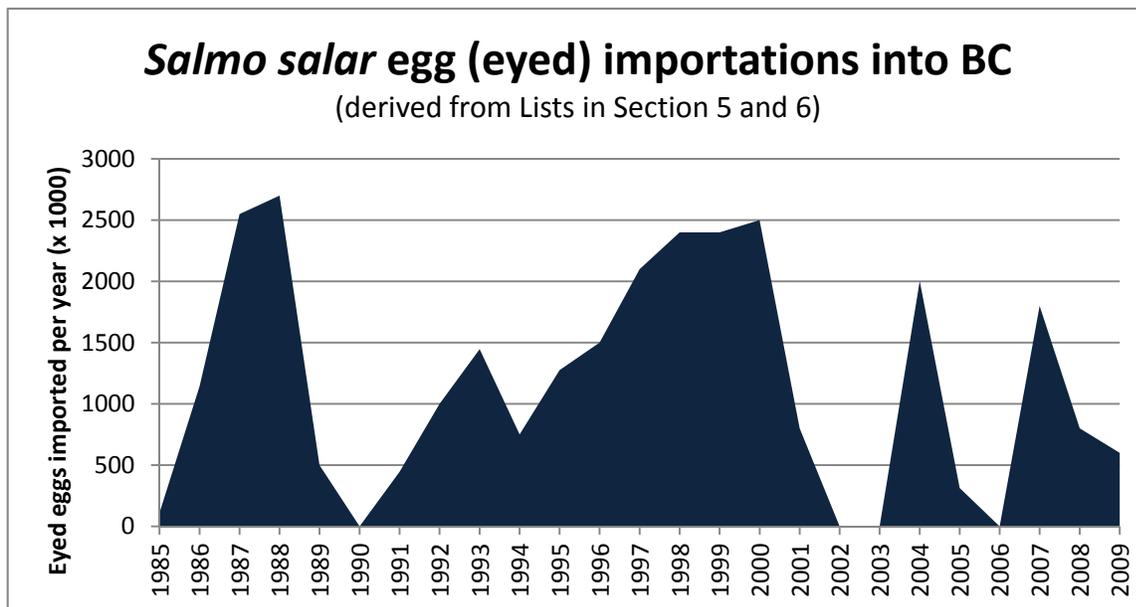


Qualitative assessment of risk, and mitigation, of importing exotic disease through eggs

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Objective: Provide a brief qualitative analysis of the risks of importing exotic diseases based on a report about the history of Atlantic salmon egg importation into the Province of British Columbia for aquaculture from 1985 to present. Assist in understanding technical issues by providing a brief literature review regarding potential imported diseases that could adversely impact wild sockeye salmon.

Lawrie Report (March 2011) Summary



Comment regarding risk components: Risk has two primary components: the probability of encountering an adverse event or effect, and the magnitude of the biological and economic consequences (World Organisation for Animal Health, 2010a). A third component is often included in discussion of risk, that is the perception of risk since an action that has relatively high probability of occurrence and severe consequence but is familiar is frequently perceived as lower risk than an unfamiliar action with very low probability but equally severe consequences. A topical example might be the risk associated with mobile phone use and cancer being far less probable than death from common daily activities, such as driving a motor vehicle, and yet one is more familiar and perceived as lower risk.

It is beyond the scope of this report to compare the relative consequences of a very low, perhaps remote, probability of disease introduction through the importation of eggs for aquaculture versus the higher (but perhaps still low) probability of introducing fish pathogens through more common but

familiar activities, such as visitors/tourists from other locations visiting farms or enhancement hatcheries, anglers visiting multiple watersheds without disinfecting, and so on. Elimination of the risk of disease introduction from imported eggs (i.e. by not importing eggs) does not mean that overall disease introduction risk is zero and in fact the overall risk of disease introduction may be almost unchanged if the egg introduction pathway contributes very little to the overall risk compared to other activities. My comments will focus on giving context to the relative probability of disease introduction through egg importation only and only briefly on the magnitude of the consequences for disease introduction since these consequences occur for any disease introduction pathway.

Comments regarding zero risk: The only measure that would absolutely prevent the risk of *any* pathogen being introduced with imported eggs, either as infected livestock or in the shipping process and equipment, is to never allow any eggs or other livestock to be imported. My comments are based on the fact that the importation of genetic lines (i.e. families or species not originally found in British Columbia) was deemed a positive and acceptable event and the only means of achieving this genetic introduction is to import live animals or gametes.

Comments regarding risk with importing eyed eggs only: Biological factors are important considerations (see OIE Aquatic Animal Health Code) when mitigating risk of importation of exotic diseases through the movement of live animals. Eyed eggs represent the lowest risk age group possible for fish importation. This assumes the eggs came from broodstock with the lowest possible probability of being infected with any vertically transmitted diseases *and* that eggs were properly surface disinfected to reduce the probability of horizontally transmitted diseases from the broodstock. Vertically transmitted diseases are normally considered those which are incorporated into the egg during development within the ovary (i.e. prior to spawning) or adsorbed to sperm from the male brood allowing the pathogen to enter the egg at fertilization. Horizontally transmitted diseases, from the perspective of egg transmission, are considered those pathogens which can be transmitted to the progeny from the broodstock through exposure to reproductive fluids (i.e. exposure to reproductive fluids from any broodstock, parents or otherwise).

Comments regarding potential for vertically transmitted pathogens: Vertically transmitted diseases are not very common in salmonids, only a few are suspected or known to be vertically transmitted. The following pathogens have been reported as possibly transmitted vertically in *Oncorhynchus* spp: IHNV, IPNV, OMV, *Herpesvirus salmonis*, *Renibacterium salmoninarum*, and *Flavobacterium psychrophilum* (Bootland et al, 1991; Brock & Bullis, 2001; Brown et al, 1997). *Piscirickettsia salmonis* has also been suggested, at least experimentally, to transmit vertically (Larenas et al, 2003). ISAV was reported to occur in first-feeding fry but this may have been horizontal transmission (Nylund et al, 1999) while extensive attempts to detect ISAV in progeny of known positive broodstock failed to confirm any evidence for vertical transmission (Melville & Griffiths, 1999). Nylund et al (2007) suggested that an avirulent genotype may circulate in salmon farming through transgenerational transmission and then mutate to virulent isolate during the grow-out phase. Vike et al (2009) suggested that Norwegian ISAV genotypes were introduced to Chile through egg importation of stock that were infected by vertical or transgenerational transmission. However, more recent molecular epidemiology studies in Norway have not supported vertical transmission of low virulent ISAV with subsequent mutation to higher virulence

as a source of ISA disease outbreaks (Lyngstad et al, 2011). Although conclusions regarding vertical transmission of ISAV are difficult to make at this time, the weight of evidence suggests that it does not occur.

Comments regarding measures required to reduce the probability of vertical transmission of disease:

Due to the fact that true vertical transmission occurs within the egg, surface disinfection is of little value (except to reduce infective particles exposed through the reproductive fluids). For this reason, the only method available to reduce the potential to have infected eggs is to reduce the potential for infected broodstock. Aside from IPNV which may attach to spermatozoa prior to entry of the egg at fertilization, infection status of the female brood is the most important determinate of potential for vertical transmission to the progeny. Consequently, pathogen detection strategies directed toward the female parent, with avoidance of eggs from positive populations is the most effective method to prevent vertically transmitted infections. This detection is best applied to the entire brood population over time with tight biosecurity and prevention of exposure to any new sources of pathogens (e.g. wild fish, surface water, or new farmed stock).

According to John Lawrie's report (2011), the Fish Health Protection Regulations (FHPR), intended to limit transfer permits to producers with biosecure conditions and subjected to intense disease testing requirements, were applied to the suppliers of eggs eventually imported into BC. These measures would have most likely detected the known pathogens of concern (such as IPNV) at the time of testing. The pathogens since discovered, primarily ISAV (orthomyxovirus) and SAV (alphavirus), are less concern for vertical transmission (Kongtorp et al, 2010). However, viral detection methods employed during the testing period and the fact that the populations did not have signs of clinical disease (a requirement for FHPR testing) resulted in imported eggs with a very low, if not extremely low, probability of infection with any pathogens of concern.

Comments regarding potential for horizontally transmitted pathogens: Theoretically, any pathogen (bacteria or virus, in this case) can be transmitted from an infected parent to its progeny provided that there is sufficient exposure. However, the imported eggs in question had the following characteristics which would minimize such horizontal transmission: 1) surface disinfection of eggs was common soon after fertilization as part of hatchery procedures, 2) surface disinfection of all egg shipments at source and on arrival at the quarantine facility (see page 5 of Lawrie's report), and 3) eggs came from FHPR tested facilities (which indicates intensive diagnostic testing of broodstock and a history of clinical disease absence in the brood population and attention to biosecurity / potential exposure to external sources of pathogens). The probability of a pathogen being introduced through horizontal transmission, with these measures in place, is extremely low.

Comments regarding consequences of pathogen introduction: Many of the pathogens discussed previously cause sufficient economic and biologic consequence that they have been listed by the World Organisation for Animal Health (<http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2011/>) for international reporting. Several of the pathogens are already endemic in the wild salmonid populations of British Columbia, namely IHNV, VHSV, and *Renibacterium salmoninarum* (not listed by OIE but important from vertical transmission perspective). VHSV has several genotypes that are not

shared geographically and therefore, introduction to BC of a different genotype could be very important. However, eggs are not implicated in its transmission risk so will not be considered further. *Piscirickettsia salmonis* is reported as present in BC (Evelyn, 1992) and so is not considered exotic. Although standard measures to prevent exotic pathogens being imported would also prevent IHNV, *R. salmoninarum*, or *P. salmonis* being imported, additional exposure of these pathogens is of little consequence since they existed in BC already. The primary concern from a biologic and economic consequence perspective is the potential to introduce ISAV, IPNV, SAV (or Pancreas Disease, PD), or Heart and Skeletal Muscle Inflammation (HSMI, associated with a novel Reovirus (Palacios et al, 2010)). As there is no evidence to support egg involvement in transmission for SAV/PD (Rodger and Mitchell, 2007) or for HSMI (Kongtorp et al, 2006), neither is considered for its potential consequence. ISAV has been devastating to Chile (Mardones et al, 2009), causing a drastic reduction in Atlantic salmon farming but has little effect on Pacific salmon (*Oncorhynchus*) species (Rolland and Winton, 2003). For this reason, the Atlantic salmon farming industry is at much greater risk of negative consequences than wild salmon populations. IPNV has not been reported in BC but has caused considerable impact on Atlantic salmon production, particularly in Europe (Roberts and Pearson, 2005). Although IPNV has not been shown to cause mortality in Pacific salmon (except in rainbow trout), the virus can affect multiple species (Hill and Way, 1995), and so may represent a potentially serious, but unproven, threat to farmed or wild naïve BC salmonid populations if it were introduced and established (i.e. not detected or contained). However, it should be noted that the quarantine practices and FHPR testing regimes mentioned previously had multiple opportunities to detect any IPNV that may have been present.

Comments regarding uncertainty in risk evaluation: Although FHPR is a respected set of regulations aimed at providing confidence that tested livestock are, and remain, free of the specific pathogens, there can be gaps in the regulations. For instance, the water supply in hatcheries which incubated eggs to the eyed stage (i.e. prior to shipping to BC) was often surface water. There was usually some type of water treatment or disinfection (e.g. ozonation or UV) but this was not as strict or as well understood in 1985-89 as it is today. For example, Lawrie's report (page 6) indicates that influent water in the quarantine facilities was UV disinfected or from fish-free sources and effluent water was chlorinated, but there is no mention of the influent water at the source hatcheries from which the eggs were imported. My understanding of many of the hatcheries in the approved sources list is that they used surface water, and this water may, or may not, have originated from a spring source that was free of fish or fish pathogens. The uncertainty raised by this fact is relatively small given that the testing regime was extensive and timed to the transfer. However, it does represent a biosecurity unknown whereby new exposures to wild fish or birds may have introduced a pathogen prior to shipping of eggs. My opinion is that the contribution of this occurrence to introduction probability is very small.

During the early years of these egg importations (e.g. 1985-1995), brood stock were usually maintained and selected from the general production population. Thus, they were usually derived from stock that had been previously exposed to other potentially infected fish (wild or farmed). The uncertainty arises in that the broodstock may have been covertly infected. Diagnostic testing in apparently healthy 'carriers' usually exhibits lower sensitivity (i.e. more false negatives) which means that FHPR testing may have missed some positive cases. However, the extensive and repeated testing of broodstock prior to

spawning and the practice of isolating broodstock from production fish over an extended time period to satisfy FHPR would have reduced the probability of false negative conclusions. Diagnostic testing in general was less proficient before the advent of PCR testing methods in routine surveillance which occurred around 1995 in salmon farming. For this reason, the earlier years (1985-1995) may have represented a slightly greater probability of generating false negative diagnostic results when testing apparently healthy fish. However, sick fish generally contain more pathogens and so the false negative issue does not apply to testing of moribund fish if they occurred.

Comments regarding risk mitigation: Three important aspects of the egg importation reduce the probability of pathogen introduction from low to extremely low. These are 1) taking eggs from FHPR approved sources, 2) restricting movement of live animals to the eyed egg stage, and 3) post-transfer quarantine with extensive diagnostic testing requirements. These actions are directed toward identifying stock that could be infected with an exotic pathogen and containing that infection if it occurred. It appears to be successful at least to the point of not identifying any exotic pathogens through the process to that stage of release from quarantine.

Summary: The probability that eyed eggs imported from 1995 to 2009 introduced any new pathogens to British Columbia is 'extremely low' to 'remote'. Although the probability of introducing any new pathogens to BC may have been higher in the 1985-1995 period due to lack of advanced diagnostic methods used today, the time period since has provided more opportunity to detect any introduced pathogens. The measures employed since 1985, namely FHPR testing of broodstock to reduce the probability of vertical transmission of pathogens to eggs, lack of clinical disease in brood stock populations (part of FHPR), egg surface disinfection practices, and quarantine of newly introduced eggs, all contributed to reducing the probability of inadvertent pathogen introduction. Pathogens not known in the early years of egg importation (e.g. ISAV, SAV) did not have testing to confirm their absence in the imported egg or their brood stock. However, the fact that these pathogens have little or no conclusive evidence of vertical transmission, absence of clinical disease in broodstock and tests that often detected other pathogens (such as virus isolation on cell lines that are quite permissive to these viruses), mean that other measures coincidentally reduced the probability of the introducing exotic pathogens, even if not yet discovered.

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