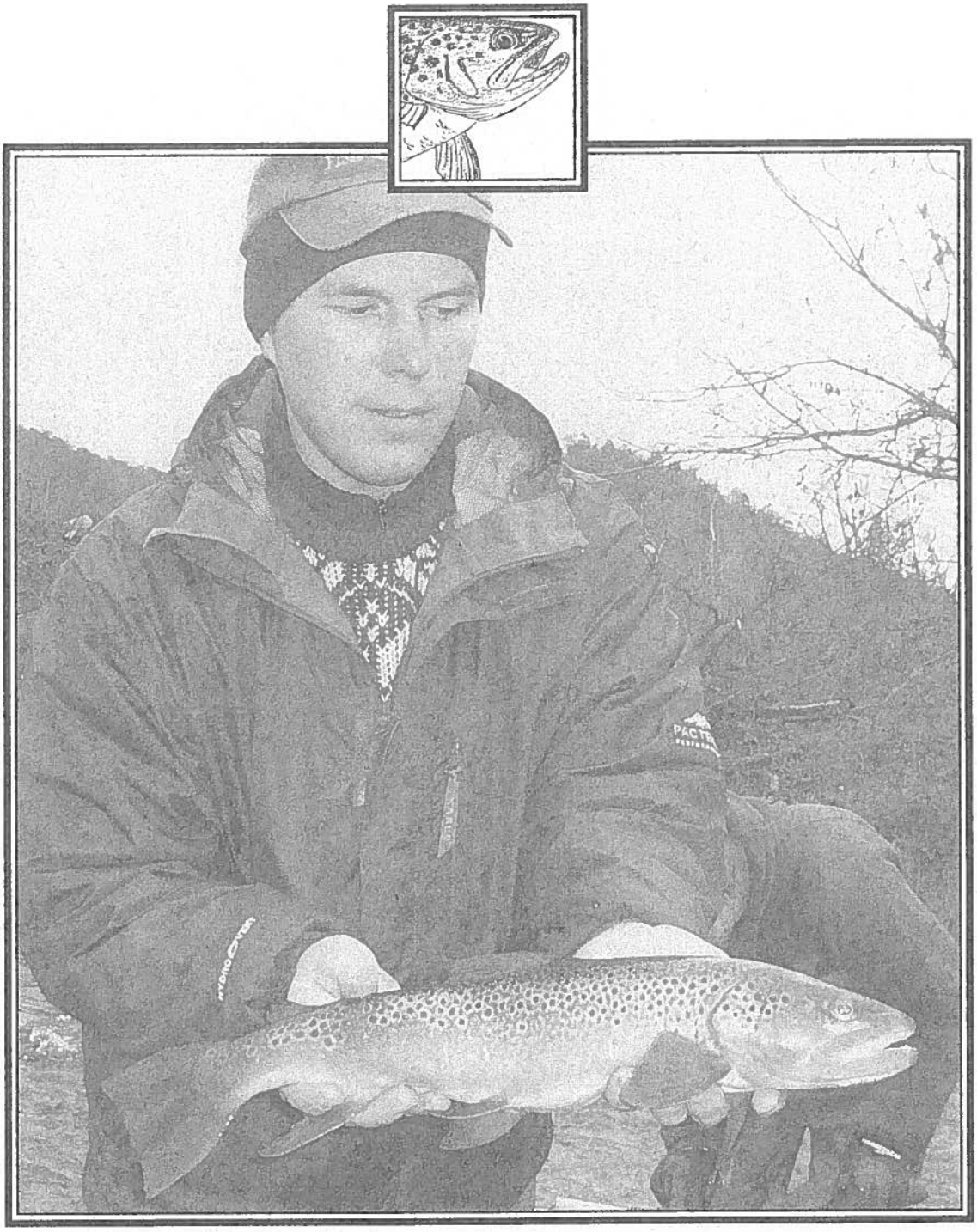

Viral Hemorrhagic Septicemia and Freshwater Fisheries:
the State of the Science

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Cover: Brown trout, a VHS-susceptible species, has been found VHS-positive in Lake Michigan but not in Wisconsin inland streams where this one was captured.
Photo courtesy of Stephanie Warnement.

ABSTRACT

This report summarizes the state of the science on viral hemorrhagic septicemia (VHS) and provides guidance for prioritizing future VHS research to support science-based fisheries management in Wisconsin. The VHS virus was first detected in Wisconsin in May 2007 and poses a threat to sport and commercial fisheries in the state. Herein we provide a brief background on VHS identification, classification and distribution, summaries of recent research on VHS and risk management pertaining to fish diseases, and the identification of VHS research gaps. Research recommendations based on the authors' interpretation of the state of the science of VHS as outlined in this report, and made in the context of practically supporting the fisheries management obligations of the Wisconsin Department of Natural Resources, include: 1) conducting a formal risk analysis on VHSV Type IVb, the new variant of VHS virus found in Wisconsin and the Great Lakes region, and on its potential impacts on fish populations in Wisconsin, 2) contributing to the development of rapid testing procedures for detecting VHSV when present and for declaring groups of fish free of VHSV when the virus is not present, 3) contributing to the development of VHS challenge models, particularly for commonly used baitfish species, 4) initiating a long-term monitoring study on the effects of VHSV on fish populations and fish communities in a selection of VHSV-positive waters, and 5) developing and parameterizing disease models for VHSV.

VIRAL HEMORRHAGIC SEPTICEMIA AND FRESHWATER FISHERIES: THE STATE OF THE SCIENCE

Matthew G. Mitro and Angela L. White

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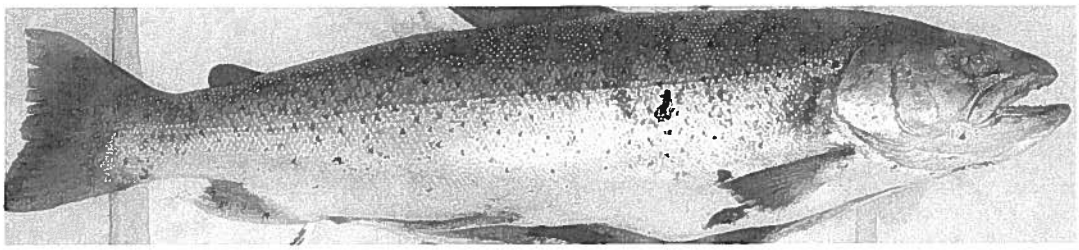
CONTENTS

VHS was first detected in Wisconsin in May 2007 in the Lake Winnebago and Lake Michigan systems. Because Lake Superior is directly connected to Lake Michigan and the Mississippi River is connected by the Illinois and Des Plaines rivers via the Chicago Sanitary and Ship Canal (not shown on map), we suspect that VHS is present in those waters, though it has yet to be confirmed.



SUSAN MARCQUENSKI

VHS was detected in one brown trout found dead on the beach in the Algoma/ Kewaunee area. No external signs of VHS were present.

INTRODUCTION

Viral hemorrhagic septicemia (VHS) is an infectious fish disease caused by the viral hemorrhagic septicemia virus (VHSV). VHS is considered a serious threat to fisheries resources because it is highly transmissible and it can result in significant mortality in a variety of fish species across many fish families (Meyers and Winton 1995; Hendrick et al. 2003; Lumsden et al. 2007). VHSV was first detected in Wisconsin in May 2007 and is considered an invasive species. Recognizing the threat VHSV poses to sport and commercial fisheries in the state, the Wisconsin Department of Natural Resources (Wisconsin DNR) has implemented a series of management rules and biosecurity protocols to prevent the spread of VHSV to other state waters.

The Wisconsin DNR recognizes the important role of science-based decision making in fisheries resource management. To that end, the Wisconsin DNR aims to make use of the best available science on VHS to manage state fisheries threatened by VHS and to support additional research on VHS where needed. The scope of this report is to summarize the state of the science on VHS as it pertains to the management of freshwater fish populations. This report provides a brief background on VHS identification, classification and distribution, summaries of recent research on VHS and risk management pertaining to fish diseases, and the identification of VHS research gaps. Appendix 1 contains a selected glossary of terms and Appendix 2 lists common and scientific names of fishes referenced in this report. The goal of this report is to provide guidance for prioritizing future VHS research to support science-based fisheries management in Wisconsin.

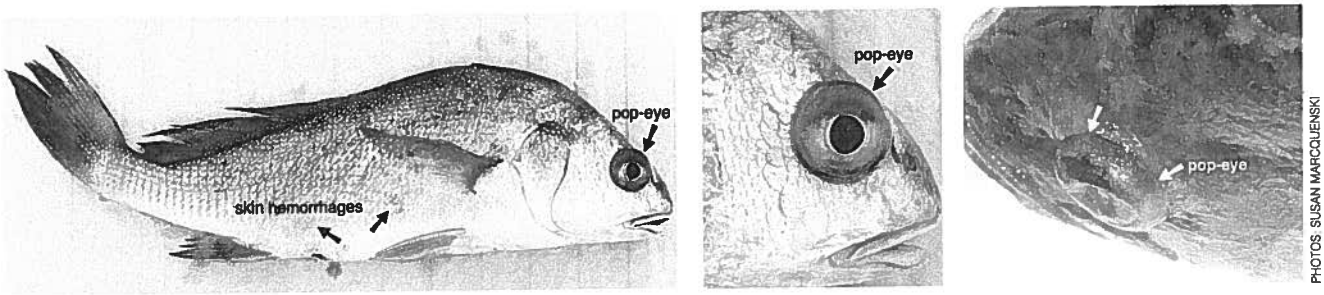
CLASSIFICATION AND DISTRIBUTION

VHSV is an enveloped, bullet shaped, single stranded ribonucleic acid (RNA) virus of the genus Novirhabdovirus in the family Rhabdoviridae (Walker et al. 2000). VHSV was historically considered a virus restricted to rainbow trout in fish farms in Europe (Enzmann et al. 1992) and was originally referred to as Egtved virus, named after a village in Denmark where the disease was first documented (Meyers and Winton 1995; OIE 2006). A related rhabdovirus that is highly virulent to trout and salmon is infectious hematopoietic necrosis virus (IHNV) (Jørgensen et al. 1991).

VHSV occurs in fish from both freshwater and marine environments and the virus can be classified into four different genotypic isolates. These four VHSV isolates vary geographically and not by fish host species or time when the isolate was sampled (Batts et al. 1993; Benmansour et al. 1997; Elsayed et al. 2006). VHSV type I occurs in European freshwater fish farms (i.e. trout farms) and has also been isolated in northern European marine fish species; type II represents isolates from the Baltic Sea, type III represents isolates from the North Sea, and type IV represents isolates from North America. VHSV type I comprises five subgroups (types Ia-e) (Thiery et al. 2002; Elsayed et al. 2006; Nishizawa et al. 2006). VHSV type IV comprises two subgroups (types IVa and b) and infects a great variety of marine and freshwater fishes across a wide geographic range in North America (Hedrick et al. 2003; APHIS 2007; Gagne et al. 2007; Groocock et al. 2007).

Meyers and Winton (1995), Skrall et al. (2005a and 2005b), AFS/FHS Blue Book (2007), Gagne et al. (2007), and Hershberger et al. (2007) review species that are susceptible to different strains of VHSV. The United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Veterinary Services also maintains a list of fish species susceptible to VHSV type IVb. As of 8 November 2007, APHIS (2007) listed 28 freshwater fish species that have been identified as susceptible to infection by VHSV under natural conditions in US and Canadian waters.

The occurrence of VHSV in North America was first documented in 1988 adult coho and chinook salmon in Washington, USA (Meyers and Winton 1995). This isolate of VHSV has been classified as type IVa and is considered enzootic in the marine environment, having not originated from Europe (Batts et al. 1993). Pacific herring have been identified as the primary reservoir of VHSV in the Northeastern Pacific Ocean, with Pacific cod acting as a secondary viral reservoir (Meyers and Winton 1995).



Left: Drum from Lake Winnebago showing pop-eye and slight skin hemorrhages.
Center: Exophthalmia (pop-eye) in drum from Little Lake Butte des Morts.
Right: Smallmouth bass from Sturgeon Bay showing pop-eye with hemorrhage.

VHSV type IVb is a freshwater variant that was first isolated from muskellunge in Lake St. Clair, Michigan in 2005 from archived samples collected in 2003 (Table 1; Elsayed et al. 2007). Fish health surveys and fish kill investigations have documented the spread of VHSV from Eastern Canadian maritime waters, through the St. Lawrence River into the Great Lakes system, and into some inland lakes within the Great Lakes watershed (Table 1; Gagne et al. 2007; Groocock et al. 2007; Lumsden et al. 2007). VHSV type IVb was first documented in the Mississippi basin in June 2008 in a muskellunge collected from Clearfork Reservoir in Ohio (Egan 2008).

In Wisconsin, VHSV type IVb has to date (June 2008) been detected in six fish species from seven locations (Table 1). Freshwater drum collected from Little Lake Butte des Morts and two locations in Lake Winnebago tested positive for VHSV in May 2007 (WDNR 2007a and 2007b); the VHSV-positive freshwater drum were part of a month-long fish kill from late April through late May involving thousands of fish. Subsequently VHSV was also detected in smallmouth bass collected from Sturgeon Bay in Green Bay, Lake Michigan, in brown trout collected in Lake Michigan near Kewaunee, and in lake whitefish collected in north Green Bay, Lake Michigan (WDNR 2007c and 2007d). Following extensive testing of fishes from targeted collections across Wisconsin, VHSV was not detected in any other state waters in 2007 (WDNR 2007d). In 2008, VHSV was detected in round gobies collected in the southern basin of Lake Michigan in Grant Park, Milwaukee, where several thousand were washed on the beach (S. Marcquenski, personal communication). VHSV was also detected in yellow perch collected in a fish survey within three miles of where the infected round gobies were found (S. Marcquenski, personal communication).

A high rate of mutation inherent in rhabdoviruses has led to the concern that more virulent strains would evolve (Meyers and Winton 1995). Of particular concern is the experience in Europe where the freshwater strain of VHSV, which is closely related to the European marine strain of VHSV, exhibits a high virulence for salmonid fishes such as rainbow trout. The North American VHSV type IVa has shown to be of low virulence for Pacific salmonids, and Meyers and Winton (1995) recommended that any occurrence of VHSV in Pacific salmonid propagation programs be quickly eradicated to prevent more virulent strains from developing. Analogous to the European experience, the newly isolated VHSV type IVb (Elsayed et al. 2006), which is closely related to the North American marine strain of VHSV, appears to be of high virulence for some freshwater species of fish.

TRANSMISSION

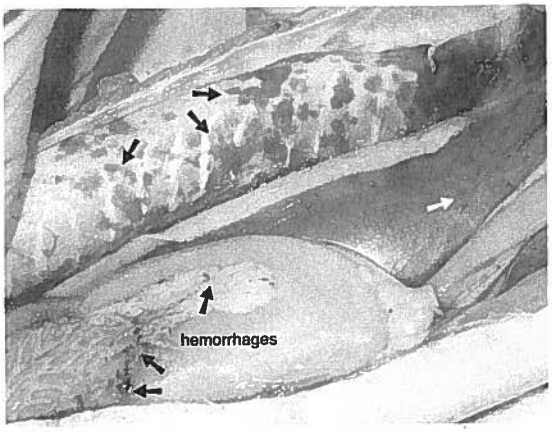
Viral transmission is in essence an invasion process whereby a novel species (here VHSV) invades a new habitat (here a fish host, possibly in a new body of water). For VHSV to successfully invade a new host, the host must have tissue with compatible receptors. The current list of fish species in which VHSV type IVb has been detected in the wild (APHIS 2007) suggests that VHSV is compatible with receptors in a wide diversity of fish species. However, the somewhat shorter list of fish species that have experienced kills attributable to VHSV type IVb suggests that the virus affects species in different ways. Most RNA-type viruses like VHSV multiply rapidly in their host, which may lead to either the death of the host or an immune response that inhibits the infection.

Hudson et al. (2008) describe a conceptual framework for the steps to invasion of a new host. The use of such a rigorous framework may aid in the assessment of invasion and transmission risks. A VHSV invasion begins when a new fish host is exposed to VHSV (step 1). VHSV must then pass any barriers to entry into the host (step 2) and become established in the host by finding tissue with compatible receptors for the virus (step 3). It is here that VHSV attempts to replicate and produce new virus for transmission (step 4). However, an acquired immune response may act to prevent the replication and shedding of virus, thereby relegating the fish host to a state of latent infection (i.e. a VHSV carrier). If the virus successfully replicates in the host, then the

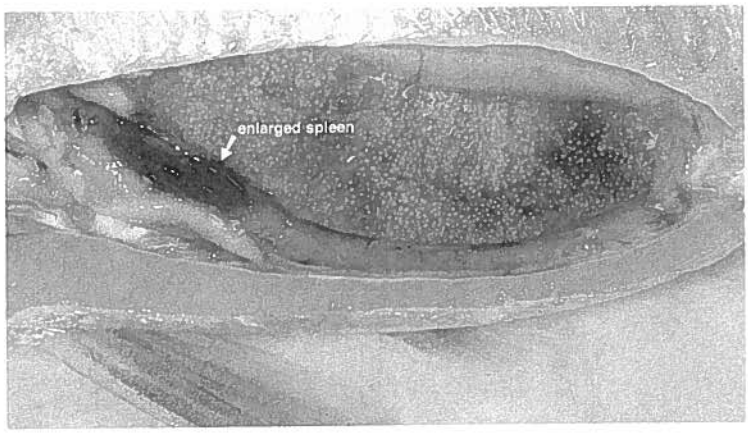
Table 1. Fish kills attributable to VHSV type IVb in fishes of Great Lakes area waters of North America. Detections of VHS-positive fish observed in surveillance monitoring (i.e. not fish kills) are also included.

Date	State or Province	Water Body	Species	Number Affected	Reference
Apr-May 2005	ON	Lake Ontario (Bay of Quinte)	Freshwater drum	20,000-30,000 [a]	Lumsden et al. 2007
			Round goby	"Large numbers"	
			Muskellunge	"Few"	
Spring 2006	MI	Lake St. Clair	Bluntnose minnow	"Large mortality"	USDA/APHIS/CEAH 2006; Whelan 2007b
			Gizzard shad		
			Muskellunge		
			Northern pike		
			Redhorse sucker		
			Yellow perch		
		St. Clair River	Gizzard shad		
		Detroit River	Gizzard shad		
			Muskellunge		
May 2006	NY	Lake Ontario (Irondequoit Bay)	Round goby	Several 1,000s	Groocock et al. 2007
		St. Lawrence River	Round goby	"Large die off"	USDA/APHIS/CEAH 2006; Whelan 2007b
			Muskellunge		
		St. Lawrence River	Smallmouth bass	"Ongoing fish kills"	S. Marcquenski, personal communication
	OH	Lake Erie	Freshwater drum	"Very large mortality"	USDA/APHIS/CEAH 2006; Whelan 2007b
			Smallmouth bass	"Mortality"	
			Walleye		
			White bass		
			Yellow perch	"Large die off" [b]	
June 2006		St. Lawrence River	Burbot	"Ongoing fish kills"	S. Marcquenski, personal communication
Aug 2006	NY	Conesus Lake	Walleye	"Fish kill" (50-100)	Kozlowski 2007
Autumn 2006	MI	Lake Huron (Swan River)	Chinook salmon	Surveillance monitoring	S. Marcquenski, personal communication
	MI	Lake Huron (Thunder Bay)	Lake whitefish	"Smaller mortality event"	Whelan 2007b
			Walleye		
Spring 2007	NY	Lake Erie (Dunkirk Harbor)	Gizzard shad	"die-offs"	Cornell University 2007
		Lake Ontario	Gizzard shad	"die-offs"	
		Skaneateles Lake	Rock bass	"Ongoing fish kill"	NYDEC 2007
			Smallmouth bass		
		St. Lawrence River	Round goby	100,000s	Cornell University 2007
Apr 2007	MI	Budd Lake	Black crappie	"Very large die off"	MDNR 2007
			Bluegill		
			Muskellunge		
Apr-May 2007	WI	Little Lake Butte des Mortes	Freshwater drum	"Fish kill"	S. Marcquenski, personal communication
May 2007	WI	Lake Winnebago (Taycheedah)	Freshwater drum	"Fish kill"	WDNR 2007b
		Lake Winnebago (Asylum Bay)	Freshwater drum	"Fish kill"	WDNR 2007b
	WI	Sturgeon Bay	Smallmouth bass	Surviellance monitoring	S. Marcquenski, personal communication
	WI	Lake Michigan (Algoma/Kewaunee)	Brown trout	A few dead fish on beach	S. Marcquenski, personal communication
	WI	Lake Michigan (North Green Bay)	Lake whitefish	Fish dying in commercial fish nets	S. Marcquenski, personal communication
	ON	Lake Ontario (Hamilton Harbour)	Freshwater drum	"Die-off"	CFIA 2008
May 2008	WI	Lake Michigan (Grant Park, Milwaukee)	Round goby	3 of 4 collected from several thousand washed on beach	WDNR 2008a
June 2008	OH	Clearfork Reservoir	Muskellunge	1 collected in surveillance monitoring	Egan 2008
June 2008	WI	Lake Michigan (Milwaukee)	Yellow perch	3 of 11 pooled samples derived from 54 fish	WDNR 2008b

[a] Model estimate of freshwater drum mortality in Lake Ontario.
[b] Yellow perch mortality observed in commercial fish traps; other nearby mortalities also observed.



Hemorrhage on body wall, swim bladder, and mesenteric fat of a lake whitefish from northern Green Bay.



PHOTOS: SUSAN MARCQUENSKI

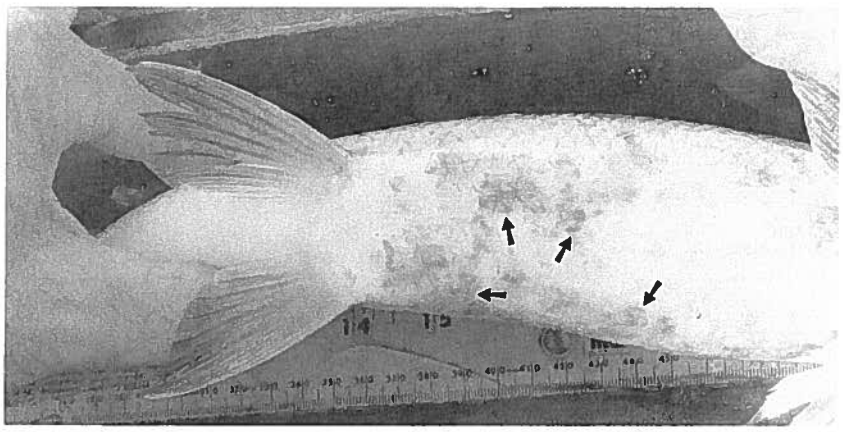
Drum from Little Lake Butte des Morts with enlarged spleen.

transmission was successful and may lead to persistence in the host fish population (step 5). The host fish may die as a result of infection, but in the process of infection may have transmitted the virus to new, susceptible hosts. The probability of infecting a new host will depend on the contact rate between the new host and the shed virus and the period of time that the infected host is infectious.

VHSV can enter an aquatic system and present an exposure opportunity in a variety of ways. An emergency prevention and response plan for VHSV produced by the National Park Service and the Grand Portage Band of Lake Superior Chippewa (NPS/GPBLSC 2008) discusses the various modes or vectors of introduction that may lead to the presence of VHSV in a water body. Modes of introduction discussed include fish stocking from hatchery or aquaculture facilities, ballast water, activities associated with commercial and subsistence fishing, the movement or migration of fish and wildlife, natural resource agency activities, and water recreation activities. Levels of risk associated with these activities are also discussed. Low-risk vectors of VHSV introduction include legal or permitted fish stocking (because biosecurity protocols are used to prevent stocking of diseased fish) and migratory birds. High-risk vectors of VHSV introduction include unauthorized fish stocking, and the use of baitfish outside of established bait-use guidelines. Moderate-risk vectors include human activities such as recreational boating on water bodies, discharge of ballast water, and wild fish migration.

VHSV is transmitted to new hosts through contact with VHSV-infected fish or VHSV-contaminated water. VHSV may be shed from a host fish into the water via urine and female reproductive fluids (OIE 2006). The primary entrance pathway for the virus to infect a new fish host is through the gills (Neukirch 1984) and a secondary pathway is through the skin at the base of fins (Harmache et al. 2006). Fish may also become infected by VHSV via consumption of infected prey (Meyers and Winton 1995). Vertical transmission of VHSV from parent to progeny has not been demonstrated, but VHSV has been isolated from the surface of eggs of infected female salmonid broodstock (Meyers and Winton 1995). Internally the virus is most abundant within the following fish tissues: encephalon, heart, kidney, and spleen (OIE 2006). The virus infects endothelial cells lining the blood vessels, and as the cells die, blood escapes the vessels and causes hemorrhages. Survivors of VHS may become lifelong carriers of the virus and act as viral reservoirs that can lead to new outbreaks (OIE 2006). Transmission of VHSV has been observed between wild fish and fish held in captivity in fish farms or aquaculture operations, with the transfer occurring in both directions (Enzmann et al. 1992; Meier et al. 1994; Knuesel et al. 2003). Outflows from fish-rearing facilities have been identified as a risk factor in VHSV transmission in Europe (Knuesel et al. 2003). Anadromous fishes returning to hatchery facilities from marine environments pose a risk in North America (Meyers and Winton 1995).

The transmission of VHSV and other fish viruses depend on characteristics of the fish host, the virus, and the environment in which they interact (Wolf 1988; Reno 1998; Hudson et al. 2008). Fish host characteristics relevant to disease transmission include species, age, and immunity. A wide range of fish species in many families has shown susceptibility to VHSV infection (Appendix 2). VHSV can infect fish of different ages, but younger fish appear to be more susceptible to infection and mortality and older fish may have developed immunity from prior exposure to the virus (Meyers and Winton 1995; Knuesel et al. 2003; OIE 2006). Characteristics of a virus that influence transmission include the ability to infect a particular species and virulence. Characteristics of the environment that affect transmission include water temperature, population density, water flow, and chemistry. We are unaware of any studies that investigated the effects of water flow on VHS, but it is hypothesized that fast flow rates, as compared to slow flow rates, may decrease the contact time between a virus and its host or more effectively distribute the virus in



KEN ROYSECK

Lake whitefish with skin hemorrhages captured in northern Green Bay.

the water medium (Reno 1998). Fish population density may affect VHSV transmission because higher fish densities may lead to more contacts between fish and virus as well as greater virus production from a greater number of infected fish.

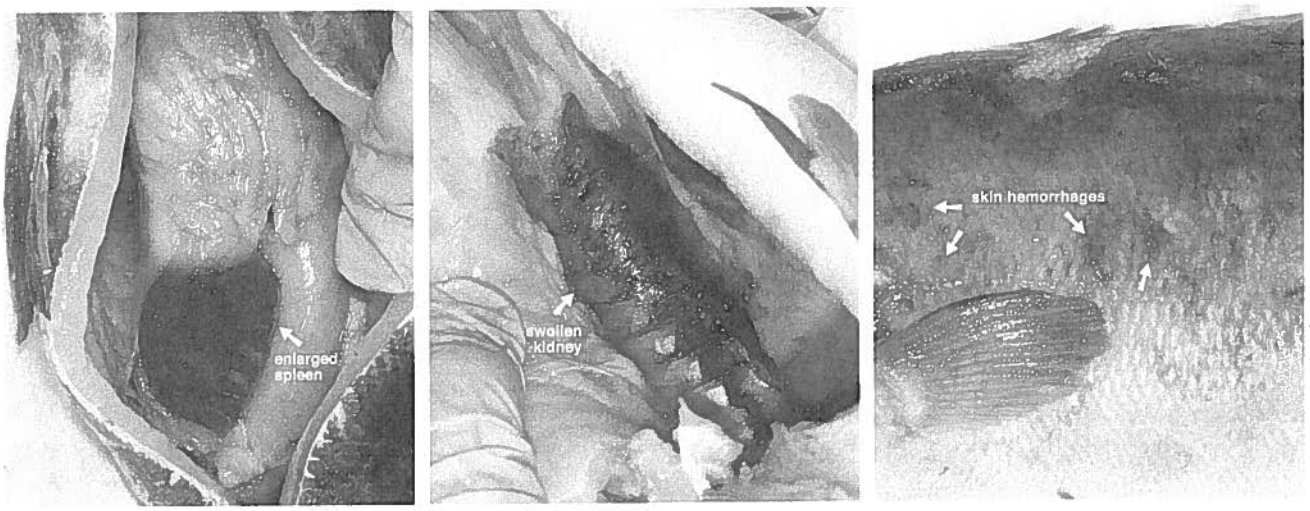
Water temperature has been shown to play an important role in the ecology of VHSV. Transmission of VHSV generally occurs between temperatures of 1°C and 12°C, and VHS outbreaks generally occur between temperatures of 4°C and 14°C (OIE 2006). Gastric and de Kinkelin (1980) noted the tendency of VHS to occur in rainbow trout when the water temperature is less than 14°C. Hedrick et al. (2003) reported a range of 4–16°C for detecting VHSV type IVa isolates in Pacific fishes.

Observations on the survival and replication of VHSV in both laboratory and field settings suggest an upper thermal tolerance for VHSV. Jørgensen (1982a) found that the persistence of VHSV in rainbow trout decreased as water temperature increased. Gastric and de Kinkelin (1984) identified an upper thermal tolerance of 18–20°C based on exposure to VHS type I by marine fishes. Hedrick et al. (2003) suggested 17–18°C as the upper tolerable range for VHSV type IVa, beyond which viral replication is inhibited. Arkush et al. (2006) performed experiments with North American strains of VHSV (type IVa) and demonstrated growth at temperatures ranging from 10 to 20°C with optimal growth at 15°C and no growth at 25°C. They also found geographic variation in temperature tolerances in which strains of VHSV from the southern range of the virus in the Pacific Ocean exhibited a higher growth rate at 20°C as compared to strains from the northern range. Arkush et al. (2006) concluded that in general temperatures 15°C and greater were inhibitive to replication of this strain of VHSV.

The upper thermal tolerance for survival and replication of VHSV type IVb in wild fish is unknown. However, laboratory studies have shown that VHSV type IVb cannot replicate in cell cultures at temperatures greater than 23°C (Winton et al. 2007), which suggests 23°C may be a thermal limit for survival of VHSV type IVb in wild fish (A. Goodwin, personal communication). If the virus cannot replicate then it cannot survive and persist in fish because VHSV does not have a latent state.

Kocan et al. (2001a) found that the survival and infectivity of VHSV type IVa may be significantly prolonged in the presence of ovarian fluids, which occur naturally during spawning. They recovered VHSV type IVa for up to 40 hours in natural filtered sea water (50% loss of infectivity after about 10 hours at 15°C). The addition of 10 ppb of North Slope crude oil did not affect survival of the virus, but the addition of 0.01% and 1.0% ovarian fluid extended recovery time up to 72 hours and 96 hours, respectively. It was thought that the extended virus survival is attributable to the high protein content of the ovarian fluids.

Given that VHSV can survive in water outside of a fish host, the transport of water from one water body to another may constitute a risk for the introduction of VHSV. VHS outbreaks have occurred in European fish farms supplied by stream water (Enzmann et al. 1992). VHSV-positive trout in streams may be shedding VHSV, which is transported in stream water to the fish farms. This hypothesis has not been tested (i.e. the concentration of VHSV carried in the stream water has not been measured). Kocan et al. (1997) showed that VHSV concentrations of 100–200 plaque-forming units (PFU) per milliliter in transported water were capable of infecting pathogen-free Pacific herring exposed to those concentrations. However, a single age-0 herring was shown to be capable of shedding 10^6 PFU per hour (Kocan et al. 1997). Therefore, it may be reasonable to conclude that the risk of introducing VHSV to a water body may be orders of magnitude greater via introducing an infected fish versus introducing some quantity of VHSV freely suspended in a volume of water. The dilution of water containing VHSV would presumably lower the risk of establishing VHSV following introduction to a water body. The risk of establishing VHSV in a water body via transporting some volume of water containing VHSV or by introducing an infected fish has not been quantified.



PHOTOS: SUSAN MARCQUENSKI

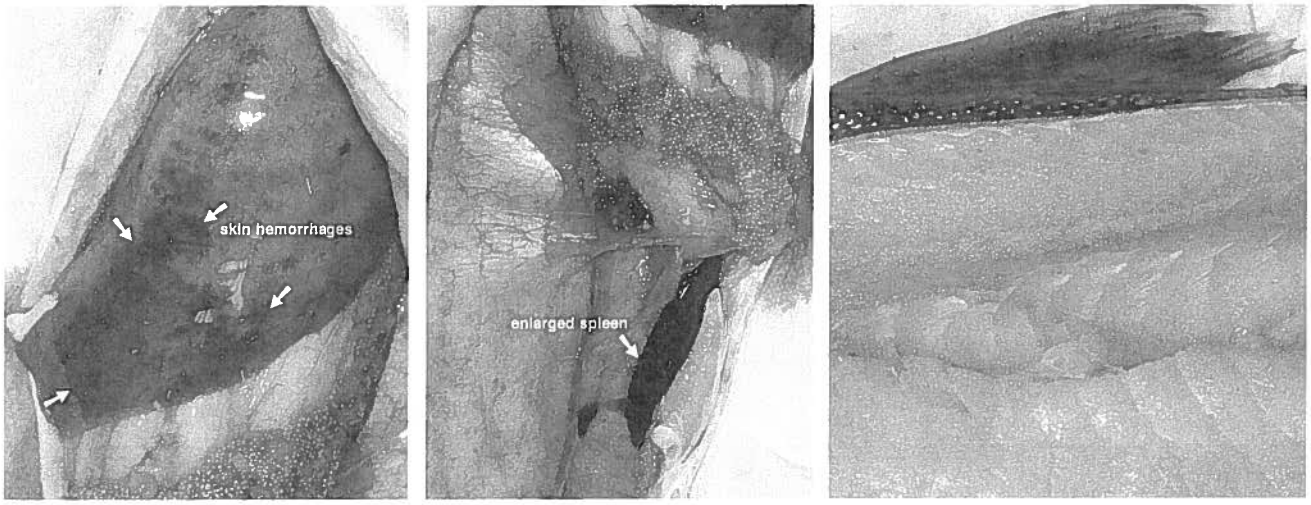
Smallmouth bass from Sturgeon Bay showing enlarged spleen (left), swollen kidney (center), and skin hemorrhages (right).

DIAGNOSIS

OIE (2006), AFS/FHS Blue Book (2007), and other texts on viral disease in fishes describe clinical signs of VHS in fish. Hemorrhaging is a common clinical sign associated with VHS and it may occur internally or externally at the base of fins, eyes, gills, or skin. Other clinical signs of VHS include anemia (pale gills), darkening of skin, distended abdomen (attributable to edema in the peritoneal cavity), exophthalmia (pop-eye), lethargy, and abnormal swimming (e.g., flashing or spiraling). Pop-eye has been the most consistent clinical sign observed in Great Lakes fish (S. Marcquenski, personal communication). Gross pathology signs of VHS include, in addition to internal hemorrhaging, a dark red kidney (or severe necrosis in moribund fish) and a pale or mottled liver. Histopathology signs of VHS include focal necrosis and degeneration in the kidney, liver, and spleen. VHS may also result in high levels of fish mortality. Mortality may occur at any age, but juvenile fish appear to be most susceptible, with mortality rates reaching up to 100% (Meyers and Winton 1995, OIE 2006). Older fish may experience significant mortality as well (25–75%; Meyers and Winton 1995), as is evident in observed fish kills attributable to VHS (e.g., Lumsden et al. 2007). Survivors of VHS can become asymptomatic carriers of the disease, however, showing no clinical signs of infection yet acting as viral reservoirs capable of infecting other fish (Meier et al. 1994).

Clinical signs of VHS as outlined above serve as the standard criteria for presumptive diagnosis of VHS but are insufficient for definitive diagnosis of VHS because many other fish diseases have the same of similar clinical signs (OIE 2006). The presence of VHSV in fish may be confirmed by isolation of the virus in cell culture followed by identification of the virus using antibody-based methods or nucleic acid-based methods. VHSV is readily isolated from samples taken from fish during or shortly after acute infection. VHSV isolation and identification is described in detail by Wolf (1988), LaPatra (1996), OIE (2006), Winton et al. (2007), and others and is summarized below. The absence of VHSV is established when no VHSV is isolated in cell cultures incubated for 28 days.

VHSV is isolated in cell cultures using susceptible fish cell lines recommended by OIE or AFS/FHS. In this procedure, fish cells are grown in monolayers on culture plates. After the monolayer is established, the cells are inoculated with fish tissues processed for virus isolation. Suitable fish tissues include the kidney, heart, encephalon, ovarian fluids (at time of spawning), and spleen; for smaller fish <4 cm, the whole body may be used. Recommended fish cell lines for isolating VHSV type IVb include epithelioma papulosum cyprinid (EPC), fat head minnow (FHM), and bluegill fry (BF-2) (AFS/FHS Blue Book 2007). Cell cultures are incubated at 15°C and pH is maintained between 7.4 to 7.8 (OIE 2006). If VHSV is present in the inoculated fish cells, morphological changes in the host cell may occur. Changes in the cell culture attributable to infection are referred to as a cytopathic effect. Inoculated cells are typically incubated for 14 days. If no cytopathic effect is observed, subcultivation procedures follow using fresh cell cultures and another 14-day incubation period. Following observation of a particular cytopathic effect consistent with rhabdoviruses at any point during the primary or secondary incubation periods, a presumptive positive diagnosis for rhabdovirus is declared and identification of the virus is required. If no cyptopathic effect is observed after 28 days then the test is declared negative for VHSV and other viruses. Some studies have found differing sensitivities among the noted cell



Brown trout from Algoma/Kewaunee area showing liver hemorrhages (left) and enlarged spleen (center) but no muscle hemorrhages (right).

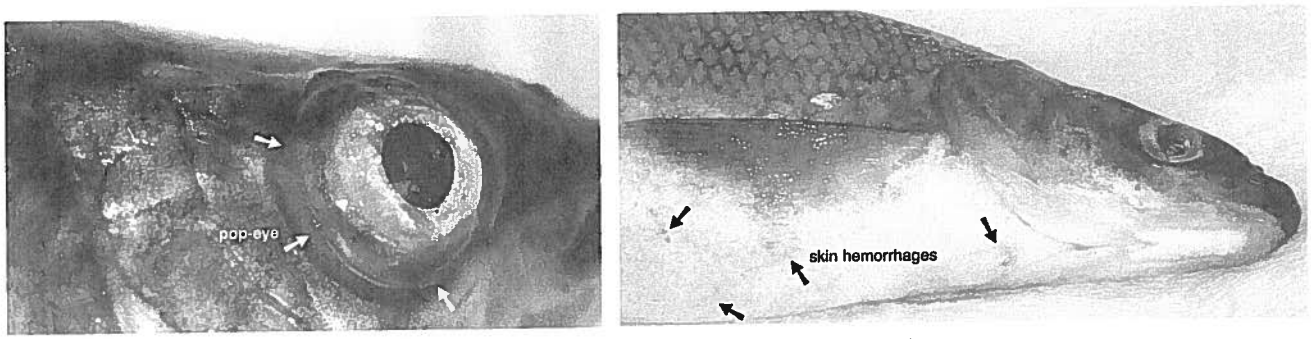
lines in their ability to detect different types of VHSV (Olesen and Jørgensen 1992; Lorenzen et al. 1999). Some researchers recommend the use of multiple cell lines to increase VHSV detection (Traxler et al. 1999).

Viral identification is performed using antibody-based detection methods or molecular techniques. Antibody-based methods include neutralization tests, indirect fluorescent antibody tests (IFAT), and enzyme-linked immunosorbent tests (ELISA). In a neutralization test, VHSV is confirmed by preventing or delaying the cytopathic effect in cell cultures using a virus suspension treated with VHSV-specific antibodies. Molecular techniques include the use of reverse-transcription polymerase chain reaction (RT-PCR), which involves the reverse transcription of viral RNA, to identify VHSV. Multiple versions of neutralization tests, IFAT, ELISA, and RT-PCR are available (LaPatra1996; Chico et al. 2005; OIE 2006; Knüsel et al. 2007).

Antibody-based detection methods and molecular techniques serve as important diagnostic tools during the acute phase of VHSV infection, and new versions of these techniques are in continual development (although few are subjected to systematic validation). Ongoing concerns in the use of antibody-based methods include sensitivity and specificity (Knüsel et al. 2007). For example, cross-reactions between VHSV and IHNV antibodies have been observed when using the ELISA method (Jørgensen et al. 1991). As a result, negative results should be viewed with caution given that there is a presumptive diagnosis of VHS, and OIE (2006) recommends that antibody-based methods not be used to detect VHSV carrier fish. Molecular techniques have, however, been shown to work for both laboratory and field samples for detecting VHSV, with sensitivity similar to the virus isolation and detection approach (Knüsel et al. 2007). The utility of these techniques for surveillance programs requires further evaluation.

Chico et al. (2005) demonstrated a quantitative real-time RT-PCR technique as a tool for detecting and quantifying a European strain of VHSV. They showed how RNA extracted from blood samples from apparently healthy (but experimentally infected) fish could be used to detect VHSV. Such an approach that does not require killing the fish may prove useful in experiments designed to follow the progression of VHS in recently infected fish. Funding was recently awarded by New York Sea Grant to researchers at Cornell University to develop a quantitative RT-PCR for VHSV type IVb. Additional funding was granted by the USDA Critical Issues Fund to support research on the sensitivity of quantitative RT-PCR for detecting VHSV. Once validated, this technique will reduce the amount of time from sampling to lab results from 28 days to at most 2–3 days, resulting in substantial savings in time and labor.

If fish have been exposed to VHSV then they may produce antibodies to VHSV that can be detected by serological methods (Knuesel et al. 2003; Knüsel et al. 2007). OIE (2006) indicates that serological methods are not accepted as a routine diagnostic method for detecting VHSV in fish because of insufficient knowledge of the serology of viral infections in fish and a lack of tested and standardized procedures. However, serological methods have been used in some VHS surveillance programs, and the USDA Critical Issues Fund grant described above for investigating the sensitivity of quantitative RT-PCR will be used to determine if serological testing can diagnose previous exposure to VHSV. Knuesel et al. (2003) used serological data along with viral isolation to document VHS, infectious hematopoietic necrosis (IHN), and infectious pancreatic necrosis (IPN) in salmonids in Switzerland. VHSV antibodies in fish are reported to be detectable about 4 weeks after infection for up to 8 months (Jørgensen 1982a; Enzmann et al. 1992).



Lake whitefish from northern Green Bay showing pop-eye (left) and skin hemorrhages (right). Commercial fishermen's observations led to this discovery.

Surveillance and Detection

OIE (2006), in a manual of diagnostic tests for aquatic animals, outlines surveillance procedures in order for a region, water body, or aquaculture facility to be recognized as free of an infectious disease. Surveillance data (both random and nonrandom), biosecurity measures, knowledge of disease agent biology, and other sources of evidence may be used to demonstrate freedom from infection. However, evidence supporting a hypothesis that VHSV is not present is logically insufficient to confirm that hypothesis (Williams et al. 2002). In other words, a detection of VHSV can refute the hypothesis that VHSV is not present and failure to find VHSV can support (but not prove) that VHSV is not present. Therefore, statistical methodologies are required to evaluate confidence in the efficacy of any surveillance program.

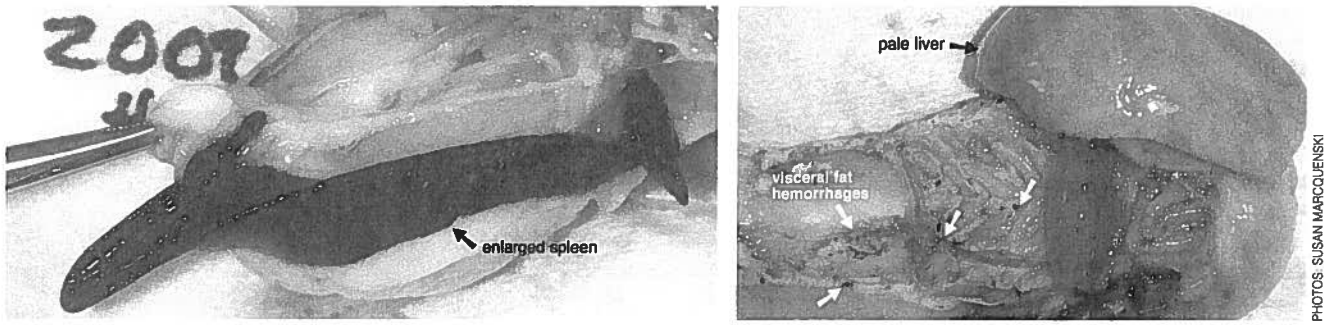
States and provinces in the Great Lakes area have implemented some forms of surveillance to begin to evaluate the prevalence of VHSV in state or provincial water bodies (e.g., WDNR 2007d; CFIA 2008). The Bilateral VHSV Surveillance Working Group (2007) has put together a working draft of a surveillance proposal for Canada and the United States which is pending approval by the U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, and the Canadian Food Inspection Agency. The National Park Service and Grand Portage Band of Lake Superior Chippewa have also put together a surveillance program for a portion of the Lake Superior basin (NPS/GPBLSC 2008).

The Wisconsin DNR currently has a VHS surveillance program that includes three components (T. Simonsen, Wisconsin DNR, personal communication):

1. A random selection of 25–30 water bodies outside the Lake Michigan basin are sampled annually. Additional water bodies identified as "high risk" are also sampled when these water bodies appear in the sampling rotation. "High risk" waters include those in the Lake Michigan basin and in proximity to the Winnebago system and waters that have been invaded by zebra mussels *(Dreissena polymorpha)*. At least 170 individual fish (in multiples of 5 from 3 or 4 species) are tested from each water body. Species in order of priority include freshwater drum, gizzard shad, yellow perch, black crappie, bluegill, emerald shiner, spottail shiner, shorthead redhorse, smallmouth bass, and walleye. Muskellunge may also be included if they exhibit clinical signs of disease infection.
2. Fish obtained from fish kills are tested for VHS. Fish turned in by anglers and exhibiting clinical signs of disease infection are also tested.
3. Wild fish used as broodstock (e.g., brook trout and brown trout in Wisconsin's wild trout stocking program) and wild fish from water bodies that serve as a source of water for hatcheries are tested for VHS.

Sampling ideally occurs when water temperatures are <60°F, and sampled fish are necropsied within 24 hours of capture.

OIE (2006) indicates that the required level of confidence in an infectious disease surveillance system must be greater than or equal to 95%. This requirement in turn will require certain levels of sampling that are sufficient to detect the presence of VHSV when the virus is present in a fish population. Typically, 60 fish are checked for disease presence in Wisconsin DNR fish health surveys. The utility of such a sample size for detecting disease in a population will depend on meeting the assumption that the sample is representative of the population of interest. If, for example, disease-positive fish are less likely to be captured in a survey, then the survey may be biased towards not detecting disease presence when the disease is in fact present in the population. We are unaware of any studies that suggest VHSV-positive fish are more or less likely to be captured than VHSV-negative fish. The utility of a particular sample size will also depend on the prevalence of the disease in the fish population. Kocan et al. (2001b) observed a prevalence of VHSV in Pacific herring of less than 1% and suggested that hundreds of fish would have to be sampled to encounter a single infected fish in populations with low disease prevalence.



Lake whitefish from northern Green Bay showing enlarged spleen (left), hemorrhages in the visceral fat and pale liver (right).

Effects of VHS on Individual Fish

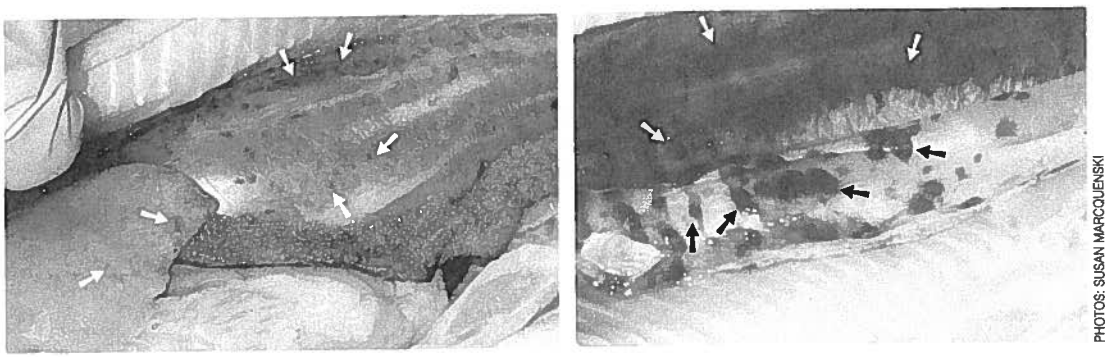
The effects of VHS on individual fish are well documented. As outlined above, VHSV infection can result in hemorrhaging and other acute physical effects (OIE 2006; AFS/FHS Blue Book 2007). Hemorrhaging occurs when endothelial cells of the blood vessel walls become infected with VHSV and weaken, allowing blood to leak from the vessels into tissues. If the kidney is infected and fails to function properly for osmoregulation, edma or swelling will occur, possibly accompanied by hemorrhaging. Fish infected with VHSV may develop antibodies to VHSV, and the detection of VHSV antibodies may be used as evidence of previous infection. Fish that are infected by VHSV may also become asymptomatic carriers and producers of VHSV. VHS may lead to mortality in fish as a direct result of infection. Infected fish may also be more susceptible to predation or unable to effectively compete for resources, thereby resulting in indirect disease-related mortality. If a fish dies before it has reproduced, then that fish will not contribute to recruitment. This may be a particular problem for spring spawning fish susceptible to VHSV.

The condition of individual fish may be a significant factor in determining the effect VHSV may have on those fish. It is well established that stress can lead to weakened immune responses to pathogens in vertebrates. Environmental factors that cause stress in fish may lead to disruptions in the fish endocrine system, resulting in suppression of the immune system and a decrease in the ability of the fish to fight infection (Anderson 1990; Rottmann et al. 1992). Fish employ a complex immune system similar to that found in other vertebrates. This system includes pathogen surveillance and antigen uptake by macrophages, transportation of antigens to the kidney and spleen by monocytes, and target destruction and antibody production by lymphocytes (Anderson 1990).

Seasonal stressors such as change in water temperature and spawning may be important factors in VHS outbreaks in fish. VHS outbreaks tend to occur in spring (Table 1) when water temperatures are typically less than 15°C (Arkush et al. 2006). Many freshwater fish species that have been involved in VHS outbreaks also spawn at this time of year. Spawning is a stressful activity that may result in natural mortality in some individuals and present an opportunity for viral disease outbreak in fish with weakened immune systems. Stress associated with fish capture, nutritional deficiency, and other disease may also contribute to VHS outbreaks (Meyers et al. 1994).

Age also is an important factor in the susceptibility of fish to viral disease (Wolf 1988). Hershberger et al. (1999) have shown that the prevalence and severity of VHS in Pacific herring decreases as fish age increases. However, Kocan et al. (1997) observed a similar prevalence and severity of VHS in specific pathogen-free juvenile Pacific herring subjected to water immersion exposure to VHSV at ages 5, 9, and 13 months. Therefore, prior exposure to VHSV may be a factor in resistance or immunity to VHS in older fish (Hershberger et al. 1999; Kocan et al. 2001b; Hershberger et al. 2007). Knuesel et al. (2003) compared the prevalence of VHSV in fish younger than 1 year versus fish older than 1 year. The prevalence of VHSV in fish younger than 1 year for samples including VHSV-positive fish was as follows: 13% of rainbow trout, 20% of Arctic char, 33% of grayling, 81% of brown trout, 86% of northern pike, and 100% of coregonid species. However, Knuesel et al. (2003) note that these percentages are confounded by the age at which many of the fish species are stocked (primarily as fingerlings but also at older ages for rainbow trout). Controlled studies or observations of VHSV outbreaks in naive (VHSV-free) populations may further elucidate issues concerning fish age and susceptibility to VHS.

Stress associated with confinement has also been shown to be a causative factor in VHS outbreaks in fish. Hershberger et al. (1999) showed that the confinement of Pacific herring in impoundments in the spawn-on-kelp fishery was correlated with increased prevalence of VHSV. Prolonged crowding increased stress in herring, resulting in increased susceptibility to VHSV infection (VHSV was present in and originated from some impounded fish). Kocan et al. (2001b) detected VHSV in less than 1% of wild-caught Pacific herring. But after confinement in a laboratory setting, VHSV prevalence increased up to 100% in herring held 14 days. Pacific sand lances captured with the herring also began to show signs of VHS after being held 3–7 days in confinement. Sand lances captured separately from herring and held in isolation from herring did not develop VHS. Sand lances in the wild were likely exposed to VHSV by herring, but it is unknown how herring were initially exposed to VHSV.



PHOTOS: SUSAN MARCQUENSKI

Lake whitefish from northern Green Bay showing swim bladder, liver, and kidney hemorrhages.

Acquired Immunity

There is some evidence for acquired immunity to VHSV. Brown trout in Europe have shown a protective immune response to VHSV following natural infection (de Kinkelin et al. 1977). Enzmann et al. (1992) documented the course of VHSV antibody development and disappearance in several distinct populations of brown trout for four years in a German stream (fish movement was completely obstructed at a few locations in the stream). This study used serological methods to detect VHSV antibodies but did not use virus isolation methods to detect VHSV. VHSV antibodies were prevalent in sampled brown trout for three consecutive years but were almost eliminated by year four. In one brown trout population, VHSV antibodies in consecutive annual surveys were found in 41%, 67%, 33%, and 0% of sampled trout. Enzmann et al (1992) concluded that VHSV infection was prevalent until a large proportion of the brown trout population became immune, after which VHSV tended to disappear.

Knuesel et al. (2003), in a survey of viral diseases in farmed and wild salmonids in Switzerland, showed that VHSV was widespread but the number of VHSV-positive fish samples had generally decreased from 35 per year in 1978 to less than 10 per year from 1993 to 2001. Viral isolation showed a small number of sites with VHSV-positive samples (8 sites in 1984-85 and 4 sites in 2000-01). Serological tests in 2000-01, however, showed a widespread distribution of fish with VHSV antibodies: 39% of streams, 41% of private fish farms, and 18% of government fish farms. VHSV antibodies were found in 13% of rainbow trout and 5% of brown trout. Discrepancies in the estimated prevalence of VHSV may have been attributable to time of survey in relation to water temperature, sensitivity of the virus isolation method, and the serological method (Knuesel et al. 2003).

Hershberger et al. (1999) and Kocan et al. (2001b), as described above, suggest that prior exposure to VHSV may be a factor in resistance or immunity to VHS in Pacific herring. Kocan et al. (2001b) demonstrated the development of resistance to VHSV in Pacific herring. However, they were unable to confirm resistance by detecting an increase in VHSV antibodies in sera. Herring serum was either toxic to EPC cells or did not allow for the detection of antibodies, depending on the dilution used. Low (currently undetectable) antibody concentrations, however, are adequate to protect fish from rhabdovirus infections (LaPatra 1996).

Disease Challenge Tests

Laboratory studies of VHSV infection may involve challenging naive fish with exposure to the virus under controlled settings. Such disease challenge tests can be used to evaluate the ability of a fish to resist a specific pathogen (Anderson 1990). Pathogens may be injected into fish or introduced to fish by immersion in water containing the pathogen. Pathogen injection into fish is the most direct way to establish infection and create fish that shed virus, ideally at a known measureable rate. Immersion of naive fish in water containing a known amount of virus or virus-shedding fish allows for experimentation with a more natural route of viral exposure to the naive fish. However, the laboratory confinement of fish is inherently unnatural and may be stressful, and therefore may facilitate the transmission and severity of VHSV (Hershberger et al. 1999; Kocan et al. 2001b). The development of a standard challenge method is an important prerequisite to further laboratory studies of VHSV pathogenesis under different environmental conditions.

Much of the research on the effect of VHSV on freshwater fish species has been conducted in controlled laboratory settings. However, these studies have typically involved VHSV types other than type IVb and their results may not be directly applicable to this type. Research grants have recently been awarded to develop laboratory challenge models for channel catfish and rainbow trout exposed to VHSV type IVb (USDA Critical Issues Fund grant awarded to researchers at Cornell University). An additional grant from the USDA Western Regional Aquaculture Center was awarded to the Western

Fisheries Research Center and Oregon State University to challenge yellow perch, rainbow trout, chinook salmon, and Pacific herring to VHSV types I, IVa, and IVb.

Dorson et al. (1991) challenged salmonid species in laboratory experiments to determine the susceptibility of salmonids and their triploid hybrids to infection by VHSV types I and III (as well as IPNV and IHNV). They found that brook trout and the brook trout × rainbow trout hybrid were resistant to VHSV types I and III (and IHNV but not IPNV), with observed mortality rates of 4–7%. Rainbow trout, however, experienced VHSV mortality rates of 68–93%. Lake trout and the lake trout × rainbow trout hybrid were both susceptible to VHSV, with lake trout showing clinical signs of infection and significant losses. Brown trout were resistant to VHSV type I (no mortality) but not VHSV type III (56% mortality).

Follett et al. (1997) challenged eight Alaskan salmonid species to VHSV type IVa (as well as IHNV) in laboratory experiments. Water immersion challenges were conducted with viral concentrations measured in plaque-forming units per milliliter (PFU/mL^{-1}) for a set timed exposure (1 hour). Rainbow trout was the only salmonid in the study to show clinical signs of VHS and experience VHS-induced mortality (12%), which occurred at an exposure concentration of 10^5 PFU/mL^{-1} (as opposed to 10^3 PFU/mL^{-1}). The authors concluded that VHSV type IVa was of low virulence towards Pacific salmonids. Meyers and Winton (1995) express a similar conclusion of low virulence of VHSV type IVa for Pacific salmonids. Similar challenge studies for VHSV type IVb have yet to be conducted.

VHSV type IVa has been observed to be enzootic in Pacific herring (Meyers and Winton 1995). Kocan et al. (1997) conducted laboratory challenge experiments to establish the causal relationship between VHSV type IVa and VHS in Pacific herring. Specific pathogen-free Pacific herring were developed and challenged to known quantities of VHSV ranging from $10^{1.5}$ to $10^{6.5}$ PFU/mL^{-1}. Challenge tests were also used to determine whether VHSV-negative herring were immune herring that had cleared the virus versus naive herring that had never been exposed to the virus (Kocan et al. 2001b). Naive herring became infected, demonstrating that the VHSV-negative herring were immune carriers of VHSV. Hershberger et al. (2007) also utilized VHSV challenge studies, in which developed laboratory challenge methods were used to study effects of VHSV on different developmental stages in Pacific herring. Fish were exposed to VHSV quantities of 1.2-5.6 × 10^4 PFU/mL^{-1}. Larval herring were susceptible to VHS with increasing cumulative mortality as the fish aged. The study also demonstrated that larval exposure to VHSV did lead to partial protection in juveniles that had survived exposure to VHSV versus naive juveniles with no prior exposure to VHSV.

Disease Dynamics in Fish Populations

Disease is a biotic factor that can potentially effect changes in fish populations. Population size may increase or decrease as a result of changes in any of four primary processes that drive population dynamics. Populations may increase as a result of reproduction and immigration, and populations may decrease as a result of mortality and emigration. Any influence of disease on population size must occur through one of these four processes (Williams et al. 2002).

Population models can provide a framework for understanding how a biotic factor such as disease influences fish population dynamics. Model structure can define the disease cycle in a fish population, providing a rigorous accounting of interactions between the virus and its host. Models of disease-host interactions can provide insight on the epidemiological process, including the course of viral infection in the host species and the dynamics of virus transmission in the host population (Oli et al. 2006). Mathematical modeling of disease population dynamics has been used extensively for infectious disease in general (Anderson and May 1979; Anderson 1994; Kakehashi 1996), but less so for infectious disease in fish (Reno 1998; Ogut and Bishop 2007).

The susceptible-infected-removed (SIR) model provides a general framework for considering how disease affects a population (Anderson and May 1979; Reno 1998; Oli et al. 2006). A population of fish N at time t can be defined as:

$$N_t = S_t + I_t + R_t$$

where the population is divided into three groups including fish susceptible to infection (S), infected fish (I), and fish removed from the population (R) because they are either dead or immune and thus no longer susceptible to infection (i.e. no longer part of the population of fish infected or potentially infected). The SIR model can be extended to a susceptible-latent-infected-removed (SLIR) model by separating latently infectious fish (L) from infectious fish (I):

$$N_t = S_t + L_t + I_t + R_t.$$

Thus VHSV-carrier fish could be explicitly modeled. The challenge is then to determine how the pathogen, in this case VHSV, affects the allocation of individuals in the population among the three or four groups. Important factors influencing infection dynamics include the transmission rate of VHSV from host to susceptible fish, the reproductive rate of the virus, the duration of infection (latency and infectious periods), fish population density, natural and disease-related mortality rates, and the development of immunity to infection. Understanding key features in how such factors influence disease dynamics may help in developing control strategies for managing fish diseases (Ogut and Bishop 2007).

The primary factor necessary for an epidemic outbreak of disease in a fish population is contact between infectious and susceptible fish and the disease transmission

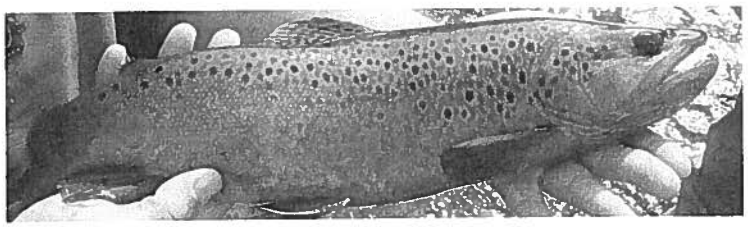
coefficient (often referred to as β). The transmission coefficient is defined as the rate at which susceptible fish become infected (Reno 1998; Ogut and Bishop 2007). A virus that is highly infectious will have a high transmission coefficient; a weak pathogen will have a low transmission coefficient. The value of the transmission coefficient depends on factors relating to the host fish, the pathogen, and the environment in which they interact. Stochastic processes also play an important role in disease transmission (Oli et al. 2006). Host fish factors affecting the transmission coefficient include species, age, and natural and induced immunity; pathogen factors include the ability to infect a particular species and virulence of the pathogen; and environmental factors include population density, temperature, and water flow and chemistry (Wolf 1988; Reno 1998).

Over the long term, the effect of a disease on a fish population will depend on the transmission coefficient and the fish population growth rate. If the transmission coefficient is greater than natural mortality then the pathogen will be persistent and enzootic with no population-level effect; if the transmission coefficient is greater than recruitment and disease mortality then the pathogen will reduce population size (Reno 1998). We are not aware of any published values of a transmission coefficient for VHSV type IVb. Therefore, the ability of VHSV type IVb to regulate a fish population over time is unknown.

Fish population density plays an important role in disease transmission. In general, a greater population density will lead to a greater probability of fish coming in contact with one another. However, here again the transmission coefficient β plays an important role in how population density interacts with the spread of disease. A larger transmission coefficient (i.e. a more virulent pathogen) will allow a disease to become established in smaller populations (Reno 1998). Fish behavior is also an important factor related to density and disease transmission. Territorial fish may have fewer contacts with conspecifics as compared to fish that exhibit schooling behavior. A given fish species may also exhibit ontogenetic shifts in behavior whereby younger fish are more likely to be in contact with one another than are older fish. Seasonal behavior may also play an important role in disease transmission. Many fish species tend to congregate to spawn in relatively confined areas, thereby increasing the likelihood of disease transmission should infectious fish be present. A longer infectious period for a virus would further decrease the critical population size for disease outbreak (Hudson et al. 2008). We are not aware of any published values for the duration of infectiousness for VHSV type IVb.

The basic reproductive rate of a pathogen is also an important factor in understanding the dynamics of the pathogen in a fish population (Reno 1998). The basic reproductive rate R_0 is defined as the rate of successful transmission of the pathogen from a host fish to a population of susceptible fish, and R_0 can be thought of as a measure of the likelihood of invasion (Hudson et al. 2008). R_0 is derived from the transmission coefficient, population size, and duration of infectiousness (Reno 1998; Hudson et al. 2008). R_0 can be increased, for example, by increasing the size of the host population. If R_0 for a virus is less than 1 then the virus will not initiate an epidemic; the virus and the disease may be eliminated from the population because it is unable to infect a sufficient number of hosts to become established in the population. If R_0 is greater than 1 then the virus may initiate an epidemic and its severity will depend on the magnitude of R_0. If R_0 is much greater than 1, the virus may infect most or all of the fish in a population and thereby eliminate itself from the population because there are no new host fish to infect. If R_0 is slightly greater than 1, the virus will persist in the fish population with a low prevalence of infected fish—a sufficient number of host fish become infected for the disease to become established, but not so many host fish such that the susceptible population becomes depleted. For example, VHSV type IVa is enzootic in Pacific herring with VHSV detected in less than 1% of free-ranging herring in a study by Kocan et al. (2001b). VHSV appears to be persistent in this population, with periodic outbreaks that affect recruitment but do not deplete the population of susceptible herring.

A practical approach to using models for understanding the dynamics of VHS in a given water body may involve the development of a SIR-type model for a fish species of interest (Oli et al. 2006). However, given that VHSV type IVb can infect multiple fish species (Table 1; APHIS 2007), the recognition that there may be multiple reservoirs of VHSV among fish species and trophic levels in any given water body will be an important consideration in any disease management strategy. Models provide a useful framework for understanding disease dynamics in fish populations and fish communities, help guide data collection and research in order to estimate critical variables in disease dynamics, and help investigate how disease management programs may influence disease dynamics in fish populations and communities.



MATTHEW MITRO

Brown trout, a VHS-susceptible species, has been found VHS-positive in Lake Michigan but not in Wisconsin inland streams where this one was captured.

Effects Of VHS On Fish Populations And Communities

Given that VHS affects individual fish, it follows that individual responses to VHSV effect changes in the dynamics of fish populations and fish communities (Wootton 1991). VHS can result in the direct or indirect mortality of individual fish as described above and mortality events attributable to VHSV type IVb have been observed in fish populations in the Great Lakes region (Table 1). Comprehensive models of VHS dynamics in fish populations have not yet been developed, but one study used models to estimate freshwater drum mortality following a fish kill in Lake Ontario (Lumsden et al. 2007) and another study described how VHS effected changes in Pacific herring recruitment and population abundance (Marty et al. 2003). Other studies have also reported population-level observations of the effects of VHS on different fish species and proposed hypotheses on VHS dynamics in fish populations as described below.

Effects of VHS on fish in the Great Lakes system have ranged from detections of VHSV or observations of individual mortalities, to large-scale fish kills measured in the thousands (Table 1). The extent to which these observed fish kills have influenced population dynamics is unknown. The largest fish kills have affected freshwater drum, gizzard shad, and round gobies. Whelan (2007b) reported that observations of fish kills have been one-time events that have not been repeated. Others have noted ongoing fish kills, particularly in New York waters (S. Marcquenski, personal communication). However, given that the first fish kills in the Great Lakes were observed in 2005, it is premature to draw conclusions about the long-term dynamics of VHS-related fish kills in these lakes and rivers.

Much attention in regard to population-level effects of VHS has been focused on Pacific herring. Marty et al. (2003) describe a six-year comprehensive epidemiological study of the Pacific herring population of Prince William Sound, Alaska. Their conclusion was that disease significantly affected recruitment and population abundance in Pacific herring. The authors used an age-structured stock assessment model modified with a disease index to estimate mortality. The disease index combined the prevalence of VHSV and ulcers in herring; prevalence of another pathogen, *Ichthyophonus hoferi*, changed with fish age but was not related to changes in fish abundance. Two important risk factors in the outbreak of disease in the herring population were poor body condition and abundant recruitment to the spawning population before spawning in spring. Outbreaks of VHS were followed by decreases in abundance, after which VHSV was no longer detected. The VHS-induced changes in abundance resulted in lower recruitment. The two lowest recruitment years (1994 and 1999) followed years with increased natural mortality in adult herring (1993 and 1998).

The link between disease and population dynamics observed in the Prince William Sound Pacific herring stock has also been suggested for other herring stocks, notably the Puget Sound herring stock (Kocan et al. 2001b). Kocan et al. (2001b) conducted laboratory tests that suggested up to 50% of a herring school in Puget Sound may have died because of VHSV, either alone or in combination with other environmental factors. This level of mortality could affect recruitment success of age-0 herring and year-class strength.

Changes in the abundance of one fish species in response to VHSV infection may alter interactions within the fish community. Fish communities are groups of fishes that directly or indirectly interact. We are not aware of any published studies that explicitly consider the effects of VHSV at the community level, but the field of infectious disease ecology, which considers the effects of disease at higher levels of organization, is receiving increasing attention (Ostfeld et al. 2008).

An example of an infectious disease altering community structure is provided in a recent study by LaDeau et al. (2007) on West Nile virus and birds in North America. The authors of this study used 26 years of Breeding Bird Survey data to investigate the effects of West Nile virus on 20 bird host species. The West Nile virus has led to changes in abundance in bird host populations—significant changes occurred in populations of seven bird species—and these changes in abundance have led to shifts in community composition and ecosystem functioning. The authors draw attention to the challenges of distinguishing disease impacts from other factors that influence population dynamics, identifying the need for abundance data both before and after disease introduction.

Many emerging diseases follow a pattern in which the disease may be held in one or more host reservoirs from which the disease is periodically transmitted to other susceptible hosts, possibly leading to disease epidemic (Hudson et al. 2008). This pattern may be expected to occur in fish communities exposed to VHSV. Many studies on Pacific herring and other fishes in Pacific marine fish communities have detected VHSV in multiple fish species and acknowledged that many species may act as reservoirs for VHSV (Meyers and Winton 1995; Follett et al. 1997; Meyers et al. 1999; Kocan et al. 2001b; Hedrick et al. 2003). Likewise VHSV has been detected in many Great Lakes fish species, which may serve as reservoirs to maintain the virus for future outbreaks (Elsayed et al. 2006). Additional research is needed to better understand how fish community composition influences the dynamics of VHSV and VHS outbreaks.

Risk Analysis for Infectious Disease in Fish Populations

Risk analysis is a formal discipline that attempts to quantify or qualitatively describe the potential negative consequences of some undesirable event and present management options for confronting the identified risk. MacDiarmid and Pharo (2003) provide an overview of the concepts of risk analysis for application in the animal health field. The steps in risk analysis include hazard identification, risk assessment, risk management, and risk communication.

Hazard identification is the first step in a risk analysis. Here the hazard identification is the recognition that the invasion of a water body by VHSV, where the water body is currently known or thought to be free of VHSV, poses a risk to the health of fish in the water body.

The risk assessment step may consist of the following steps: release assessment, exposure assessment, consequence assessment, and risk estimation. A risk assessment may be qualitative or quantitative. Both approaches may be valid, and every risk assessment is at first a qualitative exercise. It is cautioned that quantitative risk assessments are used to gain insights and not be mistakenly relied upon as providing unsubstantiated precision. Bartholomew et al. (2005) explicitly consider the risk assessment step and present a risk assessment based approach for managing infectious diseases in fish populations. They introduce and apply these concepts of risk assessment to the management of whirling disease in salmonid populations.

Risk management is the process in which management strategies are implemented to reduce risks identified and evaluated in the hazard identification and risk assessment steps. For VHSV or other infectious fish diseases, risk management may involve the implementation of disease control measures.

Risk communication is an interactive process in which information and opinions on hazards and risks are exchanged among groups. This process also involves the exchange of information generated in risk assessments and of proposed risk management measures. For VHSV or other infectious fish diseases, risk communication may involve the complex dialogs between natural resource agencies and constituents or dialogs within or among research and management agencies.

We can consider a hypothetical risk assessment about how VHSV might affect fish populations in Wisconsin waters to illustrate the risk assessment steps outlined by Bartholomew et al (2005). Questions addressed by these assessment steps include the risks that VHSV will enter an aquatic ecosystem, that VHSV will become established and proliferate, and that VHSV will manifest itself as a disease in the fish population. Following is an illustration of how a risk assessment approach might be applied to VHSV.

In a release assessment we consider the pathways to the introduction of VHSV to a water body. A primary vector for VHSV is through the transfer of live infected fish (OIE 2006; NPS/GPBLSC 2008). This can occur by way of fish stocking, fish transfers, use of live baitfish, or natural movement or dispersal of fish. The risk of any anthropogenic introduction of VHSV by way of live fish would depend on the species involved (whether or not it is a likely carrier of the virus) and the source (contaminated population or contaminated facility). The natural movement of VHSV-infected fish would depend on the proximity and connectivity of the VHSV-host population and the target population. Barriers to movement, such as artificial dams and natural waterfalls, may act to isolate and protect populations from the introduction of VHSV by way of natural fish movement. Birds may also act as a vector for VHSV. VHSV has been found to survive in regurgitated food in herons (Peters and Neukirch 1986), but birds are considered a low-risk vector for VHSV transmission (NPS/GPBLSC 2008). VHS can also be transferred by contaminated water.

The Wisconsin DNR has implemented biosecurity protocols in the state's hatchery system to address the threat of VHSV transmission. Given the previously known threat of VHSV to trout in propagation facilities experienced in Europe, steps have been in place to monitor for VHSV. For example, all trout populations used in the wild trout stocking program have been routinely (annually) checked for VHSV as well as other infectious diseases. To date, Ash Creek and South Fork of the Hay River brook trout and Timber Coulee Creek brown trout have tested negative for VHSV.

Another pathway to the introduction of VHSV to any water body is angler activity. The primary risk involves the transfer of live or dead fish, the use of live baitfish, and the transfer of water containing virus (e.g., in a live well). Wisconsin trout streams tend to be fished by wading or from the shore and not by boat, so the transfer of infected baitfish or contaminated water by boat to a trout stream might be considered a low risk. Muskellunge and walleye lakes, however, tend to be fished by boat, and the risk of VHSV transmission to such lakes might be considered high. A potential pathway for VHSV transmission in streams as well as lakes might involve harvesting an infected fish in one water body and cleaning it in another.

In an exposure assessment we consider the pathway to exposing a fish to a pathogen given that the pathogen has been introduced to the water body. The establishment and proliferation of VHSV would depend on the frequency of the introduction, the size of the introduction, the susceptibility of the fish to VHSV infection, and the exposure environment. A large stocking of infected fish might pose a high risk scenario. An isolated transfer of baitfish by an angler might pose a low risk scenario, but the collective

behavior of the angler population as a whole might pose a high risk scenario. Available data on the exposure environment for VHSV infection might suggest trout streams are at high risk. VHS outbreaks tend to occur at water temperatures between 4 and 14°C (OIE 2006). Other water bodies with more variable seasonal temperature profiles might suggest a variable risk profile. Disease outbreaks often correlate with environmental stressors such as decreasing water temperature, water pollution, or high stocking densities. The proximity of fish to one another may be an important risk factor. Risk of exposure may be greater when the density of fish in a given location is higher and an infected fish is present. Likewise, fish behavior may affect risk of VHSV transmission. For example, VHS may be transmitted in body fluids during spawning (OIE 2006). Lotic versus lentic water environments may present different risks to VHSV transmission. These are some of the many questions that should be addressed in an exposure assessment, and further research may be needed to adequately address these questions.

In a consequence assessment we consider whether a disease becomes established and what it means for the fish population. We know, for example, that all trout species in Wisconsin are susceptible to VHSV infection (but the relative degree of susceptibility is unknown). We know that all sizes and ages of susceptible fish species can become infected, and some research suggests younger age classes are more susceptible to VHSV infection (Meyers and Winton 1995; Knuesel et al. 2003; OIE 2006). We know that VHSV type I has become enzootic in European streams (Knuesel et al. 2003). There are examples of the incidence of VHS in European trout streams decreasing over time, but survivors of infection can become lifelong carriers that shed virus with urine and sex products (Enzmann et al. 1992). Eradication of VHSV may be unattainable, but the long-term dynamics of the disease and how it affects the ecology of fish populations, assemblages, and communities is unknown.

The release, exposure, and consequence assessments would be followed by risk estimation, which is an integration of the three assessment steps. Risk estimation may involve the development of a scenario tree (MacDiarmid and Pharo 2003). An example of risk estimation is a VHS prevention and response plan put together by the National Park Service and the Grand Portage Band of Lake Superior Chippewa (NPS/GPBLSC 2008). This document includes a table that provides an estimation of the risk associated with different activities that might affect the transport or introduction of VHSV. Risks are presented on a scale from low to high.

VHS Management and Control

Reno (1998) identifies four types of conceptually-possible disease intervention strategies: culling, vaccination, chemotherapy, and preventing the spread of disease to new water bodies. Not all approaches are practical for wild populations, but each is directed at reducing the reproductive rate R_0 of the virus to less than 1, a necessary requirement in eliminating the virus from any population. Preventing the introduction of VHSV to any new water body is clearly a primary goal of fisheries management, and biosecurity protocols have been implemented by federal, state, and provincial agencies to reduce the probability of spreading VHSV. Additional information on the relative risks of spreading VHSV by different activities would help guide management actions.

Research on vaccination options for managing VHSV have yet to yield any practical options for managing VHSV in wild fish populations. Intramuscular injections of a recombinant vaccine were successful at protecting rainbow trout from VHSV but application of the vaccine by immersion was unsuccessful (Lecocq-Xhonneux et al. 1994). Intramuscular injections of a DNA vaccine have also successfully protected rainbow trout from VHSV but a suitable delivery strategy for mass vaccination of fish has yet to be developed (Lorenzen et al. 1998 and 2000; Lorenzen and LaPatra 2005).

Novel applications of ecological theory may lead to new insights into disease prevention and control. Dennehy et al. (2007) discuss the use of ecological traps for eliminating virus populations. An ecological trap is a sink habitat that is perceived as a source habitat. A virus chooses host cells based on the presence of viral receptors on the host cell membrane; if the cell is unsuitable for viral replication, then the cell is functionally a habitat sink that removes the virus from the population. An increase in such sink habitats can reduce population viability by reducing the reproductive rate R_0 of a virus. This novel link between source-sink theory and the trap cell concept is demonstrated in a model showing how the use of ecological traps can lead to reduced viral fitness.

Conclusions

The Wisconsin DNR and other state fisheries management agencies have long been concerned about the threat the infectious fish disease VHS would pose to state fisheries. VHSV was first documented in North America in marine coastal fisheries in 1988. The earlier European experience showed how VHSV was particularly lethal to rainbow trout in fish farms. The coldwater fish propagation program in Wisconsin has routinely tested broodstock for VHSV and preemptively treated fish eggs with iodophor, an iodine compound, to eliminate virus transmission on

the surface of an egg should the virus be present. Now that VHSV has been documented in Wisconsin waters, the concerns previously limited to coldwater fish propagation have extended to state fisheries management as a whole.

Much of what we know about the VHS virus and disease has come from research conducted on VHSV types I-III found in Europe and type IVa found in North America. The new variant VHSV type IVb found in the Great Lakes region presents new challenges to fisheries management and propagation programs. Past experiences with other types of VHSV have indicated what we might expect with VHSV type IVb and how we might approach it. We know how VHSV may enter a water body and be transmitted among fish. We know how to diagnose clinical signs of VHS and how to isolate and identify VHSV in cell culture. We know the effects VHSV may have on some fish species. We know less about VHS disease dynamics in fish populations and the effects of VHSV on fish at the population and community levels. And we are actively learning to what extent this new variant of VHSV is similar to or different from other known variants.

Research Recommendations

VHSV type IVb is an emerging pathogen in North America and much remains to be discovered about the dynamics of VHS, how we can manage fish populations exposed to VHSV, and how we can reduce the risks of VHSV exposure to other fish populations. Following is a subjective list of research recommendations concerning VHSV type IVb. It is subjective in that these recommendations are based on the authors' interpretation of the state of the science of VHS as outlined above and are made in the context of practically supporting the fisheries management obligations of the Wisconsin DNR. We recommend:

1. Conducting a formal risk analysis on VHSV type IVb and its potential impacts on fish populations in Wisconsin. A risk analysis includes hazard identification, risk assessment, risk management, and risk communication. A risk assessment is a key component of a risk analysis and includes explicit assessments of the release, exposure, and consequences of VHSV and an estimation of risk. Such a risk assessment may serve as a guide for conceiving, selecting, and implementing management actions to prevent or respond to VHS in Wisconsin waters, while balancing the risks VHSV may pose to fisheries with the obligations for protecting and providing public fisheries.

2. Contributing to the development of rapid testing procedures for detecting VHSV when present and for declaring groups of fish free of VHSV when the virus is not present. Current testing procedures require a waiting time of 28 days to declare a group of fish VHSV-negative. This waiting period has resulted in fundamental changes to the state's fish propagation program, including fry stocking, wild fish transfers, and the wild trout propagation program. Any significant advances in VHSV testing will have practical applications to the fish propagation and management programs.

3. Contributing to the development of VHS challenge models, particularly for commonly used baitfish species. VHS challenge models are currently being developed for game species such as channel catfish, rainbow trout, and yellow perch. Challenge models can be used in controlled laboratory settings to study VHSV pathogenesis under different environmental conditions and may provide insights into fundamental questions on VHSV exposure, transmission, and effects on individual fish. We recommend a challenge model using a baitfish species because (1) the use of baitfish by anglers is a common and relatively uncontrolled activity (notwithstanding current regulations regarding the sale and use of baitfish) that may result in live fish being released and (2) releasing an infected fish into a VHSV-free water body presents a high risk of spreading VHSV.

4. Initiating a long-term monitoring study on the effects of VHSV on fish populations and fish communities in a selection of VHSV-positive waters. There is a paucity of information on population- and community-level effects of VHSV, yet such information is of critical importance to the management of fish populations. The recent occurrence of VHSV in Wisconsin waters presents the opportunity to document the dynamics of VHS from disease inception. A long-term monitoring study may include surveys of fish abundance, survival, recruitment, and growth and fish community composition. Temporal trend data on fish populations and fish communities subjected to VHSV will serve as a valuable tool for managing fisheries in newly-infected waters.

5. Developing and parameterizing disease models for VHSV. Population models can provide a framework for understanding how VHSV influences fish populations and communities, providing guidance on data collection and research for estimating critical variables in disease dynamics and for evaluating disease management programs. Models explicitly incorporate and require the parameterization of important factors influencing infection dynamics such as transmission rates, viral reproductive rates, duration of latency and infectious periods, fish population density, recruitment rates, natural and disease-related mortality rates, and the development of immunity to infection. Transmission coefficients and reproductive rates for VHSV type IVb have not yet been derived.

ANNOTATED BIBLIOGRAPHY

This bibliography includes materials from the scientific literature and books, as well as gray literature sources such as agency reports and web sites. These materials pertain to viral hemorrhagic septicemia, infectious disease epidemiology, or some aspect thereof. All citations in the VHS State of the Science Report are included here, as well as additional materials not cited in the report. A brief annotation is provided for all materials for which we were able to obtain a copy. A hyperlink is provided for all web sites. A hyperlink is also provided for papers that we were unable to obtain but for which an abstract is available online. All hyperlinks and web sites were last accessed on 24 August 2009.

The following acronyms are used freely in the annotated bibliography: VHS (viral hemorrhagic septicemia), IHN (infectious hematopoietic necrosis), and IPN (infectious pancreatic necrosis). Any of these acronyms followed by a V (e.g., VHSV) refer to the virus (viral hemorrhagic septicemia virus) as opposed to the disease (viral hemorrhagic septicemia). Some terms commonly used in the infectious disease literature are defined in Appendix 1, and scientific names for fishes referenced in the annotated bibliography are included in Appendix 2.

AFS/FHS Blue Book.

2007. Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. Fish Health Section Blue Book, American Fisheries Society, Bethesda, Maryland.

Currently published as a CD-ROM and updated annually, this book includes a chapter on VHS describing procedures for detecting and identifying VHSV in fish.

Amos, K., J. Thomas, and K. Hopper.

1998. A Case History of Adaptive Management Strategies for Viral Hemorrhagic Septicemia Virus (VHSV) in Washington State. *Journal of Aquatic Animal Health* 10:152-159.

This paper reviews the history of data and management decisions for viral hemorrhagic septicemia virus in the Pacific Northwest starting in 1988. A breakdown by year is presented to cover scientific studies and management actions taken. Some of the major findings include a discovery that VHSV is enzootic to the Puget Sound and the northeast Pacific Ocean, not an exotic import from Europe as first suspected, and occurs in a variety of marine species including cod and herring. All isolations of North American VHSV (as of 1998) have been made from fish in marine waters or adult salmon that recently exited marine waters. This supports the hypothesis that infection by VHSV occurs in the marine environment. The paper discussion speaks to how temperature plays a key role during the lifespan of an infected fish and the fish's ability to eliminate the virus (using coho salmon as an example). Management policy that changed as a result of more knowledge included altering the Co-Managers' Policy. VHSV found in anadromous salmonid adults now results in a 1-year quarantine of the watershed (changed from a 5-year period) to prevent the transfer of exposed fish from one watershed to another. This occurred because it is thought that VHSV was brought into freshwater from a marine environment and the virus would dissipate the following summer in a freshwater ecosystem containing warm water temperatures of 15°C and above.

Anderson, D.P.

1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. Pages 38-50 In S.M. Adams, editor. *Biological indicators of stress in fish.* American Fisheries Society, Symposium 8, Bethesda, Maryland.

This symposium paper reviews how environmental stressors can compromise the immune system in fish, thereby increasing the susceptibility to disease. Pathogens may be present in the fish or their environment but not provide any immunological challenge to the fish. A healthy immune system may be protecting the fish from disease. However, a change in the environment may affect fish endocrine systems and result in suppression of the immune system and a decrease in the ability of the fish to fight infection. This paper describes the physiological pathways of the fish immune system and how fish fight disease. This paper also discussed immunological stress evaluation as a tool for predicting disease outbreaks in fish.

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Ariel, E., and N.J. Olesen.

2001. Assessment of a commercial kit collection for diagnosis of the fish viruses: IHNV, IPNV, SVCV and VHSV. *Bulletin of the European Association of Fish Pathology* 21:6-11.

This paper describes an assessment of a commercial kit for the detection of four major fish viruses. The kit collection was based on fluorescence staining of infected cell cultures. Nine European VHSV isolates were tested. Five of the nine VHSV isolates did not react with the VHSV monoclonal antibodies used in the kit and thus were not detected. This paper stresses the need for proper validation before marketing VHSV test kits.

Ariel, E., and N. J. Olesen.

2002. Finfish in aquaculture and their diseases – a retrospective view on the European community. *Bulletin of the European Association of Fish Pathology* 22:72-85.

This paper presents results of annual surveys from 1995 to 2000 on finfish aquaculture and diseases in Europe. General trends in European aquaculture are covered, as well as fish diseases of critical economic importance to the aquaculture industry. Particular attention is given to the notifiable fish diseases VHS and IPN. Success is noted in the containment and control of many fish diseases in aquaculture, and this success is attributed to a thorough understanding of disease transmission and host susceptibility.

Arkush, K.D., H.L. Mendonca, A. M. McBride, S. Yun, T.S. McDowell, and R.P. Hedrick.

2006. Effects of temperature on infectivity and of commercial freezing on survival of the North American strain of viral hemorrhagic septicemia virus (VHSV). *Diseases of Aquatic Organisms* 69:145-151.

This report presents results of in vivo and in vitro experiments examining the effect of temperature on the stability and replication of the North American strain of VHSV. Studies included in vitro replication of virus isolates at varying temperatures, laboratory exposure to virus at varying water temperatures, and effect of commercial freezing on virus concentration. These studies were conducted to provide information needed to assess potential transmission risks of VHSV with movements of infected bait fishes.

Results for in vitro replication showed that temperature had an important role in virus growth. All VHSV isolates replicated at 10°C, 15°C and 20°C, but not at 25°C. Laboratory trials examining the effect of water temperature on VHSV infections in marine fishes also suggest that warmer temperatures (20°C and above) prevent the active replication and onset of disease observed at lower temperatures. Study trials of commercial freezing showed significantly reduced concentrations of infectious virus present in sardine experimentally infected with the North American strain of VHSV.

Bartholomew, J.L., B.L. Kerans, R.P. Hedrick, S.C. MacDiarmid, and J.R. Winton.

2005. A risk assessment based approach for the management of whirling disease. *Reviews in Fisheries Science* 13:205-230.

This paper presents a risk-assessment approach to managing whirling disease in trout populations. Concepts of risk assessment are introduced and related to whirling disease by showing how a model for risk assessment can be used to estimate risks and identify actions to reduce risks. These concepts are presented as applicable to infectious disease in aquatic populations in general.

Batts, W.N, C.K. Arakawa, J. Bernard, and J.R. Winton.

1993. Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. *Diseases of Aquatic Organisms* 17:67-71.

This study evaluated a series of nonradioactive DNA probes for their ability to recognize either common or unique sequences of VHSV in order to provide fish health specialists with a quick method for distinguishing the North American and European strains of the virus.

The results of this study showed that VHSV isolates from North America and Europe constitute two genetically distinct strains of the virus in which isolates from different years or species of fish on each continent were more related to each other than to isolates from the other continent. The results of this and other studies indicate that the North American strain of VHSV is enzootic in the North Pacific Ocean and is not a result of a recent importation of fish from Europe.

Benmansour, A., B. Basurco, A.F. Monnier, P. Vende, J.R. Winton, and P. Kinkelin.

1997. Sequence variation of the glycoprotein gene identifies three distinct lineages within field isolates of viral haemorrhagic septicaemia virus, a fish rhabdovirus. *Journal of General Virology* 78:2837-2846.

The authors determined sequence of the glycoprotein genes (G) of 11 North American and European isolates to further understand the genetic diversity of viral haemorrhagic septicaemia virus (VHSV).

A summary of the results includes confirmation that the fish salmonid rhabdoviruses are genetically closest to the genus *Lyssavirus*. The authors ordered VHSV strains, obtained throughout the world, into three distinct genotypes which correlate with the major geographical areas of isolation and not with the fish species or with previous serological classifications. They determined that VHSV genetic diversity and evolution fit within the model of random change and positive selection operating on quasi-species.

Betts, A. M., and D. M. Stone.

2000. Nucleotide sequence analysis of the entire coding regions of virulent and avirulent strains of viral haemorrhagic septicaemia virus. *Virus Genes* 20:259-262.

This paper presents the complete nucleotide sequences for two virulent and two avirulent strains of VHSV. The study confirmed a close genetic relationship between marine and freshwater strains of VHSV (>97.2% nucleotide sequence similarity and >98.6% amino acid similarity). The authors concluded that only a limited number of amino acid residues may determine whether or not VHSV is virulent for salmonids. This suggests marine strains pose a high risk to freshwater aquaculture.

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Bruchhof, B., O. Marquardt, and P.J. Enzmann.

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Brudeseth, B.E., J. Castric, and O. Evensen.

2002. **Studies on pathogenesis following single and double infection with viral hemorrhagic septicemia virus and infectious hematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*). *Veterinary Pathology* 39:180-189. Available on the web at http://www.vetpathology.org/cgi/content/full/39/2/180.** Accessed August 2009.

This paper describes a study in which rainbow trout were simultaneously challenged by two viruses (VHSV and IHNV) using bath immersion. Evidence supports the conclusion that simultaneous infection of a susceptible host (here rainbow trout) by VHSV and IHNV results in an interaction at the cellular level, which led to a reduced systemic distribution of IHNV in the host fish. There was also reduced replication of virus in the kidney, the prime target organ. Viral pathogenesis was documented by immunohistochemistry and histopathology.

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1998. **Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. *Canadian Journal of Fisheries and Aquatic Sciences* 2300-2309.**

This paper investigates the effect weathered crude oil has on the expression of viral hemorrhagic septicemia virus in Pacific herring. It is important to consider how an environmental stressor such as weathered crude oil can affect wild fish because many fish are asymptomatic carriers of pathogens that under normal conditions are held in check by the immune system. The authors hypothesized that sublethal concentrations of weathered oil can cause stress, resulting in immunosuppression, pathogen expression, and subsequent mortality in Pacific herring. The authors report strong correlations between oil exposure, induction of AHH, suppression of leukocytes, prevalence of VHSV, and cumulative mortality. Although causality was not proved, causal links were suggested between oil exposure and immunosuppression, immunosuppression and expression of VHSV, and VHSV and mortality in Pacific herring that were exposed to oil.

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This web-based document (last updated 6 March 2008) summarizes the results of a two-year surveillance of wild fish for VHSV in parts of Ontario and Quebec. One fish kill in 2007 was reported, involving freshwater drum in Hamilton Harbour, Lake Ontario. Tables showing the estimated risks of VHSV infection in selected watersheds is included in this document.

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2005. **Rapid detection and quantification of viral hemorrhagic septicemia virus in experimentally challenged rainbow trout by real-time RT-PCR. *Journal of Virological Methods* 132:154-159.**

This study explored the possibility of performing quantitative RT-PCR on RNA extracted from blood samples. Such a diagnosis method would be particularly useful because it would not require sacrificing the animal and would allow the analysis of blood samples from the same animal at different points in time to learn about the spread of the infection following experimental challenge. Detection of VHSV in samples from experimentally infected rainbow trout was achieved by real-time RT-PCR. Both symptomatic and asymptomatic fish were collected, and samples from spleen, kidney, liver, and blood were analyzed. In addition, real-time RT-PCR was utilized to determine whether those fish surviving VHSV challenge become virus carriers.

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Costanzi, C.
2005. **Programming and definition of prevention plans for viral haemorrhagic septicaemia (VHS) and infective haematopoietic necrosis (IHN) within the territory of the Autonomous Province of Trento and state of implementation.** *Veterinary Research Communications* 29(Supplement 2):147-151.

This paper describes a biosecurity plan to ensure the prevention of infection or dissemination of VHSV and other viral diseases in trout farms in Trento, Italy. The paper notes that the implementation of a fish health plan has not resulted in the eradication of VHSV from the province of Trento and financial constraints have resulted in protracted operations for disinfecting fish farms that have become contaminated with VHSV.

Dauber, M., H. Schutze, and D. Fichtner.
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This paper describes the development and characterization of monoclonal antibodies for a European strain of VHSV isolate. A commercial test kit had failed to identify this VHSV isolate. The authors recommend the developed monoclonal antibodies for the identification of VHSV isolates.

Dennehy, J.J., N.A. Friedenberg, Y.W. Yang, and P.E. Turner.
2007. **Virus population extinction via ecological traps.** *Ecology Letters* 10:230-240.

This paper discusses the concept of ecological traps as form of a habitat sink for viral elimination. An ecological trap is a sink habitat that is perceived as a source habitat. An increase in sink habitats can reduce population viability. In the context of a virus, an ecological trap is a host that does not function to allow viral reproduction. A virus encounters a non-functioning host and fails to reproduce, and as a result the virus population growth rate declines, ideally leading to elimination of the virus. A virus chooses host cells based on the presence of viral receptors on the host cell membrane; if the cell is unsuitable for viral replication, then the cell is functionally a habitat sink that removes the virus from the population. This novel link between source-sink theory and the trap cell concept is demonstrated in a model showing how the use of ecological traps can lead to reduced viral fitness. The authors conclude that the concept of ecological traps may contribute to the development of trap cell therapies for infectious viruses in humans. VHSV was not discussed in this paper.

Department of Agriculture, Fisheries and Forestry.
2005. **Disease strategy: Viral haemorrhagic septicaemia (Version 1.0). In:** *Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN),* **Edition 2, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT. Available on the web at http://www.daff.gov.au/__data/assets/pdf_file/0006/156129/vhs29Jun05.pdf.** Accessed August 2009.

This document outlines the Australian approach to managing viral haemorrhagic septicaemia. It reviews the nature of VHS, covering susceptible species, diagnostic criteria, resistance and immunity, and epidemiology. Also reviewed are methods to prevent the spread and eliminate pathogens as well as strategies for control and eradication. The document is meant to provide guidance based on sound analysis, linking policy, strategies, implementation, coordination, and emergency-management plans.

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This paper reports on an isolation of VHSV from Atlantic herring and molecular comparisons of the virus with other VHSV isolates.

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1991. **Comparative susceptibility of three species of char and of rainbow trout x char triploid hybrids to several pathogenic salmonid viruses.** *Diseases of Aquatic Organisms* 11:217-224.

This paper describes a study of the susceptibility of salmonid triploid hybrids (as well as non-hybrid salmonids) to infection by three common rhabdoviruses: VHSV types I and III, IPNV, and IHNV. Brook trout and the brook trout × rainbow trout hybrid were found to be resistant to VHSV (types I and III) (and IHNV but not IPNV), with observed mortality rates of 4–7%. Rainbow trout, however, experienced VHSV mortality rates of 68–93%. Lake trout and the lake trout × rainbow trout hybrid were both susceptible to VHSV, with lake trout showing clinical signs of infection and significant losses. Brown trout were resistant to VHSV type I (no mortality) but not VHSV type III (56% mortality).

Dorson, M., and C. Torhy.
1993. **Viral haemorrhagic septicaemia virus replication in external tissue excised from rainbow trout, *Oncorhynchus mykiss* (Walbaum), and hybrids of different susceptibilities.** *Journal of Fish Diseases* **16:403-408.**

Dorson, M., C. Torhy, and P. de Kinkelin.
1994. **Viral haemorrhagic septicaemia virus multiplication and interferon production in rainbow trout and in rainbow trout × brook trout hybrids.** *Fish and Shellfish Immunology* **4:369-381.**

This paper describes a study in which rainbow trout and rainow trout × brook trout triploid hybrids were challenged with type I VHSV by immersion or intraperitoneal injection. The rainbow trout × brook trout hybrids exhibited a higher resistance to type I VHSV. The hybrids appeared extremely refractory to virus infection by bath, and the virus was only detected in 3 of 80 samples (one of the three infected fish was found dead). Rainbow trout were more suceptible to waterborne VHSV infection, and 29 of 120 rainbow trout died. Results were similar for fish injected with VHSV. This study confirmed that rainbow trout surviving natural or artificial VHSV challenge (as well as hybrid trout injected with the virus) synthesize neutralizing antibodies. Neutralizing antibodies were not detected in hybrid trout subjected to immersion challenge tests. This paper also discusses the role of interferon production in viral disease.

Duffy, M.A., and L. Sivars-Becker.
2007. **Rapid evolution and ecological host-parasite dynamics.** *Ecology Letters* **10:44-53.**

This paper presents an example of how a disease epidemic can end as a result of rapid evolution of increased resistance in a host population. The authors here focus on the host-parasite system *Daphnia dentifera* and its parasite *Metschnikowia bicuspidate*. Disease epidemics can end when a susceptible host population is exhausted, but a disease can become endemic if host population reproduction continually creates new susceptible hosts. Here, the authors present an example in which *Daphnia* from lakes with recent epidemics were more resistant to infection than were *Daphnia* from naive populations. The authors concluded that rapid evolution in the host population can affect disease dynamics.

EAFP (European Association of Fish Pathologists).
2007. **Book of abstracts: the European Association of Fish Pathologists. 13th International conference of fish and shellfish diseases, 17-21 September 2007, Grado, Italy. Available on the web at http://eafp.squarespace.com/storage/publishing/Abstract%20book%20final.pdf.** Accessed August 2009.

This document includes a number of abstracts for presentations that were given on VHSV at the 2007 conference. Included are abstracts for a VHS workshop and risk factors in disease spread.

Eaton, W.D., J. Hulett, R. Brunson, and K. True.
1991. **The first isolation in North America of infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) in coho salmon from the same watershed.** *Journal of Aquatic Animal Health* **3:114-117. Available on the web at http://afs.allenpress.com/perlserv/?request=get-abstract&doi=10.1577%2F1548-8667%281991%29003%3C0114%3ATFIINA%3E2.3.CO%3B2.** Accessed August 2009.

Egan, D.
2008. **"Fish killing disease reaches inland lake near Mansfield."** *The Plain Dealer* **[Cleveland], 5 June 2008. Available on the web at http://blog.cleveland.com/sports/2008/06/fishkilling_disease_reaches_in.html.** Accessed August 2009.

First documentation of VHSV type IVb in the Mississippi basin, here observed in a muskellunge collected in a fish survey in Clearfork Reservoir, Ohio. No fish kill was observed.

Einer-Jensen, K., P. Ahrens, R. Forsberg, and N. Lorenzen.
2004. **Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus.** *Journal of General Virology* **85:1167-1179.**

This study focuses on the genetic evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus in wild reservoir hosts and in cultured fish species. To study the genetic evolution of VHSV, the entire G gene from 74 isolates was analyzed. The 74 isolates in this analysis represented 65 different fish collection sites covering wide geographic areas.

This study showed that marine lineages of VHSV in Europe and North America separated about 500 years ago. Adaptation of the virus from marine to freshwater fish is suggested to be a relatively recent event. In Europe there is a genetic linkage between VHSV in marine and freshwater environments, in which it is thought that the virus recently adapted to the freshwater environment by infecting rainbow trout in fish farms, an event occurring multiple times during the past 50 years.

Einer-Jensen, K., N.J. Olesen, N. Lorenzen, and P.E.V. Jørgensen.
1995. **Use of the polymerase chain reaction (PCR) to differentiate serologically similar viral haemorrhagic septicaemia (VHS) virus isolates from Europe and America.** *Veterinary Research* **26:464-469.**

Since 1988, VHSV has occasionally been isolated from salmonids and marine fish in USA and the isolates have been serologically indistinguishable from the European VHSV reference strain F1. However, the nucleotide sequence of an American isolate revealed a unique 20 nucleotide sequence in close proximity to the N gene. This sequence is not present in the European VHSV isolates. Through the use of polymerase chain reaction and specific primer sets for the amplification of N gene fragments, it could be demonstrated that the Makah sequence was also present in other VHSV isolates originating from the USA.

Elsayed, E., M. Faisal, M. Thomas, G. Whelan, W. Batts, and J. Winton.

2006. Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St Clair, Michigan, USA reveals a new sublineage of the North American genotype. *Journal of Fish Diseases* 29:611-619.

This paper presents information on viral haemorrhagic septicaemia virus isolated from muskellunge caught from the northwest portion of Lake St Clair, Michigan in 2003. The virus was confirmed as VHSV through testing by reverse transcriptase-polymerase chain reaction. Nucleotide sequence analysis of the glycoprotein gene demonstrated that the 2003 virus isolate from muskellunge was most closely related to the North American VHSV genotype IV and was clearly distinct from the three European genotypes. This isolate is classified as VHSV type IVb, and the previously identified North American isolates are classified as VHSV type IVa. Four genotypes of VHSV have been isolated, which are distributed geographically and not by fish species. This paper reviews the diversity of VHSV genotypes encountered in waters along the west and east coasts of North America. VHSV is probably endemic among marine fish from the Atlantic coast of North America.

The authors indicate that the absence of VHSV in historic health surveys and the presence of VHSV in many recent health surveys suggest a recent origin of VHSV in the Great Lakes system. VHSV may have been introduced via the discharge of marine ballast water or by migrating infected host fishes. Due to the fact that VHSV has been present in several species within the Great Lakes, these fishes can serve as a reservoir to maintain the virus for future outbreaks. The authors state that VHSV may represent a greater threat to the fisheries in the region than is currently understood.

Enzmann, P.J., M. Konrad, and J. Rapp.

1992. Epizootiological studies on viral haemorrhagic septicaemia in brown trout *Salmo trutta fario*. *Diseases of Aquatic Organisms* 12:143-146.

The course of a viral haemorrhagic septicaemia infection in brown trout in a southern German stream was followed over a four-year period using anti-VHS virus antibodies as the indication of infection. Trout were caught by electrofishing and the presence of anti-VHS virus antibody in the serum was determined by counter-current immunoelectrophoresis. Over the course of the study, VHSV was shown to have been eradicated from the trout populations based on the presence and subsequent absence of VHSV antibodies.

Over the study period fluctuations in the prevalence of antibodies to VHSV in several distinct populations of brown trout varied from 6 to 67%. In one population, the prevalence of antibody was found to be 41% in the first year of examination. In the following year the percentage increased to 67%, then fell to 33% and to 0% in the final year of the study. Prevalence also varied with location in the stream. In one example, prevalence decreased from 37% to 10% over a stream reach of only 3 km. The antibody data indicated that the virus persisted in the brown trout populations for a considerable period of time because antibody titres were prevalent in the trout during three successive years of sampling. In the fourth year, however, the virus appeared to have been largely eliminated from the trout as only a few fish in one population showed evidence of the presence of virus.

In areas where VHSV Type I is enzootic, prevalence of VHSV in brown trout tends to be at very low levels due to the low pathogenicity of the virus for this fish species. However, the current study demonstrated that brown trout can function as VHSV carriers for extended periods, as was predicted in earlier studies.

Evensen, O., W. Meier, T. Wahli, N.J. Olesen, P.E.V. Jørgensen, and T. Hastein.

1994. Comparison of immunohistochemistry and virus cultivation for detection of viral haemorrhagic septicaemia virus in experimentally infected rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 20:101-109. Available on the web at http://www.int-res.com/articles/dao/20/d020p101.pdf. Accessed August 2009.

This study compared virus isolation and immunohistochemistry for their ability to detect VHSV in rainbow trout experimentally infected by bath immersion. Virus isolation was found to be most sensitive to detecting VHSV. Immunohistochemistry may be used to simultaneously demonstrate virus and morphological changes and may be valuable in pathogenesis studies.

Evensen, O., and N.J. Olesen.

1997. Immunohistochemical detection of VHS virus in paraffin-embedded specimens of rainbow trout (*Oncorhynchus mykiss*): the influence of primary antibody, fixative, and antigen unmasking on method sensitivity. *Veterinary Pathology* 34:253-261. Available on the web at http://www.vetpathology.org/cgi/reprint/34/3/253.pdf. Accessed August 2009.

This study investigated the immunohistochemical detection of VHSV and the influence of primary antibody, fixative, and antigen unmasking on the sensitivity of the method. Virus cultivation and isolation was more sensitive to detecting VHSV than immunohistochemistry. Fixative and primary antibody both influenced method sensitivity and VHSV antigens concealed during fixation were difficult to reexpose.

Faustino, C.R., C.S. Jennelle, V. Connolly, A.K. Davis, E.C. Swarthout, A.A. Dhondt, and E.G. Cooch.

2004. *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. *Journal of Animal Ecology* 73:651-669.

The authors used a multistate analysis of mark-recapture data to study the impact of a pathogen on a bird population. The data indicated lower apparent survival in infected birds and the rate of recovery from

infection was greater than the rate of infection. There was also strong evidence that infected individuals were less likely to be recaptured or resighted as compared to healthy individuals. Several factors were identified as important for studies of disease in animal populations. Parameter estimation using mark-recapture models could be complicated by uncertainty in disease state assignment. And possible behavioral changes in infected individuals may have led to lower encounter rates as compared to healthy individuals. VHSV was not discussed in this paper.

Follett, J.E., T.R. Meyers, T.O. Burton, and J.L. Geesin.
1997. Comparative susceptibilities of salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). ***Journal of Aquatic Animal Health*** **9:34-40.**

Juveniles of eight Alaskan salmonid species were exposed to water immersion challenges of IHNV and North American VHSV to identify individual host susceptibilities for potential disease management purposes. (Subsequent studies have classified VHSV in Alaskan waters as type IVa.) Viral concentrations in the controlled experiments were measured in plaque-forming units per milliliter (PFU/mL^{-1}). The results of the study showed a low virulence of North American VHSV for rainbow trout, which exhibited a virus-specific mortality of about 12% with clinical signs of disease when exposed to 10^5 (versus 10^3) PFU/mL^{-1}. Other salmonid species (coho, chinook, sockeye, and pink salmon) were resistant to VHS after exposure to VHSV by water immersion.

North American VHSV (at the time this paper was published) had only been detected in Pacific cod and Pacific herring in Alaska. These two species will continue to be marine reservoirs for the virus as well as potential sources for anadromous salmonid exposure. However, the authors indicate that the likelihood of significant salmonid loss due to VHSV is low.

Gastric, J., and P. de Kinkelin.
1980. Occurrence of viral haemorrhagic septicemia in rainbow trout *Salmo gairdneri* Richardson reared in sea-water. ***Journal of Fish Diseases*** **3:21-27. Available on the web at http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2761.1980.tb00180.x.** Accessed August 2009.

Gagné, N., A.M. MacKinnon, L. Boston, B. Souter, M. Cook-Versloot, S. Griffiths, and G. Olivier.
2007. Isolation of viral haemorrhagic septicaemia virus from mummichog, stickleback, striped bass and brown trout in eastern Canada. ***Journal of Fish Diseases*** **30:213-223.**

This paper reports on the isolation of VHSV from mortalities occurring in populations of mummichog, stickleback, brown trout, and striped bass in New Brunswick and Nova Scotia, Canada.

Serum neutralization indicated the virus was VHSV (Type IVb) and sequencing identified the rhabdovirus isolates as the North American strain of VHSV. Phylogenetic analysis indicated that the isolates are closely related and form a distinguishable subgroup of North American type VHSV. VHSV Type IVb has likely been present but undetected in Atlantic Canadian waters.

Goodwin, A.E., J.E. Peterson, T.R. Meyers, and D.J. Money.
2004. Transmission of exotic fish viruses: the relative risks of wild and cultured bait. ***Fisheries*** **29:19-23.**

This paper addresses the issue that VHSV can easily be transmitted through frozen marine and freshwater baitfish. Baitfish are often caught in the wild and sent throughout the country, and freezing does not kill VHSV. Other viruses also may be present in baitfish. As of 2004 there were few regulations that would restrict transport of wild baitfish or increase the inspection methods to assess their health status before shipping. However, as a step in the right direction, many jurisdictions regulate the importation of farmed baitfish, decreasing the likelihood that pathogens and diseases will spread.

Groocock, G.H., R.G. Getchell, G.A. Wooster, K.L. Britt, W.N. Batts, J.R. Winton, R.N. Casey, J.W. Casey, and P.R. Bowser.
2007. Detection of viral hemorrhagic septicemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. ***Diseases of Aquatic Organisms*** **76:187-192.**

In May 2006 a large mortality of several thousand round gobies occurred in New York waters of the St. Lawrence River and Lake Ontario. The isolate found in these round gobies was identified to be identical to the VHSV type-IVb isolate described in muskellunge from Lake St. Clair. It is apparent from the wide genetic diversity between round gobies and muskellunge that the type-IVb isolate has a similarly broad host species range amongst freshwater fish. It is not known how VHSV was brought into the New York water system, but it is suspected that it was a combination of natural fish movement as well as human influence.

Harmache, A., M. LeBerre, S. Droineau, M. Giovannini, and M. Brémont.
2006. Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for *Novirhabdovirus*. ***Journal of Virology*** **80:3655-3659. Available on the web at http://jvi.asm.org/cgi/content/abstract/80/7/3655.** Accessed August 2009.

Hedrick, R.P., W.N. Batts, S. Yun, G.S. Traxler, J. Kaufman, and J.R. Winton.
2003. Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. ***Diseases of Aquatic Organisms*** **55:211-220.**

In this study, VHSV type IV was isolated from four new hosts including sardine, Pacific mackerel, eulachon (smelt), and surf smelt and detected by PCR from one additional host (sablefish). These detections

extended the geographic range of VHSV to include Southern California (eulachon) and Oregon (surf smelt). On at least two occasions in British Columbia, VHSV was associated with mass mortality among sardine, but in most cases the virus isolates were obtained from fish that appeared healthy. The new VHSV isolates belonged to the group of North American VHSV that has a low virulence for salmonid fishes, unlike the freshwater strains from Europe. The initial studies of these fish species indicate that each can be a reservoir for VHSV and that under certain circumstances, populations of sardine and perhaps other species can undergo mass mortality episodes.

Heppell, J., N. Lorenzen, N.K. Armstrong, T. Wu, E. Lorenzen, K. Einer-Jensen, J. Schorr, and H.L. Davis.

1998. **Development of DNA vaccines for fish: vector design, intramuscular injection and antigen expression using viral hemorrhagic virus genes as model.** ***Fish and Shellfish Immunology*** **8:271-286. Available on the web at http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%236799%231998%23999919995%23301702%23FLT%23&_cdi=6799&_pubType=J&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userId=10&md5=4c4b83b2c26311d270d23994351480af.** Accessed August 2009.

de las Heras, A.I., S. Rodriguez Saint-Jean, and S.I. Perez-Prieto.

2008. **Salmonid fish viruses and cell interactions at early steps of the infective cycle.** ***Journal of Fish Diseases*** **31:535-546. Available on the web at http://www.blackwellsynergy.com/doi/abs/10.1111/j.1365-2761.2008.00931.x.** Accessed August 2009.

Hershberger, P.K., J. Gregg, C. Pacheco, J. Winton, J. Richard, and G Traxler.

2007. **Larval Pacific herring,** ***Clupea pallasii*** **(Valenciennes), are highly susceptible to viral haemorrhagic septicaemia and survivors are partially protected after their metamorphosis to juveniles.** ***Journal of Fish Diseases*** **30:445-458.**

This paper had two study objectives. The first objective was to determine the susceptibility of Pacific herring early life history stages to VHSV during the three-month developmental period occurring between hatch and larval metamorphosis to juveniles. The second objective was to determine whether metamorphosed Pacific herring juveniles that survived a VHS epizootic as larvae demonstrated protection against subsequent exposure to VHSV. Larval herring were susceptible to VHS with increasing cumulative mortality as the fish aged. The study also demonstrated that larval exposure to VHSV did lead to partial protection to juveniles that had survived exposure to VHSV versus naive juveniles with no prior exposure to VHSV. This study underscores the importance of early life history stages in marine fish in influencing the ecology of disease processes.

Hershberger, P.K., R.M. Kocan, N.E. Elder, T.R. Meyers, and J.R. Winton.

1999. **Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Prince William Sound, Alaska, USA.** ***Diseases of Aquatic Organisms*** **37:23-31.**

This project was carried out to determine whether impoundment of herring for the closed pound spawn-on-kelp fishery was correlated with increased prevalence of VHSV and to describe the course of virus infection within the pounds. Results showed that increased prevalence of VHSV was correlated with confinement time of herring. A breakdown of how this occurred is described in the paper. The impoundment results in prolonged crowding, which increases stress and the probability of exposure to waterborne virus particles originating from impounded fish. The age of fish also influenced infection rates; older fish were more likely to have been exposed to VHSV, building resistance or immunity.

Herve-Claude, L.P., T.E. Carpenter, and R.P. Hedrick.

2008. **Risk of introducing viral hemorrhagic septicemia virus (VHSV) to the Chilean South Pacific via sardine imports from Europe.** ***Diseases of Aquatic Organisms*** **78:199-207. Available on the web at http://www.int-res.com/abstracts/dao/v78/n3/p199-207/.** Accessed August 2009.

Hetrick, F.M., J.L. Fryer, and M.D. Knittel.

1979. **Effect of water temperature on the infection of rainbow trout with infectious haematopoietic necrosis virus.** ***Journal of Fish Diseases*** **2:253–257.**

Hoffmann, B., M. Beer, H. Schutze, and T.C. Mettenleiter.

2005. **Fish rhabdoviruses: molecular epidemiology and evolution. Pages 81-118 In Z. F. Fu, editor.** ***The world of rhabdoviruses.*** **Springer, New York. Available on the web at http://books.google.com/books?id=XLMQGkBPKcAC.** Accessed August 2009.

This book chapter reviews the molecular epidemiology and phylogeography of common fish rhabdoviruses including VHSV. (The chapter section on VHSV is not included in the book preview at the above hyperlink.)

Hudson, P.J., S.E. Perkins, and I.M. Cattadori.

2008. **The emergence of wildlife disease and application of ecology. Pages 347-367 In R. S. Ostfeld, F. Keesing, and V.T. Eviner, editors.** ***Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems.*** **Princeton University Press, New Jersey.**

This chapter in a book on the ecology of infectious disease discusses emerging infectious disease in the context of ecological invasion. A parameter for the likelihood of invasion (R_0) is introduced, along with how to interpret R_0 in terms of invasion versus persistence. Some examples are provided of both RNA and DNA pathogens that affect wildlife and human, but the concepts apply to vertebrates in general. Conclusions and consequences for controlling infections are also discussed.

Isshiki, T., T. Nishizawa, T. Kobayashi, T. Nagano, and T. Miyazaki.

2001. An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder *Paralichthys olivaceus* in Japan. *Diseases of Aquatic Organisms* 47:87-99.

This is the first report of an outbreak of VHSV infection in cultured fish in Japan. The virus occurred in 1996 in farmed populations of market sized Japanese flounder in the Seto Inland Sea of Japan. VHSV was identified as the causative agent based on morphological, immunological, and genetic analyses. Diseased fish that were artificially injected with a representative virus isolate showed the same pathological signs and high mortality as observed in the natural outbreak. Diseased Japanese flounder displayed dark body coloration, expanded abdomen due to ascites, noticeable congestion in visceral organs such as the hepato pancreas, spleen, liver, and hematopoietic tissue. The congestion likely resulted from cardiac failure due to myocardium necrosis. Study results of the experimental infections indicated that VHSV is apparently pathogenic to Japanese flounder and its pathogenicity decreases with increases in the body weight of the host fish; however, mortality still occurs even in large fish weighing about 1000g.

Jencic, V., P. Hostnik, D. Barlic Maganja, and J. Grom.

2002. The spread of salmonid viral diseases in Slovenia. *Slovenian Veterinary Research* 39:197-205. Available on the web at http://www2.vf.uni-lj.si/veterina/zbornik/197_jencic_e.pdf. Accessed August 2009.

This paper provides an epidemiological analysis of the spread of viral diseases (VHS, IHN, and IPN) of salmonids in Slovenian waters (particularly fish farms). Included is an overview of diagnostic techniques for studying viral diseases in fishes.

Jørgensen, P. E. V.

1973. Artificial transmission of viral haemorrhagic septicaemia (VHS) of rainbow trout. *Rivista Italiana di Piscicoltura e ittiopatologia* 8:101-102.

This study documented that Egtved virus was shed by VHS-infected rainbow trout during the course of the disease and that VHS could be reproduced in rainbow trout by immersing trout in water containing Egtved virus. An additional conclusion was that environmental factors can exacerbate VHS in fish.

Jørgensen, P.E.V.

1980. Egtved virus: the susceptibility of brown trout and rainbow trout to eight virus isolates and the significance of the findings for the VHS control. Pages 3-7 In W. Ahne, editor. *Fish diseases*. Springer-Verlag, Berlin.

This study investigated whether the pathogenicity of Egtved virus strain 23/75 to brown trout was a unique feature of that strain or whether other Egtved virus isolates were also pathogenic to brown trout when tested by bath infection. Eight virus isolates were examined in this study. Three of the eight virus isolates were found to be pathogenic to brown trout as well as to rainbow trout. The results showed that brown trout were susceptible to some strains of Egtved virus under certain laboratory conditions.

Jørgensen, P.E.V.

1982a. Egtved virus: occurrence of inapparent infections with virulent virus in free-living rainbow trout, *Salmo gairdneri* Richardson, at low temperature. *Journal of Fish Diseases* 5:251-255.

In this study, free-living rainbow trout were caught by electrofishing at different seasons and water temperatures in a stream receiving Egtved virus from a number of infected trout farms. Captured fish were transported to a laboratory in a refrigerated container and frozen at -20°C less then 6 hours after capture. Virological examinations were conducted within four weeks. This is one of the first studies to detect Egtved virus isolates in trout outside the farm environment.

Jørgensen, P. E. V.

1982b. Egtved virus: temperature-dependent immune response of trout to infections with low virulence virus. *Journal of Fish Diseases* 5:47-55.

The pathogenicity of Egtved virus had previously been shown to be strongly reduced by in vitro passage through cell tissue. This study showed that the low pathogenicity of the virus (obtained after 20 successive passages) was a genetically stable feature and that protection against VHS induced by infection with this virus was attributable to an immune response. However, the immunogenicity of this strain was determined to be insufficient to ensure complete protection from VHS. This study also showed that the persistence of Egtved virus in rainbow trout was inversely proportional to the water temperature.

Jørgensen, P.E.V., J. Castric, B. Hill, O. Ljungberg, and P. de Kinkelin.

1994. The occurrence of virus infections in elvers and eels (*Anguilla anguilla*) in Europe with particular reference to VHSV and IHNV. *Aquaculture* 123:11-19.

This paper presents a compilation of results from virological tests of 2,092 pools of elvers and eels collected in four European countries (Denmark, United Kingdom, France, and Sweden) from 1977 to 1992. A total of 91 virus isolates were obtained; only 1 isolate was serologically related to VHSV. There was no evidence in this study or in available data that eels could be a vector for VHSV. This study concluded that the transfer of wild elvers would pose little risk for the spread of VHSV (assuming proper precautions were taken to address the possibility of surface contamination of elvers with VHSV).

Jørgensen, P.E.V., N.J. Olesen, and N. Lorenzen.
1991. **Infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicemia (VHS): detection of trout antibodies to the causative viruses by means of plaque neutralization, immunofluorescence, and enzyme-linked immunosorbent assay.** *Journal of Aquatic Animal Health* **3:100-108.**

This study investigated three serological tests for their efficacy in detecting antibodies to VHSV and IHN. The authors concluded that serological tests may be useful tools for VHSV and IHN epidemiology with the caveat that cross-reacting antibodies may be present in some sera, thereby complicating the certification process for one virus in the presence of another.

Kakehaski, M.
1996. **Populations and infectious diseases: dynamics and evolution.** *Researches on Population Ecology* **38:203-210. Available on the web at http://meme.biology.tohoku.ac.jp/POPECOL/RPcontents/AB38(2).html#Kakehashi.** Accessed August 2009.

Kaufman, J., and R.A. Holt.
2001. **Isolation of North American viral hemorrhagic septicemia virus (VHSV) from Columbia River smelt (*Thaleichthys pacificus*). *American Fisheries Society, Fish Health Section Newsletter* 29(2):1-3. Available on the web at http://www.fisheries.org/units/fhs/Newsletter_Files/V29-2_2001.PDF.** Accessed August 2009.

Kent, M.L., G.S. Traxler, D. Kieser, J. Richard, S.C. Dawe, R.W. Shaw, G. Prosperi-Porta, J. Ketcheson, and T.P.T. Evelyn.
1998. **Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. *Journal of Aquatic Animal Health* 10:211-219. Available on the web at http://afs.allenpress.com/perlserv/?request=get-abstract&doi=10.1577%2F1548-8667%281998%29010%3C0211%3ASOSPIO%3E2.0.CO%3B2.** Accessed August 2009.

King, J.A., M. Snow, H.F. Skall, and R.S. Raynard.
2001. **Experimental susceptibility of Atlantic salmon *Salmo salar* and turbot *Scophthalmus maximus* to European freshwater and marine isolates of viral haemorrhagic septicaemia virus. *Diseases of Aquatic Organisms* 47:25-31.**

In this study, Atlantic salmon parr were shown not to be susceptible to European freshwater and marine isolates of VHSV. VHSV was detected in some mortalities, however, demonstrating that viral entry and replication without manifestation of the disease can occur. The risk of European marine VHS to the Atlantic salmon culture industry in Europe appears to be low.

The turbot experiments showed the fish to be very resistant to the freshwater VHSV isolate DK-3592B, with only 3% mortality. The susceptibility of turbot to other VHSV isolates of European freshwater origin has been shown previously. For example, mortalities ranging from 16 to 68% occurred when turbot where infected with VHSV isolates taken from herring. The results of this study show that turbot are susceptible to a number of VHSV isolates that are enzootic to the European marine environment and that protection against VHSV is needed in turbot aquaculture.

de Kinkelin, P., M. Bearzotti-Le Berre, and J. Bernard.
1980. **Viral hemorrhagic septicemia of rainbow trout: selection of a thermoresistant virus variant and comparison of polypeptide synthesis with the wild-type virus strain. *Journal of Virology* 36:652-658. Available on the web at http://www.pubmedcentral.nih.gov/pagerender.fcgi?artid=353692&pageindex=1.** Accessed August 2009.

de Kinkelin, P., and J. Gastric.
1982. **An experimental study of the susceptibility of Atlantic salmon fry, *Salmo salar* L., to viral haemorrhagic septicaemia. *Journal of Fish Diseases* 5:57-65. Available on the web at http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2761.1982.tb00456.x.** Accessed August 2009.

de Kinkelin, P., J.P. Gérard, M. Dorson, and M. Le Berre.
1976. **Viral hemorrhagic septicaemia: demonstration of a protective immune response following natural infection. *Fish Health News* 6:3-4.**

de Kinkelin P., and M. Le Berre.
1977. **Isolation of a pathogenic rhabdovirus of brown trout (*Salmo trutta L.*, 1766). *Comptes Rendus de l Academie des Sciences*, Paris 284, 101-104.**

Knüsel, R., S.M. Bergmann, K. Einer-Jensen, J. Casey, H. Segner, and T. Wahli.
2007. **Virus isolation vs RT-PCR: which method is more successful in detecting VHSV and IHNV in fish tissue sampled under field conditions? *Journal of Fish Diseases* 30:559-568.**

This study compared the results of reverse transcription-polymerase chain reaction (RT-PCR) and traditional virus isolation on cell culture for detection of VHSV and IHNV. Their results indicated that RT-PCR can be successfully applied in field surveys and may also be slightly more sensitive than virus isolation at detecting VHSV. However, in a titration experiment under laboratory conditions, the sensitivity of RT-PCR was not significantly higher when compared with virus isolation.

Knuesel, R., H. Segner, and T. Wahli.
2003. **A survey of viral diseases in farmed and feral salmonids in Switzerland. *Journal of Fish Diseases* 26:167-182.**

This study provided an overview on viral disease presence in farmed and feral salmonids and compared current and historical occurrences in Switzerland. A field survey was carried out to study the occurrence and distribution of viruses causing diseases of major impact in fish farming, namely VHSV (type I), infectious haematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN) in farmed and wild fish in Switzerland. This paper presents

data on the occurrence of the three diseases from two field surveys (1984-85 and 2000-01) , as well as from routine diagnostic work performed at the Centre for Fish and Wildlife Health (FIWI) from 1978 to 2001.

Both the field and FIWI data showed a decrease in total virus isolations in Switzerland since 1978. FIWI data showed that within the 1,776 tissue samples received for routine diagnostics, 329 were positive for VHSV, mostly from rainbow trout, but also from brown trout, Arctic char, grayling, coregonids and pike. The results also indicated that age plays an important role in susceptibility of fish to viral diseases, with younger fish more likely to be infected.

Kocan, R., M. Bradley, N. Elder, T. Meyers, W. Batts, and J. Winton.

1997. North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. ***Journal of Aquatic Animal Health*** **9:279-290.**

After the massive decline in Pacific herring biomass in 1993, the Alaska Department of Fish and Game initiated an extensive field survey of spawning herring in Prince William Sound in 1994 in an attempt to identify the possible causes of the population decline.

Methods for this paper involved performing laboratory tests of waterborne exposure of a North American isolate of VHSV to pathogen-free juvenile Pacific herring. Results showed high mortality that approached 100% by 7–10 days after exposure, the fish shed large amounts of virus into the water by 3 days after exposure, and the fish also sustained extreme cellular damage apparent in the liver, spleen, kidney, and skin.

Kocan, R.M., P.K. Hershberger, and N.E. Elder.

2001a. Survival of the North American strain of viral hemorrhagic septicemia virus (VHSV) in filtered seawater and seawater containing ovarian fluid, crude oil and serum-enriched culture medium. ***Diseases of Aquatic Organisms*** **44:75-78.**

This study assessed the stability of the virus VHSV in various environments. The virus was isolated and tested in four environments: filtered seawater, and seawater supplemented with teleost ovarian fluid, crude oil, and culture medium supplemented with 10% fetal bovine serum. Significant differences existed in respect to how much of the virus could be recovered in each environment after different time periods. The authors found that the survival and infectivity of VHSV was significantly prolonged in the presence of ovarian fluids, which occur naturally during spawning. Extended stabilization of the virus was achieved in the culture medium, which may prevent virus titer loss in low titer field samples that are collected and transported unfrozen.

Kocan, R.M., P.K. Hersherger, N.E. Elder, and J.R. Winton.

2001b. Epidemiology of viral hemorrhagic septicemia among juvenile Pacific herring and Pacific sand lances in Puget Sound, Washington. ***Journal of Aquatic Animal Health*** **13:77-85.**

This study investigated the epidemiology of VHS in Pacific herring and Pacific sand lances captured from Puget Sound, Washington. The objectives of this study were to identify at what age Pacific herring were first infected with VHSV, to determine whether age-0 Pacific herring suffered significant mortality as a result of VHSV, and to determine whether juvenile Pacific herring became more resistant to VHSV as they aged.

VHSV was detected in less than 1% of free-ranging age-0 Pacific herring. However, herring that were captured and confined in laboratory tanks were shown to exhibit a prevalence of VHSV in up to 100% of the fish by 14 d post-capture, with a mortality rate of up to 50%. VHSV in the confined herring peaked and then was eradicated in surviving fish by 30 d. Similar observations were made for 18-month old Pacific herring, which tested negative for VHSV upon capture but developed active infections after 7 d in captivity. However, mortality rates for the older herring were lower (8.4%). It is suggested that a higher proportion of the older fish may have developed immunity to VHSV from prior exposure to the virus. Three months after the last observed mortalities in the laboratory fish, the herring were challenged to VHSV by immersion and no mortalities were observed. Specific-pathogen-free Pacific herring were then subjected to cohabitation with the wild Pacific herring, and VHSV was observed to have been transmitted to the nonimmune fish, resulting in infection and manifestation of the viral disease.

Kozlowski, G.

2007. New York's response to VHS. ***New York Chapter American Fisheries Society Newsletter,*** **May 2007. Available on the web at http://www.newyorkafs.org/2007may.pdf.** Accessed August 2009.

LaDeau, S.L., A.M. Kilpatrick, and P.P. Marra.

2007. West Nile virus emergence and large-scale declines of North American bird populations. ***Nature*** **447:710-713.**

This paper shows how a virus acting as an invasive species altered bird community structure across North America. The West Nile virus has led to changes in abundance in bird host populations, which has led to shifts in community composition and ecosystem functioning. The authors point out the challenges of distinguishing disease impacts from other factors that influence population dynamics, identifying the need for abundance data both before and after disease introduction. In the example presented in this paper, the authors used 26 years of Breeding Bird Survey data to investigate the effects of West Nile virus on 20 bird host species. Significant changes occurred in populations of seven bird species from four families, and these changes were attributable to West Nile virus. Two of the seven species with documented impacts by West Nile virus were observed to have recovered to pre-West Nile virus levels.

LaPatra, S.E.

1996. The use of serological techniques for virus surveillance and certification of finfish. ***Annual Review of Fish Diseases*** **6:15-28.**

Using IHNV and VHSV this paper describes various serological methods used in virus surveillance for finfish. The paper primarily compares a more traditional method, in which researchers isolate the virus and its replicating agents in order to study it, to a newer method that emphasizes detecting fish antibodies. The manuscript goes on to describe various techniques to detect such antibodies in detail, including passive immunizations, neutralization tests, and immunofluorescence, among others. These techniques are gaining in popularity in assessing and monitoring infectious diseases in the veterinary field.

Lecocq-Xhonneux, F., M. Thiry, I. Dheur, M. Rossius, N. Vanderheijden, J. Martial, and P. de Kinkelin.

1994. A recombinant viral haemorrhagic septicaemia virus glycoprotein expressed in insect cells induces protective immunity in rainbow trout. ***Journal of General Virology*** **75:1579-1587.**

This study investigated and documented the use of a recombinant vaccine to protect rainbow trout from VHSV. A recombinant vaccine was developed using the VHSV glycoprotein and was compared to an inactivated and an attenuated vaccine. The recombinant vaccine was injected intraperitoneally into rainbow trout and was shown to induce the synthesis of VHSV antibodies and to protect rainbow trout against VHSV challenge. Immunization by immersion, however, was not successful.

Levin, B. R., M. Lipsitch, and S. Bonhoeffer.

1999. Population biology, evolution, and infectious disease: convergence and synthesis. ***Science*** **283:806-809.**

This is a synthesis paper on how quantitative, population-level approaches in biology can contribute to the understanding of infectious disease. Recent work in the following areas is discussed: genetics and evolution of microparasite populations, mechanisms of pathogenesis and immune response, and consequences of medical and public health interventions in terms of population biology, ecology, and evolution.

Lopez-Vazquez, C., C.P. Dopazo, J.L. Barja, and I. Bandin.

2007. Experimental infection of turbot, ***Psetta maxima*** **(L.), with strains of viral haemorrhagic septicaemia virus isolated from wild and farmed marine fish.** ***Journal of Fish Diseases*** **30:303–312.**

This paper examined the susceptibility of turbot to infection with two strains of VHSV obtained from wild Greenland halibut and farmed turbot. Fish were infected by intra-peritoneal injection, immersion, or cohabitation and fish were maintained at two different temperatures (8° and 15°C). The trials showed that the three VHSV isolates were pathogenic for turbot fingerlings by intra-peritoneal injection at both temperatures, with high levels of mortality. Virus was recovered from most pools of dead fish that were intra-peritoneal challenged, but not from surviving fish. Although clinical signs were not produced following waterborne exposure, viral growth was obtained from some pools of surviving fish challenged by immersion with strain GH40 from Greenland halibut, which indicates that the virus can survive in sea water and infect other fish via horizontal transmission. Additionally, although low, the clinical signs and mortality observed in fish cohabitating with turbot challenged with strain GH40 confirms horizontal transmission and indicates that the passage through fish increases the virulence of this strain for turbot.

The findings in this report indicate that Greenland halibut, as well as other wild fish, may play an important role in the epizootiology of VHSV and suggest a potential risk for the turbot farming industry.

Lorenzen, E., B. Carstensen, and N.J. Olesen.

1999. Inter-laboratory comparison of cell lines for susceptibility to three viruses: VHSV, IHNV and IPNV. ***Diseases of Aquatic Organisms*** **37:81-88.**

This paper describes a comparison of the susceptibility of five cell lines to three pathogenic fish viruses (VHSV, IHNV, and IPNV). Cell lines were derived from bluegill fry (BF-2), Chinook salmon embryo (CHSE-214), epithelioma papulosum cyprinid (EPC), fathead minnow (FHM), and rainbow trout gonad (RTG-2). The study concluded that BF-2 and RTG-2 cells performed about equally well and with higher sensitivity for VHSV as compared to the other cell lines.

Lorenzen, E., K.E. Einer-Jensen, T. Martinussen, S.E. LaPatra, and N. Lorenzen.

2000. DNA vaccination of rainbow trout against viral hemorrhagic septicemia virus: a dose-response and time-course study. ***Journal of Aquatic Animal Health*** **12:167-180.**

This study evaluates the potential of DNA vaccination as a measure to protect farmed fish from VHSV. Objectives addressed in the study were to determine the dose-response relationship between the amount of vaccine administered and the level of protection and the time span during which the fish are protected against the disease after vaccination. Results from the dose-response analysis revealed that significant protection of rainbow trout fingerlings was obtained following intramuscular injection of only 0.01 μg of plasmid DNA encoding the VHSV glycoprotein gene. To further understand how the vaccine works and possibly improve its effectiveness, further studies were recommended to determine which immune mechanisms interfere with the viral infection and how they operate.

Lorenzen, N. and S.E. LaPatra.

2005. DNA vaccinations for aquacultured fish. ***Scientific and Technical Review of the International Office of Epizootics*** **24:201-213.**

This article considers the principles and perspectives

related to application of DNA vaccines in fish that are commercially cultured for food production, focusing on the DNA vaccines against fish rhabdoviruses. The paper reports on how a single intramuscular injection of microgram amounts of DNA induces rapid and long-lasting protection in farmed salmonids against economically important viruses such as IHNV and VHSV. The most efficient route of delivery is intramuscular injection, but suitable delivery strategies for mass vaccination of small fish (5 g) have yet to be developed. The paper also outlines the advantages and disadvantages of DNA vaccines.

Lorenzen, N., E. Lorenzen, K. Einer-Jensen, J. Heppell, T. Wu, and H. Davis.

1998. Protective immunity to VHS in rainbow trout (*Oncorhynchus mykiss*, Walbaum) following DNA vaccination. *Fish and Shellfish Immunology* 8:261-270. Available on the web at http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%236799%231998%23999919995%23301702%23FLT%23&_cdi=6799&_pubType=J&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=4c4b83b2c26311d270d23994351480af. Accessed August 2009.

Lorenzen, N., N.J. Olesen, and K. Claus.

1999. Immunity to VHS virus in rainbow trout. *Aquaculture* 172:41-61.

This paper covers aspects of acquired as well as innate immunity to VHSV in rainbow trout, focusing on adaptive humoral immune mechanisms, vaccination, and genetic immunity. The authors concluded that protective adaptive immunity and genetic immunity are both potential measures for reduction of disease problems caused by VHSV in cultured rainbow trout, but a better structural and functional understanding of the underlying defense mechanisms is required to fully explore these measures. In vitro assays including humoral or cellular components are valuable tools for approaching this goal but cannot be substituted for in vivo experiments where all parts of the fish immune system work together.

Lorenzen, N., N.J. Olesen, and P.E.V. Jørgensen.

1988. Production and characterization of monoclonal antibodies to four Egtved virus structural proteins. *Diseases of Aquatic Organisms* 4:35-4.

This study investigates the molecular mechanisms of virulence and immunogenesis of Egtved virus using monoclonal antibodies. Antibodies were produced for four dominant virus proteins and the reactivity of each antibody was determined by enzyme-linked immunosorbent assay (ELISA), immunoblotting, immunofluorescence, and plaque neutralization.

Lumsden, J.S., B. Morrison, C. Yason, S. Russell, K. Young, A. Yazdanpanah, P. Huber, L. Al-Hussinee, D. Stone, and K. Way.

2007. Mortality event in freshwater drum *Aplodinotus grunniens* from Lake Ontario, Canada, associated with viral haemorrhagic septicemia virus, Type IV. *Diseases of Aquatic Organisms* 76:99-111.

This paper reports on an outbreak of VHSV affecting freshwater drum in the Bay of Quinte, Lake Ontario, Canada. The mortality event happened during April and May 2005 and was responsible for the mortality of an estimated 100 metric tons of freshwater drum. Also observed were large numbers of dead round goby and a few muskellunge. Identification of VHSV was confirmed by enzyme immunoassay and PCR. Analyses showed the strain of VHSV to belong to the same lineage as the North American and Japanese isolate, genotype IV. Water temperature during the outbreak ranged between 8 and 14° C.

MacDiarmid, S.C., and H.J. Pharo.

2003. Risk analysis: assessment, management and communication. *Scientific and Technical Review of the International Office of Epizootics* 22:397-408. Available on the web at http://epicentre.massey.ac.nz/resources/acvsc_grp/docs/MacDiarmid_Pharo_2003.pdf. Accessed August 2009.

This paper provides an overview of the concepts of risk analysis for application in the animal health field. The steps in risk analysis include hazard identification, risk assessment, risk management, and risk communication. An example of risk analysis procedures is presented, with an emphasis on the utility of drawing scenario trees and influence diagrams for aiding in risk assessment and communication.

Marty, G.D., T.J. Quinn II, G. Carpenter, T.R. Meyers, and N.H. Willits.

2003. Role of disease in abundance of a Pacific herring (*Clupea pallasi*) population. *Canadian Journal of Fisheries and Aquatic Sciences* 60:1258-1265.

This paper documents how disease affects fish population abundance and recruitment. A six year comprehensive epidemiological study of the Pacific herring population of Prince William Sound Alaska involved a complete necropsy examination of 2,983 fish. Mortality was best estimated using a modified age-structured assessment model that included a disease index that combined the prevalence of VHSV with the prevalence of ulcers. Poor body condition and abundant recruitment before spawning were two important risk factors in disease outbreak. Changes in the pathogen *Ichthyophonus hoferi* with fish age were not related to changes in fish abundance. VHSV disease was observed to have disappeared from the population following changes in abundance. The authors concluded that disease affects recruitment because lower recruitment was observed following changes in abundance attributed to VHS-induced natural mortality.

McAllister, P.E.

1997. Communications: susceptibility of 12 lineages of Chinook salmon embryo cells (CHSE-214) to four viruses from salmonid fish: implications for clinical assay sensitivity. *Journal of Aquatic Animal Health* 9:291-294. Available on the web at http://afs.allenpress.com/perlserv/?request=get-abstract&doi=10.1577%2F1548-8667%281997%29009%3C0291%3ACSOLOC%3E2.3.CO%3B2. Accessed August 2009.

MDNR (Michigan Department of Natural Resources).
2007. Fish disease discovered in Budd Lake, Clare County.

Meier, W.
1981. Viral hemorrhagic septicemia in non-salmonid fishes. *Bulletin of the European Association of Fish Pathology* 1:15-17.

This paper discusses the loss of 120,000 pike fry at a hatchery on a lake in Switzerland and the diagnosis of VHSV (Egtved virus) as the cause of the mortality. The paper includes a photograph of infected pike fry showing hemorrhages and dilated abdomen.

Meier, W., and P.E.V. Jørgensen.
1980. Isolation of VHS virus from pike (*Esox lucius L.*) with hemorrhagic symptoms. Pages 8-17 In W. Ahne, editor. *Fish Diseases*. Springer-Verlag, Berlin.

Meier, W., M. Schmitt, and T. Wahli.
1994. Viral hemorrhagic septicemia (VHS) of nonsalmonids. *Annual Review of Fish Diseases* 4:359-373. Available on the web at http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%234966%231994%23999959999%23455431%23FLP%23&_cdi=4966&_pubType=J&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=d1122fa1a2423ecf11940aeaebf9ac18. Accessed August 2009.

Meyers, T.R., S. Short, and K. Lipson.
1999. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fish. *Diseases of Aquatic Organisms* 38:81-86.

This report describes the detection of the North American strain of VHSV associated with epizootic mortality in two new Alaskan host species of marine fish (Pacific hake and walleye pollock) and the possible epizootiological relationship with VHSV-infected Pacific herring.

In August 1998, thousands of dead Pacific herring, Pacific hake and walleye pollock were reported in Lisianski Inlet near Pelican, Alaska. The Pacific hake and pollock continued to die through the end of September. Virological examinations of dead fish identified the North American strain of VHSV from all three species of fish. No other primary fish pathogens were detected and there were no apparent environmental causes for the fish mortality.

Meyers, T.R., and J.R. Winton.
1995. Viral hemorrhagic septicemia in North America. *Annual Review of Fish Diseases* 5:3-24.

This paper reviews the history of VHSV in Europe and the North America. Case histories about VHSV outbreaks starting in 1988 concerning Pacific salmon, Pacific cod, and Pacific herring are covered in detail. The geographical range of reported outbreaks includes Eastern coastal states and British Columbia, Canada.

VHSV strains isolated from fish in Washington, British Columbia, and Alaska were not of European origin. The widespread occurrence of VHSV in Pacific herring indicates it is enzootic from Kodiak Island, Alaska to Puget Sound Washington. Pacific herring are common prey for both cod and salmon and were probably the source of the VHSV isolated from the adult salmon returning to spawn in rivers or at hatcheries in Washington State. The epizootiology of VHSV in herring appears to be an opportunistic pathogen triggered by stress.

Moffitt, C.M., B.C. Stewart, S.E. LaPatra, R.D. Brunson, J.L. Bartholomew, J. Peterson, and K.H. Amos.
1998. Pathogens and diseases of fish in aquatic ecosystems: implications in fisheries management. *Journal of Aquatic Animal Health* 10:95-100.

A two-day conference was convened (in Portland, Oregon, 3-4 June 1997) to address the status of information about pathogens and diseases of free-ranging fish populations. This paper provides background information about the conference, and a summary of the presentations and panel discussions.

This paper discusses the objectives of the conference, which were to (1) discuss the distribution of selected pathogens and their potential impacts on populations of fish in aquatic ecosystems, (2) discuss the factors affecting pathogen transmission between wild and cultured populations of fish and the associated risks, (3) evaluate management strategies to minimize and avoid pathogen impacts on fish in aquatic ecosystems, and (4) outline areas needing additional research.

Mortensen, H.F., O.E. Heuer, N. Lorenzen, L. Otte, and N.J. Olsen.
1998. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagenak and the North Sea. *Virus Research* 63:95-106.

This paper describes a survey of the occurrence of VHSV in the marine environment surrounding Denmark. VHSV was detected in five species that were not previously reported as hosts for VHSV.

Murray, A.G.
2006. A model of the emergence of infectious pancreatic necrosis virus in Scottish salmon farms 1996-2003. *Ecological Modelling* 199:64-72.

This paper describes the development of a simple epidemiological model of the spread of IPNV among salmon populations in Scottish fish farms. The model was applied at both a regional and national level. Disease transmission was also modeled as dependent versus independent of population size. The author concluded that IPNV prevalence was entering a state of dynamic equilibrium and would stabilize or increase only slowly with increasing population size. Recommendations were made as to how to reduce the prevalence of IPNV in salmon farms.

Murray, A.G. and E.J. Peeler.

2005. A Framework for understanding the potential for emerging disease in aquaculture. ***Preventive Veterinary Medicine*** **67:223-235.**

This paper reviews the numerous fish diseases that have emerged as serious economic or ecological problems in aquaculture species. It combines risk-analysis methods and virulence theory with historical examples (mainly from salmonid production) to identify practices that increase risks of disease emergence. The combination of factors behind the emergence of each disease is unique, but various common factors are apparent.

Diseases have emerged through pathogen exchange with wild populations, evolution from nonpathogenic micro-organisms and anthropogenic transfer of stocks. Aquacultural practices frequently result in high population densities and other stresses (such as intercurrent disease) which increase the risk of infection establishment and spread.

Neukirch, M.

1984. An experimental study of the entry and multiplication of viral hemorrhagic septicemia virus in rainbow trout, ***Salmo gairdneri*** **Richardson, after water-borne infection.** ***Journal of Fish Diseases*** **7:231-234.**

Nishizawa, T., H. Iida, R. Takano, T. Isshiki, K. Nakajima, and K. Muroga.

2002. Genetic relatedness among Japanese, American and European isolates of viral hemorrhagic septicemia virus (VHSV) based on partial G and P genes. ***Diseases of Aquatic Organisms*** **48:143-148.**

This paper reports on a study of the relationship between Japanese VHSV isolates and VHSV isolates of known North American and European origin. The study found that at least two different genotypes of VHSV exist in the western part of Japanese coastal areas. One type referred to as the Obama25 isolate was closely related to the North American VHSV. The other type was referred to as the KRRV9601 isolate and was closely related to the European VHSV. The Obama25 type of VHSV is widely distributed in the coastal areas of western Japan, is considered native, and was responsible for the occurrence of VHSV in farmed Japanese flounder. The KRRV9601 type of VHSV is considered to have been introduced from a foreign country.

Nishizawa, T., H. Savas, H. Isidan, C. Ustundag, H. Iwamoto, and M. Yoshimizu.

2006. Genotyping and pathogenicity of viral hemorrhagic septicemia virus from free-living turbot (*Psetta maxima*) in a Turkish coastal area of the Black Sea. ***Applied and Environmental Microbiology*** **72:2373-2378.**

This study was conducted to evaluate if VHSV contributed to mass mortalities of turbot larvae. VHSV isolated from Turbot was classified as type Ie and was not likely introduced from Europe, but was rather indigenous to the Black Sea. Pathogenicity tests showed that VHSV type Ie did not induce mortality in turbot larvae or juvenile rainbow trout.

Noble, A.C., and S.T. Summerfelt.

1996. Disease encountered in rainbow trout cultured in recirculating systems. ***Annual Review of Fish Diseases*** **6:65-92.**

This paper discusses diseases encountered by rainbow trout in hatchery-type environments. Recirculating systems were described as creating environments for fish culture that may provide favorable conditions for disease microorganisms. Stressful conditions in recirculating systems, such as poor water quality or high stocking densities in the culture tanks, may contribute to disease outbreaks. The authors suggested that recirculating facilities prepare a protocol for prevention and control of fish disease. VHS was briefly discussed.

NPS/GPBLSC (National Park Service/Grand Portage Band of Lake Superior Chippewa).

2008. Emergency prevention and response plan for viral hemorrhagic septicemia. National Park System Units and the Grand Portage Indian Reservation within the Lake Superior Basin.

This emergency response plan for VHS in part of the Lake Superior basin (four units of the National Park System in the Lake Superior basin and the Grand Portage Indian Reservation) covers the prevention of the spread of VHSV, the detection of VHSV in surveillance programs, and the response to VHSV detection and outbreaks should they occur. This plan provides a brief background on VHS and aquatic invasive species in general. The plan also included a detailed section on vector analysis, in which various modes of VHS introduction are discussed along with an assessment of the levels of risk.

NYDEC (New York State Department of Environmental Conservation).

2007. VHS detected in dead fish found at Skaneateles Lake. ***Environment DEC*** **(July 2007 issue). Available on the web at http://www.dec.ny.gov/environmentdec/35946.html.** Accessed August 2009.

Ogut, H., and S.C. Bishop.

2007. A stochastic modeling approach to describing the dynamics of an experimental furunculosis epidemic in Chinook salmon, ***Oncorhynchus tshawytshca*** **(Walbaum).** ***Journal of Fish Diseases*** **30:93-100.**

This paper describes the comparison of a susceptible-infected-removed (SIR) stochastic model to a susceptible-latent-infectious-removed (SLIR) stochastic model for describing the dynamics of an experimental furunculosis epidemic. Both models worked well at predicting the number of fish in each category over time for the experimental data, as well as predicting the variability in the data. The authors preferred the use of the SLIR model over the SIR model because latently infected fish were differentiated from infected fish.

OIE (The World Organization for Animal Health).
2006. *Manual of diagnostic tests for aquatic animals.* Available on the web at http://www.oie.int/eng/normes/fmanual/A_summry.htm. Accessed August 2009.

This online manual serves as a standard reference for diagnostic tests for aquatic animals. The manual includes chapters on 17 fish diseases, including VHS. The manual covers laboratory procedures for validating disease samples and also covers surveillance procedures (in order for a region, water body, or aquaculture facility to be recognized as disease-free) and disinfection protocols for aquaculture facilities.

Olesen, N.J.
1998. Sanitation of viral haemorrhagic septicaemia (VHS). *Journal of Applied Ichthyology* 14:173-177. Available on the web at http://www.blackwell-synergy.com/doi/abs/10.1111/j.1439-0426.1998.tb00638.x. Accessed August 2009.

Olesen, N.J., and P.E.V. Jørgensen.
1991. Rapid detection of viral haemorrhagic septicaemia virus in fish by ELISA. *Journal of Applied Ichthyology* 7:183-186. Available on the web at http://www.blackwell-synergy.com/doi/abs/10.1111/j.1439-0426.1991.tb00525.x. Accessed August 2009.

Olesen, N.J., and P.E.V. Jørgensen.
1992. Comparative susceptibility of three fish cell lines to Egtved virus, the virus of viral haemorrhagic septicaemia (VHS). *Diseases of Aquatic Organisms* 12:235-237.

This study investigated the susceptibility of three fish cell lines (BF-2, EPC, and CHSE-214) to Egtved virus (or VHSV). Rainbow trout tissue samples were obtained from farms suspected to have VHSV present and from a population with known carriers of VHSV. The BF-2 cell line detected two times as many VHSV carriers compared to the other two cell lines. The study also noted the value of subcultivating samples prior to final reading, because the susceptibility of a cell line may vary depending on which stock the cell line was obtained from.

Olesen, N.J., N. Lorenzen, and P.E.V Jørgensen.
1993. Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralizing monoclonal and polyclonal antibodies. *Diseases of Aquatic Organisms* 16:163-170.

This study investigated the serological variation among 127 isolates of VHSV using plaque neutralization tests.

Olesen, N.J., N. Lorenzen, and S.E. LaPatra.
1999. Production of neutralizing antisera against viral hemorrhagic septicemia (VHS) virus by intravenous injections of rabbits. *Journal of Aquatic Animal Health* 11:10-16. Available on the web at http://afs.allenpress.com/perlserv/?request=get-abstract&doi=10.1577%2F1548-8667%281999%29011%3C0010%3APONAAV%3E2.0.CO%3B2. Accessed August 2009.

Oli, M.K., M. Venkataraman, P.A. Klein, L.D. Wendland, and M.B. Brown.
2006. Population dynamics of infectious diseases: a discrete time model. *Ecological Modelling* 198:183-194.

This paper presents a framework for modeling infectious disease in discrete time using matrix population models. This model framework may be applied to any infectious disease with discrete disease states. The model allows for the estimation of important disease parameters such as disease reproductive rate. The model also estimates population growth rates of the host animal. The model requires the parameterization of vital rates such as survival and reproduction, which can be obtained from capture-recapture data if available.

Oshimalf, K.H., K.H. Higman, C.K. Arakawa, P. de Kinkelin, P.E.V. Jørgensen, T.R. Meyers, and J.R. Winton.
1993. Genetic comparison of viral hemorrhagic septicemia virus isolates from North America and Europe. *Diseases of Aquatic Organisms* 17:73-80.

This paper indicates that isolates of VHSV in North America did not originate from infected European fish or eggs because of the lack of genetic similarity between North American and European VHSV isolates. This study used the T1 fingerprinting technique to examine genetic variation among VHSV isolates and to determine the possible relatedness of the isolates from the two continents.

Ostfeld, R.S., F. Keesing, and V.T. Eviner, editors.
2008. *Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems.* Princeton University Press, New Jersey.

This book presents a series of essays on infectious disease ecology. The book is divided into three sections that focus on the effects of ecosystems on infectious disease, the effects of infectious disease on ecosystems, and the application of these ideas for disease management. A conceptual framework is developed that works to improve the integration of ecology and traditional disease biology and promote future collaboration among ecologists and other disease biologists.

Pearson, W.H., R.A. Elston, R.W. Bienert, A.S. Drum, and L.D. Antrim.
1999. Why did the Price William Sound, Alaska, Pacific herring (*Clupea pallasi*) fisheries collapse in 1993 and 1994, Review of hypotheses. *Canadian Journal of Fisheries and Aquatic Sciences* 56:711-737.

This paper sets forth hypotheses to explain the herring collapse in Price William Sound. The authors conducted a meta-analysis of literature on stock collapses and focused attention on herring. Poor nutritional status, either alone or in combination with disease or other natural factors, was suggested as the most likely cause of the 1993 herring collapse.

Peeler, E.J., A.G. Murray, A. Thebault, E. Brun, A. Giovaninni, and M.A. Thrush.

2007. The application of risk analysis in aquatic animal health management. *Preventive Veterinary Medicine* **81:3-20.**

This paper discusses the role of risk analysis in the management of aquatic animal health. VHSV is not discussed but the risk analysis applications described in the paper are applicable to managing for VHSV.

Peters, F., and M. Neukirch.

1986. Transmission of some fish pathogenic viruses by the heron, *Ardea cinerea.* ***Journal of Fish Diseases* 9(6):539-544. Available on the web at http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2761.1986.tb01050.x.** Accessed August 2009.

Quillet, E., M. Dorson, S. Le Guillou, A. Benmansour, and P. Boudinot.

2007. Wide range of susceptibility to rhabdoviruses in homozygous clones of rainbow trout. *Fish and Shellfish Immunology* **22:510-519. Available on the web at http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WFN-4KJ06V1-1&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=cafaae1bd7eb87acea95c4219019dde9.** Accessed August 2009.

Quillet, E., S. Le Guillou, P. Boudinot, A. Benmansour, and M. Dorson.

2007. Diverse range of resistance/susceptibility to VHSV (viral haemorrhagic scepticemia virus) and IHNV (infectious hematopoietic necrosis virus) evidenced in homozygous clones of rainbow trout. *Aquaculture* **(Supplement 1) 272:S302-S303.**

Raja-Halli, M., T.K. Vehmas, E. Rimaila-Pärnänen, S. Sainmaa, H.F. Skall, N.J. Olesen, and H. Tapiovaara.

2006. Viral haemorrhagic septicaemia (VHS) outbreaks in Finnish rainbow trout farms. *Diseases of Aquatic Organisms* **72:201-211.**

This paper documents phylogenetics and pathogenicity of VHSV in Finland. Phylogenetic analyses showed VHSV in Finland to be closely related to freshwater VHSV isolates from rainbow trout in Denmark and to one marine isolate from cod in the Baltic Sea. Infection experiments (water immersion and intraperitoneal injection) showed a lower pathogenicity of the Finland VHSV compared to freshwater VHSV isolates. The authors suggest that the Findland VHSV may represent an intermediate stage of VHSV that may evolve towards higher pathogenicity in rainbow trout.

Reinert, R.E., B.A. Knuth, M.A. Kamrin, and Q.J. Stober.

1991. Risk assessment, risk management, and fish consumption advisories in the United States. *Fisheries* **16:5-12.**

This article describes the issues concerning the confusion and controversy between states in regards to the safety of eating fish. Though the Food and Drug Administration is primarily responsible for issuing safety regulations, the states bear the responsibility to manage risks associated with various contaminants in fish. To help eliminate some of the confusion surrounding these issues, the paper breaks down concerns in an effort to increase fisheries professionals', as well as anglers', understanding of the distribution and interpretation of the advisories issued. This paper does not discuss VHS, but the issues surrounding fish consumption advisories and risk assessment and management are applicable to management of the VHS problem.

Reno, P.W.

1998. Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health* **10:160-171.**

This paper reviews the principles of epizootiology relating to the spreading, distribution, dynamics, and control of infectious diseases in fish populations. The dispersal of disease in fish populations has not been studied as vigorously as it has in human and other mammal populations. With this in mind the paper takes many of the same factors which affect how disease impinges on mammal populations and parallels them to fish populations. This report does not cover factors involved in how disease comes into a population. However, it does extensively cover how a disease impacts a population based on interactions with the host, agent, and environment.

Ristow, S.S., N. Lorenzen, and P.E.V. Jørgensen.

1991. Monoclonal-antibody-based immunodot assay distinguishes between viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV). *Journal of Aquatic Animal Health* **3:176-180. Available on the web at http://afs.allenpress.com/perlserv/?request=get-abstract&doi=10.1577%2F1548-8667%281991%29003%3C0176%3AMABIAD%3E2.3.CO%3B2.** Accessed August 2009.

Ross, K., U. McCarthy, P.J. Huntly, B.P. Wood, D. Stuart, E.I. Rough, D.A. Smail, and D.W. Bruno.

1994. (VHS) in turbot (*Scophthalmus maximus*) in Scotland. *Bulletin of the European Association of Fish Pathologists* **14:213-214.**

Rottmann, R.W., R. Francis-Floyd, and R. Durborow.

1992. The role of stress in fish disease. Southern Regional Aquaculture Center Publication No. 474. Available on the web at http://aquanic.org/publicat/usda_rac/efs/srac/474fs.pdf. Accessed August 2009.

This paper reviews the role of stress in fish disease, with emphasis on fish in aquaculture.

Sano, T.

1995. Viruses and viral disease of salmonids. *Aquaculture* **132:43-52.**

This article reviews and discusses viral diseases and pathologic conditions of salmonids. In addition to already well known viruses, information on new agents and the diseases they cause is included in this review. VHS is not discussed in detail and is only listed in tables.

Schlotfeldt, H.J., W. Ahne, P.E. Vestergård-Jørgensen, and W. Glende.

1991. Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – A natural outbreak. *Bulletin of the European Association of Fish Pathologists* 11:105-107.

Skall, H.F., N.J. Olesen, and S. Mellergaard.

2005a. Prevalence of viral haemorrhagic septicaemia virus in Danish marine fishes and its occurrence in new host species. *Diseases of Aquatic Organisms* 66:145-151.

This paper describes a follow-up study to that presented by Mortensen et al. (1998) concerning VHSV in marine waters surrounding Denmark. Of note is documentation of the first isolation of VHSV from dab, flounder, and plaice and the first publication on VHSV in European sand eel and sand goby.

Skall, H.F., N.J. Olesen, and S. Mellergaard.

2005b. Viral haemorrhagic septicemia virus in marine fish and its implications for fish farming – a review. *Journal of Fish Diseases* 28:509-572.

This paper reviews the phylogenetics and pathogenicity of VHSV. Also discussed is the transfer of VHSV from free-living marine fish to farmed fish and possible measures to prevent the spread of VHSV from the marine environment to aquaculture.

Skall, H.F., W.J. Slierendrecht, J.A. King, and N.J. Olesen.

2004. Experimental infection of rainbow trout *Oncorhynchus mykiss* with viral haemorrhagic septicaemia virus isolates from European marine and farmed fishes. *Diseases of Aquatic Organisms* 58:99-110.

This paper describes the susceptibility of rainbow trout to infection with various isolates of VHSV. A total of 8 experiments with rainbow trout ranging from 0.6 to 6.2 g was conducted for 139 isolates originating from wild marine fishes in European waters (115 isolates), farmed turbot from Scotland and Ireland (2 isolates), and farmed rainbow trout (22 isolates). The isolates were tested by immersion and intraperitoneal injection either as pooled or single isolates.

Results showed that VHSV isolates from wild marine fishes were generally non-pathogenic or of very low virulence by immersion challenge for rainbow trout of the size-group tested. Significant mortality with clinical signs of VHS was observed when wild marine fish isolates were given by intraperitoneal injection. All VHSV isolates from farmed rainbow trout caused significant mortality by immersion. Currently, pathogenicity trials are the only way to differentiate VHSV isolates from wild marine fishes and farmed rainbow trout. The two farmed turbot isolates did not cause mortality by immersion, supporting the view that they originated from the marine environment.

Snieszko, S.F.

1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology* 16:197-208.

This paper provides a selected literature review on the link between environmental stress and infectious disease in fish. Reviewed examples of environmental stresses coincident with infectious disease in fish include temperature, eutrophication, sewage, metabolic products of fishes, industrial pollution, and pesticides.

Snow, M.

2006. Risks to wild freshwater fisheries from viral haemorrhagic septicaemia (VHS) disease. Fisheries Research Services, Scotland. Available on the web at http://www.frs-scotland.gov.uk/Delivery/Information_resources/Information_resources_view_document.aspx?resourceId=31141&documentId=1957. Accessed August 2009.

This web-based paper published by Fisheries Research Services of Scotland discusses the risks of spreading VHSV from fish farms to wild fish populations. The author concludes from available evidence that high infection pressure would be necessary for VHS to be initiated in a wild population.

Snow, M., N. Bain, J. Black, V. Taupin, C.O. Cunningham, J.A. King, H.F. Skall, and R.S. Raynard.

2004. Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* 61:11-21.

This paper describes the phylogenetics or genetic population structure of VHSV and identifies the geographic distributions of four identified types of VHSV. Of note is the conclusion that VHSV in rainbow trout in Europe is of marine origin. The authors also discuss future control of VHSV in light of their findings and highlight the use of molecular phylogenetic analysis as a tool for addressing complex epidemiological problems.

Snow, M. and C.O. Cunningham.

2000. Virulence and nucleotide sequence analysis of marine viral haemorrhagic septicaemia virus following in vivo passage in rainbow trout *Onchorhynchus mykiss*. *Diseases of Aquatic Organisms* 42:17-26.

This paper describes a study of the susceptibility of pathogen-free juvenile rainbow trout to a representative marine isolate of VHSV. The rainbow trout showed moderate susceptibility to VHSV following intraperitoneal infection. This route of infection was chosen for in vivo passage because all marine VHSV isolates tested to date had proved to be avirulent or of low virulence to rainbow trout via water immersion. Following passage in rainbow trout, the VHSV isolate showed a higher virulence for rainbow trout as compared to unpassaged VHSV. The mechanisms responsible for the observed increase in virulence of the VHSV isolate following passage in rainbow trout remain unknown. The possibility that viral isolates may exhibit an increased virulence following passage in novel host species has important implications with regard to the epidemiology of VHSV.

Stone, M.A.B., S.C. MacDiarmid, and H.J. Pharo.

1997. Import health risk analysis: salmonids for human consumption. Ministry of Agriculture Regulatory Authority, New Zealand.

This document presents a risk analysis examining the disease risks to New Zealand associated with imports of salmonid fish for human consumption. VHSV is covered under the family rhabdoviridae starting on page 87. This section of the paper touches on the background of the disease along with the susceptibility, distribution, pathology, epidemiology, control, survival and inactivation of VHS plus IHNV.

Tafalla, C., A. Figueras, and B. Novoa.

1998. In vitro interaction of viral haemorrhagic septicaemia virus and leukocytes from trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*). *Veternary Immunology and Immunopathology* 62:359-366. Available on the web at http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%235189%231998%23999379995%2343710%23FLA%23&_cdi=5189&_pubType=J&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=916b810cd4648fddf40869a692bc150b. Accessed August 2009.

Thiéry, R., C. de Boisséson, J. Jeffroy, J. Castric, P. de Kinkelin, and A. Benmansour.

2002. Phylogenetic analysis of viral haemorrhagic septicaemia virus (VHSV) isolates from France (1971-1999). *Diseases of Aquatic Organisms* 52:29-37.

This paper describes a genetic analysis of VHSV isolates from France to delineate the diversity and spread of VHSV. Even though similar studies have been reported to classify VHSV strains from distant geographical regions of the world this was the first study on a large number of virus isolates from the same country. Genetic analysis was performed by comparison of a variable region of the glycoprotein gene, designated V2, which accumulates most of the mutations. In this study, sequencing was restricted to the V2 region of the glycoprotein gene. However, phylogenetic analysis showed that the sequence information contained in this region was sufficient to construct phylogenetic trees that were topologically similar to trees obtained after sequencing the entire open reading frame of the G gene. Therefore, V2 sequencing could provide a suitable molecular tool to investigate the origin of a local outbreak and thus would help to understand the epidemiology of the disease.

Traxler, G.S., D. Kieser, and J. Richard.

1999. Mass mortality of pilchard and herring associated with viral hemorrhagic septicemia virus in British Columbia, Canada. *American Fisheries Society, Fish Health Section Newsletter* 27(4):4-5. Available on the web at http://www.fisheries.org/units/fhs/Newsletter_Files/V27-4_1999.pdf. Accessed August 2009.

This Fish Health Section Newsletter article describes a "massive" fish kill involving Pacific herring and pilchards near British Columbia. This fish kill was attributed to VHSV. The authors stress the importance of using multiple fish cell lines in testing for VHSV to increase the detection of VHSV in fish health surveys.

USDA/APHIS (United States Department of Agriculture/Animal and Plant Health Inspection Service).

2007. Species affected by the viral hemorrhagic septicemia (VHS) federal order. Available on the web at http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/vhs_fed_order.pdf. Accessed August 2009.

In this downloadable PDF the USDA, APHIS, and Veterinary Services identified a list of species as having originated in freshwater locations in the United States and/or Canada, and as having been infected by VHS virus under natural (i.e. non-experimental) conditions of exposure; and from which VHS virus has been isolated by cell culture, with confirmation of strain identity through molecular detection. Anadromous fish species that have migrated into freshwater and from which VHS strain type IV(a) is isolated are excluded from this definition.

USDA/APHIS/CEAH (United States Department of Agriculture/Animal and Plant Health Inspection Service/Centers for Epidemiology and Animal Health).

2006. Viral hemorrhagic septicemia in the Great Lakes: July 2006 emerging disease notice. Available on the web at http://www.aphis.usda.gov/vs/ceah/cei/taf/emergingdiseasenotice_files/vhsgreatlakes.htm. Accessed August 2009.

This document provides geographical information on known VHS outbreaks. It also reviews the economical impact the virus is capable of having on fish production in the United States.

USDA/APHIS/VS (United States Department of Agriculture/Animal and Plant Health Inspection Service/Veterinary Services).

2006. Viral hemorrhagic septicemia in Great Lakes region. *APHIS Industry Alert*. Available on the web at http://www.aphis.usda.gov/publications/animal_health/content/printable_version/ia_VHS_Great_Lakes.pdf. Accessed August 2009.

This is a one page document by APHIS summarizes VHS in the Great Lakes Region and provides information on how to prevent VHS in aquaculture facilities.

WDNR (Wisconsin Department of Natural Resources).

2007a. Fish likely infected with deadly virus found in Little Lake Butte des Morts; Menasha lock closed; public asked to take steps to stop the spread. WDNR News Release (13 May 2007). Available on the web at http://dnr.wi.gov/news/BreakingNews_Lookup.asp?id=543. Accessed August 2009.

WDNR (Wisconsin Department of Natural Resources).
2007b. Lake Winnebago fish preliminarily test positive for VHS. WDNR News Release (18 May 2007). Available on the web at http://dnr.wi.gov/news/BreakingNews_Lookup.asp?id=557. Accessed August 2009.

WDNR (Wisconsin Department of Natural Resources).
2007c. Trout from Lake Michigan likely VHS positive. WDNR News Release (24 May 2007). Available on the web at http://dnr.wi.gov/news/BreakingNews_Lookup.asp?id=562. Accessed August 2009.

WDNR (Wisconsin Department of Natural Resources).
2007d. Wisconsin VHS testing of fish, 2006-2007. Available on the web at http://dnr.wi.gov/fish/documents/vhs_wifishtesting.pdf. Accessed August 2009.

WDNR (Wisconsin Department of Natural Resources).
2008a. VHS fish disease found in gobies washed ashore in Milwaukee. WDNR News Release (5 June 2008). Available on the web at http://dnr.wi.gov/news/BreakingNews_Lookup.asp?id=846. Accessed August 2009.

WDNR (Wisconsin Department of Natural Resources).
2008b. VHS fish disease found in yellow perch in Milwaukee. WDNR News Release (13 June 2008). Available on the web at http://dnr.wi.gov/news/BreakingNews_Lookup.asp?id=864. Accessed August 2009.

Whelan, G.E.
2007a. VHS—the viral invader. ANS Update 13(1):1. Available on the web at http://www.glc.org/ans/ansupdate/pdf/2007/ansUpdate-spring07.pdf. Accessed August 2009.

Whelan, G.E.
2007b. Viral hemorrhagic septicemia (VHS) briefing paper. MDNR (Michigan Department of Natural Resources). Available on the web at http://www.michigan.gov/documents/dnr/Viral-Hemorrhagic-Septicemia-Fact-Sheet-11-9-2006_178081_7.pdf. Accessed August 2009.

Whittington, R.J., and R. Chong.
2007. Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Preventive Veterinary Medicine* 81:92-116.

This paper does not specifically address VHSV but discusses risk analysis and management strategies that may be applicable to VHSV management. The purpose of this study was to review the risk and the effectiveness of risk management for disease translocation by ornamental fish. The import policies for ornamental fish between countries were compared and the efficacy of such policies was assessed using Australia as an example, because of its relatively strict policies. The role of molecular epidemiology and ecological studies of alien fish, plants and mollusks are discussed in relation to hazard identification and risk assessment, respectively. It is concluded that international trade in ornamental fish poses a threat to transboundary biosecurity, biodiversity and future development of aquaculture industries, and that this trade represents a special case for risk analysis under the Sanitary and Phytosanitary agreement, requiring an alternate approach.

Williams, B.K., J.D. Nichols, and M.J. Conroy.
2002. *Analysis and management of animal populations.* Academic Press, New York.

This book presents a comprehensive synthesis of methods used to determine the status of animal populations, including the use of models and the analysis of capture-recapture data. The book is divided into four sections that cover a methodological framework for quantifying and modeling field observations, a comprehensive framework for dynamic modeling of populations, the estimation of population vital rates using sampling data, and decision analysis and optimal management of populations.

Winton, J.R., W. Batts, R. Deering, R. Brunson, K. Hopper, T. Nishizawa, and C. Stehr.
1991. Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. Pages 43-50 In *Second international symposium on viruses of lower vertebrates.* Oregon State University, Corvallis.

Winton, J., B. Batts, and G. Kurath.
2007. Detection of viral hemorrhagic septicemia virus. USGS FS 2007-3055. Available on the web at http://wfrc.usgs.gov/pubs/factsheetpdf/vhsfs080807.pdf. Accessed August 2009.

This brief document describes procedures for detecting VHSV in samples and provides background information on the history of VHSV type IVb in North America. Also included are results on producing cell cultures of VHSV at various temperatures. VHSV type IVb grew best at 15°C, plating efficiency began declining at 20°C, and the isolate did not grow at 25°C.

Winton, J., B. Batts, and G. Kurath.
2008. Molecular epidemiology of viral hemorrhagic septicemia virus in the Great Lakes region. L. Thorsteinson, and D. Becker, editors. USGS FS 2008-3003. Available on the web at http://wfrc.usgs.gov/pubs/factsheetpdf/vhsfs2011108.pdf. Accessed August 2009.

This brief document presents a recent update of the molecular epidemiology of VHSV in the Great Lakes Region and includes a phylogenetic analysis of Great Lakes isolates of VHSV.

Wolf, K.
1976. Fish viral diseases of the USA, 1971-1975, and recent research of the Eastern Fish Disease Laboratory, USA. *Fish Pathology* 10:135-154.

Wolf, K.
1988. Viral hemorrhagic septicemia. Pages 217-249 In K. Wolf, editor. *Fish viruses and fish viral diseases.* Cornell University Press, New York.

Wootton, R. J.
1991. *Ecology of teleost fishes.* Chapman and Hall, New York.

Yamamoto, T., W.N. Batts, and J.R. Winton.
1992. In vitro infection of salmonid epidermal tissues by infectious hematopoietic necrosis virus and viral hemorrhagic septicemia virus. *Journal of Aquatic Animal Health* 4:231-239.

This report presents the results of in vitro experiments with infectious hematopoietic necrosis virus (IHNV) and VHSV infections of excised skin and gill tissues. A virulent strain of VHSV (23/75) was effectively replicated in excised gill tissues and epidermal tissues of rainbow trout and chinook salmon. The avirulent North American strain of VHSV (type IVa) replicated poorly or not at all.

The results of the study further support the hypothesis that epidermal tissue may be an important site of virus entry and early virus replication. The in vitro experiments showed that certain epithelial cells from different regions of the fish's body were capable of supporting early IHNV and VHSV replication. Therefore, the entry of these viruses into internal tissues of the fish could occur not only through gills, but also through a variety of epidermal tissues, especially those covering the fins.

APPENDIX 1.

Selected glossary of terms.

Egtved virus: The name first given to the rhabdovirus that causes viral hemorrhagic septicemia in rainbow trout; discovered in Egtved, Denmark.

enzootic: Constantly present in an animal community, but usually only affecting a small number of animals at any one time.

epidemiology: The study of factors affecting the incidence, distribution, and control of disease in a population.

epizootic: An outbreak of disease affecting many animals of one kind at the same time.

in vitro: Technique of performing a given experiment in a controlled (e.g., laboratory) environment outside of a living organism.

in vivo: Experimentation done in or on the living tissue of a whole, living organism as opposed to a partial or dead organism or in a controlled environment.

isolate: Viral material separated from host tissue. The process of virus isolation includes recovery of the virus from the host and identification of the virus.

pathogenicity: Causing or capable of causing disease.

titer: A measurement of the amount or concentration of a substance in a solution.

virology: The study of viruses and viral diseases.

virulence: The degree of pathogenicity of a microbe, or the relative ability of a microbe to cause disease.

APPENDIX 2.

Common and scientific names of fish referenced in the report and annotated bibliography.

Common Name	Scientific Name
Arctic char	*Salvelinus alpinus*
Atlantic cod	*Gadus morhua*
Atlantic herring	*Clupea harengus*
Atlantic salmon	*Salmo salar*
Black crappie	*Pomoxis nigromaculatus*
Bluegill	*Lepomis macrochirus*
Bluntnose minnow	*Pimephales notatus*
Brook trout	*Salvelinus fontinalis*
Brown trout	*Salmo trutta*
Burbot	*Lota lota*
Channel catfish	*Ictalurus punctatus*
Chinook salmon	*Oncorhynchus tshawytscha*
Coho salmon	*Oncorhynchus kisutch*
Dab	*Limanda limanda*
Eel	*Anguilla anguilla*
Emerald shiner	*Notropis atherinoides*
Eulachon (smelt)	*Thaleichthys pacificus*
Flounder	*Platichthys flesus*
Freshwater drum	*Aplodinotus grunniens*
Gizzard shad	*Dorosoma cepedianum*
Grayling	*Thymallus arcticus*
Japanese flounder	*Paralichthys olivaceus*
Lake trout	*Salvelinus namaycush*
Lake whitefish	*Coregonus clupeaformis*
Mummichog	*Fundulus heteroclitus*
Muskellunge	*Esox masquinongy*
Northern pike	*Esox lucius*
Pacific cod	*Gadus macrocephalus*
Pacific hake	*Merluccius productus*
Pacific herring	*Clupea pallasi*
Pacific mackerel	*Scomber japonicus*
Pacific sand lance	*Ammodytes hexapterus*
Pink salmon	*Oncorhynchus gorbuscha*
Plaice	*Pleuronectes platessa*
Rainbow trout	*Oncorhynchus mykiss*
Redhorse sucker	*Moxostoma* sp.
Rock bass	*Ambloplites rupestris*
Round goby	*Neogobius melanostomus*
Sand eel	*Ammodytes tobianus*
Sand goby	*Pomatochistus minutus*
Shorthead redhorse	*Moxostoma macrolepidotum*
Smallmouth bass	*Micropterus dolomieu*
Sockeye salmon	*Oncorhynchus nerka*
Spottail shiner	*Notropis hudsonius*
Sprat	*Sprattus sprattus*
Stickleback	*Gasterosteus aculeatus*
Striped bass	*Morone saxatilis*
Surf smelt	*Hypomesus pretiosus*
Turbot (1)	*Psetta maxima*
Turbot (2)	*Scophthalmus maximus*
Walleye	*Sander vitreous*
Walleye pollock	*Theragra chalcogramma*
White bass	*Morone chrysops*
Yellow perch	*Perca flavescens*

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