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Principles Underlying the Epizootiology of Viral Hemorrhagic Septicemia in Pacific Herring and other Fishes throughout the North Pacific Ocean

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Perspective:
Principles Underlying the Epizootiology of Viral Hemorrhagic Septicemia in Pacific Herring and
other Fishes throughout the North Pacific Ocean

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Abstract:

Although viral hemorrhagic septicemia virus (VHSV) typically occurs at low prevalence and intensity in natural populations of Pacific herring (*Clupea pallasii*) and other marine fishes in the NE Pacific Ocean, epizootics of the resulting disease (VHS) periodically occur, often in association with observed fish kills. Here we identify a list of principles, based on a combination of field studies, controlled laboratory experiments, and previously un-published observations, that govern the epizootiology of VHS in Pacific herring. A thorough understanding of these principles provides the basis for identifying risk factors that predispose certain marine fish populations to VHS epizootics; including the lack of population resistance, presence of chronic viral carriers in a population, copious viral shedding by infected individuals, cool water temperatures, limited water circulation patterns, and gregarious host behavioral patterns. Further, these principles are used to define the epizootiological stages of the disease in Pacific herring, including the susceptible (where susceptible individuals predominate a school or sub-population), enzootic (where infection prevalence and intensity are often below the limits of reasonable laboratory detection), disease amplification (where infection prevalence and intensity increase rapidly), outbreak (often accompanied by host mortalities with high virus loads and active shedding), recovery (in which the mortality rate and virus load decline due to an active host immune response) and refractory stages (characterized by little or no susceptibility and where viral clearance occurs in most VHS survivors). In addition to providing a foundation for quantitatively assessing the potential risks of future VHS epizootics in Pacific herring, these principles provide insights into the epizootiology of VHS in other fish communities where susceptible species exist.

46 Introduction

47 As a dominant member of the forage fish assemblage in the North Pacific Ocean, Pacific
48 herring (*Clupea pallasii*) provide a critical link in the flow of energy and nutrients from lower
49 trophic levels to higher order predators. Forage fishes in many systems, particularly those driven
50 by upwelling currents, are regarded as the major determinant of ecosystem productivity through
51 a concept commonly referred to as the ‘wasp waist hypothesis’ (Fauchald et al. 2011). In contrast
52 to bottom-up or top-down regulation of ecosystem function, the wasp waist hypothesis contends
53 that constraints on ecosystem productivity are set at the forage fish level, rather than at the basal
54 level of primary production or at the level of higher-order predators (Cury et al. 2000). As such,
55 any perturbations to the size or species assemblages of forage fish communities are expected to
56 resonate throughout both the upper and lower trophic levels. It is likely then, that functional
57 change in ecosystem structure is mediated by oscillations in forage fish species abundance.
58 Causes of these naturally-driven population fluctuations remain particularly difficult to study;
59 however, mortality from infectious and parasitic diseases is believed to be an important factor in
60 some forage fish populations (Marty et al. 1998, 2003, 2010).

61 Once thought to be solely a problem of the European rainbow trout industry, viral
62 hemorrhagic septicemia virus (VHSV) is now known to occur in freshwater and marine fishes
63 throughout the northern hemisphere (Kim & Faisal 2011), with different genotypes and subtypes
64 occurring throughout this range (Snow et al. 2004). Genotype IVa has a broad natural host range
65 in marine fishes throughout the North Pacific Ocean, including wild and cultured species in
66 North America and Asia, where it is responsible for periodic disease epizootics (Isshiki et al.
67 2001; Hedrick 2003; Garver et al. 2013). Throughout this range, Pacific herring and other forage
68 fishes are extremely susceptible to the resulting disease; as such, they play a critical role in its

epizootiology. This observation creates an apparent paradox in our understanding of the disease, whereby the highly-virulent virus persists for extended periods in populations of a highly-susceptible host, yet disease epizootics occur only infrequently (Figure 1). The intent of this manuscript is to identify the principles controlling the epizootiology of VHS in the North Pacific by combining the results of previously-published field investigations and controlled laboratory studies (primarily involving Pacific herring) with previously unpublished observations of VHS in Pacific herring. These principles are then used to describe the epizootiological stages of the disease, including the enzootic periods and the irreversible cascade of events that culminate in epizootics. Throughout the manuscript, we focus our attention primarily on Pacific herring because the vast majority of cause-and-effect relationships in marine fish populations of the NE Pacific have been established with this host species. However, the principles and concepts discussed here are broadly applicable to Pacific sardines (*Sardinops sagax*; Arkoosh et al. 2006), Pacific sandlance (*Ammodytes hexapterus*; Kocan et al. 2001a), and other susceptible forage fishes in the NE Pacific (Hedrick et al. 2003) and other VHSV enzootic areas (Altuntas et al. 2010).

Principle #1: Pacific herring are exceptionally susceptible to VHSV

In the North Pacific, Pacific herring are often infected with multiple pathogens, including VHSV, *Ichthyophonus hoferi*, erythrocytic necrosis virus (ENV), liver and intestinal coccidians, nematodes, cestodes, and sea lice. (Marty et al. 1998). While most are generally considered secondary pathogens of negligible importance as a proximate mortality factors, others (including VHSV, *Ichthyophonus sp.* and ENV) are primary pathogens of Pacific herring and can cause direct host mortality (Kocan et al. 1997, 1999; Hershberger et al. 2009). Recurring epizootics of VHS have caused fish kills in Pacific herring and other forage fishes (Garver et al. 2013). These

observations are supported by numerous controlled laboratory studies which indicate that naïve Pacific herring are highly susceptible to VHS (Kocan et al. 1997; Hershberger et al. 2007, 2010). Pacific herring, therefore, may be considered an exceptionally susceptible host species, with waterborne exposure levels as low as 10^1 virus particles (plaque-forming units, PFU) / mL capable of initiating epizootics in laboratory exposures (Hershberger et al. 2011a). Additionally, the virus is capable of infecting and killing previously-naïve Pacific herring when injected into the body cavity at a calculated dose of 0.07 PFU / fish, a level below the detection threshold of a standard viral plaque assay (Hershberger et al. 2011a).

Principle #2: Pacific herring are super-shedders of VHSV

After exposure to the virus and successful establishment of infection, Pacific herring and other species shed copious amounts of VHSV into the water (Kocan et al. 1997; Hershberger et al. 2010a; Kim & Faisal 2012). Shed VHSV can be detected in the water as early as 4-5d post-exposure, prior to the onset of host mortality from the resulting disease. Viral shedding peaks 6-10d post-exposure and high levels of shed virus are no longer detectable after 16d. During the shedding peak, each diseased host sheds an average of 500 million PFU into the water each day (Hershberger et al. 2010a). The progression of viral shedding is extremely temperature-dependent, with lower temperatures generally resulting in higher shedding levels and delayed peaks in viral shedding (Hershberger et al 2013). It is likely that low-level viral shedding continues beyond 16d, after which infectious waterborne virions are generally no longer detectable using standard virus isolation procedures. In the wild, an abundance of super-shedders in a population likely results in the rapid amplification of exogenous virus, thereby providing a critical early step in the irreversible cascade of events that culminate in a VHS outbreak.

Principle #3: Pacific herring serve as reservoirs of VHSV

Impediments to our understanding of the ecology of VHSV in the Pacific Ocean previously resulted from negative surveillance data, inadequate sampling efforts that have been directed mostly towards species of high economic value, and incorrect assumptions regarding the potential reservoirs of VHSV in the marine environment. Surveys of wild marine fishes intended to identify reservoir hosts typically returned very low VHSV infection prevalence (Kent et al. 1998; Mortenson et al. 1999; King et al. 2001; Dixon et al. 2003; Gadd et al. 2011; Matejusova et al. 2010), except for the intermittent detection of VHSV at low prevalence and intensity in Pacific herring (Kent et al. 1998) and returning adult Pacific salmonids (Winton et al. 1991). However, the low species susceptibility of Pacific salmonids indicated that they represented an inadvertent host (Follett et al. 1997) and not a reliable reservoir capable of maintaining VHSV between epizootics. Pacific herring and other highly susceptible species were initially excluded as important reservoir hosts because standard 60-fish tissue samples frequently failed to test positive. Further, when laboratory studies indicated the extreme susceptibility and high mortality of Pacific herring following exposure to VHS virus, these data appeared to be incongruous with the classic perception of a reservoir host having a relatively low susceptibility to the disease.

However, the involvement of Pacific herring and likely other highly susceptible fishes including Pacific sardines, walleye pollock (*Theragra chalcogramma*), and Pacific sandlances in the perpetuation of VHSV in the NE Pacific was recently recognized by combining lines of evidence from field observations, manipulations of wild herring, and controlled laboratory studies. Although surveys of wild herring utilizing sensitive VHSV diagnostics typically fail to return VHSV-positive tissue samples, the confinement of wild herring into net pens or laboratory tanks often results in rapid escalation of VHS epizootics that involve nearly all the confined

individuals (Kocan et al. 2001). This observation indicated that VHSV is maintained covertly in populations of wild Pacific herring at an extremely low prevalence that is often undetectable even by the highly sensitive cell culture and quantitative PCR techniques (Garver et al. 2011) on >60 fish samples; however, the virus can quickly express in the same population in response to stressful physical conditions including capture, handling, transport, and/or confinement of these individuals. The mechanism(s) of viral persistence in these populations likely involves some combination of chronic infections among neurotropic carriers (Lovy et al. 2012), active infections with viral shedding by a very small percentage of individuals (Hershberger et al. 2010a), and low-level replication of the virus in partially-immune individuals (Hershberger et al. 2010b).

Principle #4: Co-factors influence the potential for VHS epizootics

Numerous host and environmental factors influence the potential for VHS outbreaks, including ambient temperatures, weather / climatic conditions, diet, and conditions that impact infection pressures including water exchange rates, gregarious host behaviors, and proximity to VHSV reservoir species. The susceptibility of Pacific herring to VHS is inversely related to ambient seawater temperature, as evidenced by higher cumulative mortalities, greater viral shedding, and longer viral persistence in the tissues of survivors at cooler temperatures (Hershberger et al. 2013). Once shed into the water, the stability of exogenous VHSV increases with the amount of proteinaceous material in the water (especially fish spawning products; Kocan et al. 2001b), decreasing salinity, decreasing ambient temperatures (Hawley & Garver 2008), and decreased ultraviolet irradiation (Oye and Rimstad 2001). Although the effects of natural diet items on host susceptibility remain uninvestigated, the provision of various commercially-available pelleted feeds to captive herring results in host susceptibility differences

(Beaulaurier et al. 2012). Tidal and wind-driven water circulation patterns can influence the titer of waterborne VHS virus within fjords and embayments where limited water exchange may occur; additionally, advection currents from an area with a localized epizootic may result in expansion of localized epizootics to much broader areas as modeled for other aquatic viruses (Foreman et al. 2015; Salama and Rabe 2013). Finally, certain predator-avoidance behaviors of Pacific herring and other pelagic forages fishes, including the coalescence of schools and shoals into extremely tight aggregations, are conducive to transmission of VHSV.

Principle #5: Acquired Resistance is a critical determinant of VHS potential

Although immunologically naïve Pacific herring are highly susceptible to VHS, survivors of the disease develop, at optimal temperatures, a long-lived adaptive immunity that results in virus clearance and resistance to the disease. VHS progression in susceptible Pacific herring can be extremely rapid, with detectable levels of virus occurring in the tissues of exposed herring as early as 2 d post-exposure (P. E.), and mortality from the disease occurring as early as 4d P. E. (Kocan et al. 1997; Hershberger et al. 2010c). However, the pace, intensity, and outcome of the disease are highly dependent on several variables including exposure level and duration, temperature, diet, water exchange rate, and other factors (Hershberger et al. 2011a, 2013). Regardless of the disease progression and magnitude, herring surviving an active case of VHS develop solid resistance to future recurrences of the disease even after their subsequent exposure to high levels of VHSV (Hershberger et al. 2010c). Therefore, the susceptibility of Pacific herring to VHS (and the potential for VHS epizootics) typically decreases with the host age; not as a result of an increased innate immune response; rather because older cohorts are more likely to have survived prior infection and developed acquired resistance. This acquired resistance supersedes all other disease co-factors, and a resistant population will not experience a VHS

epizootic even if all other disease co-factors (i.e. exposure to high levels of the virus, cool temperatures, tight aggregations, etc.) are present. However, situations can occur where older age cohorts remain naïve to the virus and retain their high level of susceptibility to the disease (Garver et al. 2013); indeed, several VHS epizootics have been documented in wild adult herring and lifelong susceptibility is seen in laboratory colonies of adult Pacific herring that were reared under specific pathogen-free conditions.

Stages of VHS Epizootics

Taken together, these five principles provide insights into the developmental stages of VHS epizootics. These epizootiological stages include the susceptible (where susceptible individuals predominate a school or sub-population), enzootic (where infection prevalence and intensity are often below the limits of reasonable laboratory detection), disease amplification (where infection prevalence and intensity increase rapidly), outbreak (often accompanied by host mortalities with high virus loads and active shedding), recovery (in which the mortality rate and virus load decline due to a robust host immune response) and refractory stages (characterized by little or no susceptibility and where viral clearance occurs in most VHS survivors). By identifying the particular epizootiological stage of a population, insights can be gained into the potential for future epizootics.

Susceptible Stage

As there is no evidence for vertical transfer of humoral immunity from parents to progeny, all herring and other susceptible fishes are born into the VHS-susceptible stage. Although newly-hatched herring larvae are susceptible to VHS (Hershberger et al. 2007), natural

exposure to the virus does not likely occur until sometime after the 2-3 mo larval period, when newly-metamorphosed juvenile cohorts begin to venture beyond the nearshore areas and become sympatric with other reservoir fishes. As such, expansive geographic areas often experience a large influx of susceptible cohorts near the end of July / beginning of August, as massive schools of newly-metamorphosed Pacific herring juveniles begin sharing habitat with potential VHSV carriers in older age cohorts. This large annual influx of susceptibles can tip the overall balance of population resistance within geographic locations, thereby increasing the potential for epizootics.

As herring do not possess a high level of innate resistance to VHS, their susceptibility to the disease persists until after they experience infection with the virus and subsequently develop adaptive immunity. Therefore, the probability of herring converting from the susceptible stage to the refractory stage increases with host age, as the probability of surviving prior infection with the virus also increases with time. However, it should be emphasized that older herring age cohorts can remain susceptible to the disease if they have never experienced prior exposure, as is evidenced by documentation of periodic VHS epizootics involving adult herring throughout coastal waters of British Columbia, Canada (Garver et al. 2013), and documented epizootics among confined herring cohorts that were predominated by newly-recruited age 3-4 yr cohorts (Hershberger et al. 1999).

Enzootic Stage

Under typical enzootic situations, an extremely low prevalence of VHSV infections often persists-in and cycles-through susceptible schools of Pacific herring. As VHSV is not vertically passed from parents to progeny (Wolf 1988), the first infections in each susceptible school likely

occur as a result of exposure to shed virus from sympatric schools of Pacific herring, Pacific sandlance, Pacific sardines, or other natural reservoirs. Once the school is exposed, the infection prevalence often remains below the reasonable detection limits of standard field surveillance techniques. However, the existence of VHS virus in these schools is verified by the detection of shed VHS virus in the static water used to transport several thousand live individuals from these pre-patent schools back to the laboratory (Kocan et al. 2001a). Further, rapid escalation of VHS epizootics results after these individuals are confined into laboratory tanks, net pens, or other areas with limited water exchange (Kocan et al. 2001a; Hershberger et al. 1999). The enzootic prevalence of these virus-positive individuals likely changes throughout the year (Altunas & Ugut 2010), however, their presence in a population can be further confirmed by the non-random selection of symptomatic individuals from a collection. For example, during beach seining efforts in the San Juan Island regions of Puget Sound, WA during September, 2014, fisheries biologists observed that some proportion of their seine hauls contained herring with ulcerative lesions reminiscent of those associated with VHS. Samples of these symptomatic individuals were high-graded from each of two sets, frozen at -20°C, and submitted for cell culture approximately 1 month later. Even under these sub-optimal conditions for virus stability (Arkush et al. 2006), VHS virus was recovered from 27% (6/22) and 12.5% (3/24) of the selected samples, with tissue titers as high as 5×10^3 PFU / g (Hershberger unpublished data). Although the true infection prevalence in the population remained unknown, these positive surveillance data indicate that the virus was maintained in the herring schools at sub-epizootic levels.

Long-term VHS virus persistence in this enzootic stage likely involves a combination of chronic carriers and low-level transmission of virus between individuals in the school. Survivors

of VHS can develop a neurotropic stage of the disease that is characterized by the long-term persistence of virions in the brain and peripheral nerves (Lovy et al. 2013). Although this neurotropic stage provides a means of VHSV persistence within individual herring, it remains unknown whether these neurotropic carriers are capable of transmitting the virus. Continuous cycling of VHS virus within a herring aggregation occurs through a combination of viral shedding from infected individuals and the super-susceptibility of sympatric, naïve herring. Recovered individuals are also likely involved in this virus perpetuation strategy, as their re-exposure to exogenous virus can result in infection and additional virus replication, even though they are refractory to disease (Hershberger et al. 2010c). The long-term temperance of VHS virus within this sub-patent stage requires a delicate balance between the exacerbating forces (i.e. gregarious herring schooling behavior, low herd immunity, high R_0 - the basic viral reproduction number, etc.) and the alleviating forces of virus dilution that are influenced by herring vagility and hydrological patterns in coastal areas.

Disease Amplification Stage

Disease amplification is defined as the irreversible transition from the enzootic stage to the outbreak stage, characterized by elevated infection pressures or reduction in host resistance that results from a change in pathogen, host, or environmental conditions.

Changes in the pathogen can be mediated by genetic mutations affecting virulence. Because the RNA polymerases in VHSV and other RNA viruses lack proof-reading activity, their replication error rates are much higher than those of DNA viruses (Holland et al. 1982). For example, among 63 VHSV isolates recovered from coastal fishes around British Columbia, Canada over a 19 year period, genetic sequencing of the glycoprotein gene (1524 nt region)

revealed 42 sequence types with a maximum pairwise diversity of 2% (30 nt differences). The majority (72%) of the variants did not result in changes to the deduced amino acid sequence relative to the consensus wild type (Garver et al. 2013) and the ecological significance of these genetic variants remains poorly understood. Interestingly, multiple genetic variants have been detected from herring in the same school and laboratory challenge studies indicate that a single host species (Atlantic salmon) can demonstrate differences in susceptibility to these naturally-occurring variants (Traxler, Garver and Hershberger unpublished data). Therefore, it is possible that the onset of new epizootics may be initiated by the rapid emergence of new variants demonstrating increased viral fitness.

Host behavioral responses can also facilitate the transition from pre-patency to disease amplification. For example, Pacific herring and several other species of forage fish are comprised of large schools and shoals that, when confronted by a predator, often converge into extremely tight aggregations in an effort to confuse and deter the attacker(s). Although effective for its intended purpose, an unintended consequence of this behavior involves an elevated transmission potential resulting from the proximity of susceptible individuals to virus-positive and -shedding individuals within the school. An analogous proximity mechanism resulting in elevated infection pressures occurs in some Pacific herring fisheries, where wild cohorts are captured and confined into marine net pens (Hershberger et al. 1999); the magnitude of the ensuing epizootics inside the pens has generated concerns of spill-back to free-ranging populations.

Human activities as well as annual changes in population structure can also facilitate a transition from pre-patency to active VHS amplification. For example, because the potential for prior exposure to VHS virus increases as a cohort of Pacific herring ages, the potential for

population resistance also increases directly with the median age of the population. However, because protective antibodies against VHS are not passed vertically from parents to their progeny, the appearance of newly-metamorphosed juvenile herring constitutes a large influx of susceptible individuals into the system each year. Pacific herring school often by age class; nonetheless age structuring is dynamic, and schools containing multiple age classes are not uncommon (Taylor and Kieser 1982). If, at the time of recruitment and integration into the adult population (typically at the age of 2-4 years), younger age classes have not yet developed acquired resistance, then the balance of population resistance can tip towards susceptibility. Consequently, it is likely that the level of population resistance can be affected by human activities such as commercial herring roe fisheries which selectively remove the oldest, most refractory cohorts and leave the youngest, most susceptible individuals.

Another consequence of the annual influx of susceptible herring involves anthropogenic activities associated with the culture of Atlantic salmon in marine net pens. Although Pacific salmonids (*Oncorhynchus* spp.) generally demonstrate negligible susceptibility to VHS (genogroup IVa), Atlantic salmon (*Salmo salar*) are a moderately susceptible species (Lovy et al. 2013) and VHS epizootics periodically occur in farm operations throughout Washington and British Columbia (Garver et al. 2013). Newly-metamorphosed juvenile herring often venture through the mesh of Atlantic salmon net pens, and utilize the confines of the net pen as a shelter from natural predators. With an abundant supply of exogenous food inside the pens, the herring quickly attain a size that prevents their emmigration back through the mesh. This presents an optimal situation for the formation of VHS epizootics inside the pens, as a highly-susceptible species (Pacific herring) is maintained in close proximity to a moderately-susceptible species (Atlantic salmon). As a result, concurrent isolations of VHS virus from both species are typical

during documented epizootics in Atlantic salmon net pens (Garver et al. 2013). Further, laboratory exposure studies have successfully demonstrated VHSV spill-over and spill-back between Atlantic salmon and Pacific herring (Lovy et al. 2013).

Interannual changes in community structure can also affect infection pressures, thereby facilitating the transition from pre-patency to active VHS amplification. For example, Pacific sardines, a species that typically occupies warmer waters around California, periodically establish residency in the more temperate coastal waters around Washington state and Vancouver, Canada. When present, they often occur in extremely large numbers that are sufficient to justify commercial harvest (MacFarlane & Beamish 2001). As Pacific sardines also represent a super-susceptible species (Arkush et al. 2006), VHS epizootics among free-ranging fishes in British Columbia often involve mixed assemblages of both Pacific sardines and Pacific herring (Hedrick et al. 2003; Garver et al. 2013). Although investigation of these events is incapable of assigning directionality between the involved species, changes in community assemblages can certainly lead to increased infection pressures, especially when the assemblage is transiently predominated by a high percentage of naïve individuals of a susceptible species such as Pacific herring, Pacific sardines, Pacific sandlance, and walleye pollock.

Changing environmental conditions can affect infection pressures by influencing host susceptibility or characteristics of the pathogen. For example, the stability of waterborne VHSV affects infection pressures by establishing the duration of infectivity for shed virions (Hawley & Garver 2008). Therefore a transition to the disease amplification stage is more likely to occur during conditions that are conducive for virus stability, such as low water temperatures (Hawley & Garver 2008), elevated protein content and/or spawning products in the water (Kocan et al. 2001b), and periods of low ultraviolet irradiation (Oye & Rimstad 2001). Low water

temperatures constitute a further risk factor for VHS epizootics because host susceptibility is inversely related to temperature, with cooler temperatures resulting in greater cumulative mortalities, viral shedding, and viral persistence in the tissues (Hershberger et al. 2013; Goodwin & Merry 2011). In fact, the VHS epizootics involving Pacific sardines and Pacific herring in British Columbia typically occur at times or in locations with declining water temperatures (Hedrick et al. 2003). This inverse relationship with temperature is likely mediated by an enhanced host immune response at warmer temperatures where a robust type 1 interferon response occurs (Hershberger et al. 2013). Additionally, disease potential increases when large schools transiently venture into embayments or other geomorphic locations that experience limited water exchange. Low levels of shed virus can accumulate quickly in these areas, resulting in increased probability of fish exposure to exogenous VHSV.

Outbreak Stage

Once the disease amplification stage is initiated by host, pathogen, or environmental changes, the resulting epizootic cascade is often irreversible and an active disease outbreak typically ensues. Laboratory exposure studies indicate that acute mortalities occurring during the early outbreak period often occur prior to the onset of external signs of disease. Unfortunately, in populations of wild marine fishes, the majority of VHS outbreaks likely go unnoticed because of observational difficulties inherent to coastal marine ecosystems, lack of routine disease surveillance in wild marine fishes, and the occurrence of cryptic or smaller-scale VHS outbreaks that may not necessarily be accompanied by mass mortalities. Although some VHS outbreaks in marine fishes are periodically documented, especially those occurring in close proximity to populated areas or mariculture facilities (Garver et al. 2013), most are likely to occur in areas that escape human observation. Documentation of these cryptic epizootics is further hampered

by a lack of fish health surveillance programs in wild marine fishes, an information gap that remains outside the jurisdiction of state, provincial, federal, and international management authorities. Although small-scale VHSV surveys have been implemented for some herring populations in recent years, including Sitka Sound and Prince William Sound, AK, virus isolations from traditional 60 fish samples are rare during the pre-epizootic phase of VHS virus persistence. However, the early stages of a VHS epizootic were detected in a random sample of juvenile Pacific herring (mean length = 108 mm) from Sitka Sound in March 2011, when 63% (38/60) tested positive for VHSV (Hershberger unpublished data). Of the positive tissues, nearly half had high viral titers ($>40,000$ PFU / g). No mortalities or signs of abnormal behavior were observed at the time of sample collection. This aggregation of virus-positive herring was geographically distinct from aggregations of adult pre-spawn herring in the region (mean length = 232 mm), which failed to test positive for VHSV (n=60).

Recovery Stage

The recovery stage of VHS epizootics can be identified by a decrease in mortality and the appearance of survivors that demonstrate classic signs of hemorrhaging on internal organs, near the fin bases, and around the eyes. Although VHS is typically described as an acute disease - characterized by rapid disease progression and mortality, a chronic manifestation of the disease can also occur - characterized by slower disease progression, lower mortalities, and a prolonged recovery period (Hershberger et al. 2010b). The disease trajectory is likely determined by host and environmental conditions that occur during the early stages of host infection. For example, a chronic trajectory is likely to occur in a population with a significant fraction of immune individuals or during warm water periods when a robust type 1 interferon response is effective at outpacing early viral replication (Hershberger et al. 2013). This early innate response provides

adequate protection until further adaptive responses are mounted. However, an acute trajectory, characterized by a preponderance of mortalities, is expected during cooler water periods when early viral replication out-paces the delayed onset of the protective innate response. Regardless of the disease pace, herring in the VHS recovery stage often present with classic hemorrhages on internal and external surfaces as a result of hemoglobin leakage from damaged tissues. Infectious virus can be recovered from the tissues of survivors from 2 weeks to 6 months after exposure, depending on the disease trajectory and water temperature.

Refractory Stage

Pacific herring that survive infections with VHSV become refractory to the disease for extended periods, presumably for life. Protection against future outbreaks of the disease is most likely conferred by the production of specific antibodies (Hershberger et al. 2011b; Wilson et al. 2014) that remain protective at very low circulating titers. Although VHS virus is eventually cleared from the systemic tissues of recovered and refractory individuals, it can persist covertly for extended periods in the immunologically-privileged cells of the central nervous system, including the brain and peripheral nerves (Lovy et al. 2012). Further research is necessary to determine whether the disease can reactivate in these neurotropic carriers, and whether these carriers serve as a source of exogenous virus for infecting other herring. Even though VHS survivors develop strong acquired resistance and become refractory for life, re-exposure of refractory individuals can result in some additional viral replication. Therefore, even refractory individuals likely serve an important role in magnifying and perpetuating VHS virus in wild herring populations.

408 The long-term protection demonstrated by VHS survivors has important implications for
409 designing VHS surveillance strategies and forecasting future potential for epizootics in wild
410 populations. For example, predisposition of herring to VHS epizootics is influenced by
411 exposure, low ambient water temperatures, diet, and elevated infection pressures that are caused
412 by host behavioral patterns, copious viral shedding, and water circulation patterns that facilitate
413 waterborne transmission. However, these host and environmental factors provide little capacity
414 for forecasting VHS epizootics, as one or more of these factors frequently co-occurs in the
415 absence of observable host epizootics. A more quantifiable means of forecasting VHS potential
416 involves the concepts of adaptive immunity and acquired resistance, as the exposure of refractory
417 individuals to high levels of virus, in combination with any or all of the aforementioned
418 environmental co-factors, will not result in a disease epizootic. Therefore, when applied on a
419 population scale, serological assays that are capable of assessing prior host exposures to VHS
420 virus may provide a quantifiable measure of population resistance. The disease potential would
421 be greatly reduced in those populations with serological evidence of prior exposure to VHS
422 virus. Conversely, disease potential would be high in populations lacking evidence of prior
423 exposure, where disease epizootics would likely occur given the proper host and environmental
424 factors. Efforts are currently underway to develop high throughput serological assays that are
425 capable of assessing prior exposure to VHS virus. From a disease management perspective, *a*
426 *priori* knowledge of the resistance status of populations would allow resource managers to only
427 open certain herring confinement fisheries during periods of high population resistance; thereby
428 minimizing both disease risk in the confined cohorts and spillback to free-ranging populations.
429 Similarly, the results of humoral surveillances would be much more easily interpreted and
430 incorporated into population models by providing binomial data (susceptible or refractory).

Discussion

Since the time of its first documentation from freshwater rainbow trout farms in Germany in 1938 (Wolf 1988), the known range of VHS virus has expanded and different genotypes / subtypes have been described throughout the northern hemisphere. Each time a new genotype of VHS virus has been detected in a new region (i.e. Genotype IVa in the NE Pacific during the late 1980's and Genotype IVb in the Laurentian Great Lakes in the 2000's), research was immediately implemented to understand the drivers of the disease and define its epizootiological principles. However, decades of research indicate that commonalities exist in the principles of VHS, regardless of the etiological viral genotype. In hindsight, several of these principles and concepts should have been more intuitively obvious. For example, only recently was the neurotropic form of VHS detected in Pacific herring, but this stage of the disease is well-described in other VHS virus genotypes and in other close-related fish rhabdoviral diseases; indeed neurotropism is a hallmark of most rhabdoviral diseases, including rabies. Similarly, the inverse relationship between temperature and VHS severity is consistent between numerous strains of VHS virus. Therefore, it is suggested that a more holistic view of the virus could be obtained by considering the entire body of scientific literature surrounding VHS virus, rather than focusing too narrowly on genotype-specific cases. Using this expanded body of literature, we can gain insights into the disease that encompass more than 60 years of observational and empirical research.

The current state of knowledge regarding the ecology of diseases in wild marine fishes is in its infancy, primarily because of the inherent difficulties involved in observing and studying

disease mechanisms that occur in the ocean. As such, the principles described here for VHS represent perhaps our most complete ecological understanding of a disease that impacts populations of wild marine fishes. It is recommended that these principles form the foundation for new mathematical models that can be useful for assigning annual rates of disease-related mortality and for forecasting recruitment into adult spawning populations. The integration of disease information into stock assessment models for wild marine fish populations is generally under-represented or is based on gross estimations that are not based on biological data. In the case of Pacific herring and VHS in Prince William Sound, AK, annual disease mortality is estimated using an index of infection and disease prevalence (Marty et al. 2010). Unfortunately, the ecological disease principles described here provide no indication that VHSV infection prevalence data provide any capacity for forecasting future disease-related mortality. Rather, the disease principles are ideally suited for an alternative approach such as a standard susceptible – infected – recovered (SIR) model, where metrics of population resistance can be quantitatively assessed and employed as a disease forecasting tool. Each disease system is governed by a unique set of ecological principles; therefore, it is recommended that analogous ecological principles be defined for other marine diseases prior to their integration into resource management models.

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Figure 1. VHS epizootic and associated fish kill involving wild herring in Vancouver Island, B.C, Canada. Visible signs of the disease include hemorrhages along the flank of affected fish. Photo credits: Garth Traxler and Jon Richard, DFO Pacific Biological Station, Canada.