Pathogen Exchange between Wild and Farmed Finfish:

Evidence to Assess Pathogen Source and Factors Associated with Clinical Disease Occurrence

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Joy Wade, Editor

Introduction

AAC organized a series of discussions regarding pathogen exchange between wild and cultured animals. These discussions culminated in a face-to-face workshop with a panel of scientific experts which was sponsored by ACRDP. The workshop was held at the Aquaculture Canada Conference in Guleph, Ontario in 2013; the results of which are presented here.

Pathogen exchange between wild and farmed finfish: evidence to assess pathogen source and factors associated with clinical disease occurrence

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Abstract

This review describes the challenges in the assessment of the direction of pathogen exchange between wild and farmed finfish, including a consideration of the pathogen reservoir scenario. The occurrence of clinical disease depends on a complex set of interactions between the host, the pathogen, and the environment. Innate and acquired host immunity and environmental conditions (e.g. presence of co-infections, lack of feed, water temperature fluctuations, and salinity changes) that compromise the host's immune response are likely to play important roles in determining whether clinical disease occurs and what its effects might be on fish populations. The strongest evidence for pathogen exchange is provided by longitudinal studies that assess spatio-temporal prevalence patterns based on similar sampling protocols in all target populations. Infectious hematopoietic necrosis virus in salmon is used as the case study, based on outbreak and surveillance data from British Columbia, from 1992 to 2012.

Introduction

Pathogenicity (the ability to cause damage or disease) and virulence (a measure of the relative severity of the damage or disease compared with reference strains) of a microbe are traits of the microbe that can only be expressed in a susceptible (non-immune) host. For purposes of this review, we define a pathogen as a microbe (virus, bacterium, or parasite) that has the ability to cause damage or disease in a host, given appropriate circumstances. Pirofski and Casadevall (2012) consider microbial pathogenicity to be an emergent factor because it cannot be directly predicted from properties of the microorganism or from changes in the host or its environment, and because interactions among all three factors are often unpredictable. Whether a microbe causes damage or induces disease is a function of a complex set of interactions between the host, microbe, and environment (Pirofski and Casadevall, 2012). While this paper primarily discusses infectious hematopoietic necrosis virus (IHNv) in salmon species, other pathogens and species will also be used in order to make the discussion more relevant to general fish/pathogen interactions.

Microbes are extremely abundant in sea water, and it is estimated that there are 2.5×10^8 virus particles in every ml of sea water (Bergh et al. 1989, Suttle 2005). Fortunately, the vast majority of microbes are not pathogenic to salmon or most other finfish. In the marine environment, it is generally accepted that wild and farmed fish routinely interact with shared pathogens and that there can be bidirectional movement of pathogens between wild and farmed fish. However, to understand and quantify pathogen exchange between wild and farmed fish we need to know the following for each pathogen of interest:

1. The “true" source of infection
2. Pathogen/health status of wild and farmed populations
3. Relative risk of infection at the individual and population level
4. Relative susceptibility of different fish species and/or stocks to infection
5. Survival and distribution of pathogens outside of the host and routes of transmission within and between wild and farmed fish populations
6. Distribution and relative abundances of wild and farmed fish in space and time
7. Other host factors, including co-infections with pathogenic and non-pathogenic microbes

Point 1: The “true" source of infection

Although it would seem to be a relatively easy task to identify the “true” source of infection, it is exceedingly difficult under field conditions. This is because of the many challenges that complicate inferences: wild populations migrate; pathogens can be spread in the water column; the wild, at-risk population cannot be readily defined or repeatedly sampled

during narrowly-defined time (and space) windows; and there is a lag time between infection and detection of the pathogen in infected fish. In addition, it is unrealistic to expect that low prevalence in a potential source population can be readily differentiated from zero prevalence (i.e. absence of pathogen), even when large numbers of fish are sampled. This latter issue is also problematic in terrestrial animals, especially if clinical disease is a rare consequence of infection.

Point 2: Pathogen/health status of wild and farmed populations

Wild and farmed salmonids can harbour many species of pathogens, and the prevalence of different pathogens varies widely among different species, stocks and life history stages of salmon, both spatially and temporally (Arkoosh et al. 2004, Van Gaest et al. 2011, Scholz et al. 2011). Long-term datasets that contain information on the presence of pathogens and occurrences of disease are available for farmed salmonids but, unfortunately, such long-term health data sets are generally lacking for wild salmon. Without such data there will always be uncertainty with respect to the "true" source of infection and the risk of disease as a result of pathogen movement between wild and farmed fish.

Point 3: Relative risk of infection at the individual and population level

Laboratory challenge trials have been conducted for a variety of salmon pathogens (see Traxler et al. 1993). These studies have been conducted using different methods of challenge, different strains of pathogens, stocks and life history stages of salmon, with the hosts generally being held under fixed, often optimal, husbandry and environmental conditions. Although such model systems can provide data on relative host susceptibility and the outcome of infection, it is often problematic to extrapolate such laboratory results to the field. One reason is that it is impossible to replicate the complex interactions between abiotic and biotic factors that occur in the field and which can affect the development and progression of disease. For example, laboratory challenge trials with salmon alphavirus (SAV) fail to generate similar patterns of morbidity and signs of disease as observed in the field (Andersen et al. 2010). Even with modifications to the experimental system that caused sub-lethal environmental hypoxia, these authors could not reproduce naturally-occurring disease. Hence, factors not evaluated in the challenge trials are likely to have contributed to the severity of disease.

Pathogens are known to vary in their ability to infect host species and to cause disease; some are highly pathogenic while others are not. Even within a species of pathogen there is variation in pathogenicity and virulence which needs to be considered, as most routine diagnostic techniques do not differentiate between strains/genotypes of pathogens.

Infectious salmon anemia virus (ISAv) is a good example, where some strains are avirulent (e.g. HPR0) because of amino acid deletions in a highly polymorphic region (HPR) of the virus that code for the hemagglutinin-esterase glycoprotein, while other strains are virulent (e.g. HPR4) (Johnson et al. 2008. Ritchie et al. 2009).

Even the most pathogenic microbes are not ubiquitously pathogenic to all hosts. Pathogens often present highly variable host specificities that are can be extremely restrictive. In the case of infectious hematopoietic necrosis virus (IHNv), three North American isolates (genotype groups U, M and L) have been identified (Kurath et al. 2003). These genotypes are associated with different salmon species in the wild, and these associations are reflected in their patterns of host-specific virulence. For example, the U genotype is predominately found in sockeye salmon, and in laboratory challenge trials this genotype is much more virulent than the M genotype, which is found predominately in rainbow trout (Garver et al. 2006 and references therein).

Point 4: Relative susceptibility of different salmon species and/or stocks to infection

Unfortunately, due to the lack of extensive surveillance or controlled laboratory studies that investigate the distribution of pathogens amongst different potential hosts in aquatic environments, our understanding of the host specificity of many salmon pathogens is limited (Chevassus and Dorson 1990). It is, however, well documented that different salmon species vary in their susceptibility to pathogens and, in some instances, to different genotypes of pathogens. For example, laboratory challenge trials were used to determine the relative susceptibility of steelhead trout, chum, Chinook, coho and Atlantic salmon to infection with ISAv by intraperitoneal challenge (Roland and Winton 2003).

With the exception of Atlantic salmon, which suffered disease, none of the *Oncorhynchus* species showed signs of typical ISA, and no ISAv-related mortality occurred, although live virus could be isolated from fish. Whether it is possible to establish ISAv infections in *Oncorhynchus* species using more natural waterborne/cohabitation challenges is not known. As noted previously, there are marked differences between salmonid species with respect to their susceptibility to disease caused by the different genotypes of IHNv (Garver et al. 2006).

Within species there can also be marked differences in resistance to infection and disease development. It has been demonstrated that there is a strong genetic component of susceptibility of salmon to viral and bacterial pathogens such as ISAv, IHNv, VHSv and *Aeromonas salmonicida* (Grimholt et al. 2003, Quillet et al. 2007, Kjoglum et al. 2008,

Purcell et al. 2010, Drangsholt et al. 2011, Zhang et al. 2011, Odegard et al. 2011, Wargo et al. 2012).

Cipriano and Heartwell (1986) reported mortalities attributable to *Aeromonas salmonicida* of 26%, 6% and 0%, respectively, in brown trout, brook trout and rainbow trout grown in the same farm. Similarly, Fast et al. (2002) found variable sea lice infection levels in exposure studies on Atlantic salmon, rainbow trout, and coho salmon. *Tenacibaculum maritinum* (bacterial stomatitis) has been observed in farmed Atlantic salmon but not in farmed Chinook or coho (P. McKenzie pers. comm).

Even within a stock or population there is a range in susceptibility when fish are exposed to a potential pathogen. In the case of IHNv, field studies have reported widely varying levels of virus in individual fish collected from the same infected population at the same time (Mulcahy et al. 1982). Cipriano (1983) reported mortality ranging from 0-83% when different hatchery populations of rainbow trout were challenged with *Aeromonas salmonicida*. Total (100%) mortality almost never occurs, even in the most severe disease outbreaks. Within a population, susceptibility to a pathogen can vary with age or physiological changes in the host: rainbow trout are known to be more resistant to IHNv as they increase in size (LaPatra et al. 1990, Bergmann et al. 2003, Verrier et al. 2013); *Tenacibaculum maritinum* in Atlantic salmon only causes lesions in smolts, shortly after saltwater entry; and *Saprolegnia* infections in fresh water are most often evident in salmon undergoing physiological changes such as smoltification or maturation.

Within farm populations, management practices such as vaccination can greatly reduce the susceptibility of farmed populations to infection and development of disease and, thus, reduce shedding of pathogens into the environment (Sommerset et al. 2005). The efficacy of vaccines has been demonstrated in numerous laboratory studies (Boudinot et al.1998, reviewed in Leong et al. 2012); however, there are few published studies of vaccine efficacy in the field (Burnley et al. 2010). Large-scale field efficacy testing of the IHNv plasmid vaccine (APEX-IHN®) was done in British Columbia (BC), but there was no IHNv challenge at that time from wild sources (S. Saksida, pers. comm). Circumstantial evidence, based on the recent IHNv outbreaks in BC, suggests this vaccine is efficacious. In the case of wild fish, the natural occurrence of pathogens within the environment, including survivors of infection and/or disease outbreaks, may result in the development of a protective immune response that makes them less susceptible to subsequent exposure.

Point 5: Survival and distribution of pathogens outside of the host and routes of transmission within and between wild and farmed fish populations

Spread or transmission of microbes between hosts is often the result of direct or close contact; however, the aquatic environment may allow the survival of bacteria and parasites for several days or even weeks, while the survival of viruses is often of shorter duration. Hence, transmission of microbes can occur at a distance from the original source. The distance over which a microbe can travel and remain infective depends on characteristics of the water mass into which it is shed, as well as the microbe's stability outside of its host. Oceanographic conditions in nearshore environments that are strongly influenced by local conditions, especially bottom topography, river flows and winds, serve to disperse microbes both vertically and horizontally. Microbe stability and infectivity is influenced by numerous biotic and abiotic factors within the water column, including mixing depth and water clarity, as related to UV exposure, temperature, salinity, presence of fomites (organic or inorganic matter) and the makeup of the overall microbial community. For example, Pham et al. (2012) showed that viral hemorrhagic septicemia virus (VHSv) can persist while adhered to plastic (a fomite) for 30 days at 4^0C in fresh water with no loss in infectivity. In contrast, survival of non-adhered VHSv in freshwater is limited to 24 hours at 20^{o}C and five days at 4^{o}C.

The stability of IHN virus has been studied in both fresh and seawater, and found to survive in fresh water at 10^{o}C for seven weeks (Wedemeyer et al. 1978) and in seawater a 3-log decrease occurred over three weeks at 15°C (Toranzo & Hetrick 1982). Yoshimizu et al. (2005) examined the survival of IHNv in coastal waters, but the study had limitations such that inferences from findings in that study should be made with care. The survival of several important bacterial pathogens, such as *Aeromonas salmonicida* (Rose et al. 1990), and enteric bacteria (Rozen and Belkin 2001) in seawater has also been studied but, for brevity, the findings are not discussed herein.

Once a pathogen infects a host fish, there is a lag period before pathogen load exceeds the threshold that is detectable with current diagnostic methods. The duration of the lag period depends upon the replication rate of the pathogen within the host and the diagnostic sensitivity of the assay, which varies depending on pathogen load and test method. Pathogen loads are typically greater when fish are clinically affected than when they are infected but not showing clinical signs. As many salmon species actively migrate through coastal waters, this means that they may not test positive until they are at a considerable distance from the site of infection.

The incubation period, the time needed for a pathogen to induce disease following host infection, is an additional complicating factor in identifying pathogen exchange. Some pathogens have very long incubation periods (e.g. *Renibacterium salmoninarum*), lasting up to weeks or months, while others have incubation periods of less than one week (e.g., IHNv). In addition to microbial characteristics, the incubation period also depends on the

host response, especially if the host is experiencing other environmental stressors, such as starvation or water temperature fluctuations. The longer the lag time from infection to the development of signs of disease, the greater the likelihood of movement of wild hosts and a commensurately decreased ability to identify the source of infection.

Point 6: Distribution and relative abundances of wild and farmed fish in time and space.

Another complicating factor is that wild hosts (regardless of whether they are the source or recipients of infection) are mobile. Some fish have extensive migratory patterns (i.e., salmon) and, when in coastal waters, many stocks are either travelling to freshwater spawning grounds (i.e., adults) or moving offshore to feeding grounds (i.e., smolts). Although accurate estimates of salmon numbers on farms can be obtained, there are relatively few data on the abundance of wild salmon in the marine environment. Furthermore, when, and for how long, wild salmon remain in the vicinity of salmon farms or within water masses that have been in contact with salmon farms is poorly understood. Without such data, as well as data on the presence, abundance and distribution of pathogens within the environment, the risk of pathogen transfer between wild and farmed fish cannot be easily estimated.

Stock composition is another important consideration, as there are numerous wild and farmed stocks simultaneously present within the environment, and each stock may have a different history of pathogen/disease exposure, vaccination, etc. In order to identify and properly assess epidemiological questions in aquaculture settings, researchers need to know the sources of the stocks. In the case of wild stocks of fish, genetic identification is required.

Point 7 - Other host factors, including co-infections and immune status

Co-infections may positively or negatively affect susceptibility of finfish to disease. For example, it has been found that infection of rainbow trout with chum salmon reovirus prior to exposure to IHNv resulted in improved survival (LaPatra et al. 1995). In contrast, Johansen and Sommer (2001) showed that infectious pancreatic necrosis virus infection in Atlantic salmon post-smolts negatively affected the outcome of secondary infections with ISAv or *Vibrio salmonicida*. St. Hilaire et al. (2001) found that very few Chinook salmon died when exposed to IHNv, but from a few (only those co-infected with *Piscirickettsia* or *Renibacterium*) investigators were able to isolate IHNv by culture. This suggests that co-infection with other pathogens can influence the susceptibility of species to infection with IHNv.

Relationship between infection and disease

It is important to note that exposure to a pathogen does not always result in infection, infection does not always lead to disease (Figure 1), and that the source of infection may not be in the immediate vicinity when infection is detected or disease first becomes evident. For example, if clinical disease is observed in a wild population, the exposure likely occurred several days to weeks beforehand. During this time, the source and/or the recipient fish (if migratory) are likely to have travelled, and we are not likely to know the route or if there has been intermingling of stocks (or other sources of pathogens encountered) during that period. The pathogen may have also moved and no longer be present at the location where the fish were exposed to it. All of these variables are modulated by the environment.

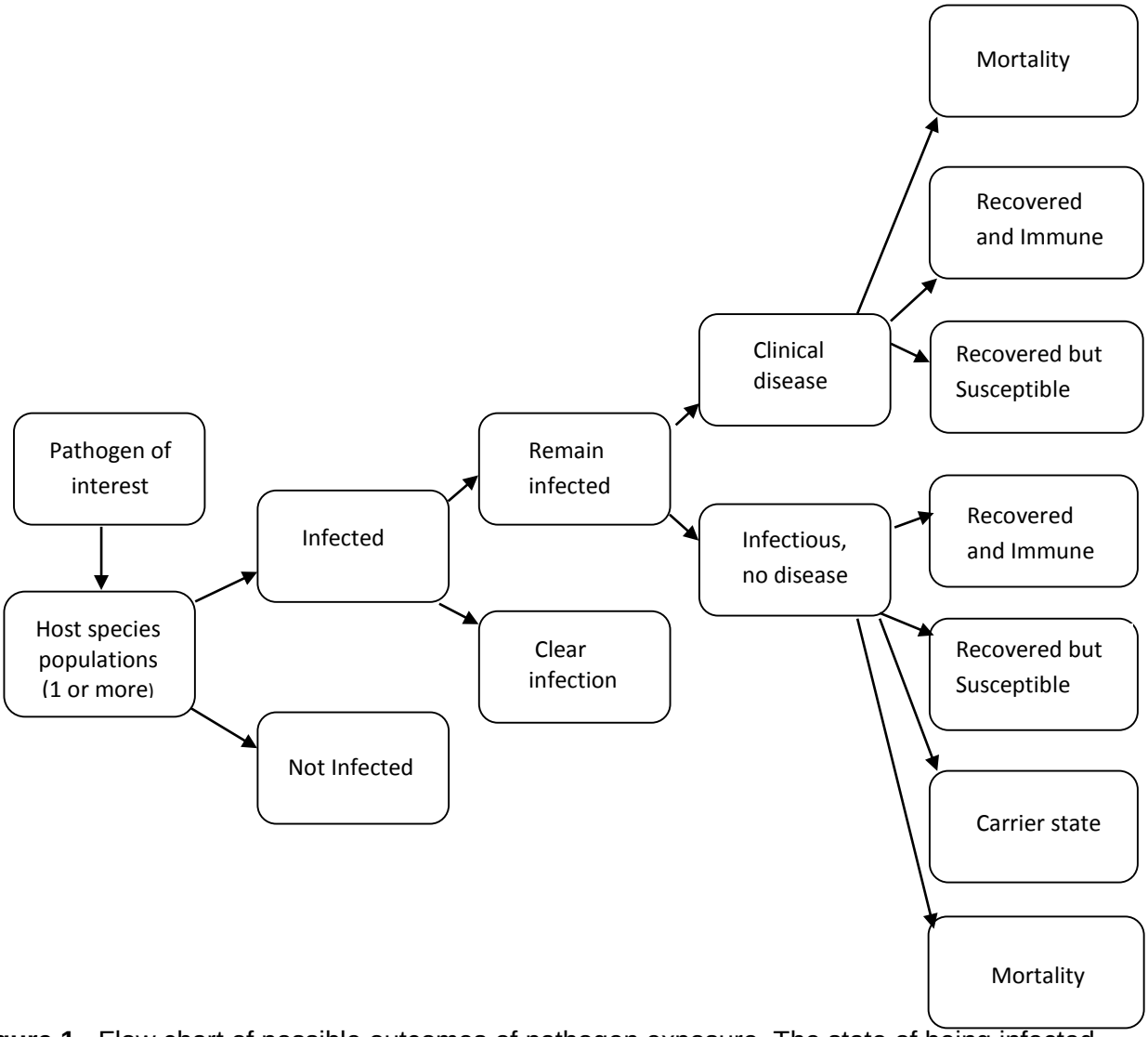

Figure 1. Flow chart of possible outcomes of pathogen exposure. The state of being infected can include latent infections.

In contrast, if a disease episode is observed in a farm population, there is at least knowledge of the history of the host (movement of fish into the population, if any, and health history). There is also some knowledge of the immediate environment, although the pathogen source could still be unknown because exposure is likely to have occurred days, if not weeks, before the index case. If wild migrating fish are the source of the pathogen, then they are likely to be some distance away at the time of disease detection. It is also possible that other sources of infection are from a distance away, such as water-borne or vector-mediated transmissions. Further complicating disease observations within the natural environment is the likelihood of secondary or concurrent diseases (infectious or non-infectious) that may modulate the incubation timeline, severity of effect, and host susceptibility.

Because farmed fish are monitored regularly, they can be used as sentinels to evaluate pathogen exposure in the aquatic environment. However, this approach only has utility if farmed fish are susceptible to the pathogen and enter the marine environment free of the pathogen of interest. In addition, occurrence of disease in farmed fish populations does not necessarily imply occurrence of the same disease in wild populations. A longitudinal study design that evaluates spatio-temporal patterns of pathogen prevalence in wild and farmed populations (with a goal of assessing direction of pathogen exchange) is theoretically possible, but the measurement system may not be adequately refined to differentiate between competing models of pathogen exchange. For example, when there are three populations, the number of possible pathways for consideration of the direction of exchange is nine (Figure 2), and the number increases exponentially with the addition of more populations.

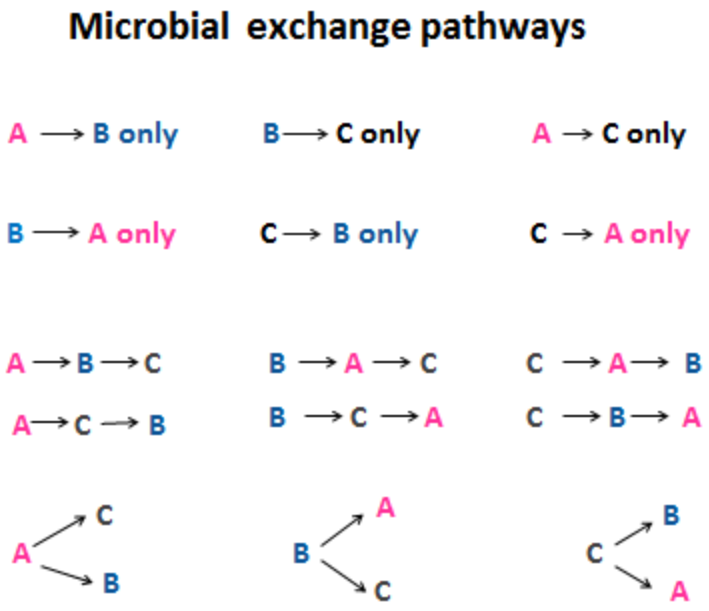

Figure 2. Possible pathways for microbial exchange when 2 and 3 populations are potential sources and recipients of microbes.

Aquatic ecosystems are complex and non-pathogenic factors (host and environment) are highly influential in affecting exposure, infection, and the consequences of infection. Further complicating our understanding is that pathogen transfer is known to be bidirectional (Kurath and Winton 2011). The strain and load of the pathogen and health/stress status of fish are critically important factors in determining whether clinical events occur after a pathogen enters a susceptible host. Hence, the most relevant question for the examination of wild/farmed fish pathogen interactions may well be: "What are the consequences of infection at the wild/farmed fish population level, and can they be mitigated in one or both populations?"

Factors associated with occurrence of clinical disease

Although infection with a pathogen (e.g. ISAv) is necessary for a disease (e.g. ISA) to occur, presence of the pathogen in host tissue may not be sufficient to induce detectable disease because of innate host immunity, immunity acquired through prior exposure or vaccination, and/or environment conditions that do not compromise the host's immune responses to control or contain the pathogen (e.g. there are no co-infections, or water

temperature fluctuations etc). Components of a sufficient cause for disease are known as risk factors.

Environmental conditions play a crucial role, not only in pathogen transfer but also as risk factors for clinical disease occurrence. Unlike mammals that regulate their internal environments, most fish are poikilotherms, meaning that they have little ability to regulate their core body temperature. In this situation, both the microbe and the host are physiologically tied to the environment and have optimal ranges for factors such as temperature, salinity, oxygen and pH for survival; above and below these ranges they become stressed and immunosuppressed. Extended periods outside the optimal range usually result in death. Examples of temperature-based immunosuppression are described by Rodrigues et al.(1998) and Nath et al. (2006), respectively, and show that carp at 6^{o}C shut off their major histocompatibility (MH) class I genes, which initiate adaptive immune responses to viruses, and trout turn off their MH class II gene expression below 2^{o}C, preventing the initiation of immune responses to bacteria. Some pathogens require temperature stress to trigger disease, including VHSv (Arkush et al. 2006, Vo et al. 2013), *Flavobacterium* (Hesami et al. 2011) and fungal infections (Bly and Clem 1992), to name a few.

Risk factor studies (e.g. case-control, cross-sectional and cohort) can be used to assess host, agent, and environmental factors associated with naturally-occurring clinical disease in farmed fish populations. Prospective cohort studies provide the strongest evidence for cause-and-effect relationships because temporality between the risk factor and the outcome can be established, and it may be possible to distinguish risk factors for the introduction of infectious agents into populations from risk factors for endemic disease, where the pathogen has been present for prolonged time periods. There are few published risk-factor studies because fish are not individually identified and cages/pens of fish are often split and moved during the production cycle, though several risk factor studies in Norway have examined skin lesions, abdominal adhesions and spinal deformities in Atlantic salmon (Vågsholm et al. 1998a, 1998b, 1999). Conducting cohort studies is difficult, though, because most diagnostic tests used in fish health require lethal sampling, which precludes the opportunity to track individuals over time.

At the individual level, it may be possible to predict with some certainty the outcomes of exposure to pathogens, but assessment of population-level effects may not be possible, especially for wild salmon. The contribution of pathogen exposure and disease occurrence to population-level effects in wild salmon is not likely to be consistent over time because of the highly variable environment in which fish live. Other, unmeasurable, abiotic and biotic factors that the fish encounter may have even larger population effects and/or change the nature of the relationship between salmon and their pathogens.

Infectious diseases are but a single component of a complex group of factors that interact cumulatively to regulate fish populations.

Inferences about risk factors being "causes" of disease or population-level declines should be evaluated using the Bradford Hill (BH) criteria, which are routinely used in human and terrestrial animal health. For infectious diseases, these are less restrictive than Koch's postulates, which depend on classical hallmarks of infection and direct and obvious mechanisms of pathogenesis (Lipkin 2008).

The most important of the BH criteria, which allow simultaneous evaluation of host, agent, and environmental factors, are:

1. **Temporal relationship**: The effect has to occur after the cause and, if there is an expected delay (e.g., incubation time plus time to detection between the cause and expected effect), then the effect must occur after that delay. As indicated previously, this is easiest to demonstrate using a prospective cohort study with repeated assessment of pathogen and health status of individual fish in netpens and of groups of fish in wild populations.
2. **Strength of association**: A small association does not mean there is not a causal effect, though the larger the association, the more likely that it is causal. In the case of detection of a pathogen in tissues with lesions, compared to those without lesions, one would expect a strong association (measured as an odds ratio) if the putative pathogen were the cause of the detectable tissue damage (Table 1). If there was no association between a microbe and histological lesions, the value of the odds ratio would be expected to be 1.

Table 1. Hypothetical example of the association between microbe A and occurrence of histological lesions, as determined by blinded evaluation of tissue samples. The large odds ratio (ad/bc) of 36 (40*45 / (5*10)) indicates a very strong association between detection of the microbe and lesion occurrence.

		Histological lesions		
		Present	Absent	Total
Microbe A	Present	40 (a)	5 (b)	45
	Absent	10 (c)	45 (d)	55
	Total	50	50	100

3. **Consistency**: Consistent findings observed by various researchers in different places with diverse samples increases the likelihood of a true effect. Lack of consistency does not preclude a causal relationship, though, because of

confounding factors such a genetic resistance to disease of certain stocks. Heritability estimates for resistance to bacterial, viral, and parasitic infections in aquaculture species are reviewed in Ødegård et al. (2011).

4. **Biological gradient/dose response**: Greater exposure (e.g., greater pathogen load, in the case of an infectious agent) should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. For many infections of finfish to be clinical, water temperatures may need to be in permissive ranges, in which case the dose-response curve would be non-linear.
5. **Biological plausibility**: A plausible mechanism between cause and effect is helpful but Hill noted that knowledge of the mechanism is limited by current knowledge.
6. **Coherence of evidence**: Consistency between field observations, experimental evidence and laboratory findings increases the likelihood of an effect. However, Hill notes that "lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations." If elimination of the putative cause results in a lower incidence of disease, this may provide additional evidence, although further proof may require randomized controlled trials.

The elimination (ruling out) of other possible explanations for disease occurrence or population-level effects may lend weight to inferences about the roles of risk factors. Specificity of the association is also mentioned as another consideration, although for infectious diseases (e.g. IHN, BKD) asymptomatic carrier fish may be present in populations, thereby negating the value of this criterion.

Infectious hematopoietic necrosis virus (IHNv): case study

In the following sections, we consider IHNv as our case study to evaluate evidence of pathogen exchange between wild and farmed salmon, and factors associated with disease occurrence. Data from experimental and observational studies, primarily from the west coast of the U.S.A and Canada, are presented. We begin with a brief introduction to IHNv, including modes of transmission.

IHNv is a rhabdovirus that is endemic in the Pacific North West of North America and has become established in Japan, Taiwan, and Europe (Hostnik et al. 2002). Clinical disease occurs in both fresh water (in wild and enhanced hatchery fish) and salt water (farmed Atlantic salmon), and the clinical signs of septicaemia are similar in all species if disease is present. IHNv appears to survive four times longer in fresh water than in estuarine or sea water, and may transmit horizontally in fresh water. Vertical transmission occurs by surface contamination of eggs. Recovered fish can become carriers.

There are three distinct genotypic groupings (clades) of IHNv: lower (California and Oregon); middle (fresh water in Idaho trout), and; upper (Washington State to Alaska) (Kurath et al. 2003). In the following sections, we focus discussion on the upper clade (U clade) of IHNv because it occurs in British Columbia (BC). In BC, IHNv has caused high mortality among wild sockeye, kokanee salmon and rainbow trout (Amend et al. 1969, Williams and Amend 1976, Traxler and Rankin 1989). Losses have occurred primarily in fresh water in either broodstock or alevin/fry.

Environment

Survival of IHNv and other viruses in the environment is affected by many physical, chemical, and biotic factors. Physical and chemical characteristics of water, such as its temperature, salinity and UV light levels, are known to at least partially influence IHNv inactivation rates (Toranzo and Hetrick, 1982; Garver et al. 2013). The presence of other microbial agents in seawater is known to increase IHNv inactivation rates compared with seawater that has been sterilized to remove microbes (Kamei et al. 2007a, Kamei et al. 2007b, Garver et al. 2013). The association of IHNv, other viruses, and other pathogens, with fomites is often linked to improved survival within the environment (Yamamoto et al. 1989, McDaniel et al. 1994).

Shedding rates from the source population, survival rates within the water column, and current hydrodynamic patterns and speeds will affect the amount and distribution of IHNv from its source. At some point, IHNv may still be present in water but at levels below the minimal infectious dose for fish.

Studies of intra- and interspecies variation in susceptibility

Susceptibility cannot be assumed to be the same among fish species or even between stocks within species. Properly-designed laboratory challenge studies are essential for determining the susceptibility of a particular host to a pathogen under predetermined and controlled environmental conditions. Such studies provide useful data on clinical disease and mortality rates, as well as information on viral shedding rates. Wherever possible, laboratory challenge models should mimic natural exposure conditions. Several IHNv laboratory challenge studies have been reported (Traxler et al. 1993, Follett et al. 1997, St-Hilaire et al. 2001, Arkush et al. 2004). The particular strains of IHNv, the routes of challenge, and other experimental conditions have not been standardized among the studies, which makes direct comparisons difficult. Further, the role of concurrent infections and different environmental factors is not typically evaluated in challenge studies.

Laboratory challenge trials using the U clade of IHNv have provided information on intra- and interspecies variation in susceptibility. Atlantic salmon are highly susceptible to water-borne infection with IHNv and the development of disease (Traxler et al. 1993). Of the Pacific salmon, sockeye salmon are the most susceptible to infection, while other species, such as Chinook, coho, chum and pink salmon are more refractory, even when challenged by injection (Traxler et al., 1993). Follett et al. (1997) conducted challenge trials in Alaska and found that Arctic char, Arctic grayling and pink salmon were resistant to IHNv while lake trout had limited mortality (5-15%). In contrast, sockeye salmon had 48- 85% mortality. Using virus culture, no virus could be recovered from the infected char and grayling, while pink salmon produced only 10 plaque-forming units (PFU)/gram. The infected lake trout and sockeye salmon shed similar titres of 10^4-10^7, which suggests that the resistance of the char, grayling and pink salmon was attributable to a lack of viral replication in those hosts, but that the difference seen in the mortality of sockeye salmon was due to differences in host resistance or immune response.

Recent studies with Fraser River, BC, sockeye salmon have demonstrated significant differences between stocks with respect to mortality, following a water-borne IHNv challenge, in which fish originating from Sakinaw Lake were found to have a lower cumulative mortality than those from Pitt River (K Garver pers comm.). Carriers have recently been identified (Muller et al. 2013) among survivors of laboratory challenge trials with IHNv, and carriers of IHNv have also been identified in juvenile Fraser River sockeye salmon caught in the Strait of Georgia, BC (S. Johnson pers comm.).

Findings from key experimental studies involving IHNv are described in the following paragraphs:

Traxler et al. (1993) compared transmission in salt and fresh water, using cohabitation of infected fish, and found that Atlantic salmon were susceptible in both water types. Sockeye salmon were susceptible to cohabitation exposure only, while the authors reported that Chinook were resistant to infection. They concluded that Atlantic salmon were more susceptible than sockeye. St. Hilaire et al. (2001) found that very few Chinook salmon died when exposed to IHNv, but from only a few (those co-infected with *Piscirickettsia* or *Renibacterium*) were they able to isolate IHNv. This illustrates the point about co-infection and secondary infection described previously, and suggests that although they show no disease, Chinook salmon are capable of acting as carriers. St. Hilaire et al. (2001) only detected anti-IHNv antibodies in two of 70 Chinook salmon experimentally exposed to the virus, so the carrier state may be rare and/or difficult to detect. This study also showed that Atlantic salmon exposed to infected Chinook salmon developed IHN, which emphasizes the need to ensure that diagnostic tests are sensitive

enough to detect infectious (i.e., capable of spreading the virus) individuals and/or populations. Older studies used virus isolation as the reference standard, but recent studies have used PCR molecular methods with higher diagnostic sensitivity. However, a positive PCR result only indicates presence of IHNv genetic material, and not necessarily live virus.

Traxler (unpublished data) repeated his work and examined the susceptibility of the five common Pacific salmon species compared with Atlantic salmon. As reported previously, Atlantic salmon were highly susceptible to IHNv, up to 100 times more susceptible than sockeye salmon. As for other species of Pacific salmon, Traxler found that pink salmon were not susceptible (with no losses and no virus recovered); chum salmon showed low susceptibility; whereas Chinook showed no losses (i.e., asymptomatic), but virus was recovered from both these species. These findings suggest that both chum and Chinook may be potential reservoirs or alternative hosts for IHNv.

Arkush et al. (2004) studied exposure of Chinook salmon to IHNv in water (3.5×10^5 $TCID_{50}$ per ml) and showed recoverable levels of virus in kidney/spleen, gill, plasma and ovarian fluid between four to 14 days post-exposure. Fish in the control group did not have detectable titres, however, there were only six fish per group (all female), a single dose, one temperature (11.4 to 12.4°C) which was permissive to infection, and the fish were starved as they were in the last stages of spawning. There were no lesions evident, even with titres in the 10^7 PFU per gram range, but it is important to note that the study had a limited number of fish.

Carriers and reservoir hosts

Returning adult sockeye have tested positive for IHNv in the Fraser River; however, it is not known if they were infected before they entered the river. The presence of IHNv carriers has been proposed but only recently have IHNv carriers been identified in juvenile Fraser River sockeye salmon in coastal waters. The IHNv genotype carried by sockeye was the same as found on salmon farms during outbreaks in 2012, which is suggestive of a marine reservoir of IHNv associated with salmon farms.

Overall, the sources and importance of reservoir hosts in the spread of IHNV has had limited investigation. Alternative or reservoir hosts are species capable of becoming infected and being infectious without showing clinical signs. While other salmonid species, such as Chinook and chum, have the potential to be reservoir hosts for key salmon diseases, there may be potential, non-salmonid, reservoir hosts. Kent et al. (1998) conducted a marine survey of wild fish in coastal waters in BC using neutralizing antibody tests and DNA probes, and detected IHNv in one Pacific herring (collected distant from

any salmon farms) and in tube-snouts and shiner perch collected from a farm experiencing an IHN outbreak. IHNv was not detected in these species collected from the same net-pen site six weeks after all Atlantic salmon had been removed from the site. Pacific herring were also examined during the outbreak, and they all tested negative (P McKenzie, unpublished data). Under laboratory conditions, Hart et al. (2012) were unable to demonstrate susceptibility of Pacific herring to overt clinical disease and noted only transient levels of IHNv in tissues of exposed fish.

Role of vectors in transmission of IHNv

Published evidence indicates that the common Mayfly and the salmon sea louse (*Lepeoptheirus salmonis*) may be potential vectors of IHNv, but there are no data to support their roles in naturally-occurring outbreaks. Shors and Winston (1989) reported detection of IHNv in common Mayflies collected from streams and an abandoned fish hatchery in Idaho. In laboratory trials, Jakob et al. (2011) reported that sea lice could acquire IHNv from infected salmon and that these lice could only transmit the infection to naïve fish when attached to them. The association of IHNv with *L. salmonis* indicates that the salmon louse likely acts as a mechanical rather than a biological vector.

Field Studies

Field monitoring of contained fish in the marine environment is far easier than the monitoring of wild populations, particularly those species with long migratory routes (i.e., salmonids). In spite of the difficulties, field studies should be longitudinal in nature in order to be able to assess data captured over several years.

Farmed salmon

In farmed Atlantic salmon in BC, there have been three IHNv outbreaks:

1. 1992-1996. This outbreak was identified as a single virus exposure that spread among farms (St Hilaire et al. 2002). Eighteen outbreaks occurred on 14 farms within an 11km radius; mortality ranged from 18-78%, and younger fish had higher mortality. Re-infection occurred on 4/14 farms. The index case occurred in summer on the east coast of Vancouver Island.
2. 2001-2003. These outbreaks were associated with two strains that were different than the previous outbreak. One strain infected fish on the west coast of Vancouver Island, and the second started in the same area as the 1992-96 outbreak but spread (evidence points to spread attributable to human activities) (Saksida 2006), with 30 outbreaks on 36 farms (no repeat outbreaks) in five management zones; mortality ranged from 20-94%. There were two index cases: the first on the east

coast of Vancouver Island in summer 2001 (same region as 1992); the second on the west coast of Vancouver Island in spring 2002.

3. 2012. Index cases were on Bainbridge Island (USA). In BC, IHN was diagnosed on the west coast of Vancouver Island in spring 2012, and then later on the Sunshine Coast of BC. The IHNv isolated from affected farms was identical to an isolate found in out-migrating sockeye in Washington State and BC rivers. The isolate from the affected farms in Washington State differed by only one base pair. There were no outbreaks in the Campbell River area (east coast) of Vancouver Island.

Since 2001, the British Columbia Ministry of Agriculture Laboratory (BCMAL), and now the Department of Fisheries and Oceans (DFO), have been conducting fish health surveillance, including testing for IHNv in farmed salmon. Outside of the three outbreaks listed above, IHNv has not been detected in these surveys, and BCMAL reports have been made available that summarize the data collected. In addition to routine fish health monitoring, most Atlantic salmon producers have been monitoring their broodstock for IHNv since the 2001 outbreak. One laboratory in BC has been conducting some of this surveillance and has tested over 31,000 samples by viral culture since 2005 with no positive IHNv results (S. Saksida unpublished data). Recently, the Canadian Food Inspection Agency (CFIA), which is responsible for the control of diseases listed by the World Organisation for Animal Health (OIE), has also been collecting test results from all laboratories that conduct diagnostic testing in order to provide evidence of pathogen freedom between these distinct outbreak periods.

We can surmise from the information collected from farms that:

- IHNv is not present on the farms except when there are outbreaks, i.e., there is no evidence of a farm acting as a reservoir population for wild fish populations.
- Index cases appear to have occurred during two distinct time periods: summer and late winter/early spring without any identified source of IHNv for the index farms.

Wild salmon

Initially, most field data about IHNv have been collected in the freshwater environment, and the most useful studies have incorporated a long time series of data (unless the report is on an unusual outbreak situation). Meyers et al. (2003) reported significant annual variation in IHNv in returning sockeye salmon in Alaskan hatcheries and little correlation with their offspring (data series from 1973 to 2000). Rudakova et al. (2007) reported similar findings in Russian sockeye salmon in fall/winter (inter-annual variation and no correlation with offspring) from 1996 to 2005. Traxler (unpublished data) also found significant inter-annual and inter-stock variation in IHNv in Weaver Creek and Nadine

River sockeye stocks between 1984 and 2007. Based on these data, there was no clear relationship between prevalence of IHNv in female broodstock and their progeny.

Traxler et al. (1997) reported the first finding of IHNv in wild sockeye salmon (7/60 virus positive on kidney samples) in the marine environment in the Alberni Inlet, BC, when there was obvious morbidity and mortality. Saksida et al. (2012) conducted a survey of juvenile pink salmon in the Broughton Archipelago and no IHNv was detected. This finding is not surprising in light of the laboratory work conducted by Follett et al. (1997) and the unpublished Traxler work. Currently, there are on-going wild fish surveillance programs; however, most (e.g., DFO Strait of Georgia study) are recent and have less than 3-4 years of data. As noted above, carriers of IHNv are present in juvenile Fraser River sockeye salmon caught in Georgia Strait and Johnstone Strait.

Is there evidence of IHNv transmission between wild and farmed fish?

Figure 3 illustrates the potential pathways of transmission of IHNv between wild and farmed fish. However, because there was no evidence to suggest that the index cases were from a farmed fish population, the sources were likely from wild fish populations carrying IHNv (spillover, Figure 3). Potential sources could include wild salmon (in the summer they return to spawn and in the spring they are out-migrating from rivers), but there could also be other sources of infection. There was no wild fish surveillance during the 1992 and 2001 outbreaks; however, during the 2012 outbreak, there was wild fish surveillance that showed genetic similarity between IHNv isolates in the wild and farmed salmon, but specific stock(s) are unlikely to be identified since incubation for the disease is about 7-10 days. So, the likelihood of an infected wild salmon source being in the vicinity of the farm at time of diagnosis would be low. While it is plausible that a non-salmonid reservoir exists, there has been limited testing of other species, such as herring, to support this hypothesis. We don't know the specific source species, but there is conjecture that it may be adult sockeye (in summer outbreak) or juvenile sockeye (in spring). Two of the authors (SS, PM) have not been able to link specific stocks to any of the outbreaks, which would require significant surveillance efforts that would not be economically viable.

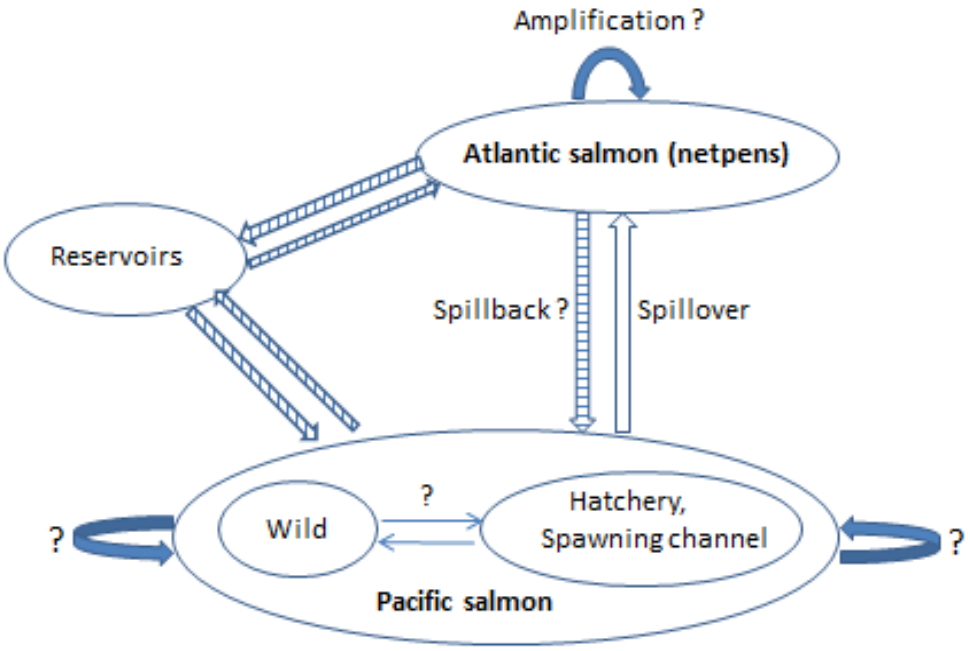

Figure 3. Possible modes of transmission of IHNv (adapted from Kurath and Winton, 2011).

Amos and Thomas (2002) reviewed five outbreaks of VHSv and IHNv in cultured salmonids. They noted that, while in many cases the use of water sources in which wild animals swam or were spawning was temporally linked to disease outbreaks in facilities where there was no previous detection of pathogens, there was no definitive evidence linking pathogens from wild animals to the hatchery outbreaks. The only strong evidence they could find was that in two successive outbreaks of IHNv at the Hoodsport hatchery in Puget Sound (1996 and 2000), a facility which releases chum, Chinook and coho smolts and collects the returning adults as broodstock, there had never been a previous positive test for the virus. In 1996, returning fall-run adult Chinook tested positive at nearly 100% prevalence, although no other fish at the facility tested positive, including the offspring of the infected adults. There was no detection of the virus at the hatchery in subsequent years until 2000, when fall-run Chinook adults again tested positive. The isolates were genotyped and found to be identical, but distinct from all other Washington State isolates. The following year, chum salmon adults returning to the facility tested positive, but that isolate was more similar to known sockeye salmon isolates from Lake Washington. No clinical disease was observed throughout these years, and there are many reports of adult animals returning from the wild with low-level infections. Amos and Thomas (2002) use this as evidence to claim that wild fish are reservoirs for pathogen

transfer to cultured animals, but their claim is not well supported by other evidence. In the cases of the outbreaks, there was no disease actually detected in wild fish, so it can only be suggested that water transmission played a role. The isolate brought back to Hoodsport, Washington in 1996 and 2000 was in fish that had been in the ocean, but it did not transfer to other stocks at the hatchery and did not cause disease. So, while the genotype was identical, the linkage was weak at best (see also Kurath and Winton 2011 and references therein).

Because of the lack of surveillance data, there is no evidence of transfer from farmed to wild salmonids. There is some evidence that there may have been some transmission of the virus to non-salmonid species living in proximity to the farm. However, the consequence of this infection is unknown and the infection was not sustained after the affected farmed fish were removed. Amos and Thomas (2002) list two cases in which it is thought enhanced populations passed pathogens to wild fish. In one case, the Cedar River hatchery, which releases sockeye salmon for stocking, the spawning adults are almost always positive for IHNv at the end of the run, with up to 100% prevalence, yet in 10 years of operation, there has been only one year in which the fry tested positive. Amos and Thomas (2002) claimed that since the eggs are incubated and hatched in virus-free spring water, the only possible source of that virus had to be river water used for a holding pond prior to release. No genotyping was performed, though, so the evidence is mostly anecdotal.

Given the nature of the evidence presented in Amos and Thomas (2002) and elsewhere, it is difficult to definitively state that the source of disease outbreaks in hatcheries is wild fish, or *vice versa*. Efforts should be made to test putative source fish and genotype any isolates from them, as well as the wild/farmed animals, to ensure that transmission is even possible. Given the other uncertainties, it would still not be definitive proof of the actual source of the pathogen but it would provide more concrete evidence consistent with the hypothesis.

The potential for spillback of IHNv from farmed to wild populations may be mitigated through vaccination, early detection of clinical cases, and interventions such as depopulation following a positive diagnosis. For example, during the most recent (2012) IHN outbreak in farmed salmon in BC, no outbreaks were observed in the Campbell River region of Vancouver Island, which contained two of the three index cases in the 1992 and 2001 outbreaks. However, during the 2012 IHN event there was no IHNv detected on farms. A possible explanation is that there was no exposure, but an equally plausible alternative is that the majority of farms affected during the last outbreaks were vaccinated against IHNv. This suggests that IHNv vaccination was highly effective in the field and consistent with controlled protection trials (Garver et al. 2005).

Consequences to wild fish depend on whether they have adequate immunity and if they have been exposed in natal waters, they may be immune. Naïve rainbow trout passively immunized with serum obtained from adult rainbow trout that survived a natural infection of IHNv showed high levels of protection to a waterborne IHNv challenge (LaPatra et al. 1993).

Data on viral shedding rates from infected Atlantic salmon, survival of IHNv within the water column under different conditions, and minimum infectious doses for Atlantic and sockeye salmon have been generated and are being used as biological variables for modelling of possible dispersal of IHNv from sites within the Discovery Islands, British Columbia. The model being used was developed by Foreman et al. (2012).

During the 2001 IHN outbreak, IHNv confirmation took, on average, 15.7 days (range, 5-21 days) but the incubation period was only 7.5 days. This indicates that increased spread could have occurred while awaiting test results, and this delay was determined to have affected IHNv spread (Saksida 2006). Current diagnostic tests, such as quantitative real-time PCR, provide results in 24 to 48 hours, thereby facilitating quicker case management (i.e., rapid depopulation), which decreases viral shedding and reduces the chance for horizontal transmission. Biosecurity practices (i.e., foot baths, limiting travel between farms), routine fish health monitoring, government surveillance programs, IHNv management agreements to depopulate index sites early in disease outbreaks, and processing plant effluent treatment (which is currently not done at wild fish processing plants), have also been implemented to limit spillback.

Some management practices, such as screening commercial aquaculture broodstock for IHNv at spawning and egg disinfection with iodophores (Batts et al. 1991), have also reduced the risk of vertical transmission of IHNv in cultured populations. Egg disinfection and disease management (i.e., culling) occurs at enhancement facilities that rear sockeye salmon, particularly in Alaska. If spawning is done in spawning channels, the fish are assessed for IHNv, but there is no intervention if virus is detected.

Summary

IHNv is a well-researched salmonid disease, and yet many unanswered questions remain. There are knowledge gaps that need to be addressed to improve the understanding of wild and farmed fish interactions for IHNv. While there is evidence of spillover, the source of infection and the risk factors associated with the events have not been determined. Evidence and the consequences for spillback have yet to be studied.

Mathematical models (i.e., stochastic or ordinary differential equation (ODE) models) of infectious diseases have become important tools for predicting effects of disease in populations, such as whether exposure to a pathogen will result in an epidemic, an endemic infection, or it will die out. Mathematical models have been used in terrestrial animal disease studies and efforts have been made to incorporate them into fish disease studies. However, models require empirical data for construction and subsequent validation, and our current, limited understanding of the aquatic environment and the apparent complicating role of water movement in pathogen transfer may limit their usefulness at this time. To our knowledge, there is only a single published model of IHNv transmission (Garver et al. 2013).

Although consideration of analogous situations involving other fish species may be a desirable tool in predicting outcomes or effects, it should be cautioned that the modes of transmission, pathogenicity, and virulence differ among microbes as do the effects they have on potential hosts, with the environment playing a significant role in modulating the whole process. Therefore, it appears that research into wild/farmed fish interactions will need to be microbe-, host-, and environment-specific to have the greatest utility.

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