Preventing Infectious Hematopoietic Necrosis in Salmonid Fish Along the Columbia River Basin

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Abstract

Infectious hematopoietic necrosis virus (IHNV) is a member of the Rhabdoviridae family and causes infectious hematopoietic necrosis (IHN) disease in many salmonid species during the juvenile or “fry” stage of life. IHNV is endemic to Western North America and occurs in the Columbia River Basin, where Steelhead and Chinook salmon are the most abundant IHNV-vulnerable species. IHNV can cause an epidemic in wild or farmed stocks, killing 90-95 percent of the fish it infects. Transmission is currently understood to occur by direct exposure through the gills, and the virus is shed typically from asymptomatic or clinically-ill carrier adults at time of spawning via eggs. To this point, control and prevention has been moderately accomplished through efficient biosecurity and sanitation. This means ensuring the waters are virus-free for incubation and rearing at hatcheries, as well as disinfecting fertilized eggs with an iodophor solution and careful surveillance of brood stock. In addition to being a significant life-sustaining source for millions of people, particularly tribal populations in the Pacific Northwest, Pacific salmon is a $3 billion dollar industry that employs thousands. Here, we articulate the need to develop strategies to minimize impacts from IHNV through vaccination and other effective prevention methods.

Problem Statement

IHN can have damaging effects on the numerous fish hatcheries along the Columbia River significantly effecting the Pacific Salmon industry and directly impairing a vital food source for the tribal populations in the Pacific Northwest.

Introduction

Infectious hematopoietic necrosis virus (IHNV), the causative agent of infectious hematopoietic necrosis (IHN), is a lethal pathogen of predominantly steelhead and Chinook salmon in the Columbia River Basin (Ferguson 2018). However, all salmonid species in the Columbia River basin are susceptible [table 1]. Entry of the Rhabdovirus is thought to occur through the gills and at the bases of fins, while the kidney, spleen and other internal organs are the sites in which the virus is most abundant over the course of infection (IOE 2018, Bootland and Leong 1998, Wolf 1988). The “fry” or juvenile stage of salmonid life is the most vulnerable to infection [figure 1] (IOE 2018). Older fish have more resistance to clinical disease but can be asymptomatic carriers of IHNV (IOE 2018). Transmission is mainly horizontal (i.e. juvenile to juvenile) with large amounts of the virus being shed in urine and external mucus (IOE 2018, Bootland and Leong 1998, Wolf 1988). Egg-associated transmission through contact with sexual fluids has been recorded, but with proper disinfection of eggs and water with iodine-based solutions (idophor) this route of transmission is diminished (IOE 2018, Bootland and Leong 1998, Wolf 1988).

Other factors that influence fish susceptibility are relevant to consider. By understanding the factors influencing population vulnerability, estimations of the likelihood of infection establishments can be made. Susceptibility to IHNV depends on factors including, fish species, virus and fish strain, life stage, weight, rearing density and environmental conditions (Dixon et al 2016). There have been a variety of studies comparing the susceptibility to IHNV between fish strains or lineages. Of the progeny produced by 16 individually paired families of sockeye salmon 52%-98% of their susceptibility to IHNV was based on genetic differences (Amend et al 1997, Dixon et al 2016). Another study using a rainbow trout model from 22 different families
the larger fry had a 10-85% mortality rate while the smaller fry had a mortality rate ranging from 65-100% (Kasai et al 1993, Dixon et al 2016). Chinook salmon fry strains round in Alaska were less susceptible to IHNV than those found in Washington State (Wertheimer et al 1982, Dixon et al 2016). As mentioned previously the juvenile, fry, stage of the fish appears to be the most susceptible age. Occasionally, there are cases of mortality in older fish such as in 14-16-month-old sockeye salmon smolts (Burke et al 1984, Dixon et al 2016) and 2-year-old kokanee (Traxler et al 1986, Dixon et al 2016). Experimentally infected, sexually mature, Chinook salmon have also reportedly died within 14 days of IHNV infection (Arkush et al 2004, Dixon et al 2016).

LaPatra et al 1998 analyzed whether age and/or weight was a determinant in mortality of rainbow trout fry infected with IHNV. In the study, fry hatched on the same date were grouped and fed based on 3 different regimens resulting in fry that were the same age, but three different weights (LaPatra et al 1998, Dixon et al 2016. Comparing fish of similar weight, but different ages (3 or 4 months), the older fish were significantly less susceptible to IHNV than the younger fish (LaPatra et al 1998, Dixon et al 2016). However, when repeated with fish at 4 or 5 months and similar weight the 5-month-old fish were more susceptible (LaPatra et al 1998, Dixon et al 2016). Leading to the conclusion that age or size alone does not determine susceptibility (LaPatra et al 1998, Dixon et al 2016). For most of the host species of IHNV an association between increased age or weight and a decrease in susceptibility has been noted (Dixon et al 2016). For the sake of planning surveillance programs, the IHNV mortalities are most abundant in juvenile fish <20g (Dixon et al 2016). This group is also the one to carry the highest pathogen loads, so is optimal for sampling (Dixon et al 2016).

Rearing density has also been associated with IHNV transmission in a susceptible fish population. Ogut et al 2004 evaluated rainbow trout vulnerability by cohabitating healthy rainbow trout with and experimentally infected IHNV donor trout. At low rearing densities, no IHNV transmission was observed (Ogut et al 2004, Dixon et al 2016). At higher rearing densities there was a higher prevalence of IHNV (Ogut et al 2004, Dixon et al 2016). In this experiment, at the highest rearing density tested (60 exposed fish in 5 repeats) between 1 and 4 fish became infected within 11 days (Ogut et al 2004, Dixon et al 2016). The authors suspect that a higher rearing density affects disease prevalence by causing a lower water quality resulting in stress and harm to the immune system and/or there is a general increase in contact between healthy and infected fish due to less space available (Ogut et al, Dixon et al 2016). Low rearing densities are greater depicted in wild fish populations while high rearing densities are normal in farms or hatcheries.
Physical handling, environmental change, and other factors causing stress are known to decrease fish resistance to disease (LaPatra et al 1998, Dixon et al 2016). During the migration upstream and annual spawning, adult sockeye salmon had an increased IHNV infection and viral load in IHNV endemic areas (Traxler et al 1997, Mulchy et al 1984, Dixon et al 2016). Reduced food supply or high fish density can increase stress levels (Follett et al 1995, Dixon et al 2016), as well as changes in the oxygen levels or exposure to high concentrations of metals, like copper and iron (Follett et al 1987, Dixon et al 2016).

Table 1. Taken from “Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review” Dixon et al 2016. Partial list of species susceptible to IHNV that are present in the Columbia River Basin. Not listed but susceptible is the pink salmon.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Listed as susceptible by EFSA</th>
<th>Listed as susceptible by OIE diagnostic manual</th>
<th>Disease commonly occurs/produces significant mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>Rainbow trout, steelhead trout</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Oncorhynchus tshawytscha</em></td>
<td>Chinook salmon</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Oncorhynchus kisutch</em></td>
<td>Coho salmon</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Oncorhynchus nerka</em></td>
<td>Sockeye salmon, kokanee</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Oncorhynchus keta</em></td>
<td>Chum salmon</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Although IHN disease has been known for over half a century, there are limited experiments dedicated to exploring the MID, or mid-infectious dose, of IHNV (Dixon et al 2016). Information regarding the MID and virus shedding rates could be used to theoretically model disease transmission in farmed fish or hatcheries (Dixon et al 2016). However, the MID seems to vary, depending on fish species and fry, between $10^2$ and $10^3$ pfu mL$^{-1}$ (Dixon et al 2016). As stated previously, susceptibility also depends on multiple factors, such as stress, environment, rearing density and others making the context of disease modeling more challenging, as exposure of susceptible fish at the MID may not result in infection (Dixon et al 2016).

Virulence of IHNV depends on the genogroup of virus and the species of susceptible fish. There are three genogroups of IHNV based on geographic regions in the Pacific northwest and they are labeled Upper (U) for the northernmost, Middle (M), and Lower (L) for the southernmost
The M genogroup is the kind prevalent in the Columbia River Basin. When compared by bath exposure of sockeye salmon, kokanee, and two strains of rainbow trout, it was noted that geographic origin of the isolates played a larger role in virulence than host origin (Kurath et al 2003, Dixon et al 2016). In sockeye salmon and kokanee, the U genogroup was highly pathogenic causing 69-100% mortality, while the M genogroup had a 0-4% mortality rate (Garver et al 2006, Dixon et al 2016). In rainbow trout the M genogroup was more fatal (25-85% mortality) and the U genogroup was less virulent (Garver et al 2006, Dixon et al 2016). As for the L genogroup, there was a moderate virulence for both sockeye (5-41%) and rainbow trout (13-53%) (Garver et al 2006, Dixon et al 2016).

IHNV is an economically important pathogen because of the challenges it poses in aquaculture industry and in conservation efforts. IHNV is endemic to the wild fish populations in the Columbia River Basin and as hatchery conditions can be disinfected and controlled for, the anadromous nature, living in both freshwater and ocean, of salmon and steelhead makes swimming in IHNV-infested waters inevitable (Cary Institute of Ecosystem Studies 2017). The damming of the Columbia River itself has caused a great decrease in amount of anadromous fish returning. The combination of dam impacts with IHN outbreaks will further decrease the salmonid population in the Columbia River. State, federal, and tribal initiatives to rebuild the wild stock, through rearing, in the Columbia River Basin incur large amounts of expenses from IHN due to fish mortality, mandatory discarding of infected fish and eggs, restriction of movement of infected fish, as well as routine surveillance and testing costs (Cary Institute of Ecosystem Studies 2017). Tribal people living along the Columbia River rely on salmon as their primary food source and as tribe populations increase the need for salmon is ever more important (CRITFC 2019). For many tribal members, fishing is still the preferred career choice and a significant source of income for tribal families. Salmon is recognized, above all, as a part of the spiritual and cultural identity of tribal people (CRITFC 2019).

This white paper will explain current vaccination and prevention efforts in the hatchery and commercial setting, propose modifications to those strategies to better fit hatchery needs and encourage the funding of IHN vaccine and prevention research to indirectly benefit workers in the salmon industry and tribal peoples.
**Background**

Many species of the Pacific salmon and steelhead are already endangered and with their declining numbers there is a loss of a food and economic resource. Washington state alone generated $1.6 billion annually from commercially harvested Pacific salmon with an estimated 16,374 employees in both recreational and commercial fisheries in 2006 (WDFW 2010, Duke’s Seafood and Chowder 2018). The same year recreational fishing of salmon and steelhead also contributed $1.1 billion to Washington’s economy as well as employing an estimated 14,655 people (WDFW 2010, Duke’s Seafood and Chowder 2018). On the other side of the Columbia river, Oregon salmon fisheries resulted in a harvest value of $8.3 million with $4.1 million attributed from the Columbia River salmon fishery (ODFW 2017). The Yakama tribe, a member of the Columbia River Inter-Tribal Commission (CRITFC), once netted $1 to $2 million in the fall period of the early 2000s. Since the price per tribal caught fish has increased over 300%, the net of that time today would be worth around $6 million (McDermott 2017, CRITFC 2019). Yakama and other CRITFC tribe fisherman depend on the return of the fish in the spring as many must deplete their savings to survive the winter (McDermott 2017). But salmon are more than economic prospects for the CRITFC tribes and others who rely on them. Salmon are a primary food source and essential to maintaining tribal culture. Previously, salmon feasts would celebrate every phase of life from births to funerals, however their already dwindling numbers has tribal members putting survival first (Fears 2015).

Currently, IHN is controlled in hatcheries through the careful sanitation and disinfection of eggs and water, surveillance and testing. There is no effective treatment or vaccine available to be used in Pacific salmon and steelhead hatcheries. The USDA has approved the use of a DNA vaccine to be used commercially for Atlantic salmon farms (USDA 2014, Newman, 1993,
Kibenge et al., 2012). However, the vaccine can only be administered through injection. The injection delivery method is not optimal for the hatchery setting. It allows for the highest level of protection but is very labor intensive (USDA 2014, Newman, 1993, Kibenge et al., 2012). Fish must be individually handled causing stress and hazards to the fish and workers. Additionally, salmonid fry are too small to receive a vaccine via injection. An orally administered vaccine, through food, is ideal as no fish need to be handled and can be dispersed with no stress. In conjunction with current sanitation methods, the development of an oral vaccine to be used in Pacific Salmon and steelhead hatcheries could eliminate the prevalence of IHNV in the Columbia River Basin.

In general, the development of an efficacious and safe oral vaccine is a challenge. However, it can be done. For the salmonid species Atlantic Salmon and Coho salmon commercial oral vaccines are available for *P. salmonis*, infectious pancreatic virus (IPNV) and infectious salmon anaemia virus (ISAV). Oral vaccines insufficient efficacy is partly attributed to antigen breakdown in the acidic gastric environment, as well as to high tolerogenic gut environments and poor vaccine design (Carmen et al 2016). In young fish, risk of tolerance induction with oral vaccination is another factor immunologist must consider (Carmen et al 2016). Also, current oral vaccines allow for weaker or shorter protection periods, so are used primarily as a booster vaccine in combination with other forms of vaccination (dip, spray or injection) in the commercial setting (Carmen et al 2016).

Because IHNV is an enveloped, single stranded virus one of its six open reading frames is for a glycoprotein (G) (Morzunov et al 1995, Regenmortal 2000, Ballesteros et al 2015). In previous studies, IHNV G protein was shown to be able to neutralize an antibody response to IHNV, therefore it is a target in DNA vaccines (Morzunov et al 1995, Regenmortal 2000, Ballesteros et al 2015). Studies using an alginate (brown algae derivative) encapsulated oral DNA vaccine in rainbow trout (*Oncorhynchus mykiss*) have shown promise. It is suggested that the properties of the alginate microparticles protect the DNA vaccine from degradation in the fish stomach which then allows vaccine to go through intestinal mucosa and disperse to internal and external organs (Ballesteros et al 2015). When a high dose of the DNA plasmid vaccine was used, several markers of the adaptive immune response were detected in the kidney and spleen in a dose-dependent manner [graph 1] (Ballesteros et al 2015). Additionally, when the vaccinated fish where challenged by immersion with live IHNV a dose-response effect was also observed (Ballesteros et al 2015). Over 50% of the fish vaccinated with high doses of the vaccine survived the challenge with a nearly undetectable IHNV gene expression in their kidneys or spleens meaning the vaccine effectively reduced the number of viruses in the tissues of survivor fishes (Ballesteros et al 2015).
Graph 1. Taken from “An oral DNA vaccine against infectious haematopoietic necrosis virus (IHNV) encapsulated in alginate microspheres induces dose-dependent immune responses and significant protection in rainbow trout (Oncorhynchus mykiss)” Ballesteros et al 2016. The graphs visualize the CD4, CD8, IgM and IgT gene expression in trout kidneys and spleens after oral immunization. There is a control group, injections levels 10, 25 and 100 μg of pIRF1A-G-(DNA plasmid) loaded alginate, and an intramuscularly injected group (im). Asterisks indicate significant differences (p<.05) between vaccinated and unvaccinated fish while black points represent significant differences between oral and intramuscular vaccinated fish.

A study done by Amar et al in 2011 investigated rainbow trout fry resistance to IHNV following ingestion of natural and synthetic carotenoids. Carotenoids have been documented to show health promoting effects in humans and other animals and were found to carry out nearly all essential roles in all biological systems (Amar et al 2011, Chew 1993, Krinski 1991). In some aquaculture species carotenoids can improve immunocompetence. It was thought that because carotenoids enhanced non-specific immune responses, such as phagocytosis, lysozyme activity and complement, it could improve pathogenic protection (Amar et al 2011, Amar et al 2000, 2001, 2004). The results of the study found that when fed a synthetic carotenoid, astaxanthin, and challenged with a low dose of virulent IHNV mortality was 22% lower and exhibited a high relative percent survival rate of 58% which was significantly different from the control (p<.05). However, when exposed to a high dose of virulent IHNV, there was no significant difference in survival (p>.05) (Amar et al 2011).
Objectives of the Report

IHNV is not the sole cause of the decline in salmon and steelhead, but its high mortality rate. Should fry become infected, this will accelerate the decline. So far, there is no oral vaccine to be used in hatcheries rearing wild salmon and, in the event, that an outbreak should happen in a controlled hatchery setting young fry are completely vulnerable.

The development of an effective oral vaccine for IHNV will ensure greater protection of the young salmon and steelhead being raised in hatcheries and/or living in IHNV endemic waters. Further, the protection of Pacific salmonids is essential to ensure financial stability for workers in the salmon industry as well as financial, health, and cultural wellbeing for tribal communities.

Based on the current research in IHNV oral vaccines, it is proposed that future research should be funded to test combining the use of both a high-dose alginate-encapsulated DNA vaccine with the synthetic carotenoid, astaxanthin. The thought is that because the alginate encapsulated DNA vaccine assists in specific, adaptive immune responses while the synthetic carotenoid can potentially enhance the non-specific innate immune system both together could overall decrease risk of contraction and mortality from IHN. The vaccine and astaxanthin should be given to salmon and steelhead fry after their yolk sac has been fully absorbed and before moving them into the sanitized rearing ponds. Another dose should be given before release back into the wild to account for the short protection time. For steelhead, incoming adults should receive the vaccine and astaxanthin every time they return [figure 2]. In addition, eggs should continue to be sanitized with an iodine solution and waters routinely tested and kept virus free. It may be a challenge to attempt a trial on salmon or steelhead because they are endangered species. As in the other studies, a rainbow trout model may be more valid.

With concerns to high-rearing density, hatcheries should consider larger, or more, rearing ponds to ensure there is enough space between fish. This may help to reduce stress from crowding and serve as a precautionary measure to ensure fewer fish are infected should an outbreak occur.
Discussion

IHNV has the potential to assist in the decline of salmonids in the Columbia River Basin, causing pathogenesis and mortality in the fry, or juvenile, stage of life. Because of IHNV, efforts to restore the wild salmonid stock are a daunting challenge. Because IHNV is endemic to the Pacific Northwest it will require constant testing of water, surveillance, and disinfecting to ensure eggs and fry are protected. In addition, susceptibility of salmonids to IHNV considers factors, such as rearing density, environment, stress, and weight. MID is an area to be further studied as it is not a specific dose for all species, it also requires consideration of susceptibility factors. IHNV has a virulence of up to 100% in some species with no available vaccine to be used in Pacific salmon or steelhead hatcheries.

Though IHNV is moderately controlled in hatcheries through disinfection of eggs and water, a vaccine, particularly an oral one, is needed to ensure immunity from the virus. An effective oral vaccine would protect the fish with minimal stress and labor. As of current research a DNA vaccine encapsulated with alginate has shown results comparative to an intramuscular vaccine typically used to deliver DNA vaccines (Ballesteros et al 2015). Another study, Amar et al 2011, tested the effects of carotenoids on assisting immunity to IHNV. There were significant findings when the fish were fed the synthetic carotenoid, astaxanthin. Based on the results of these two studies a solution combining both the encapsulated DNA vaccine and astaxanthin was proposed to be first tested in a rainbow trout model. Should the results be significant in multiple studies, trials on more salmon and steelhead should commence. Additionally, modifications to hatcheries including larger or more rearing ponds would assist in the prevention of IHNV.
Economically, salmonids make up a considerable part of Oregon and Washington’s economy and ensure the employment of thousands of people. The Pacific northwest tribal people, who also rely on salmonids for a significant source of income, see salmon as a part of their culture and people. They depend on salmon and steelhead as a primary food source and risk losing it as their populations grow. Therefore, the protection of salmon would essentially prevent unemployment, poverty, hunger, and loss of culture.
References


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