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Pathway of effects between wild and farmed finfish and shellfish in Canada: Potential factors and interactions impacting the bi-directional transmission of pathogens

Séquence des effets entre les poissons à nageoires et les bivalves sauvages et d'élevage au Canada : facteurs et interactions potentiels ayant un impact sur la transmission bidirectionnelle de pathogènes

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ABSTRACT

This paper documents the evidence in support of linkages proposed among activities, stressors and effects as they relate to the release of pathogens from aquaculture. The extent to which pathogens released from aquaculture sites are stressors requires knowledge of infection and disease in wild aquatic populations. In Canada and other jurisdictions, targeted surveillance of endemic diseases should be established. Without this knowledge an assessment of whether and to what extent pathogens are stressors cannot be made. There is scientific evidence that pathogens present in wild populations are the source of initial infections in aquaculture animals and some evidence that aquaculture animals release pathogens to their environment. However evidence of pathogen transfer from aquaculture animals and/or products to wild populations is very limited. This paper provides a background to the general principles of pathogen transfer: factors related to the host, pathogen and environment are known to form complex interactions which together influence pathogen transmission and subsequent disease development. The paper also emphasises the distinction between infection and disease. These principles are discussed using four significant aquatic pathogens that exemplify specific modes of biology or transmission among bivalves or finfish: infectious salmon anaemia virus, *Renibacterium salmoninarum*, *Haplosporidium nelsoni*, and *Aeromonas salmonicida*. Gaps in knowledge and some recommendations for future study are also discussed.

RÉSUMÉ

Cet article présente des données probantes sur les liens pouvant exister entre les activités, les facteurs de stress et les effets et la libération de pathogènes issus de l'aquaculture. Pour déterminer dans quelle mesure les pathogènes provenant des sites d'aquaculture constituent des facteurs de stress, il faut connaître les infections et les maladies existantes au sein des populations aquatiques sauvages. Au Canada et dans d'autres pays, il faudrait établir une surveillance ciblée des maladies endémiques. Sans ces connaissances, il est impossible de déterminer si les pathogènes constituent des facteurs de stress et, le cas échéant, jusqu'à quel point il en est ainsi. Il a été démontré scientifiquement que les pathogènes présents dans les populations sauvages sont à l'origine des infections initiales chez les animaux d'aquaculture et il a été établi dans une certaine mesure que les animaux d'aquaculture transmettent des pathogènes dans leur environnement. Cependant, il existe peu de données montrant le transfert de pathogènes des animaux et/ou des produits d'aquaculture, aux populations sauvages. Le présent document expose le contexte entourant les principes généraux du transfert de pathogènes : facteurs liés à l'hôte, il a été établi que le pathogène et l'environnement forment des interactions complexes qui, ensemble, influent sur la transmission de pathogènes et le développement de maladies qui s'ensuit. Ce document montre aussi la distinction à faire entre une infection et une maladie. Ces principes sont énoncés en utilisant quatre pathogènes aquatiques importants qui illustrent des modes spécifiques de biologie ou de transmission parmi les mollusques bivalves ou les poissons à nageoires : virus de l'anémie infectieuse du saumon, *Renibacterium salmoninarum*, *Haplosporidium nelsoni*, et *Aeromonas salmonicida*. Cet article aborde aussi les lacunes dans les connaissances et propose d'autres études qui pourraient être réalisées.

INTRODUCTION

The aim of this review is to provide an evidence-based discussion on the variables involved in the transmission of pathogens from wild to farmed and farmed to wild populations. It will include a brief discussion on the principles of pathogen transmission and the effects of climate factors and population dynamics on disease. Specific examples of pathogens will be discussed in the context of stressor-linkages. Stressor-linkage refers to a physical, chemical or biological stress (either positive or negative) that impacts or is linked to the susceptibility of a host or its population to the onset of disease. The pathogens chosen are of economic importance to the finfish and shellfish aquaculture industry in Canada and represent the east and west coasts of Canada. Specifically, MSX (*Haplosporidium nelsoni*), infectious salmon anaemia virus (ISAV), *Aeromonas salmonicida*, the etiological agent of furunculosis, and *Renibacterium salmoninarum,* the cause of bacterial kidney disease (BKD), will be discussed. The pathogens chosen are representative of parasite, bacterial and viral agents, in addition to residing in both intra- and intercellular host locations and are among the most well-researched microbes in peer-reviewed literature.

In the past, several scientific reviews have discussed the potential for pathogen interactions between wild and farmed fish (Brackett 1991; Håstein and Lindstad 1991; McVicar 1997; Hedrick 1998; Reno 1998; Olivier 2002; McVicar et al. 2006). Recently, the extent to which salmon louse management practices in aquaculture affect the impact of this parasite in wild salmonid populations was reviewed (Jones 2009). Consequently, this parasite will not be a focus in the present paper. Together these reviews provided no conclusive evidence attributing adverse changes in wild salmon caused by pathogen transmission from cultured populations; however, as will be discussed, outbreaks in wild populations are rarely documented, so data are limited. The present report will expand on these reviews and discuss possible abiotic and biotic variables involved in pathogen transmission between fish populations. We will identify gaps in knowledge that may limit future policy considerations relating to aquaculture activities in Canada. Additionally, we will critically analyse evidence for and against pathogen transmission between wild and farmed fish.

PRINCIPLES OF PATHOGEN TRANSMISSION

INITIAL HEALTH OF AN ECOSYSTEM AND NATURAL INFECTION LEVELS

Understanding the effects of a given condition on a population of animals requires that baseline infection levels relating to the specific variable(s) under investigation are first established. This is especially true when examining effects of a pathogen on individual fish or their populations in the wild or in captivity. Naturally occurring levels of the pathogen within the environment, rates of infection, recovery and mortality within wild populations must be established. Some evidence of the mechanisms and factors contributing to host resistance should also be investigated, thus lending insight to the natural resiliency and plasticity within a host population. Few baseline studies of pathogens in wild aquatic populations have been conducted prior to the establishment of aquaculture in Canada making it difficult to provide sound conclusions relating to the affect of aquaculture on disease impacts on wild populations. The use of non-aquaculture sites located in different geographical areas as controls for aquaculture sites is possible. However since local geography, hydrology, climate and species diversity all

impact the ecology and biology of an area, these factors must be well documented and any differences between sites may be sufficient to affect the natural diseases processes.

When examining the consequences of a pathogen on a population either natural or artificial, the ecological relevance of the infection levels must also be considered. Ecological relevance refers to the degree of impact by a pathogen exerted on a host that deviates from the natural levels. Some pathogens naturally exert a large impact on populations while others have very little impact. This concept demonstrates the importance of conducting relevant baseline studies which will be discussed in more detail below. It is also important to note that if a pathogen is detectable it does not indicate an ecologically meaningful (that is, one having or likely to have an affect at the population level that deviates from normal fluctuations) infection or that the disease will manifest and spread. Moreover, an infection does not necessarily indicate the onset of disease will occur. In this case, a host may be able to mount an immune response and remain healthy or the host may be an asymptomatic carrier. Thus, it is critical that a distinction be made between what are natural infection levels in a given population and those that are epizootic in scale (whether natural or human–induced). This information can only be determined through empirical studies conducted using solid baseline data.

In the wild, natural pathogen infections have co-evolved with their hosts to establish a delicate balance between pathogenicity and host immune responses. Adaptation selects for individual pathogens and hosts in which infectiveness and evasiveness are optimised. Thus, most natural pathogens do not result in serious disease outbreaks (McVicar 1997). Fish farms may alter this balance by influencing the contribution made by one or more factors involved in pathogen transmission and/or disease development. How these factors, and other environmental conditions influence disease susceptibility, both in the wild and in culture, will be explored. The likelihood of disease resulting from exposure of an aquatic organism to a pathogen depends on the combined influences of the pathogen (virulency, infectivity, pathogenicity, concentration, and bioavailability), the host (species, age, immunity, stress, density, nutrition, and health status) and the environment (temperature, salinity, water quality, contaminants, currents, and other hosts and carriers (Figure 1). These relationships must be considered in the interpretation of the pathogen pathway of effects diagram (Figure 2).

DEFINING A DISEASE STATE

The disease state is influenced by multiple factors and as a result any definition requires consensus from diverse expert groups including epidemiologists, pathologists and diagnosticians for the proper determination of fish health. Many factors including genetics, nutritional deficiencies and pathogens may all compromise the health of an animal. In this report, a diseased individual is one in which clinical signs are observable, repeatable and are attributable to an etiological agent including parasites, viruses and/or bacteria. For our purposes, fish display clinical disease signs when they are deemed to be a significant deviation in the natural status of an animal (Olivier and MacKinnon 1998). Additionally, an important distinction and one that is frequently overlooked and understudied is further determining at what point an infection is characterised as being in a sub-clinical or a carrier state. Often pathogens are detected in the tissues of fish, but signs fail to develop and the pathogen is rarely a problem. In this case, the pathogen lives in a balance with its host and poses no harm to it. With advances in detection techniques such as polymerase chain reaction (PCR), it becomes possible to detect small numbers of pathogen particles, which does not necessarily translate into the onset

of a diseased state. Information relating to the level of infection necessary to lead to clinical disease must be empirically derived for each pathogen and its host(s). This can be difficult to determine in wild populations, as some conditions can result in significant mortality, thus removing affected individuals from the population and therefore from surveillance.

FACTORS AFFECTING THE ABILITY TO DETERMINE RISKS ASSOCIATED WITH PATHOGEN TRANSMISSION IN THE WILD

Research on pathogen spread between wild fish populations is extremely challenged by the need for a clear understanding of fish (host) ecology (Riley et al. 2008). Factors that should be considered in comprehensive studies of disease in wild fish populations are listed below:

1. Challenging environmental conditions often hamper sampling and experimentation efforts;
2. Historical data are rarely available to provide baseline values of what is considered normal for the population (Harvell et al. 1999);
3. Most historical data only includes mortality rates rather than data relating to how or why the fish were killed (Hedrick 1998; Noakes et al. 2000);
4. Wild fish that are killed as a result of a major infection are not sampled or studied but rather are removed from the ecosystem (Bergh 2007);
5. The complexity of disease interactions with its host and the surrounding environment affects the clear interpretation of experimental results. Most evidence is obtained from single-variable studies involving the pathogen and its host where multi-factorial studies are needed (Håstein and Lindstad 1991). This leads to a rapid knowledge gain in the study of the pathogen which is more easily studied and manipulated in the laboratory and a deficiency of knowledge relating to environmental interactions. This experimental bias can further lead to the misinterpretation of data in the context of the host and its dynamic environment (Hedrick 1998);
6. Multiple and/or silent infections can lead to the exacerbation of clinical signs attributed to one pathogen;
7. Quantification of the impact of a pathogen on its host is difficult, as the presence of a pathogen does not necessarily equate to clinical signs and the outbreak of disease. Highly sensitive diagnostic tools can lead to over-assumptions relating to seriousness of disease;
8. It is difficult to quantify the level of bi-directional transfer and interaction between wild and farmed fish (LaPatra 2003);
9. Influence of non-peer-reviewed literature clouding the issue and skewing objective results obtained from empirical studies;
10. Many stake-holders have feelings of ownership over ocean and river environments which can negatively influence their objectivity;
11. Difficulty in using data obtained from sites not used by aquaculture to serve as baseline data for sites were aquaculture is active;
12. An appropriate and standardised measure of immunity or resistance to a pathogen is often lacking within wild populations.

ACCEPTED CAUSAL REASONING CRITERIA

Cause and effect relationships are normally difficult to establish in nature (McVicar 1997). However if the association is strong, no statistical tests should be necessary (Hill 1965). In the scientific community, it is accepted that in order for a correlation between a marine pathogen and disease signs can be deemed a causation, the following criteria must all be met (Olivier and MacKinnon 1998; Olivier 2002):

1. Source fish must contain pathogen;
2. Pathogen must remain present in a diseased host fish;
3. Surrounding water must contain susceptible hosts;
4. Pathogen must survive in the environment;
5. Pathogen must be exposed to a susceptible host by a route that allows infection;
6. Pathogen must be in present in biologically significant numbers to initiate a new infection;
7. Infection must spread to other hosts.

WHY STUDY PATHOGEN TRANSMISSION BETWEEN FISH POPULATIONS?

There is a high degree of public concern for fish disease in the wild and aquaculture and in particular, for the transmission of pathogens between cultured and wild aquatic organisms. Aquaculture conditions typically result in higher densities and abundance of susceptible fish or shellfish which can alter host-pathogen relationships possibly resulting in the exacerbation of natural infections under domestic conditions. The proximity to ecologically-sensitive areas may give the impression that natural infections are being spread to wild stocks. Wild stocks are often seen as ‘pristine’ while farmed fish are viewed as more likely to harbour disease-causing pathogens and have a greater impact on the surrounding ecosystem. First reports of epizootics in wild fish populations prior to the presence of cultured fish suggested the direction of pathogen transmission was from wild to cultured stocks (Håstein and Lindstad 1991). However, due to the aforementioned reasons, the reverse is also probable (Krkošek, 2010). In order to properly address these concerns, many basic science questions need to be answered. More is known about disease status in farmed aquatic animals but not wild finfish or shellfish. In enhanced finfish culture, some information is gained through pathogen-screening programs; however, little information is gathered once the fish are released into the wild. The effect of introducing fish species (non-native species culture and/or captive breeding programs) to new locations with exposure to native pathogens or the introduction of pathogens to areas with previously unexposed animals may be studied by understanding bi-directional movements of pathogens between wild and cultured finfish and shellfish populations. Data gained from these types of studies will be required to develop appropriate policy leading to the successful conservation of wild fish populations. This report will explore the probability and past documented occurrences of disease transmission in the peer-reviewed literature between both farmed and wild fish stocks and highlight the gaps in knowledge in these areas.

MODES OF PATHOGEN TRANSMISSION

Several transmission strategies are employed by aquatic pathogens: horizontal, vertical and vector-borne. Combinations of these strategies are employed by some pathogens. Horizontal transmission refers to the direct movement through the water column of a pathogen from an infected to a naïve individual. Horizontal transmission may occur

within a species group or between two individual species, where the rate of horizontal transmission is dependent on the frequency of contact between individuals, the susceptibility of the host (general health and immune ability) and the transmission coefficient (ability of the pathogen to invade, replicate and disperse) (Reno 1998). The reproductive rate (R_o) of the pathogen is expressed as the number of successful infections per unit time. The larger the R_o, the greater the ability of the pathogen to infect, reproduce and spread to susceptible hosts within or between populations. Large R_o pathogens can lead to the exhaustion of susceptible hosts and the cessation of further replication and spread. Smaller R_o pathogens have a more limited ability to cause an epizootic, as they are normally slower to replicate and transmit. As expected, intermediate R_o pathogens can persist in a population for a greater period of time. An evolutionary advantage of low and intermediate R_o pathogens is the maintenance of a high proportion of susceptible individuals in the population (Reno 1998). It is noteworthy to mention that R_o values have rarely been applied to disease infections in wild fish due to the difficulties in tracking and sampling the fish. Moreover, in the wild, long-term infections are often more difficult to investigate, as natural variations among populations with respect to environmental factors, immigration, emigration, births, deaths, other infections and mortalities due to predation have to be considered.

Vertical transmission involves the passing of a pathogen from the mother to offspring. From an evolutionary perspective, this increases the availability of future susceptible hosts and increases the chance of a pathogen successfully infecting a population. Albeit difficult to demonstrate empirically, vertical transmission will theoretically increase the transmission co-efficient and decrease the threshold density of hosts required for infection. Vertical transmission is also normally associated with longer infectious periods (Reno 1998).

The epidemiology associated with introduced pathogens (either horizontally or vertically transmitted) is altered relative to that of native pathogens. Extensive data on terrestrial vertebrate disease history shows that the introduction of a disease to a population where tolerance to that pathogen has not been established can be highly damaging to the native population and the same is most likely true of marine populations (McVicar 1997). However, before a successful infection can take place the introduced pathogen is subjected to selection pressures that are unique to the new environment. In addition to exposure to new and different environmental variables, susceptible hosts are more likely to become heavily infected, possibly leading to premature removal from the population, further decreasing the transmissibility of the pathogen to new and susceptible hosts (McVicar 1997). This process will eventually lead to the establishment of a new host-pathogen equilibrium. However, the ability of a newly-introduced pathogen to become amplified or diluted in a new environment is not well understood (Kelly et al. 2009). Amplification or dilution of a introduced pathogens appears to be dependent on many factors including species diversity, population density, infection intensity, parasite mode of transmission and host behaviour (Daszak et al. 2000; Kelly et al. 2009). The topic of introduced species is beyond the scope of this report and thus will not be further discussed.

Diseases can also be spread by a third host or vector. Vector organisms for fish can include other parasites, other fish and piscivorous animals such as birds. This facilitated transmission does not rule out the possibility that the pathogen may also use a horizontal or vertical mode of transmission. Pathogens with obligate requirements for vector-mediated transmission require more complex models and examples of such

pathogens are not discussed here although many of the basic principles apply. The complexity of these pathogen life-cycles is beyond the scope of this review which is to focus on a broader discussion of the factors affecting pathogen transmission.

ENVIRONMENTAL FACTORS AFFECTING PATHOGEN TRANSMISSION

Pathogens are a natural component of an environment and provide strong selective pressure on hosts in order to maintain healthy host populations. For finfish and shellfish, bacterial, viral and parasite pathogens all play an important role in the overall health of an ecosystem. As indicated, the onset and development of disease reflects a complex interaction between host, pathogen and the environment (Snieszko 1974). These factors are discussed on an individual basis; however, in the wild, they may act synergistically to exert multiple, compounding effects on a pathogen and on the physiology of the host both at the individual and population level. Limited data exist regarding multi-variate studies of environmental stressors and their impacts on disease susceptibility. Most of the studies described below are related only to single factor challenges which are conducted in a controlled laboratory environment.

TEMPERATURE

Temperature has a direct effect on fish and shellfish physiology and has broad-reaching implications for disease interactions between host and pathogen (Bly and Clem 1992; Harvell et al. 1999; Bowden 2008). As fish are ectotherms, temperature governs every facet of their physiology including disease susceptibility and development. Temperature also affects the survival of the pathogen in the environment and its ability to infect hosts. Most organisms function within temperature ranges suitable to the optimal physiological performance; however, perturbations can result in the alteration of biochemical pathways and thus have consequences on disease physiology. Many studies have looked at the effects of temperature on a host's immune response and only a small portion will be presented here in order to provide a framework for future discussion involving temperature effects on specific pathogens (see below).

Due to the fundamental nature of temperature, multiple physiological systems are simultaneously affected making conclusions relating to specific effects difficult. An abundance of work in this area makes it clear that interpretation of results should only be performed on individual species groups. Considerable variability is seen depending on the conditions and the species examined. Physiological responses to changes in temperature depend on several life history characteristics including immune ability, optimal temperature ranges and temperature-defence mechanisms of the species.

A number of immune-related functions are affected by temperature changes. Most studies on fish find lower temperatures are more favourable for immune function; however, adaptive responses have been noted in many species. An adaptive response worth noting is the apparent compensatory ability of the innate and humoral immune functions. A study in Atlantic salmon (*Salmo salar*) showed an increase in immunoglobin or antibody (Ig) producing cells at lower temperatures, but at higher temperatures, levels of these cell types were seen to decline while levels of neutrophils increased (Pettersen et al. 2005). This compensatory ability is also seen in Sockeye salmon in which lymphocytes were seen to increase under higher temperature conditions (12^{o}C) whereas phagocytic kidney macrophages were seen to increase at lower temperatures (8^{o}C) (Alcorn et al. 2002). In the Channel catfish (*Ictalurus punctatus*), it was illustrated that

lymphocyte and neutrophil counts were differentially modulated under low temperature (Bly and Clem 1992; Le Morvan et al. 1998). Differences seen between cellular and non-cellular responses suggest an adaptive immune response where non-specific defences are offset and function primarily under sub-optimal temperatures to continue to provide immune protection for the host over a wider range of temperature conditions (Manning and Nakanishi 1996; Alcorn et al. 2002).

Other studies also show temperature effects on immune function. In the Mozambique tilapia (*Oreochromis mossambicus*), higher temperatures were found to decrease respiratory burst activity and phagocytic activity of leukocytes (Ndong et al. 2007). Moreover, mortality increased in tilapia acclimated to extreme cold or warm temperatures when exposed to *Streptococcus iniae* where intermediate temperature groups remained unaffected (Ndong et al. 2007). Respiratory burst activity of leukocytes in Rainbow trout revealed a temperature-dependent response in animals kept at higher temperatures (Nikoskelainen et al. 2004). In Atlantic cod (*Gadus morhua*), expression of immune-related genes such as interleukin-1β in blood leukocytes increased with exposure to higher temperature (Pérez-Casanova et al. 2008). Additionally, low temperatures have been found to affect components of the non-specific immune response in Channel catfish (*Ictalurus punctatus*) including stimulants of T-cell and B-cell proliferation and cytokine production (Le Morvan et al. 1998; Bowden 2008). Some immune components, such as the complement system in Sockeye salmon, remain unaffected when exposed to lower temperatures (Alcorn et al. 2002).

Temperature can also affect carbohydrates and proteins involved in immune functions. Low temperatures have been found to alter cell membrane fatty acid compositions (e.g., increase in unsaturated fatty acids) which harbour carbohydrates involved in cell recognition (Le Morvan et al. 1998). For example, sialic acid concentrations were shown to decrease in carp (*Cyprinus carpio*) under low temperature conditions; however, it is unknown whether this translated into a detectable difference in immune competence in these fish.

Shellfish are naturally affected by dramatic temperature fluctuations owing to the seasonal and even daily fluctuations in temperatures (for example, in oysters and mussels where tidal activity greatly influences temperature exposure). As such, shellfish can be more vulnerable to pathogens during particular times of the day or year. Superoxide dismutase (SOD) is an important antioxidant defence for shellfish. Thermal stress on the clam, *Chamelea gallina*, resulted in significant modulation (both increasing and decreasing) of several distinct proteins of the SOD family (Monari et al. 2007). These differences also appear to translate into differences in survivability under higher temperatures. Clams reared at 30^{o}C had significantly lower survival rates than clams reared at either 20 or 25^{o}C (Monari et al. 2007). Haemocyte mortality increased in Pacific oysters (*Crassostrea gigas*) reared in temperatures greater than 40^{o}C (Gagnaire et al. 2006). The survival of the haemocytes under high temperature conditions (up to 40^{o}C) suggests oysters are well adapted to higher temperatures, of which they can be frequently exposed to during the summer months (Gagnaire et al. 2006). In the bivalve, *Laternula elliptica*, an increase in temperature can lead to an increase in the production of reactive oxygen species (ROS) (Heise et al. 2003). Additionally, in the mussel, *Mytilus edulis*, temperature (10^{o}C vs 15^{o}C) and copper (0.02 and 0.05 ppm) stress impaired haemocyte function resulting in a reduced ability to clear the pathogen *Vibrio tubiashii* (Parry and Pipe 2004).

Marine pathogens are thought to be greatly affected by temperature; however, little research has been conducted on species relevant to fish and shellfish pathogens. In general, temperature has a different affect on bacteria depending on the growth phase (latent or active growth). High temperatures can accelerate metabolism and thereby increase nutrient demand in a nutrient-poor environment (Sinton 2006). Many viruses appear to be relatively tolerant of moderate temperature extremes however, the loss of marine virus titres at higher temperatures is often a result of protein denaturation (Gerba 2006). Some marine viruses possess dynamic structural coat proteins which may protect against thermal challenge (Dimmock 1967).

CHEMICALS AND POLLUTANTS

The marine environment is becoming increasingly contaminated with anthropogenic chemicals (Harvell et al. 1999). Pathogens and hosts respond to chemicals in different ways potentially leading to an imbalance of host-pathogen interactions (Riley et al. 2008). Herbicides, fungicides and insecticides are major classes of chemical pollutants. These are in addition to others including chemical therapeutants, metals and aromatic hydrocarbons such as PCBs (Anderson 1996). Research on samples collected from natural populations is complicated by the co-occurrence of many types of chemicals within one individual and by the observation that the same chemical can have contrasting effects at different doses (Anderson 1996).

Environmental chemicals can interact directly with immune function and many are found to immunosuppress rather than immunostimulate (Anderson 1996; Harvell et al. 1999; Riley et al. 2008). Organochlorides can impair natural killer cell activity in a wide variety of marine species including mammals (Harvell et al. 1999). B-cells are a critical component of the vertebrate immune system with a primary responsibility of presenting immunoglobins or antibodies on cell surfaces. Antibodies recognize, bind and neutralize foreign antigens. Compounds such as endrin (a pesticide), phenol (an aromatic hydrocarbon), DDT, Aroclor 1254 (PCB) have been found to reduce the number of antibody producing cells in fish (Anderson et al. 1984; Bennett and Wolke 1987; Cleland et al. 1987; Anderson 1996). B-cell-mediated immunity can be assessed by examining the primary and secondary plaque-forming cell (PFC) responses of leukocytes. Arkoosh et al. (1994) found that Chinook salmon had a depressed PFC response when polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) were injected intraperitoneally (Arkoosh et al. 1994). Juvenile Rainbow trout have also shown decreased ability to stimulate T- and B-cell production when exposed to organotins, commonly used in industrial and agricultural practices (O'Halloran et al. 1998). Prior exposure to PAHs or PCBs increased the susceptibility of juvenile Chinook salmon to *Vibrio anguillarum* (Arkoosh et al. 1998b). Alternatively, other studies show an increase in phagocytic activity and respiratory burst in leukocytes of juvenile Rainbow trout exposed to the common pesticides chlorothalonil, cypermethrin, and pentachlorophenol (Shelley et al. 2009). However, this immunostimulation did not translate into decreased susceptibility to a disease challenge with *Listonella anguillarum*. The authors suggest that the specific immune functions measured may not be critical in the clearance of the tested pathogen; however, they also caution that deviation from the natural response of the immune response is still cause for concern and also points to the difficulties in assessing chemical impacts on host immune function, usually because defence mechanisms for specific pathogens have not been characterised. These and other results demonstrate a direct evidence-based link relating chemical exposure and increase susceptibility to marine pathogens (Sovenyi and Szakolczai 1993; Austin 2006;

Shelley et al. 2009). Putative pathways by which chemicals may modulate host defences include inhibition of Ca^{2+}-ATPase, ATP synthesis, and the production of bactericidal free radical species (Galloway and Depledge 2001). However, more research is required to elucidate the mechanisms involving the chemical-suppression of host defences.

The limited studies conducted on wild fish confirm the negative impacts of chemicals on fish immune function. For instance, the mummichog, *Fundulus heteroclitus*, exposed to pulp mill effluent displayed a significant decrease in phagocytic activity of head kidney macrophages relative to fish sampled some distance away from the effluent (Fournier et al. 2000).

The effects of organic pollutants on shellfish immunity are not well understood. Few studies have been conducted and have yielded mixed results (Dyrynda et al. 1998; Galloway and Depledge 2001). Experiments have shown that exposure to PAHs in the mussel, *Mytilus edulis* for up to 4 weeks resulted in a decline in phagocytotic cells (Grundy et al. 1996). Significant differences were also shown between sites for the generation of superoxide compounds and certain monoclonal antibodies with no differences found in total haemocyte counts, haemocyte enzyme activities and phagocytosis (Dyrynda et al. 1998). Exposure of *M. edulis* to tributyltin was found to decrease haemocyte viability and increase DNA damage (strand breaks and formation of micronuclei) with no change in phagocytic activity or antioxidant capacity (Hagger et al. 2005).

Chemicals can also impact the ability of a pathogen to survive in the environment or infect a host. Some chemicals present in the marine environment can inactivate sensitive pathogens or promote the selection of more tolerant pathogens (Reno 1998). Little research has been conducted in this area; however, it is know that antibiotics used on cultured fish populations can lead to resistant bacteria potentially re-infecting wild populations (McVicar 1997).

PHOTOPERIOD, ULTRAVIOLET LIGHT AND DIURNAL RHYTHMS

In fish, the immune system responds to photoperiods and diurnal rhythms which may also be linked to temperature fluctuations. Data are limited regarding the effects of photoperiod on finfish and shellfish. One study found an increase in immune complement activity during the day than at night in Sea bass (*Dicentrarchus labrax* L.) whereas peroxidase activity was found to peak during the morning hours (Esteban et al. 2006). In vaccinated Atlantic salmon, antibody IgM levels are lower under shorter photoperiod conditions (Bowden 2008). No research has been conducted on the affects of photoperiod on shellfish defence functions.

Ultra-violet (UV) light has long been known to inactivate bacteria and viruses in shallow waters (Downs and Blunt 1877; Sinton 2006). Time to inactivation depends on intensity of the wavelength, depth of the pathogen in the water column, turbidity, exposure period and pathogen species (Suttle and Chen 1992; Fuhrman 1999; Sinton 2006). UVB and UVC light are considered the most damaging due to the nature of the higher energy of the shorter wavelengths (280-320 nm). UVB and UVC wavelengths are optimally absorbed by atoms within DNA and other protein molecules leading to a molecular rearrangement of the bonds (Vincent and Neale 2000). UVB light intensity depends greatly on the concentration of particulates in the water which have the ability to scatter or absorb light depending on the nature of the substrate (Garcia-Pichel and Bebout

1996). UVB light can cause cross-linking of nucleotides in both viruses and bacteria (Weinbauer et al. 1997). RNA viruses are particularly vulnerable to UV damage, while DNA viruses are more resistant to UV light, as they can utilize nucleotide repair mechanisms (Gerba 2006; Sinton 2006). It has also been suggested that photoreactivation may also be a mechanism whereby blue light (300-500 nm) can activate photolyase, an enzyme which repairs nucleotide dimers and other nucleotide damage in marine viruses (Weinbauer et al. 1997). DNA repair by photoreactivation has also been documented in bacteria and algae (Weinbauer et al. 1997). Pathogen survival strategies can also include high reproduction rates to compensate for rates of decay due to UV light. It has been found that decay rates of certain marine DNA bacteriophages are 2-12 times greater under UV conditions than under control dark conditions and the rate of viral decay is proportional to the amount of radiation received (Suttle and Chen 1992). The aforementioned data may have great relevance to the persistence and maintenance of pathogen survival and infectivity in ocean communities; however, little is known regarding finfish and shellfish pathogens.

DISSOLVED OXYGEN AND OTHER GASSES

Dissolved gasses are directly affected by the temperature of the environment and some gases, such as CO_2 are interconnected with environmental pH. It is well documented that dissolved gases, and in particular dissolved oxygen, have broad implications for the health and physiology of fish. Hypoxic (low oxygen) conditions can lead to the deterioration of physiological systems involved in respiration, exercise, behaviour, digestion, reproduction and immunity (Randall 1982; Kramer 1987; Axelsson and Fritsche 1991; Schurmann and Steffensen 1997; Wu et al. 2003). Fundamentally, hypoxic conditions can affect the ability of an organism to mount an immune response by limiting the availability of energy in the form of ATP. More specifically, hypoxic conditions depress respiratory burst activity in sea bream (*Sparus aurata*) and antibody levels have been shown to decline in sea bass. In the same study, hyperoxic (high oxygen) conditions resulted in an increase in antibody levels (Bowden 2008). The induction of hypoxic conditions in the oyster, *Crassostrea virginica*, resulted in the lowered ability to produce ROS (reactive oxygen species) (Boyd and Burnett 1999). In a study where disease susceptibility was examined, the oyster, *Crassostrea virginica*, was naturally infected with *Perkinsus marinus* followed by hypoxic and pollutant challenges. Hypoxia exacerbated the effect of oyster mortality relative to the effect of the pollutant alone (Anderson et al. 1998; Mydlarz et al. 2006).

SUSPENDED SOLIDS

In fish, suspended solids may elevate hematocrit and lysozyme activity and lower leucocrit; although these effects have not been repeated they parallel other environmental impacts such as reduced oxygen availability, which may also interact (Redding et al. 1987; Bowden 2008). In one study, yearling Steelhead (*Salmo gairdneri* Richardson) challenged with *Vibrio anguillarum* and held in clear water, were found to have increased survival relative to the group held in 2.5 g/L suspended solid conditions (Redding et al. 1987). Oxygen levels were held constant in this study. Other indirect impacts include changes in behaviour and foraging success and vulnerability to predation (Wilber and Clarke 2001).

Suspended solids can protect pathogens from environmental insults such as UV light. For example, viruses can absorb onto clays, sand, and even human wastes such as

glass or plastic (Gerba 2006). Experiments involving shellfish are lacking; however, suspended solids reduce feed efficiency and prey selection and therefore likely limit energy available to devote to immune defences (Safi et al. 2007).

SALINITY

While many fish can tolerate significant deviations in environmental salinity, the effects of salinity changes on host immune defence mechanisms is poorly understood. In Rainbow trout, phagocytosis, respiratory burst activity, and lysozyme and IgM levels were found to increase in proportion to salinity (Marc et al. 1995; Delamare-Debouteville et al. 2006; Taylor et al. 2007). A compounding effect of increased salinity which has largely been under-investigated is the additional role of stress on immune and other physiological functions when fish are placed in sub-optimal salt environments.

Pacific oysters exposed to <16 ppt (originally acclimated to 34-34.5 ppt) salt showed an increased haemocyte mortality (Gagnaire et al. 2006). Salinity was shown to modulate phagocytic activity where an increase was observed under both hypo- and hypersaline conditions (Gagnaire et al. 2006). The change in ability to mount an immune defence may have implications on the host's susceptibility to pathogens on a daily basis. In *Crassostrea virginica* exposed to salinities <15 ppt, hemocytes were capable of destroying *Haplosporidium nelsoni* (Ford and Haskin 1988). The hemocyte response seen in the laboratory was rapid and prolonged exposure to lower salinities in a natural environment may be more effective at eliminating the pathogen (Ford and Haskin 1988). When previously exposed to *Vibrio tapetis,* the Manila clam, *Ruditapes philippinarum*, displayed increased susceptibility to disease progression under lower salt conditions (20%) and almost complete recovery under high salt conditions (40%) (Reid et al. 2003). The authors speculated this was due to increased haemocytes and lysozyme levels.

In bacteria, proteins and potassium ions appear to be critical in combating changes in osmotic pressure (Sinton 2006). Moreover, bacterial cell aggregation, cell wall structural components and organic matter have been shown to be protective to marine bacteria, all which may be influenced by environmental salinity (Gauthier and Le Rudulier 1990; Findlay 2003; Sinton 2006). More studies are required on the affects of inorganic ions on fish pathogens.

pH

The pH of the marine environment ranges from 7.5 to 8.5 and is influenced by dissolved gasses, temperature, pressure, microbial photosynthesis and respiration (Sinton 2006). Consistent pH levels are more important in freshwater environments, as sea water has buffering capacity. Currently, research is poor in this area for both finfish and shellfish. One study using Nile tilapia, *Oreochromis niloticus niloticus*, showed that of two groups held at pH 7.9 and pH 4.0 for 2 weeks, the lower pH group had higher lysozyme levels, but no differences were seen in IgM levels between challenge groups (Dominguez et al. 2005). Evidence suggests a dramatic change in environmental pH would also negatively affect food availability and habitat conditions of finfish and shellfish which would ultimately have an indirect effect on host susceptibility to disease (Anderson 1996; Bowden 2008).

Little research has been performed on the effect of environmental pH on pathogens. One study found that most marine viruses to be stable between pH's 5 to 9 (Gerba 2006).

DEPTH AND PRESSURE

Research relating to depth and pressure affects on immune capacity of marine hosts is poorly understood. Some research on bacteria show that bacterial protein synthesis, replication and nutrient uptake are all affected by pressure; however, the underlying mechanisms are unknown (Sinton 2006). Another study shows that hepatitis A viruses within oysters can be inactivated by hydrostatic pressure (Calci et al. 2005).

METALS

As seen in experiments involving organic pollutants, experiments involving metal toxicity in finfish and shellfish are often complicated by dose-effects (Anderson 1996; Fournier et al. 2000). For example, phagocytic activity of *Mytilus edulis* haemocytes were shown to be stimulated by copper at 0.2 ppm, but not at 0.05 or 0.5 ppm (Pipe et al. 1999). In the same study, increased copper exposure had the opposite affect on circulating haemocytes, causing the percentage of eosinophils to decrease while the percentage of basophils increased. Other studies show *M. edulis* exposed to 0.4 ppm cadmium had increased circulating eosinophil cell numbers (Pipe and Coles 1995). Copper and cadmium are widely accepted as having adverse physiological affects on marine vertebrates and invertebrates (Anderson et al. 1989; Galloway and Depledge 2001; Grosell et al. 2007). Long-term exposure to copper has also been shown to increase susceptibility to *Vibrio tubiashii* in *M. edulis*. Total mortality was higher in mussels exposed to both copper and *V. tubiashii* relative the exposure to copper alone (Pipe and Coles 1995). In Rainbow trout, *in vitro* studies show copper can have immunosuppressive effects on antibody-producing cells (Anderson et al. 1989).

CURRENTS AND GEOGRAPHY

Currents will primarily affect the spatial distribution of a pathogen in fish habitat and influence whether the two populations come into contact (Arkoosh et al. 1998a). Research is greatly lacking in this area, and there are very few data concerning pathogen tracking in the aquatic environment. It is accepted that pathogen movements will reflect local hydrography and will tend to mirror water movements. This directly influences where and at what concentration a pathogen will be in a river or ocean environment and the frequency of contact with susceptible hosts. Mathematical modelling has predicted initial point source infections of sea lice (*Lepeophtheirus salmonis*) to be dependent on factors such as local geography, wind-driven circulation and tides while depth and freshwater input into estuarine areas may have less impact (Amundrud and Murray 2009). Further empirical studies are required to validate these findings.

SEDIMENTATION

Sedimentation removes bacteria from the water column; however, turbulence can result in their re-suspension (Sinton 2006). Some bacteria can use dissolved organic matter on their outer surfaces to protective against aggregation and thereby limit sedimentation (Sinton 2006).

OTHER HUMAN IMPACTS

The adoption of chemotherapeutant and some vaccination programs has also assisted in halting the spread of many pathogens. In addition, good biosecurity practices in farms (e.g., fallowing sites to break disease cycle and testing fish prior to transfer to another facility) have mitigated the artificial transmission of disease infections (Bron et al. 1993; Ewart and Ford 1993; Costello et al. 2001). There is evidence however, that transport of fish or eggs has contributed to the global spread of Infectious salmon anaemia Virus (ISAV) (Kibenge et al. 2009, Vike et al. 2009) and that vessels used to move fish aid in transmission of the virus over shorter distances (Murray et al. 2002). Overfishing can lead to the removal of both healthy and diseased individuals from a population. This unnatural selection can result in changes in the population structure (age, longevity, body size, condition, recruitment and genetic factors) leaving an increased number of fish susceptible to disease infection (Harvell et al. 1999; Pauly et al. 2002; Berkeley et al. 2004).

DISEASE TRANSMISSION IN RELATION TO POPULATION DYNAMICS

POPULATION DYNAMICS

Studies of population dynamics in both aquatic and terrestrial environments often rely on mathematical models to help categorize and determine risk associated with specific factors including disease transmission. Mathematical equations are developed to integrate physical and biological parameters based on current knowledge of a given area, species or condition. However, considerations must be made to recognize the limitations of mathematical models, as a lack of knowledge regarding environmental parameters, host life history and pathogen survival in the natural environment are just some of the variables that limit the effectiveness and application of many models. Most ecosystems are highly integrated and dynamic and most models do not consider this complexity and therefore are extremely difficult to validate.

In an attempt to understand population dynamics, several overriding principles are used in models which help determine the ability of a pathogen to infect a population of hosts. The principle components of disease transmission include the frequency of contact between infected and susceptible individuals and how the host population interacts with the disease source (Reno 1998). Mathematical models have predicted that the frequency of contact between infected and susceptible individuals is primarily determined by the host population density (Reno 1998). Densities are more variable in the wild and are more homogeneous in culture populations. Densities in the wild can be only partly predictable, as anadromous species such as salmon spawn at specific times during the year, and at this time come in close contact in the spawning rivers creating an environment more conducive to pathogen transmission (Reno 1998). For both cultured and wild populations, a threshold density must be reached in order for a disease to be successfully transmitted between individuals. If the host density is below this threshold, an epizootic will not occur (Reno 1998). Threshold densities may be different between wild and cultured populations due to the more crowded conditions typical of cultured populations. The transmission efficiency of a pathogen is directly related to host threshold densities and is an indicator of the number of pathogens required to successfully infect a susceptible host. Parameters affecting transmission efficiency

include the virulence and invasiveness of a pathogen. If the transmission efficiency of a disease is high, a lower threshold density will be required for an epizootic whereas a pathogen with lower transmission efficiency will require a higher threshold density. The density of fish populations have also been found to have an effect on behaviour (i.e. schooling) which can lead to changes in the frequency of contact between individuals (Håstein and Lindstad 1991). Modelling has also provided some insight into the transmission of aquatic pathogens between farmed and wild fish in relation to population densities. Simple models predict a pathogen such as infectious pancreatic necrosis virus (IPNV) may be transmitted between farmed and wild populations in a density-dependent manner while the parasite *Gyrodactylus salaris* would spread through density-independent means (Anttila et al. 2008). Anttila et al. (2008) also suggest conclusions of this nature may only be applicable to the specific study site. Open recruitment models can aid in predicting transmission of such pathogens as *L. salmonis* (Amundrud and Murray 2009; Murray 2009). These models have implications for disease management procedures, allowing managers to apply more effective treatment strategies depending on the species of pathogen.

Interactions between host and pathogen populations are also dependent on the distance between host and pathogen populations and persistence of the pathogen within a host population. Proximity to a disease 'source' will determine if the pathogen is transmitted to a 'sink' host population. Wild populations are generally agreed to be the original source of infections either to other wild populations or cultured species (Håstein and Lindstad 1991). In general, greater distances between wild populations and farms or other wild hosts will prevent transmission of pathogens; however, geographical parameters play a large role. For example, water current trajectories and rates can determine how the pathogen is disseminated from the source (Reno 1998). Additionally, the initial concentration of the pathogen in the source host determines quantity of release into the water for subsequent infections (Reno 1998). The ability of a pathogen to persist in an infective state in the host can increase the probability of successful transmission. For example, the longer a fish is infectious, the greater is the transmission efficiency, thereby decreasing the population density threshold required for successful transmission. Additionally, the spread of an infection may be exacerbated by culture conditions, as weakened animals are not removed from the population. In the wild this may result in minimizing the spread of the disease (Johnson et al. 2006).

Based on mathematical models, it is generally accepted that there are three classes of hosts within a host population (Reno 1998). The first group is composed of susceptible hosts. These hosts are not infected, but have the potential to become infected. Susceptibility is determined by level of resistance that is insufficient to prevent infection by the pathogen. Resistance can be influenced by the level of previous exposure and development of protective responses through humoral or cellular immunity. A second class consists of infected hosts. These individuals have contracted the disease and have the potential to spread the disease. Within this class are two infection states; one refers to latent infections in which pathogens are not shed and transmission does not occur. The length of time associated with this phase is dependent on the biology of the host-pathogen system. The second state is where the host is actively infectious and the pathogen is readily shed. Clinical signs of infection can begin to occur in this phase and the length of time associated with this phase can determine the degree of transmission to other hosts. The duration of this phase will dictate the abundance of shed pathogen. This phase ends when the host has mounted an immune response sufficient to prevent further shedding of the pathogen or when the host succumbs to the disease. Individuals

may become re-infected if natural or acquired immunity to the pathogen wanes. A third class is composed of removed hosts. This class includes hosts that are no longer infectious and hosts that have not recovered and have succumbed to the disease. The dynamics of these interactions are based on natural infections and can become invalid when dealing with exotic or introduced species. The same may also hold true for cultured farms, as the population structures are altered. In the wild, a pathogen and host will often have co-evolved and developed a tolerance for one another and this forms the basis for extended associations host-pathogen associations (McVicar 1997).

BIOLOGY OF THE INDIVIDUAL HOST

The overall health of a host and its ability to effectively mount an immune response has direct implication on whether or not an infection will be successful. Genetics and natural physiological ability as well as environmental factors all play a part in conferring immune-competence on a host.

Development of the immune system appears to coincide with the development of the stress response in fish. Little is known about the immune response in larval fish; however juvenile (pre-smolt) fish appear to have enhanced immune ability (Schreck 1996). The innate response is more developed in larval fish where lysozyme activity has been shown to be important in the prevention of vertical transmission of *Aeromonas salmonicida* whereas other pathogens such as *Renibacterium salmoninarum* have been found to be resistant to its activity (Magnadottir et al. 2005). Rainbow trout mount a defence against *A. salmonicida* at 2-3 months of age (Zapata et al. 2006). The reasons for this are unknown. For salmonids, the smolting phase has particular implications for disease susceptibility. At this time, cortisol levels and the response to stress increase having a suppressive effect on immunity (Schreck 1996). In Coho salmon (*Oncorhynchus kisutch*), the number of splenic cells that produce antibodies are reduced during the parr-smolt transformation (Maule et al. 1987). These cells were found to be more sensitive to the suppressive effect of cortisol and the fish were found to be more susceptible to disease. In Atlantic salmon, lysozyme activity, leukocyte and lymphocyte cell numbers decrease during smolting (Muona and Soivio 1992). In the adult stage, salmonids appear to be more susceptible to disease with immunity dramatically weakening at the time of spawning (Maule et al. 1996b; Schreck 1996). This is likely due to elevated levels of cortisol and fewer antibody producing cells in the peripheral circulation.

The role of sex hormones in relation to immunity is not well understood; however, some studies have shown estradiol and testosterone correlate with antibody producing cells in females and not males (Schreck 1996). In later stages of life, testosterone appears to kill lymphocytes and androgen may act in a similar immunosuppressive manner as cortisol (Slater and Schreck 1993; Slater et al. 1995).

Under suboptimal conditions, a host's immune system will not function as effectively to defend against a pathogen. This could be due to either environmental conditions impacting immune ability or physical effects such as injuries to the skin or shell. For example, in shellfish, amoebocytes are involved in both wound healing and immunity (Acosta-Salmón and Southgate 2006). Thus, these cells will have a more limited ability to fight infection when involved in wound healing. Transient effects have been noted in molluscs subjected to physical disturbances wherein haemocyte numbers declined as

well as their ability to phagocytose. Moreover, injuries to invertebrates can lead to an entryway for pathogens into the host (Mydlarz et al. 2006).

Within a host population, natural genetic variability will allow some individuals to mount a more efficient immune defence than others (Manning and Nakanishi 1996). Studies selecting for high and low antibody-producing individuals have shown to express different classes of major histocompatibility complex (MHC) genes. MHC genes are an extensively diverse and polymorphic class of molecules responsible for self and non-self antigen presentation and recognition (Van Muiswinkel et al. 1999). Low antibody producing fish were found to be more susceptible to invading parasites and express a specific class of MHC genes (Wiegertjes et al. 1996). Correlations have also been found between MHC gene expression and disease resistance to infectious salmon anaemia virus (ISAV) and the bacterium *A. salmonicida* (Van Muiswinkel et al. 1999). These results suggest that MHC I class genes reside in a different location than MHC class II genes (viral and bacterial presenting antigens, respectively). Thus their independent locations may allow for differential immune responses that can be selected for and adapt independently of one another (Van Muiswinkel et al. 1999). Differences in susceptibility have been associated with genetic differences in native European salmon (Bakke and MacKenzie 1993). There are initiatives within the fish farming industry to select for disease-resistant strains (Gjedrem and Aulstad 1974; McVicar 1997).

BIOLOGY OF PATHOGEN

There are several ways of describing the ability of a pathogen to infect a host. The infectivity of a pathogen relates to its ability to enter, spread and reproduce within a host with or without the onset of disease (Thomas and Elkinton 2004). The terms pathogenicity and virulence are more difficult to differentiate (Steinhaus and Martignoni 1970; Lacey and Brooks 1997; Thomas and Elkinton 2004; Shapiro-Ilan et al. 2005). Earlier definitions describe pathogenicity as any ability to produce disease in a host whereas virulence is defined as the degree of disease producing power (Steinhaus and Martignoni 1970). More recently, pathogenicity has been defined as the number of dead individuals (hosts) relative to the number initially exposed to the pathogen. Similarly, virulence is defined as the number of dead individuals (hosts) relative to the total number infected, providing a more measurable degree of illness (Thomas and Elkinton 2004). Later definitions of pathogenicity and virulence would require empirical studies to be conducted for each pathogen and host combination under defined environmental conditions. Thus for the purposes of this report, more general definitions will be applied. The proper application of these terms is important in determining whether or not a pathogen possess a serious risk to a finfish or shellfish population.

Pathogens are a natural part of a healthy ecosystem. The ability of the pathogen to survive in the environment has a direct impact on its ability to colonize and infect a host. Conditions found at aquaculture sites differ from those in the wild potentially leading to differential selective pressures on pathogens (McVicar 1997). In culture conditions, environmental selection pressures characteristic of different geographic areas can lead to genetic differences amongst pathogen populations. Other survival challenges include the ability to acquire and compete for nutrient resources, avoidance of destructive environmental impacts (see above discussion), adequate reproductive rates and ability to invade a new host. Replication rates have been shown to impact viral genetic diversity and distribution, as nucleic substitution rates are directly dependent on replication rates (Nylund et al. 2007). Lower replications rates will result in lower substitution rates having

an impact on the organism's ability to adaptively respond to its changing environment (Nylund et al. 2007). The pathogenicity of an organism can also impact its survival and subsequent host infection rate. Highly pathogenic diseases may cause hosts to die quickly before the transmission has occurred whereas low pathogenic organisms may be retained longer within host populations (McVicar 1997). In addition, some studies have demonstrated differences in antibiotic resistance by bacterial strains when farms employ therapeutics such as oxolinic acid or oxytetracycline potentially leading to the alteration of population structure (Björklund et al. 1991; Spangaard et al. 1993; Ervik et al. 1994; McVicar 1997).

SELECT PATHOGENS AS EXAMPLES OF STRESSOR-LINKAGES

The pathogens discussed below represent a subset of those that contribute to economic loss in the aquaculture industry in Canada. Both shellfish and finfish pathogens are discussed as they relate to aquaculture activities on the east and west coasts of Canada. These pathogens also represent two primary transmission modes: horizontal and vertical. A brief synopsis of the known life history is presented along with an evidence-based discussion of how and if the pathogens are at risk of transmission between fish populations (both wild to cultured and cultured to wild). Knowledge gaps are also highlighted where appropriate.

SHELLFISH PATHOGENS

Shellfish immunity

Without a true circulatory system, shellfish rely on migratory immune cells, termed haemocytes, which are capable of phagocytosis. Through chemotactic mechanisms, haemocytes detect, locate and adhere to foreign bacteria (Mydlarz et al. 2006). These cells recognize and destroy foreign particles by operating a number of self versus non-self recognition systems. A primary defence mechanism involves adhesion molecules that bind to carbohydrate-containing molecules of foreign particles (Mydlarz et al. 2006). Additionally, shellfish possess lysosomal enzymes and antimicrobial peptides which have the ability to produce reactive oxygen species as an immune defence mechanism which indiscriminately aids in the elimination of unwanted bacteria (Mydlarz et al. 2006; Monari et al. 2007). Anti-viral protease-inhibiting peptides have been isolated from Pacific oysters; however, their role in viral immunity is not well understood.

Haplosporidium nelsoni (causative agent of Multinucleate Sphere X disease)

Life history of *Haplosporidium nelsoni*

Haplosporidium nelsoni is a protistan parasite termed Multinucleate Sphere X (MSX). The disease is historically also known as Haplosporidiosis or Delaware Bay disease and the pathogen was formerly classified as *Minchinia nelsoni*, but was recently reclassified in the *Haplosporidium* genus (Haskin et al. 1966). The parasite was first described in 1957 in Delaware Bay, New Jersey, USA where it was likely imported from the Pacific ocean in the 1950's with the aid of naval vessels (Haskin et al. 1966; Bower and McGladdery 2003). Sequencing analysis has determined that *H. nelsoni* likely initially originated from Asia (Burreson et al. 2000).

H. nelsoni has been detected in Europe, Asia and North America, primarily in seawater environments (Sunila et al. 2000). The original North American geographical range was from northern Florida to Massachusetts and Maine (Bower and McGladdery 2003). It has recently been reported further north in Canada. In 2002, *H. nelsoni* was identified and associated first with mortalities in cultured adult oysters from St. Patrick's Channel, Bras d'Or Lakes, Nova Scotia (Stephenson et al. 2003). The pathogen was first detected in *C. gigas* in western Canada in 2007 (International Council for the Exploration of the Sea 2008). Infections levels were deemed low, as only six out of 35 oysters were positive at one site where no disease or mortality was associated with the infection (International Council for the Exploration of the Sea 2008).

H. nelsoni is most prevalent in juvenile oysters, but occurs occasionally in adult oysters (Bower et al. 1994). Host species include *Crassostrea virginica* and *Crassostrea gigas*; however, the parasite is more virulent in adult *C. virginica* than *C. gigas* where infection intensities are higher in the former host (Burreson et al. 2000).

The route of transmission of *H. nelsoni* is unknown. An intermediate host is suspected, as direct horizontal transfer has not been established (Bower et al. 1994; Sunila et al. 1999). Horizontal transmission has only been established when uninfected susceptible oysters are exposed to naturally infected hosts using water sourced from native marine environments. Despite several attempts, researchers have been unable to establish horizontal transmission in the laboratory (Haskin et al. 1966; Canzonier 1968; Sunila et al. 2000). A study looking at disease transmission routes of *H. nelsoni* in marine water, found that spores were not observed in field samples suggesting direct transmission does not occur (Sunila et al. 2000). A second study sourced marine water filtered through 1.0 µm filters followed by irradiation and found infection was prevented in disease-free oysters; however, the use of a 150 µm filter did not (Ford et al. 2001). These results have implications on the size of the infective transmission particle (Ford et al. 2001). Moreover, the relative abundance of oysters at a given site seems to have little affect on MSX activity (Andrews 1979). This observation once again suggests that horizontal transmission is dependent on an intermediate host (Andrews 1979).

Few details are available on the mechanisms of invasion or pathology of *H. nelsoni.* The pathogen is thought to infect through the gills of the oyster, as plasmodia have been visible in this location (Ford 1985). Sporulation occurs in the digestive diverticula; however, the stimuli for this event and conditions required are unknown (Ford 1985). Additionally, sporulation of MSX is seen predominantly in juvenile oysters rather than adults (Bower et al. 1994). Death of the host begins in susceptible oysters within 3 weeks after first appearance of plasmodia in the gills (Haskin et al. 1966) and it is thought that pathogenesis involves the gradual disruption of the digestive tubule epithelia (Bower et al. 1994).

Attempts to classify *H. nelsoni* into stages based on morphological distinctions have been somewhat successful. Haskins et al. (1979) determined extensive heterogeneity exists amongst plasmodial membrane and nuclei size and abundance; however, experiments relating these morphological differences to variations in disease pathology or ecology have not been completed (Haskin et al. 1966).

Physiological responses of the shellfish host are largely unknown. External signs include local gill lesions and systemic infections (Ford 1985). In a study on Eastern oysters in Delaware Bay, USA, it was shown that gonads were slower to develop in infected hosts

relative to uninfected oysters. However, the infection did not impact the ability of the oyster to spawn (Ford 1985). Moreover, phagocytosis appears to be more effective in removing dead rather than live pathogens (Ford 1985). Haemocyte aggregation has been found to accompany chronic infections of MSX and other studies have demonstrated haemocyte abundance to be increased in disease-resistant (selected for increased survival) relative to susceptible (no previous exposure) oysters (Ford and Haskin 1982; Ford 1985; Ford et al. 1993).

Prevalence of infection appears to decline at two times during the year – the high and low temperature-seasons (Ford 1985). Most infections in oysters typically begin in the early spring and peak in the summer months when temperatures being to warm (Andrews 1979; Ford 1985). Other studies have shown a reduction of infections coinciding with the onset of cooler temperatures owing to the negative thermal impact on the parasite. Studies looking at chronic MSX infections show mortalities in infected oysters ranged between 46-64% with control oysters between 6-30% (Ford 1985). This experiment was conducted over 4 years and thus mortality rate was low overall.

Methods of treatment for MSX are largely unknown. Chemical therapeutants have not been developed. Some physical changes can be made in an attempt to mitigate the severity of infections in cultured stocks. For example, oysters can be cultured in low salinity, low temperature environments with reduced grow-out time in high temperature, high salinity environments (Bower et al. 1994). Moreover, there has been some success in culturing more disease-tolerant strains that have survived a prior exposure (Haskin and Ford 1979; Burreson et al. 2000).

Abiotic effects on *Haplosporidium nelsoni*

Since the complete lifecycle of *H. nelsoni* is unknown, determining the abiotic effects on the pathogen is difficult. Studies show infections are restricted to salinities over 15 ppt with high mortalities at 20 ppt (Bower et al. 1994). Moreover, high mortalities have been reported in Eastern oysters at temperatures greater than 20^{o}C (Bower et al. 1994). Warmer ocean temperatures have lead to outbreaks reported in more northern regions in the USA (Burreson and Ford 2004). Additionally, mathematical models suggest temperatures under 3^{o}C and salinities lower than 15 ppt restrict the growth of the pathogen (Hofmann et al. 2001).

Evidence of *Haplosporidium nelsoni* infection in farmed shellfish

H. nelsoni has been reported in farmed oysters along the Atlantic coast of North America since the advent of shellfish farming began in that area (Haskin and Ford 1979; Ford and Haskin 1982; Ford 1985; Ford et al. 2001). The prevalence of infections in seeded oysters in Delaware Bay ranged from 24 to 40% in 1973, 55 to 60% in 1974, and from 65 to 75% in 1975 (Ford and Haskin 1982). Infections have been an ongoing problem in the Delaware Bay area since the first detection of MSX in 1957 such that mortalities ranging up to 85-90% were reported (Ford and Haskin 1982).

Evidence of *Haplosporidium nelsoni* infection in wild shellfish

H. nelsoni has been identified in wild shellfish predominantly in North America and Asia. In Canada, however, documentation of outbreaks in wild shellfish is limited. *H. nelsoni* was associated with high mortalities in St. Patrick's Channel, Bras d'Or Lakes in Nova

Scotia, Atlantic Canada in the oyster *C. virginica* (Stephenson et al. 2003). *H. nelsoni* infections have been extensively documented in wild oysters in Delaware Bay and Long Island sound, USA (Ford and Haskin 1982; Ewart and Ford 1993; Sunila et al. 1999).

Evidence for pathogen transfer from wild to farmed or farmed to wild populations

Transmission between wild oysters and cultured oysters has been indirectly examined; however, these studies were conducted in the context of determining the transmission mode of *H. nelsoni* and thus qualification and quantification of transmission parameters (abundance, intensity of wild oysters) was not determined (Ford 1985; Sunila et al. 2000; Ford et al. 2001).

Evidence for impact on wild shellfish and aquaculture

Few studies involving baseline work in shellfish disease physiology has been done and therefore it is difficult to determine the direct impact on wild shellfish (Bower and McGladdery 2003). The World Organisation for Animal Health (OIE) listed MSX as a reportable disease and currently, Fisheries and Oceans Canada closely monitors the pathogen throughout the Atlantic coast. The outbreak in wild oysters in the Bras d'Or Lakes, Nova Scotia resulted in 80% mortality among *C. gigas* (Stephenson et al. 2003). The Atlantic Canadian oyster industry is estimated to contribute an average of $10 million to the local economy (Stephenson and Petrie 2005).

In both cultured and wild shellfish populations, MSX spreads quickly and over large ranges. For example, in 1960, it was documented that *H. nelsoni* spread throughout wild oysters in lower Chesapeake Bay in 1 year (Andrews, 1979). A farm in Connecticut reported a loss of 186,000 bushels (~35 L/bu) between 1984 and 1987 due to *H. nelsoni* infection (Sunila et al. 1999). Production rose again to 890,000 bushels following the implementation of monitoring and habitat restoration attempts (Sunila et al. 1999). Other farms along the Atlantic coast have reported similar losses. For example, between 1957 and 1960, 90-95% of oysters were lost to *H. nelsoni* infections resulting in a decline in production from 1-2 million bushels to a record low of 10,000 (Ford and Haskin 1982). Interestingly, there is little evidence of physiological differences between wild and cultured shellfish species as both brood and seed-stock animals are collected from the wild (Bower and McGladdery 2003). In regards to impacts on Canadian oyster populations, inferences can only be made given the large impacts on shellfish aquaculture in the USA, as little peer-reviewed research has been carried out in Canada. At present, infections in wild oysters are still restricted to the Bras d'Or Lakes, Nova Scotia, and to sites on the Atlantic coast of Cape Breton, which has natural historical links to the Bras d'Or Lakes and where oysters were transferred from the lakes prior to the implementation of movement restrictions.

FINFISH PATHOGENS

Infectious salmon anaemia virus (ISAV)

Life history of ISAV

Infectious salmon anaemia (ISA), originally known in Canada as hemorrhagic kidney syndrome (HKS), is a great concern to Canadian fish farmers in eastern Canada since the first official farm outbreak in Norway in 1984 (Thorud and Djupvik 1988). The

etiological agent for this often fatal disease belongs to the virus family *Orthomyxoviridae* (Falk et al. 1997; Mjaaland et al. 1997; Krossoy et al. 1999) and recently a new genus, *Isavirus*, has been proposed and accepted (Mjaaland et al. 1997; Krossoy et al. 1999; Kibenge et al. 2004). It is a segmented, negative sense ssRNA virus whose genome displays similarity to the influenza virus (Falk et al. 1997; Mjaaland et al. 1997; Krossoy et al. 1999; Ritchie et al. 2001; Clouthier et al. 2002). The virion is enveloped, providing it with additional virulence factors and immune-avoidance abilities.

ISA virus (ISAV) primarily infects Atlantic salmon (*Salmo salar*); however, it has been found to replicate in Brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) (Jones et al. 1999a; O'Halloran 1999; Joseph et al. 2004; Kibenge et al. 2004). Atlantic herring (*Clupea harengus*) and the American eel (*Anguilla rostrata*) may also be non-symptomatic carriers; however, work is still preliminary in this regard (Totland et al. 1996; Devold et al. 2000; Joseph et al. 2004; Kibenge et al. 2004). Pollock (*Theragra chalcogramma)* do not appear to be a reservoir for the virus despite wild populations having close contact with cultured Atlantic salmon (McClure et al. 2004a). Pacific salmon (Chum, Chinook and Coho) and Rainbow trout (*Oncorhynchus mykiss*) appear to be resistant to ISAV infections; however, ISAV has been re-isolated from ISAV-challenged salmon suggesting the possibility of future susceptibility (Rolland and Winton 2003).

Entry to the host is likely gained through the gills (Totland et al. 1996; McClure et al. 2004b). In its host, ISAV targets endothelial cells, macrophages and leukocytes (Clouthier et al. 2002; Joseph et al. 2004; Kibenge et al. 2004). Laboratory studies have determined ISAV infects cells with the aid of endosomes and lysosomes suggesting endocytosis, as opposed to membrane fusion, as a mode of entry (Eliassen et al. 2000). The mechanisms involved in this process as well as how the natural and innate host resistance influences the effectiveness of viral infection and degree of pathogenicity remain largely unknown. Once infection has been established, cell death is thought to be caused by apoptosis and/or necrosis in a cell-type specific manner such that apoptosis was noted in salmon head kidney (SHK-1) and CHSE-214 cultures and necrosis was observed in TO cells (modified SHK-1 cells) (Dannevig et al. 1995). Putative cell-death mechanisms include DNA fragmentation, HMGB1 protein production (chromatin binding factor characteristic of necrotic cells) and caspase-8 activation, resulting in the execution of cell apoptosis (Joseph et al. 2004). These data have implications in the variability observed in clinical signs in fish infected with varying strains of ISAV (Joseph et al. 2004).

Fish are infectious well before clinical signs are observed (Totland et al. 1996). Infectious-state salmon can shed viral particles within 3 days of initial infection via sloughed mucus and are thought to remain infectious for 24 hrs at 10^{o}C and pH 7.2 (Totland et al. 1996). Most laboratory-reared fish die within 2-3 weeks after an intraperitoneal injection with tissue from ISAV-inoculated fish and within 4 weeks following cohabitation with infected fish (Totland et al. 1996; Jones and Groman 2001). Fish in the wild are more difficult to assess due to the sampling difficulties previously discussed. Clinical signs displayed by an infected fish can commonly include hemorrhagic necrosis and congestion of the liver, spleen and foregut (Thorud and Djupvik 1988; Jones et al. 1999a). The whole body darkens, fish become lethargic and abdominal distension with ascites can occur (Jones and Groman 2001). Extent of clinical signs can vary depending on initial exposure type and duration making diagnosis in the wild and in culture difficult. For example, stomach lesions were only detected in injected salmon and not naturally infected fish. Moreover, temporal effects must be considered. A

rapid onset of clinical signs and mortality may preclude the development of signs normally associated with more chronic infections (Jones and Groman 2001). Following successful infection and replication, the virus is shed primarily through feces, urine and skin mucus; however, virus detected in skin mucus may be absorbed from the surrounding seawater complicating findings (Totland et al. 1996).

In Atlantic salmon, transmission of ISAV is thought to be primarily through horizontal mechanisms in both freshwater and seawater conditions (Totland et al. 1996; Jones and Groman 2001). Current trends suggest seawater-transmission may be primarily seen in farmed fish where close fish-to-fish contact is typical (McClure et al. 2004b). Transmission was also impacted by distance between farms and the infection state of neighbouring sites (Hammell and Dohoo 2005; McClure et al. 2005). Freshwater-transmission may occur predominantly in wild stocks where frequency of contact is greatest, and indeed it has been found that transmission could also occur between salmon and other species such as Rainbow trout, probably in rivers during the spawning period (Plarre et al. 2005). Further experiments are required to confirm these observations. Studies confirm transmission through seawater, as the ISAV virus has been detected directly in seawater (Jones and Groman 2001; Lovdal and Enger 2002); however, survivability and infectivity relationships are poorly understood.

Vertical transmission has not yet been established for ISAV (Thorud and Djupvik 1988). There is some evidence that the virus is present in gonadal tissue in spawning salmon (Nylund et al. 2003; Nylund et al. 2007); however, no clinical pathology or mortalities were reported. Moreover, a study using Atlantic salmon found that ISAV-free salmon injected with ISAV produced offspring with undetectable levels of ISAV in the ovarian fluid (Melville and Griffiths 1999). The possibility of vector-transmission has been briefly examined. Evidence from RT-PCR of *Caligus elongatus* suggest sea lice may be a vector of ISAV, but these results have not been repeated nor has the re-infectivity of the virus been established (Nylund et al. 1994). A non-pathogenic form of ISAV has previously been detected in *Caligus elongatus* on a fish farm in Maine, USA (International Council for the Exploration of the Sea 2006). Further examinations were not pursued. Additionally, ISA is likely not transmitted through injuries or wounds caused by sea lice (Nylund et al. 1994).

It can be difficult to assess the biological significance of ISAV infection levels; however, in the advent of molecular technologies, first attempts are being made. A well-controlled study by Workenhe et al. (2008) looked at the qPCR amplification of an ISAV RNA segment and relating it to *in vitro* $TCID_{50}$ (tissue culture infectious dose) values. While this area of research is still in its infancy, these data showed that more virulent viral strains can yield higher $TCID_{50}$ values with less copy numbers. Likewise, less virulent strains require higher copy numbers to obtain the same $TCID_{50}$. *In vivo* studies will have to be completed in order to correlate copy numbers to observed host histological lesions and recovery/mortality rates (Workenhe et al. 2008). The reasons behind the different pathological effects have yet to be elucidated.

Detection methods have become highly refined over the last decade where sensitive real-time PCR techniques have been developed for ISAV (Workenhe et al. 2008). Using these techniques it has been determined that there are two distinct subtypes of ISA, the European (EU) and the North American (NA) subtype (Nylund et al. 2007). Based on mutation rates, the NA and EU strains are thought to have separated >100-200 years ago (Krossoy et al. 2001; Nylund et al. 2003; Plarre et al. 2005) prior to the introduction

of aquaculture to North America. These data point to a wild reservoir as ISAV as a source of infection in fish farms (Plarre et al. 2005). Within Canada, genetic analysis suggests at least two separate strains with closer homology seen between the Nova Scotia strain and the Norwegian (European) strain than between the Nova Scotia and New Brunswick strains (Blake et al. 1999; Ritchie et al. 2001) suggesting the possibility of two distinct transmission occurrences. Whether these events were natural or human-induced remains to be determined. Moreover, speculation exists whether ISAV was originally introduced from Europe to North America from the transportation of sea trout (*Salmo trutta* m. *trutta*) during 1883-1884 or introduced to Europe from North America as a result of the movement of Rainbow trout (*Oncorhynchus mykiss*) in 1879 (Krossoy et al. 2001). In Chile in 2007, an outbreak of ISAV occurred in farmed Atlantic salmon where isolates were determined to have mutated and diverged from ISAV originally introduced from infected eggs imported from Norway in 1996 (Godoy et al. 2008; Kibenge et al. 2009).

Abiotic effects on ISAV

Relatively little information is known regarding environmental effects on ISAV and what is known is primarily based on laboratory studies. Studies in the wild are required in order to validate laboratory results. The environment can exert tremendous selective pressure on virus survivability which can include temperature, salinity and anthropogenic factors. Data thus far suggests ISAV is capable of survival under varying conditions indicating good long-term survival in the marine environment (Mjaaland et al. 1997). In the laboratory, ISAV is stable at temperatures ranging between 4 and 15^{o}C for approximately 2 weeks. Temperatures above 37^{o}C resulted in an 80% decline in infectivity after 6 hours (Falk et al. 1997). Moreover, replication rates were found to be higher at lower temperatures (10^{o}C verses 20^{o}C) with an optimal rate found to be 15^{o}C, reduced rates at 20^{o}C and no replication at 25^{o}C (Falk et al. 1997). ISAV was also found to retain some infectiveness for 4 days in the tissue of a deceased fish kept at 6^{o}C (Nylund et al. 1994). Data relating optimal virus performance with environmental conditions can also give insight into the distribution of the pathogen both geographically and within the water column although little research has been completed in this area.

Another environmental factor having an impact on ISAV survivability is pH. Exposure to environmental pH<4 inactivated the virus whereas between pH 5 and 9, typical of marine conditions, the virus remained infective (Falk et al. 1997). Infectivity then declined by 90% at pH 11. It is noteworthy to mention that the avian influenza virus (AIV), a closely related virus to ISA, can also survive under marine conditions between pH 6.0 and 8.5 with infectivity being highest under the higher pH conditions (Stallknecht et al. 1990). However, with the added challenge of salinity (20 ppt), the trend was reversed. Viral infectivity was higher under lower pH conditions and lower under higher pH conditions (Stallknecht et al. 1990). Studies on AIV suggest low pH conditions may induce conformational changes in the hemagglutinin glycoprotein, a key protein allowing entry of the virus into the cells via the endocytic pathway. The conformational change resulted in the loss of binding ability to receptor molecules on host cells. These data may lend insight into similar mechanisms involved in infection and pathogenicity for the ISA virus.

Anthropogenic variables present a new level of challenges to marine viruses. Contrary to universal abiotic factors where viral pathogens have co-existed and adapted over millennia, human-derived chemicals are recent additions to the marine environment to which many pathogens are mal-adapted. Currently, there is little data on the effects of

chemicals and pollutants on ISAV. *In vitro* studies found ISAV is inactive when exposed to chloroform (diluted to 33%) (Falk et al. 1997).

Tidal dispersion appears to be a viable mechanism for ISAV transmission (Ellis et al. 2005). These authors conducted a modeling analysis of disease incidence in the Quoddy region, Maine, USA and found several predictive factors related to ISAV outbreaks including whether or not a new outbreak was upstream of a farm with an outbreak in the previous 2-3 weeks. These findings may also have implications for how boundaries are defined for bay management zones.

Evidence of ISA infection in farmed fish

The virus was first observed to be associated with farmed Atlantic salmon in Norway in 1984 (Thorud and Djupvik 1988). Since then, rigorous monitoring programs have solidly documented its presence throughout farmed Atlantic salmon in Europe and eastern North America. ISAV was first described in Atlantic salmon fish farms in the Bay of Fundy, New Brunswick in 1997 (Mullins et al. 1998). Since then, ISAV has been detected in farms located in the Bras d'Or Lakes, Nova Scotia in 2002 (International Council for the Exploration of the Sea 2006) and in many other Atlantic salmon farm locations in eastern Canada (Mullins et al. 1998; Bouchard et al. 1999; Jones et al. 1999a; Lovely et al. 1999; O'Halloran 1999; Clouthier et al. 2002; McClure et al. 2004b; Nylund et al. 2007). ISAV is deemed to be wide-spread along the eastern coast of Canada and Maine. More recently, ISAV was also documented as the causative agent of diseased farmed Atlantic salmon in Maine, USA (Bouchard et al. 2001).

Studies from Jones et al. (1999) and Ritchie et al. (2001) both describe minimal pathology at the microscopic cellular level despite high mortalities in farmed Atlantic salmon from eastern Canada. Their data support several hypothesis: one, the infection level is too low to cause a clinical infection or two; these fish were in a carrier state (i.e. the infection was not a cause of the observed disease) or three, an acute infection in which mortality occurs prior to the development of significant pathology. Ritchie et al. (2001) speculate that functional differences may exist between strains resulting in varying infection intensities. Alternatively an unknown pathological factor may also have been present which contributed to the mitigation of pathological changes in the tissue, but this possibility was deemed unlikely (Jones et al. 1999a; Ritchie et al. 2001). These data also demonstrate the difficulty in conducting accurate pathological assessments, as well as determining relevant infection levels of a pathogen.

Evidence for ISA infection in wild fish populations

Relatively little research has been conducted on the prevalence and maintenance of ISA infections in wild fish populations. Infections in wild salmon are probably rare, as infected individuals are quickly removed through negative selection mechanisms (Bakke and Harris 1998). Additionally, detection in wild populations may be made difficult, as wild fish appear less susceptible than commercially-reared fish for unknown reasons (Nylund et al. 1995). Using blood samples taken from returning feral Atlantic salmon from the Connecticut and Penobscot Rivers in the eastern USA showed the presence of antibodies to ISAV suggesting they had previously been exposed to the virus (Cipriano 2009). In Canada, very little research has been conducted on wild fish populations and the extent of infection in wild populations on either the east or west coast is not known.

Information from Scotland shows that the virus was isolated from sea trout (Raynard et al. 2001). Positive RT-PCR results for ISAV were obtained from Atlantic salmon parr and adults, Brown trout, and sea trout collected either close to farm operations or in rivers distant from farm operations, but the fish did not show any clinical signs of disease (Raynard et al. 2001). In Norway the Brown trout was found to harbour the highest prevalence of the virus while Atlantic salmon showed low prevalence of the virus. No fish showed clinical signs of infection and ISAV was not cultured from any of the samples tested, but could be detected after a few weeks in disease-free salmon injected with tissue homogenates from RT-PCR-positive wild fish (Plarre et al. 2005). It is noteworthy to restate that RT-PCR can not distinguish between infective and non-infective particles, nor can it yet distinguish between virulent and non-virulent forms (Raynard et al. 2001), but some isolates are known to be non-pathogenic, e.g., HPR0 genotypes. Thus, the true nature of infection in natural populations is almost completely unknown.

Evidence for pathogen transfer from wild to farmed or farmed to wild populations

A Canadian stock assessment report published in 1998 concluded there was no evidence so far to support transmission of ISAV between wild and farmed salmon (Olivier and MacKinnon 1998). Since then, there has been little follow-up research. Only weak evidence currently exists for the possibility of ISAV transmission from either farmed or wild fish populations. Laboratory studies have shown transmission from sea trout to Atlantic salmon via injected infected blood is possible; however, this has very little relevance to conditions encountered in natural systems (Devold et al. 2000). Phylogenetic analysis suggests isolates found in farmed salmon originated from wild Atlantic salmon in Europe (Nylund et al. 2003). Their hypothesis is based on HPR (highly polymorphic region) of the genome where each mutation event could represent a new transmission from wild to farmed fish. It is largely based on speculation that one mutation event would be indicative of disease transfer. Additionally, a small number of wild fish were examined (Nylund et al. 2003). Moreover, the authors recognize that there is little documentation of transmission of ISAV between wild to farmed fish (Nylund et al. 2003).

Evidence for impact on wild fish and aquaculture

ISAV-related impact studies on wild fish populations have not been conducted, as a causative link between farmed fish-transmission has not been directly established. Impacts on cultured fish, however, are extensive if an ISAV outbreak is discovered. Infection can spread slowly increasing the time until detection and can result in 100% mortality in farms (Falk et al. 1997).

***Aeromonas salmonicida* sub. *salmonicida* (causative agent of Furunculosis)**

Life history of *Aeromonas salmonicida* subsp. *salmonicid*a

Historically known as the Great Red Plague, furunculosis was first described in 1894 (Håstein and Lindstad 1991). The causative agent, *A. salmonicida* subsp. s*almonicida,* is a non-motile, gram-negative bacterium with a morphology of coccoid rods (Emmerich and Weibel 1894). Two forms of furunculosis are recognised: typical and atypical furunculosis. Fish infected with typical furunculosis exhibit clinical signs such as swellings or furuncules on the skin, inflammation of the gut and the swimbladder-attached surface of the peritoneum (Marsh 1902; Secombes and Olivier 1997).

Decreases in blood cell counts, darkening in colour and lethargy may also be observed (Bruno 1986; Secombes and Olivier 1997). In the laboratory, clinical signs were observed in juvenile Chinook salmon at 4 days post-exposure to infected (donor) fish (Ogut and Reno 2005). Atypical furunculosis is not as clearly defined and is simply described as causing clinical signs other than those of a typical furunculosis infection. Atypical furunculosis is caused by such *Aeromonas* subspecies as *achromongenes, masoucida, smithia* and *pectinolytica* (Reith et al. 2008) which possess biochemical characteristics that are distinct from the typical strains (Paterson et al. 1980; Ishiguro et al. 1981). The taxonomy of atypical furunculosis, based on molecular methods, has yet to be agreed upon (Austin 2006; Reith et al. 2008). Atypical furunculosis appears to be more chronic in nature, causes less severe clinical symptoms and often occurs in non-salmonids.

Typical furunculosis was first described from a disease outbreak in Bavaria in 1894 (Håstein and Lindstad 1991). In North America, a bacterium known as *Bacillus truttae* was first isolated from Brook trout and was later determined to be *A. salmonicida* (Marsh 1902; Johnsen and Jensen 1994). Currently, the geographical distribution is global in both wild and farmed fish (Herman 1968; Johnsen and Jensen 1994; Bernoth 1997; Austin 2006; Bergh 2007). Furunculosis was most likely introduced to Norway from Denmark with the importation of Rainbow trout. It was likely an introduced species to Norway where wild stocks were not immune (Bergh 2007). In Canada, the pathogen occurs predominantly on the east coast where it has been detected in two major drainage basins in New Brunswick, the Saint John and Restigouche rivers, from where it subsequently spread to the Miramichi river system (Olivier and MacKinnon 1998; Wiklund and Dalsgaard 1998). Typical furunculosis has been found to occur on the west coast of Canada in wild populations of Dolly Varden (*Salvelinus malma* Walbaum) and Cutthroat trout (*Salmo clarki*) (Duff and Stewart 1933; Austin and Austin 2007). Despite some occurrences, it has not been well established as endemic to North American rivers (Austin and Austin 2007).

Typical strains of *A. salmonicida* infects a wide range of hosts from many families and orders (Marsh 1902; Wiklund and Dalsgaard 1998). In salmonids and trout, it has been reported in Atlantic salmon (*Salmo salar*), Pink salmon (*Oncorhynchus gorbuscha*), Brown trout (*Salmo trutta* m. *lacustris*), Rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), Brook trout (*Salvelinus fontinalis*), Lake trout (*Salvelinus namaycush*) and Sea trout (*Salmo trutta* m. *trutta*) (Austin 1997; Bernoth 1997). In addition to anadromous and freshwater fish, atypical strains of *A. salmonicida* have been isolated from a strictly marine fish, namely Sablefish (Evelyn 1971). Atypical furunculosis has also been found in marine species, most commonly flatfish and is associated with incidences of ulcerative disease (Austin 2006). Rainbow trout are more resistant to the disease than, for example, Brown trout (Austin and Austin 2007) whereas hybrid strains of Atlantic salmon appear to be less susceptible to the bacteria than either salmon of farmed or wild parentage (Glover et al. 2009). Atypical strains of *A. salmonicida* have also been detected in a range of possible carrier hosts including Pike (*Esox lucius*), Common carp (*Cyprinus carpio*), Yellow bass (*Morone mississippiensis*), American eel (*Anguilla rostrata*), White sucker (*Catostomus commersoni*), Turbot (*Psetta maxima*), wrasse (Family *Labridae*) and Sea bream (*Sparus aurata*) in both freshwater and seawater environments and all displayed clinical signs (Johnsen and Jensen 1994; Bernoth 1997; Olivier and MacKinnon 1998; Austin and Austin 2007).

In Rainbow trout, *A. salmonicida* has been detected on the mucus and gills, and as the infection proceeds, the bacterium appears in tissues such as the blood, kidney and spleen. Uptake is most likely via the gills, lateral line, mouth, anus or a surface injury (Effendi and Austin 1994; Austin and Austin 2007). The pathogen has been found to gain entry through the gills in Atlantic salmon reared in seawater through visual evidence where high colonization of bacteria was seen on the gill epithelium (Bruno 1986). These studies were carried out under laboratory conditions, thus the natural route of infection remains unknown (Cipriano et al. 1996). Controversy exists regarding infection of the gastrointestinal tract. Ringo et al. (2004) demonstrated that *A. salmonicida* caused significant damage to epithelial cells in the foregut region of Atlantic salmon; however, other laboratory experiments have not successfully established infection through the gut (Austin and Austin 2007). Moreover, translocation across the gut epithelium has not been conclusively demonstrated.

A. salmonicida possesses several virulence factors in order to increase its success of infection. The pathogen is able to replicate in macrophages thereby avoiding the host's immune response (Garduno and Kay 1992; Bernoth 1997; Austin 2006). Many forms of *A. salmonicida* possess a proteinaceous outer-wall known as the A-layer which may shield surface antigens from host immune detection and degradation by proteases and provide an adhesion mechanism with macrophages (Garduno and Kay 1992; Austin 2006). Moreover, the bacterium is very successful at sequestering iron in iron-poor environments (Chart and Trust 1983; Austin 2006;). Iron is an essential growth factor for bacteria and is tightly regulated in hosts by proteins such as transferrin (Chart and Trust 1983). Iron acquisition is also critical to surviving in marine conditions (Reith et al. 2008). Sequencing of the *A. salmonicida* genome has lead to further insight into other virulence factors include genes involved in secretion, adhesion, toxins, protease activity, and antibiotic resistance (Reith et al. 2008).

Once a host has been successfully infected, bacterial cells move from the host to the surrounding water (Enger 1997). Bacteria are shed into the water 24 hours post-exposure (Ogut and Reno 2005). It has also been demonstrated that in the presence of an infected dead fish, large amounts of bacterial cells become detectable in water originally devoid of the pathogen (Enger 1997). *A. salmonicida* can remain viable in a dead fish for approximately 40-49 days (Cornick et al. 1969; Austin and Austin 2007). Additionally, *A. salmonicida* has been found to be more concentrated at the water surface and in the sediment (Enger 1997), possibly due to A-layer absorption.

A. salmonicida has also been detected in non-fish hosts such as sea lice (*Lepeophtheirus salmonis*) (Nese and Enger 1993) which raises the possibility of sea lice acting as a vector for furunculosis; however, further research is necessary. It remains to be determined if successful transmission/infection can occur or if they are strictly carriers.

Several strategies are undertaken by salmon to defend against an *A. salmonicida* infection and have been extensively studied (Secombes and Olivier 1997). Non-specific humoral responses including activation of the complement system and lysosome which can indiscriminately kill *A. salmonicida* (Secombes and Olivier 1997). Anti-protease are able to neutralize *A. salmonicida* toxins and metal-binding proteins such as transferrin bind and decrease the amount of available iron for growth. Cellular defences include phagocytosis by macrophages and neutrophils and production of reactive oxygen species (ROS) (Secombes and Olivier 1997). Antibody production has been observed at

approximately 2 weeks post-infection following intraperitoneal injection of Coho salmon (Cisar and Fryer 1974). Atlantic salmon selected for resistance to *A. salmonicida* also conferred resistance to BKD and vibriosis, but reduced resistance to ISAV (Van Muiswinkel et al. 1999). As seen with other pathogens, MHC genes were implicated as being major determinants of disease resistance in salmon and these observations further confirm that MHC genes function differently in immune defence against virus and bacterial pathogens (Van Muiswinkel et al. 1999).

A. salmonicida is transmitted primarily via the horizontal route (Rose et al. 1989; Secombes and Olivier 1997). It has been demonstrated that transmission can occur through the water column from indirect contact with infected fish (McCarthy and Roberts 1980; Ogut and Reno 2005; Austin and Austin 2007). Transmission can also occur in freshwater, brackish or seawater (Bruno 1986; Johnsen and Jensen 1994; Austin 2006). Results from tests surrounding vertical transmission are inconclusive. *A. salmonicida* has been recovered from the ovaries, ova and testes of artificially infected fish; however, other studies have shown *A. salmonicida* was not present in the eggs or fry derived from infected parents (Bullock and Stuckey 1987). As with many marine pathogens, transmission of *A. salmonicida* is correlated to host density. Studies in Chinook salmon show that higher host densities result in higher cumulative mortalities and shorter mean days to mortality (Ogut and Reno 2004). The probability of disease transfer increases due to increased frequency of host-host contact. Also, higher host densities can alter host behaviour including stress, resulting in higher cortisol levels and depressed immune responses (Ogut and Reno 2004).

Abiotic effects on *Aeromonas salmonicida*

A. salmonicida appears to have good survival rates under a wide range of temperatures, but has limited survival under high salinity conditions. Optimal temperatures for *A. salmonicida* have been found to range between 10-15^{o}C (Emmerich and Weibel 1894; Cornick et al. 1969; Reno 1998), but other experiments show better survival at 4^{o}C (Wiklund and Dalsgaard 1998). Alternatively, other studies show best survival at a wide range of temperatures (5-25^{o}C) with a gradual decline in survival after 2 weeks (Effendi and Austin 1994). Experiments also show increased survival at 11^{o}C versus 20^{o}C (McCarthy and Roberts 1980). Loss of virulence was seen at high culture temperatures (>30^{o}C) under laboratory conditions; however, it is unlikely these temperatures would be encountered in the wild (Ishiguro et al. 1981).

In general, the bacterium appears to have low survival outside the host in seawater (Ferguson et al. 1995; Enger 1997). At 15^{o}C, *A. salmonicida* had greatest survival at salinities as low as 6%, with significantly lower survival at salinities greater than 10%. However, for all salinities tested, between 5 and 30%, survival decreased greatly after 2 weeks and there was virtually no survival at 35% (Effendi and Austin 1994). Optimal growth has been found in low saline conditions (2-3%) with poor survival in seawater (Novotny 1978; Ferguson et al. 1995). Other studies have shown the bacterium to survive for up to 19 days in freshwater, 25 days in brackish water (McCarthy 1983; Austin and Austin 2007) and 8 days in seawater (McCarthy and Roberts 1980). It remains to be determined if in high salt conditions, the bacterium is in a dormant stage and thus unculturable. A-layer positive strains of *A. salmonicida* can auto-agglutinate into suspended particles leading to increased survival rates (Enger 1997; Austin and Austin 2007).

Negatively charged cell-surface proteins may aid in binding and attaching to positively charged sand particles (Sakai 1986). The bacterium has been shown to survive in sterile moist soil for more than 40 days at temperatures ranging from 4-30^{o}C (Cornick et al. 1969). Additionally, *A. salmonicida* was cultured from marine sediments under a fish farm vacated 18 months earlier (Husevag and Lunestad 1995). The presence of live *A. salmonicida* persisting in sediments suggests the ability to infect new hosts.

Evidence of *Aeromonas salmonicida* infection in farmed fish

Historically, *A. salmonicida* subsp. *salmonicida* was endemic throughout fish farms in eastern Canada and Europe. Arctic charr, Atlantic salmon, Brook trout, Chum salmon, and Rainbow trout epidemics have been extensively documented (Evelyn 1971; Olivier 1992). Hatcheries in Canada have also been reported to have furunculosis infections, but as of 1992 the numbers were very low (maximum of 5 out of 19 tested were positive) due to the use of well water and vaccines (Olivier 1992). Atypical furunculosis has also been extensively examined in the salmonids in Europe including Finland, Iceland, Norway and Sweden (Wiklund and Dalsgaard 1998). In the late 1970's, farmed Chinook salmon on the west coast of the United States (Puget Sound) were found to harbour the bacterium (Novotny 1978). Additionally, low levels of *A. salmonicida* were seen in juvenile Coho salmon from the Capilano Hatchery in Vancouver, BC (Evelyn et al. 1984). In 2009, ICES did not report *A. salmonicida* as a pathogen detected in significant numbers in either farmed or wild fish populations (International Council for the Exploration of the Sea 2009).

Evidence of *Aeromonas salmonicida* infection in wild fish

In 1979, it was reported that 300-500 adult Atlantic salmon died from *A. salmonicida* in the Restigouche River in New Brunswick, which is estimated to represent 1-2% of the total run (Weber and Zwicker 1979). Further studies in a neighbouring river (Miramichi River) showed no indication of the disease. In Canada, as of 1992, *A. salmonicida*, had been detected in only one river (Restigouche) in Northern New Brunswick while rivers in Prince Edward Island, Newfoundland or Nova Scotia remained free of the disease (Olivier 1992). Moreover, a survey of disease pathogens off Nova Scotia determined wild Atlantic cod to be free of the pathogen (Morrison et al. 1986). Also, in 1992, *A. salmonicida* was sporadically detected in rivers in BC (species not listed) (Olivier 1992). Studies conducted in the early 1970's show low levels of *A. salmonicida* in wild adult Coho salmon off the coast of B.C. (Hoskins and Hulstein 1977). In 1998, bottom trawl surveys in B.C. did not find evidence of the bacteria in adult salmonids and has previously only been found when they migrate to freshwater to spawn (Kent et al. 1998). These data suggests there are either low prevalence of the bacteria or it is possible that it was not found because *A. salmonicida* is an acute disease and highly infected hosts were rapidly removed from the population.

Since the early 1900's, furunculosis infections have been documented in wild fish in Europe prior to the advent of large-scale aquaculture (Austin 2006; Austin and Austin 2007; Bergh 2007). More recent studies using molecular techniques confirm these observations and have found low infection levels (pathogen DNA detected in the absence of clear clinical signs) in wild Atlantic salmon in Irish rivers (Mooney et al. 1995). Some rivers in Norway have reportedly contained escaped farmed salmon that were infected with the bacterium (Håstein and Lindstad 1991); however, the source has yet to be confirmed. The pathogen has been found in non-salmonids such as Common

dace (*Leuciscus leuciscus*) and wrasse (*Labrus berggylta*) in Denmark and Norway (Wiklund and Dalsgaard 1998).

Evidence for pathogen transfer from wild to farmed or farmed to wild populations

Pathogens such as *A. salmonicida,* with a broad range of hosts are extremely difficult to trace and determine the source of origin. Currently, no direct evidence exists to link the transmission of *A. salmonicida* from farmed to wild salmon or vice versa, only inferences are made using data relating to the number of infected rivers in Norway (Johnsen and Jensen 1994). The authors suggest that there is a correlation between the number of occupied farms with positive infections and the number of positively infected rivers. In some counties in Norway, the disease was first discovered in farms only and not the surrounding rivers (Johnsen and Jensen 1994); however, it is likely the disease first spread from other infected farms where the disease was transmitted from wild fish. Escapes from farms in Norway are high: 10-75 tons of salmon escapes for the years 1988-1989. There is no direct evidence that these fish transmitted the disease to wild populations or to the prevalence of the disease in the escaped fish. Statistics are lacking for data relating distance of farm from spawning rivers. In the laboratory, it has been demonstrated that transmission is rare between co-habitating Atlantic salmon, Atlantic cod, halibut and wrasse (Hjeltnes et al. 1995; Austin 2006).

Evidence for impact on wild fish and aquaculture

No recent literature currently exists relating to the ecological impact of *A. salmonicida* infection on wild fish (Department of Fisheries and Oceans 1999; Ogut and Reno 2005). Historically, *A. salmonicida* has had large impacts on farmed fish including mass mortalities and culls (Austin 2006). With the advent of vaccinations, a drop in the use of antibacterial drugs (Midtlyng 1997) and increase survival of smolts to harvest has been seen (Midtlyng 1997). In Canada, in 1974 and 1991, ~50% of farmed Atlantic salmon were lost due to atypical furunculosis infections (Groman et al. 1992). For each occurrence, this resulted in a loss of approximately $500,000 in potential sales (Wiklund and Dalsgaard 1998). Data on loses relating to typical furunculosis have not been documented in the peer-reviewed literature.

***Renibacterium salmoninarum* (causative agent of bacterial kidney disease)**

Life history of *Renibacterium salmoninarum*

Renibacterium salmoninarum is the causative agent of what is more commonly known as (BKD), also known as Dee disease, Corynebacterical Kidney Disease and Salmonid Kidney Disease. *R. salmoninarum* is a slow-growing, non-motile, rod-shaped, gram-positive bacterium (Kaattari and Piganelli 1996; Austin 2006). After many previous attempts, the bacterium was successfully cultured in 1956 where following culture, *R. salmoninarum* was re-infected and subsequently re-isolated from diseased Chinook salmon (Austin 2006). This experiment demonstrated and satisfied causation criteria in determining the causative agent of BKD.

R. salmoninarum was first described in 1930 in Scotland (Austin 2006). Currently, it is endemic in wild and cultured salmonid populations (Evelyn 1993). The bacterium displays genetic and serological uniformity such that attempts to differentiate isolates are difficult (Grayson et al. 1999). Random amplified polymorphic DNA analysis has had

considerable success in distinguishing geographical isolates. Using this method on 74 isolates from around the world, three groups have been characterised. One group comprised of isolates from Canada, Scotland, England and 2 isolates from the United States. A second group includes isolates from Iceland and a third group contains the remainder of the isolates from the U.S. (Grayson et al. 1999). *R. salmoninarum* was first discovered in the U.S. in several trout species (*Oncorhynchus mykiss, Salmo trutta* and *Salvelinus fontinalis*) in Massachusetts and California. Shortly thereafter, it was discovered in hatchery-reared cutthroat trout (*Oncorhynchus clarki*) in Cultus Lake, B.C. in 1937. Presently, *R. salmoninarum* is found on both west and east coasts of Canada (Department of Fisheries and Oceans 1999). As of 1993, the bacterium was not present in Newfoundland and the Yukon Territory, but sampling efforts have been minimal.

R. salmoninarum is a salmonid pathogen primarily infecting Pacific salmon (Håstein and Lindstad 1991; Kent et al. 1998; Grayson et al. 1999). Bacterial kidney disease causes problems primarily for cultured salmonids, although it can be problematic for wild salmonids as well due to its chronic nature (Håstein and Lindstad 1991). *R. salmoninarum* can infect salmonids during both freshwater and seawater phases (Sanders et al. 1992; Maule et al. 1996a; Elliott et al. 1997; Rhodes et al. 2006). Atlantic salmon and carp are more resistant to infection (Austin 2006). Experimentally, non-salmonid fish such as herring have been infected, but little or no reports of wild infections in non-salmonids have been documented despite the reporting of other pathogens such as infectious pancreatic necrosis virus (IPNV) and *A. salmonicida* (Evelyn 1993; Austin 2006). Infected fish show external signs such as pale gills, abdominal distension, whole body darkening, skin blisters and haemorrhages. Internally, there are often creamy-white granulomatous lesions on the kidney and possibly on the spleen or liver (Evelyn 1993).

R. salmoninarum is a facultative intracellular bacterium that replicates within phagocytes. It has been reported to be resistant to lysozyme, produce catalase, is slow to reproduce, and has high cell surface hydrophobicity (Gutenberger et al. 1997). These life history traits are characteristic of intracellular pathogens. This strategy allows the pathogen to avoid detection by the host humoral defence system and can multiply for up to 4 days in Rainbow trout phagocytic cells (Kaattari and Piganelli 1996). Other studies have confirmed this and found that the bacterium survives for more than 10 days in Rainbow trout phagocytes (Gutenberger et al. 1997). Slow replication may provide an advantage in two ways. One, most antibiotics target actively replicating cells and two, the host cell will not die under slow-replicating conditions allowing the pathogen to persist in the host and host population (Gutenberger et al. 1997). Disease is typically slow to develop where a healthy host can be killed in 145-203 days when co-habited with a diseased fish (Austin 2006). On the other hand, one study found that death occurred between 12-23 days post-infection in Chinook salmon intraperitoneally injected with *R. salmoninarum* (Ordal and Earp 1956).

The bacterium displays several strategies for intracellular living. Some bacterial cells remain within the phagosome and others live in the cytoplasm. In artificially infected macrophages examined using electron microscopy, it was found that 11 to 42 bacterial cells were present in the cytoplasm per cell cross-section (Gutenberger et al. 1997). This study also demonstrated that intact, live bacteria and formalin-killed bacteria showed equal resistance to macrophage attacks. These data suggest that an innate property of the cell wall is responsible for avoidance of phagocytosis (Gutenberger et al. 1997). Additionally, both live and killed bacteria were able to move out of the phagosome into the cytoplasm of the macrophage. After 4.5 hours of infection, most of the live cells

resided in the cytoplasm. The formalin-killed cells were slower, but also moved to the cytoplasm after 96 hours. The authors suggest that the durability of *R*. *salmoninarum* may be attributed to a rare amino sugar found within the cell wall (Gutenberger et al. 1997). An additional cell wall-associated virulence factor is p57, the major soluble antigen (MSA) that is responsible for the agglutination of leucocytes in the host (Senson and Stevenson 1999). Experimental reduction of the p57 protein activity (with the use of a heat-induced protease) resulted in the inability to suppress an *in vitro* antibody response in Chinook salmon (Wood and Kaattari 1996).

R. *salmoninarum* can induce many host immune responses. The bacterium can remain infectious for life and has been suggested to be a natural part of the gut microflora (Reno 1998; Austin 2006). The pathogen can persist chronically or it can kill within a few months depending on environmental conditions and the health of the host (Gutenberger et al. 1997; Bakke and Harris 1998). There is a delicate balance between immune defence and environmental and genetic factors with respect to disease development (Grayson et al. 2002). One study using Coho salmon demonstrated a decline in plasma chloride and plasma protein levels in clinically diseased fish where healthy fish did not show the same trend (Forsyth et al. 1997). Similarly, expression of the stress protein SP-70 (HSP-70) was increased in clinically diseased fish in the liver and head kidney; however levels in sub-clinically infected fish remained unchanged (Forsyth et al. 1997). It remains possible that the induction of the heat shock protein was related to other stresses and that these results will need to be further validated. Despite the ability of *R*. *salmoninarum* to invade and avoid defence systems, macrophages have some ability to kill the bacterium with respiratory burst activity (Hardie et al. 1996). Moreover, early induction of TNF-alpha, a cytokine, may help the host suppress certain bacterial genes notably, *hly* and *rsh*, two cell membrane-associated virulence factors. In the same study, p57 was not affected (Grayson et al. 2002).

R. *salmoninarum* infections have direct ecological impacts. Juvenile Chinook salmon have been found to be more susceptible to infection (Mesa et al. 1998). Additionally, Smallmouth bass (*Micropterus dolomieui*) or Pike minnow (*Ptylocheilus oregonensis*) were observed to prey more frequently on experimentally-infected juvenile Chinook salmon (Mesa et al. 1998). These results may have relevance to the survival of wild fish infected with *R*. *salmoninarum*. The authors suggested there are large energetic demands placed on chronically *R*. *salmoninarum*-infected fish. This would lead to a decreased energetic scope and a lowered ability to avoid predation.

Transmission of *R*. *salmoninarum* is both through horizontal and vertical means (Murray et al. 1992; McVicar 1997; Reno 1998). Vertical transmission of *R*. *salmoninarum* was first discovered by Allison and colleagues in 1958 where they found evidence of a bacterial kidney infection in disease-free fish reared from eggs obtained from an infected farm (Allison 1958). It was later established that the bacterium was indeed located inside the egg (Bullock and Stuckey 1978). This result was determined using disinfected Chinook salmon eggs where the infection was successfully spread to Rainbow trout (and vice versa). *R*. *salmoninarum* produces few lethal toxins or often cause acute clinical signs when fish are naturally infected. Transmission appears to take place through the surrounding coelomic fluid (type of reproductive fluid) in the female where the role of the male in the transmission is not significant (Evelyn et al. 1986). These and other properties mentioned above allow the bacterium to survive in the host long enough for mature fish to spawn and thus be available for transmission to juvenile salmonids.

Horizontal transmission can take place in freshwater and seawater conditions (Bell et al. 1984; Murray et al. 1992). Survivability outside the host is considered limited depending on environmental conditions (e.g., amount of organic matter, water hardness) (Evelyn 1993; Austin and Austin 2007). Some studies have found the bacterium to survive up to 14 weeks with significantly reduced survival at 20 weeks in river water but not ground (Hirvela-Koski 2004). In seawater, however, studies have shown the bacterium can only survive for up to 1 week (Balfry et al. 1996). Most studies have found detectable levels in sediments rather than in water column suggesting the bacterium has an affinity for organic matter (Austin 2006). The means of dispersal and shedding *R*. *salmoninarum* into the water may involve fecal matter which was discovered to contain high levels of *R*. *salmoninarum* in infected salmonids (Balfry et al. 1996; Austin 2006). Shedding by this method by infected Chinook salmon into the water was shown to be after ~12 days (McKibben and Pascho 1999).

Austin and Austin (2006) observed that *R*. *salmoninarum* appears to be a very unaggressive pathogen yet poses a relentless problem for salmonids. The researchers have proposed that the bacterium can reside in some carrier hosts at very low levels while not causing any problems to the host fish. *R*. *salmoninarum* may be a natural resident of the fish digestive microflora forming a synergistic relationship with the host. Stress, such as that caused by adverse environmental conditions, other infections, or nutritional deficiencies may serve to cause the bacterium to replicate and cause clinical disease. However, other explanations such as a dormant or long lag-phases may also explain the inability to culture bacterial cells from some fish (Austin 2006).

The development of a treatment for *R*. *salmoninarum* infections has been challenging. Chemotherapies have proven largely ineffective due to the intracellular nature of the bacterium. Sulphur drugs and erythromycin have been found to be only temporary measures with limited effectiveness in addition to having potentially serious side-effects such as impaired kidney function (Wolf and Dunbar 1959; Evelyn et al. 1984; Evelyn et al. 1986; Evelyn 1993; Lee and Evelyn 1994; Hirvela-Koski 2004). Penicillin was also shown be ineffective at reducing infection levels in eggs (Evelyn et al. 1984). Moreover, iodophors have had little effect on disinfecting eggs (Austin 2006). These results have great implications for controlling the disease. In the past, attempts to use MSA as a target for vaccines were unsuccessful, as vaccinated Rainbow trout and Coho salmon were unprotected (Austin 2006). More recent attempts at vaccinations against the bacterium have been more successful but at present a completely effective vaccine has not been found. A commercially available attenuated vaccine (Renogen, Aqua Health) has proven somewhat effective in the protection of Chinook salmon, but other studies have concluded that there is no protective effect (Alcorn et al. 2005).

Abiotic effects on *Renibacterium salmoninarum*

R. *salmoninarum* is a poor survivor in the marine environment where local conditions can have a large impact on its abundance and concentration (Evelyn 1993). Studies have found *R*. *salmoninarum* to survive a wide range of temperatures from 8-18^{o}C such that most outbreaks occur during the colder seasons; however, other studies have shown warmer temperatures to have induce stress affects in the host thus allowing for a more conducive environment for an outbreak (Austin 2006). *In vitro* studies confirm this result showing that the pathogen grows optimally at 15-18^{o}C and not at all at 25^{o}C (Evelyn 1993). Temperature and salinity were found to have a negative affect on the probability of infection in Chinook salmon in Puget Sound. The probability of infection

declined as water salinity and temperature increased at the capture site (Rhodes et al. 2006). These reports and others suggest that *R. salmoninarum* is a more efficient pathogen in sea water despite other studies demonstrating that survivability is low in sea water (Sanders et al. 1992). These reports also note that the findings may relate to the development of a disease state of the host induced by increased environmental stress following transition from freshwater to seawater rather than survival of the pathogen in the marine environment (Frantsi et al. 1975; Sanders et al. 1992).

Evidence of *Renibacterium salmoninarum* infection in farmed fish

R. salmoninarum has been reported in many cultured fish populations around the world. In North America, *R. salmoninarum* has been reported in hatcheries on the west coast of B.C., the western slope of the Cascade mountain range, the Great Lakes, the east coast of U.S.A. (Georgia) and most Maritime provinces (Warren 1983). Hatcheries often release millions of fry which may have been exposed to *R. salmoninarum.* In 1983, a survey of the Columbia river basin found that out of 2800 salmonids (Chinook, Coho and Steelhead) sampled over 2 years, more than 20% (up to 69% of sampled fish) of each group (wild verses hatchery-origin) were infected with *R. salmoninarum* (Sanders et al. 1992). The contribution of the nearby infected hatchery-fish remains unknown, as fish were not marked. An additional study in the Columbia River and Snake River systems found Chinook salmon to have declining prevalence prior to release from hatcheries with the introduction of improved husbandry practices. Improved practices included lowered stock densities, therapeutic drug treatments, and changes in water quality monitoring (Maule et al. 1996a). In 1996, *R. salmoninarum* was discovered to be present in a fish farm on the north coast of Vancouver Island, B.C. where prevalence levels were up to 86% (Balfry et al. 1996). In Europe, low prevalence was detected in farms in Scotland between 1990 and 2002 in both Rainbow trout and Atlantic salmon farms (Bruno 2004). There have only been a few reports of infections in Norway (Bruno 2004).

Evidence of *Renibacterium salmoninarum* infection in wild fish

R. salmoninarum has been reported in many wild fish populations in North America and Europe. Surveys of aquaculture-free regions, such as Iceland, suggest carrier populations of Arctic charr and Brown trout may act as a reservoir for infection in wild fish (Jónsdóttir et al. 1998). In this study, wild Arctic charr and Brown trout had a prevalence of 46% and 35%, respectively although no gross pathological signs were reported (Jónsdóttir et al. 1998). In North America, the bacterium has been detected in fish on the north, east and west coasts as well as in inland rivers. In the Northwest Territories, a survey showed 7 out of 244 wild Arctic charr were naturally infected with the bacterium. Since there are no fish farms in the area, this is considered the natural level of the pathogen in this region (Souter et al. 1987). In 1998, a comprehensive survey of Pacific salmon on the west coast of B.C. showed by ELISA that *R. salmoninarum* was present in Chinook, Chum, Coho, Sockeye, and Atlantic salmon located >10 km away from net pens (Kent et al. 1998). Infections were considered prevalent. *R. salmoninarum* was absent in non-salmonids with the exception of low prevalence in Pacific hake (*Merluccius gayi gayi*) located away from net pens; however in these fish the level of pathogenicity, extent of clinical signs and state of infection were not reported (Kent et al. 1998). Using cell culture methods, Pacific herring (*Clupea pallasii pallasii*) that were surveyed around net-pens of infected farms in B.C. were not infected (Paclibare 1989). Another survey of Chinook salmon sampled in Puget Sound

were found to have high prevalence of *R*. *salmoninarum* which ranged from 0-83% for marked fish (presumably of a hatchery origin) and 14-50% for unmarked fish (presumably wild) from different stock origins (stream or bay areas) (Rhodes et al. 2006). In 1986, wild Chinook, Coho, Chum, Pink, Sockeye, Steelhead (*Salmo gairdneri* Richardson), and Cutthroat trout (*Salmo clarkii* Richardson) caught off the coast of Washington and Oregon, USA were surveyed for the presence of *R*. *salmoninarum* infection. Prevalence was found to be highest in Chinook at 11% and Coho at 4% with very low levels (<3%) reported in the remaining species (Banner et al. 1986). In Wyoming, USA, Brook, Brown and Rainbow trout were shown to harbour the bacterium at high levels (3-83% prevalence) with higher incidence occurring in Brook trout than Brown trout and lowest in Rainbow trout (Mitchum et al. 1979). Additionally, in 1988-1990, wild Rainbow trout and Chinook salmon in the Columbia River basin were found to have low to medium (based on ELISA detection values) infection levels (Elliott et al. 1997). On the east coast of North America, fish caught in the neighbouring tributaries of the Margaree River in Nova Scotia showed high levels of corynebacterial kidney disease in wild fish; however, the number of fish infected was low (Frantsi et al. 1975).

Evidence for pathogen transfer from wild to farmed or farmed to wild populations

Only a few studies have been conducted on possible transmission of *R*. *salmoninarum.* For example, disease-free Brook trout, Rainbow trout, and Brown trout were cultured in pens in French Creek, Wyoming, USA. This creek was known to have high incidence and severity of *R*. *salmoninarum* infections in wild Brook trout, the dominant species in the area. *R*. *salmoninarum* was diagnosed in all dead fish from the artificially-stocked Brook trout. These data suggests horizontal transmission from the wild population to cultured fish is possible (Mitchum and Sherman 1981). Moreover, Atlantic salmon and Brook trout from upstream of the Margaree Hatchery in the Margaree River, Nova Scotia, were found to harbour low levels of *R*. *salmoninarum.* The authors suggest that because released hatchery fish quickly acquire high levels of a parasite, *Diplostomulum* sp., and the wild *R*. *salmoninarum*-infected fish were free of this parasite, that the *R*. *salmoninarum*-infected fish were not hatchery-sourced. However, the possibility of horizontal transmission was not ruled out (Frantsi et al. 1975).

Evidence for impact on wild fish and aquaculture

R. *salmoninarum*, because of its high prevalence and widespread distribution, is not currently listed in the 2009 ICES report of disease trends in wild and cultured fish and shellfish (International Council for the Exploration of the Sea 2009). There are no reports of ecological impacts or costs associated with impacts in Canadian wild or farmed fish populations in the peer-reviewed literature.

CONCLUDING REMARKS

Systematic research on pathogen transmission in aquatic ecosystems is lacking in Canada. The complex interactions inherent to disease transfer between a host, pathogen and the environment demand multi-variable, large-scale, comprehensive examinations. Currently, most research is conducted in laboratories resulting in a dearth of information relating to studies in the natural environments. While it is important to describe and document pathogen transfer, the consequences of pathogen transfer and exposure to the pathogen in susceptible populations will require a much broader

ecosystem perspective. In the wild, both baseline and impact studies are required to address concerns relating to disease transmission between fish and shellfish populations.

Shared marine environments provide many opportunities for pathogen transmission and these may occur by three primary routes. While transmission through intermediate hosts is possible, pathogens are commonly transmitted horizontally or vertically. Horizontal transmission may require close contact of individuals in order to facilitate a successful infection although as discussed, many pathogens survive well in water. Overall, the possibility of disease transmission by means of direct interactions between wild and farmed fish are limited to wild fish that naturally migrate near net-pens or those populations nearby or downstream of farms as well as interactions with escaped fish. The parameters and conditions required for pathogen transmission between farmed and wild salmon will play a critical role in the success of transfer. Interactions between wild and escaped farmed salmonids would primarily occur in the freshwater stage of migration (Department of Fisheries and Oceans 1999). This ecological interaction may provide a link for transmission of pathogens and exchange of genetic material between the populations. Although not well-researched, competition for space and nutritional resources, among others, would also be of concern. Atlantic salmon escapes have spawned in the Magaguadavic and Maine River on the Atlantic coast of North America; however, the extent of disease transmission between populations is unknown (Department of Fisheries and Oceans 1999). To date, Atlantic salmon have not established wild populations in British Columbia.

The pathogens discussed here provide examples of how physical and biological variables can influence the disease susceptibility of individual fish or populations. Increases in disease susceptibility are a result of complex interactions between changing environmental conditions and interactions with other aquatic animals. Despite the lack of multi-factorial studies, it is clear from the information available, that physical factors such as temperature, light, pollutants and population densities directly impact disease susceptibility in fish which can lead to the increased pathogen shedding rates and contribute to the increased availability of pathogens for re-infection.

This report finds no significant evidence of pathogen-transfer events between wild and farmed fish populations for the pathogens discussed (namely *H. nelsoni*, ISAV, *A. salmonicida*, and *R. salmoninarum*). Based on current data, transmission of these pathogens from farmed fish does not appear to have significant impacts on wild populations. Jones (2009) concluded that while there was good evidence that farmed salmon contribute salmon lice to wild salmon populations, there was insufficient evidence to determine the magnitude with which the parasites contributed to declining abundances of wild salmon populations. The future expansion of finfish and shellfish aquaculture in Canada will place increased pressure on the policies and regulations governing aquaculture and pathogen transmission. Strong baseline and ecological impact studies will be required to support existing efforts and, with the adoption of emerging aquaculture programs, to aid in the establishment of new regulations and policies.

Specific recommendations relating to pathogen transfer among fish populations include the need for multi-disciplinary expertise (e.g., pathology, epidemiology and diagnostics) to conduct appropriate baseline studies in natural and culture environments. An emphasis should be placed on identifying underlying causes for control and spread of

pathogens, as well as the ecology and biology of host-parasite systems from individual, population and environmental perspectives. Targeted surveillance of specific endemic pathogens would lead to a better understanding of the health in wild and captive animal populations (Daszak et al. 2000). The development of non-lethal diagnostic assays and more advanced fish-tracking methods would assist monitoring of populations including those at risk.

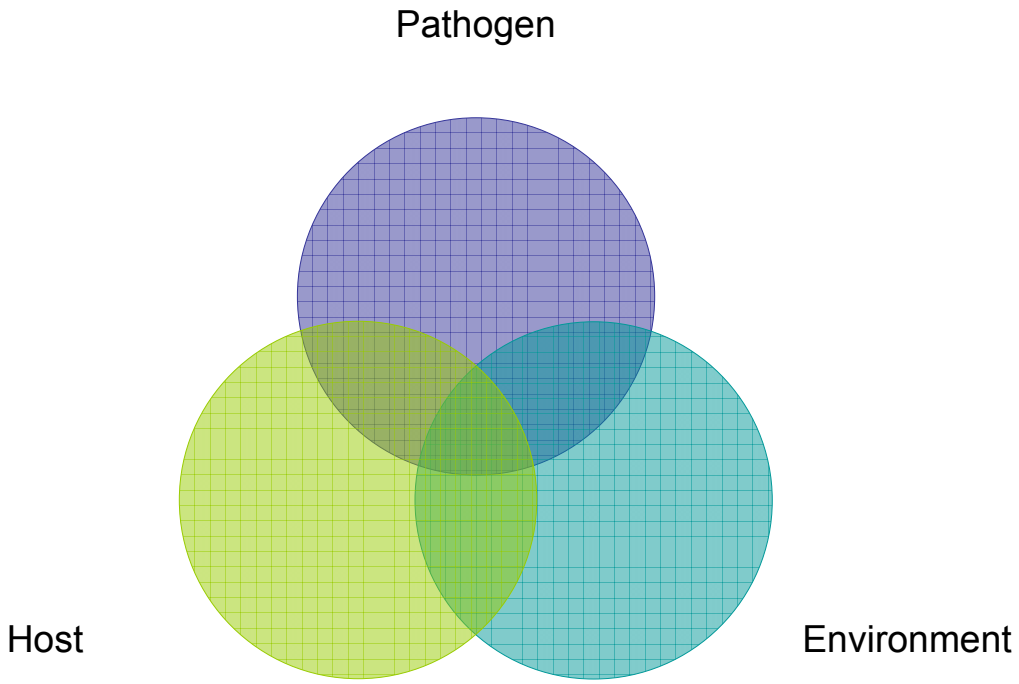

Figure 1. The relationship between the host, the pathogen, and the environment must be considered in the interpretation of the Pathways of Effects diagram.

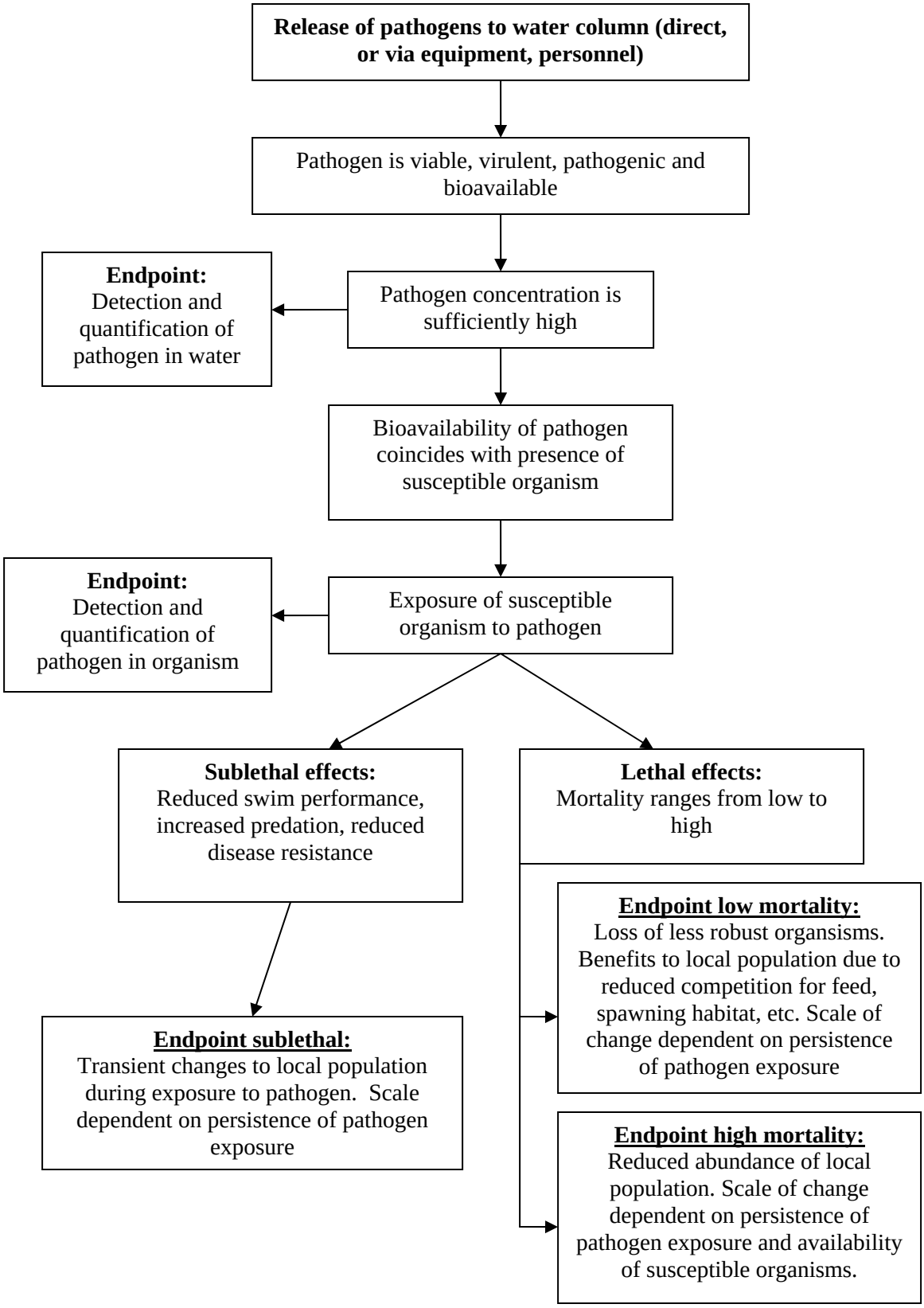

Figure 2. Pathways of effect diagram for pathogens identifying endpoints.

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