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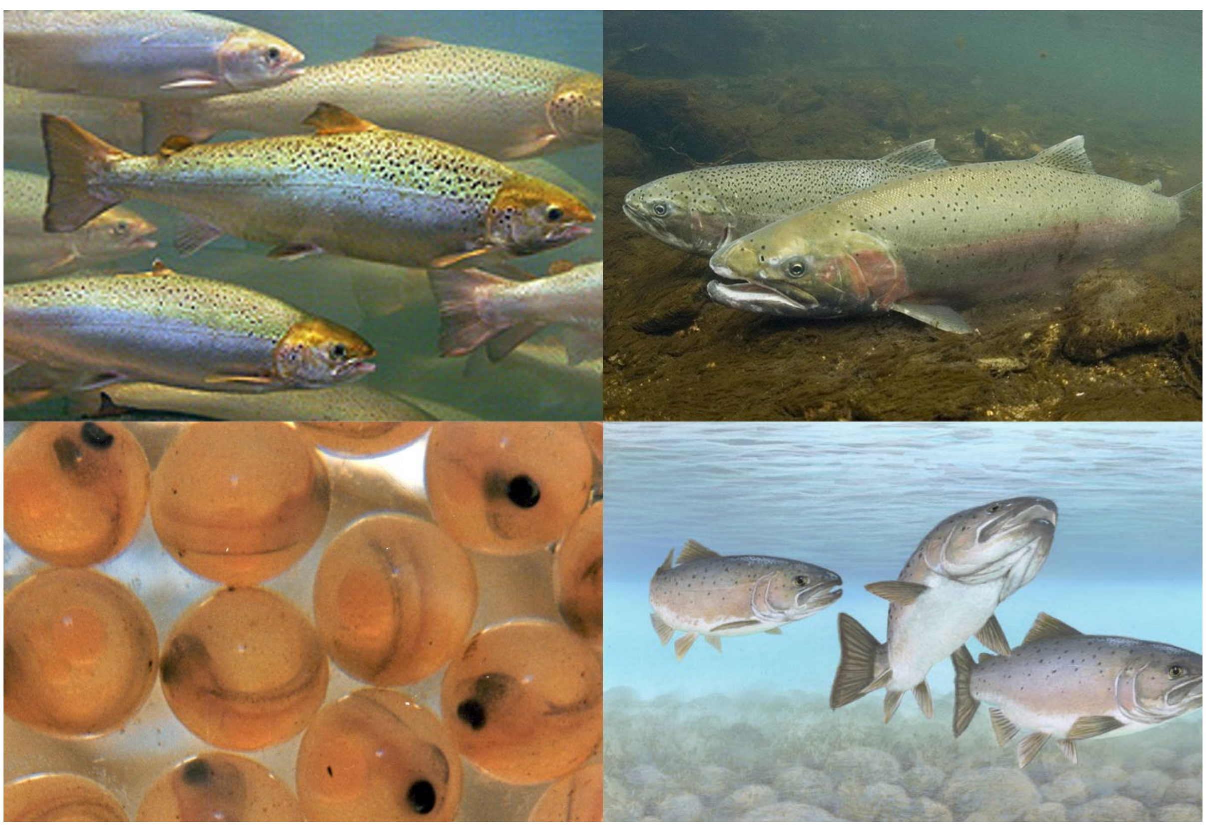
Hazard Identification: Infectious Haematopoietic Necrosis Virus (IHNV)



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Introduction

USDA APHIS VS CEAH was asked to generate a Hazard Identification for infectious haematopoietic necrosis virus (IHNV). Hazard identification is a process used to identify hazards (biological, chemical, or physical agents in, or the condition of, an animal or animal product) that may result in adverse consequences in susceptible populations.[1] The hazard identification process is used to identify pathogenic agents that may be associated with importation of a commodity (live animals, products of animal origin, genetic material, biological products, or pathological material).[1] The hazard must be relevant to the imported species, and it must be determined if the hazard is a) present in exporting countries; b) present or absent in the importing country; c) a notifiable disease or subject to control or eradication in the importing country.[1]

Subjects within the scope of this document include a description of the hazard (IHNV), identification of susceptible fish species and the geographic distribution of the hazard, and a summary of the epidemiology of the hazard. To conduct this hazard identification, we referenced World Organisation for Animal Health (WOAH) resources, subject matter expert consultation and available published data and literature relative to IHNV epidemiology. Knowledge and data gaps were present that affected complete evaluation of some tenets of this hazard identification document. Subjects that are not within the scope of this document include an assessment of potential entry and exposure pathways and summaries of consequences and overall risk. This document is intended for internal USDA APHIS VS leadership review and external distribution to industry stakeholders.

This document follows:

- The WOAH Handbook on Import Risk Analysis for Animals and Animal Products import risk analysis framework, which is accessible via a link in Appendix, Table 1.[1]
- WOAH criteria for determination of host species susceptibility as described in the WOAH Aquatic Animal Health Code and the OIE ad hoc Group on Susceptibility of Fish Species to Infection with WOAH Listed Diseases (Appendix, Table 1).[3, 4]
    - Fish species described in published literature that do not meet these criteria or in which infection was inferred using diagnostic methods that are not validated according to WOAH protocols are not included in this assessment. Briefly, species susceptibility to a pathogen requires that:
        - the experimental transmission is consistent with natural pathways of infection,
        - the pathogen is adequately identified, and
        - the presence of the pathogen in the host constitutes an infection.
- Standards in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (Appendix, Table 1)[4, 5] regarding improvement of animal health welfare, safe international trade in aquatic animals and their products, and diagnostic approaches to disease diagnosis.
- The understanding that epidemiologically, disease occurs as an interaction occurring in environmental spaces (natural and anthropogenically influenced or derived) where host and

pathogen tolerance limits for essential biotic (living) and abiotic (nonliving) environmental factors overlap.[6, 7, 8, 9]

- Definitions of animal agriculture biosecurity as:
  - A series of management steps and practices that identify, prevent, control, and mitigate introduction and spread of pathogens in an animal population, and spread of pathogens to other susceptible populations.[10]
  - Measures based on current epidemiological information and understanding of relevant knowledge and data gaps.[11, 12, 13, 14]

Key Findings

- Infectious haematopoietic necrosis virus (IHNV; salmonid novirhabdovirus) is a World Organisation for Animal Health (WOAH)-listed reportable disease.
- IHNV is included in the USDA APHIS National List of Reportable Animal Diseases - National Animal Health Reporting System (NLRAD-NAHRS) list of reportable diseases.
- IHNV infects numerous fish species, primarily salmon, but also trout and pike.
- Most of the fish species that are susceptible to IHNV are present in the United States.
- IHNV is endemically present in the North America, including the United States (Alaska to California and Pacific Coast watersheds that include Idaho).
- Spread of IHNV is believed to be the result of trade movement of IHNV-infected eggs or fry.
- Factors that contribute to IHNV infection and disease are multifactorial and incompletely understood.
- Disease with high losses have occurred in both wild stocks and enhanced stocks of sockeye salmon, and in farmed rainbow trout.
- Pathogen, host, and environmental factors are associated with occurrence of infection and disease. However, there are knowledge gaps associated with the epidemiology of IHNV.
- Confirmatory testing at the National Veterinary Services Laboratory is required following first detections.
- Some countries require testing live salmonid fish, eggs, and gametes for IHNV prior to export from the United States.
- Stringent biosecurity measures can decrease the risk of introduction.
- Re-introduction into fish farms and hatcheries can occur if the water supply to the farm is not secure.

Infectious Haematopoietic Necrosis Virus

Introduction

Infectious haematopoietic necrosis virus (IHNV; salmonid novirhabdovirus) infects numerous fish species, (primarily salmon, trout, and pike). It is a bullet- shaped, non-segmented, negative-sense, single-stranded RNA virus in the Genus *Novirhabdovirus* and Family *Rhabdoviridae*. Six proteins are encoded in the viral genome: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). The glycoprotein is a surface protein and the primary antigenic element of IHNV, to which anti-glycoprotein serum can neutralize the virus.[5, 15]

Phylogenetic analyses based on G gene sequences have identified five major genogroups of IHNV, (e.g., U, M, L, E, and J) the occurrence of which correlates with geography. Three of these genogroups (U, M, and L) are endemic in North America and were named according to their geographic occurrence in the upper, middle, and lower parts of the Pacific Coast (U for Upper, M for Middle, and L for Lower).[16, 17, 18, 19] Genogroup J isolates occur in Asia (China, Japan, and Korea).[4, 20, 21] Genogroup M strains were initially introduced to Europe and have now evolved into genogroup E isolates.[16, 20] Group U has also been found in the Russian Far East and Asia,[18, 20, 22] and M has been detected in Africa.[23]

IHNV (salmonid novirhabdovirus) is the etiologic agent of infectious haematopoietic necrosis (IHN), a disease notifiable to World Organisation for Animal Health (WOAH) (Appendix, Table 1). Disease notification requirements and requirements for self-declaration of freedom of IHNV infection for Member nations are found in the WOAH Aquatic Animal Health Code, Chapter 2.3.1. (Appendix, Table 1).[5, 24] IHNV is included in the USDA APHIS National List of Reportable Animal Diseases - National Animal Health Reporting System (NLRAD-NAHRS) list of reportable diseases (Appendix, Table 1).[25] State and Federal authorities should be contacted immediately upon suspicion or confirmation of the disease (Appendix, Table 1). Disease caused by IHNV can be highly economically impactful because it affects Atlantic salmon (*Salmo salar*), the primary salmonid species produced in aquaculture, and rainbow trout (*Oncorhynchus mykiss*).[15]

Susceptible Fish Species

Fish species that fulfill WOAH criteria for species susceptibility to infection with IHNV are summarized in Table 1.[1, 4] Nearly all of these species are present in the United States, except for marble trout and masu salmon.[26]

Table 1. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infectious haematopoietic necrosis virus (IHNV; salmonid novirhabdovirus)

Family	Scientific name	Common name
Esocidae	*Esox lucius*	Northern pike
Salmonidae	*Salmo marmoratus*	Marble trout
Salmonidae	*Salmo salar*	Atlantic salmon
Salmonidae	*Salmo trutta*	Brown trout
Salmonidae	*Salvelinus alpinus*	Arctic char
Salmonidae	*Salvelinus fontinalis*	Brook trout
Salmonidae	*Salvelinus namaycush*	Lake trout
Salmonidae	*Oncorhynchus clarkii*	Cutthroat trout
Salmonidae	*Oncorhynchus tshawytscha*	Chinook salmon
Salmonidae	*Oncorhynchus keta*	Chum salmon
Salmonidae	*Oncorhynchus kisutch*	Coho salmon
Salmonidae	*Oncorhynchus masou*	Masu salmon
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout/steelhead trout
Salmonidae	*Oncorhynchus nerka*	Sockeye salmon/kokanee salmon

Geographic Distribution

The range of IHNV in North America extends from Alaska to California and inland to Idaho. Outbreaks first occurred in sockeye salmon during the 1950s in hatcheries in Washington and Oregon, followed by outbreaks in Chinook salmon in California. IHNV was reportedly endemic in Alaska by the 1970s, in Idaho (in rainbow trout) by the late 1970s, and in salmonids in the Columbia River basin region by the early 1980s.[19, 27] Phylogenetic analyses in the Pacific Northwest region identified three major genogroups (U, M, and L). Genogroup U occurs from Alaska to the British Columbia coastal watershed, and in the Columbia River (Oregon and Washington). Genogroup M occurs in the Columbia River and Idaho. Genogroup L clusters are present in California and the southern coast of Oregon.[19, 27, 28]

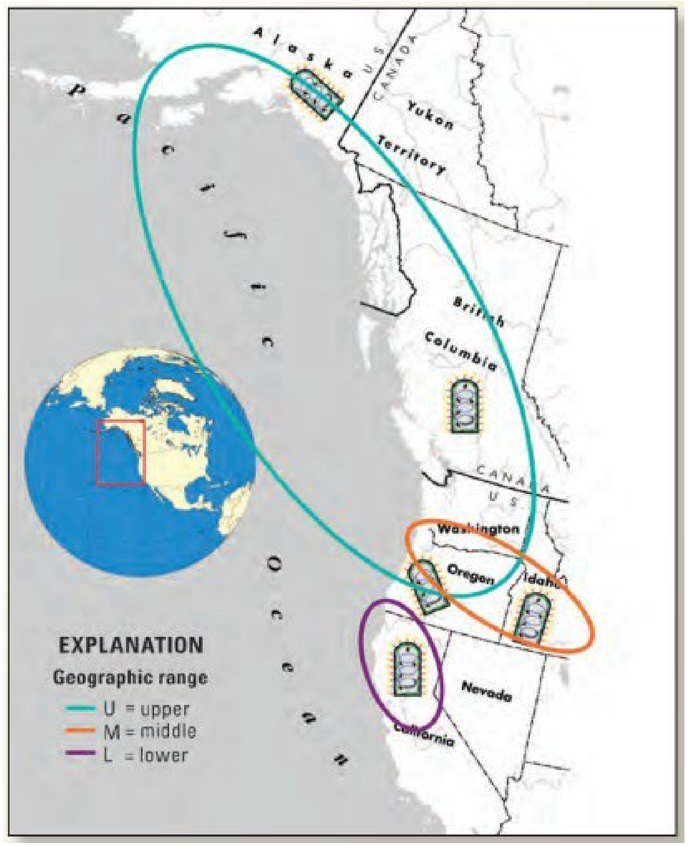

Figure 1: The geographic ranges of endemic IHNV genogroups U, M, and L in the United States[29]

The first outbreak of IHNV in British Columbia, Canada, occurred in farmed Atlantic salmon in 1992 when mortalities were observed in saltwater pens six weeks following transfer from freshwater.[30] The first detection in Japan occurred in 1971 in a kokanee salmon hatchery that imported sockeye salmon eggs from Alaska. The disease then spread throughout Japan through IHNV-contaminated fish eggs. Phylogenetic analyses of early Japanese isolates identified genogroup U; isolates after 1980 classify within genogroup J.[31, 32] In Europe, IHNV was detected for the first time in 1987 in France and Italy, and in Germany and Belgium in 1992. Sequencing analyses of European isolates identified genogroup E which likely originated from an ancestor of genogroup M from North America.[16, 17, 27, 33, 34] China and Korea reported IHNV in 1988 and 1991, respectively. Genogroup J and U occur in China[16] and genogroup J occurs in Korea.[35] The virus was reported in Russia in 2000 (genotype U)[18], and Iran in 2004 (genogroup E).[16] Other countries where IHNV has been detected are noted in the Appendix, Table 3.[5, 27] Spread of IHNV is believed to be the result of trade movement of IHNV-infected eggs or fry.[17, 27]

Public Health

There are no threats to human health as IHNV is not a zoonotic pathogen.

Epidemiology

In this section, the epidemiology of IHNV is summarized. Fundamentally, disease occurs in an environmental space (natural and anthropogenically influenced or derived) where host and pathogen tolerance limits for essential biotic (living) and abiotic (nonliving) environmental factors overlap.[6, 7, 8, 9] In general, many environmental, pathogen, and host factors of IHNV susceptibility among fish species are poorly described or understood.

Host Characteristics

Factors which contribute to IHNV infection and disease are multifactorial and incompletely understood. Disease with high losses have occurred in wild stocks and enhanced stocks of sockeye salmon. Variations in morbidity, mortality, and prevalence have been reported within the same stock.[36] Host factors (e.g., fish species and size, fish strain, life stage, age and weight, nutritional status, and presence of coinfections) appear to affect the ability of IHNV to cause disease.[15, 31, 37, 38] An experimental study in California showed that Chinook salmon fry (young fish) experienced higher mortality rates than steelhead trout fry (47–87 percent and 1.3–33 percent, respectively). Additionally, Chinook salmon mortality decreased with increasing age and water temperature.[39] Salmonid fry are likely to suffer severe disease, however, the virus does infect salmonids of all ages and subclinical infections occur.[5, 30, 38] IHNV tends to be highly pathogenic in fry and fish up to 2 months old, with 80 percent to 100 percent mortality rates, whereas 2–6-month-old fish typically experience <50 percent mortality rates. Disease and low mortalities can occur in older sockeye salmon, kokanee salmon, and rainbow trout. Atlantic salmon smolts in marine culture settings can experience mortalities over 45 percent.[27] As salmonids increase in age and size, they tend to be more resistant to developing clinical disease.[27, 38]

Environmental Characteristics

Environmental conditions for the host are also determinants of susceptibility to IHNV infection and disease.[15, 38] Environmental characteristics which affect IHNV presence and persistence in a population and viral pathogenicity include water quality parameters (e.g., temperature, salinity, and pollutants), and sanitary and biosecurity practices in aquaculture settings.[38, 39, 40] Disease outbreaks occur in water temperatures ranging from 8–14 °C/46.4–57.2 °F. Mortality rates are lower when water temperatures are above 15 °C/59 °F.[15, 38, 41] Naturally occurring epizootics occur most often during spring and autumn when water temperatures are 8–14 °C/46.4–57.2 °F and are usually not observed above 15 °C/59 °F.[27] In female sockeye salmon, IHNV prevalence is higher than that observed in males and increases from pre-spawning to spawning; however, this can vary each year.[27] Within aquaculture production settings, fish density affects transmission dynamics and prevalence of IHNV. Increasing animal density causes stress, increases contacts between animals, creates higher concentrations of pathogens, and reduces water quality, all of which can contribute to IHNV outbreaks.[27]

Pathogen Characteristics

IHNV susceptibility, virulence, and pathogenicity are incompletely understood and vary based on virus strain.[27] In cultured fish in the United States, disease has been observed in young sockeye salmon, rainbow trout, steelhead trout, Chinook salmon and kokanee salmon, with salmonid (e.g., rainbow/steelhead trout) hatcheries experiencing high losses.[28]

Genotype U has been identified in sockeye salmon, Chinook salmon, and steelhead trout. Genotype M is identified primarily in rainbow trout, and genotype L has been identified in Chinook salmon and in cultured steelhead trout. Wild and cultured fish have had identical or nearly identical IHNV genotypes when their locations were geographically close. The U group genotype occurs in the largest geographic range but has the lowest within-group genetic diversity.[28] In Idaho rainbow trout production facilities IHNV caused low virulence infections until 1977, when a highly virulent strain caused disease outbreaks that spread throughout the region, causing up to 80 percent mortality rates.[37] A California hatchery producing steelhead trout and two strains of Chinook salmon experienced mortality from IHNV in juvenile Chinook salmon but not steelhead trout, suggesting the potential for host specificity for some IHNV strains.[39, 42] In the 1950s, Washington aquaculture facilities observed 95 percent mortality in sockeye salmon but only 5 percent mortality in Chinook salmon while other outbreaks demonstrated high virulence in sockeye salmon but not for Chinook, coho salmon, or rainbow trout.[38] In Japan and Korea, IHNV was initially highly pathogenic for larvae and juvenile fish, but later, disease and losses occurred in adult and market sized rainbow trout, indicating a shift in pathogenicity for newer IHNV strains.[31, 32] Similar observations have been made in Italian rainbow trout farms.[40]

Transmission

Survivability of IHNV outside the host in fresh water and sea water is possible. Factors which influence this include temperature, ultraviolet light exposure, sedimentation, and presence of other microbes. IHNV tends to remain infectious longer in freshwater systems compared to seawater. The virus is rapidly inactivated with sunlight exposure and has been shown to be non-infectious within three hours after sunlight exposure.[5, 43, 44] IHNV survival is inversely proportional to temperature.[15] The virus can survive at 4 °C for three weeks in whole fry, four weeks in liver, and five weeks in brain.[15] Therefore, viable IHNV may exist in whole fish, tissues, and mucus, especially in cold storage settings and the risk of transmission exists in aquaculture settings with water sources, processing areas and waste, and predator bait.[15]

Spread of IHNV in aquaculture is believed to be the result of trade movement of IHNV-infected eggs or fry.[17, 27] Under natural conditions the primary mode of IHNV transmission is horizontal (waterborne) from fish to fish. Virus is shed in external mucus and reproductive fluids, and young fish shed high amounts of virus during epizootics.[15] One study measured 0.1–0.3 plaque-forming units (pfu)/mL of virus during early stages of an epizootic in steelhead trout nursery tanks and reached peaks of 1–5 pfu/mL in tanks and > 50 pfu/mL in raceways.[45] Rainbow trout fingerlings begin shedding virus within three days and are able to infect cohabitating fish.[27] Mortalities begin five to seven days post-exposure at 15 °C/59 °F.[38] Evidence suggests that horizontal transmission within and between adult salmonids occurs in fresh water and marine environments through the water.[27, 36] Fish may also become infected with IHNV via food if fed unpasteurized salmonid viscera.[15, 28] Cultured fish which are released to begin migrating to the sea may be infected with IHNV and may transmit the virus to other fish in marine waters.[27]

The mechanism of vertical transmission has not been definitively determined. IHNV can be detected in ovarian fluid and milt and is present on the surface of sperm.[27] It is stated in the literature that fry may become infected by vertical transmission (egg-associated transmission from adults). It has been observed that adult spawning fish can pass IHNV on to their offspring, and that the virus is unlikely to survive within the egg, but possibly can be transmitted due to its presence on the egg surface.[36] Other experimental studies demonstrated no vertical transmission of the virus to progeny when eggs were raised in IHNV-free water.[27, 36] Phylogenetic analyses of isolates in the first Japanese outbreaks supported the epidemiological evidence that sockeye salmon eggs shipped from Alaska were contaminated with IHNV, which resulted in fry and fingerling losses. IHNV subsequently spread throughout Japan when IHNV-contaminated eggs were transported to other salmon hatcheries.[46]

The importance of salmon lice or other vectors in the epidemiology of IHNV outbreaks in wild or farmed setting is not known. Adult salmon lice (*Lepeophtheirus salmonis*) were shown in one experimental study to be a competent vector for IHNV to Atlantic salmon. IHNV has also been detected in freshwater species such as leeches, copepods, and mayflies but their ability to transmit virus is currently unknown.[5, 15]

The maintenance of IHNV via persistence in a carrier state has been hypothesized with no definitive conclusion of the potential for reactivation of latency. Rhabdoviruses typically do not show viral latency, however, in one experimental challenge study IHNV was found in the brains of sockeye salmon that survived disease.[47] Early studies in the United States detected IHNV in sockeye salmon disease survivors once they reached maturity. However, a 1989 study showed no detections in sexually mature sockeye salmon captured as they left saltwater during spawning migration, and 90–100 percent prevalence in those that migrated on to freshwater spawning grounds in Washington. The authors of this study concluded that horizontal transmission occurred in the river during spawning, and that these infections were not due to latent IHNV.[48] Contrary to this finding, a 1997 Canadian study detected IHNV in sockeye salmon captured in marine water sites as they returned from sea to spawn. The authors concluded that the level of IHNV present in kidney tissues was high enough to indicate active replication rather than latency.[36] Other studies have detected IHNV proteins, nucleic acid, and truncated apparent rhabdovirus viral particles in tissues of rainbow trout that survived IHNV outbreaks, 1 - 2 years after virus was no longer detectable.[27]

Clinical Signs and Pathogenicity

Studies of disease progression of fish infected by waterborne IHNV show initial entry through gills, skin, fin bases, mouth, and esophagus and cardiac stomach region with viral replication occurring in epidermal cells. In rainbow trout, the gills, skin, and fin bases are the initial sites for IHNV replication before disseminating in 2–4 days to internal organs. Comparatively, the virus remains in the gills and skin of juvenile Chinook salmon for up to 39 days with no spread to internal organs. IHNV targets the kidney and spleen of young fish within 2–4 days after exposure, causing substantial necrosis, and then spreads throughout other organs.[27]

Some studies have investigated IHNV viral kinetics and infective doses. A 2013 waterborne immersion challenge experiment showed the infective dose in net pen reared Atlantic salmon smolts to be 10 pfu/ml for one hour by bath immersion. At peak infection, these same fish (demonstrating acute signs) shed 3.2 x 10^7 pfu/fish per hour.[44] A study with rainbow trout fry showed IHNV viral replication in the fin bases at 8 hours post-infection when fish were exposed to water infected with 5 x 10^4 pfu/ml by bath immersion.[49]

Clinical signs of disease typically appear in young salmonid fish within 5–19 days of exposure.[27, 37] Fish initially demonstrate lethargy, whirling, or hyperactivity, and eventually may display dark coloration, exophthalmia, distended abdomen, gill pallor, and have mucoid, opaque fecal casts. Petechial hemorrhage may be present at the fin bases and vent, and may be present in the gills, mouth, eye, skin, and muscle. Chinook salmon may develop subdermal hemorrhage caudal to the head. Sockeye salmon smolts may display clubbed, fused gill lamellae and cutaneious lesions. Two-year-old sockeye may exhibit irregular swimming movements with hemorrhage at the fin base.[27] Older fish may show fewer clinical signs. Surviving fish may demonstrate spinal curvature deformities, but these appear to be less common in rainbow trout. Fish will have a normocytic aplastic anemia with leukopenia, degenerative leukocytes and thrombocytes, low hematocrit, osmolarity, and abnormalities in the biochemical panel.[27]

Morbidity and Mortality

Acute outbreaks may show sudden mortality rate increases with no other clinical signs.[27] Mortality rates in young salmonids can reach up to 100 percent with IHNV infection.[27, 39]

Treatment

There is no medical treatment for IHNV. In some countries, vaccination may be available.

Diagnostic Testing

There are two pathognomonic features of IHNV: degeneration and necrosis of eosinophilic granular cells within layers of the alimentary tract and necrotic bodies (cellular debris) which may be observed in blood smears or kidney imprints.[27]

Gross pathological findings of IHNV infection may include darkened skin, pallor due to anemia of the liver, spleen and kidney, ascites, milky fluid filling the stomach, petechiation and hemorrhage in the adipose tissue, visceral mesentery, swim bladder, peritoneum, meninges, and pericardium, intestines filled with yellowish mucus, empty stomach, and lesions in the muscle tissue near the kidney.[27] Histopathological findings include degenerative necrosis of hematopoietic tissues, caudal kidney, spleen, liver, pancreas, and digestive tract. Macrophages and degenerative lymphoid cells may occur in the cranial kidney, and with late disease states macrophages may contain vacuolated cytoplasm, chromatin marination of nuclei. Pyknotic and necrotic lymphoid cells may appear, or the kidney may be severely necrotic. Cells in the spleen, pancreas, liver, adrenal cortex, and intestine may show nuclear polymorphism and margination of the chromatin, and necrosis.

Several diagnostic testing methods exist to identify IHNV including virus isolation (VI) using cell culture, serological assays that use IHNV-specific polyclonal or monoclonal antibodies to detect antibodies or antigens (e.g., serum neutralization, indirect fluorescent antibody test [IFAT], direct alkaline phosphatase immunohistochemistry [APIC], and enzyme-linked immunosorbent assay [ELISA]), electron microscopy (EM), and molecular methods that amplify nucleic acid from a gene or region of a gene of all known IHNV genogroups and then sequence the amplicons.[5, 27] Because IHNV is a WOAH-listed reportable disease, specific assays and definitions are required for disease confirmation. WOAH recommended protocols for targeted surveillance, presumptive and confirmatory diagnosis sampling, sample submission and diagnostic testing are described in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code.[4, 5, 24] In the United States, confirmatory testing at the USDA National Veterinary Services Laboratory (NVSL) is required following first detections (Appendix, Table 1). All suspected IHNV detections or outbreaks of IHNV are reportable to USDA APHIS VS, which is the Federal competent authority for animal health. Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL.[50]

Prevention and Control

In the context of animal agriculture, "biosecurity" is defined as a series of management steps and practices implemented to identify, prevent, control, and mitigate the introduction of infectious pathogens into an animal population, spread of the pathogen within that population, and spread of the pathogen to other susceptible populations. Measures should be based on current epidemiological information and understanding of relevant knowledge and data gaps.[11, 13, 14] Good biosecurity measures within aquaculture settings are essential in preventing exposure to IHNV and controlling disease.

Common disinfectants with active ingredients like sodium hypochlorite, iodophor, benzalkonium chloride, saponated cresol, formaldehyde, and potassium permanganate will inactivate IHNV and should be used in aquaculture facilities with susceptible species as part of a biosecurity program.[5, 51] Surface egg disinfection and utilizing IHNV-free water supplies are also important control measures in mitigating disease.[5, 39] In Japan, disinfection of eggs with iodine and disinfection of rearing water and facilities has allowed for production of IHNV-free fish and is a common method of egg disinfection because of its neutral pH, non-irritant, and relatively non-toxic properties.[5, 32] However, iodine may inhibit PCRs and may impact PCR-based test results of disinfected eggs.[5]

Experimental cross breeding of salmonids or triploid hybrids to select for increased IHNV resistance has been attempted with varying degrees of success. Specific genes which may be involved in IHNV resistance are currently unknown.[5, 15] An IHNV DNA vaccine is approved for use in Canada in farmed Atlantic salmon. This vaccine rapidly induces innate immunity and provides long-term protection (up to 2 years) in multiple salmonid species. However, it must be administered intramuscularly, which can be impractical for small fish in large production settings.[27] Additionally, it may not be possible to differentiate a vaccinated fish from an infected fish when using molecular diagnostic methods that target the viral sequence or protein of the IHNV G gene.[5] Vaccine studies demonstrate the inclusion of encoding the IHNV G gene is important for protection and antibody response.[27] Orally administered DNA vaccines are being studied[52, 53] but are not commercially available. Development of a variety of other vaccines (killed, subunit, and attenuated) have been undertaken.[27]

Farming conditions associated with risk for IHNV introduction to naïve populations include:[15]

- On-site fish processing, especially when receiving carcasses from outside farms. The risk increases over 15-fold when receiving live infected fish.
- Presence of wild fish populations or stocked fisheries with susceptible species within 5 kilometers/3.1 miles upstream.
- Receiving and storing fish waste from other farms, including mortalities and processing waste.
- Facility staff working on outside fish farms.
- Receiving eggs or stock untested for IHNV.
- Inadequate biosecurity programs for equipment and facilities.
- Receiving infected fish or egg stock.

Movement of killed fresh or frozen fish products are not considered to increase risk of IHNV introduction.

Summary

IHNV is a WOAH-listed viral disease affecting wild and farmed salmon, trout, and pike fish. It is an economically important pathogen causing clinical disease and mortalities in a wide variety of wild and farmed salmonid species, including Atlantic salmon and rainbow/steelhead trout.[15] There could be a potentially high economic impact to the United States Atlantic salmon farming industry if introduction of IHNV occurs in these populations of fish. However, in the United States, marine-based Atlantic salmon aquaculture occurs exclusively in Atlantic northeast coastal areas (e.g., Maine); therefore, the risk of natural exposure to IHNV is low. Exposure via transportation, importation, and introduction of IHNV-infected live fish or IHNV-contaminated eggs are the most likely routes of IHNV introduction for this farmed fish population. Atlantic salmon reared in inland aquaculture facilities that are not present in endemically affected regions of the United States would most likely be exposed via these routes as well. Atlantic salmon reared in inland aquaculture facilities present in endemically affected areas are at risk of exposure via these routes and may be at risk of water-borne exposure, if the influent water biosecurity measures of the aquaculture facilities are not adequate to prevent exposure. The potential economic impact to Atlantic salmon aquaculture could be high given the susceptibility of this species to IHNV.

Rainbow/steelhead trout are susceptible to IHNV infection and disease. IHNV is endemic in wild and farmed freshwater rainbow trout in Pacific Northwest watersheds that include Idaho.[54] Troyer (2002) reported that IHNV is endemic among numerous rainbow trout farms and hatcheries in the Hagerman valley region of Idaho.[54] Historical outbreaks of IHNV have resulted in serious economic losses to the Idaho trout industry.[54] There is limited data available reporting detections of IHNV in farmed steelhead trout. Breyta et al. (2017) reported that IHNV prevalence in wild steelhead trout populations in IHNV endemic regions of the United States can approach 26 percent in some areas.[55] Because steelhead trout are susceptible to IHNV infection and can develop clinical disease, it is likely that outbreaks of disease in farmed steelhead trout would be economically significant.

The United States Fish and Wildlife Service (USFWS) oversees importation of live and dead salmonid fish, eggs and gametes (Appendix, Table 1; and Appendix, Regulatory Information Associated with International Trade: The United States, Import Information).[56, 57] Requirements for importation are available in detail in the National Archives and Records Administration, Code of Federal Regulations (CFR), Title 50: Wildlife and Fisheries (Appendix, Table 1).[58] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[58] Shipments must be accompanied by a United States Title 50 Certification Form completed in the country of origin by a USFWS- certified aquatic animal health inspector. This form is valid for six months after date of issue and certifies that the fish stocks from which the shipments originated have been tested for infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), Oncorhynchus masou virus (OMV), and viral hemorrhagic septicemia virus (VHSV).[56] Eggs must be disinfected within 24 hours prior to shipment using specific protocols described in CFR, Title 50, and water used for shipping must be derived from pathogen-free water.[58] USDA APHIS does not have regulations or recommendations specific to IHNV and the international import or interstate movement of live salmonid fish, eggs, or gametes. There may be State and/or Tribal import regulation of live salmonid fish, eggs, or gametes relative to IHNV. However, such regulation is variable among States. IHNV is a WOAH-listed reportable pathogen. Therefore, detection of IHNV in cultured fish stocks in the United States would likely result in significant trade impacts.

Suggested best practices to prevent introduction of IHNV into Atlantic salmon and rainbow/steelhead trout aquaculture in the United States include development of policies that would ensure live fish, eggs, and gametes are sourced domestically and/or imported from IHNV-free sources, and that movement and importation complies with the guidelines described in the 2022 OIE Aquatic Animal Health Code.[4] It has been reported that disinfection of eggs has virtually eliminated transmission of IHNV from infected broodstock to offspring fish.[55] Cultured fish are therefore at greatest risk of IHNV exposure via virus present in the water supply when inappropriate biosecurity protocols are in place, or if there is a lapse or failure of influent water treatment methods.[55]

Limitations

In this hazard identification the characteristics and epidemiology of IHNV in susceptible hosts were summarized using available information collected from WOAH resources, subject matter experts, and available published peer-reviewed materials. Knowledge gaps and limitations identified during the data and literature review process included:

- Some factors associated with the epidemiology of IHNV are not known or fully described.
- The reservoir status of susceptible fish species is not fully elucidated.
- Factors associated with environmental persistence of the virus are not fully known.
- Capability of other aquatic organisms to function as vectors, reservoir or accidental hosts are not fully elucidated.
- Some factors associated with transmission (e.g., shedding rate, environmental conditions such as dilution, wind and current strength and direction) have not been determined.

Appendix

Tables

Table 1. Links to manuals, websites, and other resources relevant to infectious haematopoietic necrosis virus (IHNV)

Topic	Link
National Veterinary Service Laboratory National Animal Health Laboratories	USDA APHIS | Diagnostic Testing at the NVSL USDA APHIS | General NVSL Information
USDA APHIS National Animal Health Reporting System (NAHRS)	USDA APHIS | National Animal Health Reporting System (NAHRS)
USDA APHIS National List of Reportable Animal Diseases (NLRAD)	USDA APHIS | National List of Reportable Animal Diseases
USDA APHIS Veterinary Services and State Authorities	Federal and State Animal Health (usaha.org) USDA APHIS | Contact Veterinary Services
2017 OIE Report of the Meeting of the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases	a-ahg-susceptibility-of-fish-september-2019.pdf (woah.org)
World Organisation for Animal Health (WOAH) 2022 WOAH Aquatic Animal Health Code	Aquatic Code Online Access - WOAH - World Organisation for Animal Health
World Organisation for Animal Health (WOAH) 2022 WOAH Manual of Diagnostic Test for Aquatic Animals	Manual Online Access - WOAH - World Organisation for Animal Health
World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS) database	World Animal Health Information System WAHIS - WOAH - World Organisation for Animal Health
World Trade Organization, Sanitary and Phytosanitary Measures	WTO | WTO Agreements Series: Sanitary and Phytosanitary Measures
The United Nations Code of Conduct for Responsible Fisheries based upon UNCLOS and other international laws.	International Agricultural Law and Organizations Aquaculture Overview - National Agricultural Law Center (nationalaglawcenter.org)
FAO Aquaculture Regulatory Frameworks	AQUA-CULTURE REGULATORY FRAMEWORKS (fao.org)
United States Fish and Wildlife Importation Guidelines	Steps for Importing Salmonids into the United States of America | U.S. Fish & Wildlife Service (fws.gov) Information for Importers & Exporters | U.S. Fish & Wildlife Service (fws.gov) CFR-2016-title50-vol1.pdf (govinfo.gov)
USDA APHIS Import permit information	USDA APHIS | Fish, Fertilized Eggs, and Gametes
USDA APHIS International Regulations (IREGS) website	USDA APHIS | Animal and Animal Product Export Information)

Table 2. Countries for which USDA APHIS has a negotiated export health certificate to ship live salmonid fish, eggs, and gametes, and their requirements for testing for infectious haematopoietic necrosis virus (IHNV) (as of 2023)

Country	**Infectious haematopoietic necrosis virus (IHNV) Freedom Testing Required**
Argentina	Yes
Armenia	Yes
Austria	Yes
Belarus	Yes
Belgium	Yes
Bosnia-Herzegovina	No
Brazil	Yes
Bulgaria	Yes
Canada	Yes
Chile	Yes
China	No
Croatia	Yes
Cyprus	Yes
Czech Republic	Yes
Denmark	Yes
Estonia	Yes
Finland	Yes
France	Yes
Georgia	Yes
Germany	Yes
Greece	Yes

Hungary	Yes
Ireland, Republic of	Yes
Isle of Man	Yes
Israel	Yes
Italy	Yes
Kazakhstan	Yes
Kyrgyzstan	Yes
Latvia	Yes
Lithuania	Yes
Luxembourg	Yes
Malaysia	No
Malta	Yes
Mexico	Yes
Morocco	Yes
Netherlands	Yes
New Zealand	No
North Macedonia	Yes
Norway	Yes
Peru	Yes
Poland	Yes
Portugal	Yes
Romania	Yes
Russian Federation	Yes
Serbia	Yes
Singapore	No

Slovakia	Yes
Slovenia	Yes
South Africa	Yes
Spain	Yes
Sweden	Yes
Switzerland	Yes
Taiwan	Yes
Turkey	Yes
Turks and Caicos Islands	Yes
Ukraine	Yes
United Arab Emirates	Yes
United Kingdom/ Great Britain	Yes

Table 3. Countries that have reported presence of infectious haematopoietic necrosis virus (IHNV) in domestic and wild fish species per the World Organisation of Animal Health (WOAH) World Animal Health Information System (WAHIS) database (Appendix, Table 1) for years that data were available (2005–2023).

Country	**Year**	**Animal Population**	**Disease Status**
Austria	2007, 2009–2015, 2019, 2021, 2023	Domestic	Present
Belgium	2014–2015, 2023	Domestic	Present
Canada	2005–2007, 2012–2023	Domestic	Present or Suspected
	2005–2008, 2011–2023	Wild	Present
China	2005–2008	Domestic, Wild	Suspected
	2011–2023	Domestic	Present
Cote D'Ivoire	2013	Wild	Suspected
Croatia	2017–2020	Domestic	Present

Czech Republic	2006–2007, 2010–2011, 2014, 2019	Domestic	Present
Denmark	2021–2023	Domestic	Present
Estonia	2018–2021	Domestic	Present
Finland	2017–2018, 2021–2022	Domestic	Present
France	2005–2008, 2010–2011, 2014, 2016–2018, 2020–2023	Domestic	Present
	2005, 2010–2011	Wild	Present
Georgia	2023	Domestic	Present
Germany	2005–2023	Domestic	Present
Hungary	2013	Domestic	Present
Iran	2005–2007, 2015–2022	Domestic	Present
	2007	Wild	Suspected
Italy	2005–2008, 2010–2015, 2018–2023	Domestic	Present
Japan	2005–2008, 2014	Wild	Present
Kenya	2016–2017	Domestic	Present
South Korea	2005–2007	Domestic, Wild	Suspected
	2008–2009	Domestic	Present
Netherlands	2008–2009, 2011, 2016–2017, 2022–2023	Domestic	Present
	2016–2021	Wild	Present
North Macedonia	2018–2023	Domestic	Present
Poland	2008–2017, 2019, 2022	Domestic	Present
Russia	2009	Wild	Present
Slovenia	2005–2008, 2010–2011, 2013–2014, 2016–2022	Domestic	Present
Spain	2005	Domestic	Present
Switzerland	2012–2015, 2019, 2023	Domestic	Present

United States of America	2005–2008, 2010, 2011–2015, 2019–2023	Domestic	Present or Suspected
	2005–2019, 2020–2021, 2022–2023	Wild	Present or Suspected

Regulatory Information Associated with Salmonid Aquaculture

International Laws Regulating Seas and Fisheries

A comprehensive summary of all international laws regulating seas and fisheries is beyond the scope of this document. Briefly, the United Nations (UN) plays a significant role in the development of international laws. The 1982 United Nations Conference on the Law of the Sea (UNCLOS) sets offshore territorial boundaries that establish zones of exclusive economic and fisheries rights for coastal nations. This is the de facto set of guidelines for the world's oceans.[59] Some nations have not ratified this convention, resulting in different international laws among nations affecting aquaculture. The UN has also developed a Code of Conduct for Responsible Fisheries based upon UNCLOS and other international laws.[59, 60] The Food and Agriculture Organization of the United Nations (FAO) Legal papers Online: Aquaculture Regulatory Frameworks[61] also provides information summarizing significant issues related to the development and implementation of aquaculture regulatory frameworks.

Regulatory Information Associated with United States Salmonid Aquaculture

Marine and inland salmonid aquaculture systems are regulated by Federal, State, and when applicable local and Tribal governments.[62, 63] At the Federal level, "aquaculture" is defined in the National Aquaculture Act of 1980 as "the propagation and rearing of aquatic species in controlled or selected environments."[59, 64] This act calls for development of a National Aquaculture Development Plan identifying aquatic species that have significant potential for culturing on a commercial or other basis by the Secretary of Agriculture, Secretary of Commerce, and the Secretary of the Interior.[59, 65] The act also contains recommendation for aquaculture research and development, technical assistance, design and management of facilities, and coordination of national activities and resolution of legal and regulatory constrains affecting aquaculture.[59, 66, 67] The Joint Subcommittee on Aquaculture was created by enactment of the National Aquaculture Act and amended in 1985 with intention to increase effectiveness and productivity of Federal aquaculture research, transfer, and assistance programs.[59]

Federal agencies with aquaculture regulatory oversight include the Department of Health and Human Services (DHHS) Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the National Oceanic and Atmospheric Administration (NOAA), the United States Coast Guard (USCG), the United States Department of Agriculture (USDA) Animal Plant and Health Inspection Service (APHIS), the United States Army Corps of Engineers, the United States Department of the Interior (USDI) Bureau of Ocean Energy Management (BOEA), the United States Fish and Wildlife Service (USFWS).[59, 62, 63]

Marine farms must comply with regulations found in the Clean Water Act,[68] the Endangered Species Act,[69] the Fish and Wildlife Coordination Act,[70] the Magnuson-Stevens Fishery Conservation and Management Act,[71] the Marine Mammal Protection Act,[72] the National Environmental Policy Act,[73] the National Marine Sanctuaries Act.[59, 63, 74, 75] Federal agencies and regulations specific to inland aquaculture include many of the agencies described above, with exclusion of agencies specific to marine aquatic systems.

State and within State (county and local) governments regulate aquaculture activities that are permitted or licensed at the community level.[76] Generally, permits address building, community level marketing, processing and trade, fish disease testing and import, fish species certification relative to wildlife management, waste discharge, water use, and zoning.[59, 76, 77] Regulations are not uniform among States and can vary within State based on the geographic location of the aquaculture facility (coastal, inland, wetland, offshore), and associated local environmental impacts.[59, 76, 77] State agencies that provide regulatory oversite include, but may not be limited to, State Departments of Agriculture, Fish and Wildlife, and Natural Resources.[59, 78, 79]

Some States may require development of aquaculture-specific Best Management Practices designed to enhance farm biosecurity, production and minimize environmental impacts.[78, 80, 81] For example, Atlantic salmon farming operations Maine participate in the Global Aquaculture Alliance Best Aquaculture Practices program and the Maine Aquaculture Association Code of Practice, Bay Management and Biosecurity programs.[78, 82] As of October 2021, Maine also provides Aquaculture Operational Standards for Land-based Recirculating Aquaculture Systems (RAS).[78]

Regulatory Information Associated with International Trade

The Word Organisation for Animal Health

The World Organisation for Animal Health (WOAH) 2022 OIE Aquatic Animal Health Code describes international standards for protecting aquatic animal and public health.[4] Standards related to the establishment of restrictions designed to prevent introduction of animal health hazards by importing countries are included in these provisions. These standards are based on the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures.[83, 84] The SPS agreement outlines several provisions that Member countries must consider when establishing import restrictions. Members must determine the level of transmission risk, animal health measures, and biosecurity standards required to manage disease risks among live animals and animal products within the country. The level of protection deemed appropriate by a Member should be sufficient to protect human, animal, and/or plant health or life within its territory. Member countries must ensure that their sanitary and phytosanitary measures to not arbitrarily or unjustifiably discriminate between Members where identical or similar conditions prevail. Members cannot seek import restrictions that are not equivalent to those established domestically, or apply restrictions in a manner constituting a disguised restriction on international trade.[83, 84]

WOAH Import Information Specific to IHNV

WOAH import/export guidelines specific to IHNV are found in 2022 OIE Aquatic Animal Health Code.[4] Briefly,

1. When live aquatic animals or aquatic animal products are imported from a country, zone, or compartment declared free from IHNV infection, the Competent Authority of the importing country should require that the shipment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country.[4] The international aquatic animal health certificate should state that the place of production of the aquatic animal or aquatic animal products is located in a country, zone or compartment declared free from IHNV infection.[4]

2. When importing aquaculture or aquatic animals from a country, zone or compartment that is NOT free from IHNV infection, the Competent Authority of the importing country should assess the risk in accordance with the WOAH Aquatic Animal Health Code, Chapter 2.1., and consider the following risk mitigations:[4]
    a. For grow out and harvest of the imported aquatic species, there should be direct delivery and lifelong holding of the imported animals in a quarantine facility from which the animals do not leave unless they are first killed and processed. All transport water, equipment, effluent, and waste materials in this facility must be treated to inactivate IHNV in accordance with OIE Aquatic Animal Health Code, Chapters 4.4., 4.8., and 5.5.
    b. If the intention is establishment of new stock for aquaculture, the exporting country must identify potential source populations, evaluate their aquatic animal health records, test the source population(s) in accordance with the OIE Aquatic Animal Health Code, Chapter 1.4., and select a founder population (F-0) with a high health status for infection with EHVN. The importing country should import the F-0 population into a quarantine facility and determine the suitability of the population for broodstock by testing for IHNV in accordance with the OIE Aquatic Animal Health Code, Chapter 1.4. A first generation (F-1) population should be produced, cultured and tested in quarantine to establish/confirm freedom of IHNV as per the OIE Aquatic Health Code, Chapter 1.4., and the OIE Manual of Diagnostic Tests for Aquatic Animals (the Aquatic Manual), Chapter 23.1.[24] If IHNV is not detected, the F-1 population may be defined as free from IHNV infection and released from quarantine. If IHNV is detected the aquatic animals remain in quarantine until they can be killed and disposed per the OIE Aquatic Animal Health Code, Chapter 4.8.

The United States

National Import Information

The USFWS oversees importation of live and dead salmonid fish, eggs, and gametes (Appendix, Table 1).[56, 57] The United States Fish and Wildlife Service (USFWS) defines fish, including salmonids, as wildlife. This definition describes wildlife as "any wild animal, alive or dead, whether or not bred, hatched or born in captivity, and any part, product, egg, or offspring thereof."[57, 58] Per the Lacey Act of 1900, importation, and transportation of salmonid fish (live or dead), eggs, and gametes into the United States and its territories or possessions is injurious or potentially injurious to the welfare and survival of wildlife or wildlife resources of the United States, the health and welfare of human beings, and the interests of forestry, agriculture and horticulture.[56, 58, 85] These designations place importation and transportation of live salmonid fish, eggs, and gametes under the purview of USFWS which issues permits under wildlife laws and treaties at international, national, and regional levels.[86]

All live (or dead) uneviscerated fish, live fertilized eggs, or gametes of salmonid fish are prohibited entry into the United States for any purpose except by direct shipment. Imports must receive prior written approval from the USFWS Director. Requirements for importation are available in detail in the National Archives and Records Administration, Code of Federal Regulations (CFR), Title 50: Wildlife and Fisheries.[58] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[58] Shipments must be accompanied by a United States Title 50 Certification

Form completed in the country of origin by a USFWS-certified aquatic animal health inspector. This form is valid for six months after date of issue and certifies that the fish stocks from which the shipments originated have been tested for infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), Oncorhynchus masou virus (OMV), and Viral hemorrhagic septicemia virus (VHSV).[56] Eggs must be disinfected within 24 hours prior to shipment using specific protocols described in CFR, Title 50, and water used for shipping must be derived from pathogen-free water.[58]

Imported live salmonid fish, eggs, and gametes arriving at a designated port of entry must be cleared by a USFWS officer prior to department of Homeland Security (DHS) United States Customs and Border Protection (USCBP) clearance and release.[58, 85, 86] Upon release live fish, eggs, and gametes may be transported and possessed in captivity without a permit.[58] The live fish, eggs, and gametes may not be released into the wild except by a State wildlife conservation agency or persons with prior written permission from such agency.[58] In the absence of such documentation shipments are not released, and the fish, eggs, or gametes remain under detention subject to seizure and delivery to appropriate regional USFWS agents or directors for disposition as described in CFR, Title 50.[58, 85] Links to relevant information associated with USFWS regulations are found in Appendix, Table 1.

The United States Department of Agriculture (USDA) Animal Plant Health and Inspection Service (APHIS) requires import permits for live fish, eggs, and gametes from species susceptible to Spring viremia of carp virus (SVC) and Tilapia Lake virus (TiLV).[87] USDA APHIS does not have regulations or recommendations specific to IHNV and the international import or interstate movement of live salmonid fish, eggs, or gametes.

National Export Information

Exporters of fish designated as wildlife are required to obtain export permits from USFWS. Shipments must be declared and cleared by USFWS and USCBP at USFWS designated ports.[58, 85, 88, 89] Many countries of import require documentation of animal health by USDA APHIS. Country specific exportation requirements for Aquaculture/Aquatic Animals may be accessed on the USDA APHIS International Regulations (IREGS) website (Appendix, Table 1).[89, 90] Briefly, the United States has negotiated international export health certificates, completed by an accredited veterinarian and endorsed by a Veterinary Services area office, for shipments of live salmonid fish, eggs, and gametes.[90] Some countries for which USDA APHIS has negotiated an export health certificate applicable for shipment of live salmonid fish, eggs, or gametes require testing for IHNV prior to export from the United States (Appendix, Table 2).

State Import and Export Information

USFWS and USDA-APHIS do not have interstate regulations or recommendations specific to IHNV and the movement of live salmonid fish, eggs, or gametes. State, Tribal, and local governments may have importation regulations, including requirements for disease freedom testing; however, regulation and requirements among these entities may vary. Information may be accessed via individual State Departments of Agriculture, State Departments of Natural Resources (or similar agencies), or the State Veterinarian.[89, 91, 92]

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