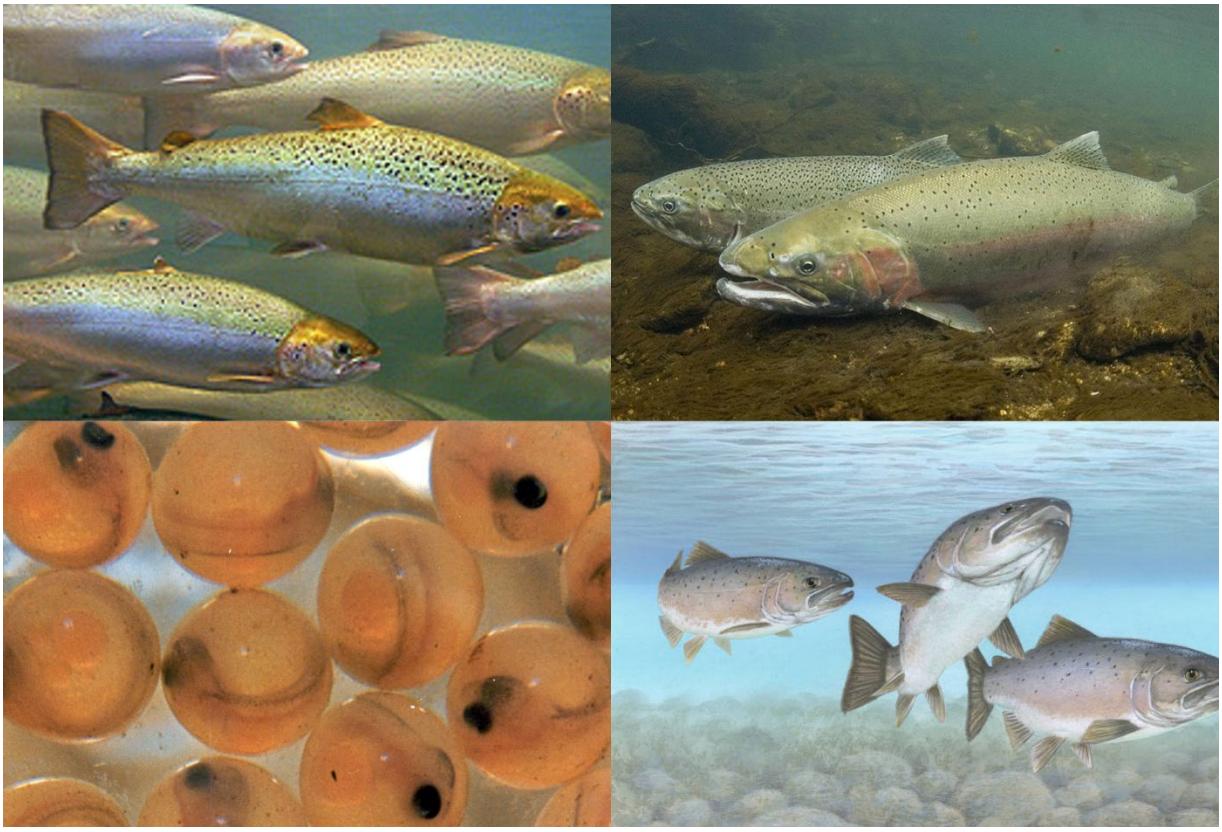
USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Pathways Assessment for Live Salmonid Fish, Eggs, and Gametes Susceptible to Six World Organisation for Animal Health Listed Pathogens



Veterinary Services | Strategy and Policy | Center for Epidemiology and Animal Health

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Cover photos (clockwise from top left): USDA ARS, NOAA Fisheries, USFWS, USFWS

Key Findings

- The United States currently produces approximately 1% of the global volume of farmed Atlantic salmon and 4.3% of the global volume of farmed rainbow trout.
- Domestic production of Atlantic salmon, rainbow trout, and steelhead trout are predicted to increase as land-based recirculating aquaculture system facilities are developed.
- Live salmonid fish and fertilized eggs are currently being imported for use in salmonid aquaculture.
    - According to United States Fish and Wildlife Service LEMIS data, imports primarily consist of live Atlantic salmon and steelhead trout fish and fertilized eggs.
- It is predicted that the number of imports and the volume of imported live salmonid fish and fertilized eggs will increase to meet rising production demands.
    - The number and volume of predicted imports, and sources of those imports, are not known.
- The likelihood, under historical and current importation conditions, that live salmonid fish, or fertilized eggs or gametes imported for use in salmonid aquaculture will result in entry of one of the six pathogens described in this assessment is **moderate** with a **low** degree of uncertainty. The risk of entry is **moderate**. Further measures to prevent or mitigate this risk should be considered.
- The likelihood that, under future importation conditions, live salmonid fish, fertilized eggs, or gametes imported for use in salmonid aquaculture will result in entry of at least one of the six pathogens described this assessment is **moderate** to **high** with a **moderate** to **high** degree of uncertainty. The risk of entry is **moderate**. Further measures to prevent or mitigate this risk should be considered.
- The likelihood, under historical and current import conditions, that live salmonid fish or fertilized eggs imported for use in salmonid aquaculture (no gametes were imported) will result in exposure of farmed Atlantic salmon, rainbow trout, or steelhead trout to of one of the six pathogens described in the Hazard Identification is **moderate** with a **low** degree of uncertainty. The risk of exposure is **moderate**. Measures to prevent or mitigate this risk should be considered.
- The likelihood that, under projected future importation conditions, live salmonid fish, fertilized eggs, or gametes imported for use in Atlantic salmon, rainbow trout, and steelhead trout aquaculture will result in exposure of farmed fish to at least one of the six pathogens described in the Hazard Identification section of this assessment is **moderate** with a **moderate** to **high** degree of uncertainty. The risk that an exposure may occur is **moderate**. Additional measures to prevent or mitigate this risk should therefore be considered.

Assessment Overview

Purpose

USDA APHIS VS CEAH was asked to generate an assessment evaluating the transboundary introduction potential of six World Organisation for Animal Health (WOAH)-listed pathogens via live salmonid fish, fertilized eggs, and gametes (e.g., reproductive cells of male or female fish) imported for use in domestic Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and steelhead trout (*O. mykiss*) aquaculture. This document is intended for internal

USDA APHIS VS leadership review and external distribution to industry stakeholders and the public.

Within Scope

Within the scope of this assessment is a review of the epidemiology associated with six pathogens of concern to salmonid aquaculture stakeholders (Table 1) and a review of the potential entry and exposure pathways associated with those pathogens and the import of live salmonid fish, fertilized eggs, and gametes for use in salmonid aquaculture (specifically Atlantic salmon, rainbow trout, and steelhead trout). All six pathogens cause diseases that are reportable to the USDA APHIS National Animal Health Reporting System (NAHRS).[1] Three of the pathogens are not endemic to the United States. Three pathogens are endemically present in specific locations within the United States.

Table 1. The six World Organisation for Animal Health (WOAH)-listed pathogens of concern to salmonid aquaculture stakeholders included in this document

Pathogen	**Endemic to the United States**	**Foreign Animal Disease**	**Reportable to the USDA APHIS National Animal Health Reporting System**
Epizootic haematopoietic necrosis virus (EHNV)	No	Yes	Reportable
Gyrodactylus salaris	No	Yes	Reportable
Infectious hematopoietic necrosis virus (IHNV)	Yes	No	Reportable
Infectious salmon anemia virus (ISAV)	Yes	No	Reportable
Salmonid alphavirus (SAV)	No	Yes	Reportable
Viral hemorrhagic septicemia virus (VHSV)	Yes	No	Reportable

Subjects within the scope of this document include:

- Overview of Atlantic salmon and rainbow trout aquaculture,
- Overview of marine and inland aquaculture systems,
- Summary of regulatory information relevant to United States aquaculture,
- Descriptions of hazards (pathogens of concern),
- Identification of fish species susceptible to each hazard,
- Geographic distribution of each hazard,
- Summary of the epidemiology of each hazard,
- Review of the entry pathway (movement of imported live salmonid fish, fertilized eggs, and gametes from a point of origin to a point of entry into the United States),

- Review of the exposure pathway (movement of live salmonid fish, fertilized eggs, and gametes from points of entry into the United States that may lead to exposure of farmed Atlantic salmon, rainbow trout, and steelhead trout),
- Review of potential consequences that introduction of any of the six pathogens may have on domestic Atlantic salmon and/or rainbow trout aquaculture,
- Summary description of the risk associated with the entry and exposure pathways,
- Summary of the assessment,
- Description of limitations that affected description of the hazards and assessment of the entry and exposure pathways, economics overview, and consequences.

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, 4] 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]

The broad nature of this request presented challenges given the number of pathogens of concern, the different types of inland- and marine-based salmonid aquaculture systems, and the endemic presence of some of the pathogens in marine and freshwater farmed, and indigenous wild salmonid and other finfish populations, present in the United States. To conduct this assessment, we referenced WOAH resources, subject matter expert consultation, and available

published data and literature. Knowledge and data gaps that affected complete evaluation of some tenets of this document included but are not limited to:

- Future projections for the United States Atlantic salmon, rainbow trout, and steelhead trout industries are hypothetical. Therefore,
  - The true trajectory of future inland aquaculture development (recirculating aquaculture system; RAS, and flow through system; FTS) is not known.
  - The true need for imported live salmonid fish, fertilized eggs, and gametes is not known.
  - The sources (countries, hatcheries, farms) for future imports are not known.
  - The epidemiology of the six pathogens in future source countries (and their hatcheries and farms) is not known.
  - Aquaculture disease management, reporting, and regulation in countries from which the United States may import live salmonid fish, fertilized eggs, and gametes in the future is unknown.
- Data deficiencies related to detection and reporting of aquatic diseases in the United States prevent complete assessment of disease status, presence, or absence.
  - There are few USDA APHIS-supported federal surveillance programs for aquaculture (other than ISAV in Maine).
    - USDA APHIS, the United States Fish and Wildlife Service (USFWS), States, Tribes, and localities may conduct surveillance (passive or case-by-case) associated with disease response or allegations (e.g., ISAV in the Pacific Northwest, VHSV outside of the Great Lakes).
  - A consolidated database tracking all detections/outbreaks of aquatic animal pathogens is lacking.
    - USFWS does conduct a National Wild Fish Health Survey to monitor the presence or absence aquatic animal pathogens in wild fish populations (Appendix, Table 1).
- Outbreak response, contingency plans, and cost estimates for prevention, eradication, and control for many aquatic animal pathogens are lacking.
- In general, factors associated with the epidemiology of all the pathogens in this assessment relative to Atlantic salmon, rainbow/steelhead trout, other salmonid fish, and other potentially susceptible non-salmonid finfish are not fully described.
  - All natural environmental, viral, and host factors associated with each pathogen are not fully known.
  - All environmental, viral, and host factors associated with each pathogen in aquaculture environments (inland and marine) are not fully known.
  - Home range distributions and movements of key carrier and susceptible wild fish species during seasons when environmental factors are optimal for occurrence of the six diseases of concern are lacking.
  - The reservoir status of susceptible farmed fish species is not known for all pathogens in this document.
  - The reservoir or transmission host status of other aquatic species (crustacean, mollusc, copepod, other fish species) has not been definitively determined for all pathogens.
  - Information specific to virus infectious dose, pathogenic mechanisms, virulence factors, and duration of infectivity are not fully described for each pathogen.
  - Many factors associated with transmission (e.g., shedding rate, environmental conditions such as dilution, wind and current strength and direction, carrier status) have not been determined for each pathogen.

- Susceptibility of United States salmonid (or other) fish stocks to some of the pathogens described in this assessment is currently lacking (e.g., EHNV, *G. salaris*, SAV).

Not Within Scope

Subjects that are not within the scope of this document include:

- Assessment of all potential pathogens that may be present in imported live salmonid fish, fertilized eggs, and gametes.
- Assessment of all potential entry pathways that could allow entry of the six pathogens into the United States. The specific entry pathway of concern identified by the requestors was imported live salmonid fish, fertilized eggs, and gametes.
- Assessment of exposure pathways that are not directly associated with imported live salmonid fish, fertilized eggs, and gametes.
- Assessment of the potential impacts to all susceptible or potentially susceptible cultured and wild indigenous fish species.

Introduction

Atlantic Salmon and Rainbow Trout Species Description and Information

Atlantic Salmon

The Atlantic salmon is the third largest member of the fish family *Salmonidae*. It is an iteroparous (capable of multiple reproductive cycles) anadromous fish (spends most of its life at sea, migrates to freshwater to spawn), and is the only salmon species indigenous to the Atlantic Ocean.[15, 16, 17] Atlantic salmon are a cold-water species, preferring temperatures ranging from 4 – 12 °C/39 – 53.6 °F (lower and upper lethal temperature limits are -0.7 °C/30.74 °F and 27.8 °C/82 °F, respectively).[18, 19] Wild Atlantic salmon hatch and live in freshwater rivers for the first two to three years of life, migrate to sea as smolts, mature for one to four years, then return to their river of origin to spawn.[20, 15] At two to three years of age fish may reach 76–100 cm/30–39 in in length and weigh from 3.5 – 5.5 kg/7.1 – 12.1 lbs. Adults that spend four or more winters at sea may be larger.[17]

Historically, wild Atlantic salmon ranged from the eastern Atlantic Ocean from the Arctic Circle to Greenland, Iceland, Russia and south as far as Portugal. Their range in the western Atlantic Ocean extended from the Arctic Circle to the Baltic Sea, through northern Quebec, Canada, and south along the Northeast coast of the United States to the Housatonic (Long Island Sound) and Connecticut Rivers.[21, 20, 17, 22] Presently, there are three anadromous populations (North American, European, and Baltic) that migrate and intermix in the North Atlantic Ocean off the coasts of Europe, Greenland, Iceland, North America, and Russia.[17, 22, 23] These populations have experienced significant historical and ongoing declines due to recreational and commercial fishing, habitat destruction, and anthropogenic factors.[24, 17, 19]

In 1984, the estimated population numbers ranged from eight to ten million fish.[25] In 2020, numbers were estimated at approximately two to three million fish, with sustainable populations present in only 14% of historical spawning rivers in North America and Europe.[26] Locally, population numbers vary considerably (0.25 million fish in some Northern European rivers to a few hundred or single individuals in other regions).[19] Some populations have been extirpated (locally extinct in specific regions) in southern Europe and North America, including in the United States.[27, 28]

In the United States, remnant anadromous populations are found only in Maine (the Gulf of Maine and the Sheepscot, Penobscot, Ducktrap, Narraguagus, Pleasant, Machias, East Machias, and Dennys rivers).[16, 17, 19, 22] In Maine and the Bay of Fundy (New Brunswick, Canada), anadromous Atlantic salmon are listed as endangered under the Endangered Species Act and the Canadian Species at Risk Act, respectively.[24, 16, 19] Stocking programs using hatchery origin fish occur in some states (Connecticut, New Hampshire, New York).[28, 29] Successful establishment of reproducing populations is low, but has been reported.[28]

Landlocked indigenous and stocked Atlantic salmon populations are found in freshwater lakes in northeastern Europe, Iceland, and North America (Canada and the United States).[30, 23] In the United States, landlocked populations were historically present in Lake Champlain, Lake Ontario, and in several lakes in Maine (Grand Lake, Green Lake, Sebago Lake, and Sebec Lake).[22] Currently, indigenous or stocked landlocked salmon are found in the Great Lakes and inland lakes in some states, including but not limited to Maine, Michigan, New Hampshire, New York, and Vermont.[31, 32, 33] Globally, introduced populations are reported in Argentina, Australia, Chile, and New Zealand.[30]

The origins of salmon farming began in Europe in the latter part of the eighteenth century.[34] In the mid-twentieth century, hatcheries were established in Canada, Japan, the USSR, and the United States. In the 1960s, the modern techniques of salmon culture in marine farms began in Norway.[34, 35] Currently, marine-based Atlantic salmon aquaculture occurs globally in coastal areas where the environment is optimal for the species (Figure 1).[36]

Note. The coastlines are all within specific latitude bands that have appropriate water temperature ranges for Atlantic salmon in the Northern and Southern Hemispheres.[37]

Figure 1. Primary coastal areas utilized for salmon farming

The Atlantic salmon farming production cycle lasts approximately three years and involves a freshwater production phase (hatchery stage) and the grow out phase.[36] The freshwater production stage is approximately 10 to 16 months long and begins in hatcheries where fertilized eggs are collected from broodstock.[37, 36] Broodstock fish are hatchery reared male and female fish selectively bred for desirable production traits (e.g., size, growth and maturation rates, feed conversion, carcass quality). These fish may spend one year in marine farms before being moved back to inland aquaculture facilities or may be reared inland for their entire life cycle.[38] Most Atlantic salmon producers rear broodstock and produce fertilized eggs for their own use. However, at times fertilized eggs may be sold to other producers.[37, 36] Fertilized eggs may also be available through various private, government, and public hatcheries. There is an international market for salmon fertilized eggs that is subject to import/export restrictions that vary by country.[37]

According to the United States Fish and Wildlife Service (USFWS) Law Enforcement Management Information System (LEMIS) database, for years in which data were available (2013 – 2023), there were 139 importations of live Atlantic salmon fertilized eggs in volumes ranging from n = 5 – 1,850,000 fertilized eggs per shipment.[39] Live fish (likely broodstock) were imported once (n = 100 fish).[40, 39] Importing entities included commercial, private, and state aquaculture hatcheries, laboratories, and conservation and environmental nonprofits located in Florida, Indiana, Kentucky, Maine, Maryland, Minnesota, Nebraska, South Dakota, Tennessee, Virginia, Washington, West Virginia, and Wisconsin.[39] Countries from which the live fish, fertilized eggs, and gametes were imported included Canada, Iceland, and Norway.[39]

Fertilized eggs are incubated through the eyed stage until hatching. Incubator types include upwelling trays that mimic natural in-stream conditions (e.g., water enters the incubator, flows under the fertilized eggs, percolates up through the fertilized eggs and substrate, then flows out of the incubator), drip incubators (fertilized eggs are placed in stacked trays and water drips down through the fertilized eggs), and jar incubators (eggs are placed in cylindrical vessels and water enters at the bottom of the vessels and exits at the top).[41, 42] Incubators may be designed so that water flows in series from one tray or jar to another or in parallel so that each individual tray or jar has an isolated water source.[42] During incubation, dead eggs are removed by hand or mechanical picking.[43, 41, 42]

After hatching, fry stage fish are moved to circular tanks, raceways, or ponds using flow-through (FTS) or recirculating aquaculture (RAS) systems when they reach the "swim-up state" (e.g., the yolk sack is absorbed and active searching for food begins).[42, 44, 40, 36] Fish remain in these systems through the smolt stage (approximately 100 – 250 g/0.22 – 0.55 lbs).[40, 36] Some facilities have the capability to produce smolts up to 1,000 g/2.2 lbs, which shortens the amount of time spent at the marine grow out stage.[40] Smolts are typically reared for a company's own use, although some may be sold to outside parties.

The grow out phase begins when smolts are transferred over land (typically by truck) from the hatchery to the grow out facilities in specialized transport tanks. Historically, grow out occurred in marine net-pen farms (see Types of Salmonid Aquaculture Systems section below). In the United States, Atlantic salmon were farmed in marine net pens in Maine and Washington until 2018. Following a large farmed Atlantic salmon escape, net pen culture of Atlantic salmon was banned in Washington.[45] Currently, all net pen production occurs along the coastal shoreline of Maine. Recently, inland aquaculture facilities have been developed that are capable of housing Atlantic salmon through the grow out phase.

The grow out phase ranges from 10 to 24 months and is dictated by host factors (e.g., size of the fish entering the grow out facility, genetics, and general health), husbandry factors (e.g., feed formulations, use of vaccines, control of concomitant pathogens and parasites, and farm biosecurity measures), and environmental conditions (e.g., daylight interval, water quality, and ambient and water temperatures).[37] Once fish reach harvestable size (4 – 5 kg/8.8 –11.0 lbs), they are transported to processing plants. After harvest, marine farms are fallowed for 2–18 months.[46, 36] Some inland aquaculture systems utilize fallowing as part of farm biosecurity.

Countries producing the greatest volume of farmed Atlantic salmon in order of proximate volume in 2018 are listed in Table 2.[47]

Table 2. Top Atlantic salmon producing countries in 2018, in order of proximate volume produced[47]

Top Salmon Producing Countries	Production (tonnes)	Share of Global production
Norway	1,282,003	53%
Chile	661,138	27%
United Kingdom	166,000	7%

Canada	123,184	5%
Faroe Islands	78,900	3%
Australia	61,227	3%
Russia	20,566	1%
United States	16,107	1%
Iceland	13,448	1%
Ireland	11,984	1%

In the United States, according to USDA National Agricultural Statistics Service (NASS) Census of Agriculture data, in 2013 and 2018 there were 12 to 14 Atlantic salmon farms involved in different stages of production (Table 3). The salmon farming industry is capital intensive, which creates industry volatility. Production costs are impacted by a biologically driven extended production cycle that is affected by external factors and market supply and demand.[37] In recent years, production costs have trended upwards due to rising feed costs, biological costs, and more stringent regulatory compliance requirements.[37] During the last several decades, the global industry has undergone consolidation and industrialization, with larger farms emerging as primary producers.[37] In marine farm systems, working capital is cyclically variable throughout the year because growth and harvest of salmon is highly impacted by seasonal seawater temperatures. This leads to seasonal variation of net working capital that typically is at a low around midsummer and peaks at the end of the year.[37] The development of inland Atlantic salmon aquaculture has created opportunity to rear Atlantic salmon through the grow out phase in production systems that utilize FTS or RAS technologies. There is potential for year-round production of Atlantic salmon in these systems due to complete control of environmental conditions required for all production stages.

Table 3. Atlantic salmon farms present in the United States in 2013 and 2018 based on USDA National Agricultural Statistics Service (NASS) 2018 Census of Agriculture

Atlantic Salmon production stage	**Number of farms**	
	2013	**2018**
Food or Market Size	4	7
Stockers	3	3
Fingerlings or Fry	1	1
Broodfish	1	NA

Eggs	3	3

Note. Data summarizing the number of fish produced or sold and total sale values were not reported in these surveys.[48]

Rainbow Trout

Rainbow trout is a salmonid fish species native to cold-water tributaries of the North Pacific Ocean region (the Amur River in eastern Asia, the extreme northeastern Russia, and the Pacific slope of North America).[49] In Canada, rainbow trout are native to the western drainages of the Pacific Coast.[50, 49] In the United States, the native range extends from Alaska to the Baja Peninsula, Mexico and includes California, Idaho, Oregon, Nevada, and Washington.[49, 51] Wild rainbow trout prefer cold water (less than 21 °C/70 °F) riverine and lake habitats.[51] Adult rainbow trout may live four to six years and typically reach an average 28 – 63.5 cm/11 – 25 in and 0.5 – 3 kg/1 – 6.6 lbs in size.[52] Spawning is partially dictated by optimal water temperatures (5.5 – 6.7 °C/42 – 44 °F).[52]

Wild caught and hatchery-reared freshwater rainbow trout have been extensively introduced for recreational and aquaculture use in approximately 70 countries on every continent except Antarctica.[49] In North America, introductions into natural environments have occurred in south central Canada, southwestern Mexico, and throughout the United States (e.g., Great Lakes, Great Plains, and East Coast regions).[51] Many introductions have resulted in establishment of wild, self-sustaining populations.[52] In some countries, including the United States, introductions have resulted in localized ecological damage to freshwater systems and some native fish populations.[52]

Globally, rainbow trout aquaculture has grown rapidly since the 1950s.[45] Countries producing the greatest volume of farmed freshwater rainbow trout in 2018, in order of proximate volume, are listed in Table 4.[53]

Table 4. Top freshwater rainbow trout producing countries in 2018, in order of proximate volume produced[53]

Top freshwater rainbow trout producing countries	Production (tonnes)	Share of Global production
Iran	173,381	32.7%
Turkey	103,192	19.4%
Peru	55,030	10.4%
China	38,606	7.3%
Russia	35,204	6.6%
Italy	32,825	6.2%
France	26,100	4.9%
Colombia	23,038	4.4%

United States	23,370	4.3%
Denmark	20,000	3.8%

In the United States, freshwater rainbow trout aquaculture began in the 1800s.[45] Currently rainbow trout aquaculture for recreational stocking and commercial food fish production is the second largest finfish aquaculture industry in the United States.[54] Hatcheries and grow out facilities are typically designed with FTS or RAS strategies (see Types of Salmonid Aquaculture Systems below). The United States industry is consolidated and vertically integrated relative to hatchery production, grow out, processing, and sales.[55] Hatcheries and feed manufacturing have retained some autonomy but may be integrated in some specific business plans. Research and development within the industry is conducted to increase production efficiency by developing technologies to increase rearing technologies, improve water use technology, improve fish genetics, improve feed formulation and conversion, and enhance sales and marketing.[42]

Data reporting the total number of rainbow trout specific hatcheries and grow out operations in the United States is not complete due to the consolidation of all trout production data in some resources. Some authors state that there are hundreds of USFWS, State agency, Tribal government, and private hatcheries propagating rainbow trout for recreational sport fishing and conservation.[51, 56] The 2018 USDA NASS Census of Aquaculture reported data for 632 rainbow trout farms. Approximately 47% (n = 297) were public or Tribal hatcheries or farms rearing trout for conservation, stocking, or recreational purposes.[48] Remaining operations were described as commercial aquaculture operations that rear rainbow trout principally as food fish, but may also sell fish for conservation, stocking, and recreation purposes.

Production capacity of farms for which data were available ranged from a few thousand to millions of kilograms/pounds of trout annually. According to the NASS surveys, 80% of production occurs in Arkansas, California, Colorado, Georgia, Idaho, Michigan, Missouri, New York, North Carolina, Oregon, Pennsylvania, Utah, Virginia, Washington, West Virginia, and Wisconsin.[48] According to other published sources, most rainbow trout hatcheries and farms (approximately 67% by volume of fish produced) are located in the Snake River region of Idaho.[57, 58, 45]

Hatcheries are the sole source of fertilized eggs used in rainbow trout aquaculture and are designed as described in the Atlantic salmon section. Fertilized eggs are usually shipped to producers when they reach the “eyed” stage (approximately halfway through the incubation period).[59] Some hatcheries also supply fry (1.9 – 7.6 cm/0.75 – 3 in long) and fingerlings (7.6 – 23 cm/3 – 9 in long) for commercial, restocking, or other purposes. Domestic shipping occurs primarily by truck. The distance that eyed eggs and live fish are moved is dependent on the number of hatcheries within a given geographic area, the production capacity of the individual hatcheries, and the proximity of the hatcheries to the producer. In some states (e.g., Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin) approximately 92% of purchased fertilized rainbow trout eggs are sourced from western state hatcheries. Hatcheries may also produce eyed eggs for export outside the United States. According to a 2017 report by Seafood Watch®, international and trans-waterbody shipments of live fish or eyed eggs comprises less than 10% of production.[55]

According to the USFWS LEMIS database, for years in which data were available (2013 – 2023) there were 115 importations of *O. mykiss* specifically identified as steelhead trout or fertilized eggs imported in volumes ranging from n = 150 –1,350,000 fertilized eggs per shipment.[60] Live fish were imported five times in volumes ranging from 121– 6,010 fish.[60] Importing entities included commercial, private, and state aquaculture hatcheries, and conservation and environmental nonprofits located in Florida, Idaho, Montana, Oregon, Washington, and West Virginia.[60] Countries from which the live fish, fertilized eggs, and gametes were imported included Canada, Croatia, Denmark, Norway, and Russia.[60]

Freshwater rainbow trout are reared from hatchery to market size in ponds, tanks, or raceways in inland farms that utilize FTS or RAS technologies (see Types of Salmonid Aquaculture Systems below).[45] Production can occur year-round in some systems. Rainbow trout reach average market size (0.45 – 0.68 kg/1 –1.5 lbs) in approximately 12 months.[61] Fish are typically transported to processing plants by truck.[61]

Steelhead Trout

Steelhead trout are anadromous iteroparous rainbow trout.[62] Juvenile steelhead live in freshwater rivers for one to three years before migrating to the sea. Steelhead return to freshwater systems as “summer-run” or “winter-run” populations in May–October or November–April, respectively.[62, 60] The survival rate after one spawning is low (approximately 10% – 20%).[52, 56] Steelhead trout are larger than freshwater rainbow trout, reaching up to 110 cm/45 in in body length, and 3.62 – 4.98 kg/8 –11 lbs on average. Fish weighing up to 9 –18 kg/20 – 40 lbs or greater have been documented.[56]

The native range includes cold-water tributaries of the Pacific Basin in Northeast Asia and North America (Canada and the United States). In the United States, wild steelhead range from Alaska to California; however, some populations are threatened or endangered due to habitat loss, blocking of waterways by dams, and other anthropogenic causes.[62] Steelhead trout have been introduced to the Great Lakes and migrate into tributaries of the Lakes to spawn. The survival rate for introduced populations in the Great Lakes can be high (70%).[62] Steelhead trout are one of the top five recreational fish species in North America and there is growing interest in aquaculture production of this fish. Steelhead are also culturally significant to many Native American tribes in the United States and Canada.[63] In response to recreational and aquaculture demand, hatcheries throughout the United States have begun to cultivate steelhead trout.

Globally, steelhead trout aquaculture produces less volume of product compared to farmed Atlantic salmon and rainbow trout.[45] Countries producing the greatest volume of farmed steelhead trout in order of proximate volume in 2018 are noted in Table 5.[53]

Table 5. Top steelhead trout producing countries in 2018, in order of proximate volume produced[53]

Top steelhead trout producing countries	Production (tonnes)	Share of Global production
Chile	78,255	41.4%
Norway	68,216	36.0%

Finland	11,119	5.9%
Denmark	9,737	5.1%
Turkey	9,235	4.9%
Iran	6,300	3.3%
United Kingdom	3,500	1.8%
Sweden	2,870	1.5%

In the United States, farmed steelhead are reared in open water (brackish riverine and marine) net pens on the Pacific and Atlantic coasts, and in inland FTS and RAS aquaculture facilities in multiple states.[60] Information describing net pen production of steelhead trout is limited, because most research and scientific data associated with rainbow trout production focuses on fish reared in freshwater systems.[45] When steelhead trout are reared in open water, the types of net pens used, site configuration, and other processes described for Atlantic salmon are largely applicable (see Types of Salmonid Aquaculture Systems below). When steelhead are reared inland, the same FTS and RAS aquaculture systems applied to rainbow trout and Atlantic Salmon are used. The popularity of steelhead trout as a farmed species is increasing due to higher growth rates in this species compared to Atlantic salmon and freshwater rainbow trout. Steelhead trout can typically reach market size (3 kg/6.6 lbs) in less than 1.5 years.[53]

Types of Salmonid Aquaculture Systems

Marine Aquaculture

Presently, most Atlantic salmon and steelhead trout are reared to harvest size in marine net pen grow out farms located offshore in coastal environments compatible to the fish. Net pens are comprised of round or square floating collars from which nets are suspended and anchored to the seafloor.[40] The netting is designed to be large enough for adequate water flow, but small enough to keep the farmed fish inside the pen.[40] The net pens are designed to withstand open water environments and weather events and rely on tides, currents, and other natural water movements for a continual high-quality water supply.[40] Large net pens may be 75 –100 m/82 – 109 yds in diameter, reach depths of 15 – 35 m/16 – 36 yds, and enclose water volumes reaching thousands of cubic meters.[36] A single generation of fish can be stocked up to maximum densities of 20kg/m3 per pen.[40, 64, 37]

Net pens are typically grouped together to form a farm site.[36] Farm sites and the number of net pens in them are based upon the environmental suitability of the location (e.g., water depth, dissolved oxygen content, exchange rates, flow rate, salinity, surface area, and temperature) and the proximity to other farms and wild fisheries.[36] Divers perform inspections on a regular basis to assess fish health, morbidity, mortality, and the condition of the enclosures (e.g., evidence of damage, fouling, holes in the nets).[40] When necessary, nets are mechanically cleaned with brushes and power washers. Video and automated equipment is used to assess

water quality and conditions, feed administration and consumption, the condition of the sea floor under and adjacent to the net pens, and other production parameters.[40]

Net pens do have some production advantages compared to land-based production systems. There is a relatively low capital cost per unit of rearing volume, land requirements are minimal, construction and capital costs are generally lower, and there are virtually no water treatment or pumping costs.[40] Primary disadvantages include lack of capability to treat effluents and solid wastes, risks of environmental damage, reduced capability to control environmental conditions, and often expensive permitting processes and regulatory oversight.[40] This production method is limited in capability to increase in scope due to site limitations, regulatory restrictions, and public opposition.[64] Biosecurity controls are challenging in open water compared to inland aquaculture systems. Fish reared in open water have the potential for exposure to a large number of bacteria, viruses, parasites, pollution, and elevated pathogen environmental DNA (eDNA). This creates the potential for increased exposure of farmed fish by aquatic animals present in the environment, and that fish in net pens may serve as potential spillback reservoirs for infectious agents.[65, 66, 40]

Inland Aquaculture

Inland aquaculture facilities include hatcheries and facilities that grow out juvenile fish to market size. These facilities utilize FTS or RAS technologies for water management.[40, 55] FTS facilities are found throughout the United States wherever high-quality water with consistent temperatures and high flow rates are available. Facilities range in size from small operations to large production level facilities capable of producing millions of fish per year. FTS has been utilized for a long time in salmonid hatcheries and in rainbow trout aquaculture.

The layout of an FTS facility consists of a source water supply that flows continuously via gravity through fish rearing structures (e.g., hatchery incubation systems, ponds, raceways, or tanks) connected in series or parallel (Figure 2 and Figure 3).[55, 53] Springs or groundwater (subterranean aquifers) are considered the ideal water sources due to the perception that they are clean, pathogen free, and temperature stable.[53] When surface waters (lakes, oceans, rivers, and streams) are utilized as water sources, producers must be cognizant of the aquatic animals, pathogens, and parasites that may be present in the water source and apply appropriate biosecurity measures to prevent introduction of these organisms into the aquaculture facility.[67]

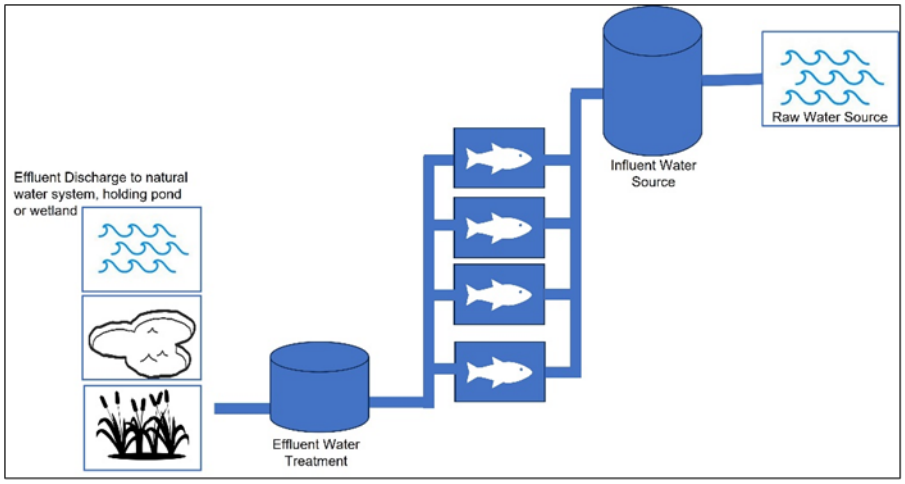

Figure 2. Diagram depicting a basic flow through (FTS) aquaculture facility designed in parallel

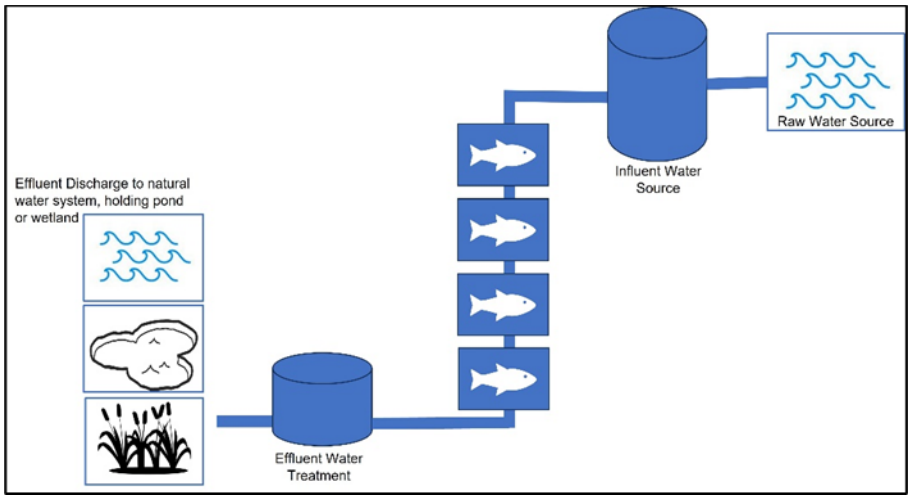

Figure 3. Diagram depicting a basic flow through (FTS) aquaculture facility designed in series

Treatment of raw influent water, regardless of source, may include aeration, filtration, sterilization, temperature control, and other measures. As water moves through fish rearing structures, waste solids (feces, uneaten feed, other wastes) settle in a section of each structure and are collected. Collected solids are placed in settling basins as part of the effluent water treatment, conveyed to a sedimentation and dewatering process, or held in a storage tank until disposal into public water works, land application as fertilizer, or other methods.[68, 53] Effluent water is treated and discharged back into the environment via a settling pond or wetland, or may be released back into the source water or another natural water body.[57, 69, 70]

Advantages to FTS include lower costs for start-up and infrastructure, lower electrical energy requirements, and lower greenhouse gas emissions compared to RAS aquaculture. The design is less complex than RAS which leads to efficiency in operation and labor. Primary disadvantages are the requirements for large consistent volumes of high-quality water and high water turnover rates, which can affect the siting and size of the facility.[57, 55] Additional disadvantages include difficulty in solid waste collection and disposal, control or treatment of dissolved wastes (e.g., nitrogen, phosphorus) in effluent discharges, environmental impacts to source waters (e.g., diversion of water from its natural course, potential effects on indigenous species composition and diversity), escape of fish in the event of overtopping or accidental releases, and potential release of pathogens or parasites. Biosecurity can be difficult to maintain at influent and effluent water source points.[57, 69, 70]

RAS aquaculture is a highly intensive land-based aquaculture production platform. RAS is not a new technology; applications have been used in home and commercial aquaria for decades. Globally, RAS has experienced significant advancements in interest and technology in response to demand for increased cultured food fish production. In the United States, inland grow out of Atlantic salmon and rainbow trout using RAS technology is a relatively new industry innovation undergoing rapid interest and development.[64, 37]

There is particular interest in utilizing RAS aquaculture as a strategy to move marine stage Atlantic salmon production to land-based facilities.[45] According to published literature, in 2020, RAS projects in production or under consideration in Canada and the United States included four Atlantic salmon producers with intention to expand production from 72 tonnes to 600 tonnes of product.[45] In 2022, over $2 billion USD was invested in inland RAS Atlantic salmon

aquaculture. In Maryland, an additional investment of $1 billion USD is predicted through 2024.[64] Large-scale projects for inland rainbow trout and steelhead trout production have also been described.[45]

There are many types of freshwater and seawater RAS systems, each with different utility.[71] Briefly, water may be sourced from some of the same sources as FTS; however, approximately 95% of the water within a RAS operating system can be reused and recirculated daily.[72] This significantly minimizes the total volume of water used in production and reduces pre- and post-aquaculture environmental impacts compared to marine and FTS aquaculture strategies. Water treatment processes performed in series or in tandem are designed to minimize water requirements, leading to concentrated, small-volume waste streams. Water that leaves the rearing structures is treated to remove solids. The water then enters a biofilter system to convert ammonia, followed by degassing, oxygenation, pH control, temperature control, and other treatments before recirculating back through the system (Figure 4). Collected solids or slurry may be discharged directly to public water treatment works or may be primarily treated and placed in settling ponds prior to discharge to public water treatment works or land applied as fertilizer. Overflow water is directly discharged, treated prior to discharge, or land-applied.[45] The types of tanks, biofilters, solids collections steps, water treatment, and other production requirements in RAS aquaculture are highly diverse and can be constructed specifically to meet the needs of the cultured species and production strategy.[72] An overview of RAS technology may be found in links located in Appendix, Table 1.

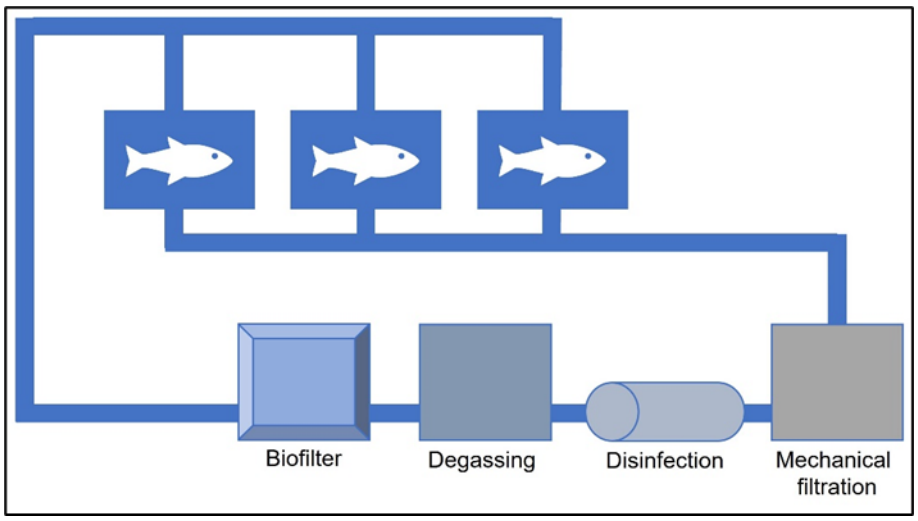

Figure 4. Diagram of a basic recirculating aquaculture system (RAS) aquaculture facility designed in series

Advantages to RAS aquaculture include capability to modify and tightly control water and other environmental parameters to optimal year-round production conditions for cultured aquatic species. This technology offers increased flexibility in aquaculture site selection, including proximity to markets, processing, and transport hubs; reduced land and water requirements compared to FTS; year-round production capability; increased efficiency of production and improved profitability; reduced wastewater effluent volume; and ability to capture and repurpose wastes.[72, 45, 492] Biosecurity is enhanced compared to marine and FTS systems. Influent water can be controlled and disinfected, which decreases likelihood that farmed fish will be exposed to pathogenic or chemical agents present in raw water sources or present in wild aquatic animal

populations. Escapes of cultured fish are unlikely and effluent water can be treated, which decrease potential impacts to wild fish populations and the environment.[45]

Disadvantages include high start-up capital, infrastructure, and operating costs; complex machinery; and high electrical energy requirements.[73, 74, 72] Staff must be highly trained in operational safety and systems controls. Equipment and operation must be well defined and standardized within a facility.[72] According to some authors, solids management, biofilter operation/management comprise, incomplete systems disinfection capability, and inappropriate or poor in-line systems design and engineering are the most common causes for RAS compromise or failures.[73, 72, 75] RAS systems consume more energy and generate greater levels of greenhouse gas emission compared to FTS aquaculture systems.[73, 76]

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.[77] 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.[77, 78] The Food and Agriculture Organization of the United Nations (FAO) Legal papers Online: Aquaculture Regulatory Frameworks[79] 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 (Appendix, Table 1).[45, 80] 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".[81, 78] 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.[82, 78] 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 constraints affecting aquaculture.[83, 84, 78] 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.[78]

Federal agencies with aquaculture regulatory oversight include:[45, 78, 80]

- Department of Health and Human Services, Food and Drug Administration (FDA),
- Environmental Protection Agency (EPA),
- National Oceanic and Atmospheric Administration (NOAA),
- United States Army, Corps of Engineers,
- United States Coast Guard (USCG),

- United States Department of Agriculture, Animal Plant and Health Inspection Service (USDA APHIS),
- United States Department of the Interior, Bureau of Ocean Energy Management,
- United States Fish and Wildlife Service (USFWS).

Marine farms must comply with regulations found in the:

- Clean Water Act,
- Endangered Species Act,
- Fish and Wildlife Coordination Act,
- Magnuson-Stevens Fishery Conservation and Management Act,
- Marine Mammal Protection Act,
- National Environmental Policy Act,
- National Marine Sanctuaries Act.

Federal agencies and regulations specific to inland aquaculture include many of same agencies, excluding those specific to marine aquatic systems.[85, 86, 87, 88, 89, 90, 80, 78]

State, within State (county and local), and Tribal governments regulate aquaculture activities that are permitted or licensed at the community level (Appendix, Table 1).[91, 92] 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.[91, 93, 78] 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.[91, 93, 78] State agencies that provide regulatory oversite include, but may not be limited to, State Departments of Agriculture, Fish and Wildlife, and Natural Resources.[94, 78, 92] Some States may require development of aquaculture-specific best management practices designed to enhance farm biosecurity, production, and minimize environmental impacts.[95, 96]

Regulatory Information Associated with International Trade

The World Organisation for Animal Health

The WOAH Aquatic Animal Health Code describes international standards for protecting aquatic animal and public health.[97] Standards related to the establishment of restrictions designed to prevent introduction of animal health hazards by importing countries, the status of exporting countries, zones, or compartments, and pathogen specific import/export recommendations are included in these provisions.[97] Import/export guidelines specific to the six pathogens included in this document are located in the Appendix, WOAH Pathogen Specific Import/Export Recommendations.

WOAH standards are based on the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement)(Appendix, Table 1).[98, 99] 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 do 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.[98, 99] The United States is a WOAH and WHO Member.

The United States

Import Information

The USFWS oversees importation of live and dead salmonid fish, fertilized eggs, and gametes (Appendix, Table 1).[100, 101] Fish, including salmonids, are defined by USFWS as wildlife. This definition identifies 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."[102, 103] Per the Lacey Act of 1900, importation and transportation of salmonid fish (live or dead), fertilized 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.[102, 104, 103] These designations place importation and transportation of live salmonid fish, fertilized eggs, and gametes under the purview of USFWS which issues permits under wildlife laws and treaties at international, national, and regional levels.[100]

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.[102] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[102] Shipments must be accompanied by a U.S. 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 the 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)(Appendix, Table 1)(Table 6).[100]

The USFWS does not require testing of imported live salmonids for epizootic haematopoietic necrosis virus (EHNV), *Gyrodactylus salaris*, infectious salmon anemia virus (ISAV), or salmonid alphavirus (SAV) prior to entry into the United States. However, Title 50, part 16.13 does require health certification of live or dead uneviscerated fish from the family Salmonidae, including pathogen testing by viral cell culture, prior to import. Both EHNV and SAV are cultivatable in the cell lines utilized for import health certification. It is within the purview of USFWS to decline an importation request for live salmonid fish, fertilized eggs, and gametes based on assessments of risk for a disease not listed in Title 50 on a case-by-case basis.[105] The USFWS does require that all fertilized salmonid eggs be disinfected within 24 hours prior to shipment using specific protocols described in *CFR,* Title 50.[102] Water and ice used for shipping must be derived from pathogen-free water and must be disposed of according to specific protocols.[102]

Imported live salmonid fish, fertilized 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.[102, 104, 103] Upon release, live fish, fertilized eggs, and gametes may be transported and possessed in captivity without a

permit.[102] In the absence of such documentation, shipments are not released, and the fish, fertilized 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.[102, 104] The live fish, fertilized eggs, and gametes may not be released into the wild, except by a State wildlife conservation agency or persons with prior written permission.[102] Links to relevant information associated with USFWS regulations are found in Appendix, Table 1.

USDA APHIS requires import permits for live fish, fertilized eggs, and gametes from species susceptible to spring viremia of carp virus (SVC) and Tilapia Lake virus (TiLV) (Appendix, Table 1).[106] There are currently no USDA APHIS regulations or recommendations specific to the international import or interstate movement of live salmonid fish, fertilized eggs, or gametes specific to the six pathogens described in this assessment.[107]

Table 6. Summary of USFWS and USDA APHIS regulatory oversight relative to importation of live salmonid fish, fertilized eggs, and gametes into the United States relative to epizootic haematopoietic necrosis virus (EHNV), Gyrodactylus salaris, infectious hematopoietic necrosis virus (IHNV), infectious salmon anemia virus (ISAV), salmonid alphavirus (SAV), and viral hemorrhagic septicemia virus (VHSV)

Pathogen	USFS	USDA APHIS
Epizootic haematopoietic necrosis virus (EHNV)	None*	None
Gyrodactylus salaris	None	None
Infectious hematopoietic necrosis virus (IHNV)	Yes	None
Infectious salmon anemia virus (ISAV)	None*	None
Salmonid alphavirus (SAV)	None*	None
Viral hemorrhagic septicemia virus (VHSV)	Yes	None

*Would be detected by the diagnostic testing methods (virus isolation/cell culture) required for IHNV and VHSV testing.

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.[102, 108, 104, 109, 100] USDA APHIS has negotiated international export health certificates, completed by an accredited veterinarian and endorsed by an USDA APHIS Veterinary Services area office, for shipments of live salmonid fish, fertilized eggs, and gametes with many countries.[110] Many of these countries require pathogen freedom testing for one or all the pathogens described in this assessment (Appendix, Table 2). Country specific exportation requirements for Aquaculture/Aquatic Animals may be accessed on the USDA APHIS International Regulations (IREGS) website (Appendix, Table 1).[110, 109]

State Import and Export Information

USFWS and USDA APHIS do not have inter- or intra-state regulations or recommendations specific to the movement of live salmonid fish, fertilized eggs, or gametes. State, within State, and Tribal governments may have importation regulations, including requirements for aquatic

animal health, import requirements, and disease freedom testing; however, regulation and requirements among these entities may vary (Appendix, Table 1). Information pertaining to the inter- and intra-state movement of live salmonid fish, fertilized eggs, and gametes may be accessed via the State Departments of Agriculture, State Departments of Natural Resources (or similar agencies), or the State Veterinarian.[111, 109]

The National Aquaculture Health Plan and Standards

The National Aquaculture Health Plan and Standards (Appendix, Table 1) was released by the USDA APHIS in 2021. This document is not a regulatory document but is intended to benefit the nation's aquaculture health by establishing guidance for national disease reporting, laboratory and testing standardization, surveillance, response, biosecurity, data management, and education and training. Additionally, it outlines health inspection options to provide consistent, verifiable methods to establish, maintain, and certify the health and safety of aquatic livestock, and lists actions USDA will employ to implement the plan. USDA will continue to collaborate with the National Oceanic and Atmospheric Administration (NOAA), the USFWS, and other Federal, State, Tribal, and local entities to ensure the health of all aquatic animals in the United States. NAHP&S will be updated biennially by USDA in consultation with stakeholder partners. The current version provides guidance for 2021–2023.[106]

The Comprehensive Aquaculture Health Program Standards

The Comprehensive Aquaculture Health Program Standards (CAHPS) (Appendix, Table 1) is a voluntary nonregulatory framework established to facilitate trade and improve and verify the health of farmed aquatic animals produced in U.S. commercial aquaculture industry sectors. Principles of CAHPS may be used by veterinarians, industry, Federal, State, Tribal, and other regulatory and private stakeholders to guide a) strategies for early disease detection, surveillance, reporting, and response; b) control of aquatic animal pathogens (especially those listed by WOAH); and c) prevent pathogen dissemination via movement and trade of aquatic animals. Any aquaculture producer (public or private) may participate in this voluntary program.[112]

Economics Brief

A complete economic analysis of global, North American, and United States salmonid aquaculture is not within the scope of this assessment. Briefly, aquaculture is the fastest growing animal-based food production sector in the world due in part to the diversity of the sector (e.g., farmed species, farming systems, and farming environments). In 2018, approximately 622 species (e.g., 387 finfishes, 111 molluscs, 64 crustaceans, 7 frogs and reptiles, 10 miscellaneous aquatic invertebrates, and 43 aquatic plants) were reared in aquaculture systems.[113] In 2018, approximately 20.5 million people worked in aquaculture globally.[113]

From 2000 to 2018, aquaculture production in fresh, brackish, and marine water increased at a compounded annual growth rate of 5.7%, 7.7%, and 5.2%, respectively (total growth rate of total aquaculture production = 5.6%). Marine aquaculture contributed the most to global production (55.5 million tonnes), while freshwater finfish had the highest production (46.0 million tonnes). In 2020, aquaculture accounted for production of 86 million tonnes of fish destined for human food production. Comparatively, wild fisheries accounted for 73 million tonnes of fish.[37]

The human population is estimated to grow globally at a rate of 9% from 2020 – 2029, resulting in an increasing need for sustainable protein production.[37] During this period, the FAO estimates

that per capita consumption of aquaculture products will increase by approximately 4%.[37] Per capita consumption rate increases are expected to be greatest in developing countries. In developed countries the per capita volume of aquaculture products is expected to increase.[37] Aquaculture production is expected to increase by approximately 12% during this period because the global supply for all seafoods is shifting toward an aquaculture-based paradigm as wild fisheries are stagnating or declining.[37] By 2029, it is estimated that global aquaculture will be supplying an additional 20 million tonnes of product.[37]

The United States is the largest importer of all fish and fishery products due to low domestic production and supply. In 2018, 90% of the seafood products consumed in the United States were imports (approximate value $23.7 billion USD). Approximately 50% of those seafood products were aquaculture reared.[45, 19] In contrast, United States exports of fish and fishery products were valued at approximately $6 billion USD.[45] Channel catfish is the dominant cultured finfish in the United States, followed by Atlantic salmon and rainbow trout.[45]

The expected expansion of domestic salmonid aquaculture production will require increased production of hatchery-reared fish, fertilized eggs, and gametes, increased investment in aquaculture facilities and supportive infrastructure, and will likely promote job creation and local, regional, and national economic development. Globally and domestically, there has been significant interest in technological advancements to support increased inland aquaculture production.[45] In 2020, there were at least four Atlantic salmon producers in Canada and the United States exploring RAS aquaculture with the intention to expand production from 72 tonnes to 600 tonnes of product.[45] By 2022, approximately $2 billion USD has been invested in inland RAS Atlantic salmon aquaculture. In some states, such as Maryland, investments of up to $1 billion USD are predicted through 2024. Large-scale projects for inland rainbow trout and steelhead trout production have also been described.[55, 45]

Atlantic Salmon

In 2018, countries producing the greatest volume of farmed Atlantic salmon in order of proximate volume included Norway, Chile, the United Kingdom, Canada, the Faroe Islands, Australia, Russia, the United States, Iceland, and Ireland (Table 2).[47] In 2022, top exporters of edible Atlantic salmon product in order of proximate value included Norway, Sweden, Chile, Canada, the United Kingdom, Denmark, the Faroe Islands, Australia, Iceland, Finland, Switzerland, the Netherlands, the United States, France, and Germany (Table 7).[114]

Table 7. Top Atlantic salmon (edible product) exporting countries in 2022 in order of proximate value[114]

Top Exporting Countries in 2022	Value (USD)	Percentage of total value
Norway	$8.2 billion	47.6%
Sweden	$3.8 billion	22.0%
Chile	$1.0 billion	6.1%
Canada	$722.3 million	4.2%

United Kingdom	$715.7 million	4.2%
Denmark	$566.1 million	3.3%
Faroe Islands	$502.2 million	2.9%
Australia	$285.5 million	1.7%
Iceland	$285.0 million	1.7%
Finland	$245.4 million	1.4%
Switzerland	$187.7 million	1.1%
Netherlands	$184.6 million	1.1%
United States	$177.4 million	1.0%
France	$130.3 million	0.8%
Germany	$76.1 million	0.4%

In 2022, top importers of edible Atlantic salmon product in order of proximate value included Sweden, the United States, Poland, France, Denmark, Brazil, China, the United Kingdom, Italy, Spain, German, the Netherlands, Finland, South Korea, and Lithuania (Table 8).[114]

Table 8. Top Atlantic salmon (seafood product) importers in 2022 in order of proximate value[114]

Top importing countries in 2022	**Value (USD)**	**Percentage of total value**
Sweden	$3.8 billion	23.7%
United States	$1.5 billion	9.2%
Poland	$1.4 billion	8.9%
France	$1.2 billion	7.5%
Denmark	$936.9 million	5.8%
Brazil	$746.2 million	4.6%
China	$745.3 million	4.6%
United Kingdom	$683.9 million	4.2%
Italy	$516.0 million	3.2%

Spain	$512.6 million	3.2%
Germany	$478.1 million	3.0%
Netherlands	$467.3 million	2.9%
Finland	$387.8 million	2.4%
South Korea	$313.2 million	1.9%
Lithuania	$302.8 million	1.9%

Relative to the global salmonid industry (all salmonid species), the produced volume of aquaculture-reared human consumable product surpassed that of wild fisheries in 1999.[37] In 2020, the total global supply of all farmed salmonids exceeded 2.57 million tonnes, while the total catch volume of wild salmonids totaled approximately 0.51 million tonnes. Chum, pink, and sockeye salmon were the predominant species in the wild catch volume.[37]

Presently, Atlantic salmon comprises over 50% of the total global salmon market. Approximately 80% of this volume is farmed.[37] Worldwide, Atlantic salmon production increased by 7% per year from 1995 to 2010 and by 6% per year from 2011 to 2020.[64, 37, 36] Projected future global growth from 2020 to 2024 is 4% annually, and future harvest volumes are estimated to exceed 1 million tonnes of product per year.[37] Demand is projected to increase in the United States, the Asia Pacific region, Brazil, Germany, Italy, Russia, and the United Kingdom.[37, 115]

The United States is the largest global consumer of Atlantic salmon (over 450,000 tonnes in 2018). To meet this demand, most product is imported from Chile, Norway, and the United Kingdom.[37] Consumer demand is greatest for fresh (filleted, steaked, whole), frozen, and smoked products.[37] Factors driving the increased demand include the changing dietary habits of consumers, the health benefits associated with eating salmon, and rising consumer interest in sustainable, resource efficient, easily consumable food products.[116, 37]

The U.S. Atlantic salmon farming industry is the third largest aquaculture sector domestically but comprises only a small percentage of the global industry. In 2018, the total estimated economic impact of Atlantic salmon production in the United States was over $1 billion USD.[117, 64] It has been estimated that future production could increase by 3,500% over 2018 levels as inland salmon farming is developed.[64] In the United States, Atlantic salmon have historically been farmed in marine net pens in Maine and Washington. However, following a large farmed Atlantic salmon escape, culture of Atlantic salmon was banned in Washington in 2018.[45] Virtually all current production occurs along the coastal shoreline of Maine. Fish are primarily reared for consumption; however, a small percentage of fish are reared for recreational stocking or conservation efforts.[45]

Rainbow Trout and Steelhead Trout

Countries producing the greatest volume of freshwater farmed rainbow trout in 2018 in order of proximate volume are listed in Table 9.[53] Top steelhead trout producing counties in 2018 in order of proximate volume are noted in Table 10.[53]

Table 9. Top freshwater rainbow trout producing countries in 2018, in order of proximate volume produced[53]

Top Producing Countries	Production (tonnes)	Share of Global production
Iran	173,384	32.7%
Turkey	103,192	19.5%
Peru	55,030	10.4%
China	38,606	7.3%
Russia	35,204	6.6%
Italy	32,825	6.2%
France	26,100	4.9%
Colombia	23,038	4.3%
United States	22,370	4.2%
Denmark	20,000	3.8%

Table 10. Top steelhead trout producing countries in 2018, in order of proximate volume produced[53]

Top Producing Countries	Production (tonnes)	Share of Global production
Chile	78,255	41.4%
Norway	68,216	36.0%
Finland	11.119	5.9%
Denmark	9.737	5.1%
Turkey	9.235	4.9%
Iran	6,300	3.3%
United Kingdom	3,500	1.8%
Sweden	2,870	1.5%

Review of the literature did not identify any resources summarizing the volumes of imported and exported edible rainbow trout and steelhead trout products, as described above for Atlantic salmon. This may be related to the aggregation of rainbow trout and steelhead trout product with that of other trout species and the practice by some countries (e.g., China, Turkey) to label edible trout products as “salmon.”

In the United States, rainbow trout and steelhead trout production data are typically aggregated with data for other farmed trout species.[48, 55] Because freshwater rainbow trout constitutes the bulk of domestic trout production, the aggregated data may still be used to approximate production statistics.[55] In 2000, the total value of all aggregated trout sales (live fish, fertilized eggs, and gametes) was approximately $75.8 million USD.[55] Live fish and fertilized eggs from State and Federal hatcheries reared for conservation, recreation, and restoration purposes accounted for approximately 80% of that value ($60.9 million USD).[55] Idaho was the leading trout producing state (53% of total value of trout sold), followed by Pennsylvania, North Carolina, and California.[55]

Freshwater rainbow trout production is the second largest aquaculture sector in the United States. Outputs of freshwater rainbow trout aquaculture include food production; stocking for conservation, recreation, and restoration purposes; and domestic and international sales to other hatcheries or farms.[42] Most of the rainbow trout reared domestically for human consumption is sold within the United States. The exported volume of fresh and frozen rainbow trout product in 2012 was 807.2 million tonnes valued at $5.8 million USD.[48, 55] Canada is the leading importer of U.S. sourced rainbow trout (98% of volume exported).[48, 55]

Imports of edible rainbow trout products have been increasing. In 2012, the value of imported rainbow trout ($72 million USD) was proximate to that of domestic production.[48, 55] Approximately 80% of 2012 imports were from Chile and Canada ($49.9 and $8.6 million USD, respectively).[48, 55] In 2015, the value of imports ($104 million USD) surpassed the value of domestic production ($96.5 million USD). Most of the imports (80%) were from Chile and Norway ($65.2 and $19.4 million USD, respectively).[48, 55]

Hazard Identification

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.[118] 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).[118] 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.[118]

In this assessment, six pathogens of concern to Atlantic salmon and rainbow trout aquaculture stakeholders constitute the hazards reviewed. Assessment of all potential pathogens that might be present in imported live salmonid fish, fertilized eggs, and gametes is not within the scope of this document.

Epizootic Haematopoietic Necrosis Virus

Introduction

Epizootic haematopoietic necrosis (EHN, Nillahcootie redfin virus, Redfin virus) is a disease present in Australia that affects redfin (European) perch (*Perca fluviatilis*) and freshwater rainbow trout. The WOAH Manual of Diagnostic Tests for Aquatic Animals (Appendix, Table 1) defines EHN as disease caused by infection with genomically identified epizootic haematopoietic necrosis virus (EHNV, Family Iridoviridae, genus *Ranavirus*) specific to Australia.[119, 120, 121, 122, 5, 97]

EHN is a foreign animal disease in the United States and is included in the USDA APHIS National List of Reportable Animal Diseases (NLRAD) and National Animal Health Reporting System (NAHRS) lists of reportable diseases (Appendix, Table 1).[1, 123] All animal health professionals, including accredited veterinarians, should coordinate with their State Animal Health Official and Area Veterinarian in Charge (AVIC) upon suspicion or confirmation of NLRAD listed diseases. Confirmed cases of NLRAD disease should be reported in accordance with NLRAD Standards. Reporting under NLRAD does not supersede State requirements or notification processes for foreign animal emerging disease incidents or other regulated/high-priority endemic disease reporting requirements (Appendix, Table 1). EHN is a WOAH listed notifiable disease.[124, 125] Disease notification requirements and requirements for self-declaration of freedom of EHNV infection for Member nations are found in the WOAH Aquatic Animal Health Code, Chapter 2.3.1 (Appendix, Table 1).[126, 120, 4, 97] EHN is listed as an exotic disease by the European Union health directive and is a reportable disease in Canada.[127, 128]

Susceptible Fish Species

Fish species identified by WOAH as susceptible to EHNV are summarized in Table 11.[118, 129] In the United States, rainbow trout are the farmed salmonid species of greatest economic concern relative to infection with EHNV.

Table 11. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to epizootic haematopoietic necrosis virus (EHNV).[118, 129]

Genus species	Common Name
Ameiurus melas	Black bullhead
Bidyanus bidyanus	Silver perch
Esox lucius	Northern pike
Galaxias olidus	Mountain galaxias
Gambusia affinis	Mosquito fish
Gambusia holbrooki	Eastern mosquito fish
Macquaria australasica	Macquarie perch
Melanotaenia fluviatilis	Crimson spotted rainbow fish

Oncorhynchus mykiss	Rainbow trout
Perca fluviatilis	European (redfin) perch
Sander lucioperca	Pike-perch

Geographic Distribution

EHN is endemic only to Australia (Appendix, Table 3).[124, 126, 125, 130, 129] In 1986, the disease emerged in wild redfin perch in New South Wales and subsequently spread to other wild redfin perch populations in the Australian Capital Territory, Victoria, and South Australia.[126, 131, 120] Outbreaks of EHN have also occurred in farmed rainbow trout in New South Wales.[124] EHN has remained contained within these regions, causing discontinuous, discrete outbreaks followed by long lapses in occurrence.[125] A search of the WOAH World Animal Health Information System (WAHIS, Appendix, Table 1) database for years that data were available (2005 to 2021) identified reports of EHNV presence in Australia and in Kuwait in 2009–2012 (this occurrence could not be verified via a search of scientific literature).[129]

According to the European Union Reference Laboratory for Fish and Crustacean Diseases, EHNV has never been detected in Europe.[125, 128, 129] Additionally, EHNV has never been detected in North America, including the United States.[127, 128, 129] Review of the literature did identify manuscripts describing detection of fish iridioviruses in North America (e.g., white sturgeon iridovirus[132] and Santee-Cooper ranavirus[133, 134]). Santee-Cooper ranavirus includes three virus strains (doctor fish virus, DFV; guppy virus 6, GV-6; and largemouth bass virus, LMBV) which were originally described as viruses similar to EHNV and European catfish virus (ECV).[132, 135, 136, 137, 133, 138, 139, 140] These viruses are genetically distinct from EHNV.

Public Health

EHNV is not a zoonotic pathogen. There are no threats to human health.[124, 141]

Epidemiology

In this section, the epidemiology of EHN in the natural host species (e.g., redfin perch, rainbow trout) is summarized. In general, many environmental, pathogen, and host factors of EHNV susceptibility among fish species are poorly described or understood.

Host Characteristics

Redfin perch

Redfin perch are highly susceptible to EHNV infection. The disease is highly fatal in juvenile fish compared to adults.[142, 120] Infection of fertilized eggs and early life stages (larvae and fry up to approximately 5 g in weight) are not reported in published literature or the WOAH Manual of Diagnostic Tests for Aquatic Animals.[5] Initial emergence of EHN in this species lead to collapse of the recreational redfin perch fishery and caused severe economic losses when outbreaks occurred in redfin perch aquaculture.[143, 124] Experimental challenge studies have demonstrated high rates of susceptibility following a low virus dose challenge of 0.08 $TCID_{50}$ mL^{-1} (50% tissue culture infective dose per milliliter) via immersion bath or intraperitoneal inoculation.[144, 126] Disease outbreaks in wild populations are often followed by years of disease absence.[145] The epidemiology of this pattern of disease occurrence is not fully described but appears related to the dynamic relationship between host population density and environmental conditions optimal

for host-virus interaction.[146, 147, 130] High mortality (95%) outbreaks have been observed during the summer in fingerling and juvenile perch, while adult fish are largely unaffected.[148, 143, 124, 125] Factors contributing to the high mortality in young perch may include the inability of young fish to mount adequate immune responses to EHNV and behavioral differences between young and adult fish. Young perch tend to reside and feed in shallow warm waters which may increase susceptibility to EHNV infection. Adult fish typically reside and feed in deeper, cooler waters.[146, 143, 126, 120, 141]

There is lack of sufficient data to fully describe the potential transmission capability, duration of subclinical infection, or carrier status in wild perch.[149, 150, 125, 130, 120] Virus has been infrequently isolated from wild juvenile and adult perch following natural disease outbreaks.[150, 125, 130, 141] In experimental studies, both resistance to reinfection and lack of isolation of EHNV from individual fish after experimental challenge have been reported.[149, 150, 130, 141] If wild perch are capable of functioning as subclinical carriers, this could contribute to the spread of EHNV and the irregular occurrences of EHN outbreaks in natural water systems.[125] Differences in susceptibility between Australian and European redfin perch stocks following experimental challenge have been described in the literature.[151, 152, 150, 125, 120] It is unknown if this reflects differences in the fish stocks or factors related to the design of the studies (e.g., the challenge strain and dose of EHNV used).[151, 125, 130, 120]

Rainbow Trout

The epidemiology of EHN in rainbow trout is not fully described.[147, 141] Infection can occur at all ages; however, in general rainbow trout appear to be relatively resistant to EHNV infection and the resulting disease is less severe that than observed in redfin perch.[124, 5] Infection of fertilized eggs and early life stages (larvae and fry up to approximately 5 g body weight) are not reported.[97] In experimental studies, the immersion bath and intraperitoneal challenge dose required to infect rainbow trout ($1 \times 10^{2.2}$ $TCID_{50}$ mL^{-1}) was greater than that required to infect redfin perch.[144, 126] Typically, only a small proportion of individuals in a population become infected or develop clinical disease.[146, 147, 142] In farmed trout, disease surveillance strategies that incorporate routine moribund sampling would improve detection of EHNV due to the low number of fish exhibiting clinical signs of illness and the low level of mortality observed in this species.[126, 131] Clinical disease is most commonly observed in young fingerlings (up to 125 mm fork length),[124, 120] and is rarely observed in grower and broodstock fish.[147] Rates of EHNV detection via virus isolation during outbreaks in farmed trout range from 60% – 80% in moribund and dead fish, and 0%–4% in clinically normal appearing fish.[146, 147, 126, 120] Post-outbreak, surviving fish appear to develop long-lasting immunity and virus is rarely detected.[146, 147, 120] There is a lack of consensus among researchers regarding the presence of a carrier state in naturally infected rainbow trout.[153, 154, 155, 120] Anti-EHNV antibodies have been detected at low prevalence (0.2% – 3.7%), which some authors suggest indicate the capability of surviving fish to function as carriers.[147, 120, 145] A literature search did not identify any reports or transmission studies verifying that trout surviving EHNV infection were capable of infecting other fish.

There is currently no evidence that an amphibian reservoir exists.[125] It is currently unknown if other reservoir hosts (other aquatic animals, vectors) may maintain EHNV presence in aquatic environments.[125, 130]

Environmental Characteristics

Environmental factors that appear related to outbreaks of EHN in endemic areas include seasonal variation in water temperature and quality, as well as other factors. Outbreaks in wild redfin perch tend to occur at intervals lasting two to three weeks during summer months, and appear associated with the above described environmental factors and food availability which affect behavior.[146, 120, 145] In farmed rainbow trout, EHN occurrence is associated with environmental factors such as high stocking rates, low water quality and exchange rates, sudden changes in water temperature (low to high), water temperatures ranging from 11 – 20 °C/51.8 – 68 °F, and concomitant presence of parasitic, protozoal, fungal, or systemic bacterial infections.[146, 147, 124, 126, 145]

The incubation periods for EHNV are inversely proportional to water temperature in fish challenged by intraperitoneal injection.[125] The incubation period for redfin perch ranged from 10 – 28 days at 12 – 18 °C/53.6 – 64.4 °F and 10 – 11 days at 19 – 21 °C /66.2 – 69.8 °F.[144, 124, 150, 125, 120] In rainbow trout, the incubation period ranged from 14–32 days at 8 –10 °C/46.4 – 50 °F and 3 –10 days at 19 – 21 °C/66.2– 69.8 °F.[144, 124, 126, 125, 120] Viral replication in infected fish is also temperature dependent. Peak viral replication was documented in experimentally infected northern pike (*Esox lucius*) at 3 and 7 days post-experimental challenge via bath exposure at 22 °C/71.5 °F and 12 °C/53.6 °F, respectively.[156, 125]

Pathogen Characteristics

Environmental persistence of EHNV appears to be a key factor in the epidemiology of disease occurrence.[143] Under natural conditions, EHNV appears to be highly resistant to drying. According to WOAH, for these reasons it should be presumed that EHNV is capable of persisting for months to years on fish farms in water and sediment, and plausibly on plants, equipment and other fomites.[153, 124, 126, 97] Experimentally, the virus remains environmentally stable in distilled water for 97 days. [153, 124, 126] Infectivity persists for approximately 97 and 300 days in water stored at 15 °C/59 °F and 4 °C/39 °F, respectively, 110 days in dried fish tissues, 113 days in dried tissue culture spots stored at 15 °C/59 °F, and for over 300 days in cell cultures stored at 4 °C/39 °F. [153, 124, 126] Viability has been documented for two years in fish tissues frozen at -20 °C/-4 °F and for approximately one year in frozen fish carcasses.[124, 120, 5]

Transmission

Factors associated with transmission are not fully understood. According to the literature, EHNV enters the water column from carcasses and the tissues of infected fish.[125, 130, 120, 157] Movement of EHNV suspended in water and via movement of infected redfin perch are thought to be methods of local and regional spread in rivers, lakes, and ponds.[143, 158, 11, 125, 12, 120] Under natural conditions, EHN outbreaks have been documented in rainbow trout farms using influent water sourced from areas where infected redfin perch were present.[146, 147, 159, 125] Movement of virus suspended in water and the presence of subclinically infected individual fish are thought to be the primary methods of disease spread in farmed rainbow trout.[143]

Horizontal transmission has been documented experimentally via immersion bath.[153, 126, 150, 120] Potential modes of horizontal transmission include oral ingestion of virus present in water and the tissues of infected or dead fish, and contact exposure via the gills or skin lesions.[144, 124, 126, 125] Anthropogenic transmission of EHNV via translocation of subclinically infected fish for aquaculture purposes and the accompanying transport water has been described as a route of introduction into trout farms.[142, 159, 125, 120, 141] Activities of recreational fishermen (use of raw or

frozen/thawed fish bait, movement of live fish and transport water) have been implicated in introduction of EHNV to susceptible wild fish populations.[143, 147, 130] Other potential transmission routes include mechanical transmission via fomites (e.g., boats, fishing gear, farm equipment, clothing) and wildlife (e.g., piscivorous birds).[143, 126, 141] Vertical transmission has not been verified in the field or under experimental conditions. Langdon et al. (1987) was unable to isolate EHNV from wild redfin perch eggs or yolk-sac fry,[149] and EHNV has not been detected in rainbow trout ovarian tissues or broodstock.[124, 126, 120] According to the WOAH Manual of Diagnostic Tests for Aquatic Animals, infections in eggs and early life stages (larvae and fry up to approximately 5 g body weight) are not reported.[5]

Clinical Signs and Pathogenicity

Clinical signs are non-specific and may include abnormal swimming behavior at the water surface, abdominal distension, anorexia, darkened skin color, gill hemorrhages, lethargy, loss of equilibrium, opercula flaring, petechial (pinpoint) hemorrhages, reddening at the base of fins, and skin ulcers.[153, 144, 124, 125, 121] Most clinically affected fish die within a few weeks.[130] All ages are susceptible to infection; however, clinical signs are typically most apparent in fingerlings and juvenile fish.[124, 120] Adult fish are most likely to develop clinical signs when the disease is first introduced into a naïve population.[121]

Clinical signs of EHN are often inapparent or observed at low frequency in naturally infected farmed rainbow trout.[146, 147] Development of disease in this species is often associated with poor husbandry (e.g., high stocking density, poor water quality) and the presence of concurrent disease (e.g., external parasites, focal and systemic bacterial infections).[146, 147, 126] Differential diagnosis for EHN include toxicities and other bacterial, fungal, parasitic, or viral pathogens that cause non-specific signs of illness.

Morbidity and Mortality

In naïve redfin perch, rates of infection, morbidity, and mortality in natural outbreaks can approach 95%.[148, 149, 143, 124, 129] In populations where the disease is recurrent, the highest rates of morbidity and mortality are observed in fingerling and juvenile fish, while adult fish are rarely affected.[143, 126] In rainbow trout, under natural farm conditions, the infection rate is low. When individual fish do become infected, they typically die from the disease. Infection is often present on a farm but goes unnoticed because the low infection rate leads to low daily observed morbidity and mortality rates that are within expected standard loss rates (0.2% daily mortality, up to 4% total mortality).[147, 124, 126, 125, 120] Mortalities are most common in fingerlings (up to 125 mm fork length and 500 g body weight) and can reach 90% in this age group.[160, 153, 146, 147, 125] Infection has not been confirmed in broodstock.[146, 147, 129]

Treatment

There is no treatment or vaccine.[120] There have been no formal EHNV resistance breeding programs for redfin perch or rainbow trout.[125, 120]

Diagnostic Testing

EHNV infects a wide range of cell types, including the hematopoietic cells, hepatocytes, and the endothelial cells of organs. Target organs include kidney, liver, and spleen. Capability of EHNV to infect reproductive tissues (i.e., gonadal tissues, milt, ovarian fluid) and the suitability of these tissues for surveillance of EHNV in broodstock is unknown.[120]

Gross lesions may not be present in some affected fish. When present, lesions are more often observed in redfin perch[124] and may include gastrointestinal ulceration; pale focal necrosis in the

liver; petechial hemorrhages or redness at the base of the fins; petechial hemorrhages on the viscera; serosanguineous peritoneal effusion; and swelling of the kidney, spleen, and/or liver.[124, 125, 121] The spleen may also appear small and pale.[124] Histological lesions include basophilic intra-cytoplasmic inclusion bodies in hepatocytes adjacent to necrotic foci in the liver; fibrinous exudate; hemorrhage, hyperplasia, multifocal necrosis, and thrombosis in gill tissues; focal to extensive necrosis in the hematopoietic kidney, liver, spleen, heart, lamina propria of the intestine, and pancreas; and degenerate vascular endothelial cells and necrotic hematopoietic cells in organs and blood vessels.[119, 159, 125, 130, 141]

Clinical signs and gross pathological lesions caused by EHNV infection are not pathognomonic. Definitive diagnosis must be confirmed using laboratory diagnostic assays that utilize genomic sequencing to differentiate this EHN from disease caused by other closely related iridoviruses.[120] Direct diagnostic test methods include histological examination of fixed tissues, polymerase chain reaction (PCR) and genetic sequencing, and virus isolation (VI) in cell culture.[5] In the United States, Title 50 diagnostic testing methods include use of virus isolation/culture methods using cell lines sensitive to EHNV infection.

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.[120, 5, 97] Briefly, because infection can go unnoticed due to low daily and total mortality rates, surveillance sampling should be focused on moribund fish and fresh mortalities.[97] This testing strategy is also encouraged by USDA APHIS Comprehensive Aquaculture Health Program Standards (CAHPS) (Appendix, Table 1). In the United States, confirmatory testing at the USDA National Veterinary Services Laboratory (NVSL) is required following first detections (Appendix, Table 1). All suspected EHNV detections or outbreaks of ENV are reportable to USDA APHIS VS as 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.[161]

Prevention and Control

Biosecurity measures are the most important control measures available to prevent the introduction and spread of EHNV through infected fish, including apparently healthy carriers and survivors of disease outbreaks.

Recommended import biosecurity measures include pre-import certification of live fish, fertilized eggs, and gametes, and their source for EHNV freedom.[125] Under some circumstances, countries may implement more stringent measures. For example, Australia enforces quarantine restrictions on the importation of some fish species because of the risk of EHNV introduction.[125, 141] Suggested farm biosecurity measures include sourcing fish stocks only from sources demonstrated to be free from EHNV.[125] On farm quarantine should be a standard practice for all incoming fish.[125]

Water is one of the most common introductory pathways of aquatic pathogens into aquaculture establishments.[158, 11, 12] Therefore, disinfection of influent water is recommended to prevent exposure of farmed fish to pathogenic agents.[125] Treatment of effluent water prior to release is recommended to prevent downstream exposure of susceptible aquatic animals (including downstream farmed fish) to pathogenic agents. Additional disease control measures include maintaining good husbandry practices and optimal environmental conditions (water quality, flow,

and temperature) and minimizing physiological stressors (bacterial and fungal pathogens, external parasites, high stocking densities, inadequate nutrition).[146, 147, 126, 120, 162]

EHNV is highly resistant to drying, desiccation, and many of the physical conditions and chemical agents used to inactivate other aquatic viruses.[153, 124, 126] Equipment should be thoroughly cleaned to remove biofilms and debris prior to disinfection.[124] The virus is temperature tolerant across a wide thermal range, but is inactivated when heated to 40 °C/104 °F for 24 hours or 60 °C/140 °F for 15 minutes.[153, 125] On dry surfaces, the EHNV is resistant to sodium hypochlorite, but can be inactivated by application of 70% ethanol for 120 minutes wet contact time.[124, 120] On wet surfaces or in liquid suspensions, sodium hypochlorite (200mg/L) and other disinfectants in the 4.0 to 12.0 pH range (chlorhexidine 150 mg/mL for 1 minute; potassium peroxymonosulfate (Virkon®) 200 mg/L) are effective.[124, 126, 125] Ultraviolet sterilization units may have some inactivation efficacy.[141] According to the literature, lime may be used to disinfect earthen ponds or raceways.[124] Disinfection protocols for fertilized eggs and larvae have not been validated.[120]

Prevention and control measures should include development of risk-based surveillance strategies for susceptible farmed and wild fish populations, and contingency plans for EHNV containment and eradication if introduction occurs. Whittington et al. (2009) recommends sampling of unexpected mortalities in redfin perch and “routine” mortalities of rainbow trout, instead of random samples of live fish.[131, 125] According to Whittington, given the low prevalence of pathogen prevalence in apparently healthy subclinically infected fish, certification practices based on random sampling of apparently healthy fish may lead to misclassification of the population as EHNV-free.[131] WOAH recommends that Members consider use of passive surveillance strategies to identify zones free from infection to facilitate the trade of live fish.[125, 97]

In the United States, EHN is a reportable foreign animal disease. Reporting of EHNV is required under USDA APHIS NLRAD and WOAH notifiable disease reporting requirements.[1] If EHNV is suspected or detected via diagnostic testing, the State veterinarian and Federal veterinary officials should be contacted, and samples collected and submitted under the guidelines provided by the NVSL.[1] Control measures utilized by USDA APHIS may include controlling the movements and humane destocking of infected farmed fish, and cleaning, disinfection, and quarantine of affected premises according to WOAH protocols.[124, 120, 97] Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of EHN introduction. A summary of WOAH import/export guidelines specific to EHN, U.S. regulations, and other regulatory information related to aquaculture in the United States is summarized in the Appendix, WOAH Pathogen Specific Import Export Recommendations.

Summary

EHNV is a WOAH-listed viral disease affecting wild and farmed redfin perch and farmed rainbow trout. The economic impact to redfin perch aquaculture can be high given the high rates of susceptibility and mortality in this species. The economic impact to the rainbow trout industry is relatively low, due to the low infection rate and level of morbidity that occurs in this species.[129] In Australia, where EHNV is endemic, control of disease occurrence is difficult in wild fish populations (redfin perch) once introduction has occurred. Farmed rainbow trout are less susceptible to EHNV infection, which may go undetected due to low associated rates of mortality. The impact that EHN may have on wild rainbow trout populations is not known.

In the United States, there are no Federal regulations specific to the import of live salmonid fish, fertilized eggs, or gametes and EHNV. Pre-import testing for EHNV is not specifically required for USFWS import health certification. However, EHNV 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 disinfection of fertilized salmonid eggs prior to import; however, there are no validated disinfection protocols for fertilized fish eggs relative to EHNV.[120] Suggested best practices to prevent introduction of EHN into rainbow trout aquaculture in the United States include development of policies that would ensure live fish, eggs, and gametes are imported from EHN-free sources and that importation complies with the guidelines described in the 2022 WOAH Aquatic Animal Health Code.[97] Information describing State and Tribal regulation of live salmonid fish, fertilized eggs, or gametes relative to EHNV that guide local aquatic animal health and import requirements is found in Appendix, Table 1.

The potential impact that EHNV introduction may have on the U.S. rainbow trout industry is unknown due to lack of susceptibility testing of native farmed rainbow trout in the United States, as well as other knowledge gaps and data deficiencies. The impact that EHNV introduction may have on wild or stocked rainbow trout populations is likewise unknown. The susceptibility of other farmed and wild fish species in the United States is also unknown. EHNV is a WOAH-listed reportable disease. Therefore, detection of EHNV in cultured or wild fish stocks in the United States would likely result in significant trade impacts.

Gyrodactylus salaris

Introduction

Gyrodactylus salaris is an environmentally and economically significant pathogen of wild Atlantic salmon and farmed rainbow trout in Europe. The WOAH Manual of Diagnostic Tests for Aquatic Animals (Appendix, Table 1) defines the disease gyrodactylosis as infection with the pathogenic trematode (flatworm) ectoparasite *Gyrodactylus salaris* (salmon fluke; Family Gyrodactylidae, class Monogenea, genus *Gyrodactylus*).[5, 97]

The genus *Gyrodactylus* is comprised of a large diverse group (approximately 400 species) of viviparous (live bearing) ectoparasites capable of parasitizing fish at high population densities for long periods of time.[163, 164, 18, 165] In general, *Gyrodactylus* spp. are considered obligate (requires a host to complete the life cycle) and host specific, parasitizing only one host or closely related host species.[165] Most *Gyrodactylus* spp. do not cause disease in host fish; however, clinical signs of disease, morbidity, and mortality are associated with some North American *Gyrodactylus* spp.[166, 165]

Gyrodactylus salaris was first described in the 1950s as an ectoparasite of Baltic Atlantic salmon which display tolerance to the parasite.[167, 168] In the 1970s, hatchery reared Baltic salmon were translocated to Norway, resulting in introduction of *G. salaris* into freshwater river systems.[169] By 2002, 45 river systems were infested, leading to catastrophic losses of native Atlantic salmon populations and highly impactful ecological and economic consequences.[170, 171, 172, 173] In some rivers, over 80% of juvenile Atlantic salmon may die due to infestation, and reductions in wild salmon fisheries catches have been estimated at over 40%.[174] In 2021, detections were reported in 51 rivers, 13 Atlantic salmon hatcheries or farms, and 26 rainbow trout hatcheries or farms.[175] Negative impacts on Atlantic salmon populations in rivers on the Swedish west coast and in the Keret river in Russia have been reported as well.[175] Detections of

pathogenic and non-pathogenic strains have also been detected on Arctic char (*Salvelinus alpinus*).[175]

Gyrodactylus salaris is a reportable disease in the United States and is included on the USDA APHIS NAHRS and NLRAD lists or reportable diseases (Appendix, Table 1).[1, 123] State and Federal authorities should be contacted upon suspicion of or detection of gyrodactylosis (Appendix, Table 1).[1] Gyrodactylosis is a WOAH listed notifiable disease.[97] Requirements for self-declaration of freedom from infection with *G. salaris* and maintenance of free status are described in the WOAH Aquatic Animal Health Code (Appendix, Table 1).[97]

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 *G. salaris* are found in Table 12.[5, 97] In the United States, migratory and landlocked Atlantic salmon and steelhead trout, and wild and farmed rainbow trout represent the species of greatest economic concern relative to gyrodactylosis.

Table 12. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infestation by *Gyrodactylus salaris*[5, 97]

Genus species	Common Name
Salvelinus alpinus	Arctic char
Salmo salar	Atlantic salmon
Salveinus fontinalis	Brook trout
Salmo trutta	Brown trout
Thymallus thymallus	Grayling
Onchorhynchs mykiss	Rainbow trout

Geographic Distribution

The native range extends from the eastern regions of the Baltic Sea to the Karelian isthmus and drainages of Onega and Ladoga lakes in Russia.[97, 18] The parasite may also occur naturally in low numbers in some Swedish and Finnish rivers that drain into the Baltic Sea.[176, 177] Introductions into other countries have occurred via import and translocation of infested fish. Search of the WOAH WAHIS database (Appendix, Table 1) for years that data was available (2005–2022) identified reports of *G. salaris* in unidentified farmed and wild fish species in Costa Rica, Finland, Norway, Sweden, and Vietnam.[129] Other countries reported in the literature include Denmark, Estonia, Georgia, Germany, Italy, Latvia, North Macedonia, Poland, Romania, Russia, and Ukraine.[177] There have been no reports of this parasite in North America, including the United States.

Public Health

Gyrodactylus salaris is not a zoonotic parasite. There are no threats to human health.

Epidemiology

In this section, the epidemiology of *G. salaris* in susceptible host species (e.g., salmonid fish) is summarized. However, all epidemiological factors associated with this parasite are not fully described in available literature or data sources.

Host Characteristics

Gyrodactylus salaris infestation has been most extensively studied in salmonid fish species. Occurrence of clinical disease has only been reported in Atlantic salmon. However, all salmonid species that inhabit freshwater, or migrate to and from the ocean, are considered potentially susceptible to infestation.[5, 97, 18]

Atlantic Salmon

Gyrodactylus salaris infestation occurs under natural conditions on Atlantic salmon that reside in or return to freshwater environments.[177, 129] All life stages are susceptible to infection. Prevalence and abundance of the parasite and development of clinical disease are greatest in fry and parr stages and may be related to lack of immunity to the parasite.[178, 97] Differences in susceptibility to infestation and development of clinical disease among Atlantic salmon populations has been observed.[171, 179, 172] Baltic Atlantic salmon are described in the literature as both tolerant (the host is unharmed with no direct negative effects on the parasite) and resistant (the host is protected at the expense of the parasite), likely due to co-evolution of the parasite and salmon population. [171, 179, 172] Atlantic salmon in other European regions are highly susceptible to infestation and development of clinical disease under natural and experimental conditions (e.g., Norway, Denmark, the United Kingdom). [171, 179, 172] Review of available peer reviewed literature did not find published research describing the susceptibility of North American wild or farmed Atlantic salmon to *G. salaris*.

Rainbow Trout

Gyrodactylus salaris is widely distributed in wild and farmed rainbow trout in Europe.[180, 179, 177, 174] In some areas (e.g., Italy), gyrodactylosis is a common and economically significant disease.[181, 177] In other regions (e.g., Sweden) infestation occurs, but clinical disease is rare.[182] The cause of this variability is unknown, but may be due to host factors or environmental conditions specific to the different farms or regions in Europe. [182] Rainbow trout exhibit reservoir host potential in that infestation, while often self-limiting, has been observed to persist for over 90 days.[171, 182, 177] The susceptibility of wild and farmed rainbow trout populations present in North America has not been described in published literature.

Arctic Char

Arctic char are capable of serving as long-term reservoir hosts.[171, 183, 184, 185] Asymptomatic infestation under natural conditions is common in some rivers and lakes where this species is present.[183, 184, 177] Variable susceptibility to infestation has been reported. In some endemic areas, infestation in the absence of other suitable hosts has been observed for approximately 20 years.[186, 185]

Brook Trout

In Europe (e.g., Romania), *G. salaris* infestation in the absence of clinical disease has been reported in farmed brook trout.[187] Experimentally, this species develops self-limiting infestation that was almost fully resolved by 70 days (the end point of the experimental study).[188, 189] Review of published literature did not identify any studies examining the susceptibility of brook trout in North America.

Brown Trout

Gyrodactylus salaris infestations of wild and farmed brown trout in Europe have been reported in the literature.[187, 177, 174] Susceptibility to infestation is described as limited. In Norway, infestation was noted in rivers during the first year of epizootics when infestation pressure was high and brown trout were observed feeding on dead or moribund Atlantic salmon.[182] In subsequent years, low to zero levels of infestation were detected. [182] Experimentally, some population-based variability in susceptibility has been observed.[190, 182] Some fish were observed to be resistant to infestation while others maintained parasite presence for over 100 days.[190, 182] The susceptibility of North American wild or farmed brown trout to *G. salaris* is not reported.

Grayling

Grayling are susceptible to *G. salaris* infestation.[180, 174] Under experimental conditions, infestation was self-limiting up to 35 days (the end date of the study).[180] Grayling are also susceptible to infestation with *G. thymalli*, which is similar morphologically to *G. salaris*.[180] Molecular assay is required to differentiate between the two parasites.[191]

Other Fish

Review of published literature finds references to detection or experimental infestation of *G. salaris* in other freshwater fish species including North American lake trout (*Salvelinus namaycush*), freshwater eels (*Anguilla anguilla*), Adriatic trout (*Salmo obtusirostis*), and European flounder (*Platichthys flesus*).[192, 193, 190, 182, 177] Infestations in these species were self-limiting and did not result in clinical disease.

Environmental Characteristics

Gyrodactylus salaris is a cold-water-adapted parasite that lives in freshwater rivers and lakes. Environmental conditions influencing the presence, growth, and mortality rates of the parasite include salinity and water temperature.[172, 182, 18] Freshwater is the optimal environment; however, the parasite can survive in brackish water for salinity and temperature dependent intervals of time. Experimentally, adult parasites exposed to water salinity levels ranging from 10–20 ppt survived for 42–240 hours at low water temperatures (1.4 °C/34.5 °F), and for 10 –72 hours at water temperatures of 12 °C/53.6 °F.[194, 172, 18] *Gyrodactylus salaris* is incapable of survival when water salinities approach that of seawater (35 ppt).[194, 174, 18] Reproduction is water salinity and temperature dependent (5 – 6 ppt and 2.5 –19 °C/36.5 – 66.2 °F, optimal temperature, 10 °C/50 °F, respectively).[182, 97] Survival rates of detached parasites are also temperature dependent, ranging from 132 hours at 3 °C/37.4 °F to 24 hours at 19 °C/66.2 °F.[195, 196, 97] Survivability at temperatures above 25 °C/77 °F is not reported.[197] Survival of the parasite on dead Atlantic salmon is also temperature dependent. Experimentally, survival times of 72, 142, and 365 hours have been observed at water temperatures of 18 °C/64.5 °F, 12 °C/53.5 °F, and 3 °C/37.5 °F, respectively.[129]

Pathogen Characteristics

Most *G. salaris* research has focused on the distribution and pathogenicity of this parasite in salmonid populations present in Europe. More recently, genetic studies using mitochondrial DNA analysis have identified numerous *G. salaris* clades (a group of organisms believed to have evolved from a common ancestor), strains (isolates described by a combination of biological and genetic characteristics), and haplotypes (genotypes identified based on genetic testing). The different mitochondrial clades, strains, and haplotypes correspond to geography (i.e., *G. salaris* from different watersheds are genetically different).[191, 182] Additionally, these

clades, strains, or haplotypes are often linked to host specificity (e.g., haplotypes from salmon are not found on grayling and vice versa).[171, 195, 198] Some haplotypes have strains that differ in virulence. For example, haplotype F has strains that are either pathogenic or non-pathogenic to rainbow trout.[191, 195, 182] According to Mo (2020), this indicates that there is no established correlation between genetic strains or haplotypes and pathogenicity. This author and others also state that there is lack of research to develop markers that can unambiguously identify pathogenic strains, studies evaluating the pathogenicity/nonpathogenicity of the different strains and haplotypes in genetically diverse salmonid fish stocks is lacking, and that such research is required to fully elucidate which *G. salaris* strains are pathogenic in different salmonid species.[163, 187, 182] By WOAH's definition in the Manual of Diagnostic Tests for Aquatic Animals, all *G. salaris* strains found on Atlantic salmon are highly pathogenic to that species, while strains recovered from Arctic char in Norway and rainbow trout in Denmark are not pathogenic.[163, 199, 182, 5] Review of the literature did not identify any research exploring the susceptibility of North American Atlantic salmon or other salmonid fish to any of the identified *G. salaris* clades, haplotypes, or strains.

Genetic testing has resulted in synonomization of *G. salaris* with *G. thymalli*, a gyrodactylid parasite found exclusively on grayling.[200, 182, 177] According to the WOAH Manual of Diagnostic Tests for Aquatic Animals, *G. thymalli* has never been observed on Atlantic salmon and does not appear to be pathogenic in this species based on experimental trials.[191, 176, 5] As of 2023, WOAH has not accepted this synonymization[182, 5]; therefore, it will not be described further in this hazard identification

Gyrodactylus salaris is a social (group living) ectoparasite that lives and feeds on the surface of freshwater fish hosts. Preferential feeding sites are the fins, skin, and gills (less common).[5, 18] The distribution of the parasites in these preferential sites is affected by the intensity of the infestation.[190, 5] Unlike many *Gyrodactylus* spp., *G. salaris* has a relatively broad host range. Most host species exhibit some symbiosis or resistance to the parasite and do not develop clinical disease associated with infestation.[187] Atlantic salmon (except for Baltic Atlantic salmon) are the only salmonid fish that develop clinical disease. According to Paladini et. al., 2014, *G. salaris* may transiently attach to any freshwater fish species for a short period of time.[190] Reproduction may occur on some of these fish, but at levels insufficient for development of persistent infection.[190] When not attached to a host, *G. salaris* is not parasitic. It can survive for 5–6 days, floating in bottom sediments or in the water column, opportunistically seeking a host.[190, 18] The parasite has no known predators.

This parasite is hermaphroditic, asexually viviparous (bears live young), and has a direct life cycle involving only one host.[200, 172, 196, 129] The parasite gives birth to one live young at a time. The young parasite is almost as large as the parent parasite, and already has a developing embryo inside of it.[172, 182] This reproduction strategy creates a short generation time, rapid population growth, and potential for a single parasite to elicit an epizootic under appropriate environmental and host conditions.[200, 196] Reproduction is temperature dependent and occurs throughout the year. When temperatures are optimal the gestation is period is 24 hours. This can lead to a very high infection intensity of several thousand parasites on a single fish host.[182, 196, 18] At the upper end of the optimal temperature range, populations can double in four days. Gestation and reproductive rates decrease in the winter due to direct effects of low temperature on the parasite and indirect host effects (decreased activity and metabolism). [182, 196, 18]

Gyrodactylus salaris attaches to its host using an attachment organ (the opisthapor) that has two median anchors and 16 marginal hooks.[172, 196] The mouth is located at the opposite end of the parasite. When feeding the parasite uses cephalic glands to attach to the host, everts its pharynx through its mouth, and releases a digestive solution containing proteolytic enzymes which dissolve the host's skin.[18, 201] Mucus and dissolved skin are then ingested. The attachment and feeding sites result in wounds in the host's epidermis that can subsequently lead to osmoregulatory failure, secondary infections, debilitation, and death.[202, 182, 201]

Transmission

Transmission is horizontally direct via contact with infested live or dead fish, detached parasites in the water column, and parasites present in or attached to bottom sediments and substrates.[195, 182, 97] Risk of transmission via these routes is greatest in waterbodies that are hydrologically and/or geospatially proximate and natural movement or migration of infested fish may occur.[182, 129] Release of effluent water from aquaculture facilities where infested fish are present may also be a source of introduction into proximate areas.[187]

Long distance translocation of *G. salaris* is thought to occur via anthropogenic activity, because *G. salaris* is not capable of long-distance movement independent of a host.[203] Translocation of infested fish is considered the primary route of introduction throughout natural watershed and aquaculture facilities in Europe.[193, 172, 187, 177, 129] Rainbow trout are most often associated with the spread of *G. salaris* in Europe, however, any fish that the parasite attaches to can serve as a transport vector.[129] Indirect transmission may also occur because *G. salaris* can survive for several days on damp materials (e.g., boats, packaging materials, recreational and commercial fishing gear, waders, and other fomites).[129]

Clinical Sign and Pathogenicity

In most host species infestation follows a pattern of initial exposure, an increase in the number of parasites present on the host, and a variable interval during which parasites decrease in number until disappearance.[182] An exception to this pattern is the fulminant infestation leading to clinical disease and mortality observed in Atlantic salmon.[182] Prevalence of infestation is variably dependent on environmental factors (e.g., water quality, salinity, and temperature, seasonal factors, geographic location), the fish species, the age and health status of individual fish, and fish population factors (e.g., population density).[172, 176, 129] In susceptible wild and farmed Atlantic salmon prevalence can reach 100%.[129] In wild Baltic Atlantic salmon, prevalence is highly variable (0%–70%) based on environmental factors and geographic location.[204] Prevalence in other susceptible species is similarly variable. In farmed rainbow trout, prevalence rates of less than 10% have been reported.[204]

G. salaris primarily infests the dorsal, pectoral and pelvic fins, and skin.[182, 129, 185] When the parasite burden is high, parasites may also be found on the gills and head, including the eyes and nostrils.[182] Development of clinical signs in susceptible fish species is associated with the parasite burden and the damage to the host tissues that occurs via the repetitive attachment and feeding of the parasites.[172] Some fish species (e.g., Arctic char, farmed rainbow trout) may be infested with low numbers of parasites for years without exhibiting any clinical signs of disease.[182, 205, 185] In highly susceptible Atlantic salmon, especially those in the parr stage, the parasite burden can reach thousands of parasites.[182, 174] Clinical signs may take several weeks to appear, and can include anorexia, behaviors such as flashing (rubbing against substrates or net pen surfaces), darting and erratic swimming, erosion of the fins, epithelial hyperplasia, focal

areas of redness, irritation of the skin which leads to increased mucous production and gives affected fish a grayish color, lethargy, lesions and ulcerations on the skin, and osmoregulatory impairment.[187, 182, 129] Moribund fish may lie on the bottom or congregate in areas with low water flow rates.[182] Development of secondary bacterial, viral or fungal (e.g., *Saprolegnia* spp.) infections is a common sequelae.[182, 196, 129]

Morbidity and Mortality

Morbidity and mortality vary among different fish species and populations. In many susceptible species (e.g., Arctic char, Baltic Atlantic salmon, farmed rainbow trout, grayling), morbidity and mortality rates are negligible to low.[129] In highly susceptible Atlantic salmon, high rates of morbidity and mortality (85% –100%) are frequently observed in fry, parr, and smolt.[196, 129]

Treatment

There are no vaccines, chemotherapeutic, or immunostimulation therapies available.[182] Gyrodactylosis can be controlled in aquaculture facilities using commonly used bath treatments containing high salinity salt water, or compounds containing chlorine or iodine, and formaldehyde (see Prevention and Control).[182, 97] Total eradication of the parasite requires depopulation of the affected fish population, drying the fish rearing structures, and instituting a fallow period.[170, 175, 174] Chemicals such as rotenone or aluminum sulphate are used to treat natural water bodies (e.g., lakes, rivers) to completely eradicate affected fish populations (see Prevention and Control).[170, 175] Experimentally, in Europe, selective breeding of Atlantic salmon and rainbow trout has resulted in increased survival rates among offspring.[182] However, fish are still susceptible to infection.[182, 97]

Diagnostic Testing

Gyrodactylus salaris cannot be visualized on affected fish without magnification. Observable gross lesions include cutaneous ulcers, epidermal thickening, excess mucus on the skin giving a grayish appearance, frayed fins that may appear eroded, white and thickened, and sloughing of the skin.[196, 97] Secondary fungal infections (*Saprolegnia* spp.) may be observed.[97] There are no definitive histopathological signs.[196]

All *Gyrodactylus* spp. are similar morphologically.[170, 182] Confirmatory diagnosis requires morphological identification of the parasite under magnification in combination with molecular testing (PCR and DNA sequencing of the ribosomal internal transcribed spacer region [ITS]).[170, 187, 5] WOAH recommended protocols for specimen selection, sample collection, transport and handling, and diagnostic methods are described in the WOAH Manual of Diagnostic Tests for Aquatic Animals and the WOAH Aquatic Animal Health Code (Appendix, Table 1).[5, 97] 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).[161] Experimentally, environmental DNA assays have been used for surveillance in natural water bodies.[206, 207]

Prevention and Control

Control of the spread of *G. salaris* should include application of risk-based approaches for surveillance and control in natural waterbodies and in aquaculture systems, and development of regulations to safeguard susceptible fish populations.[182, 174] In Europe, legal and illegal translocation and importation of infested fish are identified as the most significant pathways for introduction of *G. salaris* into naïve ecosystems and aquaculture facilities.[170, 190, 182] The next

most significant potential pathway is introduction via equipment used for fishing and recreational water sports (e.g., bait, boats, canoes, kayaks, fishing tackle, nets, paddle boards, waders).[182]

Control efforts in some European Union countries (including those that are currently *G. salaris* free) include regulatory standards such as:[187, 208, 177, 174]

- Controls on transfer, local movement, and international importation of fish,
- Surveillance programs for farmed and wild fish populations,
- Eradication plans for natural waterbodies,
- Eradication plans for hatcheries and fish farms,
- Regulations for drying or disinfection of boating, fishing, and diving equipment prior to movement between or within watershed systems,
- Guidelines for use of live or dead bait in certain regions,
- Requirements for gutting, cleaning of fish or discharging of fish waste in natural waters,
- Controls for disposal of water anywhere other than where it was collected,
- Contingency plans that include outbreak control measures, movement restrictions, establishment of buffer zones, treatment plans, eradication measures, and public outreach frameworks.

In the EU, trade of live fish species susceptible to gyrodactylosis is only permitted between countries, zones, or compartments of equivalent health status (or from higher to lower status).[187, 177] Many countries also have prohibitions on the transport of live fish to rivers containing wild Atlantic salmon unless the source of the fish is known to be *G. salaris* free.[197] Surveillance plans are applied to natural waterbodies and aquaculture facilities. The intent is to document freedom from *G. salaris* in unaffected areas and aquaculture facilities, to detect and trace spread of the parasite from natural areas or aquaculture facilities where it is present to new sites, and to evaluate post-eradication disease freedom.[208]

There are currently no requirements for routine *G. salaris* surveillance in United States aquaculture facilities. The USFWS does conduct routine surveillance on wild fish populations and maintains a database which catalogues testing data by species, year, and location (Appendix, Table 1). Surveillance protocols are not focused specifically on *G. salaris*; however, they do involve examination for external parasites. Additionally, the USDA and other Federal agencies periodically conduct structured surveillance for research or regulatory purposes that include sampling fish with susceptibility to *G. salaris*. There may be relative State and Tribal regulations with additional local aquatic animal health and import requirements that would detect *G. salaris* or other external parasites (Appendix, Table 1). When required by importing countries, evaluation for *G. salaris* is conducted by APHIS-approved laboratories or accredited veterinarians, who are obligated to report to the Federal and State animal health officials in their region. As of 2023, USDA APHIS has negotiated health certificates for the export of live salmonid fish, fertilized eggs, and gametes with around fifty-eight countries; at least forty-six of these countries include pre-export testing requirements for *G. salaris*.

Gyrodactylus salaris is capable of surviving without a fish host for several days in damp environments but is susceptible to desiccation and temperatures outside of its optimal thermal range.[182, 205, 129] Boats, equipment, fishing gear, nets and other potential fomites should be completely dried for several days and can be disinfected by placing in water at temperatures ranging from 20 °C/68 °F for 24 hours, 40 °C/104 °F for 5 minutes, or 50 – 60 °C/122 –140 °F for one minute, freezing at -18 °C/-0.4 °F for 24 hours, or can be treated with disinfectants

efficacious for *G. salaris*.[195, 197, 182, 196, 129] Recommended disinfectants include Virkon S® (1% for 15 minutes), iodine-based compounds (e.g., Wescodyne®), and sodium hydroxide-based compounds (e.g.,Biosolve™ Plus).[197, 169, 196, 129] In some EU member states, equipment must be accompanied by a certificate of disinfection issued by a competent professional in the country of origin.[174]

There are no drugs that demonstrate efficacy against *G. salaris*. Most of the insecticide or parasiticide treatments tested also exhibit toxicity to the fish hosts.[182, 201] Chemical bathing of fish in formalin (0.017% – 0.025% for 30 minutes) or high salinity water (200 – 250 ppt x 30 minutes) will remove *G. salaris* from infested fish. These treatments do not eradicate the pathogen; therefore, repeated treatments are required to control the parasite in aquaculture facilities.[182] Experimentally hydrogen peroxide (H_2O_2) treatment have been used to control infestations on host fish.[209, 182] The WOAH Manual of Diagnostic Tests for Aquatic Animals stipulates that treatment of farmed fish populations with bath treatments reduces the prevalence and abundance of *G. salaris* on affected fish; however, fish may remain infested, and detection of the parasite will become more difficult.[5] Iodine-containing compounds have been used to disinfect fertilized eggs that may be surface contaminated via contact with contaminated water.[5]

Because there are no efficacious treatments for *G. salaris*, eradication of infested hosts is the recommended control measure. In Norway, rotenone and acidified aluminum sulphate have been used to eradicate *G. salaris* from infested river systems (via killing all the fish present), followed by restocking with fertilized eggs and fry from *G. salaris*-free hatcheries.[170, 197, 182, 208, 196] Experimentally, low doses of sodium hypochlorite (200 µg Cl/L) have been used in some areas.[182] Other control methods include use of physical or electric barriers to stop the movement of fish from infested to uninfested rivers.[170, 210, 197, 208] As of 2021, these efforts resulted in eradication of *G. salaris* from 39 of 44 infested rivers.[175] In fish hatcheries and farms, eradication of all affected fish hosts is followed by disinfection of the farm, and a period of fallowing followed by restocking with fertilized eggs, fry, and fish from *G. salaris*-free hatcheries.[175] In Norway, application of such measures resulted in eradication of *G. salaris* from all affected hatcheries and fish farms by 2021.[175]

Recommended aquaculture facility biosecurity measures include:[170, 193, 197, 187, 182, 97]

- Siting farms rearing susceptible fish in areas where *G. salaris* is not present in wild fish populations,
- Use of wells or springs for water sources,
- Treatment of influent water prior to use,
- Sourcing live fish, fertilized eggs, and gametes from sources known to be *G. salaris* free,
- Use of preventative surveillance, use of disinfectants and other cleaning methods known to be efficacious for the parasite,
- Use of designated PPE,
- Use of site designated equipment,
- Maintenance of fish health (avoid overstocking, maintain good water quality and temperature),
- Treatment of effluent water prior to release.

Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of *G. salaris* introduction. A summary of WOAH import/export guidelines specific to *G. salaris*, U.S. regulations, and other regulatory information related to aquaculture in the United

States is summarized in the Appendix, WOAH Pathogen Specific Import Export Recommendations.

Summary

Gyrodactylosis is an economically important WOAH-listed parasitic disease affecting salmonid fish. Atlantic salmon are highly susceptible to this parasite and develop clinical signs of disease with subsequent high morbidity and mortality. Other salmonids (e.g., Arctic char, grayling, rainbow trout) are susceptible to infestation, but rarely develop clinical disease. Importation and translocation of sub-clinically infested rainbow trout has been associated with translocation of *G. salaris* throughout Europe.

In EU states where *G. salaris* is present in wild salmonid populations, control is difficult once introduction has occurred. Eradication measures require application of chemicals to natural water systems that result in the death of all wild fish present, with likely ecosystem and local economic consequences. Control in fish farms and hatcheries requires eradication of all fish hosts, followed by disinfection and fallowing, with economic consequences to the producer, and the local economy. Presence of *G. salaris* has resulted in trade regulations relative to the movement and importation of susceptible fish.

The susceptibility of wild, stocked, and farmed salmonid species in the United States is not known. However, it may be assumed that farmed, stocked, and wild Atlantic salmon in the Atlantic Northeast, and farmed, stocked, and wild rainbow trout populations will exhibit similar patterns of susceptibility described in European fish. It may also be expected that control and eradication of this parasite in wild and farmed fish populations would be similarly complex, expensive, and impactful.

In the United States, there are no Federal, State, local, or Tribal import regulations specific to *G. salaris*, although the USFWS does require disinfection of salmonid fertilized eggs prior to import. The USFWS does perform routine disease surveillance in wild fish stocks that includes sampling for external parasites. State and Tribal entities may also have local requirements for external parasite sampling. USDA and other Federal agencies may periodically conduct structured surveillance that includes external parasite sampling for research or regulatory purposes. Best practices to prevent introduction of *G. salaris* into the United States should include development of policies and contingency plans to ensure that imported all live fish, fertilized eggs, and gametes are imported form *G. salaris*-free sources and that importation complies with the guidelines described in the WOAH Aquatic Animal Health Code. Because the parasite can potentially live on any freshwater fish species, regulatory measures would ideally control movement of all fish species from areas where *G. salaris* is present. *G. salaris* is a WOAH-listed reportable pathogen. Therefore, detection of gyrodactylosis in cultured fish stocks in the United States would likely result in significant trade impacts.

Infectious Hematopoietic Necrosis Virus

Introduction

Salmonid novirhabdovirus, commonly known as infectious haematopoietic necrosis virus (IHNV), infects numerous fish species, primarily salmon, but also 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.[211, 97]

Five major genogroups have been identified (U, M, L, E and J). Occurrence of these genogroups correlates with geography, not host species. Three 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).[212, 213, 214, 215] Genogroup J isolates occur in Asia (China, Japan, and Korea).[216, 3, 217] Genogroup M strains were initially introduced to Europe and have now evolved into genogroup E isolates.[216, 215] Group U has also been found in the Russian Far East and Asia,[213, 218, 216] and M has been detected in Africa.[219]

The disease (infectious haematopoietic necrosis, IHN) caused by infection with salmonid novirhabdovirus is notifiable to 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).[120, 97, 4] 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).[1] State and Federal authorities should be contacted immediately upon suspicion or confirmation of the disease (Appendix, Table 1).

Susceptible Fish Species

Fish species that fulfill the WOAH criteria for species susceptible to infection with IHNV are summarized in Table 13. Nearly all of these species are present in the United States, except for marble trout and masu salmon.[33] IHN could be highly impactful to aquaculture in the United States because it affects Atlantic salmon and rainbow trout.[211]

Table 13. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infectious hematopoietic necrosis virus (IHN)[97, 4]

Scientific name	Common name
Esox lucius	Northern pike
Salmo marmoratus	Marble trout
Salmo salar	Atlantic salmon
Salmo trutta	Brown trout
Salvelinus alpinus	Arctic char
Salvelinus fontinalis	Brook trout
Salvelinus namaycush	Lake trout
Oncorhynchus clarkii	Cutthroat trout
Oncorhynchus tshawytscha	Chinook salmon

Oncorhynchus keta	Chum salmon
Oncorhynchus kisutch	Coho salmon
Oncorhynchus masou	Masu salmon
Oncorhynchus mykiss	Rainbow trout/steelhead trout
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 (*Oncorhynchus nerka*) during the 1950s in hatcheries in Washington and Oregon, followed by outbreaks in Chinook salmon in California in the United States. IHNV was reportedly endemic in Alaska by the 1970s, in Idaho (rainbow trout) by the late 1970s, and in salmonids in the Columbia River basin region by the early 1980s.[220, 221] 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.[212, 221]

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.[222] The first detection in Japan occurred in 1971 in a sockeye salmon hatchery that imported fertilized sockeye salmon eggs from Alaska. The disease then spread throughout Japan through IHNV-contaminated fertilized fish eggs. Phylogenetic analyses of early Japanese isolates identified genogroup U; isolates after 1980 classify within genogroup J.[223, 224] 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.[225, 226, 214, 221, 215] China and Korea reported IHNV in 1988 and 1991, respectively. Genogroup J and U occur in China [215] and genogroup J occurs in Korea.[227] The virus was reported in Russia in 2000 (genotype U)[213] and Iran in 2004 (genogroup E).[215] Other countries where IHNV has been detected include Taiwan, Austria, the Czech Republic, Poland, the Netherlands, Croatia, Kuwait, Pakistan, Spain, Switzerland, Georgia, North Macedonia, Finland, Denmark, Estonia, Slovenia, and Bolivia.[221, 228] Spread of IHNV is believed to be the result of trade movement of fertilized IHNV-infected eggs or fry.[214, 221]

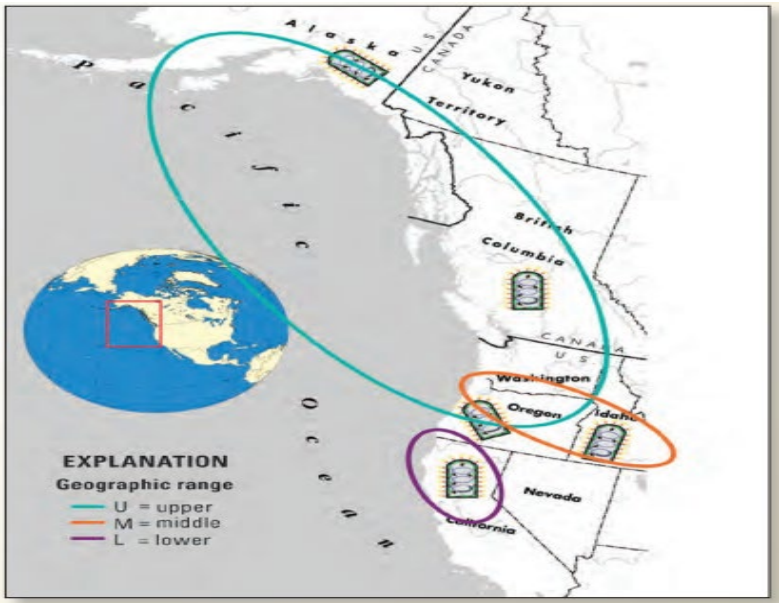

Figure 5. The geographic ranges of endemic IHNV genogroups U, M, and L in the United States[229]

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. 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.[230] Host factors (e.g., fish species and size, fish strain, life stage, age and weight, nutritional status, and presence of coinfections) affect the ability of IHNV to cause disease.[231, 232, 223, 211] An experimental study in California showed that Chinook salmon fry experienced higher mortality rates than steelhead trout fry (47% – 87% and 1.3% – 33%, respectively). Additionally, Chinook salmon mortality decreased with increasing age and water temperature.[233] Young salmonid fish (fry) are likely to suffer severe disease, however, the virus does infect salmonids of all ages and subclinical infections occur.[4, 231, 222] IHNV tends to be highly pathogenic in fry and fish up to 2 months old, with 80% –100% mortality rates, whereas 2 – 6-month-old fish typically experience <50% 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%.[221] As salmonids increase in age and size, they tend to be more resistant to developing clinical disease.[231, 221]

Environmental Characteristics

Environmental conditions for the host are also determinants of susceptibility to IHNV infection and disease, including stress and fish density.[231, 211] 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.[231, 228, 233] 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/49 °F.[234, 231, 211] 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/49 °F.[221] In female sockeye salmon, IHNV prevalence is higher than that observed in males and increases from pre-spawning to spawning and then post-spawning, but this can vary each year.[221] Within aquaculture production settings, fish density affects transmission dynamics and prevalence of IHNV. Increasing animal density causes stress, increased contacts between animals, higher concentrations of pathogens, and reduced water quality, all of which can contribute to IHNV outbreaks.[221]

Pathogen Characteristics

IHNV virulence is incompletely understood and is not reliably predicted based on virus strain at this time.[221] Additionally, evidence supports that susceptibility to IHNV varies with virus strain. 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 (rainbow and steelhead trout) hatcheries experiencing high losses.[212] 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, but also in contained 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.[220] Virulence of IHNV varies within genogroups, further demonstrating differences in strain as an important factor in pathogenicity. In Idaho rainbow trout production facilities IHNV caused low virulence infections until 1977, when a highly virulent strain caused disease outbreaks which spread throughout the region, causing up to 80% mortality rates.[232] A California hatchery producing steelhead trout and two strains of Chinook salmon experienced mortality from IHNV primarily in juvenile Chinook salmon but not the steelhead trout, suggesting the potential for host specificity for some IHNV strains.[235, 233] In the 1950s, Washington aquaculture facilities observed 95% mortality in sockeye salmon but only 5% mortality in Chinook salmon while other outbreaks demonstrated high virulence in sockeye salmon but not for Chinook or coho salmon, or rainbow trout.[231] In Japan and Korea, IHNV was initially highly pathogenic for larvae and juveniles, but later, disease and losses occurred in adult and market sized rainbow trout, indicating a shift in pathogenicity for newer IHNV strains.[223, 224] Similar observations have been made in Italian rainbow trout farms.[228]

Transmission

Survivability of IHNV outside of a host in fresh water and sea water is possible. Factors which influence this include temperature, UV 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.[236, 129] IHNV survival is inversely proportional to temperature.[211] The virus can survive at 4 °C for three weeks in whole fry, four weeks in liver, and five weeks in brain.[211] Therefore, viable IHNV may exist in whole fish, tissues, and mucus, especially in cold storage settings and risk of transmission exists in the aquaculture setting with

water sources, processing areas and waste, and predator bait.[211] The virus is inactivated at high temperatures.

Spread of IHNV in aquaculture is believed to be the result of trade movement of fertilized IHNV-infected eggs or fry.[214, 221] Under natural conditions the primary mode of IHNV transmission is horizontal (waterborne) from fish to fish. Virus is shed in external mucus and sexual fluids, and young fish shed high amounts of virus during epizootics.[211] One study measured 0.1 – 0.3 plaque-forming units (pfu)/mL 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 Burrows ponds (raceways).[237] Rainbow trout fingerlings begin shedding virus within 3 days and are able to infect cohabitating fish.[221] Mortalities begin 5 – 7 days post-exposure at 15 °C/59 °F.[231] Evidence suggests that horizontal transmission within and between adult salmonids occurs in fresh water and marine environments through the water.[230, 221] Fish may also become infected with IHNV via food, if fed unpasteurized salmonid viscera.[212, 211] 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.[221]

The mechanism of vertical transmission is unclear. 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.[230] Other experimental studies demonstrated no vertical transmission of the virus to progeny when fertilized eggs were raised in IHNV-free water.[230, 221] Phylogenetic analyses of isolates in the first Japanese outbreaks supported the epidemiological evidence that fertilized sockeye salmon eggs shipped from Alaska were contaminated with IHNV, which then resulted in fry and fingerling losses and subsequently spread of IHNV throughout Japan when IHNV-contaminated fertilized eggs were transported to other salmon hatcheries.[238] IHNV can be detected in ovarian fluid and milt and is present on the surface of sperm.[221]

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 study to be a competent vector for IHNV to Atlantic salmon. IHNV has been detected in freshwater species such as leeches, copepods, and mayflies but their ability to transmit virus is currently unknown.[211, 129]

The maintenance of IHNV by persisting 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.[239] Early studies in the United States detected IHNV in sockeye salmon disease survivors once they reached maturity. However, a 1989 study showed 0% detections in sexually mature sockeye salmon captured as they left saltwater during spawning migration, and 90% – 100% prevalence in those which migrated on to freshwater spawning grounds in Washington. The authors concluded that horizontal transmission occurred in the river during spawning, and that these infections were not due to latent IHNV.[240] Contrary to this finding was a 1997 Canadian study which captured migrating sockeye salmon returning from the sea to spawn. In this study, IHNV was detected in fish captured in marine water sites, and the authors concluded that the level of IHNV present in kidney tissues was high enough to indicate active replication rather than latency.[230] Others have detected IHNV proteins, nucleic

acid, and truncated apparent rhabdovirus viral particles in tissues of rainbow trout, which survived IHNV outbreaks, 1–2 years after virus was no longer detectable.[221]

Clinical Signs and Pathogenicity

Studies of disease progression with fish infected by waterborne IHNV show initial entry through gills, skin, fin bases, mouth, and esophagus and cardiac stomach region with viral replication 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.[221]

Clinical signs of disease typically appear in young salmonid fish within 5 –19 days of exposure to IHNV.[232, 221] 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, also sometimes in the gills, mouth, eye, skin, and muscle. Chinook salmon may show subdermal hemorrhage caudal to the head. Older fish may show fewer clinical signs. Two-year-old kokanee salmon demonstrated irregular swimming movements with hemorrhage at the fin base. Sockeye salmon smolts may display clubbed, fused lamellae and cutaneious lesions.[221] 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.[221]

Morbidity, Mortality, and Prevalence

Acute outbreaks may show sudden mortality rate increases with no other clinical signs.[221] Mortality rates in young salmonids can reach up to 100% with IHNV infection.[221, 233]

Treatment

There is no medical treatment for IHN. Experimental cross breeding of salmonids or triploid hybrids to select for increased IHNV resistance has been attempted with varying degrees of success.[211, 129] An IHNV DNA vaccine is approved for use in Canada in farmed Atlantic salmon.

Diagnostic Testing

Pathognomonic features of IHN include 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.[221]

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.[221] 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 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, and molecular methods, which amplify nucleic acid from a gene or region of a gene of all known IHNV genogroups and then sequence the amplicons.[221, 5] Because IHN 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.[120, 5, 97] In the United States, Title 50 describes testing protocols for IHNV. All suspected indigenous IHNV detections or IHN outbreaks are reportable to USDA APHIS VS as the Federal competent authority for animal health. Confirmatory testing at the USDA National Veterinary Services Laboratory (NVSL) is required following first detections (Appendix, Table 1). Samples should be collected and submitted under the direction of State and Federal authorities via guidelines provided by NVSL.[161]

Prevention and Control

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 therefore should be used in aquaculture facilities with susceptible species as part of a biosecurity program.[241, 5] Surface egg disinfection and utilizing IHNV-free water supplies are important control measures in mitigating disease.[233, 5] In Japan, disinfection of fertilized 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.[224, 5] However, iodine may inhibit PCRs and may impact PCR-based test results of disinfected fertilized 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.[211, 5] 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.[221] 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.[221] Orally administered DNA vaccines are being studied[242, 243] but are not commercially available. Development of a variety of other vaccines (killed, subunit, and attenuated) have been undertaken.[221]

Farming conditions associated with risk for IHNV introduction to naïve populations include:[211]

- 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 km/3.1 mi upstream,

- Receiving and storing fish waste from other farms, including mortalities and processing waste,
- Facility staff working on outside fish farms,
- Receiving fertilized 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

IHN 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.[211] There is potential high economic impact to the U.S. Atlantic salmon farming industry if introduction of IHNV occurs in these populations of fish. 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 fertilized eggs is the most likely route of disease 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 are most likely to 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.[244] Troyer 2002 reported that IHNV is endemic among numerous rainbow trout farms and hatcheries in the Hagerman valley region of Idaho.[244] Historical outbreaks of IHNV have resulted in serious economic losses to the Idaho trout industry.[244] There are 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% in some areas.[245] 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 USFWS oversees importation of live and dead salmonid fish, fertilized eggs and gametes (Appendix, Table 1 and Appendix, Regulatory Information Associated with International Trade: The United States, Import Information).[100, 103] 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).[102] Briefly, persons engaged in importation or exportation of wildlife must obtain an import/export license prior to importing or exporting a shipment of wildlife.[102] Shipments must be accompanied by a U.S. 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).[100] Fertilized 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.[102] 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, fertilized eggs, or gametes relative to IHNV (Appendix, Table 1).

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, fertilized 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.[97] It has been reported that disinfection of fertilized eggs has virtually eliminated transmission of IHNV from infected broodstock to offspring fish.[245] 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.[245]

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 deleted (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.[124, 246, 247, 5] The nucleotide sequences of these eight genome segments encode approximately ten proteins.[248, 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).[249, 5]

Gene insertion, recombination, and reassortment with potential links to virulence have been identified throughout the evolution of the virus.[249, 250, 251, 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).[252, 249, 5] In the segment 6 HE gene, a highly polymorphic (HPR) region characterized by variations in sequence length is present.[253, 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.[252, 249, 254, 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.[251, 255, 247, 5] The EU clade is most common to 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.[95, 256, 255, 5] Within each clade, and the EU clade subgroups, multiple different HPR0 and HPRΔ genotype variants can be identified.[257, 258]

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.[259, 260, 261, 262] To date, all ISAV variants associated with clinical disease (HPRΔ variants) contain gaps in the HPR sequence.[263, 5] These HPRΔ variants are hypothesized to arise via deletions from a full-length precursor gene.[263, 95] 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.[264, 259, 265, 261, 262, 250, 95] However, these reports are rare, suggesting that this is likely a low but not negligible probability event.[266, 250, 267, 256] It has also been suggested that HPR0 variants might occasionally derive from attenuation or genetic insertions in HPRΔ variants.[252, 267, 268]

[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).[1, 123] 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). [1] ISA is a WOAH listed notifiable disease.[95, 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).[263, 246, 269] ISA is a reportable disease in Canada.[270, 271, 14]

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 14.[5, 97] In the United States, marine-farmed Atlantic salmon and steelhead trout represent the species of greatest economic concern relative to infection with ISAV.

Table 14. Fish species identified by World Organisation for Animal Health (WOAH) as susceptible to infection with ISAV HPR0 and HPRΔ variants[5, 97]

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).[129] 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, 129] In Maine, the last ISA disease detection of HPRΔ ISAV occurred in 2006.[95] Subsequently, localized HPRΔ detections responsive to control measures have been occasionally reported in Canada and Maine.[251, 14] HPR0 variants are also periodically detected at marine and freshwater sites in Maine and Maritime Canada.[95, 272]

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.[248, 247, 4] However, disease outbreaks are primarily reported only in Atlantic salmon marine life stages. Susceptibility is likely affected by environment as well as 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).[273, 248, 274, 95, 5] Persistent infection in individual fish has not been confirmed.[129] Anecdotally, differences in ISAV susceptibility between individual fish and among farmed Atlantic salmon family groups have been reported in the literature.[274, 246, 129]

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.[275] 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.[275] 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.[276, 277, 278, 279, 205] 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.[274, 247]

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.[129] ISAV has been detected by RT-PCR in seawater sampled at farming sites where ISAV-positive Atlantic salmon are present.[95, 247] Virus detections and disease outbreaks appear can occur throughout the year, but are often seasonally associated with cold water temperatures ranging from 10 –15 °C/50 – 59 °F.[274, 247, 129] 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.[273, 274, 95, 255, 247, 129] HPR0 and HPRΔ variants have been detected in freshwater hatcheries, broodfish farms, and smolt farms utilizing flow-through and RAS water handling technologies.[280, 255] 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.[255]

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.[259, 265, 260, 261, 95, 255,129] The prevalence is variable, ranging in some field studies from individual fish to 100% in some populations.[253, 249, 274, 255] In farmed salmon, the rate of HPR0 detection is greater than that of HPRΔ.[248, 266, 281, 129] 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.[260, 266, 255, 14, 129] HPRΔ variants are associated with the occurrence of clinical disease in Atlantic salmon.[282, 129] The primary route of infection is most likely gill epithelium; however, exposure via the skin and intestine is also suggested.[129] HPRΔ variants target the endothelial cells of blood vessels in all tissues and organs, leukocytes, macrophages, and red blood cells.[14, 129] Because endothelial cells are the primary target cells, HPRΔ replication can occur in any organ.[248, 129] Concurrent detections of both HPR0 and HPRΔ variants in marine-reared Atlantic salmon and in individual fish have been reported.[259, 248, 266, 250, 281, 129] This has been hypothesized by some authors as an emergence link between non-pathogenic HRP0 and pathogenic HPRΔ genotypes.[274, 281, 282, 129]

Optimal ISAV replication temperatures in cell culture range from 10 –15 °C/50 – 59 °F.[274, 120, 5] According to the literature, replication ceases at 25 °C/77 °F, and inactivation occurs when temperatures reach 56 °C/133 °F for 30 minutes.[274, 95, 247, 5] 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.[283, 5] 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.[274, 284, 285, 95, 5] Infected wild Atlantic salmon are considered a likely source of exposure for farmed Atlantic salmon (and vice versa).[279, 275, 274, 285, 95, 247, 14] 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.[286, 284, 129]

The primary route of transmission is horizontal.[274, 284, 255, 287, 247, 5] Direct horizontal transmission occurs via close contact between infected and susceptible fish.[288, 278, 289, 255] Indirect horizontal

transmission occurs via movement of ISAV in the water column.[290, 291, 255, 247, 14] Indirect transmission prior to or during movement or transport of infected live fish, fertilized eggs, or gametes has been reported.[292, 14, 129] Vertical transmission has not been confirmed; however, fertilized eggs and gametes may be horizontally infected during collection, preparation for transport, and by exposure to contaminated water.[284, 129]

Transmission via vectors, such as salmon lice and sea lice, has been suggested but not definitively proven.[293, 294, 95, 5] 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).[295, 274, 95, 247, 14] 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.[296, 290, 273, 297] 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).[298, 299, 255, 300] Introductions via aerosols and sea spray has also been suggested.[301, 302, 255] 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.[255, 247, 129] 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.[303, 304, 284, 256, 255] Infectivity was not confirmed in those reports because the HPR0 could not be isolated 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.[284] In a separate study, Christiansen et al. (2021) described detection of HPR0 in the ovarian fluid of 12% of HPR0 infected farmed broodfish.[255] 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.[255] 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.[295, 255, 247] This represents an epidemiological transmission pathway that requires continued investigation.

Clinical Signs and Pathogenicity

Atlantic salmon infected with HPR0 variants develop a transient infection in gill epithelium, but do not develop clinical disease.[260, 287, 5] Exposure pathways for HPRΔ ISAV are thought to include gill tissue, skin, and oral ingestion.[305, 95, 247, 14] Atlantic salmon infected with HPRΔ variants develop clinical disease, which is typically observed during marine life stages.[274, 14, 129] Subclinical HPRΔ infections can occur, and may be accompanied by anemia and circulatory disturbances in some fish.[274, 14]

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).[274, 246, 247, 14] Clinical signs during the final stages of the disease are attributed to severe anemia (hematocrit less than 10) and circulatory collapse.[248, 274, 95] 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.[290, 273, 293, 95] Differential diagnoses include other causes of anemia and hemorrhages, including winter ulcer (*Moritella viscosa*) and bacterial septicemias.[274] 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).[306, 290, 307, 308, 309, 310]

Morbidity, Mortality, and Prevalence

Morbidity and mortality rates vary by location among sea pens, farms, and season (higher rates in early summer and winter).[274, 95, 129] The disease course can be prolonged, occurring over months. Daily morbidity and mortality rates are typically low (0.5% –1%).[274, 129] 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.[95, 287, 247, 129]

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.[274, 246, 97] 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.[305, 274, 247, 97] 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.[274, 247] Significant clinical pathology includes anemia ranging from 2%–10% or greater. Serum biochemistry changes indicate hepatic and renal compromise.[305, 274, 247]

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 virus identification via genomic sequence analyses.[282, 311, 97, 5] 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.[289, 95, 5]

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, 97] 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).[161]

Prevention and Control

Stringent biosecurity measures can decrease the risk of ISAV introduction via importation of live salmonid fish, fertilized eggs, and gametes, transmission from wild Atlantic salmon to farmed salmon (and vice versa), between sea pens in marine settings, and among farms.[11, 95, 13, 247] Import biosecurity measures utilized by many countries include pre-import certification of live fish, fertilized 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 fertilized 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, fertilized eggs, and gametes based on assessments of risk for a disease not listed in Title 50 on a case-by-case basis.[105] In the United States, Maine has implemented broodstock testing and egg disinfection recommendations for ISAV prevention and control (see the WOAH Aquatic Animal Health Code, Chapter 4.4).[274, 97, 5] Other States, Tribes and local entities may have regulatory requirements relative to ISAV and the inter- and intra-state movement of salmonid fish, fertilized 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:[274, 95, 247]

- Acquisition of live fish, fertilized eggs, and gametes from sources tested free of ISAV,
- Disinfection of fertilized eggs,
- Quarantine of incoming fish, fertilized eggs, and gametes,

- Avoid transferring live fish, fertilized 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,
- 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).[95, 5] 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).[274, 95, 5] 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.[274, 95, 5]

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.[97] 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.[95] 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.[274, 289, 95, 247] 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, 97]

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. Disease outbreaks have only been observed in marine farmed Atlantic salmon; however, susceptibility to infection has been observed in other salmonid species and environments. 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, fertilized eggs, or gametes. However, the USFWS does require disinfection of fertilized salmonid eggs prior to import. Information describing State (other than Maine and Washington) or Tribal regulation of live salmonid fish, fertilized eggs, or gametes relative to ISAV is available via links in Appendix, Table 1.

Salmonid Alphavirus

Introduction

Salmonid alphavirus (SAV; family Togaviridae, genus *Alphavirus*) is a single stranded, positive-sense, enveloped virus approximately 60 – 70 nm in diameter. SAV is phylogenetically distinct from mammalian alphaviruses and does not require an arthropod vector for transmission.[312, 313, 97, 5] The genome codes four capsid glycoproteins (E1, E2, E3, and 6K), four nonstructural proteins (nsP1 – 4), and contains four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD).[314, 5] Glycoprotein E2 is considered the site of most neutralizing epitopes, while E1 contains conserved, cross-reactive epitopes.[314, 5] SAV is typically divided into six genotypes (SAV1 – SAV6) based on phylogenetic analysis of glycoprotein E2 and nsP3.[315, 313, 5] However, recently published whole-genome sequencing of SAV isolated from Ballan wrasse suggests an additional genotype (SAV7).[316, 317] All genotypes are antigenically similar, leading to serological relatedness and antibody cross-reactivity.[318, 319, 5]

Differences in virulence, and environmental/geographical distribution of the genotypes are reported. SAV1 and SAV3 are described as more virulent than the other subtypes.[320, 321, 322] SAV1 and SAV2 are reported to cause disease in freshwater and marine fish, while SAV3 – SAV 6 are reported to cause disease only in marine species (Table 1, Susceptible Fish Species Section).[129] Geographically, SAV1, 2, 4 – 6 have been detected in the United Kingdom. In Norway, SAV2 and SAV3 are found in separate enzootic zones.[323, 324, 325, 326]

Salmonid alphavirus disease (also referred to as SAV) is an economically important disease of farmed Atlantic salmon and fresh- and seawater reared rainbow trout. The WOAH Manual of Diagnostic Tests for Aquatic Animals defines infection with SAV as infection with any SAV genotype.[5, 97] SAV infection causes pancreas disease (PD) in marine-reared Atlantic salmon and rainbow trout (steelhead trout), and sleeping disease (SD) in fresh-water reared rainbow trout and Arctic char (*Salvelinus alpinus*).[327, 312, 328, 329, 5] SAV is a WOAH listed notifiable disease. SAV is reportable in the United States is included on the USDA APHIS NAHRS and NLRAD lists of reportable diseases which are available via the links in the Appendix, Table 1. State and Federal authorities should be contacted upon suspicion or detection of SAV. Links to contact information for State and Federal authorities are found in the Appendix, Table 1.

Susceptible Fish Species

Fish species fulfilling WOAH criteria for listing as susceptible to SAV infection are summarized in Table 15.[97, 5] In the United States, Atlantic salmon and rainbow trout are the species of greatest economic concern and highest likelihood of SAV infection.

Table 15. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infection with salmonid alphavirus[97, 5]

Genus species	Common Name	SAV Genotype
Salvelinus alpinus	Arctic char	SAV2
Salmo salar	Atlantic salmon	SAV1,2,3,4,5,6
Oncorhynchus mykiss	Rainbow trout Steelhead trout	SAV1,2,3
Limanda limanda	Common Dab	SAV5

Geographic Distribution

SAV is present in Europe and has been detected in countries that export susceptible fish and fish products to the United States. SAV infection in Atlantic salmon (PD) was first described in Scotland and Norway in 1976 and 1989, respectively. Countries reporting presence in Atlantic salmon via the WOAH WAHIS database for years that data are available (2014–2019) include Ireland, Norway, and Spain.[129] SAV infection in rainbow trout (SD) was first reported in France in the 1990s, and has subsequently been reported in Croatia, Germany, Italy, Spain, and the United Kingdom (England, Scotland).[330, 327, 331] Search of the WOAH WAHIS database did not identify any reports of SAV in rainbow trout from 2014 – 2019. The literature reports one SAV detection in farmed Arctic char in Austria, in 2018;[328] however, there are no WAHIS database reports relative to Arctic char.[129] As of 2023, SAV has not been detected in United States. In 1987, Kent and Elston published a report of a PD-like event in farmed Atlantic salmon in Washington.[332] Gross and histopathological changes were suggestive of PD; however, no etiological agent was identified via diagnostic testing.[332, 314] This is the only report of a PD-like condition outside Europe.[314]

Public Health

SAV is not a zoonosis. There are no threats to human health.

Epidemiology

In this section, the epidemiology of SAV is summarized. Many of the epidemiological factors, including environmental, pathogen and host factors, associated with SAV are poorly described or understood.

Host Characteristics

Susceptibility to SAV has been most comprehensively documented in Atlantic salmon and rainbow trout (including steelhead trout).[333] SAV infection in these economically important species is associated with mortality losses, chronic morbidity, poor growth, reduced production, carcass downgrading, and economic losses to aquaculture.[129]

Rainbow Trout

All life stages are susceptible to infection; however clinical disease and mortality occur with greatest frequency in fingerlings (10 –16 g).[327] Older fish may exhibit clinical signs of SD or may be subclinically infected.[334] Steelhead trout exhibit clinical signs of PD, which is described below.[312]

Atlantic Salmon

All Atlantic salmon and steelhead trout life stages are susceptible to infection; however, PD is typically observed in first year smolts after transfer from freshwater to marine pens.[320, 335, 336] According to Kristoffersen et al.(2009), autumn smolts are at greater risk for SAV infection.[337] Occurrence of outbreaks in a given area appears related to the density and proximity of net pens, and local spatiotemporal and hydrological factors.[338, 339, 13] Differences in susceptibility among Atlantic salmon family groups have been observed (such data are not available for steelhead trout).[340, 317, 129]

Arctic Char

Arctic char is a cold-water salmonid species native to the circumpolar north that has been cultured for aquaculture purposes globally, including in Canada.[328] SAV2 infection, described as SD, was first reported in farmed Arctic char in Austria in 2018.[328]

Common Dab

SAV2 infection in wild common dab (*Limanda limanda*) was first reported in 2010.[341] Subsequent surveys in Scotland and Ireland identified SAV1, 2, and 5 prevalence rates in this species ranging from 3.3% – 25%.[342, 335, 343, 317] In 2014, common dab derived SAV5 was cultured in a salmonid cell line.[342, 317] Phylogenetic studies suggest that transmission of SAV4 between common dab and farmed Atlantic salmon have occurred.[344] This species does meet the WOAH criteria required to confirm species susceptibility.[3]

Other Wild Caught Flatfish Species

SAV has been detected in other wild-caught marine flatfish (Ballan wrasse [*Labrus bergylta*], European plaice [*Pleuronectes platessa*], long rough dab [*Hippoglossides platessoides*]) in Scotland and Ireland (the Scottish sea, and the Irish and Celtic seas, respectively).[341, 342, 317] Published whole-genome sequencing of the SAV isolated from an asymptomatic Ballan wrasse suggests an additional subtype (SAV7).[316] Detections of SAV in these flatfish species is well reported in the literature; however, the role of these species in the epidemiology of SAV has not been determined. As of 2023, the WOAH criteria for species susceptibility have not been met for any of these fish.[3]

Potential Reservoir Hosts

Recurrence of SAV in sea- and fresh-water aquaculture facilities and SAV introductions into farmed salmon from unknown sources have been documented, suggesting the presence of reservoir hosts.[317] SAV has been detected in wild flatfish; however, the significance of the detections and the capability of these fish species to serve reservoir or accidental hosts is not known.[337, 342] SAV has been recovered from sea lice (*Lepeophtheirus salmonis*) collected from Atlantic salmon during PD outbreaks; however, viral replication and transmission capability have not been demonstrated.[312] The potential for crustacean and mollusc species to function as reservoir or accidental hosts has not been determined. The potential for farmed Atlantic Salmon, rainbow trout, and steelhead trout to function as reservoir hosts has not been determined. In farmed Atlantic salmon and rainbow trout at all production stages, SAV infection results in a brief viremia, followed by development of humoral immune responses.[345] Following the viremic period, viral RNA is detectable in individual fish by reverse transcription-polymerase chain reaction (RT-PCR) and virus isolation.[346, 333, 345] Some authors and WOAH state that these findings, and reports of repeated occurrences of SAV at farm sites, are supportive evidence for long-term carrier or reservoir host status.[333, 345, 347, 129] Other authors report that these finding

may only represent low levels of residual RNA remaining in host tissues after infection, that the host's immune response is significant enough to prevent recrudescence of the disease, and that evidence to confirm a long-term carrier status is incomplete.[348, 346, 341]

Environmental Characteristics

Many of the extrinsic environmental factors (e.g., dissolved oxygen, presence of other infectious agents and parasites, salinity, suspended organic matter, ultraviolet radiation, water currents, wind) that may be associated with the epidemiology of SAV are not well described.[333, 347, 349] In natural conditions, the temperature range associated with SAV occurrence is 9 – 15 °C/48.2 – 59 °F.[330] Extrinsic factors associated with aquaculture (e.g., biosecurity, environmental controls, feeding regimens, fish movement, handling and sorting of fish, health management, management practices, movement and sharing of boats, equipment and personnel, population density, proximity to fish processing, proximity of net pens and farms, stocking density, vaccination, and other factors) also affect SAV introduction, occurrence, and duration of infection.[333, 344] The capability for SAV to persist in biofilms, organic matter, and sediments has not been confirmed.

In the laboratory, optimal growth of SAV in cell culture occurs at 10 –14 °C/50 – 57.2 °F.[330] Differences in optimal growth rates among serotypes within this temperature range have been reported.[330, 350] However, infectivity (capability of virus to infect cells) is maintained at temperatures up to 37 °C/98.6 °F.[351, 330] Virus survivability in sterile water, organically loaded salt water, and cell culture ranges from 5.7, 35, and 56 days at 10 °C/50 °F, 20 °C/68 °F, and 4 °C/39.2 °F, respectively[352, 338, 342, 344, 129] SAV isolated in cell culture and in serum/plasma samples remains viable for years without a significant decline in virus titer when stored at -80 °C/-112 °F.[129] It has been reported that infectivity is lost at or below pH 3.0 and is reduced at pH 11.0.[330]

Pathogen Characteristics

SAV is highly infectious and causes large economic losses in countries where it is highly prevalent. However, information specific to infectious dose, pathogenic mechanisms, virulence factors, how long SAV maintains infectivity is limited.[349] Jarungsriapisit (2016) and Moore (2017) reported that 7 $TCID_{50}$ L^{-1} (50% tissue culture infective dose per liter) of SAV3 in seawater is sufficient to induce infection in Atlantic salmon smolts challenged by bath immersion (6 hours immersion in static water).[353, 349, 317] The molecular determinants of virulence have not been identified but appear to be SAV subtype variable. A small number of cell culture studies suggest that genome replication, transcription efficiency, cell receptor binding, and amino acid substitutions in the E2 glycoprotein are associated with virulence and activation of severe inflammatory responses that generate severe pathological damage in infected fish.[354]

Transmission

Transmission of SAV in the field is not fully described because many factors associated with transmission (e.g., shedding rate, environmental conditions such as dilution, wind and current strength and direction) have not been determined.[349] Experimentally, viral shedding from infected fish coincides with the viremic period of infection.[333, 355] Virus is shed into the water in feces and mucus.[320, 356, 355] In farmed rainbow trout, it is reported that SAV is transmitted directly from infected resident or introduced rainbow trout or via virus present in the water column, and indirectly by contaminated equipment and personnel.[330, 313, 336, 129] In Norway, transmission

among farmed Atlantic salmon is thought to occur via self-sustaining direct transmission events that lead to disease outbreaks and virus present in the water column.[357, 325, 317]

Hydrodynamic, spatiotemporal, and statistical transmission models suggest that ocean currents and water contact time between farms are the variables that best correlate with local PD outbreaks.[314, 358, 338, 336, 359, 360] Long distance outbreaks are thought to occur primarily through the transport or introduction of infected live farmed fish.[358, 336, 359, 360] However, identification of phylogenetically related SAV isolates, in marine salmon farms separated by large geographical distances, unexplainable outbreaks, and recurrence of PD in fallowed Atlantic salmon net pens and farms have been reported.[341, 357, 344, 360, 317] It has been suggested that this indicates there are other modes by which the virus enters or moves through the water, or that there are other marine SAV reservoirs through which bidirectional transmission (e.g., escaped farmed Atlantic salmon, other wild fish species, animal vectors) or unilateral transmission (e.g., biofilms, organic matter, sediments) occurs.[341, 342, 357, 344, 343, 360, 317] For example, in 2014, Skjold et al. (2013) reported that SAV can be detected in the lipid film found on the surface of the water around salmon farms and suggested that this oil layer could serve as a protective fomite for SAV transmission between net pens and salmon farms.[355, 336]

Vector transmission of SAV has not been demonstrated. SAV has been detected by RT-PCR in salmon lice (*Lepeophtheirus salmonis*) during SAV outbreaks.[361, 129] However, transfer of SAV from salmon lice to susceptible fish has not been reported.[361, 335, 317, 129] Other potential methods of SAV introduction include farm management activities (e.g., fish movement and handling, shared equipment and other fomites, lack of biosecurity measures to prevent transmission between sites), fish slaughter practices, and the use of unpasteurized fish, fish meal, or fish products in feed.[317] Sufficient evidence for vertical transmission in rainbow trout and Atlantic salmon is lacking.[303, 347, 344, 325, 129]

Clinical Signs and Pathogenicity

Viremia precedes development of clinical signs.[355] During viremia, a substantial quantity of virus is detectable in the serum.[5] Primary target organs include the heart and pancreas; however, virus is also found in brain, kidney, spleen, gills, mucous, and feces.[324, 5] Subclinically infected fish exhibit no clinical signs of disease. When clinical signs do occur, they are not pathognomonic.[331, 328] Initially fish exhibit decreased appetite. As the disease progresses, clinical signs include anorexia, exophthalmos, lethargy, swelling of the abdomen, decreased and slow swimming activity, and “sleepy behavior” (inactivity and laying on their sides on the bottom of enclosures).[330, 312, 331, 313, 328] Increased numbers of fecal casts may be observed. Growth and rate of gain are reduced in later stages of the disease.[312] Fish surviving infection appear stunted, slender, and have poor body condition.[312] Differential diagnoses include infectious pancreatic necrosis (IPN), heart and skeletal muscle inflammation disease (HSMI), cardiomyopathy syndrome (CMS), and nutritional myopathies.[5]

Morbidity and Mortality

The duration of SAV outbreaks range from 1 – 32 weeks.[345, 321, 129] Increased mortality rates typically begin 1 – 2 weeks after the onset of an outbreak. Mortality rates are affected by virus subtype and host (fish species, age, overall health), environmental (season, temperature, water quality), and anthropogenic factors (farm management, husbandry, biosecurity).[320, 323, 129] Cumulative mortality at the farm level typically ranges from 3%–50%.[333, 323, 129] However, rates

may approach 80% or greater if populations are stressed or concurrent disease or parasitism is present.[312] Chronic morbidity may be observed in fish that survive outbreaks.[312, 325]

Treatment

There is no treatment. According to the literature, cumulative mortality may be reduced during outbreaks by minimizing handling and cessation of feeding.[129] Preventative vaccines are commercially available in countries where disease is present, and have been shown to reduce the risk of infection, viral shedding, cumulative mortality during outbreaks, and downgrading of carcasses at slaughter.[355, 129] Atlantic salmon breeding programs in Ireland and Norway have demonstrated some success in introducing increased SAV resistance.[340, 129]

Diagnostic Testing

Gross pathological findings in Atlantic salmon and rainbow trout are not pathognomonic and include ascites, exophthalmos, pale myocardial tissues, petechial hemorrhages in tissues, reddening of the pancreatic area near the pyloric caeca, scale pocket edema, and yellow mucoid content in the gastrointestinal tract. Pale heart muscle or cardiac ruptures may be present.[312, 129] Histopathological changes develop sequentially. Inflammatory cell infiltration and necrosis of exocrine pancreatic tissues are the first lesions to appear. Within two weeks post-infection inflammatory cell infiltration and myocarditis are observed in the heart. At approximately three weeks, inflammatory myositis is present in skeletal muscles.[312, 362] Late in the disease pancreatic peri-acinar and skeletal muscle fibrosis or regeneration may be detected. Occasionally, inflammatory lesions in the peripancreatic fat, and eosinophilic cytoplasmic granules in the kidney are observed.[314, 312, 129]

Diagnostic tests to confirm SAV infection include VI and PCR assays. WOAH recommended diagnostic tests and protocols for SAV specimen selection, sample collection, transport and handling are available in the WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.8. and the WOAH Aquatic Animal Health Code, Chapters 1.4. and 10.5.(Appendix, Table 1).[5, 97] In the United States, Title 50 diagnostic testing methods include use of virus isolation/culture methods using cell lines sensitive to SAV infection. 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.[161] Relevant information, including sample submission instructions are in the Appendix, Table 1.

Prevention and Control

Risk factors associated with SAV outbreaks in farmed fish include a previous history of SAV infection, high feeding rates, concomitant disease or parasitism, and use of autumn smolts.[358, 337, 324, 129] In Norway, where SAV is endemic, a combination of vaccination, avoidance of farm practices that increase stress (frequent movement of fish, overhandling, overcrowding), stringent biosecurity, depopulation measures, and geographical separation of net pens and farm sites are used to reduce the occurrence of PD outbreaks.[355, 325] Other preventative husbandry practices include acquisition of fish, fertilized eggs, and gametes from SAV-free sources, generational segregation of fish, prompt removal of sick and dead fish, regular cleaning of tanks and net pens, fallowing of farm sites, prevention and control of other parasites and pathogens, and use of site-dedicated equipment and personnel.[129] If use of site-specific equipment is not practical, equipment should be thoroughly cleaned and disinfected before use. Thorough cleaning and disinfection of ponds, raceways, net pens, and equipment followed by fallowing

should be implemented as control measures following outbreaks.[97] Fish processing is described in published literature as a potential pathway for pathogen introduction.[13, 129] Risk of exposure is proportional to the proximity of processing facilities to hatcheries and fish farms (inland and marine). Ideally processing plants should be located as far as possible from fish farming sites. Processing effluent, solid and sludge wastes should be disinfected and disposed of using methods to prevent pathogen introduction.

Commercially available disinfectants containing alcohol ethoxylate, iodine, potassium peroxymonosulfate (Virkon™ S), and quaternary-based compounds (Virex®) with efficacy against other alphaviruses are effective in inactivating SAV.[363, 364, 97] Ultraviolet light, temperatures greater than 60 °C/140 °F, and pH extremes (less than or equal to 4.0, equal to or greater than 12.0) are also reported as effective.[363, 347, 129] The presence of organic matter decreases the effectiveness of disinfectants; therefore, surfaces should be cleaned prior to application of the disinfectant.[363] Standard egg disinfection protocols are considered sufficient to prevent surface contamination of fertilized eggs.[363, 97]

The USFWS does not require pre-import testing of imported live salmonids for SAV. However, SAV is cultivable in the cell lines that are utilized in other required USFWS import health certifications and would likely be detected. It is within the purview of USFWS to decline an importation request for live salmonid fish, fertilized eggs, and gametes based on assessments of risk for a disease not listed in Title 50 on a case-by-case basis.[105] The USFWS does require that all fertilized salmonid eggs be disinfected within 24 hours prior to shipment using specific protocols described in CFR, Title 50.[102] Water and ice used for shipping must be derived from pathogen-free water and must be disposed of according to specific protocols.[102]

Many countries utilize import/export regulations and recommendations in effort to limit or control the risk of SAV introduction. A summary of WOAH import/export guidelines specific to SAV, U.S. regulations, and other regulatory information related to aquaculture in the United States is summarized in the Appendix, WOAH Pathogen Specific Import Export Recommendations.

Summary

SAV is an economically important WOAH-listed viral disease affecting marine-reared (Atlantic salmon, steelhead trout) and freshwater-reared (rainbow trout) food fish. The most pronounced consequences of SAV are reduced fish welfare, high morbidity, and mortality, reduced feed conversion ratios, reduced growth and gain in affected fish, and reduced carcass quality. Impacts associated with outbreaks include the costs of outbreak mitigation and control which result in local economic and labor effects. SAV is a WOAH listed disease; therefore, introduction and outbreaks of SAV are likely to have national economic consequences relative to trade.

In regions where SAV is endemic, control of disease occurrence is difficult once introduction has occurred. Reoccurrence of disease has been reported in marine and freshwater fish farms after outbreak mitigation and site fallowing. Currently, there are gaps in the epidemiology of this disease that limit capability to discern why recurrences happen. In marine settings, SAV has been detected in some wild fish species, leading to speculation that wild reservoir hosts may be present in the environment. In both marine- and freshwater settings, presence of chronically infected farmed fish, or presence of biofilms, organic matter, or other environmental niduses have been considered potential sources of SAV reintroduction.

Best practices to prevent introduction of SAV into aquaculture include development of policies that would ensure live fish, fertilized eggs, and gametes are imported from SAV-free sources and that importation complies with the guidelines described in the WOAH Aquatic Animal Health Code.[97]

Viral Hemorrhagic Septicemia Virus

Introduction

Viral hemorrhagic septicemia (VHS) is a viral disease of wild and farmed marine and freshwater teleost finfish. The etiological agent is viral hemorrhagic septicemia virus (VHSV; genus *Novirhabdovirus*, family Rhabdoviridae)(Appendix, Table 1).[243, 97] VHS has been associated with high mortality (greater than 90%) disease outbreaks in farmed and wild fish globally.[365] This disease is described in peer-reviewed published literature as a disease with potential to cause serious economic and environmental impacts to aquaculture, indigenous susceptible fish species, and natural resources.[366, 367]

This virus is an enveloped, bullet-shaped, non-segmented, negative-sense, single-stranded RNA virus.[243, 368, 97] The linear genome encodes six proteins (e.g., glycoprotein G, matrix protein M, non-virion protein NV, nucleoprotein N, phosphoprotein P, and RNA polymerase protein L).[368, 97] The genes are separated by conserved gene junctions with di-nucleotide gene spacers.[368] The NV protein is unique to the genus and can suppress apoptosis in early stages of viral infection.[369, 368] The other five proteins are common among rhabdoviruses.[368] The G glycoprotein comprises the neutralizing surface antigen, and is a key component of host cellular receptor adhesion and insertion, viral replication, evasion of host immune responses, infection emergence, and cross-species transmission.[368, 97] Phylogenetic analyses infers that the virus is of marine origin, and that there are four geographically distributed genotypes (I–IV), and several genotype I and IV sublineages.(Table 16).

Table 16. Viral hemorrhagic septicemia virus (VHSV) genotypes and sublineages[370, 371, 372, 373, 367, 374, 375, 243, 368, 97, 5]

Genotype 1	
This genotype is found in Europe and is comprised of six sublineages that correspond to specific geographic regions. This genotype is capable of infecting multiple species of fish. It is highly virulent in freshwater farmed rainbow trout and is often associated with high mortality (up to 100% in fry) disease outbreaks. Phylogenetic data suggest this genotype originated from wild marine fish, with several host species jumps prior to adaptation to rainbow trout.	
Sublineage Ia	Found in terrestrial freshwater bodies in continental Europe. It was the first sublineage associated with the European aquaculture industry (freshwater farmed rainbow trout), and it continues to be the primary isolate associated with disease outbreaks in that species. This sublineage can be further divided into VHSV-Ia-1 and VHSV-Ia-2, each with distinct geographic distributions.
Sublineage Ib	The prevalent genotype in marine environments in Northern Europe (e.g., the Baltic Sea, Kattegat, Skagerrak, the North Sea, and the English Channel, and as far north as latitude 70°N in Norway). This sublineage is

	detected in wild marine fish but has not been associated with clinical disease outbreaks. In 1998 and 2000, evidence of transfer between wild fish and farmed steelhead trout was reported in Sweden. Historically, there was a single foreign introduction in Japan.
Sublineage Ic	Is found in freshwater bodies in continental Europe, including in mainland lakes of Germany, Austria, and Denmark.
Sublineage Id	Found marine and freshwater bodies extending from Scandinavia to the Baltic Sea. Has been detected in farmed rainbow trout reared in fresh- and brackish-water in Norway and Finland. Experimentally, this sublineage is pathogenic but less virulent than sublineage VHSV-Ia to rainbow trout.
Sublineage Ie	Is described in the literature as a marine isolate from the Baltic Sea. It has been isolated from farmed rainbow trout in freshwater and marine environments.
Genotype II	
This genotype is present in wild marine fish in the Baltic Sea, including the Gulf of Bothnia and Gulf of Finland. It has also been detected in lamprey in Gulf of Bothnia tributary rivers. This genotype has not been associated with disease outbreaks or mortalities.	
Genotype III	
This genotype has been detected in wild and farmed marine fish in the North Atlantic Sea from the Flemish Cap near Newfoundland to Norway, and the North Sea (the British Isles, Skagerrak, and Kattegat). In 2007, this genotype was associated with a disease outbreak in farmed steelhead trout in Norway.	
Genotype IV	
This genotype is found in North America (Atlantic Ocean coastal areas, the Great Lakes region, and the Pacific Northwest). Detections have also been reported in Asia (Japan, South Korea). This genotype contains four sub-lineages, which can be highly virulent in susceptible marine and freshwater fish species (mortalities ranging from 20%–80% in some outbreaks). Salmonids are susceptible to infection with this genotype; however, the virulence of this genotype and its sublineages are low and clinical disease is rare.	
Sublineage IVa	Has been detected in wild marine finfish in the Pacific Ocean waters of western North America from Alaska to California, and in Japan and South Korea. Virulence is variable among fish species. Infected fish may be asymptomatic or may exhibit clinical signs of disease. Pacific and Atlantic salmon, rainbow trout, and steelhead trout are susceptible to infection; however, infection rates are low, and pathogenicity is minimal. This

	genotype is sporadically detected in Pacific salmon and has historically been detected in farmed Atlantic salmon in the Pacific Northwest.
Sublineage IVb	Is endemically present in freshwater fish in the Laurentian Great Lakes and associated lakes and rivers in North America. It has a broad host range and has been associated widespread epidemics and large die-offs in numerous fish species.
Sublineage IVc	Is present in North American Atlantic coastal and brackish estuarine waters of New Brunswick and Nova Scotia, Canada. It does share some genetic sequence homology with genotype IVb.
Sublineage IVd	Was recently identified in Iceland in wild and sea-farmed lumpfish (*Cyclopterus lumpus*).

VHS is a reportable disease in the United States and is a WOAH listed notifiable disease.[95, 97] This disease is included on the USDA APHIS NAHRS and NLRAD lists of reportable diseases (Appendix, Table 1).[1, 123] State and Federal authorities should be contacted upon suspicion or detection of VHS (Appendix, Table 1). Information relevant to the importation of fish species susceptible to VHSV is in the Appendix, Regulatory Information Associated with Salmonid Aquaculture.

Affected Fish Species

Fish species meeting WOAH criteria for listing as susceptible to infection with VHSV are summarized in Table 17.[97, 5] Fish species described in published literature that do not meet these criteria or in which VHSV infection was inferred using diagnostic methods that were not validated according to WOAH protocols are not included in this assessment. In the United States, there are many susceptible marine and freshwater wild and farmed fish species, including Atlantic salmon, rainbow trout, and steelhead trout.

Table 17. Fish species identified by the World Organisation for Animal Health (WOAH) as susceptible to infection with viral hemorrhage septicemia virus (VHSV)[97, 5]

Genus and species	Common Name	Associated genotypes		
Coregonus lavaretus	Common whitefish	Ia		
Esox Lucius	Northern pike	Ia		IVb
Oncorhynchus mykiss	Rainbow trout	Ia-e	III	IVb
Oncorhynchus mykiss X Oncorhynchus kisutch hybrids	Rainbow trout X coho salmon hybrids	Ia		
Salmo marmoratus	Marble trout	Ia		

Salmo trutta	Brown trout	Ia-b				
Salmo salar	Atlantic salmon	Ia-b	II	III		IVa
Salvelinus namaycush	Lake trout	Ia			IVa	IVb
Thymallus thymallus	Grayling	Ia				
Clupea harengus	Atlantic herring	Ib		III		
Gadus morhua	Atlantic cod	Ib		III		
Limanda limanda	Common dab	Ib				
Micromesistius poutassou	Blue whiting	Ib		III		
Platichthys flesus	European flounder	Ib				
Pomatoschistus minutus	Sand goby	Ib				
Scophthalmus maxima	Turbot	Ib		III		
Sprattus sprattus	European sprat	Ib				
Trisopterus esmarkii	Norway pout	Ib		III		
Alosa immaculata	Pontic shad	Ie				
Engraulis encrasicolus	European anchovy	Ie				
Gaidropsarus vulgaris	Three-bearded rockling	Ie				
Mullus barbatus	Red mullet	Ie				
Merlangius merlangus	Whiting	Ie				
Raja clavate	Thornback ray	Ie				
Trachurus mediterraneus	Mediterranean horse mackerel	Ie				
Uranoscopus scaber	Atlantic stargazer	Ie				
Lampetra fluviatilis	River lamprey		II			
Centrolabrus exoletus	Rock cook wrasse			III		

Ctenolabrus rupestris	Goldsinny wrasse	III		
Labrus bergylta	Ballan wrasse	III		
Labrus mixtus	Cuckoo wrasse	III		
Pleuronectes platessus	European plaice	III		
Solea senegalensis	Senegalese sole	III		
Symphodus melops	Corkwing wrasse	III		
Ammodytes hexapterus	Pacific sand lance		IVa	
Clupea pallasii pallasii	Pacific herring		IVa	
Cymatogaster aggregata	Shiner perch		IVa	
Danio rerio	Zebra fish		IVa	
Gadus macrocephalus	Pacific cod		IVa	
Oncorhynchus kisutch	Coho salmon		IVa	
Oncorhynchus tshawytscha	Chinook salmon		IVa	IVb
Paralichthys olivaceus	Bastard halibut		IVa	
Sardinops sagax	South American pilchard		IVa	
Scomber japonicus	Pacific chub mackerel		IVa	
Thaleichthys pacificus	Eulachon		IVa	
Ambloplites rupestris	Rock bass			IVb
Ameiurus nebulosus	Brown bullhead			IVb
Aplodinotus grunniens	Freshwater drum			IVb
Coregonus artedii	Lake cisco			IVb
Coregonus clupeaformis	Lake whitefish			IVb
Dorosoma cepedianum	American gizzard shad			IVb

Esox masquinongy	Muskellunge	IVb		
Lepomis gibbosus	Pumpkinseed	IVb		
Lepomis macrochirus	Bluegill	IVb		
Micropterus dolomieu	Smallmouth bass	IVb		
Micropterus salmoides	Largemouth bass	IVb		
Morone americana	White perch	IVb		
Morone chrysops	White bass	IVb		
Morone saxatilis	Striped bass	IVb	IVc	
Neogobius melanostomus	Round goby	IVb		
Notropis atherinoides	Emerald shiner	IVb		
Notropis hudsonius	Spottail shiner	IVb		
Perca flavescens	Yellow perch	IVb		
Pimephales notatus	Bluntnose minnow	IVb		
Pimephales promelas	Fathead minnow	IVb		
Pomoxis nigromaculatus	Black crappie	IVb		
Sander vitreus	Walleye	IVb		
Fundulus heteroclitus	Mummichog		IVc	
Gasterosteus aculeatus	Three-spine stickleback		IVc	
Cyclopterus lumpus	Lumpfish			IVd

Geographic Distribution

VHSV has been reported in fish present in marine and freshwater bodies throughout the Northern Hemisphere (Northern Europe, North America, and North Asia). Distributions of VHSV and the various sublineages are summarized in Table 17. Countries reporting presence via the WOAH WAHIS (Appendix, Table 1) database for years that data are available (2005–2022) include Austria, Belgium, Canada (British Columbia, Newfoundland, Nova Scotia, Ontario, and Quebec), Croatia, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Iceland, Iran, Italy, Japan, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, South Korea,

Sweden, Switzerland, Turkey, the United Kingdom (England, Scotland), and the United States.[129] VHSV has never been reported in the Southern Hemisphere.[129]

North America

VHSV-IVa was first identified in the 1980s in Washington in asymptomatic adult Coho salmon (*Oncorhyncus kisutch*) and Chinook salmon (*Oncorrhynchus tshawytscaha*) returning to hatcheries.[376, 243, 368, 377] Subsequently, the virus has been detected in multiple marine and anadromous fish species (Table *17*), including other Pacific salmon (wild and hatchery reared) and farmed Atlantic salmon, that exhibit varying degrees of susceptibility to the virus and clinical signs ranging from asymptomatic infection to epizootic disease outbreaks.[376] Presently, VHSV-IVa is endemically present at low prevalence and intensity throughout the Pacific Northwest from Alaska to California (including British Columbia).[376, 378] The origin of VHSV in this region is unclear. In British Columbia, there are historical reports dating to the 1940s of VHSV-like mortality events in Pacific herring (*Clupea pallasii*) and Pacific sardine (*Sardinops sagax*).[376]

VHSV-IVb was first identified in Lake St. Claire, Michigan in 2003. The virus has subsequently been detected in all major water bodies of the Great Lakes system, including the St. Lawrence River and inland lakes in Michigan, New York, Ohio, Ontario, and Wisconsin, and has caused high mortality outbreaks in multiple wild fish species.[379, 366, 291, 380, 243, 368] There have been no detections of VHSV-IVb in freshwater farmed or managed fish in this region.[381] The route of introduction into the Great Lakes has not been definitively identified. Ballast water, natural fish migrations, translocations of fish (including baitfish), and recreational fishing have all been proposed as potential pathways of entry.[382, 383] In the years following introduction, the virus has undergone genetic divergence and diversification among the Upper, Central, and Lower Great Lakes, resulting in declining virulence and occurrence of outbreaks.[384, 375] Presently, it is estimated that approximately 30 wild fish species in the Great Lakes region are susceptible to VHSV (Table 17).[385, 386, 383, 387] In 2000, VHSV-IVc was detected in four wild fish species (Table 17), including brown trout (*Salmo trutta*) in the northeastern Atlantic coastline of North America (e.g., New Brunswick and Nova Scotia, Canada).[388, 373]

Public Health

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

Epidemiology

Factors associated with the epidemiology of VHSV are not fully described and there are differences among genotypes relative to the geographic distribution and host susceptibility to disease. Therefore, generalizations on host susceptibility, environmental characteristics, and pathogen virulence and pathogenicity should be interpreted with caution.[389]

Host Characteristics

Seventy fish species are recognized by WOAH as susceptible to infection with VHSV (Table *17*).[97, 5] Comparatively, the number of fish described as susceptible in the peer-reviewed literature approaches 140 species. However, this estimate must be interpreted with caution because it includes species of fish experimentally challenged with VHSV using methods that do not meet WOAH criteria for natural infection (e.g., intraperitoneal injection).[3]

Host factors affecting susceptibility to VHSV infection include fish species, age, overall physiological condition (e.g., immune status, nutritional status, general health, presence of concomitant infection or parasitism, population density), and the presence of certain behaviors

(e.g., spawning).[366, 367, 375, 376, 378] Susceptibility has been observed to decrease with age in some fish species, potentially due to acquired immunity from prior VHSV exposures.[376, 378]

According to WOAH, the potential long-term reservoir, carrier, and transmission capacity of susceptible fish species in natural environments are not clearly defined.[97] Subclinical infection at low prevalence rates (1% –17%) with host specific genotypes has been observed in some wild marine and freshwater fish species, which suggests that some fish species are capable of serving as long-term reservoir hosts.[390, 391, 392, 367, 393, 378] The duration of the potential carrier status in wild fish is unknown, but has been hypothesized to be lifelong, with virus shedding occurring intermittently.[390, 391, 393, 378] Factors associated with recrudescence of shedding are largely unknown but may be influenced by pathogen, environmental, and host factors.[394]

The duration of viral shedding by experimentally challenged fish varies by fish species, experimental challenge method (bath immersion versus intraperitoneal injection), virus genotype, and challenge dose. Periods of viral shedding in published literature range from four days up to 60 weeks.[395, 396, 386, 392, 397, 398, 374, 376] Experimentally it has been demonstrated some carrier fish resume shedding after exposure to stress (e.g., handling stress) for up to 15 weeks.[392, 393] Greater rates of shedding have also been documented as water temperatures decreased from 15 °C/59 °F to 8 °C/46.5 °F.[399, 393]

Host susceptibility to infection varies by fish species and VHSV genotype and sublineage (Table *18*).[97] Some hosts are susceptible to infection with one genotype, while others are susceptible to multiple genotypes and/or sublineages.[400, 399, 376, 243, 97] Nine salmonid fish are identified by WOAH as variably susceptible to infection by different VHSV genotypes (Table *18*).[97, 5]

Table 18. Susceptibility of specific salmonid species to infection with various viral hemorrhage septicemia virus (VHSV) genotypes, as identified by the World Organisation for Animal Health (WOAH)[97, 5]

Genus and species	Common Name	Associated genotypes
Oncorhynchus mykiss	Rainbow trout	
*Steelhead trout**	Ia-e	III* IVb
Oncorhynchus mykiss X Oncorhynchus kisutch hybrids	Rainbow trout X coho salmon hybrids	Ia
Salmo marmoratus	Marble trout	Ia
Salmo trutta	Brown trout	Ia-b
Salmo salar	Atlantic salmon	Ia-b II III IVa
Salvelinus namaycush	Lake trout	Ia IVa IVb
Thymallus thymallus	Grayling	Ia

Oncorhynchus kisutch	Coho salmon	IVa	
Oncorhynchus tshawytscha	Chinook salmon	IVa	Ivb

Rainbow Trout (Freshwater)

Freshwater rainbow trout are susceptible to infection by VHSV Ia–Ie.[371, 97, 5] The pathogenicity of the subtypes in this species is variable.[243, 368] For example, VHSV-Ia is highly virulent in rainbow trout, and has been associated with severe disease outbreaks in Europe.[373, 367] Published literature states the pathogenicity of VSHV-Ib–e is low in this species.[372, 367] VHSV-II and VSHV-III are marine isolates. Freshwater rainbow trout are unlikely to be exposed to these genotypes under natural conditions, and exhibit low susceptibility to infection experimentally.[372, 401] Rainbow trout are relatively resistant to VHSV-IVa–c; however, low rates of susceptibility to infection and pathogenicity have been observed experimentally.[373, 402]

Steelhead Trout (Marine-farmed and Anadromous Rainbow Trout)

In general, steelhead trout exhibit the same susceptibility to infection with VHSV genotypes observed in freshwater rainbow trout. However, in 2007, disease outbreaks caused by VSHV-III occurred in marine-farmed steelhead in Norway.[373, 401] Subsequently, this genotype has been detected in net-pen reared steelhead trout in other regions (Finland).[401, 403]

Atlantic Salmon

Atlantic salmon are susceptible to infection with VHSV-I–III but are refractory to development of clinical disease.[404, 97, 5] VHSV-IVa has been periodically detected in farmed Atlantic salmon in Canada (British Columbia) since 1996, and the United States (Washington, when Atlantic salmon farming was present).[373, 397, 376] Detections have occurred in apparently healthy fish during routine surveillance and in association with low-level clinical disease and mortality.[376] Detections often are concurrent to detections in pelagic fish species found near the affected net pens.[376] Natural infection with VHSV-IVb has not been described. Experimentally, Atlantic salmon demonstrate susceptibility to infection with VHSV-IVb following experimental challenge via intraperitoneal injection (challenge dose 106 pfu (plaque forming units)/fish). Clinical and gross pathological signs of disease and low rates of mortality were observed in some challenged fish (54% and 2%, respectively). Variable levels of virus (101–107 mean viral RNA copies detected/µg total RNA in each fish) were detectable by quantitative reverse transcription polymerase chain reaction (qRT-PCR) up to day 49 post-challenge (termination of the study).[405] However, results of this study should be interpreted with caution, given that the challenge method used does not meet WOAH criteria for natural infection.[3] Review of the literature did not find publications describing detection of VHSV-IVc in farmed Atlantic salmon on the East coast of the United States or experimental studies exploring the susceptibility of Atlantic salmon to this genotype.

Pacific Salmonids

Review of the literature did not identify reports of natural infection of Pacific salmonids (*Oncorhynchus* spp.) with VHSV-I – III, and experimental challenge studies examining the susceptibility are generally lacking. Emmenegger et al. (2013) did report that Chinook salmon exhibited susceptibility to VHSV-Ia and VHSV-IVb following experimental challenge by intraperitoneal injection; however, these results should be interpreted with caution.[373, 3] Pacific salmonids are susceptible to infection with VHSV-IVa, but typically do not develop clinical

disease.[373, 400, 376] Infections are intermittently detected during routine surveillance of hatchery reared fish returning to freshwater to spawn.[376] Natural infection with VHSV-IVb and VHSV-IVc have not been reported. Limited published research is available describing VHSV-IVb experimental challenge studies in Pacific salmon. Review of the literature did not find publications describing experimental studies exploring the susceptibility of Pacific salmon to VHSV-IVc.

Other Salmonid Fish

Susceptibility to VSHV has been reported in other salmonid fish. Lake trout (*Salvelinus namaycus*) are reported susceptible to infection with VHSV-Ia, VHSV-IVa, and VSHV-IVb.[373, 402, 376] Brown trout are reported as susceptible to infection with VHSV-Ia – b,[97, 5] and Gagne et al. (2007) describes isolation of VHSV-IVb from brown trout mortalities.[388]

Other North American Fish Species

In the Pacific Northwest, several marine fish species (e.g., Pacific hake [*Merluccius productus*], Pacific herring, Pacific sand lance [*Ammodytes hexapterus*], Walleye Pollock [*Gadus chalcogrammus*], yellow perch [*Perca flavescens*]) are highly susceptible to VHSV-IVa infection, and can develop clinical disease leading to high mortality outbreaks (Table *17*).[376] Published literature states that approximately 30 freshwater fish species in the Great Lakes Region are susceptible to infection with VHSV-IVb (Table *17*).[385, 406, 384] VHSV-IVc has been detected in marine/estuarine fish (e.g., mummichog [*Funduus heteroclitus*], stickleback [*Gasterosteus aculeatus aculeatus*], brown trout; and striped bass [*Morone saxatilis*]) in the Atlantic coastal region of North America (e.g., New Brunswick and Nova Scotia, Canada).[388, 407, 373]

Other Animals

VHSV has been detected in other aquatic animal species (e.g., amphipods [*Hyalellea* spp. and *Dipporeia* spp.], common snapping turtle [*Chelra serpentina*], leech [*Myzobdella lugubris*], northern map turtle [*Grapetymys geographicas*], and water flea [*Moina macrocopa*]).[408, 409, 380, 394] However capability of these species, piscivorous birds, or terrestrial wildlife that frequent water and/or scavenge fish to serve as transport or transmission vectors in natural environments has not been definitively proven.

Environmental Characteristics

Environmental factors affecting the length of time that VHSV remains viable in fresh- or sea-water include, but may not be limited to microbial content, organic load, water salinity and temperature, and exposure to ultraviolet light.[410, 399, 394, 376, 411] The presence of bacteria decreases virus stability. Experimentally, virus survival is reduced when bacteria are present in the water and increases when water is autoclaved and filtered through a 0.22 µm membrane.[411, 129] Experimentally, virus stability improves when organic materials such as aqueous proteins (e.g., ovarian fluids, blood products, bovine serum) are added to water samples.[399, 129] VHSV is capable of surviving for several weeks in soil-based sediments; however, survival is affected by the sediment composition.[412] Experimentally, the virus is capable of surviving on stainless steel surfaces for approximately 42 days at temperatures ranging from 4 – 25 °C/39 –77 °F.[412] On plastic surfaces the virus can survive for 6–21 days at temperatures between 4 –37 °C/39.2 – 98.6 °F.[402, 412] Environmental pH affects virus characteristics. The virus is stable at low pH (5.0); however, replication does not occur until the pH ranges from 7.4 –7.8.[243]

All VHSV genotypes appear to be more stable in freshwater versus saltwater.[390, 391, 410, 389, 403, 243] Experimentally, in raw freshwater, the virus can persist for 13 and 28 – 25 days when stored at

15 °C/60 °F and 4 °C/39.2 °F, respectively.[389, 374, 403, 129] In filtered freshwater stored at 4 °C/39.2 °F, viability can be maintained for over one year.[403, 129] In seawater stored at 15 °C/60 °F virus viability is reduced by 50% after 10 hours and inactivation (99.9%) occurs after 4 days.[389, 374, 403, 129]

Water temperature affects host factors (e.g., infection rate, development and duration of clinical disease, virus shedding, longer virus persistency in tissues, and mortality) and VHSV characteristics (pathogenicity, replication, and survival in the environment).[216, 399, 376, 413] In marine environments, VHSV outbreaks are most commonly observed in winter and spring months when water temperatures are fluctuating or rising.[399] In inland freshwater environments, most outbreaks occur in spring and early summer when water temperatures are rising and spawning is occurring.[366] The optimal water temperature is consistent among genotypes and ranges from 4 –15 °C/39.2 – 59 °F.[414, 415, 416] Within this range, optimal viral viability and pathogenicity, and replication occurs at 9 –12 °C/48.2 – 53.6 °F and 14 –15 °C/57.2 – 59 °F, respectively.[366, 374, 375, 243] At temperatures below or above the optimal range, virus survival is lower, host transmission and infection rates decrease, and if disease occurs, the course is short with low mortality.[389, 399, 367, 243] Natural outbreaks typically do not occur once water temperatures reach 18 – 20 °C/64.4 – 68 °F.[366, 374, 403, 243] VHSV can be propagated in cell cultures at temperatures up to 20 °C/68 °F. Virus stocks remain infectious for long periods and fish tissues frozen at -20 °C/-4 °F or lower, and can withstand freeze-thaw cycles.[389, 129, 416] The virus undergoes inactivation when exposed to ultraviolet irradiation (sunlight).[399, 403, 412] Other water quality characteristics (i.e., dissolved oxygen) may affect the occurrence of VHS disease and stability of the virus as well.

Pathogen Characteristics

VHSV genotypes are cumulatively capable of infecting a large number of marine and freshwater fish species (Table *17*). Virulence and pathogenicity are host specific and vary among genotypes.[417, 291, 401, 418, 399, 367, 376] The length of time that VHSV may remain viable in freshwater and marine environments is dependent upon the amount of virus shed into the environment by infected hosts, the density of infected hosts present in the environment, genotype, and environmental factors affecting virus stability.[399, 367, 376]

In general, VHSV isolated from marine species causes low to no mortality in freshwater and anadromous rainbow trout and other salmonids, and vice versa.[399, 129] However, genetic diversification appears to have allowed some genotypes to make species jumps from marine to freshwater and anadromous fish.[371, 419, 367] In Europe, VHSV-Ia is hypothesized to have evolved from a marine ancestor in association with the historical practice of including unpasteurized raw marine fish in the diets of farmed rainbow trout.[420, 371, 376] In 2007, sequence analysis of the VHSV genotype III responsible for a VHS outbreak in farmed steelhead trout showed that the virus responsible for the outbreak was closely related to marine strains that were not considered pathogenic for trout.[421, 367]

The infectious dose and level of exposure required to establish infection in susceptible hosts are dependent upon host susceptibility. For example, in experimental challenge studies, Pacific herring exhibited high susceptibility to low challenge doses of VHSV-IVa administered via bath immersion (101 pfu/mL VHSV for 24 hours in seawater).[396, 376] In other studies, Chinook, Coho, Pink (*Onchorhynchus gorbuscha*), and Sockeye (*Onchorhynkus nerka*) salmon exhibited

resistance to VHSV-IVa infection following high dose experimental challenge via freshwater bath immersion (103 and 105 pfu/mL for one hour).[422, 376]

Transmission

The transmission dynamics associated with VHSV are not fully described. VHSV is transmissible to fish of all ages and can occur bi-directionally from wild to farmed fish.[374, 376] Transmission rates are affected by virus genotype, viral shedding rates by individual fish and the fish population per unit time, the prevalence of infection in the affected population over the course of the disease outbreak, the minimum infectious dose required to elicit infection in susceptible fish, virus dilution and movement in the water column, and other host, environmental and pathogen factors.[13, 412]

Direct transmission occurs via exposure to virus shed into the water in the mucus, urine, feces, and reproductive fluids (milt and ovarian fluid) of infected fish.[415, 375, 403, 376, 377] Portals of entry for the virus are thought to include the gills, wounds on the body, and potentially the gastrointestinal tract.[393, 129] Potential sources of waterborne virus include VHSV-infected wild fish, VHSV-positive fish farms and hatcheries, and processing plant effluent, liquid, and other wastes.[382, 11, 13, 403]

Oral transmission of virus in infected prey, bait, baitfish, or feed has been described in the literature as potential transmission pathways.[423, 424, 394, 376] Experimentally, VHSV has been transmitted to naïve fish via feeding of infected fish and fish tissues.[158, 423] Anecdotally, in Denmark in 1985, the number of rainbow trout farms experiencing VHSV outbreaks declined after incorporation of marine fish meal in rainbow trout diets was prohibited, suggesting that VHSV was being orally transmitted via the feed.[368] Vertical transmission has not been definitively proven. However, VHSV is present in the reproductive fluids (ovarian fluid and milt) which can lead to contamination of the surface of fertilized eggs during spawning.[425, 426, 389, 399, 375, 129]

Indirect transmission may occur via fomites (e.g., aquaculture equipment, boats, ballast water, fishing tackle and other materials).[402, 399, 368] Anthropogenic movement and translocation of live fish, fertilized eggs, and gametes are considered primary methods of VHSV introduction into Europe, Iran, and the Great Lakes region of the United States.[373, 427, 368] Movement and use of baitfish has been also been suggested as a probable pathway of introduction of VHSV into the Great Lakes and other inland waters.[423] Transmission via animal vectors has been suggested but not definitively confirmed.[374] Experimentally, various freshwater turtle species have been found capable of harboring VHSV for up to 20 days after feeding on fish experimentally infected with VHSV.[414, 374] Virus has been detected in invertebrates such as leeches (*Mzyobdella lugubris*), amphipods (*Diporeia* spp.) and cladocerans (*Moina macrocopa*) as well.[408, 409, 428] Fish-eating birds and wildlife that access areas where VHSV-infected fish are present may be capable of introducing VHSV into areas by acting as mechanical vectors

Clinical Signs

VHS should always be considered a disease rule out when suspect clinical signs are observed in susceptible fish species found in environmental conditions and geographic areas where VHS occurs.[389] Primary portals of entry are thought to be the epithelial cells of the gills, skin, and gastrointestinal tract.[397, 365] Target organs include the brain, endothelial cells of blood vessels and heart, gills, hematopoietic tissues in the spleen and kidney, fibroblasts in the dermis and at the base of fins, liver, and muscle.[389, 399, 374, 243] The incubation period is water temperature

dependent. When water temperatures range between 1 –12 °C/34 – 54 °F the incubation period is 1–2 weeks. In colder and warmer water temperatures the incubation may be shortened or extended up to four weeks, respectively.[243]

Clinical signs are not pathognomonic and vary among individual fish and fish species.[366, 397, 368] Some fish exhibit no clinical signs, while others develop acute, chronic, or neurological manifestations of disease.[390, 366, 394, 243] Clinical signs associated with acute infection include abdominal distention, anorexia, darkened skin color, exophthalmia (popeye), lethargy, petechial hemorrhages in the eyes, internal organs, musculature and skin, and rapid onset of high mortality with no clinical signs. Affected fish may exhibit abnormal behaviors (e.g., abnormal swimming, crowding at enclosure edges or water outlets, do not attempt to escape netting, flashing, isolation from schools/shoals, and spiraling).[394, 243, 129] Rainbow trout specifically may present with darkened color, exophthalmia, lethargy, and may stay near the edges or the outlet of enclosures.[129] Skin lesions are frequently described as common clinical signs in cod, haddock, and herring.[429, 420, 430] In freshwater fish and halibut infected with VHSV-IVb pale gills are a common clinical sign.[431, 374] Clinical signs observed in chronic infection may include symptoms noted in acute infection, and anemia (pale gills), uncoordinated and/or spiral swimming, and significant cumulative mortality over time.[374, 394, 243] Fish affected by the neurological form exhibit severe abnormal swimming behavior (flashing and spinning), and low cumulative mortality.[394, 243] Fish that survive VHSV infection develop a strong antibody response.[432, 374]

Morbidity and Mortality

Mortalities typically begin antecedent to or shortly after clinical signs appear.[389, 243] Morbidity and mortality rates vary depending on environmental, pathogen, and host factors.[389, 367, 243] For example, juvenile rainbow trout infected with VHSV-Ia develop severe disease with mortality rates approaching 100%. Comparatively, mortality rates associated with VHSV-Ia infection and disease in older fish are lower (25% – 75%).[420, 417] Infection of this rainbow trout and other salmonids (Pacific salmonids, Atlantic salmon) with VHSV-IVa and VSHV-IVb results in zero to low (10%) cumulative mortality while high mortality (up to or greater than 90%) outbreaks may be observed in wild marine and freshwater fish species, respectively.[420, 433, 434, 365] Chronic fish losses resulting in low daily but high cumulative mortality rates are observed at low water temperatures (less than 5 °C/ 41 °F).[374] When water temperatures range from 9 –12 °C/48.2 – 53.6 °F, mortality rates increase (up to or greater than 90%)[435, 243] Mortality rarely occurs once water temperatures reach or exceed 15 °C/59 °F.[435, 374, 243]

Treatment

There are no treatments for VHS. There is currently no commercial vaccine available. Resistance to VHSV has not been established. However, potential additive genetic variation in rainbow trout for resistance to VHSV infection has been demonstrated.[436, 437]

Diagnostic Testing

A presumptive diagnosis can be made based on clinical signs, and gross and histopathological findings.[374] However, laboratory confirmation of infection is required for definitive diagnosis.

Gross pathological lesions include ascites, and/or edema in the peritoneal cavity, exophthalmia, gill pallor, hemorrhage in the eyes and under the skin around the pectoral and pelvic fins, lack of food in the gastrointestinal tract, hyperemia, multifocal hemorrhages, swelling or necrosis of the kidney, and the liver may be pale, mottled, or contain multifocal hemorrhages. The dorsal

muscles, internal organs, and skin should be examined for petechial hemorrhages. Fish affected by the chronic and neurological forms of the disease may exhibit no gross pathological signs.[366, 389, 374, 129]

Histopathological findings include extensive focal necrosis and degeneration in kidney, liver, and spleen, hemorrhagic myocarditis, hemorrhage and necrosis in the thymus and/or pancreas, and widespread subtle to severe vasculitis in internal organs and the skeletal muscle.[389, 374, 243, 129] Degeneration of peripheral nerves and optic nerves may be observed in fish affected by the neurological form of the disease.[374] Distribution of the lesions can vary dependent on the VHSV genotype and fish species. Rainbow trout infected with VHSV-Ia typically have necrotizing lesions in the kidney and liver, but may also have lesions in the brain, heart, spleen and other tissues.[389] Severe lesions in the myocardial tissues with accompanying changes in the liver and hematopoietic tissues were the most prevalent histopathological findings in Great Lakes freshwater fish infected with VHSV-IVb and turbot in Asia infected with VHSV-Ib.[438, 439, 389] If histological changes are absent, viral proteins may be visualized by immunohistochemical staining.[439, 440, 374]

Available diagnostic assays include antibody-based assays (e.g., enzyme linked immunosorbent assay (ELISA), indirect fluorescent antibody testing (IFAT), reverse transcription loop-mediated isothermal amplification (LAMP), or molecular assays (RT-PCR, qRT-PCR)) followed by sequencing, transmission electron microscopy (TEM), and/or virus isolation (VI) in cell culture.[389] Because VHS is WOAH-listed reportable disease, specific diagnostic assays, are required for disease confirmation.[97, 5] WOAH recommended protocols for targeted surveillance, presumptive and confirmatory diagnosis sampling, sample submission and diagnostic testing are described in the 2022 WOAH Manual of Diagnostic Tests for Aquatic Animals and the 2022 WOAH Aquatic Animal Health Code (Appendix, Table 1).[5, 97] The USFWS does require pre-import testing of salmonids for VHSV using methods described in Title 50. The USFWS and individual States, Tribes, and other localities engage in surveillance strategies to monitor the presence of VHSV in indigenous fish populations (Appendix, Table 1).[291] In the United States, confirmatory testing at the National Veterinary Services Laboratories (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.[161] Relevant information, including sample submission instructions, may be accessed via the NVSL link in Appendix, Table 1.

Prevention and Control Measures

Risk factors associated with the introduction and spread of VHSV in wild and cultured fish populations, and measures to prevent and control the introduction, spread, and impacts of VHSV are well described in published peer-reviewed literature, and include but are not limited to:[382, 441, 291, 11, 12, 403]

- Presence of wild VHSV-infected and/or VHSV-susceptible fish populations in waterbodies near hatcheries and fish farms,
- Hydrologic connectivity between waterbodies and water sources where VHSV-infected and naïve susceptible fish species (wild and farmed) are present,
- Exposure of susceptible wild and farmed fish species to personnel and fomites, boats, equipment, or fish wastes from known VHSV-positive areas,

- Unregulated translocation or import of live fish, fertilized eggs, and gametes from VHSV-endemic areas,
- Lack of appropriate farm biosecurity and best management practices,
- Insufficient regulatory infrastructure for fish health oversight,
- Lack of passive surveillance for VHSV (including genotype identification),
- Proximity of susceptible wild and farmed fish populations to fish processing plants and associated effluent water and other wastes,
- Release of fish farm effluent water that was not treated at levels required to inactivate VHSV.

Measures to prevent and control VHS introduction, spread, and impacts should be designed to prevent introduction of the virus to a) areas where the disease is not currently present, b) naïve wild and farmed fish populations, and may include:[382, 441, 291, 11, 12, 403]

- Pre-import testing of salmonid fish, fertilized eggs, and gametes per USFWS requirements,
- Surveillance and pre-import testing requirements at State, Tribal, and other regional levels,
- Identification VHSV endemic areas or zones,
- Equipment disinfection requirements for aquaculture, fisheries, and recreational fishing,
- Identification of areas with aquatic environments that are optimal for VHSV introduction,
- Identification VHSV-susceptible (and/or infected) wild fish species in natural water bodies with potential hydrological connectivity to the hatcheries or fish farms,
- Identification of hatcheries and farms that rear VSHV-susceptible species in areas where VHSV is endemically present,
- Restriction of broodstock collection locations, fish culture and stocking locations, and fish transfers,
- Active and passive surveillance for VHSV (wild and farmed fish populations) that includes genotype identification,
- Development of databases that allow disease trace back,
- Establishment of VHSV areas or zones and VHSF-free status designations for fish farms and hatcheries, recreational fishing, bait industries, and commercial fish processing,
- Implementation of farm biosecurity and best management practices to prevent VHSV introduction, including, but not limited to:
    - Use of specific pathogen free (SPF) stock, including fertilized eggs
    - Quarantine of shipments of live fish, fertilized eggs, or gametes,
    - Testing of live fish, fertilized eggs, and gametes for VHSV prior to release from quarantine,
    - Implementation of “all in, all out” policies for fish stocks, when possible,
    - Use of appropriate personal protective equipment (PPE), foot baths and other personnel biosecurity measures,
    - Use of disinfectants appropriate for inactivation of VHSV,
    - Fallowing of fish rearing structures after disinfection,
    - Use of spring or well water instead of ground water (rivers, streams, lakes),
    - Use of recirculating aquaculture systems (RAS) rather than flow through (FTS) or open water rearing systems,

- Avoidance of farming practices that create stress (high population densities, overfeeding, poor water quality management),
- Avoidance of polyculture, especially with VHSV-susceptible species,
- Treatment of influent water appropriate for inactivation of VHSV,
- Treatment of effluent water appropriate for VHSV inactivation prior to release,
- Development of contingency plans, including eradication and movement restrictions, in the event VHSV introduction occurs.

Disinfectants with VHSV efficacy include chlorine, chloroform, ether, formalin, glycerol, hydrogen peroxide (Peroxigard™), iodophors, potassium bisulfate (Virkon® Aquatic), sodium hypochlorite, and sodium hydroxide.[425, 442, 376, 129] Heat inactivation temperatures described by Bovo et al. (2005) include 30 °C/86 °F for 24 hours, 50 °C/122 °F for 10 minutes, and 70 °C/158 °F for 1 minute.[442] VHSV can also be inactivated by ultraviolet irradiation (280–200 nm wavelength), desiccation, and exposure to pH levels less than 2.4 or greater than 12.2.[425, 442, 389, 243] Newly fertilized and eyed eggs may be disinfected with iodophors.[425, 442, 389, 129] Disinfectants containing high salt concentrations and concentrated ammonium sulphate solution may not be effective.[443, 129]

In the United States, VHS is a reportable disease, endemically present in some wild fish populations.[1, 123] Detection of VHSV is required under USDA APHIS NLRAD and WOAH notifiable disease reporting requirements.[1, 123, 97, 5] At present, individual States are responsible for VHS regulatory oversight which may include measures such as import/export controls, VHSV testing and disease-free certification of hatcheries/farms, wild fish surveillance, and public outreach.[444, 366, 376, 387, 445, 446, 447] These requirements vary by State (Appendix, Table 3).[445]

If VHSV is suspected or detected via diagnostic testing the State veterinarian and Federal veterinary officials should be contacted and samples collected and submitted under the guidelines provided by the NVSL (Appendix, Table 1).[161] Control measures utilized by individual States and/or USDA APHIS may include controlling the movements and humane destocking of infected farmed fish, and cleaning, disinfection, and quarantine of affected premises according to WOAH protocols. Internationally, many countries utilize import/export regulations and recommendations in effort to limit or control the risk of VHSV introduction (Appendix, Table 2). A summary of WOAH import/export guidelines specific to VHS may be found in the Appendix, WOAH Pathogen Specific Import Export Recommendations.

Summary

VHS is an economically significant disease of susceptible wild fish, and farmed fish reared for commercial, recreational, and restocking purposes. Farmed fish reared in net pens or in land-based FTS farms that utilize surface water (e.g., lakes, rivers, streams) are at greatest risk of exposure to VHSV. Fish reared in land-based FTS or RAS aquaculture systems that utilize ground water are least likely to be exposed. Stringent biosecurity and influent water treatment measures are recommended for all land-based aquaculture systems to decrease the risk of VHSV introduction.[389]

The significance of VHSV is illustrated by its broad host range across all genotypes and apparent persistent prevalence in apparently healthy carrier fish. The historical adaptation of VHSV-I from marine to freshwater fish, the evolution of VHSV-III as a pathogen in steelhead trout, and the more recent adaptation of VHSV-IVb from marine to freshwater fish species and its rapid dissemination throughout the Great Lakes region demonstrates that VHSV genotypes

have the potential for environmental and host adaptation with highly impactful outcomes.[379, 373, 367] Thus, assessment of pathways of movement and introduction for indigenous (IVa, IVb, and IVc) and non-indigenous genotypes (I, II, III, and IVd), programs to monitor the epidemiology of VHSV in the United States, and measures to prevent introduction and translocation of VHSV into new areas are important.[373, 376]

Entry Assessment

An entry assessment describes the pathway from points of origin to points of entry that might allow introduction of the hazard into a particular environment and estimates the likelihood and uncertainty of that happening.[2] Definitions of likelihood, uncertainty, and risk used in this assessment are found in Appendix, Tables 4–6. Data supporting these assessments of likelihood, uncertainty, and risk are found in the Appendix, Entry Pathway Supplemental Materials.

In this assessment, the entry pathway of concern is imported live salmonid fish, fertilized eggs, and gametes. The points of origin consist of countries and producers from which the United States has historically, currently, and may potentially import live salmonid fish, fertilized eggs, and gametes. Ports of entry are the USFWS designated internationals ports of entry where the imported live fish, fertilized eggs, and gametes would enter the United States. Assessment of other potential entry pathways that might allow introduction of the six pathogens into the United States is not within the scope of this assessment.

Historical and Current Importation of Live Salmonid Fish, Eggs, and Gametes

The likelihood, under historical and current importation conditions, that live salmonid fish or fertilized eggs (no gametes were imported) imported for use in Atlantic salmon, rainbow trout, and steelhead trout aquaculture will result in entry of one of the six pathogens described in this assessment is **moderate** with a **low** degree of uncertainty.

The **moderate** likelihood indicates that a transboundary introduction of one or more or the six pathogens is nearly as likely to occur as to not occur. The **low** degree of uncertainty is based upon the quantity and reliability of the available data associated with historical and current importation of live salmonid fish and fertilized eggs, WOAH reporting, the disease monitoring and prevention measures present in countries from which the United States imports live salmonid fish, fertilized eggs, and gametes, and the disease translocation risk awareness of salmonid producers in the United States.

The risk that under historical and current importation conditions, that imported live salmonid fish or fertilized eggs may result in entry of one of the six pathogens described is **moderate**. A **moderate** level of risk suggests that the risk is of a sufficient magnitude that measures to prevent or mitigate the risk should be considered. A **moderate** risk is greater than a **low** risk due to a greater likelihood of occurrence, greater consequences, or a combination of both.

The levels of likelihood, uncertainty and risk are based on the decades-long history of importation of salmonid fish and fertilized eggs for aquaculture purposes. Historically, despite the presence of one or more of the pathogens of concern in each country that the United States has imported live salmonid fish, fertilized eggs, and gametes from (Table 19, Table 20), there have been no confirmed reports of disease outbreaks associated with this entry pathway. Review of the WOAH WAHIS database did not identify any reports or immediate notifications of introduction of the three pathogens that are foreign animal diseases (EHNV, *G. salaris*, and

SAV) into United States domestic salmonid aquaculture via imported live salmonid fish, fertilized eggs, or gametes.[129] Likewise, there were no reports of non-United States origin IHNV, ISAV, and VHSV introduced into United States salmonid aquaculture via imported live salmonid fish and fertilized eggs.

Countries that the United States currently imports live salmonid fish and fertilized eggs from are all WOAH Member countries. Members are obligated to report disease events, specifically those caused by WOAH-listed pathogens of animal health significance.[448, 449] Additionally, countries that the United States currently imports live salmonid fish, and fertilized eggs from (for which data were available) are either a) members of the European Union and must meet requirements for disease control, surveillance, and freedom as described in Directive 2006/88/EC – Animal Health Requirements for Aquaculture Animals[453, 454] and Article 5 of Regulation (EU) 2016/429[452] (Appendix, Table X) or b) have implemented country-specific disease surveillance, control, and freedom measures.[450] Those measures variably include establishment of zones or compartments of disease freedom, disease freedom testing of broodstock, fertilized eggs, and juvenile fish, disinfection protocols for fertilized eggs, and other control measures implemented at hatchery and grow out stages of production.[451] An internet search of some producers of live salmonid fish, fertilized eggs, and gametes in the exporting countries identified specific disease control biosecurity measures in use by producers that include disease-free certification of broodstock, live fish, fertilized eggs, and gametes.

Historical and current oversight of live salmonid fish and egg imports has been largely successful in preventing entry of foreign disease pathogens into the United States (the exception is ISAV) and introduction of endemically present pathogens into aquaculture. The USFWS, per the Code of Federal Regulations (CFR), Title 50, part 16.13 a) prohibits the importation of certain fish species susceptible to some of the pathogens described in this document (e.g., European perch [*Perca fluviatilis*], and zander [Pike-perch {*Sander lucioperca*}]), b) requires surface disinfection of fertilized salmonid eggs prior to import, c) requires health certification of live or dead un-eviscerated salmonid fish, and d) requires that fish stocks from which imported shipments originate are tested prior to import for IHNV and VHSV using viral cell culture methods that would detect EHNV, ISAV, and SAV.[102] USDA APHIS currently has no regulations relative specific to the six pathogens described in this assessment.[107] Individual States, Tribal governments, and other agencies variably require that live salmonid fish and fertilized eggs meet disease-freedom requirements, including for some of the pathogens summarized in this assessment (e.g., IHNV, VHSV, ISAV)(Appendix, Table 1).

Table 19. World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS) data for countries that countries that are top producers of Atlantic salmon that the United States imports live fish, fertilized eggs, and gametes from[129, 39]

Country		Pathogen	Years Reported	Reported Presence
Canada:	WOAH Member	IHNV	2012	Unspecified farmed fish species, wild Chinook salmon, sockeye salmon and unspecified fish species

	All three pathogens are reportable	ISAV	2007–2009, 2012–2020	Farmed Atlantic salmon and unspecified fish species
		VHSV	2007–2009, 2016–2018, 2020–2022	Farmed Atlantic salmon and unspecified fish species
Iceland	WOAH Member Associated with the EU via European Economic Area Membership	ISAV	2014–2022	Farmed Atlantic salmon and unspecified fish species.
		VHSV	2005, 2022	Unspecified wild and domestic fish species
Norway	WOAH Member Associated with the EU via European Economic Area Membership and the European Free Trade Association	GS	2014–2015	Unspecified wild fish species
		ISAV	2005–2022	Farmed Atlantic salmon and unspecified fish species
		SAV	2015–2021	Farmed Atlantic salmon and unspecified fish species
		VHSV	2007–2008	Unspecified farmed fish species

Table 20. World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS) data for countries that are top producers of rainbow trout and steelhead trout that the United States imports live fish, fertilized eggs, and gametes from[129, 39]

Country		Pathogen	Years Reported	Reported Presence
Canada	WOAH Member All three pathogens	IHNV	2012	Unspecified farmed fish species, wild Chinook salmon, sockeye salmon and unspecified fish species
		ISAV	2007–2009, 2012–2020	Farmed Atlantic salmon and unspecified fish species

	are reportable	VHSV	2007–2009, 2016–2018, 2020–2022	Farmed Atlantic salmon and unspecified fish species
Croatia	WOAH Member EU Member	IHNV	2013–2020	Farmed rainbow trout and unspecified fish species.
		SAV	2011, 2013–2020	Farmed rainbow trout
		VHSV	2013–2018	Unspecified farmed fish species.
Denmark	WOAH Member EU Member	IHNV	2021–2022	Farmed rainbow trout.
		VHSV	2005–2009	Farmed rainbow trout, brown trout, and unspecified fish species.
Norway	WOAH Member Associated with the EU via European Economic Area Membership and the European Free Trade Association	GS	2014–2015	Unspecified wild fish species
		ISAV	2005–2022	Farmed Atlantic salmon and unspecified fish species
		SAV	2015–2021	Farmed Atlantic salmon and unspecified fish species
		VHSV	2007–2008	Unspecified farmed fish species
Russia	WOAH Member	GS		No reports to WOAH. According to the literature is present in some rivers in Russia
		IHNV	2009	Unspecified wild fish species

In summary, live salmonid fish, fertilized eggs, and gametes have historically been, and currently are being, imported from countries where presence of at least one of the six pathogens of concerns is present. To date, entry of these pathogens via this entry pathway has not occurred. This may be associated with a) the disease control programs present in countries from which the United States imports live salmonid fish, fertilized eggs, and gametes; b) the current (albeit variable) level of aquatic health oversight by Federal, State, Tribal and other agencies; c) the awareness of United States producers of the presence or absence of diseases of concern in countries from which they import; and d) the biosecurity and other preventative

measures that United States producers utilize to diminish risk. The United States salmonid industry appears to be aware of the risk of disease introduction and the potential industry-wide consequences that may result if outbreaks of reportable diseases occur. There is also risk awareness (albeit variable) among individual producers.

Future Importation of Live Salmonid Fish, Fertilized Eggs, and Gametes

The likelihood that, under future importation conditions, live salmonid fish, fertilized eggs, or gametes imported for use in Atlantic salmon or rainbow trout (including steelhead trout) aquaculture will result in transboundary introduction of at least one of the six pathogens described in this assessment is **moderate** to **high** with a **moderate** to **high** degree of uncertainty. The risk that a transboundary introduction of one or more of the six pathogens may occur in the future via this entry pathway is **moderate**.

A **moderate** to **high** likelihood indicates that a transboundary introduction of one or more or the six pathogens would be likely to occur. The **moderate** to **high** degree of uncertainty is based upon the quantity, quality, completeness, reliability, and interpretability of the available data, and the lack of some data required to fully assess this pathway. The **moderate** risk indicates that there is increased potential for transboundary disease introduction, with associated negative consequences to United States salmonid producers. Further measures to prevent or mitigate this risk should therefore be considered.

The levels of likelihood, uncertainty and risk are based on the possibility that the United States may begin to import greater volumes of live salmonid fish and fertilized eggs (and potentially gametes) to meet increased demand as domestic and global consumer demand for salmonid products continues to increase, the total volume of fish produced to meet this demand increases, and as more marine-based and inland FTS and RAS aquaculture systems are developed for production purposes. The United States will likely continue to import live salmonid fish, fertilized eggs, and gametes from international suppliers it has used historically. It is also likely that the United States may begin to import live salmonid fish, fertilized eggs, and gametes from new international sources to meet increasing demand. This section of the Entry Assessment assumes that the regulatory oversight described above will continue as described.

The new sources from which the United States may import live fish, fertilized eggs, and gametes are not known, which contributes to the assigned levels of uncertainty and risk. Potential sources may include countries that are top producers of Atlantic salmon and rainbow trout/steelhead trout (Table 21). All countries that are top producers of Atlantic salmon, rainbow trout, or steelhead trout, except for Colombia and Peru, have reported presence of at least one of the six pathogens described in this assessment (Table 21). Some of these countries are WOAH Members (e.g., Australia, Chile, Colombia, Finland, France, Germany, Italy, and Sweden) and must comply with requirements to report disease events of animal health significance caused by WOAH-listed pathogens.[449] Some countries are members of the European Union (e.g., Finland, France, Germany, Italy, the Netherlands, and Sweden) and must also comply with control and prevention measure for disease of aquatic animals as described in Directive 2006/88/EC – Animal Health Requirements for Aquaculture Animals[453, 454] and Article 5 of Regulation (EU) 2016/429.[452] Other countries, such as the Faroe Islands, comply with all EU mandates and directives associated with aquaculture.[455] Some top producing countries state in various resources (e.g., government websites, published literature) that aquatic disease surveillance and control measures, including monitoring for the presence of disease in

broodstock, fertilized eggs, fish of all life stages are utilized; however, detailed information is not accessible. Top producing countries for which some data were available include Australia, Chile, China, and the United Kingdom.[217, 456] Data were unavailable for some countries regarding disease status and disease control measures (e.g., Colombia, Iran, Turkey, Peru, and Switzerland). There may be additional unidentified sources of imported live fish, fertilized eggs, and gametes that emerge based on availability.

Table 21. Data for additional countries that are top producers of farmed Atlantic salmon and rainbow trout/steelhead trout[129, 448]

Country		Species the country is top producer of	Pathogen	Years reported	Species detection occurred in
Australia	WOAH Member	Atlantic Salmon	EHNV	2012, 2021–2022	Wild redfin perch and unspecified fish species
Chile	WOAH Member	Atlantic Salmon	ISAV	2007–2010, 2012–2015, 2017–2019	Unspecified farmed fish species
China	Some regulatory data available	Rainbow trout Steelhead trout	IHNV	2008, 2011–2013, 2017–2022	Farmed rainbow trout and unspecified fish species
Colombia	WOAH Member	Rainbow trout Steelhead trout	No reports in the WOAH WAHIS database		
Faroe Islands	Complies with EU mandates and directives	Atlantic Salmon	ISAV	2016–2022	Farmed Atlantic salmon and unspecified fish species
Finland	WOAH Member EU Member	Atlantic Salmon	*Gyrodactylus salaris*	2005, 2007, 2008, 2010	Unspecified wild and domestic fish species.
			IHNV	2017–2018, 2021–2022	Farmed rainbow trout and unspecified fish species.
			VHSV	2005–2010, 2012	Farmed rainbow trout, brown trout, and unspecified fish species

France	WOAH Member EU Member	Atlantic Salmon	IHNV	2006–2008, 2010, 2014, 2016–2018, 2022	Farmed rainbow trout and unspecified fish species.
			VHSV	2005–2012	Farmed rainbow trout, northern pike, and unspecified fish species
Germany	WOAH Member EU Member	Atlantic Salmon	IHNV	2005–2022	Farmed rainbow trout, brook trout, brown trout, and unspecified fish species
			VHSV	2007–2010, 2012, 2014, 2016–2021	Farmed rainbow trout and unspecified fish species
Iran	WOAH Member	Rainbow trout Steelhead trout	IHNV	2005–2006, 2015–2021	Farmed rainbow trout, and unspecified fish species.
			VHSV	2005, 2013–2021	Farmed rainbow trout, brown trout, and unspecified fish species
Italy	WOAH Member EU Member	Rainbow trout Steelhead trout	IHNV	2005–2008, 2010–2015, 2018–2022	Farmed rainbow trout, brown trout, and unspecified fish species
			VHSV	2005–2016, 2018–2021	Farmed rainbow trout, brown trout, marble trout and unspecified fish species
Netherlands	EU Member	Atlantic Salmon	IHNV	2009, 2011	Unspecified farmed fish species
			VHSV	2011	Unspecified farmed fish species
Turkey	No regulatory data available	Rainbow trout Steelhead trout	VHSV	2006–2007	Unspecified farmed fish species
Peru	No regulatory data available	Rainbow trout Steelhead trout	No reports in the WOAH WAHIS database		

Sweden	WOAH Member EU Member	Atlantic Salmon	*Gyrodactylus salaris*	2005, 2015–2016	Unspecified farmed and wild fish species
			VHSV	2015–2016	Unspecified farmed and wild fish species
Switzerland	No regulatory data available	Atlantic Salmon	IHNV	2012–2015, 2019	Unspecified farmed fish species
			VHSV	2006–2010, 2012, 2014–2015, 2017, 2019	Farmed rainbow trout and unspecified fish species
United Kingdom	Complies with EU mandates and directives	Atlantic Salmon	ISAV	2009–2010 2021–2022	Farmed Atlantic salmon and unspecified fish species
			VHSV	2006, 2008, 2012–2013	Unspecified fish species

Lack of information on future potential sources of imported live salmonid fish, fertilized eggs, and gametes is a data deficiency that increases the levels of likelihood, uncertainty, and risk. Other data deficiencies affecting this assessment include but are not limited to:

- When increased demand for imported live salmonid fish, fertilized eggs, and gametes may occur is not known,
- The volumes of live fish, fertilized eggs, and gametes that may be imported in the future are not known,
- The types of aquaculture systems (e.g., RAS vs. FTS vs. marine aquaculture) that the imported live fish, fertilized eggs, and gametes will be placed in are not known,
- The level of precaution at which United States producers will approach importation from new resources is not known,
- It is unknown if Federal, State, Tribal or other agencies will change existing or implement new regulatory oversight relative to aquatic animal diseases.

In summary, it is predicted that the salmonid industry will begin to import increased volumes of live fish and fertilized eggs (and potentially gametes) from new resources as projected increases in production occur. To meet demands, producers may begin to import large volumes (currently unknown) of live fish, fertilized eggs, and potentially gametes from new resources (currently undefined). The potential increase in production, the increased but unknown factors associated with sourcing and importing live fish, fertilized eggs, and potentially gametes, importation, increases the level or likelihood, uncertainty, and risk that entry of one of the six pathogens described in this assessment may enter the Unites States.

Exposure Assessment

An exposure assessment describes the pathway from the port of entry that could result in exposure of a vulnerable population (animal or human) to a hazard and estimates the likelihood and uncertainty of that happening.[2]

In this assessment, the exposure pathway of concern is imported live salmonid fish, fertilized eggs, and gametes that have passed through a port of entry into the United States and enter a hatchery or fish farm. The vulnerable populations of concern are live salmonid fish, fertilized eggs, and gametes present in the hatcheries and fish farms receiving the imports. The hazards are the six pathogens identified in the Hazard Identification section of this assessment. Assessment of other potential exposure pathways that might result in exposure of vulnerable populations to the hazards is not within the scope of this assessment. Definitions of likelihood, uncertainty, and risk are found in Appendix, Tables 4–6.

Potential Exposure of Farmed Atlantic Salmon, Rainbow Trout, and Steelhead Trout to the Six Pathogens of Concern Via Imported Live Salmonid Fish, Fertilized Eggs, and Gametes

Current Importation Conditions

The likelihood that, under current import conditions, imported live salmonid fish, fertilized eggs, or gametes will result in exposure of farmed Atlantic salmon, rainbow trout or steelhead trout to one of the six pathogens described in the Hazard Identification is **moderate** with a **low** degree of uncertainty. The risk of exposure via this entry pathway is **moderate**.

The **moderate** likelihood indicates that introduction and exposure to one or more or the six pathogens is nearly as likely to occur as to not occur. The **low** level of uncertainty is based upon the reliability, and completeness of the available data. The **moderate** level of risk indicates that there is potential for exposure, with associated negative consequences. Measures to prevent or mitigate this risk should therefore be considered.

The levels of likelihood, uncertainty and risk are based on:

- Historical evidence indicating that imported live salmonid fish and fertilized eggs leaving a port of entry have not been associated with exposure of farmed salmonids to the pathogens of concern.
    - It should be noted that the historical occurrence of ISAV suggests that this is a potential exposure pathway. The original exposure pathway for ISAV introduction into domestic Atlantic salmon aquaculture was not confirmed. It has been hypothesized that exposure may have occurred via wild fish or other reservoirs present in the aquatic environment. It has also been hypothesized that the exposure may have occurred via movement of imported hatchery reared fish.[306, 272]
- Historical evidence that the Federal, State, Tribal and other regulations associated with the inter- and intra-state movement of live salmonids and fertilized eggs have been predominantly successful in preventing exposure of farmed salmonids to the pathogens summarized in this document.

- The awareness of salmonid producers to the risk of pathogen exposure occurring in populations of farmed fish via introduction of live fish and fertilized eggs.
- Many hatcheries and fertilized have best management practice plans (BMPs) in place that include biosecurity measures intended to decrease the risk of pathogen introduction. However, BMPs and biosecurity plans are not standardized and there is no database reporting on the number of farms and hatcheries that utilize them. If a hatchery or farm receiving the imported shipment of live fish and fertilized eggs is not risk averse, and does not utilize stringent biosecurity measures, the likelihood, uncertainty, and risk of this exposure pathway occurring are increased.

Potential Future Importation Conditions

The likelihood that, under projected future importation conditions, live salmonid fish, fertilized eggs, or gametes imported for use in Atlantic salmon, rainbow trout, and steelhead trout aquaculture will result in exposure of farmed fish to at least one of the six pathogens described in the Hazard Identification section of this assessment is **moderate** with a **moderate** to **high** degree of uncertainty. The risk that an exposure to one or more of the six pathogens may occur in the future is **moderate**.

A **moderate** likelihood indicates that an exposure of one or more or the six pathogens would be nearly as likely to occur as to not occur. The **moderate** to **high** degree of uncertainty is based upon the quantity, reliability, and interpretability of the available data, and the lack of some data required to fully assess this pathway. The **moderate** risk indicates that there is increased potential for exposure to occur, with associated negative consequences to United States salmonid producers. Additional measures to prevent or mitigate this risk should therefore be considered.

Because gametes (unfertilized ova, milt/sperm) are not typically imported for use in Atlantic salmon, rainbow trout or steelhead trout production, these materials will not be included in the rest of this exposure assessment. If these materials are imported in the future, it is possible milt or unfertilized ova could serve as an exposure pathway for the pathogens summarized in this document under the same specifics described for live fish and fertilized eggs.

Most imported salmonid fish are broodstock.[105] Therefore, hatcheries are the likely recipients of most imported live salmonid fish. Hatcheries are also the recipients of imported fertilized salmonid eggs. Additionally, hatcheries account for most of the movement of live salmonid fish and fertilized eggs within the industry (approximately 80%).[40, 55] Hatcheries therefore, are the locations associated with greatest risk relative to the six pathogens summarized in this document. In the United States, hatcheries are comprised of land-based facilitates that utilize FTS or RAS technologies.[40, 55]

The potential for infected imported live fish, or fertilized eggs to enter a hatchery or farm level is affected by:

- The factors summarized in the entry pathway,
- The level of regulatory oversight by Federal, State, Tribal, or other agencies regarding post-port of entry inter- or intra-state movement or translocation of live fertilized fish eggs,

- The level of regulatory oversight by Federal, State, Tribal, other agencies and producers regarding aquatic animal health and disease surveillance,
- The biosecurity practices of the United States hatchery or farm receiving the shipment of live fish and fertilized eggs (biosecurity in hatcheries is typically high but can vary),
- Frequency at which imported live fish, fertilized eggs, or gametes may be sorted or intermingled with other live fish, fertilized eggs, or gametes present in the hatchery or farm,
- The type of aquaculture system in use by the hatchery or farm (e.g., RAS vs. FTS).

Once live fish or fertilized eggs are released at ports of entry, there is limited Federal regulation relative to inter- and intrastate movement. The USFWS does not require permitting if live fish, fertilized eggs, or gametes are to be transported or possessed in captivity.[102] The USFWS does regulate release of imported live fish, fertilized eggs, or gametes into the wild by a State wildlife conservation agency or persons with prior permission.[102] USDA APHIS currently has no regulations or recommendations relative to inter-state or intra-state movement of live salmonid fish, fertilized eggs, or gametes specific to the six pathogens described in this assessment.[107] State, Tribal, and other agencies may have specific regulations related to inter- and intra-state movement of live fish, fertilized eggs, and gametes (Appendix, Table 1).

There is limited Federal oversight regarding post-import disease surveillance for the six pathogens described in the Hazard Identification section. The USFWS requires that fish stocks from which imported shipments originate are tested for IHNV and VHSV (and IPN).[102] Testing for EHNV, *G. salaris*, ISAV, and SAV is not required. USDA APHIS currently has no regulations relative specific to the six pathogens described in this assessment.[107] Individual States, Tribal governments, and other agencies may variably require that live salmonid fish and fertilized eggs meet disease-freedom requirements, including for some of the pathogens summarized in this assessment prior to entry into the state or locality, release, or stocking in marine net pens (e.g., IHNV, VHSV, ISAV)(Appendix, Table 1).

Levels of likelihood, uncertainty, and risk of pathogen introduction leading to exposure of vulnerable populations in the facility are lowest for hatcheries and farms that are risk averse and have stringent biosecurity measures that include:

- Requirements that imported live fish and fertilized eggs are sourced from certified disease-free sources,
- Disinfection of fertilized eggs from outside sources upon receipt by the hatchery at a designated egg disinfection station in the receiving area prior to entering the hatchery proper,
- Quarantine of live fish and fertilized eggs in systems separate from the rest of the facility (including water sources),
- Disease surveillance testing of live fish and fertilized eggs during quarantine,
- Holding and/or rearing of imported live fish and fertilized eggs in isolation from other live fish or fertilized eggs for sufficient time to establish disease freedom,
- Disinfection and appropriate disposal of transport water accompanying shipments and water used for rearing the imported live fish and fertilized eggs.

Many hatcheries and farms have best management practice plans (BMPs) in place that include biosecurity measures. However, BMPs are not required, are not consistent within the industry,

and there is no data reporting on the number of facilities that utilize BMPs. If a hatchery or farm receiving the imported shipment of live fish and fertilized eggs is not risk averse, and does not utilize stringent biosecurity measures, the likelihood, uncertainty, and risk of this exposure pathway occurring are increased.

Ideally hatcheries have designated receiving areas for shipments of imported fertilized eggs. Fertilized eggs and transport water and ice should be disinfected using appropriate protocols when the shipment enters this designated area. If transport water and ice accompanying imported fertilized eggs is not disinfected and disposed of in a biosecure manner (water does not enter the water system used by the hatchery or farm) upon receipt, it is possible that other fish or fertilized eggs in the hatchery or farm may be exposed to a pathogenic agent. If imported potentially infected or contaminated fertilized eggs are not disinfected properly prior to import and are not disinfected upon receipt by the hatchery or farm, it is possible that introduction of a pathogenic agent could occur.

The likelihood, uncertainty, and risk are increased if the imported fertilized eggs are intermingled with other fertilized eggs. The likelihood, uncertainty, and risk are further increased if the hatchery utilizes FTS systems in which water flows contiguously through multiple hatching vessels, trays, or racks. During the egg incubation stage, fertilized trout and salmon eggs are picked mechanically or by hand to remove dead fertilized eggs or fertilized eggs with signs of fungal infection.[43, 41] If infected or contaminated fertilized eggs are present, and sorting equipment is not disinfected appropriately between hatching vessels or trays, the likelihood, uncertainty and risk of pathogen introduction or contamination during the picking process are increased.

Once fertilized eggs hatch, and the hatched fry reach the “swim up stage” and are actively searching for food, they are moved to the first of several rearing structures (indoor or outdoor tanks, ponds, raceways, or similar structures) for grow out. During this phase, fish are graded and sorted up to five times to keep the size of the fish in each rearing structure as uniform as possible.[44] The process typically involves mechanically removing the fish from a rearing structure onto a horizontal surface grid. The fish then fall through differently sized slots based on size.[44] The sorted fish are then mechanically transported back to new rearing structures. Occasionally, in ponds and raceways, bar graders may be used to sort the fish before netting or mechanically moving them.[44] The sorting process is likely stressful and could result in cutaneous injury to some fish, which may increase the potential that subclinically infected fish may develop disease, and that naïve fish may be more susceptible to pathogen exposure. The likelihood, uncertainty, and risk for pathogen exposure are increased if imported infected fish, or fish hatched from infected or contaminated fertilized eggs, are intermingled with other fish. The likelihood, uncertainty, and risk for pathogen exposure are decreased if the imported fish, or fish hatched from imported fertilized eggs, are sorted separately from other fish in the hatchery and maintained as separate cohorts.

It is plausible that water from the rearing structures holding different populations of fish may be intermixed during the sorting process. If one of the pathogens of concern is present in the water, this could provide an exposure pathway. The likelihood, uncertainty, and risk for pathogen exposure are increased if one of the pathogens of concern is present in the water, if water from different rearing structures is intermixed, and if sorting equipment is not adequately disinfected during and after the sorting process. The likelihood, uncertainty, and risk for pathogen exposure

are decreased if the imported fish or fish hatched from imported fertilized eggs are sorted separately from other fish, if water from the rearing structures housing imported fish or fish hatched from imported fertilized eggs is not allowed to mix with water from other rearing structures, and if sorting equipment is adequately disinfected during and after the sorting process.

The type of aquaculture system used by the hatchery or grow out facility affects the likelihood of disease introduction occurring among cohorts of fertilized eggs and fish. Facilities with FTS systems are at greatest risk of pathogen introduction or spread among cohorts. Flow through systems connected in series are most vulnerable as water flows through one rearing structure to another, sometimes multiple times, before exiting the system. The risk of pathogen exposure among cohorts in RAS designed hatcheries and farms is lower than that of FTS systems but is not zero. Recirculating systems do favor the growth of some disease-causing organisms and can facilitate spread of disease and parasites among cohorts of fish within the system.[457] The high population densities of fish reared in RAS systems, the build-up of biofilms and sediments within the water handling system (tanks, sumps, pipes, and mechanical or biological filtration components), and the slow turnover of water can contribute to a) maintenance and spread of pathogenic agents, and b) water quality fluctuations, such as temporary increases in ammonia or nitrite which affect fish health and increase susceptibility to pathogens.[457, 458, 459]

It is unlikely that imported fertilized eggs or gametes would be placed into a marine aquaculture environment. Therefore, this will not be considered a plausible exposure pathway in this assessment. It is plausible, under some circumstances, that live fish (e.g., Atlantic salmon or steelhead trout) may be imported and placed directly into a marine aquaculture system. If live fish infected with one of the six pathogens are imported and are placed into a marine aquaculture system without first being quarantined and tested for the six pathogens described in this assessment, it is plausible that introduction of those pathogens may occur. Such an introduction could affect other fish being reared in the net pen housing the imported fish, fish present in other pens present in the marine farm, and susceptible fish present in the marine environment. The likelihood, uncertainty, and risk for pathogen exposure are increased if imported fish are infected with one of the six pathogens of concern and if the transport water is contaminated and is transferred into the marine environment. The likelihood, uncertainty, and risk for pathogen exposure are decreased if the imported fish are not infected with one of the six pathogens of concern and if the transport water is not contaminated or transferred into the marine environment.

In summary, live salmonid fish and fertilized eggs have historically been, and currently are, being imported from countries where presence of at least one of the six pathogens of concern is present. These imported live salmonids and fertilized eggs have been reared in hatchery and farm systems with no documented introduction of the pathogens via this exposure pathway. This supports the assessment that under historical and current conditions, the likelihood, uncertainty, and risk of exposure occurring via this pathway is **low**.

If production demands increase, and live salmonid fish, fertilized eggs, and gametes are a) imported from countries that are not WOAH members and/or do not have sufficient disease control, surveillance, and mitigation measures in place; b) pass through the entry pathway; and c) enter a hatchery or farm, there is increased likelihood, uncertainty and risk that one of the six

pathogens summarized in this assessment will be introduced via this exposure pathway. The levels of likelihood, uncertainty and risk are dependent upon:

- The level of biosecurity and disease prevention measures required at Federal, State, and regional or local levels,
- The biosecurity and disease prevention practices of the individual farm or hatchery at all levels of production (e.g., incubation and handling of fertilized eggs, rearing and sorting of fish; and disinfection of equipment),
- The water handling system (e.g., FTS vs. RAS) of the facility.

The potential for pathogen introduction is lower for facilities that are highly risk averse and practice stringent biosecurity that includes:

- Disinfection of fertilized eggs upon receipt prior to entry into the hatchery,
- Quarantine and rearing of live imported fish and fish hatched from imported fertilized eggs as individual cohorts throughout all stages of the production process,
- Routine disease surveillance,
- Disinfection of equipment and tanks between movement or handling of fish cohorts,
- Measures to avoid intermixing of fish and water among fish cohorts.

Hatcheries and farms with FTS water handing systems constructed in parallel are at lower likelihood and risk of pathogen exposure among fish cohorts compared to FTS facilities constructed in series. Facilities with RAS water handling systems are at lower likelihood and risk of pathogen spread among cohorts than facilities with FTS systems; however, the likelihood and risk are not zero.

Consequence Assessment

A consequence assessment describes the relationship between the exposures to a pathogen and the various consequences of such exposures. Consequences may be evaluated at the local, regional, or national level, and may include such things as:

- Direct consequences, such as production losses or public health impacts,
- Indirect consequences, such as prevention and control costs or trade losses.

Definitions for levels of consequence are found in Appendix, Table 7.

In the absence of control measures, the economic consequences associated with introduction and/or an outbreak of one of the six pathogens summarized in this assessment may be **moderate** to **high** depending on the pathogen and the population of farmed fish involved.

A **high** level of consequence occurs if the morbidity and mortality associated with a disease introduction are great enough to threaten the economic viability of a sector for a lengthy period and if the effects of the exposure may not be reversible. A **moderate** level of consequence occurs when the morbidity and mortality associated with the pathogen are great enough to impose moderate production losses and if the effects of exposure may not be reversible (Appendix, Likelihood, Consequence, Uncertainty and Risk Categories, Table 4).

The direct and indirect consequences of EHNV introduction would likely be **high**. EHNV is not present in the United States and is a WOAH-listed reportable foreign animal disease. In Australia, rainbow trout appear to be relatively resistant to infection; however, outbreaks of

disease in farmed rainbow trout described as economically impactful have been reported (the reports lacked specific data) in the literature.[147, 124] Disease is most common in young fingerlings.[147] The consequences of EHNV introduction into farmed rainbow trout operations are **high** because detection would likely result in significant trade impacts and direct consequences affecting hatcheries and grow out producers. Secondary impacts would affect supporting industries (e.g., feed, transport, labor) and conservation, recreational, and private stocking programs. Additional impacts would include the costs of outbreak control, mitigation, and trade losses.

The direct and indirect consequences of *G. salaris* introduction would likely be **high** for Atlantic salmon, steelhead trout, and rainbow trout reared in freshwater. The direct and indirect consequences associated with rearing steelhead trout in marine-based aquaculture systems is likely to be **moderate**, given the sensitivity of the parasite to high salinity aquatic environments. Gyrodactylosis is not present in the United States and is a WOAH-listed reportable foreign animal disease. Detection of *G. salaris* would likely result in trade impacts. The direct and indirect consequences of *G. salaris* introduction would likely be **high** for Atlantic salmon, steelhead trout, and rainbow trout reared in freshwater. The direct and indirect consequences associated with Atlantic salmon and steelhead trout in marine-based aquaculture systems is likely to be **moderate**, given the sensitivity of the parasite to high salinity aquatic environments. Epidemiological factors that make this pathogen potentially impactful include its capability to infest any freshwater fish host. Occurrence of clinical disease has only been reported in Atlantic salmon in freshwater. However, farmed and wild rainbow trout are considered reservoir hosts, can be chronically infested, and rarely exhibit clinical signs of disease.[171, 181, 190, 185] In Europe, affected rainbow trout farms are often located in watershed where *G. salaris* has been introduced, which exemplifies the difficulty in maintaining biosecurity for this pathogen. The economic consequences associated with introduction of this parasite may be significant. In Norway, direct costs associated with rainbow trout farm and watershed eradication efforts (surveillance, preventing spread of the parasite, removal of wild fish, watershed conservation, restoration, and restocking efforts) are estimated to cost approximately $9.7 million USD annually. Annual economic losses (loss of farm production and associated costs, local economic effects) are estimated to range from $36.5 to $42.9 million USD.[460] It is plausible that the costs to mitigate introduction of *G. salaris* may be similar in the United States, and that the direct and indirect impacts to the affected salmonid industry sector and the supporting industry and labor sectors may be significant. Detection of *G. salaris* would potentially result in negative trade impacts.

The direct and indirect consequences of IHNV introduction into farmed Atlantic salmon, rainbow trout, and steelhead trout aquaculture systems would likely be **high**. IHNV is endemic to the Pacific Northwest of North America (including the United States) and has been reported in wild and farmed Pacific salmonid stocks. Internationally, IHNV has historically been associated with significant economic losses to salmonid aquaculture (e.g., Atlantic salmon, steelhead trout, rainbow trout). In the United States, the historical consequences associated with IHNV detection has been economically impactful. For example, from 1981 to 1995, approximately 70 million IHNV-infected rainbow trout fish and fertilized eggs were destroyed in hatcheries in Idaho. Associated direct and indirect economic losses to the hatcheries and commercial and recreational fisheries were approximately $350 million USD.[461] It is plausible that future

detections may have similar direct and indirect consequences, and that trade impacts could occur.

The direct and indirect consequences of ISAV introduction would likely be **high**. ISAV is endemically present along the Atlantic Northeast of North America (including the United States). Globally, economic losses associated with ISAV outbreaks are estimated to be in the billions of dollars.[462] Outbreaks of ISA between 1999 and 2003 in Maine and Maritime Canada caused devastating economic losses to the national industries and global markets of both countries.[463] During the initial ISAV outbreak in Chile, production of Atlantic salmon decreased by 60% and approximately 8,400 direct jobs were directly impacted.[463] It is plausible to expect that there would be similar direct, indirect, and trade impacts if detection or outbreaks or ISAV were to occur in the future.

The direct and indirect consequences of SAV introduction would likely be **high**. SAV is not present in the United States and is a WOAH-listed reportable foreign animal disease. There is a general lack of published data regarding the direct and indirect economic costs of this pathogen in countries where this pathogen is present. However, it is likely that introduction of SAV into the United States would result in negative direct consequences to Atlantic salmon, rainbow trout, and steelhead trout industries, negative indirect consequences to supporting commodity and labor sectors, and negative trade impacts. In regions in Europe where SAV is endemic, control of the disease is difficult once introduction has occurred.

Viral hemorrhagic septicemia virus (VHSV-IVa, VHSV-IVb, VHSV-IVc) is a WOAH-listed reportable pathogen that is endemically present in North America (including the United States). The direct and indirect consequences of VHSV detection in salmonid aquaculture would likely be **high**. For example, in Europe it is estimated that farmed rainbow trout losses associated with VHSV-Ia-e control exceeds 40 million pounds of fish annually.[464] VHSV outbreaks on two Danish fish farms in 2000 resulted in approximately 50% cumulative mortality and economic losses of approximately $230,000 USD per farm.[390] Other consequences associated with VHSV outbreaks include disruption and adverse economic impacts on commercial, recreational, and subsistence fish industries and degradation of natural fish ecosystems. It is reasonable to expect that detection of this pathogen in farmed salmonid aquaculture systems in the United States could result in similar direct and indirect impacts, and that there may be negative impacts to trade.

In summary, introduction of any of these pathogens into the United States that results in exposure of farmed fish could result in direct negative consequences to the affected hatchery or fish farm. Initially, the most direct consequences would be associated with occurrence of the disease, such as reduced fish welfare (poor health, poor growth) and production losses (mortality losses, reduced production, carcass downgrading, reduced weight gain, and costs associated with poor food conversion ratios). Following disease detection, producer, local economy, and labor consequences would be associated with, and impacted by, production losses, loss of income, and costs associated with implementation of control measures (e.g., depopulation of all fish hosts followed by cleaning and disinfection and a fallowing period). Costs associated with restocking and subsequent disease-freedom confirmation testing would be additional economic consequences to the producer. Potential long-term consequences may be affected by implementation of trade restrictions by other countries relative to export of live fish, fertilized eggs, and gametes, and potentially, salmonid products. According to published

literature, in countries where these diseases are endemic in aquaculture systems, once introduced into farmed and wild fish populations some of these pathogens are difficult to eradicate. If any of the six pathogens become established in aquaculture and/or in natural watersheds, eradication or control of the pathogen may be difficult, expensive, and time consuming. The length of time that these long-term consequences may be in effect cannot be predicted. There are no public health consequences associated with the six pathogens summarized in this assessment.

Risk Estimation

A risk estimation is defined as the combination of the likelihood and uncertainty of the entry and/or exposure pathways and the consequences of exposure. Definitions of risk are found in Appendix, Table 6.

Historically and contemporarily, the risk that there is an entry pathway for one or more of the six pathogens summarized in the assessment has been **moderate**. A **moderate** risk indicates that the potential for transboundary disease introduction is of a sufficient magnitude, and that there would be associated negative consequences to United States salmonid producers. Measures to prevent or mitigate this risk should therefore be considered. Factors contributing to this risk designation are summarized in the Entry Assessment.

Based upon projected future importation conditions, the risk is **moderate** to **high** that live salmonid fish, fertilized eggs, and gametes may serve as an entry pathway for one or more of the six pathogens summarized in this assessment. A **moderate** to **high** risk indicates the consequences of pathogen introduction may significantly impact salmonid aquaculture at regional or national levels. Therefore, further measures to prevent or mitigate this risk should be considered. Factors contributing to this risk designation are summarized in the Entry Assessment.

The risk that, under historical and current import conditions, imported live salmonid fish and fertilized eggs will result in hatchery- or farm-level exposure of farmed Atlantic salmon, rainbow trout, or steelhead trout to of one of the six pathogens described in this assessment is **moderate**. This **moderate** risk indicates that there is potential for exposure, and that the magnitude of the risk is sufficient for consideration of preventative or mitigation measures. Factors contributing to this risk designation are summarized in the Exposure Assessment.

The risk that, under projected future importation conditions, live salmonid fish, and fertilized eggs imported for use in Atlantic salmon, rainbow trout, and steelhead trout aquaculture will result in exposure of hatchery- or farm-reared fish to at least one of the six pathogens described in this assessment is **moderate**. A **moderate** risk indicates that there is increased potential for exposure to occur, with associated negative consequences to United States salmonid producers. Additional measures to prevent or mitigate this risk should therefore be considered. Factors contributing to this risk designation are summarized in the Exposure Assessment.

Assessments Summary

The United States is the largest global consumer of Atlantic salmon (over 450,000 tonnes [GWT] in 2018). Factors contributing to the increased demand include the changing dietary habits of consumers, the health benefits associated with eating salmon, and rising consumer interest in sustainable, resource efficient, easily consumable food products.[116, 37] To meet this

demand, most product is imported.[37] The Atlantic salmon farming industry is the third largest aquaculture sector in the United States. In 2018, the total estimated economic impact of Atlantic salmon production in the United States was over $1 billion USD.[117, 64] It has been estimated that future production could increase by 3,500% over 2018 levels to meet consumer demand. It is predicted that a predominant proportion of production will occur inland in hatcheries and grow out facilities that utilize FTS and RAS aquaculture.[64]

Freshwater rainbow trout production is the second largest aquaculture sector in the United States. Outputs of freshwater rainbow trout aquaculture include food production, and stocking for conservation, recreation, and restoration purposes, and domestic and international sales to other hatcheries or farms.[42] According to FAO data total sales of rainbow trout have increased slightly since 2013; however, production has fluctuated.[45] There are limited published data describing the steelhead trout industry in the United States. It is plausible that information related to this salmonid sector is generalized with all rainbow trout production parameters. However, given that all imported *O. mykiss* fish and fertilized eggs reported in the USFWS LEMIS database from 2013 to 2023 were identified as steelhead trout[39], it is plausible that this sector of the salmonid industry is undergoing expansion and development.

The United States salmonid industry has historically, and currently is, importing live salmonid fish and fertilized eggs from countries where one or more of the six pathogens described in this assessment are present. Despite this history, there have been no documented reports of transboundary disease entry or hatchery- or farm-level exposure to one of the six pathogens via imported live salmonid fish or fertilized eggs (or gametes).[129]

Factors contributing to the lack of pathogen introduction likely include but are not limited to:

- The number of producers in the United States has been relatively small compared to global statistics,
- The number of countries that export live salmonid fish, fertilized eggs, and gametes to the United States is small (n = 6),
- Exporting countries are WOAH members, members of the European Union, follow European Union guidelines for aquaculture disease regulation, or have control measures in place relative to the six pathogens summarized in this assessment,
- The global salmonid industry is relatively risk averse due to historical experience with disease outbreaks, and the economic importance of the salmonid industry locally, nationally, and internationally,
- The United States salmonid industry is relatively risk averse due to historical negative impacts caused by diseases endemically present in North America (e.g., IHNV, ISAV, VHSV),
- Regulatory oversight by States,
- Biosecurity practices utilized by domestic salmonid producers.

Atlantic salmon production is projected to increase as large inland RAS (and a to a lesser extent, FTS) aquaculture facilities are developed. Increased production of freshwater rainbow trout and steelhead trout are also predicted to increase. If production increases at predicted rates, it is unlikely that domestic hatcheries will be able to meet the increased demand for live fish and fertilized eggs. It is therefore likely that the volume of live salmonid fish, fertilized eggs (and potentially gametes) imported annually will increase to meet this demand. It is plausible that the six countries from which the United States has historically, and is currently, importing

live salmonid fish and fertilized eggs from will be unable to meet the projected increased demand. Domestic producers would then likely begin to import live fish and fertilized eggs (and potentially gametes) from other countries.

Translocation and movement of infected fish and fertilized eggs are one the most common pathways of pathogen introduction in aquaculture.[441, 11] For this assessment, the most plausible pathway of pathogen entry and exposure is importation of infected or contaminated live salmonid fish or fertilized eggs, and the accompanying transport water, from a country that does not have adequate aquatic pathogen surveillance, control, and reporting regulation, and/or from a hatchery or farm that does not practice proactive biosecurity and disease prevention and monitoring programs. The expected increased demand and volume of imports, and increased sources of imported live fish, fertilized eggs (and potentially gametes) increase the likelihood, uncertainty and risk of a transboundary pathogen entry and exposure occurring via this pathway.

The increased likelihood, uncertainty, and risk are balanced to some degree by the awareness of the U.S. salmonid industry of the risks and consequences of a foreign animal pathogen introduction and disease outbreak. However, if producers in the United States are not risk averse or are complacent regarding biosecurity and disease prevention and monitoring, the potential for transboundary entry and exposure are further increased.

There is limited regulatory oversight at the Federal level relative to the entry pathway (e.g., imported live salmonid fish, fertilized eggs, and gametes entering the United States), although USFWS does have capability to impose requirements for pathogens not listed in Title 50 based upon risk assessment.[105] Despite this limited oversight, there are no records of the six pathogens summarized in this assessment entering the United States via this pathway. However, based on the **moderate** risk ranking for projected future imports, it may be beneficial to consider implementation of some Federal regulatory oversight for these commodities.

There is limited regulatory oversight at the Federal level relative to inter- and intra-state movement of imported live salmonid fish, fertilized eggs, and gametes destined for private use. States, Tribes, and local government entities do have regulatory oversight; however, requirements are variable (Appendix, Table 1). Due to the **moderate** risk ranking for projected future imports, it may behoove States, Tribes, and local government entities in which inland salmonid aquaculture is being developed to consider implementation of some regulatory oversight, to include disease freedom requirements.

Limitations

In this assessment, primary pathways of entry and exposure were identified, and associated levels of likelihood, uncertainty and risk were estimated using available data and literature relative to the epidemiology of the six pathogens, current import, export and production practices, and existing biosecurity measures. To characterize the risk more accurately, additional information is needed. Some of these needs include, but are not limited to:

- Future projections for the United States Atlantic salmon, rainbow trout, and steelhead trout industries are hypothetical. Therefore,
    - The true trajectory of future inland aquaculture development (recirculating aquaculture system; RAS, and flow through system; FTS) is not known,

    - The true need for imported live salmonid fish, fertilized eggs, and gametes is not known,
    - The sources (countries, hatcheries, farms) for future imports are not known,
    - The epidemiology of the six pathogens in future source countries (and their hatcheries and farms) is not known,
    - Aquaculture disease management, reporting, and regulation in countries from which the United States may import live salmonid fish, fertilized eggs, and gametes in the future is unknown.
- Data deficiencies related to detection and reporting of aquaculture diseases in the United States:
    - There are few USDA APHIS-supported federal surveillance programs for aquaculture (other than ISAV in Maine),
        - USDA APHIS, USFWS, States, Tribes, and localities may conduct passive or case-by-case surveillance associated with disease response or allegations (e.g., ISAV in the Pacific Northwest, VHSV outside of the Great Lakes),
    - A consolidated database tracking all detections/outbreaks of aquatic animal pathogens in aquaculture is lacking,
        - USFWS does conduct a National Wild Fish Health Survey to monitor the presence or absence aquatic animal pathogens in wild fish populations (Appendix, Table 1);
- Outbreak response, contingency plans, and cost estimates for prevention, eradication, and control for many aquatic animal pathogens are lacking.
- In general, factors associated with the epidemiology of all the pathogens in this assessment relative to Atlantic salmon, rainbow/steelhead trout, other salmonid fish, and other potentially susceptible non-salmonid finfish are not fully described,
    - Experimental challenge studies using challenge methods that approximate natural exposure are generally lacking for some pathogens in this assessment,
    - All natural environmental, viral, and host factors associated each pathogen are not fully known,
    - All environmental, viral, and host factors associated with each pathogen in aquaculture environments (inland and marine) are not fully known,
    - Home range distributions and movements of key carrier and susceptible wild fish species during seasons when environmental factors are optimal for occurrence of the six diseases of concern are lacking,
    - The reservoir status of susceptible farmed fish species is not known for all pathogens in this document,
    - The reservoir or transmission host status of other aquatic species (crustacean, mollusc, copepod, other fish species) has not been definitively determined for all pathogens,
    - Information specific to virus infectious dose, pathogenic mechanisms, virulence factors, and duration of infectivity are not fully described for each pathogen,
    - Many factors associated with transmission (e.g., shedding rate, environmental conditions such as dilution, wind and current strength and direction, carrier status) have not been determined for each pathogen,

- Gaps in the epidemiology of some pathogens limit capability to discern why recurrences of disease at specific farm sites or disease epizootics happen,
- Susceptibility of United States salmonid (or other) fish stocks to some of the pathogens described in this assessment is lacking.

Appendix

Tables

Table 1. Links to manuals, websites, and other resources relevant to the pathogens included in this assessment

Topic	Link
Guide to State and Tribal aquaculture regulations	USDA APHIS | Interactive Maps
National Veterinary Service Laboratories National Animal Health Laboratories	USDA APHIS | Diagnostic Testing at the NVSL USDA APHIS | General NVSL Information USDA APHIS | Laboratory Information and Services USDA APHIS | Laboratories
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
World Organisation for Animal Health (WOAH) 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) Help Center Articles - Do I Need a Permit? (servicenowservices.com)
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 APHIS has a negotiated export health certificate that can used to ship live salmonids (fish or fertilized eggs) that require testing for some of the pathogens described in this assessment

Country	Pathogen Freedom Testing Required Prior to Export from The United States					
	Epizootic Haematopoietic Necrosis	***Gyrodactylus salaris***	**Infectious Haematopoietic Necrosis**	**Infectious Salmon Anemia**	**Salmonid alphavirus**	**Viral Hemorrhagic Septicemia**
Argentina	Yes	Yes	Yes	Yes	No	Yes
Armenia	Yes	No	Yes	Yes	No	Yes
Austria	Yes	Yes	Yes	Yes	No	Yes
Belarus	Yes	No	Yes	Yes	No	Yes

Belgium	Yes	Yes	Yes	Yes	No	Yes
Bosnia-Herzegovina	No	No	No	No	No	No
Brazil	Yes	No	Yes	Yes	Yes	Yes
Bulgaria	Yes	Yes	Yes	Yes	No	Yes
Canada	Yes	Yes	Yes	Yes	Yes	Yes
Chile	Yes	Yes	Yes	Yes	Yes	Yes
China	Yes	Yes	No	Yes	No	Yes
Croatia	Yes	Yes	Yes	Yes	No	Yes
Cyprus	Yes	Yes	Yes	Yes	No	Yes
Czech Republic	Yes	Yes	Yes	Yes	No	Yes
Denmark	Yes	Yes	Yes	Yes	No	Yes
Estonia	Yes	Yes	Yes	Yes	No	Yes
Finland	Yes	Yes	Yes	Yes	No	Yes
France	Yes	Yes	Yes	Yes	No	Yes
Georgia	Yes	Yes	Yes	Yes	Yes	Yes
Germany	Yes	Yes	Yes	Yes	No	Yes
Greece	Yes	Yes	Yes	Yes	No	Yes
Hungary	Yes	Yes	Yes	Yes	No	Yes
Ireland, Republic of	Yes	Yes	Yes	Yes	No	Yes
Isle of Man	Yes	Yes	Yes	Yes	No	Yes
Israel	Yes	Yes	Yes	Yes	Yes	Yes
Italy	Yes	Yes	Yes	Yes	No	Yes
Kazakhstan	Yes	No	Yes	Yes	No	Yes
Kyrgyzstan	Yes	No	Yes	Yes	No	Yes

Latvia	Yes	Yes	Yes	Yes	No	Yes
Lithuania	Yes	Yes	Yes	Yes	No	Yes
Luxembourg	Yes	Yes	Yes	Yes	No	Yes
Malaysia	Yes	No	No	No	No	No
Malta	Yes	Yes	Yes	Yes	No	Yes
Mexico	Yes	Yes	Yes	Yes	No	Yes
Morocco	Yes	Yes	Yes	Yes	No	Yes
Netherlands	Yes	Yes	Yes	Yes	No	Yes
New Zealand	Yes	No	No	No	No	Yes
North Macedonia	Yes	Yes	Yes	Yes	No	Yes
Norway	Yes	Yes	Yes	Yes	No	Yes
Peru	Yes	No	Yes	Yes	Yes	Yes
Poland	Yes	Yes	Yes	Yes	No	Yes
Portugal	Yes	Yes	Yes	Yes	No	Yes
Romania	Yes	Yes	Yes	Yes	No	Yes
Russian Federation	Yes	No	Yes	Yes	No	Yes
Serbia	Yes	Yes	Yes	Yes	No	Yes
Singapore	Yes	No	No	No	No	No
Slovakia	Yes	Yes	Yes	Yes	No	Yes
Slovenia	Yes	Yes	Yes	Yes	No	Yes
South Africa	Yes	Yes	Yes	Yes	Yes	Yes
Spain	Yes	Yes	Yes	Yes	No	Yes
Sweden	Yes	Yes	Yes	Yes	No	Yes
Switzerland	Yes	Yes	Yes	Yes	No	Yes

Taiwan	Yes	Yes	Yes	Yes	Yes	No
Turkey	Yes	Yes	Yes	Yes	No	Yes
Turks and Caicos Islands	Yes	Yes	Yes	Yes	No	Yes
Ukraine	No	No	Yes	No	No	Yes
United Arab Emirates	Yes	Yes	Yes	Yes	Yes	Yes
United Kingdom	Yes	Yes	Yes	Yes	No	Yes

Table 3. Countries in which presence of the six World Organisation for Animal Health (WOAH)-listed pathogens have been reported historically (wild and or farmed fish species)

Note. This table presents summary data from 2010 through 2022. The WOAH WAHIS database (Appendix, Table 1), should be consulted for information regarding current country status.[129, 97]

Country	Epizootic haematopoietic necrosis virus	*Gyrodactylus salaris*	Infectious hematopoietic necrosis virus (IHNV)	Infectious salmon anemia virus (ISAV)	Salmonid alphavirus (SAV)	Viral hemorrhagic septicemia virus (VHSV)
Australia	Yes	–	Yes	–	–	–
Austria	–	–	–	–	Yes	Yes
Belgium	–	–	Yes	–	–	Yes
Canada	–	–	Yes	Yes	–	Yes
Chile	–	–	–	Yes	–	–
China			Yes			
Costa Rica	–	Yes	–	–	–	–
Croatia	–	–	Yes	–	Yes	Yes
Czech Republic	–	–	Yes	–	–	Yes
Denmark	–	Yes	–	–	–	Yes
Estonia	–	Yes	–	–	–	Yes

Faroe Islands	–	–	–	Yes	–	–
Finland	–	Yes	–	–	–	Yes
France	–	–	Yes	–	–	Yes
Georgia	–	Yes	–	–	–	–
Germany	–	Yes	Yes	–	Yes	Yes
Iceland	–	–	–	Yes	–	Yes
Iran	–	–	Yes	–	–	Yes
Ireland	–	–	–	–	Yes	–
Italy	–	Yes	Yes	–	Yes	Yes
Japan	–	–	Yes	–	–	Yes
Latvia	–	Yes	–	–	–	–
Netherlands	–	–	Yes	–	–	Yes
North Macedonia	–	Yes	–	–	–	–
Norway	–	Yes	–	Yes	Yes	Yes
Poland	–	Yes	Yes	–	–	Yes
Romania	–	Yes	–	–	–	Yes
Russia	–	Yes	Yes	–	–	–
Slovakia	–	–	–	–	–	Yes
Slovenia	–	–	Yes	–	–	Yes
South Korea	–	–	Yes	–	–	Yes
Spain	–	–	Yes	–	Yes	–
Sweden	–	Yes	–	–	–	Yes
Switzerland	–	–	Yes	–	–	Yes
Turkey	–	–	–	–	–	Yes

Ukraine	–	Yes	–	–	–	–
United Kingdom (England, Scotland)	–	–	–	–	Yes	Yes
United Kingdom (Scotland)	–	–	–	Yes	–	–
United States	–	–	Yes	Yes	–	Yes
Vietnam	–	Yes	–	–	–	–

WOAH Pathogen Specific Import/Export Recommendations

WOAH import/export guidelines specific to each of the following pathogens are found in WOAH Aquatic Animal Health Code.[97] Briefly,

Epizootic Haematopoietic Necrosis Virus

1. When live aquatic animals or aquatic animal products are imported from a country, zone, or compartment declared free from EHNV 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.[97] 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 EHNV infection.[97]

2. When importing aquaculture or aquatic animals from a country, zone or compartment that is NOT free from EHNV 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:[97]

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 EHNV in accordance with WOAH 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 WOAH Aquatic Animal Health Code, Chapter 1.4, and select a founder population (F-0) with a high health status for infection with EHNV. 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 EHNV in accordance with the WOAH 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 EHNV as per the

WOAH Aquatic Health Code, Chapter 1.4, and the WOAH Manual of Diagnostic Tests for Aquatic Animals (the Aquatic Manual), Chapter 23.1.[120] If EHNV is not detected, the F-1 population may be defined as free from EHNV infection and released from quarantine. If EHNV is detected the aquatic animals remain in quarantine until they can be killed and disposed per the WOAH Aquatic Animal Health Code, Chapter 4.8.

Gyrodactylus salaris

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with *G. salaris*. The importing country's Competent Authority should require that the consignment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country. The international aquatic animal health certificate should state that, based on the procedures described in the WOAH Aquatic Animal Health Code, the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with *G. salaris*.

2. Importing aquatic animals or aquatic animal products from a country, zone, or compartment NOT declared free from infection with *G. salaris*. The importing country's Competent Authority should assess the risk as described in the WOAH Aquatic Animal Health Code, and consider the following risk mitigation measures: a) direct delivery and lifelong holding of the imported aquatic animals in a quarantine facility; and treatment of all transport water, equipment, effluent and waste materials sufficient to inactivate *G. salaris*; or b) immediately prior to movement require that the aquatic animals are held in water with a minimum salinity of 25 parts per thousand (ppt), and have no contact with other susceptible aquatic animal species.

3. Importing fertilized eggs for aquaculture from a country, zone, or compartment not declared free from infection with *G. salaris*. Fertilized eggs should be disinfected using a method demonstrated to be effective against *G. salaris* and post-disinfection should not come into contact with anything that may affect their health status.

4. Importing aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from *G. salaris*. The importing country's Competent Authority should a) require deliver of the consignment directly to authorized quarantine facilities where the animals will be held; b) all water (including ice), equipment, containers and packaging materials used in transport are treated to ensure inactivation of *G. salaris* and disposed of in a biosecure manner as described in the WOAH Aquatic Animal Health Code, Chapters 4.4, 4.8, and 5.5; c) all effluent and waste materials from the quarantine facilities are disposed of in a biosecure manner or treated to ensure inactivation of *G. salaris*; and d) all carcasses are disposed of as described in the WOAH Aquatic Animal Health Code, Chapter 4.8.

Infectious Haematopoietic Necrosis Virus

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with IHNV. 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, based on the procedures described in WOAH Aquatic Animal Health Code, Chapter 10.4, Articles 10.6.5., 10.6.6., or 10.6.7. and 10.6.8., the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with IHNV.

2. Importing aquatic animals or aquatic animal products from a country, zone or compartment that is NOT declared free from IHNV. The importing country Competent Authority should assess the risk as described in the WOAH 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 WOAH 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 IHNV in accordance with WOAH 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 IHNV in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4, and select a foundation population (F0) of animals with a high health status for infection with IHNV. The importing country should import the F0 population to a quarantine facility and test for IHNV as described in WOAH Aquatic Animal Health Code, Chapter 1.4 to determine the suitability of the population for broodstock. A first generation (F1) population should be produced in quarantine, cultured under conditions conducive for clinical expression of IHNV infection, and sampled and tested for IHNV as described in WOAH Aquatic Animal Health Code, Chapter 1.4, and WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.5. If IHNV is not detected in the F1 population, it may be defined as free from IHNV infection and released from quarantine. If IHNV is detected, the F1 population should not be released from quarantine, and should be killed and disposed of in a biosecure manner as described in WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 4.8.

Infectious Salmon Anemia Virus

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). 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, based on the procedures described in WOAH 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. 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, based on the procedures described in WOAH 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 WOAH 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 the WOAH 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 WOAH 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 WOAH Aquatic Animal Health Code, Chapter 1.4, and select a foundation population (F0) of animals with a high health status for infection with ISAV. The importing country should import the F0 population to a quarantine facility and test for ISAV as described in WOAH Aquatic Animal Health Code, Chapter 1.4 to determine the suitability of the population for broodstock. A first generation (F1) 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 WOAH Aquatic Animal Health Code, Chapter 1.4, and WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.5. If ISAV is not detected in the F1 population, it may be defined as free from ISAV infection and released from quarantine. If ISAV is detected, the F1 population should not be released from quarantine, and should be killed and disposed of in a biosecure manner as described in WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 4.8.

4. Importing disinfected fertilized eggs for aquaculture from a country, zone, or compartment that is NOT declared free from ISAV infection (either HPR0 and/or HPRΔ variants). 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 fertilized 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 fertilized eggs be disinfected prior to importing in accordance with WOAH Aquatic Animal Health Code, Chapter 4.5 recommendations, and that the fertilized 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 fertilized 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 fertilized eggs upon arrival in the importing country.

Salmonid Alphavirus

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with SAV. The importing country's Competent Authority should

require that the consignment be accompanied by an international aquatic animal health certificate issued by the exporting country's Competent Authority. The international aquatic animal health certificate should state that, based on the procedures described in WOAH Aquatic Animal Health Code, Chapter 10.4, Articles 10.5.5., 10.5.6., 10.5.7., and 10.5.8., the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with SAV.

2. Importing aquatic animals for aquaculture from a country, zone, or compartment NOT declared free from infection with SAV. The importing country's Competent Authority should assess the risk in accordance with the WOAH 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 for the life span of the animals. Before leaving quarantine (either the original quarantine facility or after biosecure transport to another quarantine facility) the animals should be humanely killed and processed into one or more of the aquatic animal products described in the WOAH Aquatic Animal Health Code, or products authorized by the Competent Authority. All transport water, packaging materials, equipment, effluents, and wastes must be treated to inactivate SAV in accordance with WOAH 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 potential source populations and evaluate their aquatic animal health records; test the identified source populations in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4., and select a foundation population (F0) of aquatic animals with a high health status for infection with SAV. The importing country should import the F0 population into a quarantine facility and test for SAV in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4. to determine suitability of the F0 population as broodstock. A first generation (F1) population should be reared in quarantine under conditions that are conducive to the clinical expression of SAV infection. During this period, sampling and testing of the F1 populations for SAV should be performed in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4. and WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.6. If SAV is not detected in the F1 population, it may be defined as free from SAV infection and may be released from quarantine. IF SAV is detected, the F1 population should not be released from quarantine and should be killed and disposed of in a biosecure manner in accordance with WOAH Aquatic Animal Health Code, Chapter 4.8.

3. Importing aquatic animals intended for use in authorized laboratories or zoos from a country, zone, or compartment declared free from infection with SAV. The importing country's Competent Authority should ensure that the consignment is delivered directly to and held in quarantine facilities in the authorized laboratories or zoos. All transport water and ice, equipment, containers and packaging material, facility effluents, wastes and animal carcasses should be treated to ensure inactivation of SAV, or disposed of in a biosecure manner in accordance with WOAH Aquatic Animal Health Code, Chapters 4.4., 4.8., and 5.5.

4. Importing disinfected fertilized eggs for aquaculture from a country, zone, or compartment that is NOT declared free from SAV infection. Prior to importation, the importing country's Competent Authority should assess at minimum, the likelihood that the water used during disinfection of the fertilized eggs is or may be contaminated with SAV, and the prevalence of

SAV infection in the broodstock (including evaluation of results of testing milt and ovarian fluid). If it is determined that importation is acceptable, the importing country's Competent Authority should mitigate risk of SAV introduction by requesting that the fertilized eggs be disinfected prior to importation in accordance with recommendations in WOAH Aquatic Animal Health Code, Chapter 4.5., and that during the interval between disinfection and importation, the fertilized eggs do not contact with anything that may impact their health status. The importing country's Competent Authority should require that the consignment of fertilized eggs be accompanied by an international aquatic animal health certificate issued by the exporting country's Competent Authority certifying that the risk mitigation procedures were conducted. The importing country's Competent Authority should also consider internal measures such as additional disinfection of the fertilized eggs upon arrival in the importing country.

Viral Hemorrhagic Septicemia Virus

1. Importing aquatic animals or aquatic animal products from a country, zone, or compartment declared free from infection with VHS. 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, based on the procedures described in the WOAH Aquatic Animal Health Code, Articles 10.10.5., 10.10.6., or 10.10.7 (as applicable) and 10.10.8, the production site of the aquatic animals or aquatic animal products is a country, zone, or compartment declared free from infection with VHSV.

2. Importing aquatic animals or aquatic animal products from a country, zone or compartment that is NOT declared free from VHS infection. The importing country Competent Authority should assess the risk as described in the WOAH 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 for the duration of the animals' lifetime. Before leaving quarantine (wither in the original facility or via biosecure transport to another quarantine facility) the animals are killed and processed into one or more products described in the WOAH Aquatic Animal Health Code, Article 10.10.3, or authorized by the Competent Authority. All water (transport, effluent, waste) equipment and waste materials are treated to inactive VHSV as described in the WOAH 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 VHS in accordance with WOAH Aquatic Animal Health Code, Chapter 1.4, and select a foundation population (F0) of animals with a high health status for infection with VHSV. The importing country should import the F0 population to a quarantine facility and test for VHS as described in WOAH Aquatic Animal Health Code, Chapter 1.4 to determine the suitability of the population for broodstock. A first generation (F1) population should be produced quarantine, cultured under conditions conducive for clinical expression of VHSV infection, and sampled and tested for VHSV as described in WOAH Aquatic Animal Health Code, Chapter 1.4, and WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 2.3.5. If VHSV is not detected in the F1 population, it may be defined as free from infection and released from quarantine. If VHSV is detected, the F1 population should not be released from

quarantine, and should be killed and disposed of in a biosecure manner as described in WOAH Manual of Diagnostic Tests for Aquatic Animals, Chapter 4.8.

3. Importing disinfected fertilized eggs for aquaculture from a country, zone, or compartment that is NOT declared free VHSV. Prior to importation, the importing country Competent Authority should assess at minimum, the likelihood that water used during disinfection of the fertilized eggs may be contaminated with VHSV, and the prevalence of VHSV infection in the broodstock (including evaluation of testing of ovarian fluid and milt). If the risk is acceptable, risk mitigation measures should be applied. The fertilized eggs be disinfected prior to import as described in the WOAH Aquatic Animal Health Code, Chapter 4.5. During the interval between disinfection and importation, the fertilized eggs should not contact anything that may impact their health status. The importing country Competent Authority should require that the consignment of fertilized 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 fertilized eggs upon arrival in the importing country.

Entry Pathway Supplemental Materials

Atlantic Salmon

According to USFWS LEMIS data, import of live Atlantic salmon fish, fertilized eggs, and gametes does occur (n = 140 total imports over 10 years; 2013–2023). Imports from 2013 to 2023 included live fish (n = 2 shipments) and fertilized eggs (n = 138 shipments). The number of live fish imported in the two shipments was 100 and 250 fish, respectively. The volume of imported fertilized eggs was reported by weight or by number of fertilized eggs and ranged from 5–360 kg/11–793.7 lbs and 545–1,850,000 fertilized eggs per shipment, respectively. Importing entities ranged from commercial, private, and State aquaculture hatcheries, laboratories, and conservation and environmental nonprofits located in 13 States (Florida, Indiana, Kentucky, Maine, Maryland, Minnesota, Nebraska, South Dakota, Tennessee, Virginia, Washington, West Virginia, and Wisconsin).[22] Countries from which the live fish, fertilized eggs, and gametes were imported included Canada, Iceland, and Norway.[22] One or more of the pathogens of concern summarized in this assessment are present in each of those countries.

Rainbow Trout/Steelhead Trout

There were 150 total imports of *O. mykiss* (identified in the LEMIS data base as steelhead trout) during the same time interval (2013–2023). Fertilized eggs comprised the greatest number of shipments (n = 145) and ranged between 150 to 1,350,000 fertilized eggs per shipment. There were five shipments of live fish (n = 121–6,010 fish per shipment). Importing entities included commercial, private, and State aquaculture hatcheries, and conservation and environmental nonprofits located in six States (Florida, Idaho, Montana, Oregon, Washington, and West Virginia).[60] Countries from which the live fish, fertilized eggs, and gametes were imported included Canada, Croatia, Denmark, Norway, and Russia.[60] One or more of the diseases included in this assessment are present in salmonid populations in each of these countries.

EHNV is not present in any of the counties from which the United States has historically imported live salmonid fish, fertilized eggs, or gametes from. The other five pathogens are present in two or more of the exporting countries.

Canada is a WOAH Member.

- IHNV is a reportable pathogen in Canada.[465] The virus has been identified in wild finfish, freshwater and marine life stages of wild Sockeye salmon, and sporadically in marine farmed Atlantic salmon in the Pacific Ocean watershed of British Columbia.[236, 466] The Canadian Food Inspection Agency has a specific response plan for IHNV that includes zoning for the presence and absence of IHNV in susceptible finfish species. Zones identified IHNV infected areas include the Pacific Ocean, Pacific Ocean Watershed of British Columbia, and the Pacific Ocean Watershed of Yukon.[467] In 2011, the salmonid industry in Canada developed a Salmon Farming Industry Viral Disease Management Plan (Viral Management Plan; VMP) that is shared with the CFIA. Included in the VMP are standard procedures relevant to prevention of vertical transmission of infectious pathogens including adherence with importation guidelines, disinfection of hardened fertilized eggs, and the agreement that gametes from broodstock populations that have viruses present that adversely affect fish health will not be used. The standard also requires that all companies will vaccinate for IHNV in all production areas.[467] Beginning in 2015, the British Columbia Introductions and Transfers Committed required that fish transferred within and between zones be tested for Fish Health Protection Regulations Schedule II diseases that includes IHNV.[467] Through the use of broodstock screening, egg disinfection, and virus free water to rear fish, the BC Atlantic Salmon aquaculture industry has maintained an IHNV-free status during the freshwater stage of the production cycle.[236]
- ISAV is a reportable pathogen in Canada that was first detected in New Brunswick in 1996.[271] Areas where ISAV is present include Quebec, New Brunswick, Prince Edward Island, Nova Scotia, Newfoundland and Labrador, and the Atlantic Ocean (the belt of sea within the Atlantic Ocean that extends from the landward baseline of the territorial sea with an outer limit of 24 nautical miles as defined in the Oceans Act of Canada).[465] Historically most detections have occurred in marine stage Atlantic salmon; however, there have been occasional detections in hatchery reared smolts prior to transfer to marine pens.[468, 469]
- VHS is a federally reportable disease. VHSV-IVa has been detected in the Pacific Ocean watershed of British Columbia in wild marine finfish, marine farmed Atlantic salmon, and hatchery-reared Pacific salmon returning to freshwater to spawn.[470] VHSV-IVb is present in the Canadian Great Lakes and associated watersheds (VHSV-IVb; Lake Ontario, Lake Erie, Lake Huron, Lake Simcoe, Lake Superior, the Lower Thames River, and the St. Lawrence River west of the Moses-Saunders dam).[470, 471, 472] Two management zones have been established encompassing sections of Lake Ontario and Lake Huron in response to this detection. VHSV-IVc has been detected in brackish water finfish in the Atlantic Ocean watersheds in New Brunswick and Nova Scotia.[472] VHSV-IVc is has been detected in wild estuarine and brackish water fish species present along the Atlantic coast.[470, 471, 472] Beginning in 2015, the British Columbia Introductions and Transfers Committed required that fish transferred within and between zones be tested for Fish Health Protection Regulations Schedule II diseases including VHSV.[467] Review of available literature did not identify reports of VHSV surveillance or detection in hatcheries that rear salmonid fish in British Columbia or elsewhere.

Croatia is a WOAH Member. Croatia is also a member of the European Union (since 2013) and must comply with control and prevention measure for disease of aquatic animals (including IHNV, SAV, and VHSV) as described in Directive 2006/88/EC – Animal Health Requirements for Aquaculture Animals[453, 454] and Article 5 of Regulation (EU) 2016/429.[452]

- IHNV has been detected in farmed freshwater rainbow trout, including fry collected at hatcheries.[473, 474, 331] Review of the literature did not identify specific reports describing the distribution of the disease within farmed rainbow trout industry in Croatia or country-specific regulatory or eradication measures. There have been no reports of IHNV submitted to WAHIS since 2013.[129]
- SAV was detected in farmed freshwater rainbow trout fry in 2011.[331] Subsequent detections were reported to WOAH in 2013–2020.[129] Review of the literature did not identify specific reports describing the distribution of the disease within the rainbow trout industry or regulatory or eradication measures.
- VHSV has been reported farmed freshwater rainbow trout; however, there is limited data available regarding detection and control of this disease. Review of the literature did not identify country specific regulatory oversight regarding surveillance, zoning, compartmentalization, or control of this disease specific to rainbow trout. According to the WOAH WAHIS database VHSV was detected in 2013–2018 in unidentified farmed and wild fish species (n = 25 detections and n = 4 detections, respectfully).[129]

Denmark is a WOAH Member. Denmark has been member of the European Union since 1973, and must comply with control and prevention measure for disease of aquatic animals (including IHNV and VHSV) as described in Directive 2006/88/EC – Animal Health Requirements for Aquaculture Animals[453, 454] and Article 5 of Regulation (EU) 2016/429.[452]

- IHNV was detected in asymptomatic hatchery-reared rainbow trout fry at two hatchery/farms in May 2021.[475, 476] Prior to detection, fish had been exported from these farms to a number of European countries (Austria, Germany, the Netherlands, and Finland).[475]
- VHSV has been historically reported present in farmed rainbow trout. Review of the WOAH WAHIS database for years that data was available identified detections of VHSV from 2005 to 2009.[129] Following implementation of an EU Commission approved VHSV eradication plan in 2009 there have been no detections of VHSV.[477, 367]

Iceland is a WOAH member. Iceland is not a European Union Member. However, it is associated through its membership of the European Economic Area.

- ISAV was detected in a marine Atlantic salmon farm (Reyðarfjörður fjord, East Iceland) in 2021. The Icelandic Food and Veterinary Authority (MAST) responded to this detection via lethal control. ISAV has not been detected in Atlantic salmon hatcheries or smolt farms.[478, 479]
- VHSV-IVd was isolated from wild lumpfish in Iceland in 2018. There have been no reports of VHSV in farmed Atlantic salmon or other salmonid fish.[480, 418] Review of available literature did not identify reports of VHSV surveillance or detection in hatcheries that rear salmonid fish. VHSV has not been reported in Norway in Atlantic salmon.

Norway: is a WOAH Member. Norway is not a European Union Member. However, it is associated through its membership of the European Economic Area, and the European Free Trade Association.

- *Gyrodactylus salaris* is a listed notifiable pathogen in Norway present in some freshwater rivers where wild salmon populations are present.[481] The Norwegian Veterinary Institute

coordinates a surveillance plan on the behalf of the Norwegian Food Safety Authority and publishes annual reports on its website.[481] From 1975 to 2020, pathogenic *G. salaris* were detected on Atlantic salmon in 51 river and in 13 hatcheries.[481] In 2021, the parasite was confirmed eradicated in 39 rivers and in all Atlantic salmon and rainbow trout hatcheries and fish farms. In 2022, *G. salaris* was still confirmed present in eight river systems.[481]

- ISAV is a reportable pathogen in Norway that was first detected in 1984. Annual ISAV detection reports are available through the Norwegian Food Safety Authority. Following implementation of control measures to limit the spread of the disease the number of annual outbreaks dropped from 80 cases in 1990 to 1 to 23 annually. The Norwegian Food Safety Authorities (NFSA) has identified and received approval for ISA-free zones and compartments based on historical disease freedom and targeted surveillance for ISAV (HPR0 and HPRΔ) in accordance with requirements in the European Union Council directive 2006/88/EF.[482, 483] Surveillance is based on inspections by the NFSA and health controls performed by Fish Health Services (FHS) at all phases of Atlantic salmon aquaculture (e.g., land-based hatcheries, broodstock sites, marine grow out farms).[483] Since implementation of the targeted surveillance program, ISAV has been detected in some broodfish, smolt farms, and at marine farms.[483, 255]
- SAV has been a notifiable disease in Norway since 2007 and is currently present in two separate endemic zones (e.g., SAV2 on the northwest and mid-Norwegian coast, and SAV3 on the southwestern coast). The Norwegian Food Safety Authorities (NFSA) oversees national a national surveillance program to monitor the status of SAV in freshwater hatcheries and marine-stage fish farms.[483, 484, 485] In 2017, the NFSA surveillance program detected SAV in 2 hatcheries, and 130 and 12 marine farms located within and outside of the endemic zones, respectfully.[483] The source of SAV in the hatcheries was not stated in the report;[483] however, the NFSA has assessed the risk of vertical SAV transmission as insignificant.[325, 486]

Russia: is a WOAH Member. Russia is not a member of the European Union.

- *Gyrodactylus salaris* has been detected on Atlantic salmon in river systems in the southern regions of the Russia including the White Sea basin, Lake Kuito, the River Kem, River Pisto, the River Lizhma, the Lake Onega basin, and in Lake Ladoga and the River Keret.[487, 488, 491, 489] Detections are reported in farmed rainbow trout in Karelia.[489] Rainbow trout farms in this area typically source fingerling trout from neighboring regions in Russia (e.g., the Leningrad Region, and Republic of North Ossetia-Alania) and from Finland.[489] Review of available literature did not identify reports of *G. salaris* surveillance or detection in hatcheries that rear salmonid fish.
- IHNV has been detected in wild and cultured sockeye salmon (e.g., wild and hatchery) spawning adults and juvenile sockeye salmon in Russia (e.g., on the Kamchatka Peninsula [2001] and the Bolshaya watershed [1995–2005]).[213] According to the WOAH WAHIS database for years in which data were available (2005–2022), there was one report of IHNV in wild fish (species unspecified) in 2009.[129] Review of the literature did not identify any other materials relevant to IHNV detections, surveillance, or control efforts in salmonid fish (wild or domestic).

Likelihood, Uncertainty, Risk, and Consequence Categorization for Risk Assessment

For this assessment, we have assigned qualitative likelihoods for expressing likelihood, uncertainty, consequence, and risk. Terms and definitions for provided in the following tables.

Table 4. Definition of likelihood categories for risk assessment

Term	Definition
Negligible	This event would almost certainly never occur
Low	This event would be unlikely to occur
Moderate	This event would be nearly as likely to occur as to not occur
High	This event would be likely to occur
Very High	This event is almost certain to occur

Table 5. Definition of uncertainty levels

Term	Definition
Low	Available data is well supported, reliable, complete, and accessible from multiple sources or published references, and in general agreement.
Moderate	Data available, but with high interpretability issues, potential biases, reliability issues, and/or underreporting.
High	Some data available but may be incomplete, unreliable, from a small number of published sources, and/or demonstrates conflicting evidence. Includes the combination of anecdotal evidence, personal communications, and expert opinion with available published data, if all sources are in general agreement.

Table 6. Definition of risk levels

Term	Definition
Negligible	Suggests that the risk is low enough that it need not be considered, and no further mitigations are necessary.
Low	Suggests resources to further evaluate or mitigate this risk should be considered. A low risk is greater than a negligible risk due to a potential likelihood of occurrence, associated consequences, or a combination of both.

Moderate	Suggests that the risk is of a sufficient magnitude that measures to prevent or mitigate the risk should be considered. A moderate risk is greater than a low risk due to a greater likelihood of occurrence, greater consequences, or a combination of both.
High	Suggests that the risk is of sufficient magnitude that measures to prevent or mitigate the risk are necessary and the consequences will have significant impact at the regional or national level. A high risk is greater than a moderate risk due to a greater likelihood of occurrence, greater consequences, or a combination of both.

Table 7. Definition of consequence levels[490]

Term	**Definition**
Negligible	The consequences of exposure are so low as to be undetectable
Low	Minor increases in morbidity/mortality and some decreases in production. Effects of exposure are controllable or reversible.
Moderate	Morbidity/mortality are great enough to impose moderate production losses. Effects of exposure may not be reversible.
High	Morbidity and mortality are great enough to threaten the economic viability of the sector for a lengthy period. Effects of exposure may not be reversible.

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