Self-declaration of historical freedom from Epizootic Hematopoietic Necrosis Virus (EHNV) for the United States of America

1. Introduction

This article is to formally declare historical freedom from Epizootic Hematopoietic Necrosis Virus (EHNV), as defined in the World Organisation for Animal Health (WOAH) *Aquatic Animal Health Code* (*Aquatic Code*) for the United States of America (USA).

This designation meets all requirements of WOAH self-declarations of historical freedom, except concurrent submission by neighboring countries; however, EHNV is reportable and considered absent in Canada and in Mexico, the two countries that share watersheds to varying degrees with the USA.

The USA is considered historically free from EHNV and conducts surveillance in compliance with the EHNV-specific articles of the *Aquatic Code* (10.1.5) and the WOAH *Manual of Diagnostic Tests for Aquatic Animals* (2.3.2). This self-declaration is also compliant with the following relevant articles in the *Aquatic Code:*

- Chapter 1.4
  - Articles 1.4.4–1.4.8
  - Article 1.4.12
  - Article 1.4.15

2. History of absence

2.1 EHNV has never been detected in the USA

EHNV has never been detected in the USA or its surrounding waters. The USA has been an active WOAH member country since 1976, and its responsibility toward consistent and accurate reporting of notifiable diseases is evidenced in regular contributions to the World Animal Health Information System (WAHIS).

3. Compliance with basic biosecurity condition requirements of the WOAH *Aquatic Code*

3.1. The United States of America Competent Authority

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is granted the authority to govern the prevention, detection, control, and eradication of animal diseases under the Animal Health Protection Act, in the *Code of Federal Regulations* (CFR) Title 7 Chapter 109 where *animal* is defined as any member of the animal kingdom (excluding humans).

USDA APHIS is the Competent Authority for diseases of aquatic livestock in the USA. USDA APHIS Veterinary Services (VS) houses the Chief Veterinary Officer who serves as the USA's delegate to WOAH. The U.S. National Oceanic and Atmospheric Association (NOAA), the U.S. Fish and Wildlife Service (USFWS), the U.S. Food and Drug Administration (FDA), the U.S. Environmental Protection Agency (EPA), and other Federal, State, and Tribal entities with aquatic animal jurisdiction work jointly to expand outreach, surveillance, response, and legislation to further protect the health of all aquatic animals in the USA.

3.2 WOAH-listed diseases are notifiable

USDA APHIS Accredited Veterinarians and APHIS-approved laboratories have an obligation to report the suspicion and detection of any occurrence of listed or emerging diseases to USDA APHIS, the Competent Authority for animal health in the USA. Accredited Veterinarians are required by law (Title 9 CFR Chapter 161.4) to immediately notify Federal authorities of any confirmed or suspected findings of WOAH-listed diseases not known to exist in the USA. APHIS-approved laboratories are required by law to report non-negative results under the authority for laboratory approval. USDA APHIS National List of Reportable Animal Diseases (NLRAD) provides standards for the reporting of suspect or confirmed cases of FADs, program diseases, or diseases not known to exist in the United States. Currently, all States are required to report FADs and evidence of emerging domestic animal diseases to USDA APHIS.

USDA APHIS develops and maintains case definitions for WOAH-listed diseases and other diseases of concern. These case definitions are required under the National List of Reportable Animal Diseases (NLRAD) and follow WOAH guidance.

3.3 Training and awareness programs

USDA APHIS administers the National Veterinary Accreditation Program (NVAP) for veterinarians licensed to practice in the USA. This program accredits and authorizes veterinary practitioners to work cooperatively with Federal veterinarians and State Animal Health Officials. Regulations in 9 CFR 161 outline the requirements and standards for Federally Accredited Veterinarians, which include disease reporting. Specifically, Accredited Veterinarians are required to immediately report to the Federal and State Animal Health Official all diagnosed or suspected cases of a communicable animal disease for which USDA APHIS has a control or eradication program, and all diagnosed or suspected cases of any animal disease not known to exist in the USA. Accredited Veterinarians must renew their accreditation every three years. State veterinary licenses also require continuing education earned through courses, meetings, conferences, and online trainings specific for animal health and drug use. Veterinary accreditation is required to conduct certain activities at the State and Federal levels, including the issuance of international export health certification of aquatic animals. There are approximately 70,000 Accredited Veterinarians in the USA and its Territories. The NVAP provides four training modules specifically devoted to aquatic animal health. Additionally, the American Fisheries Society (AFS) provides certification for Aquatic Animal Health Inspectors to serve in aquatic animal health professional roles, not all of whom are veterinarians. AFS certified Aquatic Animal Health Inspectors also conduct health inspections for private and public aquaculture entities.

Awareness and education for producers, veterinarians, and the public, is distributed through various methods including the USDA APHIS website for aquaculture, aquaculture industry webpages, extension and outreach services, industry meetings, professional conferences, and technical documents and resources. Many State aquaculture associations have designed training modules specifically for producers. Examples include Aquaculture/Fish Culture - University of Wisconsin-Stevens Point (uwsp.edu); Fish Health Medicine Program – AAFV FishVets.org; Aquaculture Training (themaineaquaculturist.org); Educational Resources | Maine Aquaculture; and Workforce Development - Aquaculture Research Institute (umaine.edu).

USDA APHIS administers training and professional development to Federal and State Veterinarians, Accredited Veterinarians, diagnosticians, animal health technicians, epidemiologists, port veterinarians, foreign veterinary medical officers, USDA APHIS program specialists, and others whose responsibilities require updated knowledge of animal disease diagnosis and identification. USDA APHIS conducts annual training for Federal employees in a wide variety of topics, including FAD diagnostics, agriculture emergency response, and animal identification.

3.4 Laboratory capacity in the USA

The National Veterinary Services Laboratories (NVSL) serves as the national veterinary diagnostic reference and confirmatory laboratory for foreign and emerging diseases of significant concern for animal health. APHIS also oversees APHIS-approved laboratories to conduct frontline testing of aquatic animal pathogens. APHIS-approved laboratories include the National Animal Health Laboratory Network (NAHLN), which is a nationally coordinated network and partnership of Federal, State, and university-associated animal health laboratories. The NAHLN enhances the capability to diagnose specific endemic and foreign high-consequence livestock pathogens in animals and environmental samples. Aquatic animal pathogens currently covered under the NAHLN are spring viremia of carp virus (SVCV), infectious salmon anemia virus (ISAV) and viral hemorrhagic septicemia virus (VHSV). In addition to the NAHLN, APHIS approval is also required of laboratories conducting diagnostic testing to support export health certification. A list of all APHIS-approved laboratories, accompanied by a summary of the diseases for which the laboratories are approved to test, is maintained and available to the public, as is a map of the NAHLN laboratories, depicted alongside the diseases for which they are authorized to test. In addition, the American Fisheries Society Fish Health Section (AFS FHS) provides guidance and inspection for fish health laboratory quality management/quality assurance. Laboratories conducting tests of wild fish, for domestic movement and stock enhancement, generally follow published guidelines.

3.5 Diagnostic tests in the USA

Diagnostic tests available to identify EHNV include virus isolation (Fryer, 1994), genomic sequencing, and polymerase chain reaction (PCR); screening is typically done by virus isolation. Per the WOAH *Aquatic Manual,* "EHNV replicates well in many fish cell lines including BF-2 (bluegill fry; Wolf, 1966), FHM (fathead minnow; Gravell, 1965), EPC (epithelioma papulosum cyprini; Fijan et al., 1983), and CHSE-214 (Chinook salmon embryo cell line; Lannan et al., 1984) at temperatures ranging from 15 to 22°C."

3.6. Measures to prevent the introduction of EHNV into the USA

USDA APHIS is granted the authority to govern the prevention, detection, control and eradication of animal diseases under the Animal Health Protection Act. The USFWS administers the Lacey Act, which specifies that it is unlawful for any person to market, transport or acquire fish or wildlife in violation of any law, treaty, or regulation of the USA.

The Code of Federal Regulations (CFR), Title 50, part 16.13 (1) prohibits the importation of certain fish species which include the European perch (*Perca fluviatilis*) and the zander (also known as Pike-perch, *Sander lucioperca*); (2) requires health certification of live or dead un-eviscerated fish from the family Salmonidae, including pathogen testing by viral cell culture, prior to import; and (3) requires surface disinfection of salmonid eggs prior to import. Though

EHNV testing is not specifically required for USFWS import health certification, EHNV is cultivable in the cell lines that are utilized (EPC, CHSE-214). Any suspicion or detection of EHNV in the exporting country would trigger reporting of this WOAH-listed pathogen by Member Countries (WAHIS). A 10-year review (2012 through 2022) of USA port authority records identified that all imports of EHNV-susceptible species were imported into the USA from WOAH Member Countries. None of those source countries have known occurrences of EHNV.

Additionally, Title 50 (CFR) part 16.13 broadly prohibits release of *any* fish species into surface waters without express permission of the receiving State, and APHIS restricts the movement of diseased animals under Title 9 (CFR) 71.2 and 71.3.

State regulations guide additional aquatic animal health and import requirements locally.

4. Passive Surveillance in the USA

4.1. Broad oversight and extent

The reach and functionality of the USA's passive surveillance infrastructure for aquatic animal health is evidenced by several data sources. As described in Section 3.2, Accredited Veterinarians and APHIS-approved laboratories are required to report suspected or confirmed cases of FADs, or any diseases not known to exist in the USA. Suspect cases compatible with a WOAH-listed pathogen may trigger Federal investigations, guided by USDA APHIS VS policy. Between 2018 and 2023, USDA APHIS conducted 62 disease investigations in aquatic species, 46 of which were FAD investigations. Regional laboratories also investigate disease concerns upon producer request. APHIS-approved laboratories conducted over 570 finfish diagnostic investigations in 2022 and 2023 combined.

Reach and functionality is also evidenced through data collected by natural resource agencies. The USFWS operates 70 public hatcheries distributed throughout the country that together rear over 100 species of fish, including rainbow trout (*Oncorhynchus mykiss*), northern pike (*Esox Lucius*), and mosquitofish (*Gambusia affinis*), for conservation or stock enhancement. All facilities are inspected, with fish tested biannually, and any unexplained mortality events are promptly investigated. The USFWS conducted over 285 disease investigations in public hatcheries in 2022 and 2023 combined. State natural resource agencies similarly conducted over 1,310 disease investigations in 2022 and 2023 combined. These tallies, though not species-specific, further demonstrate an active and broad disease detection capacity for aquatic animals in the USA. USFWS testing follows guidance in the Aquatic Animal Health Policy and Handbook, and employs cell lines considered sensitive to EHNV (BF-2, FHM, EPC, or CHSE-214).

The APHIS National Animal Health Reporting System's (NAHRS) voluntary database compiles State-level designations of pathogens of concern from participating State Animal Health Officials and captures information on both farmed and wild animals. Between 2018 and 2023, 46 States formally designated EHNV as absent. Furthermore, APHIS collects reports of endemic pathogen detections through NAHRS and direct reports from laboratories, State and Federal agencies, and private producers. Between 2018 and 2023, several non-EHNV disease reports were made in EHNV-susceptible species: 1 in eastern mosquitofish (*Gambusia holbrooki*) and at least 16 in rainbow/steelhead trout (*Oncorhynchus mykiss*). EHNV has never been reported in NAHRS's history (established in 2004).

4.2. Conditions are conducive to clinical expression

The WOAH *Aquatic Code* lists the following species as susceptible to EHNV: northern pike (*Esox Lucius*), mountain galaxias (*Galaxias oldies*), black bullhead (*Ameiurus melas*), crimson spotted rainbowfish (*Melanotaenia fluviatilis*), European perch (*Perca fluviatilis*), pike-perch (*Sander lucioperca*), macquarie perch (*Macquaria australasica*), eastern mosquitofish (*Gambusia holbrooki*), mosquitofish (*Gambusia affinis*), rainbow trout (*Oncorhynchus mykiss*), and silver perch (*Bidyanus bidyanus*). Rainbow trout (*Oncorhynchus mykiss*) and European (aka redfin) perch (*Perca fluviatilis*) are the most well-described hosts for this pathogen.

Species that occur naturally in the USA include northern pike (*Esox Lucius*), black bullhead (*Ameiurus melas*), mosquitofish (*Gambusia affinis*), eastern mosquitofish (*Gambusia holbrooki*), and rainbow trout (*Oncorhynchus mykiss*). Northern pike (*Esox Lucius*), rainbow trout (*Oncorhynchus mykiss*), and mosquitofish (*Gambusia affinis*) are also farmed in the USA. USFWS refugia holding EHNV-susceptible species are distributed widely throughout the USA (Figure 1). European perch (*Perca fluviatilis*) do not occur in the USA.

Figure 1. US Fish and Wildlife Service refugia with managed populations of EHNV-susceptible species. Maps generated by USFWS.

Rainbow Trout (*Oncorhynchus mykiss*)

Black Bullhead (*Ameiurus melas*)

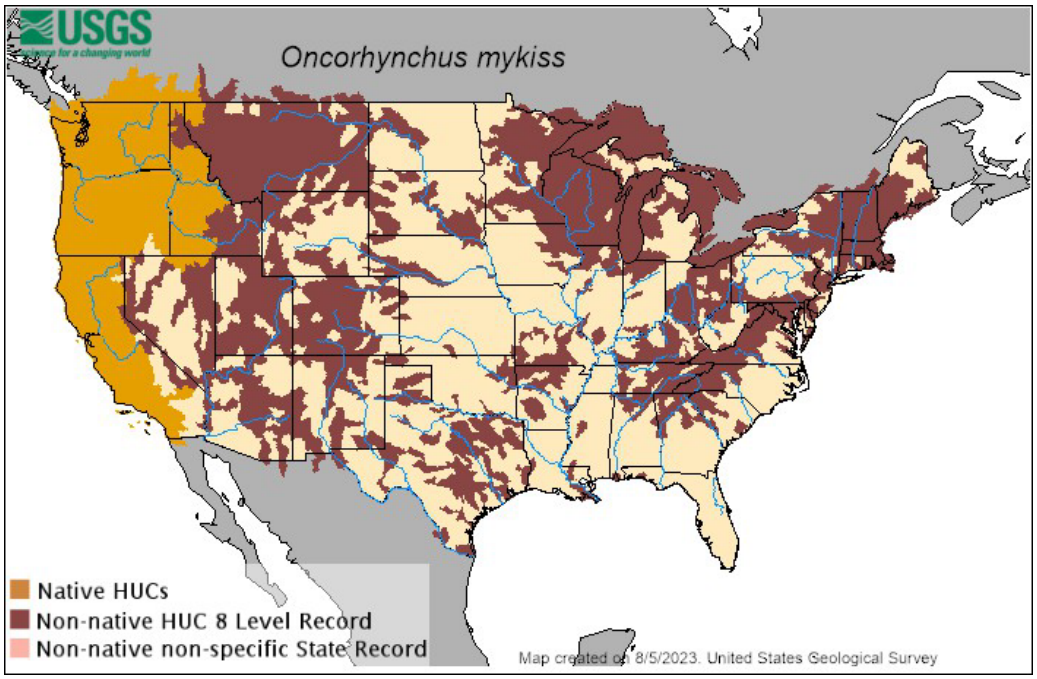
Northern Pike (*Esox Lucius*)

Mosquitofish (*Gambusia affinis*)

Disease outbreaks in farmed rainbow trout (*Oncorhynchus mykiss*) occur at temperatures ranging from 52 to 63°F (11 to 17°C; Whittington et al., 1994). Rainbow trout (*Oncorhynchus mykiss*) are widely distributed in the USA (Figure 2), survive in waters up to 70°F (21°C), and

prefer temperatures between 55 to 64°F (13 to 18°C). Northern pike (*Esox Lucius*) prefer similar temperatures to rainbow trout (*Oncorhynchus mykiss*), while black bullhead (*Ameiurus melas*) tolerate a wider temperature range. Susceptibility in northern pike (*Esox Lucius*) and black bullhead (*Ameiurus melas*) has only been demonstrated experimentally.

Figure 2. Geographic range data for rainbow trout (*Oncorhynchus mykiss*) in the USA mainland (native range includes Alaska). *HUC* stands for hydrologic unit code. Map generated by U.S. Geological Survey (USGS).



Seasonal variability across much of the USA affords the opportunity to observe disease events in a wide range of conditions. Rainbow trout (*Oncorhynchus mykiss*) populations infected with EHNV may exhibit only low-grade mortality, a situation that is most easily recognized in farmed populations under frequent observation. Many farms also undergo routine disease surveillance which improves the likelihood of detection at lower pathogen prevalence levels; however, observational capacity to detect low-grade events associated with EHNV will be less reliable in free-ranging conditions. Federal agency data supplement this historical absence argument in wild populations (Section 5).

4.3. USA populations are not vaccinated for EHNV

Globally, there are no vaccines developed for EHNV protection. Additionally, there are no licensed or approved vaccines being used in the USA for EHNV. The current list of USDA approved aquatic animal vaccines is found here.

5. Structured Surveillance

5.1 USFWS wild fish health survey

USFWS conducts routine surveillance on wild fish populations and maintains a database that catalogues testing data by species, year, and location. Since 1997, and through 2023, USFWS's regional health programs (currently 7 mainland centers) have tested over 10,259 wild-caught fish of EHNV-susceptible species (2,213 from 2021 through 2023). Screening protocols

consist of pooling tissue from one to five fish and testing by virus isolation in cell lines sensitive to EHNV replication, with follow-up molecular testing as needed for virus detection and identification. Regional USFWS programs also periodically conduct additional testing that is not captured in the wild fish database. The described totals above are conservative estimates of national testing efforts. EHNV has never been detected.

5.2 Other Federal testing efforts

USDA and other Federal agencies periodically conduct structured surveillance for research or regulatory purposes. Notable examples target different pathogens but use screening tests sensitive to EHNV detection. A long-running example is the Northwest Indian Fisheries Commission (NWIFC), which in conjunction with the State of Washington and USFWS, conducts regional surveillance in hatchery and returning salmonid stocks annually; EHNV has never been detected. A broad yet historic example is a USDA APHIS-led (2007 through 2012) surveillance effort focused on VHSV (Gustafson et al., 2014). Twenty-nine states participated in active sampling, which included rainbow trout and northern pike. The Canadian Food Inspection Agency led a parallel effort in the Great Lakes region of Canada over a similar time frame. EHNV was never detected.

5.3 State testing efforts

Many States require pre-movement testing for aquatic animals moving inter- or intra-state. This testing often follows AFS Blue Book standards with virus isolation using cell lines conducive to EHNV infection, and typically includes evaluation of 12 to 30 tissue pools of 5 fish. EHNV has never been detected. The majority of interstate movement testing is conducted at laboratories that have American Association of Veterinary Laboratory Diagnosticians (AAVLD) certification, International Organization for Standardization (ISO) certification, and/or APHIS approval for aquatic health testing. State natural resource agencies also conduct routine health inspections in their public hatcheries and most follow AFS Blue Book standards.

5.4 International movement testing

When required by importing countries, testing for EHNV is conducted by APHIS-approved laboratories obligated to report any detections to the Federal and State Animal Health Official in their region. As of 2023, USDA APHIS has negotiated health certificates for the export of live salmonid fish, eggs, and gametes with around 58 countries; at least 56 of these countries include pre-export testing requirements for EHNV.

6. Measures implemented to maintain freedom

The described authorities, passive surveillance, and basic biosecurity conditions have been in place for over a decade and will remain in place for the foreseeable future.

APHIS also recently completed a “Hazard Identification for EHNV” and a “Pathways Assessment for Live Salmonid Fish, Eggs, and Gametes Susceptible to Six World Organisation for Animal Health Listed Pathogens,” which once released, will provide further guidance on risks and relevant mitigations.

The United States currently has in the rule-making process a proposed Federal rule for the National List of Reportable Animal Diseases (NLRAD), which provides legal obligation to report detections of listed diseases. Also, in the early stages of rulemaking is the Comprehensive Aquaculture Health Program Standards (CAHPS), a system to support and verify aquatic livestock health management for participating producers.

Under the NLRAD, reporting detections is divided into pathogens that must be immediately reported and those that must be reported monthly. EHNV is categorized as an FAD and immediately notifiable, meaning that veterinarians and laboratories must immediately report any suspicion or confirmation of EHNV. In turn, APHIS would then submit an immediate notification of confirmed EHNV detections to WOAH.

7. References

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