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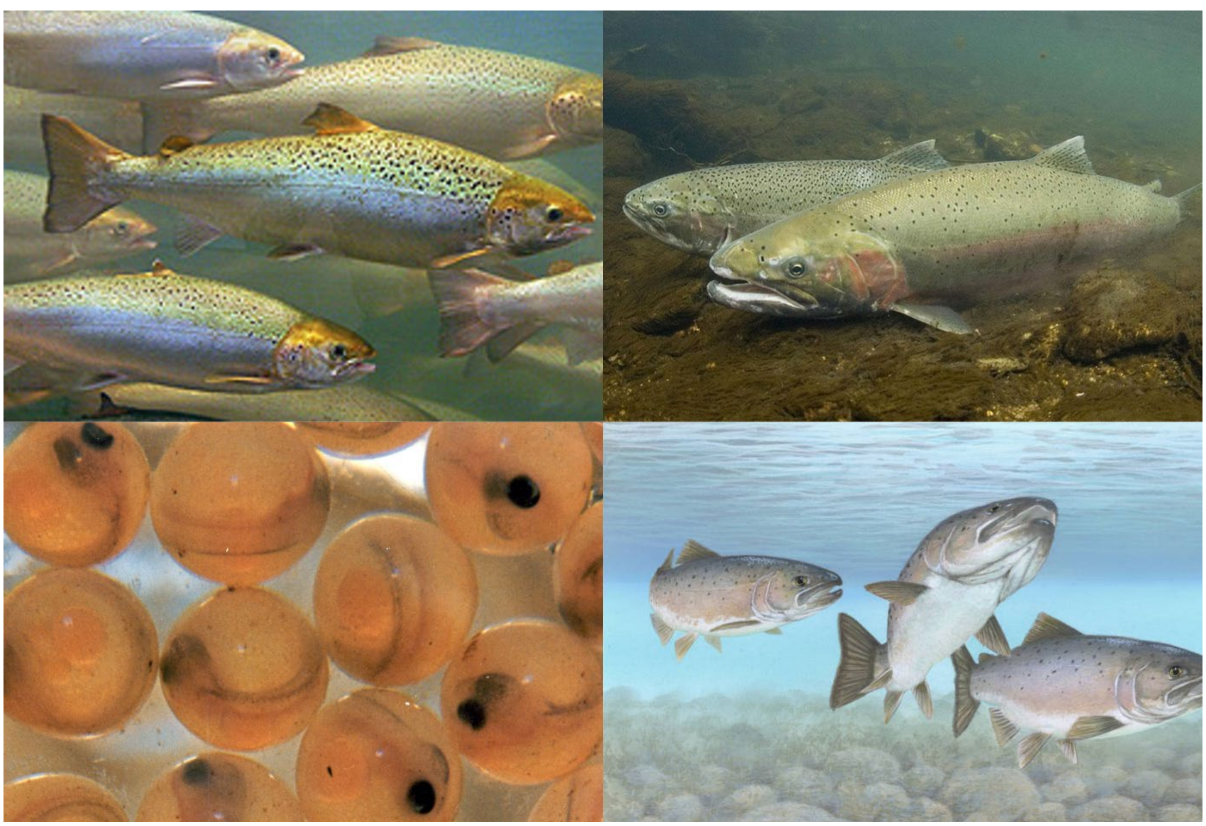
Hazard Identification:
Infectious Salmon Anemia Virus (ISAV)



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Introduction

USDA APHIS VS CEAH was asked to generate a Hazard Identification for infectious salmon anemia (ISA). 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; and 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 (ISA), 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 ISA epidemiology. Knowledge and data gaps were present that affected complete evaluation of some tenets of this hazard identification document.

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.[2]
- 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 OIE 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)[5, 6] 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.[7, 8, 9, 10]
  - 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.[11]

    - measures based on current epidemiological information and understanding of relevant knowledge and data gaps.[12, 13, 14]

Subjects that are not within the scope of this document include an assessment of potential entry and exposure pathways and summaries of likelihood, uncertainty, consequences and overall risk. This document is intended for internal USDA APHIS VS use and distribution to external stakeholders.

Key Findings

- Infectious salmon anemia (ISA) is a World Organisation for Animal Health listed notifiable disease.
- ISA is a reportable disease in the United States.
    - Confirmatory testing at the National Veterinary Services Laboratory is required following first detections.
- ISA is caused by infectious salmon anemia virus (ISAV) and has occurred in the United States.
- ISA is an economically important disease of farmed Atlantic salmon.
    - Natural disease outbreaks have only been observed in farmed Atlantic salmon.
    - Susceptibility to infection has been observed in other salmonid species.
    - Farmed Atlantic salmon infected with HPR0 genotypes do not develop clinical disease.
    - Farmed Atlantic salmon infected with HPRΔ genotypes may develop clinical disease or may be subclinically infected.
- There are no Federal regulations specific to ISA and the international import or interstate movement of live salmonid fish, eggs, or gametes. In general, eggs must be disinfected 24 hours prior to import
    - Maine and Washington have ISAV specific import and monitoring regulations in place.
    - Most countries require testing live salmonid fish, eggs, and gametes for ISA prior to export from the United States.
- Transmission routes and risks for inland Atlantic salmon aquaculture farms and hatcheries, are poorly described.
    - The presence of wild reservoir hosts (other than wild Atlantic salmon and brown trout) is unknown.
- Natural and anthropogenic (farming related) environmental factors influence development of ISAV infection and clinical disease.
- The time interval between infection and detection in the susceptible populations, and the rate at which outbreak response and control efforts are initiated may also affect the rate and risk of transmission.
- Cumulative morbidity and mortality rates vary dependent on environmental, host, and pathogen factors, the time at which detection and intervention occurs, and the duration of the outbreak.
- Stringent biosecurity measures can decrease the risk of ISAV introduction.

Infectious Salmon Anemia Virus

Introduction

Infectious salmon anemia (ISA) is an important disease of farmed Atlantic salmon caused by infectious salmon anemia virus (ISAV). ISAV infection is defined by the WOAH Manual of Diagnostic Test for Aquatic Animals (Appendix, Table 1) as infection with the pathogenic agent highly polymorphic region (HPRΔ, HPR-deleted) ISAV genotype or the non-pathogenic (HPR0, non-deleted HPR) ISAV genotype.[5]

ISAV (Family Orothomyxoviridae, genus *Isavirus*) is a 100–130 nm diameter, enveloped virus. The genome consists of eight single-stranded RNA genome segments.[5, 15, 16, 17] The nucleotide sequences of these eight genome segments encode approximately ten proteins.[18, 5] Segments 1, 2, and 4 encode viral polymerases PR2, PB1, and PA, respectively.[5] Segments 3, 8, 6, and 5 respectively encode four major structural proteins (68 kDa nucleoprotein, a 22 kDa matrix protein, 42 kDa haemagglutinin-esterase (HE) protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa surface glycoprotein fusion (F) protein).[19, 5]

Gene insertion, recombination, and reassortment with potential links to virulence have been identified throughout the evolution of the virus.[19, 20, 21, 5] In the Segment 5 F protein there are two distinctive molecular features a) either an insertion derived from other segments of the virus, or transpositions of sequences among the same segment, and b) a change in the primary sequence between two specific amino acids in position 266 (Q/L).[5, 19, 22] In the segment 6 HE gene, a highly polymorphic (HPR) region characterized by variations in sequence length is present.[23, 5] Segment 7 contains an open reading frame (ORF1) which encodes a protein with type 1 interferon antagonistic properties.[5] Segment 8 encodes a matrix protein and has an ORF2 that appears to encode a nuclear export protein (NEP) and a RNA-binding structural protein with type-1 interferon antagonistic properties.[5] The variability in these features appears correlated to the virulence potential of HPRΔ variants. HPR0 variants, by comparison, conserve the entire HPR epitope, consistently display Q in position 266 in segment 5, and lack the insertions or transpositions observed in HPRΔ variants.[22, 19, 24, 5]

Two distinct clades (the European (EU) clade and the North American (NA) clade) have been identified based on analysis of sequence data from ISAV segments 2, 5, 6, and 8.[21, 25, 17, 5] The EU clade is commonly detected in Europe and contains three geno-groups (EU-G1, EU-G2, and EU-G3) based on phylogenetic studies of virus surface glycoprotein gene sequences. The NA clade has not been similarly subdivided because it exhibits less variability.[26, 27, 25, 5] Within each clade, and the EU clade subgroups, multiple different HPR0 and HPRΔ genotype variants can be identified.[28, 29]

HPR0 variants have an intact genome, do not have any gaps in the HPR sequence, and are not associated with clinical disease. These variants have been identified in salmon production regions globally.[30, 31, 32, 33] To date, all ISAV variants associated with clinical disease (HPRΔ variants) contain gaps in the HPR sequence.[34, 5] These HPRΔ variants are hypothesized to arise via deletions from a full-length precursor gene.[34, 26] Emergence of HPRΔ variants from HPR0 variants has been hypothesized based on detections of HPR0 variants prior to, concurrent with, and subsequent to HPRΔ detection at affected sites and occasionally in single fish, and identification of phylogenetic and temporal relationships between HPR0 and HPRΔ variants at some affected locations.[35, 30, 36, 32, 33, 20, 26] However, these reports are rare, suggesting that this is likely a low but not negligible probability event.[37, 20, 38, 27] It has also been suggested that HPR0 variants might occasionally derive from attenuation or genetic insertions in HPRΔ variants.[22, 38, 39]

In North America, during the original ISA outbreaks in Canada (Brunswick) and the United States (Maine), the NA clade was commonly detected.[35, 26] Beginning in 1996, both EU and NA HPR0 and HPRΔ variants have been detected.[21, 35] Since 2006, EU clade genotypes have been become the most commonly detected ISAV variants.[5]

ISA is a reportable disease in the United States and is included on the USDA APHIS NAHRS and NLRAD lists of reportable diseases (Appendix, Table 1).[40, 41] All non-negative detections of any genotype (HPR0 and HPRΔ) or outbreaks of ISA must be reported to USDA APHIS VS and State authorities (Appendix, Table 1).[40] ISA is a WOAH listed notifiable disease.[26, 4] Disease notification requirements and requirements for self-declaration of freedom of ISAV infection for Member nations are found in the WOAH Aquatic Animal Health Code, Chapter 10.4 (Appendix, Table 1).[4] In Europe ISA is classified by the EU fish health directive as a Category C disease (a disease of relevance for which measures are needed to prevent it from spreading).[16, 34, 42] ISA is a reportable disease in Canada.[14, 43, 44]

Susceptible Fish Species

Fish species listed in the WOAH Manual of Diagnostic Tests for Aquatic Animals that meet the WOAH Aquatic Animal Health Code criteria for listing as susceptible to infection with ISAV are listed in Table 1.[5, 45] In the United States, marine-farmed Atlantic salmon and steelhead trout represent the species of greatest economic concern relative to infection with ISAV.

Table 1. Fish species identified by World Organisation for Animal Health (WOAH) as susceptible to infection with ISAV HPR0 and HPRΔ variants.[5, 45]

Genus species	**Common Name**
Oncorhynchus mykiss	Rainbow trout, Steelhead trout
Salmo salar	Atlantic salmon
Salmo trutta	Brown trout, Sea trout

Geographic Distribution

ISA was first identified in Norway in the mid-1980s. Search of the WOAH WAHIS database (Appendix, Table 1) for years that data were available (2007–2021) identified reports of ISA (HPR0, HPRΔ, or both variants) in Canada, Chile, the Faroe Islands, Iceland, Norway, the United Kingdom (Scotland), and the United States (Maine only).[14, 46] In North America, ISA occurs on the eastern coast of Canada (Labrador, New Brunswick, Nova Scotia, Prince Edward Island) and on the northeast coast of the United States (Maine).[14, 46] In Maine, the last ISA disease detection of HPRΔ ISAV occurred in 2006.[26] Subsequently, localized HPRΔ detections responsive to control measures have been occasionally reported in Canada and Maine.[14, 21] HPR0 variants have also been periodically detected at marine and freshwater sites in Maine and Maritime Canada.[26, 46]

Public Health

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

Epidemiology

In this section, the epidemiology of ISA in the natural host species (e.g., Atlantic salmon) is summarized. Some factors associated with the epidemiology of ISA are not fully described.

Host Characteristics

In naturally susceptible species, all life stages from yolk-sac fry to marine stage fish are susceptible to ISAV infection.[4, 17, 18] However, disease outbreaks are primarily reported only in Atlantic salmon marine life stages. Susceptibility is likely affected by host factors such as age, immune status, nutritional status, overall health, reproductive status, vaccination status, and factors that contribute to stress (e.g., fish handling and sorting, population density, splitting or movement of sea pens).[5, 18, 26, 47, 48] Persistent infection in individual fish has not been confirmed.[46] Anecdotally, differences in ISAV susceptibility between individual fish and among farmed Atlantic salmon family groups have been reported in the literature.[16, 46, 48]

Information describing ISAV infection in wild reservoir hosts is incomplete. Maintenance and transmission of ISAV (HPR0 and HPRΔ) among wild Atlantic salmon is likely; however, virus prevalence, persistence, and transmission characteristics are unknown.[49] It has been suggested that detections of ISAV (HPR0 and HPRΔ) and ISA outbreaks in farmed Atlantic salmon are associated with the migration patterns of wild Atlantic salmon.[49] A limited number of published studies have reported detections of ISAV via RT-PCR in wild sea trout (brown trout, *Salmo trutta*) and farmed steelhead trout.[50, 51, 52, 53, 54] Under experimental conditions, ISAV has been detected by PCR in salmon lice (*Lepeophtheirus salmonis*) and sea lice (*Caligus rogercresseyi*), however, the capability of these species to serve as reservoir hosts or transmission vectors has not been determined.[17, 48]

Environmental Characteristics

Environmental factors (e.g., presence of organic material, salinity, temperature, ultraviolet radiation) appear to influence virus persistence in the environment and host, and development of ISAV infection and clinical disease.[46] ISAV has been detected by RT-PCR in seawater sampled at farming sites where ISAV-positive Atlantic salmon are present.[17, 26] Virus detections and disease outbreaks appear seasonally associated with cold water temperatures ranging from 10–15 °C/50–59 °F.[17, 46, 48] The concomitant presence of other pathogens or parasites, concentration of organic materials suspended in the water column, water currents, host population dynamics (e.g., the length of time that fish have been in seawater, stocking density), and intensity and duration of natural ultraviolet (UV) radiation may contribute to disease occurrence.[17, 25, 26, 46, 47, 48] HPR0 and HPRΔ variants have been detected in freshwater hatcheries, broodfish farms, and smolt farms utilizing flow-through and RAS water handling technologies.[25, 55] In a study by Christiansen et al. (2021), inland farms with the most frequent detections were often using freshwater mixed with low concentrations of seawater, which suggests a transmission pathway and/or that water salinity may influence host susceptibility or virus infectivity.[25]

Pathogen Characteristics

ISAV is pleomorphic (capable of altering morphology, biological functions, replication modes, and virulence in response to environmental conditions). Factors related to virus infectivity, persistence, and viability in natural hosts and environments are not completely understood. HPR0 variants exhibit tissue tropism for gill epithelial cells. HPR0 variants and genomic material are detected seasonally and transiently in apparently healthy wild and marine-farmed Atlantic salmon globally, including in the United States (Maine) and Canada.[25, 26, 30, 31, 32, 36, 46] The prevalence is typically not homogenous, ranging in some field studies from individual fish to 100 percent in some populations.[17, 19, 23, 48] In farmed salmon, the rate of HPR0 detection is greater than that of HPRΔ.[18, 37, 46, 56] There is no direct evidence linking the presence of HPR0 variants or detections of HPR0 genomic material to pathological signs or clinical outbreaks of ISA.[14, 25, 31, 37, 46] HPRΔ variants are associated with the occurrence of clinical disease in Atlantic salmon.[46, 57] The primary route of infection is most likely gill epithelium; however, exposure via the skin and intestine is also suggested.[46] HPRΔ variants target the endothelial cells of blood vessels in all tissues and organs, leukocytes, macrophages, and red blood cells.[14, 46] Because endothelial cells are the primary target cells, HPRΔ replication can occur in any organ.[18, 22, 46] Detections of both HPR0 and HPRΔ variants in marine-reared Atlantic salmon and in individual fish have been reported.[18, 20, 37, 46, 56] This has been hypothesized by some authors as an emergence link between non-pathogenic HRP0 and pathogenic HPRΔ genotypes.[46, 48, 56, 57]

Optimal ISAV replication temperatures in cell culture range from 10 –15 °C/50 – 59 °F.[5, 48] According to the literature, replication ceases at 25 °C/77 °F, and inactivation occurs when temperatures reach 56 °C/133 °F for 30 minutes.[5, 17, 26, 48] Experimentally, infectivity has been retained in ISAV recovered from whole fish frozen for several years at -20 °C/-4 °F, in tissue homogenates stored for six months at -80 °C/-112 °F, and in suspensions held at 4 °C/39 °F and 15 °C/59 °F for 14 days and 10 days, respectively.[5, 58] ISAV is sensitive to UV irradiation, ozonation, and to pH less than 5.7 or greater than 9.0.[5]

Transmission

The transmission dynamics associated with ISAV are not fully described. ISAV is introduced into the water column via the blood, feces, mucus, skin, urine, or carcasses of infected fish.[5, 26, 48, 59, 60] Infected wild Atlantic salmon are considered a likely source of exposure for farmed Atlantic salmon (and vice versa).[14, 17, 26, 48, 49, 53, 60] Infected farmed salmon also serve as sources of virus for other farmed salmon. Release of raw or improperly treated blood, offal, and production wastes from salmon harvest operations and processing plants have been implicated as sources of exposure as well.[46, 59, 61]

The primary route of transmission is horizontal.[5, 17, 25, 48, 59, 62] Direct horizontal transmission occurs via close contact between infected and susceptible fish.[25, 52, 63, 64] Indirect horizontal transmission occurs via movement of ISAV in the water column.[14, 17, 25, 65, 66] Indirect transmission prior to or during movement or transport of infected live fish, eggs, or gametes has been reported.[14, 46, 67] Vertical transmission has not been confirmed; however, eggs and gametes may be horizontally infected during collection, preparation for transport, and by exposure to contaminated water.[46, 59]

Transmission via vectors, such as salmon lice and sea lice, has been suggested but not definitively proven.[5, 26, 68, 69] Other potential vectors have not been identified. Transmission may be associated with some Atlantic salmon farming practices (i.e., carrying over or stocking multiple year classes of fish at one site or within a hydrologically connected region), certain handling and harvesting methods, and fomites (e.g., shared divers, employees, equipment, boats).[14, 17, 26, 48, 70] Aerosol transmission has been proposed by some authors as a plausible transmission pathway, and is an area that requires further research.

In marine aquaculture settings, transmission rates and risks increase when sources of the virus are hydrologically and/or spatiotemporally proximate to susceptible Atlantic salmon populations.[47, 65, 71, 72] The time interval between infection and detection in the susceptible populations, and the rate at which outbreak response and control efforts are initiated may also affect the rate and risk of transmission. Transmission routes and risks for inland Atlantic salmon aquaculture farms and hatcheries, are poorly described. Inland farms that utilize flow-through water methodologies may be at risk via water-borne transmission. Repeated ISAV detections in inland farms operating with RAS technology may indicate a) direct transmission between fish in the farm, or b) transmission from an unidentified nidus of ISAV in the RAS environment (e.g., biofilms, sediments).[25, 73, 74, 75] Introductions via aerosols and sea spray has also been suggested.[25, 76, 77] Transmission routes and risks for inland salmonid aquaculture represents an area requiring further investigation.

Per the WOAH Manual of Diagnostic Tests for Aquatic Animals, vertical transmission has not been confirmed, but cannot be excluded as a potential transmission pathway.[17, 25, 46] Over the last 40 years, a small number of published reports have described RT-PCR detection of HPR0 or HPRΔ sequences in broodfish, fertilized and unfertilized eggs, ovarian fluid, and smolts.[25, 27, 59, 78, 79] Infectivity was not confirmed in those reports because the HPR0 could not be grown in cell culture or because assays to confirm infectivity of the detected HPRΔ variants were not performed. Marshall et al. (2014) reported detection of a HPRΔ variant via quantitative RT-PCR (qRT-PCR) and cell culture in ovarian fluid and eggs collected from two apparently healthy broodfish submitted for routine ISAV surveillance.[59] In a separate study, Christiansen et al. (2021) described detection of HPR0 in the ovarian fluid of 12 percent of HPR0 infected farmed broodfish.[25] However, the author stated that cross-contamination during collection could not be ruled out as a source of virus in the ovarian fluid. In the same study, HPR0 variants were detected in broodstock and smolts housed at different inland fish farms; however, phylogenetic and statistical analyses did not identify genetic links between those variants.[25] According to the authors, horizontal or aerosol transmission were the most likely routes of ISAV introduction into the inland fish farms involved in the study. Other published studies have not been successful in repeating or confirming these findings.[17, 25, 70] This represents an epidemiological transmission pathway that requires continued investigation.

Clinical Signs and Pathogenicity

Exposure pathways for HPRΔ ISAV are thought to include gill tissue, skin, and oral ingestion.[14, 17, 26, 80] Atlantic salmon infected with HPR0 variants develop a transient infection in gill epithelium, but do not develop clinical disease.[5, 31, 62] Atlantic salmon infected with HPRΔ variants do develop clinical disease, which is typically observed during marine life stages.[14, 15, 46] Subclinical HPRΔ infections can occur, and may be accompanied by anemia and circulatory disturbances in some fish.[14, 15]

Clinical signs include abnormal behaviors such as lethargy and swimming close to the water surface or the sides of the sea pen, anorexia, blood in the anterior chamber of the eye, darkened skin, distended abdomen, exophthalmia (popeye), jaundice on the ventral portion of the body, lethargy, pale gills, petechial (pinpoint) hemorrhages on skin, organs, and tissues, scale pocket edema, and yellow to blood-tinged ascites (fluid in the abdomen).[14, 16, 17, 48] Clinical signs during the final stages of the disease are attributed to severe anemia (hematocrit less than 10) and circulatory collapse.[15, 18, 26] The severity of clinical signs are dependent on the HPRΔ variant and the infective dose, environmental and host factors, and time from infection to detection, and from detection to initiation of outbreak responses.[26, 47, 65, 68] Differential diagnoses include other causes of anemia and hemorrhages, including winter ulcer (*Moritella viscosa*) and bacterial septicemias.[48] The onset of clinical ISA can occur over several months in some net-pens and is influenced by host factors (the length of time the fish have been in saltwater, fish density, immune status, nutritional status, vaccination status), environmental factors (water quality and temperature, presence of sea lice), factors associated with farm management (coordination of production activities, hydrographic delineation of management areas, rigorous biosecurity, single year-class stocking of sites, synchronized fallowing within management areas), and disease detection and response (surveillance, speed of infected net-pen removal).[65, 81, 82, 83, 84, 85]

Morbidity, Mortality, and Prevalence

Morbidity and mortality rates vary by location among sea pens, farms, and season (higher rates in early summer and winter).[15, 25, 46] The disease course can be prolonged, occurring over months. Daily morbidity and mortality rates are typically low (0.5%–1%).[15, 46] Cumulative mortality rates vary (1%–90%) dependent on environmental, host, and pathogen factors, the time at which detection and intervention occurs, and the duration of the outbreak.[17, 26, 46, 62]

Treatment

There are no treatments for this disease. Preventative vaccines have been used in many countries including Canada and the United States; however, vaccine efficacy is variable and does not provide complete protection from infection with HPR0 or HPRΔ variants.[16, 45, 48] There are currently no formal ISAV resistance breeding programs; however, differences in susceptibility among different Atlantic salmon family groups have been anecdotally reported.

Diagnostic Testing

Gross pathological lesions include yellow to blood-tinged (serosanguinous) ascites, enlargement and swelling of the spleen (splenomegaly), fibrin deposition on the surface of the liver, hemorrhagic lesions in the gastrointestinal tract, petechial hemorrhages in skeletal muscle, the swim bladder, internal organs and other tissues, swim bladder edema, and swelling and congestion of the kidney (renomegaly) with fluid effusing from cut surfaces.[17, 45, 48, 80] Histopathological findings include erythrophagocytosis in the spleen, filamental sinus congestion in the gills, focal, multifocal, or confluent hemorrhagic congestion and necrosis in the blood vessels, heart, liver, spleen and other internal organs, interstitial renal hemorrhage and tubular necrosis, and pathological changes consistent with anemia and circulatory collapse.[17, 48] Significant clinical pathology includes anemia ranging from 2%–10% or greater. Serum biochemistry changes indicate hepatic and renal compromise.[17, 48, 80]

Diagnostic tests include RT-PCR (conventional gel-based and real-time), immunofluorescence antibody test (IFAT), and virus isolation (VI) in cell culture (applicable only to HPRΔ variants, except for a single report, HPR0 variants have not be isolatable in cell culture), and VI via genomic sequence analyses.[5, 45, 57, 86] Genotyping and genogrouping of the isolated variants are important for phylogenetic tracing which may help identify the source and geographic distribution of the identified variant.[5, 26, 64]

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, Chapter 2.3.1. and the WOAH Aquatic Animal Health Code, Chapters 1.4. and 10.4.[5, 45] In the United States, confirmatory testing at the USDA APHIS NVSL is required following first detections. Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL (Appendix, Table 1).[87]

Prevention and Control

Stringent biosecurity measures can decrease the risk of ISAV introduction via importation of live salmonid fish, eggs, and gametes, transmission from wild Atlantic salmon to farmed salmon (and vice versa), between sea pens in marine settings, and among farms.[11, 26, 13, 17] Import biosecurity measures utilized by many countries include pre-import certification of live fish, eggs, and gametes, or their source for ISAV freedom. Currently, there are no USDA APHIS or USFWS international import regulations specific to ISA. However, ISAV is cultivable in the cell lines that are utilized in other required USFWS import health certifications and would likely be detected. The USFWS does require that salmonid eggs be disinfected prior to importation to the

United States. It is within the purview of USFWS to decline an importation request for live salmonid fish, eggs, and gametes based on assessments of risk for a disease not listed in Title 50 on a case-by-case basis.[88] In the United States, Maine has implemented broodstock testing and egg disinfection recommendations for ISAV prevention and control (*see* WOAH Aquatic Animal Health Code, Chapter 4.4).[5, 45, 48] Other States, Tribes and local entities may have regulatory requirements relative to ISAV and the inter- and intra-state movement of salmonid fish, eggs, and gametes (Appendix, Table 1).

Federal and State ISA biosecurity requirements for Atlantic salmon farming in Maine are summarized in the USDA APHIS VS Infectious Salmon Anemia Virus Control Program Standards. Washington employs measures for ISAV which are available via the Washington Department of Fish and Wildlife (WDFW) website (Appendix, Table 1). Farm level biosecurity measures are important for ISAV detection, control, management, and prevention. Basic measures should include:[17, 26, 48]

- acquisition of live fish, eggs, and gametes from sources tested free of ISAV,
- disinfection of eggs,
- quarantine of incoming fish, eggs, and gametes,
- avoid transferring live fish, eggs, and gametes between sites,
- farm only one age group of fish at a time,
- implementation of a passive surveillance plan,
- utilization of an all-in-all-out farming strategy,
- synchronized fallowing sites between production cycles,
- utilization of bay management areas,
- prompt removal of sick and dead fish,
- keep equipment clean and disinfected,
- do not share employees, including divers, between sites or farms,
- do not share equipment between sites or farms,
- control access, including boat traffic, to sites and farms, and
- coordination of biosecurity measures among sites and farms.

Disinfectants with ISAV efficacy include formaldehyde (1.0% for 16 hours), formic acid (for 24 hours), iodophor (100ppm for 10 minutes or 250ppm for a few seconds), potassium peroxymonosulfate (Virkon® S, 2% solution for 10 minutes) sodium hydroxide (for 7 hours), and sodium hypochlorite (100mg/mL free chlorine for 15 minutes).[5, 26] In cell culture, the virus is inactivated when exposed to temperatures equal to or greater than 56 °C/133 °F for 30 minutes, and pH 4 and pH 12 for 24 hours.[5] In seawater, the virus is susceptible to ozonation (8 mg/mL for 3 minutes, corresponding to a 600–750 redox potential).[5, 26, 48] Experimentally, a 3-log reduction in infectivity of ISAV suspended in sterile fresh water and seawater occurred following treatment with ultraviolet irradiation (UVC) at doses of 35 J/M^2 and 50 J/M^2, respectively.[5, 26, 48]

Prevention and control measures should include implementation of risk-based surveillance plans for susceptible farmed and wild Atlantic salmon populations. WOAH recommends that Members consider use of passive surveillance strategies to identify zones free from infection to facilitate the trade of live fish.[45] Extension of surveillance to other WOAH identified susceptible species indigenous to North America (i.e., rainbow trout) may be warranted considering the development of inland Atlantic salmon farming operations. All suspected ISAV detections or outbreaks of ISA are reportable to USDA APHIS VS as the Federal competent authority for animal health.[25] In the event of an outbreak, USDA APHIS may enact control measures humane sanitary depopulation of infected fish, movement controls, and quarantine on ISA affected, suspected, and neighboring farms.[17, 25, 48, 64] Specific regulatory measures for sanitary slaughtering, and disinfection of offal and wastewater from fish slaughterhouses and processing plants may also contribute to reduced risk of disease introduction.[11, 13, 45] Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of ISAV introduction. A summary of WOAH import/export guidelines specific to ISAV, U.S. regulations and other regulatory information related to aquaculture in the United States is summarized in the Appendix.

Summary

ISA is an economically important disease of farmed Atlantic salmon, caused by ISAV. Natural outbreaks have only been observed in farmed Atlantic salmon; however, susceptibility to infection has been observed in other salmonid species. Detections of HPR0 and HPRΔ variants in Atlantic salmon farmed in Maine do occur. Salmon farms in Maine are required to follow Federal and State ISA biosecurity requirements which are summarized in the USDA APHIS VS Infectious Salmon Anemia Virus Control Program Standards. Atlantic salmon farms in Washington are required to comply with preventative regulations enforced by the WDFW.

In the United States, there are no Federal regulations specific to ISAV and the import of live salmonid fish, eggs, or gametes. However, the USFWS does require disinfection of salmonid eggs prior to import. Information describing State (other than Maine and Washington) or Tribal regulation of live salmonid fish, eggs, or gametes relative to ISAV is available via links in Appendix, Table 1.

Appendix

Tables

Table 1. Links to manuals, websites, and other resources relevant to the ENV and other resource materials associated with aquaculture and aquatic animal diseases.

Topic	Link
Guide to State and Tribal aquaculture regulations	USDA APHIS | Interactive Maps
National Veterinary Service Laboratory National Animal Health Laboratories	USDA APHIS | Diagnostic Testing at the NVSL USDA APHIS | General NVSL Information USDA APHIS | Laboratory Information and Services
USDA APHIS Comprehensive Aquaculture Health Program Standards (CAHPS)	USDA APHIS | Comprehensive Aquaculture Health Program Standards
USDA APHIS National Animal Health Reporting System (NAHRS)	USDA APHIS | National Animal Health Reporting System (NAHRS)
USDA APHIS National Aquaculture Health Plan & Standards (NAHP&S): 2021–2023	USDA APHIS | National Aquaculture Health Plan & Standards (NAHP&S): 2021–2023
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) Aquatic Animal Health Code	Aquatic Code Online Access - WOAH - World Organisation for Animal Health
World Organisation for Animal Health (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 National Fish Health Survey Mapper	National Wild Fish Health Survey Mapper | U.S. Fish & Wildlife Service (fws.gov)
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) Import/Export Requirements for Aquaculture Products (fdacs.gov)

Table 2: Countries for which USDA APHIS has a negotiated export health certificate that can be used to ship live salmonid fish, eggs, and gametes, and their requirements for testing for infectious salmon anemia (as of 2023)

Country	**Pre-export testing for Infectious Salmon Anemia required**
Argentina	Yes
Armenia	Yes
Austria	Yes
Belarus	Yes
Belgium	Yes
Bosnia-Herzegovina	Yes
Brazil	Yes
Bulgaria	Yes
Canada	Yes
Chile	Yes
China	Yes
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	No
United Arab Emirates	Yes
United Kingdom/ Great Britain	Yes

WOAH Import/Export Recommendations For ISA

The World Organisation for Animal Health (WOAH) *2022 OIE Aquatic Animal Health Code* describes international standards for protecting aquatic animal and public health.(WOAH 2022) 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 (Appendix, Table 1).[89, 90] 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.[89, 90]

WOAH import/export guidelines specific to ISA vary depending on the status of the exporting country, zone, or compartment, and are found in *2022 OIE Aquatic Animal Health Code.*[91] Briefly, when:

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with ISAV (either HPR0 and/or HPRΔ variants).
    a. The importing country Competent Authority should require that the consignment be accompanied by an international aquatic animal health certificate issued by the exporting country Competent Authority. The international aquatic animal health certificate should state that, on the basis of the procedures described in *2022 OIE Aquatic Animal Health Code, Chapter 10.4, Articles 10.4.5., 10.4.7., or 10.4.9.* and *10.4.11*., the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with ISAV.

2. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from HPRΔ variant ISAV, but not necessarily free from infection with HPR0 variant ISAV.
    a. The importing country Competent Authority should require that the consignment be accompanied by an international aquatic animal health certificate issued by the exporting country Competent Authority. The international aquatic animal health certificate should state that, on the basis of the procedures described in *2022 OIE Aquatic Animal Health Code, Chapter 10.4, Articles 10.4.6., 10.4.8., or 10.4.10.* and *10.4.12*., the place of production of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with HPRΔ ISAV.

3. Importing aquatic animals or aquatic animal products from a country, zone or compartment that is NOT declared free from ISAV infection (either HPR0 and/or HPRΔ variants). The importing country Competent Authority should assess the risk as described in the *2022 OIE Aquatic Animal Health Code, Chapter 2.1* and consider the following risk mitigation measures:
    a. If the intention is to grow out and harvest the imported aquatic animals the aquatic animals should be delivered directly to a quarantine facility and held there throughout the animals' lifespan. The animals may not leave this quarantine facility or be transported to another quarantine facility unless they are first killed and processed onto one or more of the aquatic animal products described in *2022 OIE Aquatic*

Animal Health Code, Chapter 10, Article 10.4.3, or products authorized by the Competent Authority. All transport water, effluents and waste materials must be treated to inactivate ISAV in accordance with *2022 OIE Aquatic Animal Health Code, Chapters 4.4, 4.8*, and *5.5.*

b. If the intention is to establish a new stock for aquaculture, the exporting country should identify source populations, evaluate their aquatic animal health records, test the identified source populations for ISAV in accordance with *2022 OIE Aquatic Animal Health Code, Chapter 1.4*, and select a foundation population (F_0) of animals with a high health status for infection with ISAV. The importing country should import the F_0 population to a quarantine facility and test for ISAV as described in *2022 OIE Aquatic Animal Health Code, Chapter 1.4* to determine the suitability of the population for broodstock. A first generation (F_1) population should be produced in quarantine, cultured under conditions conducive for clinical expression of ISAV infection, and sampled and tested for ISAV as described in *2022 OIE Aquatic Animal Health Code, Chapter 1.4,* and *2022 OIE Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.5.* If ISAV is not detected in the F_1 population, it may be defined as free from ISAV infection and released from quarantine. If ISAV is detected, the F_1 population should not be released from quarantine, and should be killed and disposed of in a biosecure manner as described in *2022 OIE Manual of Diagnostic Tests for Aquatic Animals, Chapter 4.8.*

4. Importing disinfected eggs for aquaculture from a country, zone, or compartment that is NOT declared free from ISAV infection (either HPR0 and/or HPRΔ variants).
   a. Prior to importation, the importing country Competent Authority should assess at minimum, the prevalence of ISAV infection in the broodstock (including evaluation of test results on milt (seminal fluid) and ovarian fluid), and the likelihood that the water used to disinfect the eggs may be contaminated with ISAV. If it is determined that importation is acceptable, the importing country Competent Authority should mitigate risk of ISAV introduction by requesting that the eggs be disinfected prior to importing in accordance with *2022 OIE Aquatic Animal Health Code, Chapter 4.5* recommendations, and that the eggs do not contact anything that may impact their health status in the interval between disinfection and importation. The importing country Competent Authority should require that the consignment of eggs be accompanied by an international aquatic animal health certificate issued by the exporting country Competent Authority certifying that the risk mitigation procedures were conducted. The importing country Competent Authority should consider internal measures such as additional disinfection of the eggs upon arrival in the importing country.

United States Import/Export Recommendations For ISA

Import Information

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."[92, 93] 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.[92, 93, 94] 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.[93]

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.[92] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[92] 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), Viral hemorrhagic septicemia virus (VHSV).[93] Eggs must be disinfected within 24 hours prior to shipment using specific protocols described in CFR, Title 50.[92] Water used for shipping must be derived from pathogen-free water.[92]

The USFWS does not require testing of imported live salmonids, eggs or gametes for ISAV. 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.[92, 93, 94] Upon release live fish, eggs, and gametes may be transported and possessed in captivity without a permit.[92] 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.[92] 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.[92, 94] Links to relevant information associated with USFWS regulations are found in the 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).[95] USDA APHIS does not have regulations or recommendations specific to ISAV and the international import or interstate movement of live salmonid fish, eggs, or gametes.

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.[92, 93, 94, 96, 97] 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).[97, 91] 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.[91] 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 ISAV 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 ISAV 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.[97, 98]

The state of Maine has import and monitoring regulations in place relative to ISA, and utilizes the USDA APHIS VS *Infectious Salmon Anemia Virus Control Program Standards* (the *Standards*) as guidance for producers, APHIS-accredited veterinarians, fish heath and laboratory personnel, and regulators to prevent and contain ISA in farm-raised Atlantic Salmon (Appendix, Table 1).[26] The Standards were developed in response to an ISA outbreak that in Maine in 2001, and include recommendations for administrative procedures, biosecurity and quarantine, depopulation, disease control, investigation, reporting, and surveillance, fish health, indemnity, laboratory testing, management and communication coordination among marine farming operations, notification guidance for laboratory and VS personnel, and risk identification and mitigation.[26, 48]

In Washington State, the Washington State Department of Natural Resources (WDNR), regulates interstate importation of live salmonid fish, eggs, and gametes.[99] Regulations include required testing of live fish, eggs, and gametes for ISAV and other specific finfish pathogens at hatcheries and on aquatic farms. In 2011, genetic material suggestive of ISAV was detected by reverse transcription polymerase chain reaction (RT-PCR) assay in two wild juvenile Sockeye salmon (*Oncorhynchus nerka*) collected from marine waters in British Columbia, Canada.[100] However, confirmatory testing in concordance with WOAH guidelines was not performed.[64] Subsequent extensive and systematic surveillance of wild and farmed salmon in Washington State and British Columbia did not result in detection of ISAV.[64, 101] In Washington, Maine, and other states, individual Atlantic salmon producers may utilize Best Management Practices (BMP) and/or international certification protocols that employ ISA management measures that align with the *Standards*.[26]

References

1. WOAH. (2022). "World Organisation for Animal Health Terrestrial Animal Health Code. World Organisation for Animal Health." from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=sommaire.htm

2. Bruckner, G., S. MacDiarmid, N. Murray, F. Berthe, C. Muller-Graf, K. Sugiura, C. Zepeda, S. Kahn, and G. Mylrea. (2010). "Handbook on import risk analysis for animals and animal products." Office International des Epizooties, Paris.

3. WOAH. (2017). "OIE Report of the Meeting of the OIE ad hoc Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases. World Organisation for Animal Health." from https://www.woah.org/app/uploads/2021/10/a-ahg-susceptibility-of-fish-april-2017.pdf.

4. WOAH. (2022). Aquatic Animal Health Code, Chapter 10.4, Infection with Infectious Salmon Anemia Virus, World Organisation for Animal Health.

5. WOAH. (2022). "OIE Manual of Diagnostic Tests for Aquatic Animals 2022. World Organisation for Animal Health." from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/

6. Hedrick, R. (1998). "Relationships of the host, pathogen, and environment: implications for diseases of cultured and wild fish populations." *Journal of Aquatic Animal Health* 10(2): 107–111.

7. Jiménez-Valverde, A., A. T. Peterson, J. Soberón, J. Overton, P. Aragón, and J. M. Lobo. (2011). "Use of niche models in invasive species risk assessments." *Biological invasions* 13(12): 2785–2797.

8. Engering, A., L. Hogerwerf and J. Slingenbergh. (2013). "Pathogen–host–environment interplay and disease emergence." *Emerging microbes & infections* 2(1): 1–7.

9. James, T. Y., L. F. Toledo, D. Rödder, D. da Silva Leite, A. M. Belasen, C. M. Betancourt-Román, T. S. Jenkinson, C. Soto-Azat, C. Lambertini, and A. V. Longo. (2015). "Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research." *Ecology and Evolution* 5(18): 4079–4097.

10. Hovingh. (2017). "Biosecurity Fundamentals." from https://extension.psu.edu/biosecurity-fundamentals#:~:text=What%20is%20biosecurity%3F,to%20other%20herds%20or%20flocks.

11. Oidtmann, B. C., E. J. Peeler, M. A. Thrush, A. R. Cameron, R. A. Reese, F. M. Pearce, P. Dunn, T. M. Lyngstad, S. Tavornpanich, and E. Brun. (2014). "Expert consultation on risk factors for introduction of infectious pathogens into fish farms." *Preventive Veterinary Medicine* 115(3–4): 238–254.

12. Sitjà-Bobadilla, A. and B. Oidtmann. (2017). Integrated pathogen management strategies in fish farming. Fish diseases, Elsevier: 119–144.

13. Oidtmann, B., P. Dixon, K. Way, C. Joiner, and A. E. Bayley. (2018). "Risk of waterborne virus spread–review of survival of relevant fish and crustacean viruses in the aquatic environment and implications for control measures." *Reviews in aquaculture* 10(3): 641–669.

14. Romero, J. F., I. A. Gardner, L. Hammell, D. Groman, D. Whelan, N. O'Brien, L. J. Hawkins, H. Burnley, and K. Thakur. (2022). "Descriptive epidemiology of variants of infectious salmon anaemia virus in four Atlantic salmon farms in Newfoundland and Labrador, Canada." *Journal of Fish Diseases* 45(6): 919–930.

15. Spickler. (2007). Epizootic Hematopoietic Necrosis. Center for Food Security and Public Health, Iowa State University.

16. Gervais, O., A. Barria, A. Papadopoulou, R. Gratacap, B. Hillestad, A. Tinch, S. Martin, D. Robledo, and R. Houston. (2021). "Exploring genetic resistance to infectious salmon anemia virus in Atlantic salmon by genome-wide association and RNA sequencing." *BMC genomics* 22(1): 1–14.

17. Pyecroft, S. B., E. Beynon, J. J. Mahadevan, and R. S.-M. Chong. (2022). Infectious salmon anemia. Aquaculture Pathophysiology, Elsevier: 177–184

18. Rimstad, E., O. B. Dale, B. H. Dannevig, and K. Falk. (2011). "Infectious salmon anemia." *Fish diseases and disorders* 3: 143–165

19. Markussen, T., C. M. Jonassen, S. Numanovic, S. Braaen, M. Hjortaas, H. Nilsen, and S. Mjaaland. (2008). "Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus." *Virology* 374(2): 515–527

20. Cardenas, C., M. Carmona, A. Gallardo, A. Labra, and S. H. Marshall. (2014). "Coexistence in field samples of two variants of the infectious salmon anemia virus: a putative shift to pathogenicity." *PLoS One* 9(1): e87832

21. Gagne, N., and F. LeBlanc. (2018). "Overview of infectious salmon anaemia virus (ISAV) in Atlantic Canada and first report of an ISAV North American-HPR 0 subtype." *Journal of fish diseases* 41(3): 421–430.

22. Kibenge, F. S., M. J. Kibenge, Y. Wang, B. Qian, S. Hariharan, and S. McGeachy. (2007). "Mapping of putative virulence motifs on infectious salmon anemia virus surface glycoprotein genes." *Journal of General Virology* 88(11): 3100–3111.

23. Cunningham, C. O., A. Gregory, J. Black, I. Simpson, and R. S. Raynard. (2002). "A novel variant of the infectious salmon anaemia virus (ISAV) haemagglutinin gene suggests mechanisms for virus diversity." *Bulletin-European Association Of Fish Pathologists* 22(6): 366–374.

24. Cárdenas, C., N. Ojeda, Á. Labra, and S. H. Marshall. (2019). "Molecular features associated with the adaptive evolution of Infectious Salmon Anemia Virus (ISAV) in Chile." *Infection, Genetics and Evolution* 68: 203–211

25. Christiansen, D. H., P. E. Petersen, M. M. Dahl, N. Vest, M. Aamelfot, A. B. Kristoffersen, M. D. Jansen, I. Matejusova, M. D. Gallagher, and G. Jónsson. (2021). "No Evidence of the Vertical Transmission of Non-Virulent Infectious Salmon Anaemia Virus (ISAV-HPR0) in Farmed Atlantic Salmon." *Viruses* 13(12): 2428.

26. USDA–APHIS (2017). Maine Infectious Salmon Anemia Virus Control Program Standards. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS).

27. Nylund, A., J. Brattespe, H. Plarre, M. Kambestad, and M. Karlsen. (2019). "Wild and farmed salmon (Salmo salar) as reservoirs for infectious salmon anaemia virus, and the importance of horizontal-and vertical transmission." *Plos one* 14(4): e0215478.

28. Kibenge, M. J., T. Iwamoto, Y. Wang, A. Morton, R. Routledge, and F. S. Kibenge. (2016). "Discovery of variant infectious salmon anaemia virus (ISAV) of European genotype in British Columbia, Canada." *Virology journal* 13(1): 1–17.

29. Cárdenas, M., S. Michelson, D. R. Pérez, M. Montoya, J. Toledo, Y. Vásquez-Martínez, and M. Cortez-San Martin. (2022). "Infectious Salmon Anemia Virus Infectivity Is Determined by Multiple Segments with an Important Contribution from Segment 5." *Viruses* 14(3): 631.

30. Kibenge, F. S., M. G. Godoy, Y. Wang, M. J. Kibenge, V. Gherardelli, S. Mansilla, A. Lisperger, M. Jarpa, G. Larroquete, and F. Avendaño. (2009). "Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences." *Virology journal* 6(1): 1–16

31. Christiansen, D. H., P. S. Østergaard, M. Snow, O. B. Dale, and K. Falk. (2011). "A low-pathogenic variant of infectious salmon anemia virus (ISAV-HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (Salmo salar L.) in the Faroe Islands." *Journal of General Virology* 92(4): 909–918

32. Lyngstad, T., M. Hjortaas, A. Kristoffersen, T. Markussen, E. Karlsen, C. Jonassen, and P. Jansen. (2011). "Use of molecular epidemiology to trace transmission pathways for infectious salmon anaemia virus (ISAV) in Norwegian salmon farming." *Epidemics* 3(1): 1–11.

33. Godoy, M. G., M. J. Kibenge, R. Suarez, E. Lazo, A. Heisinger, J. Aguinaga, D. Bravo, J. Mendoza, K. O. Llegues, and R. Avendaño-Herrera. (2013). "Infectious salmon anaemia virus (ISAV) in Chilean Atlantic salmon (Salmo salar) aquaculture: emergence of low pathogenic ISAV-HPR0 and re-emergence of virulent ISAV-HPRΔ: HPR3 and HPR14." *Virology Journal* 10(1): 1–17.

34. EFSA. (2012). "Scientific Opinion on infectious salmon anaemia (ISA)." *EFSA Journal* 10(11): 2971

35. Gustafson, L., S. Ellis, D. Bouchard, T. Robinson, F. Marenghi, J. Warg, and C. Giray. (2008). "Estimating diagnostic test accuracy for infectious salmon anaemia virus in Maine, USA." *Journal of fish diseases* 31(2): 117–125

36. Aldrin, M., T. Lyngstad, A. Kristoffersen, B. Storvik, Ø. Borgan, and P. Jansen. (2011). "Modelling the spread of infectious salmon anaemia among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates." *Journal of The Royal Society Interface* 8(62): 1346–1356

37. Lyngstad, T. M., A. B. Kristoffersen, M. J. Hjortaas, M. Devold, V. Aspehaug, R. B. Larssen, and P. A. Jansen. (2012). "Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming." *Diseases of aquatic organisms* 101(3): 197–206.

38. LeBlanc, F., S. Leadbeater, M. Laflamme, and N. Gagné. (2018). "In vivo virulence and genomic comparison of infectious Salmon Anaemia Virus isolates from Atlantic Canada." *Journal of fish diseases* 41(9): 1373–1384.

39. Rimstad, E. and T. Markussen. (2020). "Infectious salmon anaemia virus—molecular biology and pathogenesis of the infection." *Journal of Applied Microbiology* 129(1): 85–97

40. USDA–APHIS. (2022). "Voluntary 2022 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections and Infestations List. United States Department of Agriculture (USDA), Animal Plant and Health Inspection Service (APHIS) National List of Reportable Animal Diseases (NLRAD)." from https://www.aphis.usda.gov/animal_health/nahrs/downloads/nlrad-nahrs-disease-list.pdf.

41. USDA–APHIS. (2022). "National Animal Health Reporting System (NAHRS). United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) ", from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/sa_disease_reporting/ct_usda_aphis_animal_health.

42. Stubgaard. (2022). "Infectious Salmon Anaemia (ISA)." from https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals/isa.

43. CFIA. (2022). "Facts about infectious salmon anemia (ISA). Government of Canada, Canadian Food Inspection Agency." from https://inspection.canada.ca/animal-health/aquatic-animals/diseases/reportable-diseases/isa/facts/eng/1327198930863/1327199219511.

44. CFIA. (2022). "Infectious Salmon Anaemia. Government of Canada, Canadian Food Inspection Agency." from https://inspection.canada.ca/animal-health/aquatic-animals/diseases/reportable-diseases/isa/eng/1327197013896/1327197115891.

45. WOAH. (2022). "Aquatic Animal Health Code. World Organisation for Animal Health ", from https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/

46. WOAH. (2022). "World Animal Health Information System (WAHIS) database. World Organisation for Animal Health." from https://wahis.woah.org/#/home.

47. Gustafson, L., S. Ellis, T. Robinson, F. Marenghi, P. Merrill, L. Hawkins, C. Giray, and B. Wagner. (2007). "Spatial and non-spatial risk factors associated with cage-level distribution of infectious salmon anaemia at three Atlantic salmon, Salmo salar L., farms in Maine, USA." *Journal of fish diseases* 30(2): 101–109

48. Spickler. (2011). Infectious Salmon Anemia. The Center for Food Security and Public Health. Iowa State University College of Veterinary Medicine, Ames Iowa.

49. Plarre, H., M. Devold, M. Snow, and A. Nylund. (2005). "Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway." *Diseases of Aquatic Organisms* 66(1): 71–79

50. Nylund, A., S. Alexandersen, J. Rolland, and P. Jakobsen. (1995). "Infectious salmon anemia virus (ISAV) in brown trout." *Journal of Aquatic Animal Health* 7(3): 236–240.

51. Nylund, A. and P. Jakobsen. (1995). "Sea trout as a carrier of infectious salmon anaemia virus." *Journal of Fish Biology* 47(1): 174–176

52. Rolland, J., and A. Nylund. (1998). "Sea running brown trout: carrier and transmitter of the infectious salmon anemia virus (ISAV)." *Bulletin-European Association Of Fish Pathologists* 18: 50–55.

53. Raynard, R., A. Murray, and A. Gregory. (2001). "Infectious salmon anaemia virus in wild fish from Scotland." *Diseases of Aquatic Organisms* 46(2): 93–100.

54. Alarcón, M., T. Moldal, M. Dverdal Jansen, M. Aamelfot, H. Sindre, T. M. Lyngstad, and K. Falk. (2021). "Infectious salmon anemia virus detected by RT-qPCR in Norwegian farmed rainbow trout, Oncorhynchus mykiss (Walbaum, 1792)." *Journal of Fish Diseases* 44(4): 479–481.

55. Jansen. (2020). The Surveillance Programme for Infectious Salmon Anaemia Virus HPR0 (ISAV HPR0) in Norwegian hatcheries 2019. N. F. S. Authority, Norwegian Veterinary Institute.

56. Christiansen, D. H., A. J. McBeath, M. Aamelfot, I. Matejusova, M. Fourrier, P. White, P. E. Petersen, and K. Falk. (2017). "First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus." *Journal of General Virology* 98(4): 595–606.

57. Ditlecadet, D., C. Gautreau, L. Boston, R. Liston, E. Johnsen, and N. Gagné. (2022). "First report of successful isolation of a HPR0-like variant of the infectious salmon anaemia virus (ISAV) using cell culture." *Journal of Fish Diseases* 45(3): 479–483

58. Smail, D. and R. Grant. (2012). "The stability of infectious salmon anaemia virus infectivity at− 80° C in tissue homogenate and dry-stored tissue from clinically diseased Atlantic salmon, salmo salar L." *Journal of Fish Diseases* 35(10): 789–792

59. Marshall, S. H., R. Ramírez, A. Labra, M. Carmona, and C. Muñoz. (2014). "Bona fide evidence for natural vertical transmission of infectious salmon anemia virus in freshwater brood stocks of farmed Atlantic salmon (Salmo salar) in Southern Chile." *Journal of virology* 88(11): 6012–6018

60. Vike, S., H. Duesund, L. Andersen, and A. Nylund. (2014). "Release and survival of infectious salmon anaemia (ISA) virus during decomposition of Atlantic salmon (Salmo salar L.)." *Aquaculture* 420: 119–125

61. Vågsholm, I., H. O. Djupvik, F. V. Willumsen, A. M. Tveit, and K. Tangen. (1994). "Infectious salmon anaemia (ISA) epidemiology in Norway." *Preventive Veterinary Medicine* 19(3-4): 277–290

62. Weli, S. C., H. Tartor, B. Spilsberg, O. B. Dale, and A. Lillehaug. (2021). "Evaluation of charged membrane filters and buffers for concentration and recovery of infectious salmon anaemia virus in seawater." *PloS one* 16(6): e0253297

63. Totland, G. K., B. K. Hjeltnes, and P. R. Flood. (1996). "Transmission of infectious salmon anaemia (ISA) through natural secretions and excretions from infected smolts of Atlantic salmon Salmo salar during their presymptomatic phase." *Diseases of Aquatic Organisms* 26(1): 25–31

64. Amos, K. H., L. Gustafson, J. Warg, J. Whaley, M. Purcell, J. Rolland, J. Winton, K. Snekvik, T. Meyers, and B. Stewart. (2014). "US response to a report of infectious salmon anemia virus in western North America." *Fisheries* 39(11): 501–506

65. Gustafson, L., S. Ellis, M. Beattie, B. Chang, D. Dickey, T. Robinson, F. Marenghi, P. Moffett, and F. Page. (2007). "Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon (Salmo salar L.) farms in the Quoddy region of Maine, USA and New Brunswick, Canada." *Preventive veterinary medicine* 78(1): 35–56

66. Gustafson, L., M. Remmenga, O. S. Del Valle, R. Ibarra, M. Antognoli, A. Gallardo, C. Rosenfeld, J. Doddis, R. E. Sais, and E. Bell. (2016). "Area contact networks and the spatio-temporal spread of infectious salmon anemia virus (ISAV) in Chile." *Preventive Veterinary Medicine* 125: 135–146

67. Murray, A. G., R. J. Smith, and R. M. Stagg. (2002). "Shipping and the spread of infectious salmon anemia in Scottish aquaculture." *Emerging Infectious Diseases* 8(1): 1

68. Valdes-Donoso, P., F. Mardones, M. Jarpa, M. Ulloa, T. Carpenter, and A. Perez. (2013). "Co-infection patterns of infectious salmon anaemia and sea lice in farmed Atlantic salmon, Salmo salar L., in southern Chile (2007–2009)." *Journal of fish diseases* 36(3): 353–360

69. Oelckers, K., S. Vike, H. Duesund, J. Gonzalez, S. Wadsworth, and A. Nylund. (2014). "Caligus rogercresseyi as a potential vector for transmission of Infectious Salmon Anaemia (ISA) virus in Chile." *Aquaculture* 420: 126–132

70. Lyngstad, T. M., P. A. Jansen, H. Sindre, C. Jonassen, M. Hjortaas, S. Johnsen, and E. Brun. (2008). "Epidemiological investigation of infectious salmon anaemia (ISA) outbreaks in Norway 2003–2005." *Preventive veterinary medicine* 84(3-4): 213–227

71. Jarp, J. and E. Karlsen. (1997). "Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon Salmo salar." *Diseases of Aquatic Organisms* 28(2): 79–86

72. Scheel, I., M. Aldrin, A. Frigessi, and P. A. Jansen. (2007). "A stochastic model for infectious salmon anemia (ISA) in Atlantic salmon farming." *Journal of the Royal Society Interface* 4(15): 699–706

73. Nazir, J., R. Haumacher, A. C. Ike, and R. E. Marschang. (2011). "Persistence of avian influenza viruses in lake sediment, duck feces, and duck meat." *Applied and environmental microbiology* 77(14): 4981–4985

74. Ramey, A. M., A. B. Reeves, J. Z. Drexler, J. T. Ackerman, S. De La Cruz, A. S. Lang, C. Leyson, P. Link, D. J. Prosser, and G. J. Robertson. (2020). "Influenza A viruses remain infectious for more than seven months in northern wetlands of North America." *Proceedings of the Royal Society* B 287(1934): 20201680

75. Ramey, A. M., A. B. Reeves, B. J. Lagassé, V. Patil, L. E. Hubbard, D. W. Kolpin, R. B. McCleskey, D. A. Repert, D. E. Stallknecht, and R. L. Poulson. (2022). "Evidence for interannual persistence of infectious influenza A viruses in Alaska wetlands." *Science of the Total Environment* 803: 150078.

76. Stewart, J. E. (1998). "Sharing the waters: an evaluation of site fallowing, year class separation and distances between sites for fish health purposes on Atlantic salmon farms."

77. Reche, I., G. D'Orta, N. Mladenov, D. M. Winget, and C. A. Suttle. (2018). "Deposition rates of viruses and bacteria above the atmospheric boundary layer." *The ISME journal* 12(4): 1154–1162

78. Nylund, A., H. Plarre, M. Karlsen, F. Fridell, K. Ottem, A. Bratland, and P. Saether. (2007). "Transmission of infectious salmon anaemia virus (ISAV) in farmed populations of Atlantic salmon (Salmo salar)." *Archives of virology* 152: 151–179

79. Vike, S., S. Nylund, and A. Nylund. (2009). "ISA virus in Chile: evidence of vertical transmission." *Archives of virology* 154(1): 1–8.

80. Cottet, L., A. Rivas-Aravena, M. Cortez-San Martin, A. M. Sandino, and E. Spencer. (2011). "Infectious salmon anemia virus—Genetics and pathogenesis." *Virus research* 155(1): 10–19

81. Ellis, S., L. Gustafson, C. Giray, T. Robinson, F. Marenghi, and P. Merrill. (2005). "Hydrographics and the Epidemiology of ISA: Findings from a High-Risk Region in Maine and New Brunswick." *Water Movement and Aquatic Animal Health* 105(1): 44

82. Murray, A. G., L. A. Munro, I. S. Wallace, B. Berx, D. Pendrey, D. Fraser, and R. S. Raynard. (2010). "Epidemiological investigation into the re-emergence and control of an outbreak of infectious salmon anaemia in the Shetland Islands, Scotland." *Diseases of Aquatic Organisms* 91(3): 189–200

83. Mardones, F., P. Jansen, P. Valdes-Donoso, M. Jarpa, T. Lyngstad, D. Jimenez, T. Carpenter, and A. Perez. (2013). "Within-farm spread of infectious salmon anemia virus (ISAV) in Atlantic salmon Salmo salar farms in Chile." *Diseases of aquatic organisms* 106(1): 7–16

84. Gustafson, L., M. Antognoli, M. L. Fica, R. Ibarra, J. Mancilla, O. S. Del Valle, R. E. Sais, A. Perez, D. Aguilar, and E. Madrid. (2014). "Risk factors perceived predictive of ISA spread in Chile: applications to decision support." *Preventive veterinary medicine* 117(1): 276–285

85. Mardones, F., B. Martinez-Lopez, P. Valdes-Donoso, T. Carpenter, and A. Perez. (2014). "The role of fish movements and the spread of infectious salmon anemia virus (ISAV) in Chile, 2007–2009." *Preventive veterinary medicine* 114(1): 37–46

86. Warg, J. (2022). Personal Communication.

87. USDA–APHIS. (2022). "General NVSL Information. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Program (APHIS) ", from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/SA_About_NVSL/CT_About_nvsl.

88. Bader, J. (2023). National Coordinator for Aquatic Animal Health, Aquaculture and Technology. United States Fish and Wildlife Service.

89. WTO. (1998). "Understanding the WTO Agreement on Sanitary and Phytosanitary Measures. The World Trade Organization." from https://www.wto.org/english/tratop_e/sps_e/spsund_e.htm.

90. WTO. (2010). "The WTO Agreements Series: Sanitary and Phytosanitary Measures. The World Trade Organization." from https://www.wto.org/english/res_e/booksp_e/agrmntseries4_sps_e.pdf.

91. USDA–APHIS. (2020). "Animal and Animal Product Export Information. United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS)." from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/export.

92. USGPO. (2016). "Code of Federal Regulations, Title 50: Wildlife and Fisheries: Parts 1 to 16. United States Government Publishing Office (USGPO), Office of the Federal Register National Archives and Records Administration, Washington DC." from https://www.govinfo.gov/content/pkg/CFR-2016-title50-vol1/pdf/CFR-2016-title50-vol1.pdf.

93. USFWS. (2022). "Steps for Importing Salmonids into the United States of America. United States Fish and Wildlife Service (USFWS)." from https://www.fws.gov/service/steps-importing-salmonids-united-states-america

94. CLS. (2022). "19 CFR § 12.26 - Importations of wild animals, fish, amphibians, reptiles, mollusks, and crustaceans; prohibited and endangered and threatened species; designated ports of entry; permits required. Cornell Law School, Legal Information Institute." from https://www.law.cornell.edu/cfr/text/19/12.26.

95. USDA–APHIS. (2022). "Fish, Fertilized Eggs, and Gametes. United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS)." from https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/live-animal-imports/aquatic-animals/fish-eggs-gametes

96. GovInfo. (2018). "Code of Federal Regulations, Title 50 - Wildlife and Fisheries, Volume 1, Part 14 - Importation, Exportation, and Transportation of Wildlife. GovInfo." from https://www.govinfo.gov/content/pkg/CFR-2018-title50-vol1/xml/CFR-2018-title50-vol1-part14.xml#seqnum14.91

97. FDACS. (2022). "Import/Export Requirement for Aquaculture Products, Florida Department of Agriculture and Consumer Services, Division of Aquaculture." from https://www.fdacs.gov/content/download/78858/file/FDACS-P%E2%80%9301785-ImportExportRequirements.pdf.

98. WDFW. (2022). "Atlantic Salmon (Salmo salar). Washington Department of Fish and Wildlife." from https://wdfw.wa.gov/species-habitats/invasive/salmo-salar.

99. WDFW. (1999). "Atlantic Salmon in Washington State: A Fish Management Perspective. Washington Department of Fish and Wildlife." from https://wdfw.wa.gov/publications/00922.

100. Kibenge, M. J., T. Iwamoto, Y. Wang, A. Morton, R. Routledge, and F. S. Kibenge. (2016). "Discovery of variant infectious salmon anaemia virus (ISAV) of European genotype in British Columbia, Canada." *Virology journal* 13(1): 1–17

101. Gustafson, L., M. Remmenga, I. Gardner, K. Hartman, L. Creekmore, A. Goodwin, J. Whaley, J. Warg, S. Gardner, and A. Scott. (2014). "Viral hemorrhagic septicemia IVb status in the United States: Inferences from surveillance activities and regional context." *Preventive veterinary medicine* 114(3-4): 174–187