Mar 22	J	94 (8)	13% (8/60)	0% (0/60)	5% (3/60)	ADF&G #09-0543 & MMFS #AK 09-1
		Snug Corner Cove	April 13	A	217(27)	26% (16/62)	ND	ND	ADF&G #09-0543 & MMFS #AK 09-1
		Unknown Location	April 4-9	A		45% (27/60)	ND	ND	ADF&G #09-0547
		Port Gravina	Nov 15	A	179 (17)	12% (7/60)	0% (0/60)	0% (0/60)	ADF&G 10-0529 & MMFS AK 09-1B
		Elrington Pass	Nov 17	A	216 (19)	17% (10/60)	0% (0/60)	0% (0/60)	ADF&G 10-0529 & MMFS AK 09-1B
		Simpson Bay	Nov 19	J	87 (14)	5% (3/60)	0% (0/60)	3% (2/60)	ADF&G 10-0529 & MMFS AK 09-1B
		Eaglek Bay	Nov 14	J	98 (4)	3% (1/29)	0% (0/29)	16% (5/31)	ADF&G 10-0530
		Lwr. Herring Bay	Nov 16	J	99 (4)	0% (0/14)	0% (0/14)	21% (3/14)	ADF&G 10-0530
		Simpson Bay	Nov 19	J	70 (12)	5% (1/20)	0% (0/20)	0% (0/33)	ADF&G 10-0530
	Cook Inlet	Kamishak Bay	May 8	A	ND[4]	3% (2/60)	ND	ND	
		Kamishak Bay	May 21	A	ND[4]	2% (1/60)	ND	ND	
	Sitka Sound	Guide Island	Feb 15-16	A	256 (15)	40% (32/80)	ND	ND	ADF&G #09-0540
		Unknown	Mar 24-27	A	270 (19)	46% (20/44)	0% (0/44)	ND	ADF&G #09-0545 & MMFS AK 09-2
		St. John Babtist Bay	Mar 24-27	A	248 (23)	31% (21/67)	0% (0/67)	0% (0/67)	ADF&G #09-0545 & MMFS AK 09-2
		Unknown	Mar 24-27	J	175 (7)	4% (3/69)	0% (0/69)	0% (0/69)	ADF&G #09-0545 & MMFS AK 09-2
	Lynn Canal	Cohen Isl. Amalga Trench	Feb 11-12	A	203 (15)	7% (3/44)	ND	ND	ADF&G #09-0539
		Fritz Cove, Outer Pt, Lena Pt	Mar 18-19	A	ND	13% (8/60)	ND	ND	ADF&G #09-0541
		Gull Isl. & Benj. Isl. Trench	Nov 24	A	210 (14)	18% (11/60)	ND	ND	MMFS #AK09-4
		Benj. Isl. Trench & Fritz Cv.	Dec. 7	A		8% (5/60)	ND	ND	MMFS #AK09-4
	Puget Sound	Port Orchard (Yukon H.)	Feb 2	A	170 (9)	3% (2/60)	ND	ND	MMFS #PS 09-1
		Skagit Bay	Feb 2	A	166 (23)	18% (11/60)	ND	ND	MMFS #PS 09-1
		Port Gamble	Feb 12	A	169 (12)	27% (16/60)	ND	ND	MMFS #PS 09-1
		Holmes Harbor	March 18	A	193 (20)	22% (13/60)	ND	ND	MMFS #PS 09-1
		Skagit Bay	June	J	122 (11)	ND	ND	55% (33/60)	MMFS #VEN FF09
		Skagit Bay	July	J	125 (10)	ND	ND	32% (19/60)	MMFS #VEN FF09
		Skagit Bay	Aug 12	J	121 (18)	ND	ND	4% (2/54)	MMFS #VEN FF09
		Skagit Bay	Oct 12	J	105 (18)			17% (10/60)	MMFS #VEN FF09
	San Fran. Bay[5]	Pt. Chauncey	Feb 11	A	155 (15)	0% (0/81)	ND	ND	MMFS #Ich 09-3B
		Pt Chauncey	Feb 25	A	149 (18)	0% (0/60)	ND	ND	MMFS #Ich 09-3C

[4]Herring lengths in Cook Inlet were recorded as standard length, not fork length.
[5]Additional samples from San Francisco Bay included 69 longfin smelt (Jan 6-13) and 70 striped bass (May 15); none tested positive for *Ichthyophonus.*

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2010	PWS	Port Gravina	Mar 16	A	213 (14)	18% (11/60)	0% (0/60)	2% (1/60)	ADF&G #10-0536 & MMFS # AK10-1
		Port Fidalgo	Mar 19	A	200 (15)	23% (14/60)	0% (0/60)	3% (2/60)	ADF&G #10-0536 & MMFS # AK10-1
		Simpson Bay	Mar 20	J	109 (23)	13% (8/60)	2-5%[6]	10% (6/60	ADF&G #10-0536 & MMFS # AK10-1
		Cordova Harbor	June 2-13	J	85 (12)	35% (17/49)	0% (0/49)	71% (38/48)	MMFS #AK 10-3
		Cordova Harbor	Aug 18	J	44 (3)	0% (0/18)	0% (0/54)	0% (0/17)	MMFS #AK 10-3
		Cordova Harbor	Sept 28 -Oct 7	J	50 (6)	0% (0/22)	0% (0/22)	0% (0/21)	MMFS #AK 10-3
		Simpson Bay	Nov. 3	J	73 (7)	0% (0/38)	ND	6% (2/36	MMFS #AK 10-3
		Port Fidalgo	Nov. 4	J	77 (4)	0% (0/22)	ND	5% (1/22)	MMFS #AK 10-3
		Eaglik	Nov. 5	J	90 (9)	0% (0/34)	ND	26% (8/31)	MMFS #AK 10-3
		Whale Bay	Nov 10-11	J	95 (33)	3% (2/58)	2% (1/60)	18% (10/55)	MMFS #AK 10-3
	Cook Inlet	Kamishak B	May 4	A	ND[7]	2% (1/60)	ND	ND	
		Kamishak B	May 18	A	ND[7]	3% (2/60)	ND	ND	
	Sitka Sound	Indian River	Mar 22-24	A	242 (22)	27% (16/60)	0% (0/60)	2%	MMFS #AK10-2
		Boarder / Sitka Rocks	Mar 22-24	A	209 (28)	15% (9/60)	ND	3%	MMFS #AK10-2
		Mountain Point Kruzof Island	Mar 22-24	A	241 (25)	37% (22/60)	0% (0/60)	0%	MMFS #AK10-2
	Lynn Canal	Shelter Isl	Mar 15-16	A	202 (20)	5% (3/56)	ND	ND	MMFS #AK10-4
		Bridget Cove	April 26	A	212 (11)	13% (5/40)	ND	ND	MMFS #AK10-4
	Puget Sound	Squaxin Pass	Jan 28	A	140 (12)	3% (2/60)	ND	ND	MMFS #PS10-1
		Holmes Harbor	March 23	A	171 (15)	28% (17/60)[8]	ND	ND	MMFS #PS10-1
		Hood Canal[9,10]	May 25&27	A	140 (24)	44% (43/97)	ND	ND	MMFS #PS10-1

[6]A single pooled sample containing tissues from 3 fish tested positive (n=60) for VHSV. Therefore, the prevalence was 1-3 / 60.
[7]Herring lengths in Cook Inlet were recorded as standard length, not fork length.
[8]*Ichthyophonus* prevalence was 6% (1/17) in Pacific staghorn sculpin and 78% (28/36) in American shad.
[9]Biased sample: largest fish were removed from this sample for other purposes prior to determination of *Ichthyophonus* prevalence.
[10]Sample consisted of post-spawn adult herring

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2011	PWS	St. Matthew's B	April 2	A	246	≥12%[11] (7/60)	0% (0/60)	0% (1/60)	ADF&G #11-0538 & MMFS# AK10-3
		Port Gravina	April 4	A	219	27% (16/60)	0% (0/60)	2% (1/60)	ADF&G #11-0538 & MMFS #AK10-3
		Hell's Hole	April 6	A	253	47% (28/60)	0% (0/60)	2% (61/60	ADF&G #11-0538 & MMFS #AK10-3
		Port Gravina	Nov 21	A[12]	205	63% (19/30)	0% (0/60)	3% (1/30)	ADF&G #12-0524 & MMFS #AK11-8
		Port Gravina	Nov 22	A[12]	157	13% (4/30)	0% (0/60)	0% (0/30)	ADF&G #12-0524 & MMFS #AK11-8
		Lwr Herring B	March 11	J	96	2% (1/59)	0% (0/60)	23% (14/60)	MMFS #AK 11-1
		Eaklek	March 15	J	113	5% (3/60)	0% (0/60)	2% (1/59)	MMFS #AK 11-1
		Port Fidalgo	March 16	J	76	10% (6/58)	0% (0/60)	13% (8/60)	MMFS #AK 11-1
		Simpson B	Oct 13	J	52	ND	0% (0/47)	ND	MMFS #AK 11-6
		Simpson B	Nov 15	J	60	ND	0% (0/60)	?	MMFS #AK 11-9
		Whale B	Nov 20	J	83	0% (0/60)	0% (0/60)	?	MMFS #AK 11-9
		Simpson B	Dec 13	J	60	0% (0/60)	0% (0/60)	?	MMFS #AK 11-10
	Cook Inlet	Kamishak B	May 4	A	ND[13]	0% (0/60	ND	ND	
		Kamishak B	May 13	A	ND[13]	2% (1/60)	ND	ND	
	Sitka Sound	Bear Cove	Mar 24	J	108 (11)	2% (1/60)		3%	MMFS #AK 11-4
		Long Isl	Mar 22	A	232 (16)	18% (11/60)		0%	MMFS #AK 11-4
		Salisbury Snd	April 6	A	228 (20)	20% (12/60)			MMFS #AK 11-4
	Lynn Canal	Halibut Cove	Jan 12	A	ND	2% (1/60)	ND	ND	
		Amalga Tr	Jan 28	A	ND	10% (6/60)	ND	ND	
		Amalga Tr	Apr 9	A	ND	18% (11/60)	ND	ND	
		Auke Bay	Apr 18, June 4	A	202 (15)	18% (11/60)	ND	ND	
	Puget Sound	Squaxin Pass	Jan 28	A	140 (12)	3% (2/60)	ND	ND	MMFS #PS10-1
	British Columbia, Canada	Little Qualicum	March 17	A	189 (14)	8% (5/60)	ND	ND	MMFS #BC11-1
		Sydney Inlet	March 23	A	183 (16)	20% (12/60)	ND	ND	MMFS #BC11-1
		Prince Rupert	March 23	A	167 (18)	22% (13/60)	ND	ND	MMFS #BC11-1
		Kwakshua Inlet	March 24	A	194 (16)	27% (16/60)	ND	ND	MMFS #BC11-1
		Haida Gwaii	March 26	A	191 (12)	8% (5/60)	ND	ND	MMFS #BC11-1
		Haida Gwaii	March 30	A	192 (13)	5% (3/60)	ND	ND	MMFS #BC11-1

[11]*Ichthyophonus* cultures were frozen by the airline, killing the parasite; therefore, the true population prevalence was likely greater than the reported prevalence.
[12]Both groups of fish from Gravina were from the same school; the first 30 were high graded for larger fish; the second 30 were representative of the population.
[13]Herring lengths in Cook Inlet were recorded as standard length, not fork length.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2012	PWS	Port Gravina	Mar 28	A	218	42% (25/60)	0% (0/60)	0% (0/60)	ADF&G #12-0533 & MMFS# AK12-4
		Port Gravina	Mar 31	A	215	40% (24/60)	0% (0/60)	0% (0/60)	ADF&G #12-0533 & MMFS# AK12-4
		Fidalgo B	April 2	A	231	35% (21/60)	0% (0/60)	0% (0/60	ADF&G #12-0533 & MMFS# AK12-4
		Port Gravina	Nov 15	A	159	3% (2/60)	0% (0/60)	0% (0/60)	MMFS #AK12-8
		Simpson B	Jan 11	J	57	0% (0/28)	0% (0/60)	?	MMFS #AK12-1
		Simpson B	April	J	ND	3% (1/30)	0% (0/30)	ND	MMFS #AK 12-3
	Cook Inlet	Kamishak Bay	May 7	A	ND	2% (1/60)	ND	ND	
	Sitka Sound	N Khasiana Isl	April 3	A	232 (23)	20% (12/60)			ADF&G #12-0534 & MMFS# AK12-5
		St John Bay	April 4	A	214 (24)	32% (19/60)			ADF&G #12-0534 & MMFS# AK12-5
		Sitka breakwall	April 4	A	225 (22)	10% (6/60)			ADF&G #12-0534 & MMFS# AK12-5
	Lynn Canal	Tee Harbor	June 8	A	176 (13)	0% (0/60)	ND	ND	MMFS #AK 12-6

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2013	PWS	Port Gravina	Mar 27	J	147	3% (2/60)	0% (0/60)	0% (0/60)	ADF&G #13-0537 & MMFS# AK13-2
		Port Gravina	Mar 31	A	232	34% (20/59)	0% (0/60)	0% (0/60)	ADF&G #13-0537 & MMFS# AK13-2
		Port Gravina	April 1	A	225	32% (19/60)	0% (0/60)	0% (0/60	ADF&G #13-0537 & MMFS# AK13-2
		Lwr Herring B	Nov 9	J	93	5% (3/60)	0% (0/60)	12% (7/59)	MMFS #AK13-4
		Port Gravina	Nov 13	J	90	0% (0/39)	0% (0/39)	18% (7/39)	MMFS #AK13-4
		Cordova Hbr	Nov 20	J	70	0% (1/61)	0% (0/61)	7% (4/61)	MMFS #AK13-4
	Sitka Sound	Apple Islands	March 29	A	246 (28)	18% (11/60)	0% (0/60)	0% (0/60)	ADF&G #13-0538 MMFS #AK13-3
		Silver Bay	March 30	A	251 (16)	18% (11/60)	0% (0/60)	0% (0/60)	ADF&G #13-0538 MMFS #AK13-3
		Unknown	March 30	A	226 (26)	18% (11/60)	0% (0/60)	0% (0/60	ADF&G #13-0538 MMFS #AK13-3
	Craig	Diamond Point	Feb 20	A	214 (23)	22% (13/60)	ND	ND	MMFS #AK 13-1
	Puget Sound	Hood Canal[14]	May 19	A	171 (18)	57% (25/44)	ND	ND	MMFS #PS 13-1

[14]Hood Canal sample consisted of post-spawn herring.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2014	PWS	Sheep B	Mar 26	A	217	24% (15/60)	2-8%[15]	0% (0/60)	ADF&G #14-0534 & MMFS #AK14-1
		Fidalgo B	Mar 28	A	218	22% (13/60)	0% (0/60)	0% (0/60)	ADF&G #14-0534 & MMFS #AK14-1
		Snug Corner	Mar 29	A	242	32% (19/60)	0% (0/60)	0% (0/60	ADF&G #14-0534 & MMFS #AK14-1
		Simpson	Nov 15-23	J	78 (12)	2% (1/60)	0% (0/60)	ND	MMFS #AK14-4
		Beartrap	Nov 16	J	70 (5)	2% (1/61)	0% (0/61)	ND	MMFS #AK14-4
		Eaglek	Nov 19	J	96 (4)	3% (2/61)	0% (0/61)	ND	MMFS #AK14-4
	Cook Inlet	Kamisha k Bay	April 30	A	ND	0% (0/60)	0% (0/60)	2% (1/60) [16]	ADF&G #14-0078 & MMFS #AK14-3
			May 13	A	ND	0% (0/60)	0% (0/59)	0% (0/60)	ADF&G #14-0078 & MMFS #AK14-3
	Sitka	Causeway	Mar 26	A	245 (26)	25% (15/60)	0% (0/60)	2% (1/60)	ADF&G #14-0533 & MMFS #AK14-2
		Middle Island	Mar 27	A	241 (31)	20% (12/59)	0% (0/60)	0% (0/60)	ADF&G #14-0533 & MMFS #AK14-2
		Inner Point	Mar 28	A	222 (20)	27% (16/60)	0% (0/60)	0% (0/60)	ADF&G #14-0533 & MMFS #AK14-2
	Puget Sound[17]	Lopez Isl.	Sept 11	J	ND	ND	27% (6/22)	ND	MMFS #PS4-1
		Waldron Isl.	Sept 12	J	ND	ND	13% (3/24)	ND	MMFS #PS4-1

[15]CPE was detected in a single pooled sample containing tissues from 5 fish after the 3rd passage; therefore, the infection prevalence was 1-5 / 60. Viral titer was below 10^1 PFU / g. VHSV was confirmed in the cell culture supernatant by nested cPCR.

[16]VEN inclusions graded at a 3+ infection severity from Fish #38.

[17]Herring samples were collected from two locations in the San Juan Islands during Chinook salmon beach seining efforts. VHSV symptoms observed included a bloody exterior, with an increase to 10% of observed fish showing these symptoms over the course of the summer. Symptomatic herring were high-graded (i.e. not a random sample), frozen -20°C, and sent to the USGS – Marrowstone for VHSV testing (plaque assay). Warm water temperatures were noted, as well as unusually large numbers of age 0+ herring and unusually low numbers of age 0+ sandlance. Positive sampled were confirmed by qPCR using VHSV-specific primers.

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Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2015	PWS	Gravina Pt	April 3	A	228 (17)	25% (15/60)	0% (0/60)	0% (0/60)	ADF&G #15-0533 & MMFS #AK15-2
		Simpson Bay	Nov 6	J	ND	2% (1/46)	0% (0/46)	ND	MMFS #AK15-4
		Lwr Herring B.	Nov 11	J	85 (5)	2% (1/54)	0% (054)	ND	MMFS #AK15-4
		E Whale Bay	Nov 12	J	89 (7)	3% (2/60)	0% (0/60)	ND	MMFS #AK15-4
	Cook Inlet	Kamishak Bay	April 27	A	ND	2% (1/60)	0% (0/60)	0% (0/60)	ADF&G #2015-0048
	Sitka	Beili Rock	Mar 20	A	239 (26)	10% (6/60)	0% (0/60)	0% (0/60)	ADF&G 15-0532 &MMFS #AK15-1
		Beili Rock	Mar 22	A	250 (22)	13% (8/60)	0% (0/60)	0% (0/60)	ADF&G 15-0532 &MMFS #AK15-1
		Beili Rock	Mar 22	A	231 (24)	20% (12/60)	0% (0/60)	0% (0/60)	ADF&G 15-0532 &MMFS #AK15-1
	Ketchican[18]	Near Craig	Dec 17	A	193 (17)	ND	0% (0/76)	ND	MMFS #AK15-5

[18]Submitted by Eric Coonradt (ADF&G): concerns of a possible disease event occurring near Craig AK. Symptoms observed included a bloody exterior, with possible causes hypothesized to include infection or predator marks. Affected individuals comprised approximately 1 out of every 100; those with obvious symptoms were removed from the larger sample and photographed (see photo below). Samples were shipped frozen to MMFS, a 5 gallon bucket of frozen herring that were stored 60 herring were stored at -20°C. VHSV was not detected.

Year	Stock	Location	Collection Date	A/J	Mean Length mm (SD)	*Ichthyophonus* Prevalence	VHSV Prevalence	VEN Prevalence	Diagnostic Lab & Ref#
2016	PWS	Red Head Pt	April 7	A	205 (28)	24% (15/60)	0% (0/60)	0% (0/60)	ADF&G #16-0539 & MMFS #AK16-2
		Knowles Head E	April 8	A	212 (22)	29% (17/59)	0% (0/60)	0% (0/60)	ADF&G #16-0539 & MMFS #AK16-2
		Snug Corner C	Mar 29	A	234 (21)	47% (28/60)	0% (0/60)	2% (1/60)	ADF&G #16-0539 & MMFS #AK16-2
		Simpson Bay	Oct 29	J	82 (4)	25% (15/60)	0% (0/60)	2% (1/60)	MMFS #AK 16-3
		Eaglek Bay	Oct 30	J	95 (5)	3% (2/60)	0% (0/60)	2% (1/60)	MMFS #AK 16-3
		Lower Herring B	Nov 2	J	96 (4)	10% (6/60)	0% (0/60)	0% (0/60)	MMFS #AK 16-3
	Sitka	S. Salsbury Anika	Mar 21	A	218 (23)	10% (6/60)	0% (0/60)	0% (0/60)	ADF&G #16-0537 & MMFS #AK16-1
	Sound	N. Crest	Mar 22	A	215 (13)	18% (11/60)	0% (0/60)	0% (0/60)	ADF&G #16-0537 & MMFS #AK16-1
		Pt. Brown	Mar 22	A	217 (24)	22% (13/60)	0% (0/60)	0% (0/60)	ADF&G #16-0537 & MMFS #AK16-1
	Puget	Dabob Bay	Feb 18	J	128	23% (28/120)	ND	ND	MMFS #PS16-1
	Sound	S. Lopez	Feb 19	J	112	0% (0/30)	ND	ND	MMFS #PS16-1
		Dallas Bank	Feb 19	J	113	2% (1/60)	ND	ND	MMFS #PS16-1
		Gulf of Georgia	Feb 24	J	121	5% (3/60)	ND	ND	MMFS #PS16-1
		Squamish Harbor	April 4	J	126	15% (9/60)	ND	ND	MMFS #PS16-1
		Dabob Bay	April 4	J	131	13% (8/60	ND	ND	MMFS #PS16-1
		Squamish Harbor	April 4	J	115	18% (9/50)	ND	ND	MMFS #PS16-1
		S. Saratoga	April 5	J	117	2% (1/60)	ND	ND	MMFS #PS16-1
		N. Saratoga	April 6	J	123	2% (1/58)	ND	ND	MMFS #PS16-1
		Oak Bay	April 5	J	143	11% (7/62)	ND	ND	MMFS #PS16-1
		E. Pt Angeles	April 13	A	164	0% (0/60)	ND	ND	MMFS #PS16-1
		Yukon Harbor	April 18	J	143	7% (4/60)	ND	ND	MMFS #PS16-1
		Nisqually / Drayton	April 20	J	146	0% (0/60)	ND	ND	MMFS #PS16-1
		Colvos Passage	April 19	J	149	3% (2/60)	ND	ND	MMFS #PS16-1
		Nisqually / Drayton	June 4	A	155	13% (8/60)	ND	ND	MMFS #PS16-1
		N Saratoga	June 8	A	172	7% (4/60)	ND	ND	MMFS #PS16-1
		Gulf of Georgia	June 15	J	126	12% (7/60)	ND	ND	MMFS #PS16-1
		Squamish Harbor	Aug 25	J	93	5% (3/60)	ND	ND	MMFS #PS16-1
		Dabob Bay	Aug 24	A	177	40% (24/60)	ND	ND	MMFS #PS16-1
		S. Lopez	Aug 29	J	91	2% (1/60)	ND	ND	MMFS #PS16-1
		N Saratoga	Aug 25	A	150	5% (3/60)	ND	ND	MMFS #PS16-1
		President Channel	Aug 16	J	89	0% (0/60)	ND	ND	MMFS #PS16-1
		President Channel	Oct 6	J	102	2% (1/60)	ND	ND	MMFS #PS16-1
		Yukon Harbor	Oct 11	A	173	5% (3/60)	ND	ND	MMFS #PS16-1
		Gulf of Georgia	Oct 6	A	173	7% (4/60	ND	ND	MMFS #PS16-1
		N Saratoga	Oct 16	A	154	7% (8/120)	ND	ND	MMFS #PS16-1
		Colvos	Oct 16	J	117	0% (0/60)	ND	ND	MMFS #PS16-1
		Oak Bay	Oct 18	J	106	15% (9/59)	ND	ND	MMFS #PS16-1
		Dabob Bay	Oct 16	J	146	53% (32/60)	ND	ND	MMFS #PS16-1
		N. Saratoga	Dec 16	A	177	13% (8/60)	ND	ND	MMFS #PS16-1
		Dallas Bank	Dec 13	J	100	2% (1/60)	ND	ND	MMFS #PS16-1

Chapter 2: Experimental Studies Involving *Ichthyophonus* spp.

2.1 The Parasite *Ichthyophonus* in Pacific Herring

A review of *Ichthyophonus* survey results since 2007 was published (Hershberger et al. 2016a). Infection prevalence in local Pacific herring stocks varied seasonally and annually, and a general pattern of increasing prevalence with host size and/or age persisted throughout the NE Pacific. An exception to this zoographic pattern occurred among a group of juvenile, age 1+ year Pacific herring from Cordova Harbor, AK in June 2010, which demonstrated an unusually high infection prevalence of 35%. Reasons for this anomaly were hypothesized to involve anthropogenic influences that resulted in locally elevated infection pressures. Inter-annual declines in infection prevalence from some populations (e.g. Lower Cook Inlet, AK; from 20-32% in 2007 to 0-3% during 2009-2013) or from the largest size cohorts of other populations (e.g. Sitka Sound, AK; from 62.5% in 2007 to 19.6% in 2013) were likely a reflection of selective mortality among the infected cohorts. All available information for *Ichthyophonus* in the NE Pacific, including broad geographic range, low host specificity, and presence in archived Pacific herring tissue samples dating to the 1980's, indicate a long-standing host-pathogen relationship.

2.2 Infecting Pacific Herring with *Ichthyophonus* in the Laboratory

The route(s) whereby Pacific herring and other planktivorous fishes become infected with *Ichthyophonus* remains unknown. Several methods for establishing *Ichthyophonus* infections in laboratory challenges were examined. *Ichthyophonus* sp. infections were most effectively established after intraperitoneal injections with suspended parasite isolates from culture or after repeated feedings with infected fish tissues (Hershberger et al 2015). Among groups that were offered infected fish tissues, infection prevalence was greater after multiple feedings (65%) than after a single feeding (5%). Additionally, among groups that were exposed to parasite suspensions prepared from culture isolates, infection prevalence was greater by intraperitoneal injection (74%) than by gastric intubation (12%); infections were not established in any experimental herring by flushing parasite suspensions over the gills. Although the consumption of infected fish tissues is not likely the primary route of *Ichthyophonus* sp. transmission in populations of wild Pacific herring, this route may contribute to abnormally high infection prevalence in areas where juvenile herring have access to infected offal.

Table 2. Prevalence of *Ichthyophonus* sp. infection in Pacific herring after single and multiple feedings with infected tissues.

Treatment Group	Infection Prevalence - Mortalities	Infection Prevalence - Survivors	Infection Prevalence - Total
Single Exposure	5.0% (1/20)	4.4% (2/45)	4.6% (3/65*)
Multiple Exposures	38% (9/24)	96% (21/22)	65% (30/46)
Negative Control	0% (0/13)	0% (0/16)	0% (0/29)

*Five additional fish were subsampled from the single-exposure treatment 1d post-exposure to assess whether *Ichthyophonus* sp. was detectable in histological sections of the stomach bolus.

2.3 Persistence of External Signs in Pacific herring *Clupea pallasii* with Ichthyophoniasis

The progression of external signs of *Ichthyophonus* infection in Pacific herring *Clupea pallasii* was highly variable and asynchronous after intraperitoneal injection with pure parasite preparations; however, external signs generally persisted through the end of the study (429 d post-exposure). Observed signs included 'sandpaper skin,' open lesions, pigmented ulcers and / or bleeding ulcers. The prevalence of external signs plateaued 35 d post-exposure and persisted in 73-79% of exposed individuals through the end of the first experiment (147 d post-exposure). Among a second group of infected herring, external signs completely resolved in only 10% of the fish after 429 d. The onset of mortality preceded the appearance of external signs (Fig. 1). Histological examination of infected skin and skeletal muscle tissues indicated an apparent affinity of the parasite for host red muscle. Host responses consisted primarily of granulomatous inflammation, fibrosis, and necrosis in the skeletal muscle and other tissues. The persistence and asynchrony of external signs and host response indicated that they were neither a precursor to host mortality nor did they provide reliable metrics for hind-casting the date of exposure. However, the long-term persistence of clinical signs in Pacific herring may be useful in ascertaining the population-level impacts of ichthyophoniasis in regularly observed populations. These results were published in Hart et al. (2016).

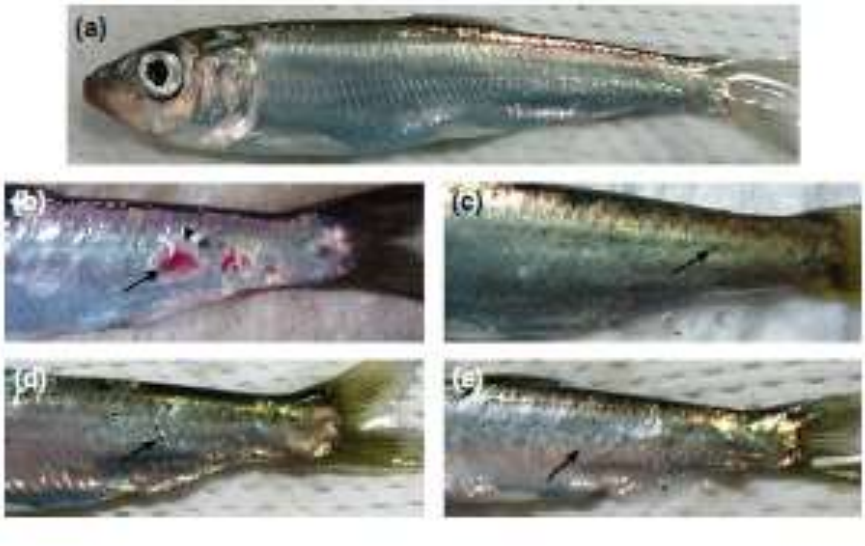

Figure 1. Representative external signs observed in Pacific herring after treatment with *Ichthyophonus* by i.p. injection. A. No external signs. B. Large bleeding ulcers (arrow) and blood infused white tuft (arrowhead). C. Small pigmented ulcers (arrowhead) with suspected scarring (arrow) from healing lesions. D. Open lesions (arrow). E. Sandpaper skin (arrow) with pigmented nodules along complete flank. F. Mild external signs with one pigmented lesion and one small open lesion (arrows). G. Inconspicuous small black nodules (arrow).

2.4 Detection of *Ichthyophonus* by Chromogenic in Situ Hybridization

Ichthyophonus-like organisms have been reported in amphibians, reptiles, birds and invertebrates and may have been incorrectly classified under a single type species, *I. hoferi*. Although less sensitive than other detection techniques such as explant tissue culture, histopathological examination is effective for simultaneously evaluating host response and severity of *Ichthyophonus* infections. Histological sections showing positive periodic acid-Schiff (PAS) staining of multinucleate organisms 50-250 µm in diameter can be presumptive for *Ichthyophonus*, but lack of a definitive confirmatory test may lead to misdiagnosis, particularly when the organism is not cultured. We developed a chromogenic in situ hybridization (CISH) procedure that specifically detected *Ichthyophonus* ribosomal DNA in histological sections thereby complementing the histological diagnosis by providing highly specific molecular confirmation of the observed organism (Fig. 2). A digoxigenin-labeled oligonucleotide probe was designed to target conserved portions of the 18S small subunit ribosomal gene of known *Ichthyophonus* species *I. hoferi* and *I. irregularis.* Formalin-fixed, paraffin-embedded tissues from naturally infected Chinook salmon (*Oncorhynchus tshawytscha*) and red-spotted newt (*Notophthalmus viridescens*), and experimentally infected Pacific herring, rainbow trout (*O. mykiss*) and Pacific staghorn sculpin (*Leptocottus armatus*) were analyzed by CISH and PAS staining. Probe hybridization was indicated by dark purple precipitates and correlated with the distribution and morphology of parasites observed in PAS-positive tissues and also identified *Ichthyophonus* developmental stages in the presence of PAS-positive host cells. The CISH probe hybridized with PAS-positive, *Ichthyophonus*-like organisms in all host species except the red-spotted newt, supporting the hypothesis that the organism infecting amphibians is taxonomically distinct from fish-associated *Ichthyophonus*. The CISH has utility for both diagnostic and research applications. These results are published in Conway et al. (2015).

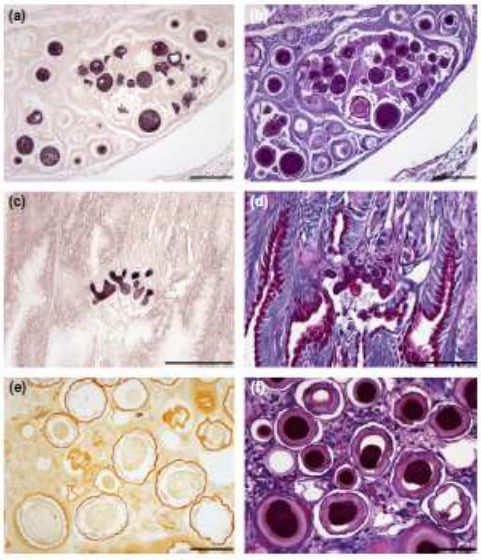

Figure 2. *Ichthyophonus* identified in (a) Pacific herring epicardial connective tissue and (c) rainbow trout stomach tunica propria by hybridization with an oligonucleotide probe targeting an *Ichthyophonus* 18s rDNA sequence. Staining with periodic acid-Schiff (PAS) revealed PAS-positive schizonts in Pacific herring (b) and amoeboid cells in rainbow trout (d). Schizonts present in red-spotted newt skeletal muscle did not hybridize with the *Ichthyophonus*-specific probe (e) (section counter-stained with Bismarck brown Y for visibility), but stained PAS-positive (f). Scale bars = 100 µm.

2.5 Identification of the Infectious Stage of *Ichthyophonus* sp. and Description of a Circulating Blood Stage

Small amoeboid cells, believed to be the infectious stage of *Ichthyophonus*, were observed in the bolus (stomach contents) and tunica propria (stomach wall) of Pacific staghorn sculpins and rainbow trout shortly after they ingested *Ichthyophonus*-infected tissues (Fig. 3)**.** By 24-48 hrs post-exposure the parasite morphed from the classically reported multi-nucleate thick walled schizonts to two distinct cell types; a larger multinucleate amoeboid cell surrounded by a narrow translucent zone and a smaller spherical cell surrounded by a "halo" and resembling a small schizont. Both cell types also appeared in the tunica propria, indicating that they had recently penetrated the columnar epithelium of the stomach. No *Ichthyophonus* pseudo-hyphae ("germination tubes") were observed in the bolus or penetrating the stomach wall. Simultaneously, *Ichthyophonus* was isolated in vitro from aortic blood, which was consistently positive from 6 -144 hrs post-exposure, then only intermittently for the next four wks. Small PAS-positive cells observed in blood cultures grew into colonies consisting of non-septate tubules (pseudo-hyphae) terminating in multinucleated knob-like apices similar to those seen in organ explant cultures. Organ explants were culture-positive every day, however typical *Ichthyophonus* schizonts were not observed histologically until 20-25 days post-exposure. From 20 to 60 days p.e. schizont diameter increased from $\leq$ 25µm to $\geq$ 82µm. Based on the data presented here, a life cycle within the piscivorous host is proposed and published in Kocan et al. (2013).

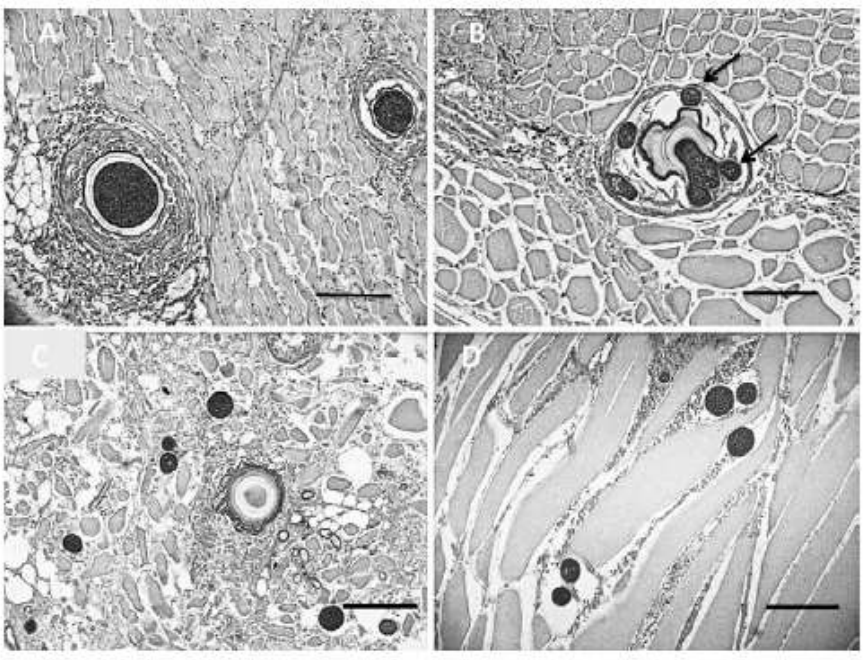

Figure 3. Transformation of *Ichthyophonus* schizonts in the stomach (bolus) of Pacific staghorn sculpins, *Leptocottus armatus,* following ingestion of infected herring tissue. (A) Normal multinuclear spherical schizonts surrounded by a granuloma (0 hr - infected homogenate). (B) Small amoeboid cells (arrows) budding from parent schizont (48 hr post consumption). (C) Empty schizont surrounded by dispersing amoeboid cells (48 hr post-consumption). (D) Amoeboid cells dispersed throughout digesting herring muscle (48 – 96 hr post consumption). (Bar - 50µm) Stain - Periodic acid-Schiff (PAS) reagent.

2.6 Viability and Infectivity of *Ichthyophonus sp.* in Post-Mortem Pacific Herring

Ichthyophonus-infected Pacific herring decomposed in flowing seawater, then serially sampled for 4 wk and examined for the presence of *Ichthyophonus* as determined by in vitro culture and single plane histology (Fig. 4). The same tissues were fed to *Ichthyophonus*-free Pacific staghorn sculpins, *Leptocottus armatus*, to determine the duration of parasite infectivity. *Ichthyophonus* sp. was viable in decomposing herring viscera and muscle for at least 4 wk post-mortem and remained infectious for sculpins for up to 5 days post-mortem. Many of the morphologic changes observed were similar to those previously reported to occur during the first 24 hr following death of the host, but also included novel forms not previously described. The significance of extended survival and progressive morphologic transformation in the post-mortem host is unknown, but it could be inferred that it has survival value for the parasite. These results are published in Kocan et al. (2014).

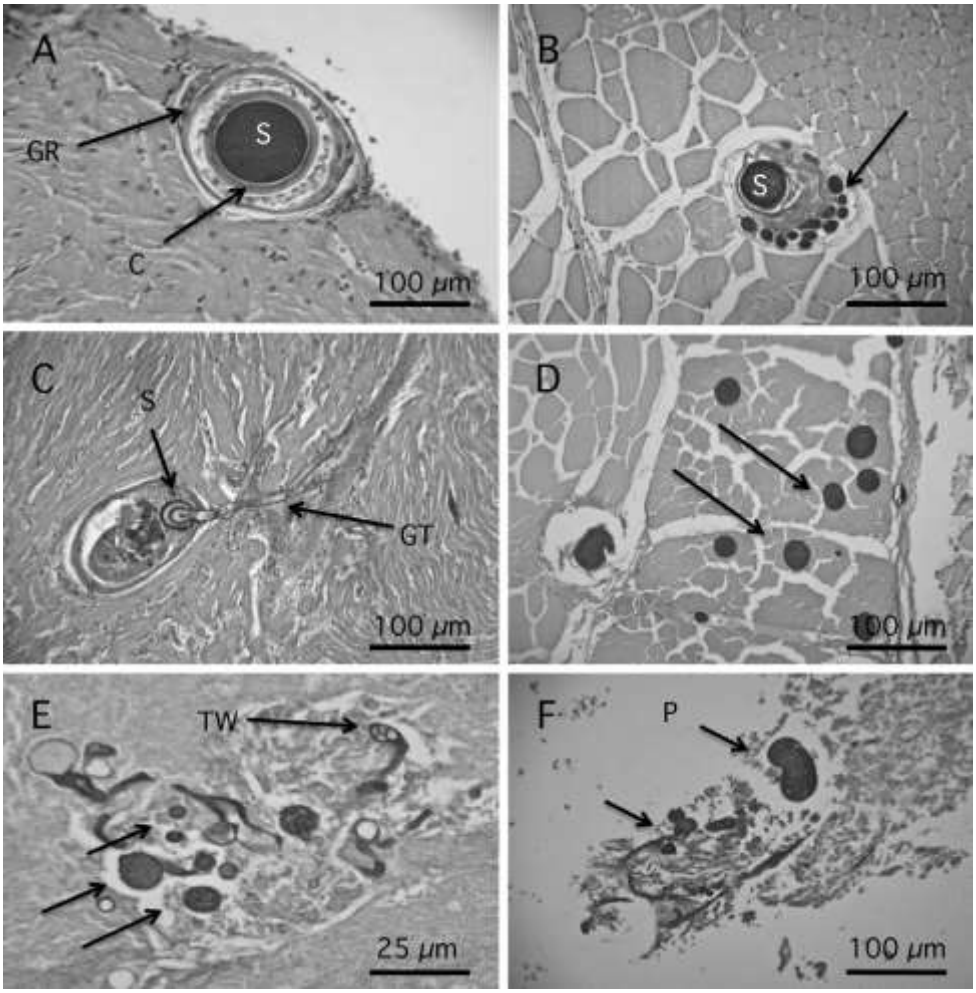

Figure 4. Periodic Acid-Schiff (PAS) stained histologic sections of post-mortem herring (*Clupea pallasii*) tissue. (**A**) (0 hr) Typical multinuclear schizont (S) with multilaminate capsule (C) and granuloma (GR). (**B**) (24 hr) Shrunken schizont (S) and multiple plasmodia (arrow). (**C**) (48 hr) Empty schizont capsule (S) in granuloma with germ tubes (GT) penetrating surrounding tissue. (**D**) (36-48 hr) Multiple un-encapsulated plasmodia (arrows) migrating into dark muscle. (**E**) (15 days) Thick walled cells in apical tip of germ tube (TW), small plasmodia (arrows), and unidentified structures. (**F**) (22 days) Un-encapsulated plasmodium (P) and unidentified structures.

2.7 *Ichthyophonus* Phylogeny Based on ITS rDNA Structure Prediction and Alignment Identifies Six Clades, with a Single Dominant Marine Type

A molecular phylogenetic study was undertaken to examine whether different genetic types of *Ichthyophonus* spp. exist. *Ichthyophonus* spp. isolates from fish hosts in the Atlantic and Pacific Oceans, several rivers, and aquaculture sites in North America, Europe, and Japan separated into six genetic clades, based on consensus sequences of the internal transcribed spacer rDNA region (Fig. 5). Species-level genetic differences were identified in each clade, however a single *Ichthyophonus* species accounted for a majority (71 of 98) of parasite isolations. This ubiquitous type occurred in 13 marine and anadromous hosts. A single *Ichthyophonus* species occurred in all samples from freshwater aquaculture, despite great geographic separation of the hosts. This ITS genotype, previously shown to be adapted to freshwater, was also encountered in a minority of the clones from two marine hosts. The remaining *Ichthyophonus* species generally occurred in a single host; however sample sizes were too small to determine if they are specialists in these species or are just rarely encountered. These results are published in Gregg et al. (2016). Further research is necessary to describe phenotypic differences between the *Ichthyophonus* species identified, and more variable molecular genetic markers are necessary to understand intra-specific transmission process of the ubiquitous marine form.

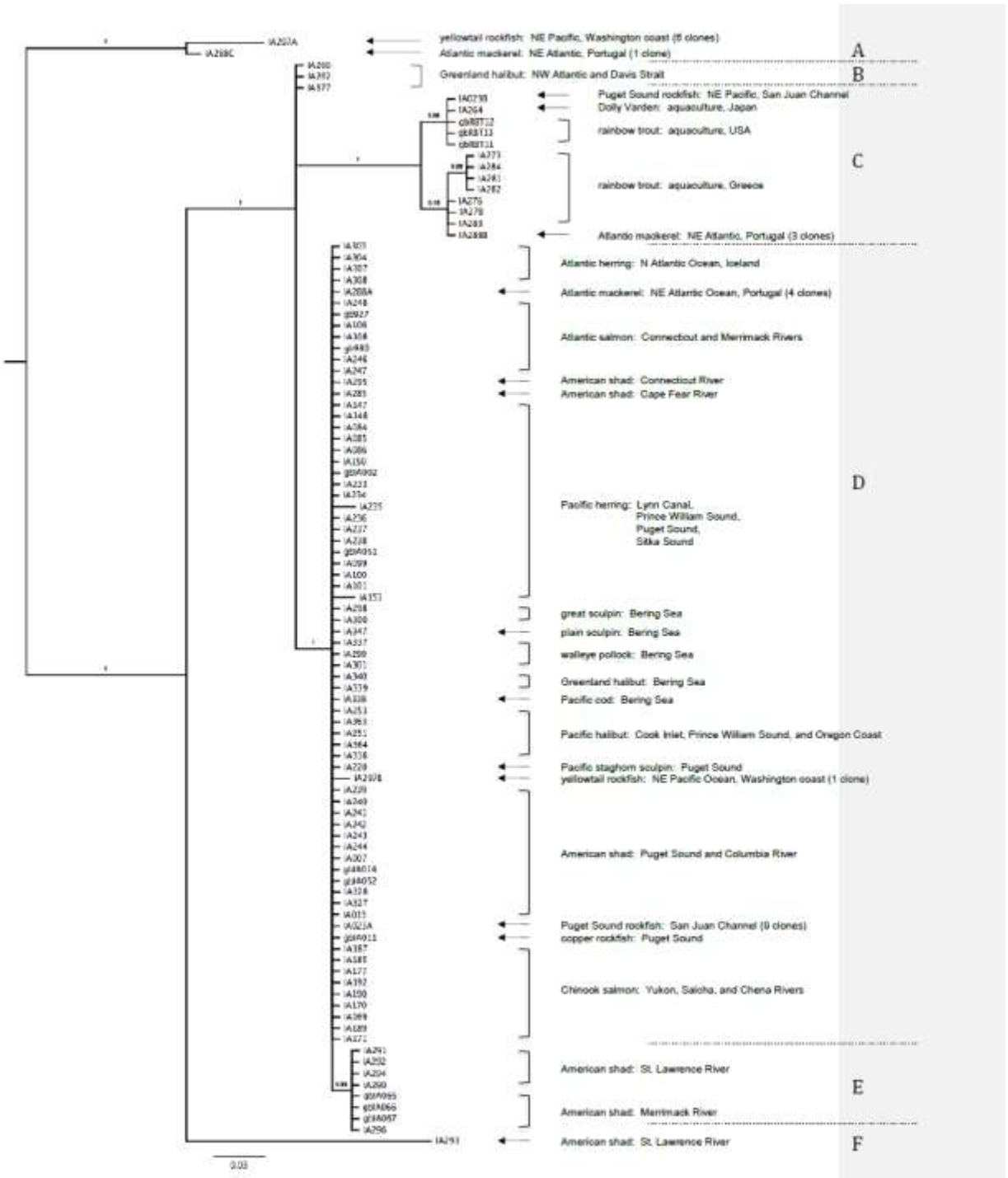

Figure 5. Phylogeny of *Ichthyophonus* based on Bayesian MCMC analysis of ITS rDNA consensus sequences under a six-partition model. Posterior probabilities are indicated above branches. Tree has an arbitrary midpoint root. Host and location of each isolate indicated right of Isolate ID. Isolates with gb prefix were obtained from GenBank. Three isolates (IA023, IA297, and IA288) contained variant haplotypes and are indicated with A, B, and C suffixes. Clades are labeled arbitrarily A through F.

2.8 Analytical and Diagnostic Performance of a qPCR Assay for *Ichthyophonus* spp. Compared to the Tissue Explant Culture 'Gold Standard'

Parasites of the genus *Ichthyophonus* infect many fish species and have a non-uniform distribution within host tissues. Due in part to this uneven distribution, there has been much debate as to whether molecular-based detection methods can be as sensitive and accurate as culture for estimating parasite prevalence in wild populations. We evaluated the analytical and diagnostic performance of an existing qPCR assay in comparison to the 'gold standard' culture method using Pacific herring with known disease history in a controlled environment (White et al. Accepted). It was determined that the assay is suitable for use on this previously untested host, and diagnostic specificity was consistently high (>98%) in both heart and liver tissues. Diagnostic sensitivity could not be fully assessed due to low infection rates in *Ichthyophonus*-inoculated fish, but our results suggest that qPCR is not as sensitive as culture under all circumstances. qPCR diagnostic sensitivity relative to culture is likely affected by the amount of sample processed, as prevalence estimates were not significantly different when the assayed sample sizes were equal. This sensitivity issue would be most problematic for fish with light infections. Although qPCR does not detect the presence of a live organism, DNA-based pathogen detection methods from preserved samples provide the opportunity for alternate testing strategies when culture is not possible.

Table 3. Diagnostic sensitivity (DSe) and specificity (DSp) of the *Ichthyophonus* qPCR test on experimentally inoculated *Ichthyophonus*-exposed (ICH) and control (PBS) *Clupea pallasii*. Cross-classified culture (C) and qPCR (Q) results are presented individually for fish heart and liver tissues at two different qPCR limits of detection for parasite DNA in fish tissue with associated DSe and DSp. Prevalence estimates are listed for each detection method with chi-square p-values from comparing each pair. Prevalence values are percentages (95% binomial proportion CI).

Inoculum/ Tank			---Number of samples---					---Infection prevalence (%)---			Diagnostic performance	
	Tissue	qPCR LOD	n	C+ Q+	C+ Q-	C- Q+	C- Q-	Culture	qPCR	P-value	DSe	DSp
PBS	Heart	≥ 3	139	0	0	0	139	0	0	-	NA	100
		≥ 1		0	0	0	139	0	0	-	NA	100
	Liver	≥ 3	139	0	0	0	139	0	0	-	NA	100
		≥ 1		0	0	0	139	0	0	-	NA	100
ICH	Heart	≥ 3	298	17	2	3	276	6.4 (4.1-9.8)	6.7 (4.4-10.1)	0.868	89.5 (69-97)	98.9 (97-100)
		≥ 1		18	1	4	275	6.4 (4.1-9.8)	7.4 (4.9-11.0)	0.627	94.7 (75-99)	98.6 (96-99)
	Liver	≥ 3	296	21	25	1	249	15.5 (11.9-20.1)	7.4 (5.0-11.0)	0.002	45.7 (32-60)	99.6 (98-100)
		≥ 1		23	23	4	246	15.5 (11.9-20.1)	9.1 (6.3-12.9)	0.018	50 (36-64)	98.4 (96-99)

Chapter 3: Experimental Studies Involving Viral Hemorrhagic Septicemia Virus (VHSV)

3.1 Principles Underlying the Epizootiology of Viral Hemorrhagic Septicemia in Pacific Herring throughout the North Pacific Ocean

A synthesis paper was published describing our current understanding of VHS epizootiology in Pacific herring (Hershberger et al.2016b). Although viral hemorrhagic septicemia virus (VHSV) typically occurs at low prevalence and intensity in natural populations of Pacific herring and other marine fishes in the NE Pacific Ocean, epizootics of the resulting disease (VHS) periodically occur, often in association with observed fish kills (Fig. 6). Here we identify a list of principles, based on a combination of field studies, controlled laboratory experiments, and previously unpublished observations, that govern the epizootiology of VHS in Pacific herring. A thorough understanding of these principles provides the basis for identifying risk factors that predispose certain marine fish populations to VHS epizootics, including the lack of population resistance, presence of chronic viral carriers in a population, copious viral shedding by infected individuals, cool water temperatures, limited water circulation patterns, and gregarious host behavioral patterns. Further, these principles were used to define the epizootiological stages of the disease in Pacific herring, including the susceptible, enzootic, disease amplification, outbreak, recovery, and refractory stages. In addition to providing a foundation for quantitatively assessing the potential risks of future VHS epizootics in Pacific herring, these principles provide insights into the epizootiology of VHS in other fish communities where susceptible species exist.

Figure 6. VHS epizootic and associated fish kill involving wild herring in Vancouver Island, B.C, Canada. Visible signs of the disease include hemorrhages along the flank of affected fish. Photo credits: Garth Traxler and Jon Richard, Department of Fisheries and Oceans, Pacific Biological Station, Canada.

3.2 Influence of Temperature on Viral Hemorrhagic Septicemia (Genogroup IVa) in Pacific Herring, *Clupea pallasii*

An inverse relationship between water temperature and susceptibility of Pacific herring to VHS was indicated by controlled exposure studies where cumulative mortalities, viral shedding rates, and viral persistence in survivors were greatest at the coolest exposure temperatures. Among groups of specific pathogen-free (SPF) Pacific herring maintained at 8, 11, and 15°C, cumulative mortality after waterborne exposure to VHSV was 78%, 40%, and 13%, respectively (Fig. 7). The prevalence of survivors with VHSV-positive tissues 25d post-exposure was 64%, 16%, and 0% (at 8, 11 and 15°C, respectively) with viral prevalence typically higher in brain tissues than in kidney/spleen tissue pools at each temperature. Similarly, geometric mean viral titers in brain tissues and kidney/spleen tissue pools decreased at higher temperatures, and kidney/spleen titers were generally 10-fold lower than those in brain tissues. This inverse relationship between temperature and VHS severity was likely mediated by an enhanced immune response at the warmer temperatures, where a robust type I interferon response was indicated by rapid and significant upregulation of the herring Mx gene. The effect of relatively small temperature differences on the susceptibility of a natural host to VHS provides insights into conditions that preface periodic VHSV epizootics in wild populations throughout the NE Pacific. These results were published in Hershberger et al. (2013a).

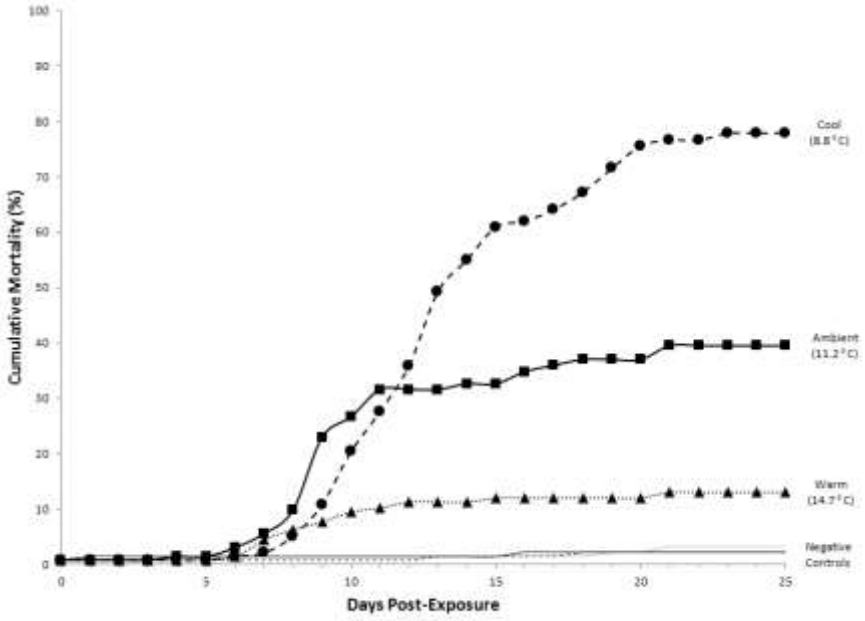

Figure 7. Effects of temperature on the cumulative mortality of VHSV exposed herring. Data points represent back-transformed percentages corresponding to the means of the arcsine-transformed proportions from triplicate tanks (31 herring/tank).

3.3. Development of a Blocking Enzyme-Linked Immunosorbent Assay and Virus Neutralization Assay to Detect Antibodies to VHSV

Detection of VHSV currently relies on virus isolation, which is lethal to fish and only indicates current infection status. A serological method is required to ascertain prior exposure. Here, we report the development of two serologic tests for VHSV that are non-lethal, rapid, and species-independent: a virus neutralization assay (VN) and a blocking enzyme-linked immunosorbent assay (ELISA). Serum was collected from 34 uninfected fish (VHS negative group) and 28 fish that survived VHS virus infection (VHS positive group). The VN did not detect neutralizing antibodies in the serum of any of the 34 VHSV negative fish, demonstrating a test specificity of 100%. Neutralizing antibodies were detected in 12 of 28 VHS positive fish, indicating a test sensitivity of 42.9%. The anti-nucleocapsid blocking ELISA detected non-neutralizing VHSV antibodies in four of the 34 fish in the VHS negative group, indicating a specificity of 88.2%. Non-neutralizing antibodies were detected in 27 of 28 VHS positive fish, indicating a sensitivity of 96.4%. Used in parallel, the VN and ELISA correctly identified all survivors of VHSV infection and unexposed fish. Our VN and ELISA are valuable tools for assessing exposure to VHSV and should improve detection and surveillance efforts for both wild and commercial fish populations. However, further testing indicated that the ELISA was only capable of detecting VHSV antibodies in Pacific herring that were hyper-immunized against VHS; the assay was incapable of detecting antibodies in survivors of lower-level exposures. Therefore, further efforts were made to develop and optimize a plaque neutralization assay (see section 3.4). These results are published in Wilson et al. (2014).

3.4 Optimization of a Plaque Neutralization Test to Identify the Exposure History of Pacific Herring to Viral Hemorrhagic Septicemia Virus.

Methods for a plaque neutralization test (PNT) were optimized for the detection and quantification of VHSV neutralizing activity in the plasma of Pacific herring. The PNT was complement-dependent, as neutralizing activity was attenuated by heat inactivation; further, neutralizing activity was mostly restored by the addition of exogenous complement from specific pathogen-free Pacific herring. Optimal methods included the overnight incubation of VHSV aliquots in serial dilutions (1:16 – 1:256) of whole test plasma containing endogenous complement. The resulting viral titers were then enumerated using a viral plaque assay in 96 well micro plates. Serum neutralizing activity was virus-specific, as plasma from VHS survivors demonstrated only negligible reactivity to infectious hematopoietic necrosis virus (IHNV), a closely-related rhabdovirus. Among Pacific herring that survived VHSV exposure, neutralizing activity was detected in the plasma as early as 37d post-exposure and peaked approximately 64 d post-exposure (Fig. 8). The onset of neutralizing activity was slightly delayed at 6.0 ^{0}C relative to warmer temperatures (8.5 and 12.0 ^{0}C); however, neutralizing activity persisted for at least 345 d post exposure in all temperature treatments. It is anticipated that this novel ability to assess VHSV neutralizing activity will enable retrospective comparisons between a priori VHS exposures and year class recruitment failures. Additionally, the optimized PNT is expected to be employed as a forecasting tool capable of identifying the potential for future VHS epizootics in

wild Pacific herring populations. These results were published in Hart et al. 2017a.

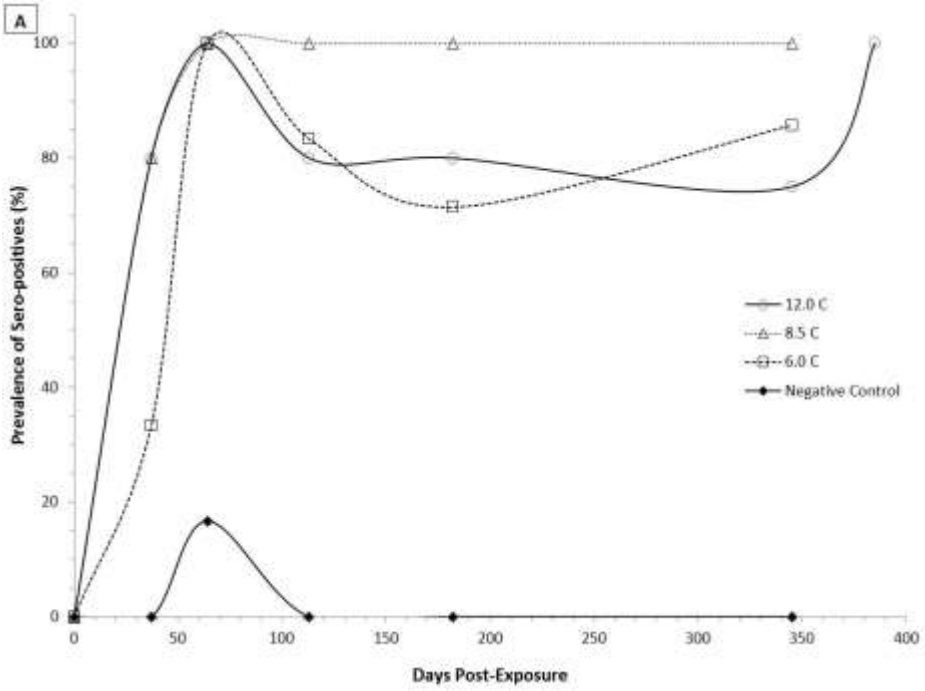

Figure 8. Kinetics of virus neutralizing activity in the plasma of VHSV survivors (n = 5 – 13 d^{-1} $group^{-1}$). Fish were considered sero-positive when neutralizing activity was detected at plasma dilutions $\geq$1:16.

3.5 Virulence of Viral Hemorrhagic Septicemia Virus (VHSV) Genotypes Ia, IVa, IVb, and IVc in Five Fish Species

The susceptibility of yellow perch (*Perca flavescens*), rainbow trout, Chinook salmon, koi (*Cyprinus carpio*), and Pacific herring to 4 strains of VHSV was assessed. Fish were challenged via intraperitoneal injection with high (1 x 10^6 plaque-forming units, PFU) and low (1 x 10^3 PFU) doses of a European strain (genotype Ia), and North American strains from the West Coast (genotype IVa), Great Lakes (genotype IVb), and the East Coast (genotype IVc). Pacific herring were exposed to the same strains, but at a single dose of 5 x 10^3 PFU / mL by immersion in static seawater. Overall, yellow perch were the most susceptible, with cumulative percent mortality (CPM) ranging from 84-100%, and 30-93% in fish injected with high and low doses (Fig. 9), respectively. Rainbow trout and Chinook salmon experienced higher mortality (47-98% CPM) after exposure to strain Ia than to other genotypes. Pacific herring were most susceptible to their endemic strain (IVa) with an average CPM of 80%, and were moderately susceptible (42-52% CPM) to the other genotypes. Koi had very low susceptibility (<5% CPM) to all 4 VHSV strains. These results are published in Emmenegger et al. (2013).

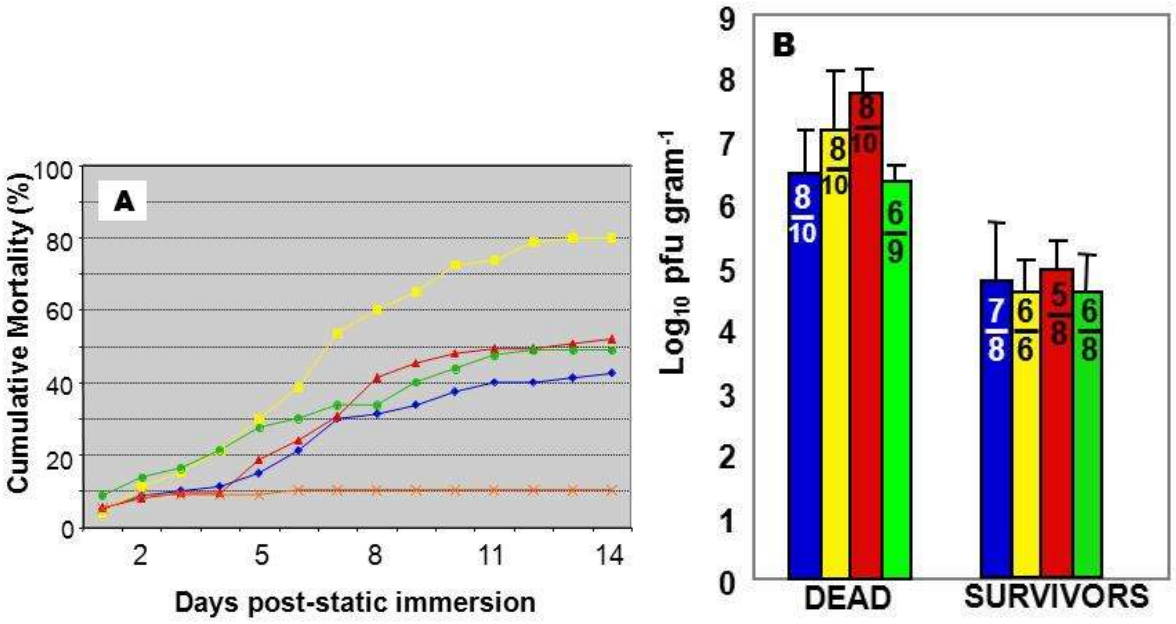

Figure 9. Susceptibility of Pacific herring to VHSV genotypes. (A) Mean Cumulative mortality of Pacific herring after immersion exposure to a dose of 5.0 x 10^3 PFU/mL of VHSV (blue - European Ia; yellow - North American West Coast IVa; red - North American Great Lakes IVb; green – North American East Coast IVc) or mock treatment (orange). (B) Virus concentrations (geo mean of virus-positive fish) in dead and surviving fish (14d post-challenge) with prevalence shown within the columns as the number of virus-positive fish / number tested. Error bars indicate SD of the virus titers.

3.6. Demonstration of Atlantic Salmon as a Host and Reservoir of Viral Hemorrhagic Septicemia Virus Type IVa

In British Columbia (BC) the farming of non-native Atlantic salmon (*Salmo salar*) in ocean net pens leads to problems with disease because salmon are exposed to pathogens occurring in the environment. VHSV is enzootic in BC and causes serious disease in wild Pacific herring, which often enter and remain in salmon netpens. Isolation of VHSV from farmed Atlantic salmon has been previously documented, but the effects on the health of farmed salmon and the wild fish sharing the environment are unknown. To determine Atlantic salmon susceptibility to VHSV, fish were infected with a mixture of VHSV isolates originating from farmed Atlantic salmon by IP-injection or through a route including immersion in virus and cohabitation with VHSV-infected Pacific herring. Transmission of virus from Atlantic salmon to Pacific herring was also tested. Measurements of disease included mortality, clinical signs, histopathology, immunohistochemistry, expression of interferon-related genes, and viral plaque assay. The results demonstrated that VHSV caused disease in Atlantic salmon infected by both methods. Fish had gross disease signs including darkening of the dorsal skin, bilateral exophthalmia, light cutaneous hemorrhage, and lethargy (Fig. 10). The virus replicated within endothelial cells causing endothelial cell necrosis leading to extensive hemorrhage in anterior kidney. Infected fish activated the type I interferon system as seen by up-regulation of genes for IFNα, Mx, and ISG15. Both IP-injected and immersion infected salmon were able to transmit the virus to SPF Pacific herring. The results demonstrate that farmed Atlantic salmon can develop clinical VHS

and virus can persist for at least 10 weeks in this host. With risks of VHS in farmed salmon and the potential for further host adaptation and spread of VHSV to native wild fishes sharing the environment, precautions should be taken to avoid the introduction of VHSV into salmon farms. These results are published in Lovy et al. (2013).

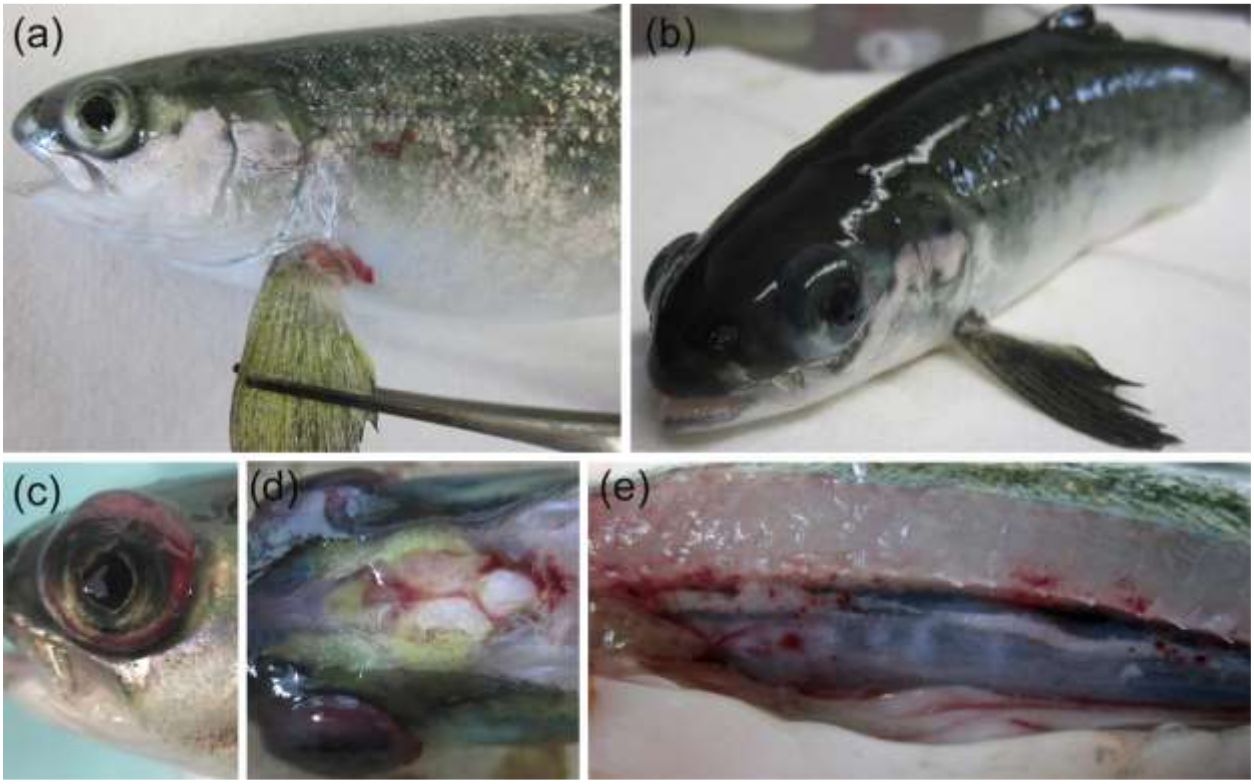

Figure 10. Gross clinical signs of VHSV in Atlantic salmon; similar signs appear in fish infected by IP-injection and immersion; shown in (a) through (d) are fish infected by immersion and (e) is a fish infected by IP-injection. Common signs included (a) skin hemorrhage frequently near the pectoral fin, (b) dark dorsal coloration and bilateral exophthalmia, (c) hemorrhage occurring within exophthalmic eyes, (d) hemorrhage in the brain cavity, and (e) hemorrhage on the swim bladder.

3.7. Influence of Temperature on the Efficacy of DNA Vaccines against Viral Hemorrhagic Septicemia in Pacific Herring

Homologous and heterologous (genogroup Ia) DNA vaccines against viral hemorrhagic septicemia virus (VHSV – genogroup IVa) conferred partial protection in Pacific herring. Early protection at 2 wk post vaccination (PV) was low and occurred only at elevated temperature (12.6^0C, 189 degree days - DD), where the relative percent survival (RPS) following viral exposure was similar for the two vaccines (IVa and Ia, respectively) and higher than that of negative controls at the same temperature. Late protection at 10 wk PV was induced by both vaccines but was higher with the homologous vaccine at both 9.0^0C and 12.6°C. Virus neutralization titers were detected among 55% of all vaccinated fish at 10 wk PV. The results suggest that the immune response profile triggered by DNA vaccination of herring was similar to that reported for rainbow trout where interferon responses occur in the early days PV and

transition to adaptive response at later time points. However, the protective effect was far less prominent in herring, possibly reflecting different physiologies and or adaptations of the two fish species. These results were published in Hart et al. 2017b.

Table 4. Relative percent survival (RPS) 15 d after exposure of Pacific herring to viral hemorrhagic septicemia virus. All values are relative to a negative control group that was injected with saline (9.0 ^{0}C) in lieu of vaccine.

Vaccine treatment	Temperature	RPS (%)	
		2 wk post vaccination	10 wk post vaccination
Homologous IVa	9.0°C	0.0	40.4
	12.6°C	21.9	51.1
Heterologous Ia	9.0°C	0.9	11.1
	12.6°C	22.7	27.3
Plasmid	9.0°C	0.0	2.2
	12.6°C	5.7	4.2
Saline	12.6°C	7.9	48.3

Saline controls at 10 wk post-vaccination were comporomised due a co-infections with ENV that resulted in cross-protection against VHS.

Chapter 4: Experimental Studies Involving Viral Erythrocytic Necrosis (VEN).

4.1. Molecular Identification of Erythrocytic Necrosis Virus (ENV) from the Blood of Pacific Herring

A conventional polymerase chain reaction (PCR) technique was developed to detect ENV from the blood of infected Pacific herring. Presently, VEN is diagnosed by observation of typical cytoplasmic inclusion bodies in stained blood smears from infected fish. The causative agent, ENV, is unculturable and a presumed iridovirus by electron microscopy. *In vivo* amplification of the virus in cultured Pacific herring and subsequent virus concentration, purification, DNA extraction, and high-throughput sequencing methodologies were applied to obtain genomic ENV sequences. Fragments with the highest sequence identity to the family *Iridoviridae* were used to design four sets of ENV-specific polymerase chain reaction (PCR) primers. Testing of blood and tissue samples from experimentally and wild infected Pacific herring as well as DNA extracted from other amphibian and piscine iridoviruses verified the four assays were specific to ENV. Sensitivity testing determined a limit of detection of 0.0003 ng. Preliminary phylogenetic analyses of a 1448 bp fragment of the putative DNA polymerase gene supported inclusion of ENV in a proposed sixth genus of the family *Iridoviridae* that contains other erythrocytic viruses from ectothermic hosts (Fig. 11). This study provides the first molecular evidence of ENV's inclusion within the *Iridoviridae* family and offers a conventional PCR assay as a means of rapidly surveying the ENV-status of wild and propagated Pacific herring stocks. These results are published in Emmenegger et al. (2014).

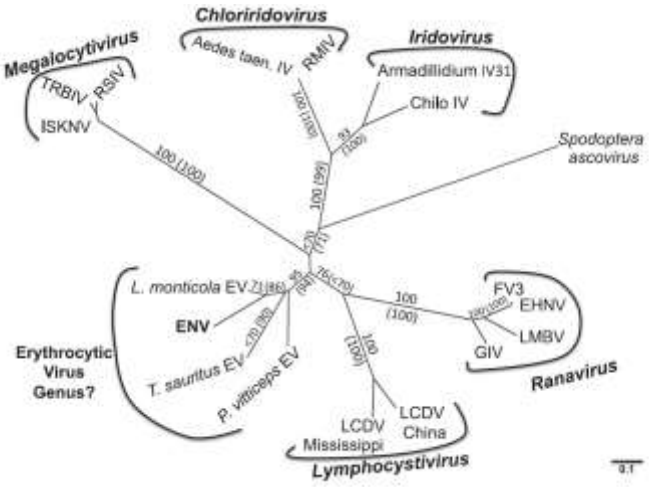

Figure 11. Maximum likelihood (ML) phylogenetic tree for ENV based on the predicted DNA dependent DNA polymerase amino acid sequences (174 - 572) of a 17 virus MUSCLE alignment. The tree was rooted to the outgroup *Spodoptera ascovirus*. ML bootstrap percent values of over 60 for the branchings from 500 re-samplings are displayed along with neighbor-joining (NJ) bootstrap percentages shown in parentheses. Erythrocytic necrosis virus (ENV) is highlighted in bold. Iridoviral genera are delineated with bracket arcs.

4.2. Identification of the Major Capsid Protein of Erythrocytic Necrosis Virus (ENV) and Development of Quantitative Real-Time PCR Assays

A highly sensitive quantitative PCR (qPCT) technique was developed that is capable of providing a quantitative assessment of ENV load in the tissues of infected Pacific herring. The technique will be capable of diagnosing ENV infections and VEN epizootics in the absence of available blood films. Phylogenetic analysis of the ENV DNA polymerase grouped ENV with other erythrocytic iridoviruses from snakes and reptiles. In the present study, we identified the gene encoding the ENV major capsid protein (MCP) and developed a quantitative PCR (qPCR) assay targeting this gene. Phylogenetic analysis of the MCP gene sequence supported the conclusion that the ENV does not group with any of the currently described iridovirus genera (Fig. 12). Because there is no information regarding genetic variation of the MCP gene across the reported host and geographic range for ENV, we also developed a second a qPCR assay for a more conserved ATPase-like gene region. The MCP and ATPase qPCR assays showed good analytical and diagnostic sensitivity and specificity based on samples from laboratory challenges of Pacific herring. The qPCR assays had similar diagnostic sensitivity and specificity as light microscopy of stained blood smears for the presence of intraerythrocytic inclusion bodies. However, the qPCR assays may detect viral DNA early in infection prior to the formation of inclusion bodies. Both qPCR assays appear suitable for viral surveillance or as a confirmatory test for ENV in Pacific herring from the Salish Sea. These results were published in Purcell et al. (2016).

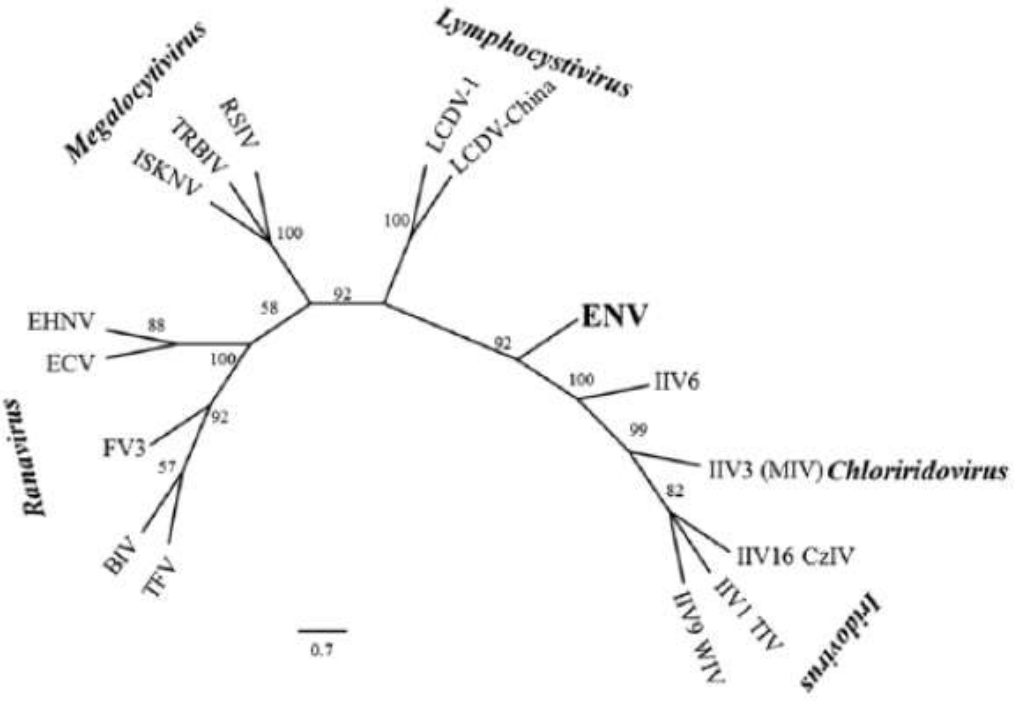

Figure 12. Relationship of erythrocytic necrosis virus (ENV) to other iridoviruses based on partial sequencing of the major capsid protein (MCP). Evolutionary relationships were inferred using the maximum likelihood with Poisson correction model. The numbers above the nodes represent bootstrap support after 1000 replicates.

Chapter 5: Additional Studies

5.1 Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society

A review describing the impacts of climate change on marine diseases was published (Burge et al. 2014), including a section on ichthyophoniasis (Table 5). Infectious diseases are common in marine environments, but the effects of a changing climate on marine pathogens are not well understood. Here, we focused on reviewing current knowledge about how the climate drives host-pathogen interactions and infectious disease outbreaks. Climate-related impacts on marine diseases are being documented in corals, shellfish, finfish, and humans; these impacts are less clearly linked to other organisms. Oceans and people are inextricably linked, and marine diseases can both directly and indirectly affect human health, livelihoods, and well-being. We recommended an adaptive management approach to better increase the resilience of ocean systems vulnerable to marine diseases in a changing climate. Land-based management methods of quarantining, culling, and vaccinating are not successful in the ocean; therefore, forecasting of conditions that lead to outbreaks and designing tools/approaches to affect these conditions may be the best tool to manage marine diseases.

Table 5. Reported ichthyophoniasis epizootics in wild fishes.

Time period	Location	Affected fish	Impacts	Reference(s)
1913–1914	Gulf of St. Lawrence, Canada	Atlantic herring	Dead fish "in great numbers were washed ashore on beaches or sand reefs, skirting the coast, or in quiet coves"	Cox 1914
1931–1932	Gulf of Maine	Atlantic herring	Infection prevalence reached 70% during the peak of the epizootic, then declined to 18%	Daniel 1933, Fish 1934
1940–1943	British Isles	Mackerel	Infection prevalence was as high as 100%, and the disease was described as fatal	Sproston 1944
1947	Gulf of Maine	Atlantic herring	No information	Scattergood 1948
1954–1956	Gulf of Saint Lawrence, Canada	Atlantic herring	At least half of the mature herring in the western Gulf of St. Lawrence were killed	Sindermann 1956, Tibbo & Graham 1963
1966–1970	Western North Atlantic	Yellowtail flounder	Infection prevalence was as high as 25–57%, and "there can be no question that many [affected flounder] must succumb directly to the infection"	Powles et al. 1968, Ruggieri et al. 1970
1991–1993	Eastern North Atlantic (Denmark, Sweden, Norway, and Iceland)	Atlantic herring	Total *Ichthyophonus*-induced mortality in the North Sea was estimated at 12.8–36.8%	Rahimian & Thulin 1996, Mellergaard & Spanggaard 1997
1990s–2000s	Prince William Sound, Alaska	Pacific herring	The disease was a possible contributor to the population decline and failed recovery	Marty et al. 2010
Early 2000s	Yukon River, Alaska and Canada	Chinook salmon	The disease was a possible contributor to prespawn mortality	Kocan et al. 2004
2007	Columbia River, Washington and Oregon	American shad	Infection prevalence peaked at 72% and declined concomitantly with shad abundance	Hershberger et al. 2010
2008–2011	Iceland	Atlantic herring	Infection prevalence was as high as 70%	Oskarsson & Pálsson 2009

5.2 Infectious Diseases of Fishes in the Salish Sea

A review of the primary infectious and parasitic diseases affecting wild marine fishes in the Salish Sea was published (Hershberger et al. 2013b). As in marine regions throughout other areas of the world, fishes in the Salish Sea serve as hosts for many pathogens, including: nematodes, trematodes, protozoans, protists, bacteria, viruses, and crustaceans. Here, we review some of the better-documented infectious diseases that likely contribute to significant losses among free-ranging fishes in the Salish Sea and discuss the environmental and ecological factors that may affect the population-level impacts of disease. Demonstration of these diseases and their impacts to critical and endangered resources provides justification to expand pathogen surveillance efforts and to incorporate disease forecasting and mitigation tools into ecosystem restoration efforts.

5.3 Molecular Characterization of Hepatic Coccidiosis and a Morphological Report of a Novel Intestinal Coccidia.

Surveillance for pathogens of Atlantic herring, including VHSV, *Ichthyophonus hoferi*, and hepatic/intestinal coccidians, was conducted from 2012 to 2015 in the NW Atlantic Ocean, New Jersey, USA. Neither VHSV nor *I. hoferi* was detected from any samples. *Goussia clupearum* was found in the livers of 40-78% of adult herring in varying parasite loads; however, associated pathological changes were negligible (Fig. 13). Phylogenetic analysis placed *G. clupearum* most closely with other extraintestinal, liver coccidia from the genus *Calyptospora*, though the *G. clupearum* isolates had a unique nucleotide insertion between 604-729 bp that did not occur in any other coccidian species. *G. clupearum* oocysts from Atlantic and Pacific herring were morphologically similar, though differences occurred in oocyst dimensions. Comparison of the small subunit 18S ribosomal RNA gene of *G. clupearum* from Atlantic and Pacific herring revealed four nucleotide substitutions and two gaps in a 1749 bp region, indicating some divergence in the geographically separate populations. Intestinal coccidiosis, possibly attributed to more than one species, was described for the first time from juvenile and adult Atlantic herring. A novel intestinal coccidian species was detected based on morphological characteristics of exogenously sporulated oocysts. A unique feature in these oocysts was the presence of three long (15.1 ± 5.1 µm) spiny projections on both ends of the oocyst. The novel morphology of this coccidian led us to tentatively name this parasite *Eimeria echinata* n. sp. These results were published in Friend et al. (2016).

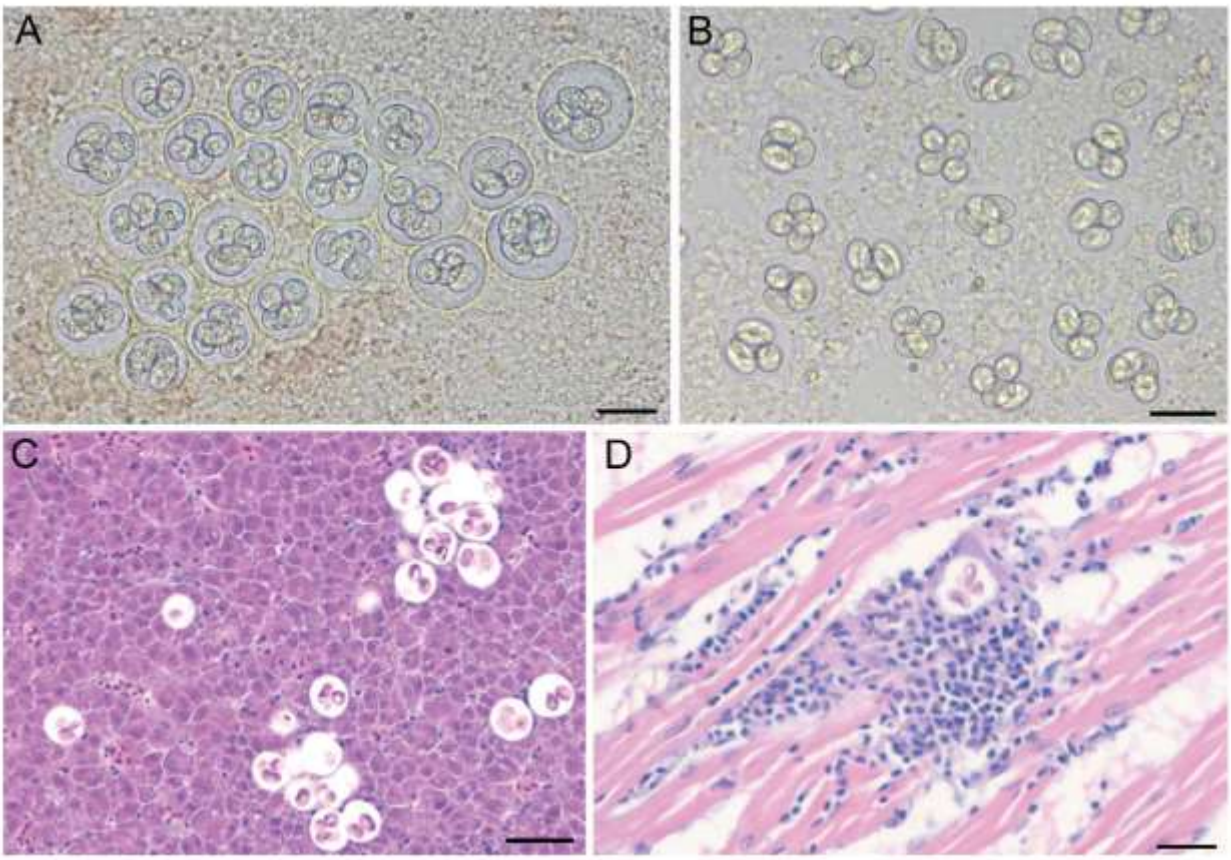

Figure 13. *Goussia clupearum*, bar=20µm (A-B) Wet mounts of fresh, homogenized liver tissue with (A) oocysts in Atlantic herring showing size variation of oocysts, and (B) oocysts in Pacific herring. (C-D) Histology of Atlantic herring stained with H&E showing (C) aggregates of oocysts in liver tissue and (D) Atlantic herring heart infected with coccidia resembling *Goussia clupearum* showing inflammatory response.

List of Scientific Products and Literature Cited

Scientific publications:

White, V.C., P.K. Hershberger, C.S. Friedman. *Accepted*. Analytical and diagnostic performance of a qPCR assay for *Ichthyophonus* spp. compared to the tissue explant culture 'gold standard'. Diseases of Aquatic Organisms.

Hart, L.M., M.K. Purcell, R. Powers, A. MacKenzie, P.K. Hershberger. 2017a. Optimization of a plaque neutralization test to identify the exposure history of Pacific herring to viral hemorrhagic septicemia virus. Journal of Aquatic Animal Health 29: 74-82.

Hart, L.M., N. Lorenzen, K. Einer-Jensen, M. Purcell, P.K. Hershberger. 2017b. Influence of temperature on the efficacy of homologous and heterologous DNA vaccines against viral hemorrhagic septicemia (VHS) in Pacific herring. Journal of Aquatic Animal Health 29: 121-128.

Purcell, M.K., S. Pearman-Gillman, R.L. Thompson, J.L Gregg, L.M. Hart. J.R. Winton, E.J. Emmenegger, P.K. Hershberger. 2016. Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of quantitative real-time PCR assays for quantification of ENV DNA. Journal of Veterinary Diagnostic Investigation 28: 382-391.

Friend, S.E., J. Lovy, P.K. Hershberger. 2016. Disease surveillance of Atlantic herring: molecular characterization of hepatic coccidiosis and a morphological report of a novel intestinal coccidian. Diseases of Aquatic Organisms 120: 91-107.

Gregg, J.L., R.L. Powers, M.K. Purcell, C.S. Friedman, P.K. Hershberger. 2016. *Ichthyophonus* parasite phylogeny based on ITS rDNA structure prediction and alignment identifies six clades, with a single dominant marine type. Diseases of Aquatic Organisms 120: 125-141.

Hart, L.M., C.M. Conway, D.G. Elliott, P.K. Hershberger. 2016. Persistence of external signs in Pacific herring *Clupea pallasii* with ichthyophoniasis. Journal of Fish Diseases 39: 429-440.

Hershberger, P.K., J.L. Gregg, L.M. Hart, S. Moffitt, R. Brenner, K. Stick, E. Coonradt, T. Otis, J. J. Vollenweider,K. A. Garver, J. Lovy, T.R. Meyers. 2016a. The parasite *Ichthyophonus* sp. in Pacific herring. Journal of Fish Diseases 39: 309-410.

Hershberger, P.K., K.A. Garver, J.R. Winton. 2016b. Principles underlying the epizootiology of viral hemorrhagic septicemia in Pacific herring and other fishes throughout the North Pacific Ocean. Canadian Journal of Fisheries and Aquatic Sciences. 73: 853-859.

Hershberger, P.K., L.M. Hart, A.H. MacKenzie, M.L. Yanney, C. Conway, D. Elliott. 2015. Infecting Pacific herring with *Ichthyophonus* sp. in the laboratory. Journal of Aquatic Animal Health 27: 217-221.

Conway, C.M., M.K. Purcell, D.G. Elliott, P.K. Hershberger. 2015. Detection of *Ichthyophonus* by chromogenic *in situ* hybridization. Journal of Fish Diseases 38: 853-857.

Kocan, R., L. Hart, N. Lewandowski, P. Hershberger. 2014. Viability and infectivity of *Ichthyophonus sp.* in post-mortem Pacific herring, *Clupea pallasii*. Journal of Parasitology 100: 790-796.

Emmenegger, E.J., J.A. Glenn, J.R. Winton, W.N. Batts, J.L. Gregg, P.K. Hershberger. 2014. Molecular identification of erythrocytic necrosis virus (ENV) from the blood of Pacific herring (*Clupea pallasii*). Journal of Veterinary Microbiology 174: 16-26.

Wilson, A. E., T. L. Goldberg, S. V. Marquenski, W. J. Olson, R. F. Goetz, P. K. Hershberger, K. L. Toohey-Kurth. 2014. Development and evaluation of a blocking enzyme-linked immunosorbent assay and virus neutralization assay to detect antibodies to viral hemorrhagic septicemia. Clinical and Vaccine Immunology 21: 435-442.

Burge, C. A., C. M. Eakin, C. S. Friedman, B. Froelich, P. K. Hershberger, E. E. Hofmann, L. E. Petes, K. C. Prager, E. Weil, B. L. Willis, S.E. Ford, C. D. Harvell. 2014. Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science 6: 249-277.

Emmenegger, E.J., C.H. Moon, P.K. Hershberger, G. Kurath. 2013. Virulence of viral hemorrhagic septicemia virus (VHSV) genotypes Ia, IVa, IVb, and IVc in five fish species. Diseases of Aquatic Organisms 107: 99-111.

Kocan, R, S. LaPatra, P. Hershberger. 2013. Evidence for an amoeba-like infectious stage of *Ichthyophonus* sp. and description of a circulating blood stage: a probable mechanism for dispersal within the fish host. Journal of Parasitology 99: 235-240.

Hershberger, P.K., M. K. Purcell, L.M. Hart, J.L. Gregg, R.L. Thompson, K.A. Garver, J.R. Winton. 2013a. Influence of temperature on viral hemorrhagic septicemia (Genogroup IVa) in Pacific herring, *Clupea pallasii* Valenciennes. Journal of Experimental Marine Biology and Ecology 444: 81-86.

Hershberger, P.K., L. Rhodes, G. Kurath, J. Winton. 2013b. Infectious diseases of fishes in the Salish Sea. Fisheries 38: 400-409.

Lovy, J., P. Piesik, P.K. Hershberger, K. A. Garver. 2013. Experimental infection studies demonstrating Atlantic salmon as a host and reservoir of viral hemorrhagic septicemia virus type IVa with insights into pathology and host immunity. Veterinary Microbiology 166: 91-101.

Invited Seminars:

2016: School of Aquatic and Fishery Sciences, University of Washington
Departmental Seminar (Nov 3, 2016): "Forecasting and Mitigating Disease Impacts in Wild Fishes"

2016: USGS Monthly Wildlife Disease Coordination Call
Webinar: "Current Research at the USGS – Marrowstone Marine Field Station"

2016: School of Aquatic and Fishery Sciences, University of Washington: FISH 404.
Guest Lecture and Facility Tour (April 14, 2016) "How Does Science Really Work?"

2014: Cordova Weekly Seminar Series (November 24, 2014)
Ecology of Disease in Pacific herring

2014: National Science Foundation, Research Coordination Network
Invited Presentation - Pathogen Persistence and Perpetuation Strategies in Marine Fishes: Perspectives from Pacific Herring (August 16, 2014). Friday Harbor, WA.

2014: School of Aquatic and Fishery Sciences, University of Washington: FISH 404.
Guest Lecture and Facility Tour (May 30, 2014) "How Does Science Really Work? The Frustration of Dead Ends and the Satisfaction of Tiny Advancements."

2014: Tribal Climate Change Webinar Series
Invited Webinar (May 21, 2014): Climate Change and Marine Issues
Shifting Ocean Currents and Infectious / Parasitic Diseases of Marine Fishes

Co-hosted by the Institute for Tribal Environmental Professionals – Northern Arizona University,– Pacific Northwest Tribal Climate Change Project - University of Oregon, and North Pacific Landscape Conservation Cooperative

2013: University of Southern Mississippi, Gulf Coast Research Laboratory
Invited Seminar (Nov 6)
Ecology of Diseases in Wild Marine Fishes

2013: Alaska Herring Managers Meeting
Invited Presenter (Nov 4-5)
Diseases of Pacific herring in Alaska

2013: University of Vermont (April 29)
Invited Seminars:
Biology Department: "Ecology of Infectious and Parasitic Diseases in Marine Fishes"
Experimental Program to Stimulate Competitive Research Committee: Research on Adaptation to Climate Change: "Impacts of Climate Change on Diseases of Marine and Anadromous Fishes"

Presentations at Scientific Meetings

Hershberger, P.K., L. Hart, A. MacKenzie, R, Powers, M. Purcell. January 23-27, 2017. Poster. Quantifying the potential for disease impacts to Pacific Herring. Alaska Marine Science Sympisium. Anchorage, AK.

Sitkiewicz, S., B. Harris, P. Hershberger, N. Wolf. January 23-27, 2017. Poster. Effects of the parasite *Ichthyophonus* (sp.) on groundfish growth and condition. Alaska Marine Science Symposium. Anchorage, AK.

Hart, L.M., M.K. Purcell, R.L. Powers, A.H. MacKenzie, P.K. Hershberger. June 26-30, 2016. Poster. Optimization of a plaque neutralization test capable of assessing the exposure history of Pacific herring to viral hemorrhagic septicemia virus. 2nd International Conference of Fish & Shellfish Immunology. Portland, ME.

Gregg, J.L., R.L. Thompson, M.K. Purcell, C.S. Friedman, P.K. Hershberger. November 5-8, 2015. Phylogeny of *Ichthyophonus* parasites indicates majority of global impacts can be attributed to a single ubiquitous marine species. Western Society of Naturalists – 96th Annual Meeting. Sacramento, CA.

Elliott, D.G., C.L. McKibben, C.M. Conway, A. MacKenzie, P.K. Hershberger. September 7-11, 2015. Platform. Differential susceptibility of Yukon River and Salish Sea Chinook salmon (*Oncorhynchus tshawytscha*) stocks to *Ichthyophonus*. 17th International Conference on Diseases of Fish and Shellfish. Las Palmas de Gran Canaria, Spain.

Hart, L.M., P.K. Hershberger. August 16-20, 2015. Platform. Integration of disease information into population assessments: the case of VHS and Pacific herring. American Fisheries Society 145th Annual Meeting. Portland, OR.

Hershberger, P.K., J.L. Gregg, A.H. MacKenzie, M.L. Yanney, C. Conway, D.Elliott. June 2-4, 2015. Poster. Infecting Pacific herring (*Clupea pallasii*) with *Ichthyophonus* in the laboratory. 56th Annual Western fish Disease Workshop. Steamboat Springs, CO.

Gregg, J.L., C. Dykstra, P.K. Hershberger. November 9-14, 2014. Platform. Epizootiology of *Ichthyophonus* sp. in Pacific Halibut (*Hippoglossus stenolepis*) in the Northeast Pacific Ocean and Bering Sea. 9th International Flatfish Symposium. Cle Elum, WA.

Conway, C.M, M.K. Purcell, D.G. Elliott, P.K. Hershberger. August 31 – September 4, 2014. Poster. Detection of *Ichthyophonus* by Chromogenic *In Situ* Hybridization. 7th International Symposium on Aquatic Animal Health. Portland, OR.

Garver, K.A., J. Lovy, P. K. Hershberger. August 31 – September 4, 2014. Platform. Trafficking of Viral Hemorrhagic Septicemia Virus from wild to farmed fish. 7th International Symposium on Aquatic Animal Health. Portland, OR.

Hart, L.M. C. Conway, D. Elliott, P.K. Hershberger. August 31 – September 4, 2014. Platform. A qualitative assessment of the progression of ichthyophoniasis related external signs and distribution of host response and parasite morphology in somatic tissues of Pacific herring *Clupea pallasii.* 7th International Symposium on Aquatic Animal Health. Portland, OR.

McKibben, C.L., P.K. Hershberger, M.K. Purcell, C.M. Conway, D.G. Elliott. August 31 – September 4, 2014. Poster. Influence of Temperature and Fish Stock on Progression of *Ichthyophonus* Infections in Chinook Salmon (*Oncorhynchus tshawytscha*). 7th International Symposium on Aquatic Animal Health. Portland, OR.

R.M. Kocan, P.K. Hershberger. August 31 – September 4, 2014. Platform. Impact of *Ichthyophonus* sp. on pre-spawn Yukon River Chinook salmon. 7th International Symposium on Aquatic Animal Health. Portland, OR.

Grenier, C., L. Cornick, P. Hershberger, B. Harris. January 20-24, 2014. Poster. Quantifying *Ichthyophonus* prevalence and intensity in Pacific halibut (*Hippoglossus stenolepsis*) in Cook Inlet, Alaska. Alaska Marine Science Symposium. Anchorage, AK.

Wilson, A.E., T.L. Goldberg, S. Marquinski, W. Olson, F. Foetz, P. Hershberger, L. Hart. October 17-23, 2013. Platform. Development and evaluation of a blocking enzyme-linked immunosorbent assay and virus neutralization assay to detect antibodies to viral hemorrhagic septicemia virus (VHSV). 56th American Association of Veterinary Laboratory Diagnosticians and United States Animal Health Association Meeting. San Diego, CA.

Kocan, R., S. LaPatra, L. Hart, P. Hershberger. June 18-20, 2013. Platform. Multiple routes of transmission for *Ichthyophonus*: myth or fact? 54th Western Fish Disease Workshop and AFS Fish Health Section Meeting. Port Townsend, WA.

Hart, L.M. M.K. Purcell, S.M. Badil, R.L. Thompson, P.K. Hershberger. June 18-20, 2013. Platform. Development of an Enzyme-linked immunosorbent assay for detection of VHSV specific antibodies in Pacific herring. 54th Western Fish Disease Workshop and AFS Fish Health Section Meeting. Port Townsend, WA.

Gregg, J.L., R.L. Thompson, M.K. Purcell, C.S. Friedman, P.K. Hershberger. June 18-20, 2013. Platform. Phylogeny of parasites in the genus *Ichthyophonus* and their prevalence in several host species. 54th Western Fish Disease Workshop and AFS Fish Health Section Meeting. Port Townsend, WA.

Garver, K.A., J. Lovy, P.K. Hershberger. September 2-6, 2013. Platform. Viral hemorrhagic septicemia virus in British Columbia, Canada: transmission from wild to farmed fish. 16th International Conference on Diseases of Fish and Shellfish; European Association of Fish Pathologists. Tampere, Finland.

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