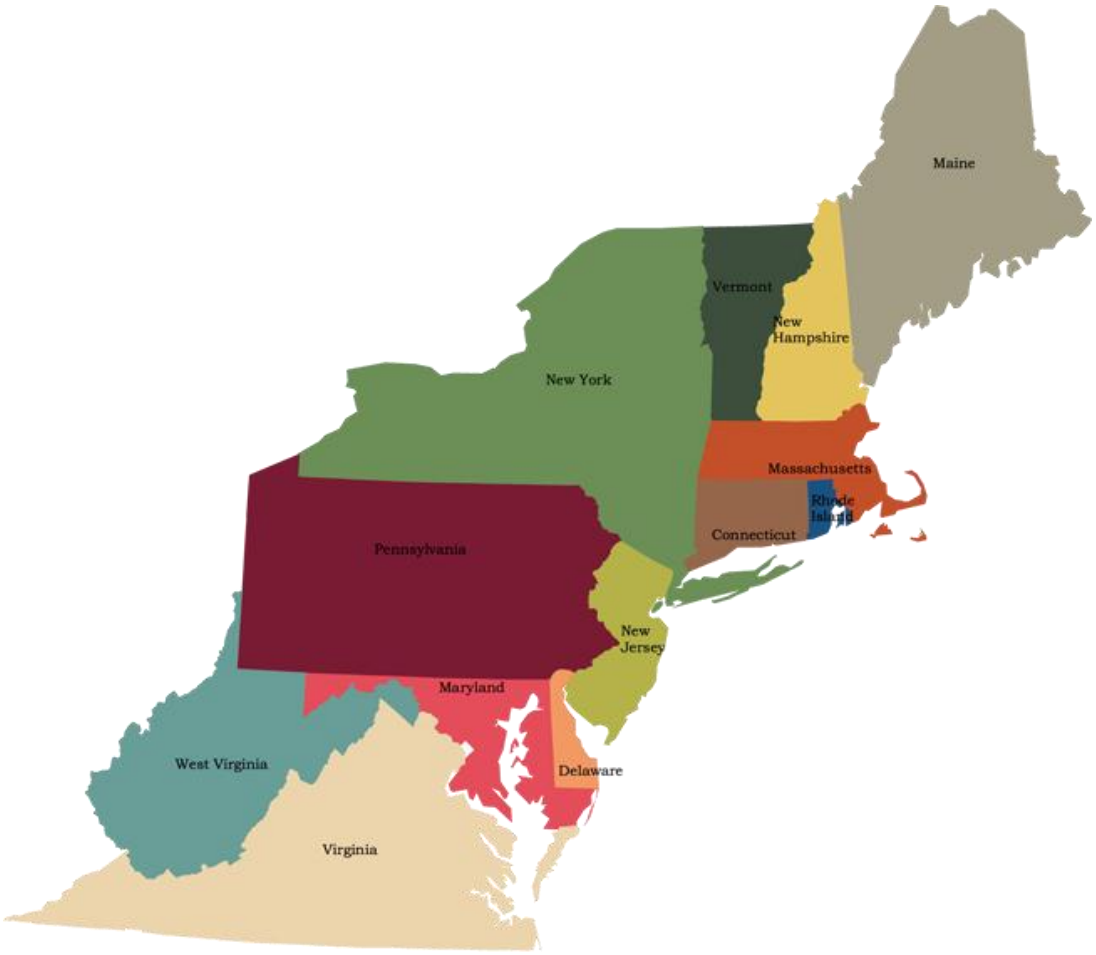
Guidelines for Fish Health Management in Northeastern States



Northeast Fish Health Committee

A subcommittee of the Northeast Fisheries Administrators Association

Approved October 27, 2015

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Resolutions of the Northeast Fisheries Administrators Association Concerning Fish Health Management

WHEREAS, the transfer of fish can transfer pathogens and diseases; and

WHEREAS, fish diseases have caused serious losses in wild and cultured populations; and

WHEREAS, disease problems have resulted in reduced survival of wild and stocked fish, and has caused significant losses of fish and diminished economic returns; and

WHEREAS, efficient propagation of fishes may be severely affected by the occurrence of fish diseases and major disease outbreaks, and have caused serious fish losses at aquaculture facilities; and

WHEREAS, introduction of fish pathogens hitherto not found in the northeast states can be prevented or curtailed through adequate inspection and risk assessment protocols; and

WHEREAS, it is technically possible to restrict or eliminate pathogens already introduced; and
WHEREAS, existing diagnostic techniques are adequate to identify fish pathogens; and

WHEREAS, the Northeast states include the following jurisdictions (with the exception being those waters under the jurisdiction of the Model Program for Fish Health Management in the Great Lakes Basin) Connecticut, Delaware, District of Columbia, Maine, Maryland, Massachusetts New Hampshire, New Jersey , New York, Pennsylvania, Rhode Island, Vermont, Virginia and West Virginia, as well as federal agencies with natural resource mandates for the region including the National Marine Fisheries Service and United States Fish and Wildlife Service.

NOW, THEREFORE, BE IT RESOLVED that the Northeast Fisheries Administrators Association encourages state and federal fish and wildlife agencies to:

Maintain a Northeast Fish Health Committee of fishery professionals to annually review the fish health status of the Northeast states and develop regional guidelines that enable agencies to:

- Prevent the importation or transfer among member states of fish infected with the pathogens listed herein.
- Require appropriate health inspections of all imported fish.
- Develop rules, regulations, and/or protocols to manage fish importation in ways that minimize the transfer of pathogens based on these guidelines that include health inspections of all imported fish.

Northeast Fish Health Committee Overview and Guidelines

Section A: Introduction

The Northeast Fish Health Committee (NEFHC) is charged with coordinating fish health management among the Northeast Association of Fish and Wildlife Agencies (NEAFWA) member states and agencies. The NEFHC serves under the auspices of the NEAFWA Northeast Fisheries Administrators Association. Its primary responsibilities include oversight of fish health issues affecting the region and providing relevant management recommendations. A main focus has been development of the Northeast Fish Health Guidelines.

The NEFHC is comprised of fishery professionals, including experts in fish health, from member state and federal government agencies. Leadership is provided by a chair or co-chairs who serve on an ad hoc basis. The NEFHC conducts business via dedicated working groups, conference calls and meetings. An annual meeting is held in conjunction with the Northeast Fish Culture Chief Working Group.

Section B: Northeast Fish Health Committee Objectives

The NEAFWA Northeast Fisheries Administrators Association has charged the NEFHC with three primary objectives.

- Assess fish health issues related to the importation and transfer of fish into and within member states.
- Encourage communication and education of fishery professionals and administrators of member agencies on the importance of fish health.
- Develop workable approaches and recommendations for fish health management that can be fully integrated with fisheries management strategies within member agencies.

Objective 1: Importation and Transfer of Fish

These guidelines were developed to serve as a model for health management of fish that are imported, cultured or otherwise subject to fisheries management actions within member states. These guidelines set forth the essential elements for the prevention and control of certain fish pathogens. The guidelines will be revised as necessary in order to remain current. Each member agency is encouraged to develop and promulgate appropriate policies, rules and regulations, as well as fish health management plans that are consistent with these guidelines. Nothing in these

guidelines shall prevent member agencies from applying additional measures for the control and management of fish pathogens.

These guidelines apply to fish imported or transferred into any member state that may be placed into the waters of a member state or held in waters discharged into its waters, including the following:

- The interstate and interbasin transfer of wild-acquired fish and their transport water.
- The interstate transfer of cultured fish and their transport water.
- Intra-state transfer of fish and their transport water.

***The provisions of these guidelines do not apply to the following*:**

- Fish or water in transit through member states that are not released from their original shipping containers.
- Fish destined for a state-approved quarantine facility or to a diagnostic laboratory.
- Fish transported for the purpose of restaurant or grocery store sales provided that the fish and/or untreated transport water will not be released or discarded into member state waters.
- Fish used in the pet trade or destined for a public aquarium facility, provided that the fish and/or untreated water will not be released to the member state waters.

<u>Wild-Acquired Fish Transfer</u>

The transfer of fish acquired from the wild environment may pose a significant risk to fisheries resources. Therefore, the NEFHC encourages all member agencies not to transfer wild-acquired fish. When transfer of wild-acquired fish is necessary it should only be conducted under the protocols as set forth in these guidelines.

***Therefore, the NEFHC recommends the following*:**

- No interstate or inter-basin transfer of wild-acquired fish.
- A risk assessment model should be used as a guide (Appendix V) if a wild-acquired fish transfer is necessary that is not consistent with these guidelines.
- A fish health component is to be included in member agencies fisheries management plans and strategies whenever wild-acquired fish are involved.

<u>Cultured Fish Transfer</u>

Cultured fish are an important component of the fisheries management plans for all member agencies. Although fish pathogens can be more effectively monitored and controlled in a fish culture station than in the wild, there still is a potential risk that their transfer could introduce pathogens to receiving waters. Therefore, the NEFHC suggests that cultured fish transfers should be considered a potential source of fish pathogens and be managed appropriately to reduce this risk.

***Therefore, the NEFHC recommends the following*:**

- No member agency will knowingly extend the range of a fish pathogen beyond its current range. The NEFHC recommends that member agencies accomplish this by:
    - Adopting and maintaining a fish health testing program for cultured fish that are consistent with these guidelines (Appendix I).
    - Adopting and maintaining fish health management plans and a fish pathogen classification system that is consistent with these guidelines.
- A risk assessment model (Appendix V) should be used as a tool to help determine the risk if a cultured fish transfer is necessary that is not consistent with the above recommendations.
- A fish health component should be included as part of each member agency's fisheries management plans that involve cultured fish.

Objective 2: Communication and Education as a Component of Fish Health Management

The NEFHC believes that communication and education are essential components for promoting and implementing an effective fish health management strategy. However, the diverse aquatic resources and fisheries management practices of member agencies pose a major challenge for the NEFHC to effectively advocate for a universal fish health management strategy among all members. The NEFHC's has approached this issue by promoting fish health education among the fishery professionals and administrators of member agencies through written and oral communication, meetings and technical presentations. It also has developed an on-line educational module on fish health management. The NEFHC plans to continue these efforts both within the NEFHC and with member agency fishery professionals.

The NEFHC recommends the following:

- Reporting any unknown agent causing clinical disease signs or cytopathic effects to the NEFHC.
- Sponsoring fish health-related symposia on a regular basis at annual NEAFWA meetings.
- Continue holding a joint annual meeting of the NEAFWA fish health and fish culture committees.
- Continue outreach efforts by developing educational workshops for fish health professionals, fishery managers and fisheries administrators designed to facilitate integration of fish health and fisheries management programs and policies.

Objective 3: Recommend Relevant Approaches to Fish Health Management

NEFHC members have a wide range of expertise and knowledge in fish health, fish culture and fishery management. The NEFHC can provide expert advice to member agencies on matters pertaining to fish health as it relates to all fisheries management issues and policies as requested.

The NEFHC recommends the following:

- The NEFHC will provide advice and recommendations to the Northeast Fisheries Administrators Association on issues related to fish health management.
- The NEFHC will serve as the advisory board for a fish health risk assessment when requested by a member agency.

Section C: Glossary of Terms and Definitions

APPL: Assumed Pathogen Prevalence Level (APPL) is the level, as a percentage, of the population in which the pathogen is present. (Ex. In a population of 500 fish, a pathogen prevalence of 5% would mean 25 fish are infected, however to test this population for the 5% APPL, 55 fish would be required to provide a 95 % confidence that at least one infected fish would be included in that sample.

Baitfish: A fish that is a source of food for another fish. Fish in the families Cyprinidae, Clupeidae, Osmeridae, Fundulidae, Percidae, Centrarchidae and Catostomidae are commonly used as bait by anglers for catching other fish.

Basin: An area as defined by the United States Geological Service (USGS) as a hydrologic unit code category two (HUC 2). Distinct basins under the jurisdiction of member states are: New England (St John, Penobscot, Kennebec, Merrimack, Connecticut rivers). Mid-Atlantic (Hudson, Delaware, Susquehanna, Potomac, James rivers), Great Lakes (Lake Ontario, Lake Erie, and St. Lawrence River), Ohio River (Allegheny, Monongahela, Kanawha and Ohio rivers), South Atlantic (Pee Dee and Roanoke rivers), and Tennessee (Clinch and Holston rivers). See Appendix VII for details.

Coldwater Fish: Fish species whose optimal temperature range for growth and survival in a culture environment is 10^{o}C to 15^{o}C and will not thrive or survive long-term in temperatures that exceed 20^{o}C. Coldwater fish are typically reared at temperatures less than 12^{o}C. Coldwater fish species primarily include the family Salmonidae, but may also include members of the families Osmeridae, Gadidae and Cottidae.

Coolwater / Warmwater Fish: Fish species that are typically reared at temperatures between 12^{o}C and 20^{o}C. Coolwater/warmwater fish species primarily include the family Esocidae, Percidae, Centrarchidae, and Ictaluridae.

Cultured Fish: Fish that spend their entire life cycle in a fish culture facility until release into the wild environment.

Clinical Sign: Visually apparent abnormalities in fish behavior and/or morphology.

Cytopathic Effect: Changes in the morphology and/or metabolism of tissue culture cells.

Disease: A condition that impairs normal functioning of the fish and may be manifested by distinct clinical signs.

Extensive Facility: An open fish culture facility with limited ability for observation and husbandry of fish species (e.g., earthen pond system). Typically animal densities are low to moderate, and rearing units are not in close proximity to one another.

Fish: All life stages of fish and all sexual products of fish including sperm and eggs

Fomite: An inanimate object such as a net, brush, or clothing on which a pathogenic microorganism may be transmitted from one animal to another.

Importation: The relocation of fish from one jurisdiction into another jurisdiction for the purposes of trade or use. For these guidelines, jurisdictions are considered member states.

Intensive Facility- An open fish culture facility with adjacent rearing units that allow for direct observation and husbandry of fish species (e.g., standard hatchery with raceways and tanks). Typically animal densities are high and rearing units are in close proximity to each other.

Isolation Facility: A structure that maintains a group of fish without any contact with other fish or water sources in order to allow observation for a specified length of time and, if appropriate, testing and treatment. The effluent waters are not treated.

Listed Pathogen: Certain infectious pathogens of fish (Appendix I) caused by viral, bacterial, or parasitic agents which are transmissible, directly or indirectly, from one fish to another.

Lot: Fish of the same species and age that originated from the same spawning stock which have shared a common water supply throughout their life history.

Member States: Connecticut, Delaware, Maine, Maryland, Massachusetts New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia.

Member Agencies: State fish and wildlife agencies as established by NEAFWA to include Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey , New York, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia; and federal agencies with natural resource mandates including National Marine Fisheries Service and United States Fish and Wildlife Service.

Pathogen: Any micro or macro organism that is capable of causing a disease.

Quarantine Facility: A completely enclosed, locked structure with a given room or space allotted to only one captive population. Water is either supplied by a well or is disinfected (ozone/ultraviolet light). It must be operated by a written standard operating plan, with the highest level of sanitation, including but not limited to: restricted personnel access; dedicated equipment, such as brooms, nets, etc.; dedicated external outerwear, such as rain coats and boots;

disinfection foot baths; landfill disposal of carcasses. Effluent is disinfected by ultraviolet light sterilization or strong oxidation, (i.e. chlorination system).

B Isolation- Same recommendations as for quarantine except the isolation area need not be totally enclosed. It should be physically separated from the rest of the production area and the effluent should never flow into production areas.

C Isolation- Same recommendations as B isolation except multiple lots of fish may be housed.

Sub-Basin: An area as defined by the USGS as a hydrologic unit code category four (HUC 4). See Appendix VII for details.

Transfer: A human-induced relocation of fish.

Vertical Transmission: Transference of pathogens from parents to progeny through their gametes.

Wild-acquired Fish: Fish that have spent any portion of their life cycle in the wild environment.

Section D: Acknowledgements

The NEFHC team that developed and wrote these guidelines consisted of Patricia Barbash (U.S. Fish and Wildlife Service), Tom Jones (Vermont Fish and Wildlife Department), Megan Kirchgessner (Virginia Department of Game and Inland Fisheries), Jan Lovy (New Jersey Division of Fish and Wildlife), Joe Marcino (Maryland Department of Natural Resources), Adam Miller (Vermont Fish and Wildlife Department), Chris O'Bara (West Virginia Division of Natural Resources), Ken Simmons (Massachusetts Division of Fisheries and Wildlife), Jason Smith (New Hampshire Department of Fish and Game), and Colby Wells (Maine Department of Inland Fisheries and Wildlife. Members that provided technical input and editorial comments included Brian Richardson (Maryland Department of Natural Resources), Katherine Zipfel (West Virginia Division of Natural Resources), Coja Yamashita (Pennsylvania Fish and Boat Commission), Sarah Friend (New Jersey Division of Fish and Wildlife), Todd Langevin (Maine Department of Inland Fisheries and Wildlife), Ron Southwick (Virginia Department of Game and Inland Fisheries), David Bean (National Marine Fisheries Service), and Rick Van Nostrand (Connecticut Department of Energy and Environmental Protection).

Appendix I

Pathogens, Testing Protocol, and Pathogen Based Classification System

Section A: Listed Fish Pathogens and Classification System

The NEFHC has determined that these pathogens (Pathogen List) are of particular concern for member states when importing or transferring wild or cultured fish. Listed fish pathogens are categorized out into four main groups: Emergency, Limited A, Limited B, and Restricted pathogens. The list includes pathogens of both salmonid and non-salmonid fishes (Tables I-1 through I-4). The Pathogen List has been classified by the risk and severity of the consequences of importing or transferring infected fish. In order to reduce the risk associated with importing or transferring these pathogens, all fish importations and transfers should be accompanied by an up-to-date fish health inspection report issued by a fish health inspector that demonstrates appropriate tests were conducted with valid results (Section B).

Amendments to the Pathogen List may be proposed by member agencies at any time by notification to the committee chair. Amendments may only be adopted following review by the NEFHC, and consensus is reached among member agencies.

EMERGENCY: Pathogens that have not been detected in wild or cultured fishes in member states.

- No fish testing positive for any pathogens contained within the Emergency pathogen list should be imported into any member state**.** Importation and transfer can lead to epizootics resulting in spread of the pathogen beyond its enzootic range, high mortality of stock, and large-scale die-off of wild stocks.
- No fish originating from regions enzootic for pathogen(s) on the Emergency pathogen list should be imported or transferred into any member state without first conducting a risk assessment (Appendix V), an established isolation and biosecurity program at the receiving site (Appendix IV) and an enhanced post-importation disease monitoring plan. Eggs from regions enzootic for pathogen(s) on the Emergency pathogen list are permitted provided these are imported from a fish culture facility with a minimum of ten consecutive years of negative Emergency pathogen detections and a biosecurity plan that ensures the integrity of the fish health certification.
- Detection within member states shall lead to immediate notification of the NEFHC Chair as well as implementation of disease contingency protocols by the member agency (See Appendix VI).
- Fertilized eggs which originate from a broodstock source testing positive for *Ceratomyxa shasta* and/or *Tetracapsuloides bryosalmonae* are permitted provided that they are disinfected in accordance with Appendix III.

Table I-1. List of Emergency pathogens and associated disease.

Emergency Pathogens		
Pathogen Code	**Pathogen Name**	**Disease**
IHNV	Infectious Hematopoietic Necrosis Virus[1]	Infectious Hematopoietic Necrosis
VHSV-NIVB	Viral Hemorrhagic Septicemia (non-IVb)[1]	Viral Hemorrhagic Septicemia
CS	*Ceratomyxa shasta*[2]	Ceratomyxosis
SV	Spring Viremia of Carp Virus[1]	Spring Viremia of Carp
PKD	*Tetracapsuloides bryosalmonae*[2]	Proliferative Kidney Disease
[1] Notification of OIE authorities is required with detection. **[2] Inspections within the member state need not include these pathogens unless there have been known importations of fish (excluding gametes) from endemic areas.**		

*To notify OIE authorities of specific pathogen detection a member agency is recommended to contact either their USDA APHIS veterinarian services district office or the USDA APHIS veterinarian services area veterinarian.

LIMITED A: Pathogens that have been detected in wild and cultured fishes in specific sub-basins of member states.

- Fish testing positive for any Limited A pathogen should not be imported or transferred into sub-basins in member states which are not known to be enzootic for that pathogen. Importation and transfer can have adverse effects on cultured and wild stocks, including epizootic events causing mortality.
- No fish originating from regions enzootic for pathogen(s) on the Limited A pathogen list should be imported or transferred into other sub-basins within a member state without a risk assessment (Appendix V), an established isolation and biosecurity program at the receiving site (Appendix IV), and enhanced post-importation disease monitoring.
- Detection of Limited A pathogens from a location that is outside an established enzootic range requires notification of the NEFHC Chair and member agencies, as well as implementation of disease contingency protocols by the member agency (Appendix VI). A future task of the NEFHC will be to develop an enzootic regional list of Limited A pathogens.
- Fertilized eggs which originate from a broodstock source testing positive for *Myxobolus cerebralis* are permitted provided that they are disinfected in accordance with Appendix III.

Table I-2. List of Limited A pathogens and associated disease.

Limited A Pathogens		
Pathogen Code	**Pathogen**	**Disease**
MC	*Myxobolus cerebralis*	Whirling Disease
ISAV	Infectious Salmon Anemia virus[3, 4]	Infectious Salmon Anemia
KHV	Koi Herpesvirus[3, 4]	Koi Herpesvirus
VHSV-IVB	Viral Hemorrhagic Septicemia virus (IVb only) [4]	Viral Hemorrhagic Septicemia

3 Inspections within member states need not include this pathogen unless there has been an epidemiological link to specific pathogen-positive or suspect fishes.

4 Notification of OIE authorities is required with detection

LIMITED B: Pathogens that have been detected in wild and cultured fishes in specific member states, but whose geographic range may be limited or undetermined.

- Appropriate action should be taken by member agencies to restrict and further reduce pathogen transmission (Appendix II, Appendix V).
- A risk assessment is recommended to determine appropriate fish transfers when a Limited B pathogen is detected.
- Fertilized eggs which originate from a broodstock source testing positive for *Aeromonas salmonicida* and/or *Yersinia ruckeri* are permitted provided that they are disinfected in accordance with Appendix III.

Table I-3. List of Limited B pathogens and associated disease.

Limited B Pathogens		
Pathogen Code	**Pathogen**	**Disease**
IPNV	Infectious Pancreatic Necrosis virus	Infectious Pancreatic Necrosis
LMBV	Largemouth Bass virus	Largemouth bass virus
RS	*Renibacterium salmoninarum*	Bacterial Kidney Disease
AS	*Aeromonas salmonicida*	Furunculosis
YR	*Yersinia ruckeri*	Enteric Redmouth Disease

RESTRICTED: Pathogens which have caused epizootics under very specific circumstances, within limited species and situations, but for which a member agency may wish to expand inspection sampling to include so as to reduce the risk of adverse effects resulting from importation or transfers.

- A status review of each restricted pathogen will occur as additional information is gained on life history, etiology and detection methods.
- Appropriate action should be taken by member agencies to restrict and further reduce pathogen transmission (Appendix III and V).

Table I-4. List of Restricted pathogens and associated disease.

Pathogen Code	Pathogen	Disease (Acronym)
EEDV	Lake trout herpesvirus	Epizootic epitheliotrophic disease
NS	*Nucleospora salmonis*	Nucleospora
WSHV	White Sturgeon Herpesvirus	White Sturgeon Herpesvirus
WSIV	White Sturgeon Iridovirus	White Sturgeon Iridovirus
CCV	Channel Catfish Virus	Channel Catfish Virus
ESC	*Edwardsiella ictaluri*	Enteric Septicemia of Catfish
BA	*Bothrioscephalus acheilognathi*	Asian Tapeworm
ELSV	Lymphosarcoma Virus	Esocid Lymphosarcoma Virus
PLO	*Piscerickettsia*-like organism	Muskie Pox
HSP	*Heterosporis*	Heterosporisis
Any other OIE listed fish pathogens are included in this category. Notification of OIE authorities is required with detection.		

Section B: Sampling and Testing Protocols

The NEFHC recommends that fish health inspections (screening and confirmatory testing and sampling methods) be performed according to the methods detailed in the most recent editions of the 'Fish Health Section Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens' (USFWS & AFS-FHS) and the 'Manual of Diagnostic Tests for Aquatic Animals' (OIE – World Organization of Animal Health). The NEFHC recommended standards for guiding fish transfer decisions, including inspector qualifications, fish culture facility or wild population inspection frequency and inspection history are also included in this Section.

Fish health inspectors shall submit copies of all fish health inspection reports to the appropriate member agency under whose jurisdiction the inspected fish culture facility lies, as well as to the appropriate state and/or federal agencies that require the information for a proposed importation.

Inspector Qualifications:

No owner or employee with direct supervisory authority over a facility may serve as an inspector for their fish culture facility.

Individuals that collect samples for a fish health inspection must be one of the following:

- An accredited and licensed veterinarian: a veterinarian holding a current veterinary license who has also fulfilled the accreditation requirements of the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA/APHIS).

- A certified aquatic animal health inspector or fish pathologist: an individual certified by the American Fisheries Society/Fish Health Section (AFS/FHS) as an Aquatic Animal Health Inspector or Fish Pathologist.
- A person recognized by a member agency with responsibility and training for fish health inspections in the state from which the fish originate.

Acceptable sampling methods for detection of pathogens listed in Section A

Sampling should be carried out in such a way as to provide the best likelihood that sample results will be representative of the population (Table I-5). The sample should include any moribund fish or fish showing signs of disease, if available. See Tables I-6 through I-8 for additional guidelines for sampling of each pathogen.

Every susceptible lot of fish held at a fish culture facility must be inspected annually for all of bacterial, viral, and parasitic pathogens listed in section A (the exception is *Myxobolus cerebralis:* only one lot of the most susceptible species on each water source needs to be tested).

Table I-5. Guidance on sampling based on type of inspection.

Fish Type of Inspection	Lethal	Ovarian Fluid
Annual Facility / Water Body Inspection	60 fish/lot max or 5% APPL	N/A
Domestic Broodstock Inspection[1]	60 fish/lot max or 5% APPL	5% APPL
Recommended Wild Broodstock Inspection[2]	5% APPL for Males, All Mortalities	All Females
Alternate Wild Broodstock Inspection[2, 3]	All Mortalities	All Females
Baitfish Inspection[4]	150 fish/facility, No less than 60 fish/species (semi-annually)	N/A

[1]If a domestic broodstock lot has undergone 3 consecutive annual inspection with no listed pathogen detection, the number of fish needed for testing could be reduced to the 10% APPL.

[2] It is recommended that a wild fish health assessment be conducted annually for all broodstock source waters. If a broodstock source water tests positive for a listed pathogen, then a risk assessment should be conducted.

[3]If it is not feasible to 100% lethally sample wild broodstock, then 100% ovarian fluid sampling is recommended with as much lethal sampling as possible.

[4] Baitfish facilities should be inspected twice annually to encompass pathogens which are detected on a seasonal basis. All fish species on station should be equally represented in the sample. Intervals between inspections should be at a minimum five (5) months apart.

*For recommended sample numbers based on an assumed pathogen prevalence level in the population of 10%, 5 %, or 2% (based on a 95% confidence level) for different lot sizes please refer to "Chapter 2.2 Sampling" on the AFS Fish Health Section Blue Book website:http://www.afsfhs.org/bluebook/inspection-index.php.

** Monitoring samples from broodstock populations and production lots using moribund and/or dying fish throughout the year may count toward the annual /broodstock inspection for the pathogens the moribund and/or dying fish were tested for.

Acceptable detection methods of pathogens listed in Section A:

- Protocols for presumptive and confirmatory diagnosis listed in the *Fish Health Section Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens*.
- Protocols listed in the OIE Manual of Diagnostic Tests for Aquatic Animals for each of the OIE-listed diseases under "Section 4.3. Agent detection and identification methods".

The most recent editions of the 'Fish Health Section Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens' (USFWS & AFS-FHS) and the 'Manual of Diagnostic Tests for Aquatic Animals' Section 2.3: Diseases of fish (OIE – World Organization of Animal Health) must be followed. They can be accessed on the following websites:

http://www.afs-fhs.org/bluebook/bluebook-index.php
http://www.oie.int/international-standard-setting/aquatic-manual/access-online/

Other methods described in peer reviewed journals are not recommended unless specified herein.

The following table provides additional guidelines for sampling to optimize detection of pathogens listed in Section A. References are provided for information on sampling for pathogens which are not presently described in the AFS/FHS Bluebook or OIE Aquatic Manual.

Table I-6. Additional guidelines for sampling of bacterial pathogens listed in Section A.

Bacterial Pathogens				
Organism	**Common Name of Disease**	**Pathogen Classification**	**Species to be Screened**	**Additional Considerations**
Aeromonas salmonicida	Furunculosis	Limited-B	Any freshwater fish	Egg disinfection blocks vertical transmission
Piscirickettsia-like organism	Musky Pox	Restricted	Esocidae	See Thomas & Faisal, 2009 for methods specific to Musky Pox.
Renibacterium salmoninarum	Bacterial Kidney Disease (BKD)	Limited-B	Salmonidae	Vertically transmitted
Yersinia ruckeri	Enteric Redmouth Disease (ERM)	Limited-B	Any freshwater fish	Egg disinfection blocks vertical transmission

Table I-7. Additional guidelines for sampling of parasitic pathogens listed in Section A.

Parasitic Pathogens				
Organism	**Common Name of Disease**	**Pathogen Classification**	**Species to be Screened**	**Additional Considerations**
Bothriocephalus acheilognathi	Asian Tapeworm	Restricted	Cyprinidae	Not vertically transmitted
Ceratanova (Ceratomyxa) shasta	Ceratomyxosis	Emergency	Salmonidae	Not vertically transmitted
Heterosporis sp.	Heterosporis	Restricted	Percidae, Esocidae, Centrarchidae	Sample fish with epizootiological link.
Myxobolus cerebralis	Whirling Disease	Limited-A	Salmonidae	Rainbow trout are most sensitive, lake trout least; Not vertically transmitted

Table I-7. continued

Nucleospora salmonis	Nucleospora	Restricted	Salmonidae	Potential vertical transmission
Tetracapsula bryosalmonae	Proliferative Kidney Disease (PKD)	Emergency	Salmonidae	Not vertically transmitted
Edwardsiella ictaluri	Enteric Septicemia of Catfish	Restricted	Ictaluridae	Optimal screening temperature: 20 to 30 ^{o}C

Table I-8. Additional guidelines for sampling of viral pathogens listed in Section A.

Viral Pathogens				
Organism	**Common Name of Disease**	**Pathogen Classification**	**Species to be Screened**	**Additional Considerations**
Epizootic Epithieliotrophic Disease	EEDV	Restricted	Lake trout	See Korobe et al. 2009. Sample fish with epizootiological link to pathogen.
Infectious Hematopoietic Necrosis	IHN	Emergency	Any freshwater fish	Vertically Transmitted. Optimal screening temperature: <15^{o}C
Infectious Pancreatic Necrosis	IPN	Limited-B	Any freshwater fish	Vertically transmitted
Infectious Salmon Anemia	ISA	Limited-A	Atlantic salmon	Vertically transmitted. Sample any fish species with an epizootiological link to pathogen.
Koi Herpesvirus	KHV	Limited-A	Carp (all strains)	Vertically transmitted; optimal screening temperature: 16-28^{o}C
Largemouth Bass Virus	LMBV	Limited-B	Centrarchidae	
Lymphosarcoma	Esocid Lymphosarcoma	Restricted	Esocidae	Confirm histologically if lesions are present. See Coffee et al. 2013.

Table I-8. continued

Spring Viremia of Carp Virus	SVCV	Emergency	Cyprinidae	Vertically transmitted; Optimal screening temperature: 10-18^{o}C; Cannot be detected at temps exceeding 25°C.
Viral Hemorrhagic Septicemia (IVb strain)	VHSv-IVb	Limited-A	Any freshwater fish	Potential vertical transmission
Viral Hemorrhagic Septicemia (non-IVb strains)	VHSv	Emergency	Any freshwater fish	Potential vertical transmission
White Sturgeon Herpesvirus	WSHv	Restricted	Acipenseridae	
White Sturgeon Iridovirus	WHIv	Restricted	Acipenseridae	
Channel Catfish Virus	CCV	Restricted	Ictaluridae	Young life stages (<1 yr) most susceptible; Optimal screening temperature: > 25 ^{o}C

Section C: Pathogen Based Classification System

Fish Culture Station Pathogen-Based Classification

The NEFHC has established a pathogen classification system based on fish health inspection results and regular monitoring of fish on a culture station or an isolation facility within a fish culture station. NEFHC's goal is that member agencies operate all fish culture stations and facilities under their jurisdiction in a manner that will maintain or improve the station or facility classification. The NEFHC recommends that the following guidelines be followed when designating the classification of a fish culture station or facility.

Class A Fish Culture Station or Facility: Fish culture stations or facilities are assigned an **A** classification if the following criteria are met: 1) all lots on the station or facility have been inspected annually and found negative for the Emergency, Limited A, and Limited B pathogens, 2) three consecutive negative annual inspections, and 3) documentation that all fish brought onto the facility originated from Class A or AW sources.

To maintain a Class A Certification for a fish culture station:

- All fish lots must be tested in accordance with Appendix I and found free of Emergency, Limited A, and Limited B fish pathogens.
- All wild-acquired fish brought onto a Class A fish culture station or facility must be kept in isolation in accordance with Appendix II.
- All wild-acquired fish must be tested in accordance with the Recommended Wild Broodstock sampling procedures and found free of Emergency, Limited A, and Limited B fish pathogens (Appendix I).
- All fertilized eggs must be disinfected. Recommended methods for egg disinfection are outlined in Appendix III.
- Fish from a Class A facility that has an up-to-date fish health report may be transferred to another facility or water body without affecting the classification of the receiving facility or water body.

To maintain a separate Class A Certification for a facility within a fish culture station:

- An incubation/rearing facility within a fish culture station can maintain a separate Class A certification provided :
    - The water source is free of Emergency, Limited A, and Limited B fish pathogens.
    - It is completely enclosed and physically separated from the outside environment (i.e. predators, visitors, etc.).
    - The biosecurity measures outlined in Appendix IV are followed.
    - Fish are sampled according to Appendix I prior to release or transfer to another fish culture station.

Class B Fish Culture Station or Facility: Fish culture stations or facilities are assigned a **B** classification when one or more of the listed fish pathogens in Appendix I have been detected. The pathogen codes listed in Appendix I should be added to the **B** classification as an identifier.

Examples: B – RS (positive for bacterial kidney disease)
B – RS, AS (positive for bacterial kidney disease and furunculosis)

The pathogen code will remain part of the fish culture station or facility's classification until the facility undergoes three consecutive annual inspections without the pathogen being detected.

Class C Fish Culture Station or Facility: Fish culture stations or facilities are assigned a **C** classification if the following conditions are present:

- Has an unknown pathogen history,
- Has not been inspected for all Emergency, Limited A, and Limited B pathogens,
- Has not undergone an annual fish health assessment for three consecutive years, or
- Has received fish from an uninspected source.

Wild Population Pathogen-Based Classification

The NEFHC recommends a pathogen wild fish classification system based on the results of fish health assessments of a wild population similar to the fish culture station pathogen classification system. The NEFHC intent is that classifications of wild fish population be used in association with fish culture activities, especially related with the use of wild broodstock and fisheries management programs. The following guidelines should be used when designating a wild fish population classification.

Class AW Population: Wild fish populations will be assigned an **AW** classification if an annual fish health assessment has been conducted following procedures in accordance with Appendix I and found negative for the Emergency, Limited A and Limited B pathogens. Three consecutive negative annual inspections are required for a Class **AW** classification.

Class BW Population: Wild fish populations are assigned a **BW** classification when one or more of the listed fish pathogens in Appendix I were detected. The pathogen codes listed in Appendix I should be added to the BW classification as an identifier.

Examples: BW – RS (positive for bacterial kidney disease)
BW – RS, AS (positive for bacterial kidney disease and furunculosis)

The pathogen code will remain part of the population until the population undergoes three consecutive annual inspections without the pathogen being detected.

Class CW Population: A **CW** classification shall be assigned to a wild population that has an unknown pathogen history, has not been inspected for all Emergency, Limited A, and Limited B pathogens, or has not undergone an annual fish health assessment for three consecutive years.

There is no classification for the restricted pathogens listed in Appendix I. However, the NEFHC recommends including any of these pathogens on a fish health inspection report if they have been detected at a fish culture station, facility, or in a wild population. If a restricted pathogen is detected from a fish culture station, facility, or wild population after a fish inspection report has been issued, the NEFHC recommends issuing an amended inspection report to reflect the finding.

Section D: References

AFS-FHS (American Fisheries Society-Fish Health Section). 2014. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2014 edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.
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Sutherland, D., and Stelzig, P. 2005. Host-specificity, modes of transmission and pathogenicity of the parasite *Heterosporis* sp. (Microsporida:Pleistophoridae) in yellow perch (*Perca flavescens*) from the Bay of Quinte in Lake Ontario and inland lakes of Minnesota and Wisconsin[online]. Available from http://www.glfc.org/research/reports/Sutherland.html [accessed 08 August 2014].

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OIE Aquatic Animals Commission. 2009. OIE manual of diagnostic tests for aquatic animals, 6th ed. World Organization for Animal Health, Paris, France.

Appendix II

Fish Health Management

Section A: Introduction

Sound fish health management practices are important for proper management and protection of the freshwater fisheries and fish culture facilities of member agencies. This Appendix is a framework for fish health management for member agencies to integrate into their fisheries management and fish culture programs, to reduce the risk of increasing the range of existing listed fish pathogens, and to help prevent introduction of new fish pathogens of concern to the region. These guidelines follow the basic principles of fish health management - prevention, containment, reducing and, where possible, eradication of the fish pathogens of concern listed in Appendix I.

Member agencies are encouraged to develop fish health management plans for fish culture facilities within their jurisdictions that are consistent with these guidelines. However, the NEFHC recognizes that given the broad range of fisheries resources and management programs of member agencies it is not possible, and some cases even feasible, to conduct a fish health assessment that is in full compliance with these guidelines. In those cases, the member agency is encouraged to conduct a risk assessment to assess the risk that is posed by this situation (Appendix V) and to utilize the information from the risk assessment to develop a best management plan to minimize any risks before proceeding with the program or management activity. Member agencies also are encouraged to develop biosecurity and disease management contingency plans for the fish culture facilities and fisheries management programs within their jurisdiction that are consistent with these guidelines (Appendices IV and VI).

Member agencies represent states in the Northeastern and Mid-Atlantic regions of the United States with diverse fisheries resources and widely varying fishery management and fish culture programs and fish culture facilities. For example, some states have warmwater and coldwater fish culture programs (e.g. New Jersey, Virginia and West Virginia), others have both coolwater and coldwater (Vermont) and other states only have coldwater culture programs (e.g. Massachusetts, New Hampshire and Maine). Some states are also responsible for management

of the fisheries resources on river systems that originate in or flow into non-NEFHC member states or countries. For example, the Ohio River of Pennsylvania and West Virginia, which has more than 190 fish species, also flows through or borders five other states before draining into Mississippi River. West Virginia also has other river systems that drain into the Chesapeake Bay. Both Vermont and Maine also have river systems that drain into multiple basins, including at least one from each state that drains into Canada.

The coldwater fish culture and stocking operations of member states are generally less complex than warmwater and coolwater culture and stocking operations. Coldwater fish culture facilities of member states generally have well defined fish production goals that have been developed for facilities that produce a limited number of salmonid species in intensive, relatively biosecure culture environments. These facilities also utilize biosecure commercially produced pelleted fish feed. In contrast, coolwater and warmwater culture programs often involve a larger number of species and multiple lots in an extensive pond culture system, often all within a single growing season. Warmwater and coolwater culture facilities also rely on live forage fish purchased commercially from a variety of farmed sources. Additionally, some member states may also share warmwater and coolwater fish culture facilities with culture programs for mussel restoration programs, further complicating biosecurity and fish health management at these facilities.

Fish health management goals set forth in this appendix:

- Encourage each member agency to review their current fish culture and fisheries management practices and to develop program-specific fish management plans that are integrated with sound fish health management and biosecurity practices.
- Encourage each member agency to inspect and assign a classification to each fish culture facility and waterbody under their jurisdiction that is consistent with these guidelines.
- Reduce the risk of introducing new fish pathogens of concern to the waters and fish culture facilities of member states.
- Encourage each member agency to develop contingency plans consistent with Appendix VI for management of fish disease agents and affected fisheries.

Section B: Integrated Fish Health Management Plans

It is recommended that each member agency develop fisheries management and fish culture facility operational plans that are based on good fish health management principles and are

consistent with these guidelines. If cultured fish are part of a fishery management plan, then there should be full integration of the fish culture production cycle and stocking into the plan. The plan should include clearly defined production goals that include the type of stock (eggs, fry, fingerling, broodstock, etc.) and sources required to meet those goals. It should also include an assessment of the fish health status of the stock and whether it includes adult broodstock, fingerlings, yearlings, fry or eggs. If the fish health history of the stock source is incomplete or inconsistent with these guideline recommendations, a risk assessment (Appendix V) should be included as an integral part of the plan before deciding if the stock is to be transferred to the receiving facility. If the fish require live forage, then the plan should also include a biosecurity assessment (Appendix IV) of the source and health status of the forage fish. The management plan should also include a detailed plan for fish transportation and stocking of the fish. The transportation and stocking plan should include a full biosecurity assessment to prevent introduction of fish pathogens and aquatic nuisance species during the transport and stocking.

Section C: Wild-Acquired Fish Collection and Use of Wild-Acquired Fish

The NEFHC recommends that the use of wild-acquired fish be conducted in a sound manner. In many cases, the use of wild-acquired fish is not appropriate because adequate safeguards cannot be met or the risk of transferring pathogens or aquatic nuisance species is high. In other cases the use of wild-acquired fish is important (i.e. use of wild broodstock) for effective fishery management programs.

If the use of wild-acquired fish is warranted, the NEFHC recommends at minimum the following criteria be met:

- A fish health assessment should be conducted on the target fish species from the source waters following the procedures in Appendix I. The NEFHC realizes that in some cases it is not possible to conduct a fish health assessment of the target species for all source waters. At this time, the NEFHC recommends that surrogate species should only be used in limited cases where target species cannot be sacrificed for biologically-sound reasons. The NEFHC warns that the reliability of testing surrogate species is still unknown, thus results should be interpreted as a beneficial screening assessment, but not a fish health certification for a given water body or species. Table II-1 includes a list of suggested surrogate species for this purpose. The NEFHC recommends that a cooperative multi-agency research project be conducted to determine the reliability of testing surrogate species as a means of circumventing this problem.

- Inter sub-basin wild-acquired fish should be placed in an isolation facility, tested in accordance with the sampling and testing procedures from Appendix I, and found free of Emergency, Limited A, and Limited B fish pathogens prior to transfer.
- Intra sub-basin wild-acquired fish transfers can be conducted pending a risk assessment.

Wild fish should not be used at any time if one or more of the following conditions are met:

- The water body is outside the basin of the proposed recipient water body or fish culture station or facility.
- There is an on-going fish die-off or other aquatic animal die-off or such die-offs have occurred within the current growing cycle in the source water.

Section D. Release of Fish

The release of cultured or wild-acquired fish is a common fishery management practice. Care must be given prior to their release.

The NEFHC recommends the following be met:

- No fish culture facility should release fish into shared waters of member states until a current fish health inspection report for that facility has been issued in compliance with these guidelines (Appendix I) or a risk assessment (Appendix V) has been conducted in accordance with these guidelines.
- No fish known to be infected with the Emergency, Limited A, and/or Limited B pathogens (Appendix I) may be released into the waters of member states unless a risk assessment is in accordance with Appendix V.
- No fish with clinical signs of any disease listed in Appendix I may be released into the waters of member states.
- Fish obtained from a wild population for which a fish health inspection study has not been completed for three consecutive years prior to the proposed transfer should not be released or transferred into the water of member states until a risk assessment is completed in accordance with Appendix V.

Section E. Recommendations for Forage Fish Used in Fisheries Management Activities

Member agencies that utilize forage fish in fish culture operations or forage fish augmentation activities should have the following requirements at a minimum for the use of forage fish:

- All forage fish should be raised at a fish culture facility; no wild forage fish should be utilized in member agency fish culture facilities.
- All fish culture facilities that raise forage fish should undergo certified semi-annual fish health inspections.

- No forage fish should be imported or utilized that are known to be infected with any of the Emergency, Limited A or Limited B pathogens listed in Appendix I.
- Fish culture facilities that supply forage fish should have a biosecurity plan to ensure that there are no aquatic nuisance species of concern for the receiving state present on the facility.
- Non-target fish species that are mixed with forage fish should be at a level that is acceptable for the receiving state and facility.
- All applicable laws regarding species, fish health certificates, and importation and transportation requirements should have been met.
- A bio-secure location should be established at or near the facility that the forage fish delivery truck must go to for a pre-delivery inspection prior to its acceptance by the receiving station.

It is recommended that the staff of the receiving facility inspect all forage fish deliveries prior to acceptance onto the facility to determine the following:

- Review of the vehicle load sheet to determine that all fish species and transport water on the vehicle are in compliance with the specifications set forth in the contract with the vendor for the fish and their condition upon delivery.
- Review of the fish health certificate that is included on the delivery truck to confirm that the fish health certification of the fish is current.
- Verification from the delivery truck driver on the source of the fish and the transport water.
- If these conditions are not met, then the shipment should be rejected.

Section F. Isolation of Fish Rearing Units

Isolation of Wild Fish

Wild fish introduced into a fish culture facility must be isolated from other facility operations. Isolation at a minimum should include physical separation from other production stocks as well as preventing the effluent water from mixing with the production water. When this isolation is maintained, the classification of the facility remains independent of the pathogen status of the associated wild fish and shipments of production stock may be made. Whenever the newly introduced wild fish cannot be isolated from the production fish, no shipments of any kind can be made until the appropriate fish health testing has been completed and an appropriate classification has been assigned to the entire facility.

Separate Designations of Fish Culture Station

A fish culture station may have a separate incubation/rearing facility (such as a hatch house) that is isolated from other fish rearing facilities on the station such that separate fish health classifications may be applicable to the individual facilities located on the station. In order for the individual facilities to receive a separate fish health classification, they must each be inspected and accepted by qualified fish health personnel of the agency granting the classification.

The following requirements must be met in order for one rearing facility within a culture station to be considered separate from other rearing facilities at the same station for the purposes of fish health classification:

- The facility must be physically separate from the other fish culture activities and facilities at the station; it must be completely enclosed and secure to prevent entry of birds, animals and unauthorized personnel.
- The water supply to the facility must be free of Emergency, Limited A, and Limited B fish pathogens.
- Access to the facility should be limited to essential personnel. Footbaths with PVP iodine at 250-mg/1 or other appropriate disinfectant must be properly used and maintained at all entrances to the facility. The facility must be equipped with all essential equipment so that it will not be necessary to move any equipment from the facility to other locations on the facility. All equipment (unless new and unused) must be thoroughly disinfected before being brought into the facility.
- Effluent water from the separate, enclosed facility must be properly disinfected and verified to be free of listed pathogens or otherwise be completely independent of the rest of the facility. If the effluent from the isolated facility does not meet this requirement, any disease classification of the isolated facility will also apply to the facility that receives the effluent.
- A biosecurity plan to maintain the separate fish health status of each facility within the culture station should be developed. Elements of the biosecurity plan should be consistent with the biosecurity recommendations in Appendix IV.

Table II-1. Suggested surrogate species for fish health assessment among the coolwater and warmwater species cultured by NEFHC member agencies. The NEFHC warns that the reliability of testing surrogate species is still unknown, thus results should be interpreted solely as a beneficial screening assessment.

Cultured Species	**Status of possible surrogate species for fish health assessment**			
	Walleye	**Sauger**	**Yellow perch**	**Esocids**
Walleye	N/A	Yes	Yes	Yes
Sauger	Yes	N/A	Yes	Yes
Yellow perch	Yes	Yes	N/A	Yes
Cultured Species	**Muskellunge**	**Northern pike**	**Chain Pickerel**	**Yellow perch**
Chain pickerel	Yes	Yes	N/A	Yes
Muskellunge	N/A	Yes	Yes	Yes
Tiger musky	Yes	Yes	Yes	Yes
Northern pike	Yes	N/A	Yes	Yes
Cultured Species	***Micropterus* sp.**	***Lepomis* sp.**	***Pomoxis* sp.**	***Ambloplites* sp.**
Bluegill	Yes	Yes	Yes	Yes
Hybrid sunfish	Yes	Yes	Yes	Yes
Redear sunfish	Yes	Yes	Yes	Yes
Largemouth bass	Yes	Yes	Yes	N/A
Smallmouth bass	Yes	Yes	Yes	N/A
Black crappie	Yes	Yes	Yes	Yes
White crappie	Yes	Yes	Yes	Yes
Cultured Species	**Blue catfish**	**Channel catfish**	**Flathead catfish**	**Bullheads**
Blue catfish	N/A	Yes	Yes	Yes
Channel catfish	Yes	N/A	Yes	No
Brown bullhead	Yes	Yes	Yes	N/A
Cultured Species	**White bass**	**Striped bass**	**White perch**	**Yellow bass**
Hybrid striped bass	Yes	Yes	Yes	Yes
Striped bass	Yes	N/A	Yes	Yes
White bass	N/A	Yes	Yes	Yes
Cultured Species	**Gizzard shad**	**Threadfin shad**	**Blueback herring**	**Alewife**
American shad	Yes	Yes	Yes	Yes
Hickory shad	Yes	Yes	Yes	Yes
River herring	Yes	Yes	Yes	Yes
Cultured Species	**Gars**	**Bowfin**	**Northern Snakehead**	**American eel**
Atlantic sturgeon	Yes	Yes	Yes	Yes
Shovelnose sturgeon	Yes	Yes	Yes	Yes
Paddlefish	Yes	Yes	Yes	Yes

Appendix III

Egg Collection and Disinfection

Section A: Introduction

Iodophor egg disinfection has been widely accepted as an important biosecurity component for preventing infection by bacteria, viruses, fungi or parasites. The iodophors most commonly used for disinfection are povidone or polyalcoholic complexes of iodine where the solubilized iodine confers a broad spectrum germicidal activity but is not as corrosive or irritating as the elemental form.

The NEFHC strongly encourages member agencies to develop and incorporate egg disinfection protocols for all fish gamete collection programs. While egg disinfection procedures have been well established for salmonids (trout, salmon, and char), the recent emergence of Viral Hemorrhagic Septicemia in the Great Lakes Basin has prompted fisheries management agencies to research and employee egg disinfection protocols for other fish species including, but not limited to, percids and esocids.

Section B: Limitations and General Procedures

Iodophor egg disinfection reduces the probability of egg surface pathogen transmission, but does not completely kill all microbes. A number of factors act to reduce the effectiveness of the iodophor. These include the presence of the pathogen within the yolk of the egg (thereby preventing the iodophor from contacting the pathogen), the masking effect of organic matter on the egg, improper pH or iodine concentration or specific resistance characteristics of the pathogen. Therefore, the NEFHC recommends that iodophor should not be solely relied on to prevent vertically transmitted pathogens.

A product made specifically for fish egg disinfection should be used according to product label instructions. The egg disinfection station in the receiving area of a fish culture facility must be isolated from the incubation and rearing areas to prevent cross-contamination. Two people should always be involved in the egg disinfection process; one person should be in charge of receiving and disinfecting the eggs, and the second person should handle the eggs after disinfection. It is important that neither individual enters the other's work area.

The NEFHC recommends the following general egg disinfection procedures:

Ensure the use of clean, non-production water during all gamete collection, disinfection, and egg transportation activities. Do not use water from rivers or lakes.

- Freshly mix the iodophor just before the egg disinfection process begins; avoid reusing the iodophor to maximize its disinfection properties. Eggs are very sensitive to changes in pH, dissolved oxygen and temperature. Always monitor these parameters to ensure consistency during the egg disinfection procedure.
  - Water temperature during disinfection should not be allowed to change more than 3^{o} Celsius at any time.
  - The pH of the solution must be monitored and maintained between 7.0 and 7.5. Total alkalinity of the disinfecting water should be above 100 mg/L. To maintain the minimum alkalinity, the solution should be buffered by adding sodium bicarbonate (NaHC03) at 0.01 percent to prevent egg toxicity effects of low pH drift (<6.0).
- The ratio of egg volume to iodophor volume should be a minimum of 1:4 (1 part egg/4 parts solution.
- Avoid direct sunlight if disinfecting outdoors as it will photo-degrade the iodophor solution.
- Disinfection of eyed eggs that are less than 5 days from hatching will cause excessive mortality and/or premature hatch.

Section C: Salmonid Egg Disinfection

<u>General Procedure for Egg Disinfection of Newly Fertilized Eggs during the Water-Hardening Process</u>

The NEFHC recommends a 50-100 mg/L, 30 minute iodophor treatment during water hardening. The treatment should be initiated during the first stage of water hardening so that the iodophor is drawn into the perivitelline space of the egg.

***The following procedures are recommended*:**

1. Disinfect the fish's vent with a 1:100 mg/L solution of iodophor and wipe the vent surface dry with a clean paper towel. Spawn eggs into a colander and separate the ovarian fluid. Ovarian fluids may contain high levels of bacteria that potentially can infect eggs during the fertilization and water hardening process. Removal of the ovarian fluid also removes proteins, blood cells, organics, etc., all of which can interfere with the fertilization process by blocking the micropyle. These substances

can also combine with iodophor and thereby reduce the iodophor concentration during treatment.

2. Gently transfer the eggs to a dry container. Be careful not to introduce water or organic material (mucous, feces, etc.) during steps 1 and 2.
3. Add milt and gently stir each container using a clean instrument. Immediately add pathogen-free water (same temperature that brood stock are maintained) to just cover the top of egg mass. Gently swirl the container. Fertilize for two to five minutes.
4. Rinse the eggs with a 50-100 mg/L iodophor solution, discarding the rinse. Repeat this procedure until the rinse solution is relatively clear of organic material. This will remove excess milt, blood etc.
5. Add fresh 50 mg/L or 100 mg/L iodophor solution. The volume ratio of egg to solution should be a minimum of 1:4. Gently stir to ensure even distribution of iodine. Disinfect the eggs for 30 minutes.
6. Gently rinse the iodophor from the eggs using clean, non-production water.
7. Eggs will continue to water harden for up to 90 minutes. Finish water hardening the eggs in clean, non-production water. Clean, disinfect and dry all potentially contaminated equipment used in the disinfection process.

General Procedure for Standard Surface Disinfection of Eyed Eggs

For salmonids, the NEFHC recommends that all eggs be disinfected following transfer to a receiving fish culture station and prior to coming in contact with fish culture station water, equipment, and rearing units. Eyed eggs should be disinfected at 100 mg/L for 10 minutes.

***The following procedures are recommended*:**

1. If the eggs are shipped without water, place them in pathogen-free water for 30-60 minutes before adding iodophor to replenish water loss during shipping.
2. Completely drain water from the eggs.
3. Immerse the eggs in freshly prepared iodophor and gently stir to ensure the solution is mixed through the eggs. The ratio of eggs to solution should be a minimum of 1:4.
4. Disinfect eggs for 10 minutes.
5. Remove the eggs from the solution and place into the flowing incubator. Discard the disinfectant solution.
6. Clean, disinfect and dry all potentially contaminated equipment used in the disinfection process.

Section D: Cool Water Fish (Walleye, Northern Pike and Muskellunge) Egg Disinfection

General Procedure for Egg Disinfection

The following recommendations for cool water egg disinfection are based on the best available information and should be considered a minimum disinfection methodology. These recommendations will be updated as new information becomes available.

The following procedures are recommended:

1. Disinfection of fertilized cool water fish eggs should be conducted during water hardening whenever possible; and, when disinfection during water hardening is not possible, the eggs should be surface disinfected after they are water hardened.
2. Procedures for cool water egg disinfection:
    a. During water hardening, a 50 mg/L concentration of iodophor solution should be used for 30 minutes to kill pathogens and prevent them from entering the egg; pathogen-free water from a protected source should be used for water hardening, egg rinsing, and egg transport.
    b. If disinfection during water hardening is not possible, or if pathogen-free water is not used during water hardening, egg rinsing and/or egg transport, a 100 mg/L concentration of iodophor solution should be used for 10-15 minutes to kill pathogens adhering to the surface of eggs prior to the eggs being transferred into an agency hatchery building.
    c. If eyed eggs are transferred to another fish production facility, a 100 mg/L concentration of iodophor solution should be used for 10-15 minutes to kill pathogens adhering to the surface of eggs prior to their being transferred into a hatchery building at the receiving facility.
3. When eggs are disinfected, the pH should be buffered to ensure it does not change by more than 0.3 units and remains between 7.0 and 7.5.

General Procedure for Egg Disinfection of Newly Fertilized Eggs during the Water Hardening Process

1. Disinfect the fish's vent with a 1:100 iodophor solution and wipe the vent surface dry with a clean paper towel. Spawn eggs into a dry pan and add an appropriate amount of milt to fertilize the eggs. Gently mix the eggs and milt to ensure full distribution of the milt throughout the mass of eggs. Add clean, non-production water and mix to ensure milt activation.
2. If the eggs are adhesive and require use of a de-adhesive agent (i.e., walleye), add tannic acid or Fullers earth from a stock solution and mix gently, but thoroughly. Stir for approximately 2 minutes. **Caution:** Fuller's earth and tannic acid have been commonly used as an anti-clumping agent for cool water species. Published research

suggests that when tannic acid is combined with iodophor, tannic acid destroys the ability of either compound to effectively inhibit VHS, Type IVB. **Thorough rinsing of both de-adhesive agents is required to ensure that it does not interfere with the disinfectant properties of iodophor.**

3. Gently pour off the solution and gently rinse eggs with clean, non-production water.
4. Immediately but gently add the prepared solution of iodophor (50 mg/L) and gently mix to ensure even distribution of iodine to the egg mass. Disinfect for 30 minutes.
5. Gently remove eggs from the solution and place into clean, non-production water to complete water-hardening.
6. Clean, disinfect and dry all potentially contaminated equipment used in the disinfection process.

Section E: Equipment Disinfection

The NEFHC recommends disinfection of all equipment used in the spawning, water-hardening and handling of eggs; including boats, nets, raingear, footwear, clothing, egg containers, tables, etc.

- Equipment should be disinfected with either a 10% chlorine bleach solution for 10 minutes or a 200 mg/L iodophor solution for 30 minutes.
- Thoroughly rinse all spawning fomites with pathogen-free water.
- Clothing used during spawning and egg handling should be washed and machine dried (house-hold washer and dryer is acceptable) before being used again for spawn collection.
- Dispose of the iodophor and chlorine solutions according to state regulations. Both chemicals can be neutralized using sodium thiosulfate. Take precaution to avoid chlorine contact with fish.

Section F: Human Safety

To avoid human safety concerns associated with use of either chlorine or iodophor disinfection, the NEFHC recommends the following:

- Always use appropriate safety precautions. Wear non-porous personal protection equipment, including gloves, raingear, boots and splash-proof eye protection. These protective measures are important when handling concentrated solutions of either chlorine or iodophor. Iodophor solutions should never be atomized due to documented respiratory and hypersensitivity problems.
- Chlorine is a strong oxidization/reduction agent and will damage skin and metal equipment.

Section G: References and Resources

World Organization for Animal Health, (OIE), Manual of Diagnostic Tests for Aquatic Animals, 2014, Section 1.1.3

Great Lakes Fishery Commission, Model Program for Fish Health Management in the Great Lakes, 2014

USFWS Iodophore Disinfection Protocol for Fish Eggs, USFWS Handbook of Aquatic Animal Health, Procedures and Protocols

Alaska Sockeye Salmon Culture Manual, Alaska Department of Fish & Game, 1994

Egg Disinfection & Incubation Procedures for Salmonids (Salmon, Trout, Whitefish), Ontario Ministry of Natural Resources, Bulletin 2009, 01

Egg Disinfection Procedures for Muskellunge & Walleye, Ontario Ministry of Natural Resources, Bulletin 2010, 01

Appendix IV

Biosecurity Approaches

Section A: Introduction

Biosecurity is defined as measures taken to prevent exposure to harmful biological, chemical or physical agents which may cause adverse health effects in humans or animals. These agents include infectious microorganisms, such as bacteria, viruses, and parasites, and also non-infectious entities, such as toxins, contaminants and poor water quality. Biosecurity practices are often initiated in aquaculture facilities in order to meet economic, public health, production and fish health objectives.

Specifically, certain biosecurity practices seek to:

- Reduce the risk of pathogen introduction into a facility;
- Minimize the risk of disease spread throughout a facility;
- Minimize the risk of disease spread out of the facility through cultured product;
- Reduce conditions that increase the risk of stress and disease susceptibility in a population;
- Promote overall fish health;
- Protect economic investment and reputation; and
- Protect human health.

Section B: Development of a Biosecurity Plan

There are several critical points where pathogens may enter a system and pose a hazard to susceptible fish. These include, but are not limited to, imported or transferred fish, source water, commercial feeds, food, fomites or vectors such as humans or animals. Potential threats and pathogens that have been historically diagnosed on-site should be identified prior to drafting a biosecurity plan. Additionally, the most significant threats to the biosecurity of a facility (i.e., untreated surface water, importation of commercially raised fish for rearing or forage on-site, transfer of fish between state hatcheries, equipment that is used in multiple systems or shared between hatcheries, nearby piscivorous bird nesting site, proximity to water body with aquatic nuisance species, etc.) should be identified and specifically addressed by the four essential elements of any biosecurity plan: 1) disease prevention, 2) security precautions, 3) cleaning and disinfection and 4) disease surveillance.

Biosecurity plans should be tailored to each individual facility. Staffing levels, budget constraints, estimated risk, and available equipment all need to be considered. Biosecurity plans are dynamic documents that should be reviewed on a regular basis and amended when situations change. The first step in drafting a biosecurity plan is to critically examine each portion of the facility and all aspects of production to identify potential biosecurity risks or hazards. The guidelines should then be developed to minimize each potential risk to an acceptable level. While there is a certain level of risk associated with all biosecurity plans, the goal is to create a workable, enforceable, and practical biosecurity plan with an *acceptable* level of risk.

Identification and Alleviation of Biosecurity Threats

Water Supply

- Although deep wells and municipal water sources are much less likely to harbor significant levels of pathogens when compared to surface water or shallow wells, deep wells located near rivers can be contaminated by river water either through normal conditions or during storms or flooding. Dye studies may be used to determine if ground water is contaminated with surface waters. If possible, deep water and municipal waters should be utilized prior to contamination from surface runoff or shallow well water.
- Water with known pathogen contamination should be disinfected via ultraviolet radiation, ozone, or chlorine (followed by neutralization) (Table IV-1).
- Ultraviolet dose should be tailored to the susceptibility of the target organism to ultraviolet radiation, flow rate, and water clarity. Quartz or glass sleeves of ultraviolet sterilizers should be cleaned and ultraviolet bulbs replaced to maintain appropriate UV dosage for target organisms. Maintenance schedules may vary depending on manufacturer specifications, water chemistry, temperature, etc. UV dosage can be monitored on a regular schedule with a UV meter.
- If high gas levels have historically been a problem or necropsy results suggest gas bubble disease, gas levels should be monitored with a saturometer. Use of a low head oxygenator (LHO), passive mechanism, or vacuum degassing will help to decrease total dissolved gases and increase oxygen saturation. This will help reduce susceptibility of fish to opportunistic pathogens.

Feed and Nutrition

- High quality feed from a reputable source should be fed to all fish.
- Feed should be stored in a temperature controlled, insect- and rodent-free, and humidity-free environment.
- Feed should be stored only in water-proof, insect- and rodent-proof containers. Feed should be kept off the floor on pallets.
- Proper pest control techniques should be used to eliminate pest infestations.

- Expired or abnormal appearing feed should be discarded and not fed out.
- All food containers should be cleaned and disinfected regularly (Table IV-1).
- Live or frozen fish or invertebrates fed to early life stages or carnivorous fish can either be tested for common pathogens prior to feeding or pathogen load may be reduced via a pre-feeding treatment (i.e., rinsing newly hatched animals with clean water before feeding, ultraviolet radiation, ozone, hydrogen peroxide, etc.).

Discharge

- A waste management plan should ensure that chemical therapeutants, solids, toxicants, exotic aquatic species, marking agents, etc. are released in compliance with appropriate wastewater discharge codes and standards. The waste management plan should address treatment of pathogens or disinfection of effluent from the facility (if appropriate) if the effluent enters directly into an open water body.

Vectors and Fomites

- *Predators:* Access to rearing units and water supply from potential predators should be minimized via the use of nets, fencing or appropriate legal means.
- *Visitors to the facility:* Public visitation is a common occurrence at many fish culture stations. However, humans may act as vectors of infectious pathogens. To help avoid contamination, visitation should be limited to certain locations of the facility with well-established boundaries and parking locations. Signs depicting these locations must be clearly displayed. Footbaths should be placed at all public entrances to any buildings or areas. Additional signage must be posted to make visitors aware of biosecurity measures that must be followed while visiting the facility. Examples include but are not limited to: no pets, no hands in the water, wash hands before feeding fish, feed only designated fish feed, no fishing/swimming clothing or boots permitted. Visitors that have visited either an aquaculture facility or a laboratory that handles pathogens within the past 24 hours should not be allowed on-site.
- *Vehicles and Work Equipment (e.g., dip nets, buckets, brooms, brushes, aerators, weighing scales)*
    - The sharing of work equipment between hatcheries is prohibited.
    - All stocking trucks and equipment returning from stocking native waters or visiting another hatchery should be power washed, cleaned, and disinfected prior to entrance into the facility (Table IV-1).
    - All stocking trucks and equipment arriving from another hatchery should be power washed, cleaned, and disinfected prior to entrance into the facility (Table IV-1).
    - Any equipment (bobcat, feeder, steam Jenny, nets, aerators, etc.) that is loaned out for use in native wild waters or another hatchery should be power washed, cleaned, and disinfected immediately upon return to the hatchery (Table IV-1).
    - Work equipment designated for use in one particular area of the hatchery should be clearly marked for that location.
- *Personal protective equipment (e.g., waders, hip boots, rubber boots, raingear, gloves)*

    - The sharing of PPE between hatcheries is prohibited.
    - PPE should be disinfected post-stocking in native waters (Table IV-1).
    - PPE should be disinfected between separate hatchery areas (Hatchery building, outdoor ponds, spawning building, etc.)
- *Cleaning Equipment*
    - Mops and buckets should be clearly designated for use in one particular room.
    - Disinfectant solution should be replaced on a regular basis so as to not compromise product efficacy.
- *Disinfectable Surfaces*
    - Whenever possible, wooden structures (i.e., dam boards, etc.) should be replaced with non-porous, disinfectable materials (i.e., metal, plastic, etc.).
    - Dirt-lined raceways should be replaced with non-porous raceways or tanks.
    - In order to properly sanitize and disinfect, organic matter must first be manually removed. The surface or piece of equipment should then be scrubbed with a detergent or soap. After rinsing, a disinfectant should be applied with the appropriate contact time. After rinsing the disinfectant off, the object should be allowed to dry completely (Table IV-1).

Biosecurity Standard Operating Procedures for Fish Culture Stations and Facilities

Some biosecurity Standard Operating Procedures (SOPs) are common to all three types of production systems (hatchling/fingerling, production, broodstock areas), whereas others are particular to a specific system.

Common biosecurity SOPs include:

- Staff should be trained and updated regularly regarding biosecurity measures and the reasoning behind them.
- Rearing units should be cleaned on a regular basis.
- Separate or sterilized equipment (nets, brushes, waders, etc.) should be used for each segregated rearing unit. Equipment should be clearly marked for use in a specific unit. Disinfecting tubs/brushes should be maintained near rearing units.
- Rearing units that are temporarily empty of fish should be drained, cleaned, and disinfected prior to the introduction of new fish (Table IV-1).
- Mortalities should be removed daily and more frequently during disease events.
- Mortalities should be disposed of in a manner that ensures clean stocks are not exposed to potential pathogens.
- A work flow plan should be developed; clean, healthy fish should be handled first, then diseased or clinically abnormal fish and quarantined fish should be handled last. Strict biosecurity measures should be followed after working with diseased fish and before working with quarantined fish.

- Strict biosecurity measures should be followed when working between production and quarantine systems (ex. rain gear specific for quarantine area, boots, etc.).

Hatching/Fingerling Area

- All equipment used for eggs and fry should be used exclusively for fry and eggs only.
- Due to the increased susceptibility of fry and fingerlings to infectious diseases, visitors should be prohibited from entering hatching/fingerling rearing rooms or buildings. A viewing window can be utilized by the public to see rearing operations.
- Floors should be mopped weekly, with a mop and bucket that is designated for use in the hatching/fingerling room only, with a proper disinfectant (please refer to the AFS-FHS "Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture" and the OIE Manual "Methods for Disinfection of Aquaculture Establishments" chapter).
- Disinfection foot baths and hand cleaners should be placed at all entrances/exits to the hatching/fingerling room(s) (Table IV-1). Baths should be changed twice weekly, at minimum (more often if there is organic material present in the foot bath).
- If possible, separate staff should be assigned to the hatching/ fingerling rearing facility. If staffing levels do not permit this, then ideally work should be completed within the hatching/fingerling rearing area before working with production or brood stock populations.
- Lights and feeders above tanks should be periodically cleaned.
- Vaccination (generic or autogenous) of appropriately aged fish should be performed for pathogens that have a vaccine approved for use in aquaculture and whose presence in the facility has negatively affected fish health.
- Depending upon the species and available information, eggs should be disinfected during the water-hardening process and prior to entering the facility's egg incubation area.

Fish Production Area(s)

- Raceways should be worked in order, starting at the unit nearest the headwaters and ending with the unit furthest downstream. Equipment and PPE does not need to be disinfected when working between units of the same raceway unless one must return to a unit upstream.
- If located indoors, foot baths should be placed at all entrances to the production area. All footbaths should be changed twice weekly, at minimum (more often if there is organic material present in the foot bath).

Broodstock

- Maintenance of domestic broodstock reduces the risk of pathogen introduction from wild broodstock brought into the facility each season for spawning.
- Broodstock observed to be in poor body condition or exhibiting any clinical disease signs should not be spawned and culled from the population.

Grounds

- The entire production facility should be fenced, with access controlled through lockable gates.

Potential Standard Operating Procedure Hazards

Sick Fish

- The cause of morbidity and mortality should be identified via complete necropsies, skin scrapes, gill biopsies, clinical signs, and/or sample submission for parasitological, bacterial, and virological diagnostics. Treatment should then be tailored to a proper diagnosis.
- If the cause of morbidity and/or mortality is not easily identified, fish culture staff should contact the designated fish health specialist.
- Some pathogens are common in aquaculture and opportunistically cause disease when fish are stressed or environmental conditions are poor. Minimizing stress by maintaining adequate nutrition, keeping densities low, maintaining proper oxygen levels, minimizing handling, and/or eliminating predation, helps to prevent morbidity and mortality from opportunistic pathogens.
- Antibiotics should be administered at the prescribed dosage and treatment duration.
- Expired antibiotics should be properly discarded and not fed out.
- Antibiotics or medicated feed should not be used for disease prevention.
- Mortalities from affected units should be collected last.
- Units of sick fish should not be moved, graded, or split (unless thinning is used as part of the treatment) while diseased or debilitated.
- Equipment and PPE used in affected units shall be disinfected daily and use should be restricted to affected units (Table IV-1).
- After contact with sick units of fish, staff should wash and disinfect hands (Table IV-1).
- If feasible, designated staff should work only with the sick fish.

Dead Fish

- Mortalities should be removed daily or more often if possible, from rearing units. Units with sick fish should have their mortalities removed last, and mortalities should be collected from headwaters first and then collected downstream. Personnel working with sick fish should not work with healthy fish again that work day.
- Proper disposal of dead fish through incineration or burying is necessary. Mortality pits should be located away from any fish rearing units to prevent contamination of nearby ponds via runoff or movement of fish back into the facility by scavengers.
- Personnel should wash hands or use hand sanitizer after collecting and disposing of dead fish.

Aquatic Animal Transfers

- Pre-approval from the fish health specialist, agency veterinarian, and/or program director should be required for any transfer of aquatic animals into a hatchery.
- Diagnostic testing for pathogens of concern should be required for all imported aquatic organisms.

- All equipment used to off-load shipments of aquatic organisms should be cleaned and disinfected properly after use (Table IV-1).
- Aquatic animals held temporarily for transfer or with an unknown health status should be held in the most downstream outdoor raceway or in an isolation unit, if possible. The area should be drained, cleaned, and disinfected after the aquatic animals are removed (Table IV-1).
- Stress reduction in post-transport fish may be achieved via 0.5% salt bath for one to three days post-arrival.
- Quarantine of newly arrived aquatic organisms protects resident fish populations from potential exposure to pathogens carried by the new arrivals. Additionally, quarantine allows newly arrived organisms to acclimate to water, feed, and new management and to recover from handling and transport. Stress from transport to a new facility may increase susceptibility to opportunistic pathogens. Properly designed quarantine areas physically separate incoming organisms from the rest of facility's population, and also divert discharges from the new organism away from the resident population. Untreated quarantine effluent should not flow directly into surface waters.

Disease Surveillance

- Regular staff educational training concerning common hatchery diseases (clinical signs, treatment, and associated stress factors, methods to minimize or prevent disease outbreaks) is the most important preventative measure for disease transmission. Timely notification and response after identification of a potential disease outbreak is essential to minimize the time between identification of a disease problem and initiation of appropriate treatment.

  Annual fish health inspection data contributes to a historical database documenting the presence or absence of pathogens of concern.

Section C: Summary

Good adherence to an effective biosecurity plan will reduce the risk of catastrophic losses from infectious diseases and low-level, chronic losses that add up over time. Good fish husbandry practices and alleviation of stress factors help to reduce disease outbreaks. As mentioned previously, there is an element of risk in all biosecurity plans; however, with careful consideration and planning, an effective plan that takes into account hatchery-specific variables can be developed to minimize production losses and threats to fish health and the environment.

Section D: References

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Appendix V

Risk Assessment for the Introduction or Transfer of Fish and Associated Pathogens into Waters of the Northeast States

Section A: Introduction

Transfer of fish has been, and continues to be, the cornerstone of many fishery conservation and restoration programs within the United States. Often, pathogens have invaded new geographic ranges as a result of fish importation or stocking, resulting in negative consequences for fish populations. Numerous examples can be found such as the incidence of whirling disease in the intermountain west (Bartholomew and Reno 2002). Outbreaks of emerging diseases in wild and cultured fishes within geographic regions of the United States (such as *Heterosporis* sp., largemouth bass virus, *Piscirickettsia* sp., *Nucleospora salmonis*, and viral hemorrhagic septicemia virus) indicate that a more quantifiable protocol is needed when assessing the pathogen risk of potential introductions or transfers of fish. In light of the potential disease risks associated with such animal transfers and inherent limits to the number of animals that can be sampled for testing, other methods must be used to assess the risks associated with inter-facility fish transfers, transfers between private fish culture stations or interbasin fish transfers. The following procedures establish guidelines for member agencies to assess and document the risks associated with fish propagation without unduly jeopardizing the fish population in question, the health of other fish on neither the premises nor the ecosystem into which the subject population is transferred at a later date.

Quantitative risk assessment (probability models) can be performed when a given stressor (physical, chemical, or biological) is evaluated and sufficient data on the stressor are available. It is unlikely that fish propagation health risk assessments will have the necessary focus (a single stressor/pathogen) or sufficient pathogen data for a quantitative approach. Therefore, the following guidance will permit aquatic animal health officials to formulate a qualitative risk assessment with a rating of low, moderate or high risk being assigned to a given fish population's transfer. This rating will be used to formulate recommendations regarding the transfer of the fish in question.

National and international agencies have developed a standard, science-based process to accurately assess pathogen introduction risks associated with fish transfer, collectively called Import Risk Analysis (IRA) (Amos 2004; Bondad-Reantaso 2004; Hine 2004; Kanchanakhan and Chinabut 2004; Olivier 2004; Perera 2004). Guided by this widely accepted process of IRA for fish importation and transfers, the GLFHC and NEFHC have adopted a Risk Assessment (RA) process in compliance with the World Animal Health Organization Aquatic Code (OIE 2013), the International Council for the Exploration of the Sea Code (ICES 2004), the Food and Agriculture Organization of the United Nations (Bartley et al., 2006), and the U.S. Fish and Wildlife Service Handbook of Aquatic Animal Health Procedures and Protocols.

Specifically, the NEFHC seeks to:

- Develop a general risk assessment framework that the NEFHC will follow to reach recommendations regarding introductions or transfers for which no standard procedures are established, or which fall outside of, or in conflict with the Northeast Fish Health Guidelines.
- Archive each risk assessment for review and evaluation when similar cases arise in the future.

The NEFHC risk assessment (NEFHCRA) is designed to determine the likelihood of pathogen introduction into a fish culture rearing, facility or watershed, or the potential extension of the range of a pathogen within the member states jurisdiction with associated fisheries management actions such as fish and aquatic organism transfers. The NEFHCRA will also document likely risks of such actions and provide fisheries managers with NEFHC recommendations about how to minimize any identified risks using the best available information at the time the NEFHCRA is performed. The NEFHCRA will not address any issues outside of the aquatic animal health considerations of any proposed introduction.

The NEFHC strongly recommends that a risk assessment be conducted well in advance of the planned importation or transfer of fish or other aquatic organisms, particularly when the Northeast Fish Health Guidelines do not provide clear guidance to fisheries managers on minimizing potential aquatic animal health risks in receiving fish culture stations, facilities, and waters. This assessment is designed to support and assist in the decision record for the proposed fisheries management action. Based on all available information, the NEFHC will review, evaluate and provide recommendations on the proposed introduction exclusively focused on the

potential aquatic animal health risks to the receiving fish culture station, facility, or water body from the proposed management action.

Section B: NEFHCRA Objectives

- Identify pathogen(s) of concern that may be introduced or transferred into waters of member states as a result of the proposed introduction of fish or aquatic organism, including their gametes.
- Document potential aquatic organism disease issues to include epizootic risk associated with the proposed action.
- Determine the most likely aquatic organism disease risks, to include the likelihood of such risks, associated with the proposed transfer or introduction of fish or aquatic organisms and their gametes into waters, fish culture stations, or facilities of member states.
- Develop and provide member agency‘s fisheries managers with recommendation as to whether or not the proposed action to import or transfer fish or other aquatic organisms should proceed from a fish health perspective.
- Develop and provide member agency‘s fisheries managers with risk management options to eliminate or reduce the effects of the proposed action.
- Facilitate responses to fish and aquatic organism disease questions from member agency’s administrators and other entities on the proposed fish management action including the NEFHCRA process, supporting documentation, and recommendations.

Section C: NEFHCRA Procedure

The NEFHCRA is to be used in the following situations:

- The Northeast Fish Health Guidelines do not provide clear guidance, or
- A proposed action is in direct conflict with the Northeast Fish Health Guidelines.

When one of these situations arises, the NEFHC Chairperson should be contacted by the affected member agency’s representative to initiate the NEFHCRA process. Once contacted, the NEFHC Chairperson will work with the requesting member agency to select the appropriate NEFHCRA form (NEFHCRA-1, NEFHCRA-2 or NEFHCRA-3) and to complete a preliminary risk assessment. The NEFHC Chairperson will share the preliminary risk assessment with the entire NEFHC and solicit input from members to develop a final NEFHCRA report.

<u>Final Assessment of the Pathogen Risk Potential</u>

The process results in a numerical score, which is placed into one of three categories of risk: low, moderate, or high. The NEFHC will provide a summary report (Form NEFHCRA-4) which will focus and summarize the most critical information that was used in the process, including its

recommendation, documentation of fish health risks to naturally occurring populations of native or naturalized species, important fisheries or aquaculture resources, biological communities and habitats which may be impacted by a proposed action, and potential options for mitigation (if applicable). The final summary report will be provided to all member agencies after review from the affected member agency.

Risk Communication

Risk communication represents the interactive exchange of information about risk among risk assessors, risk managers, and other interested parties. It begins when a risk assessment is requested and continues after the implementation of a recommendation regarding the possible translocation of a pathogen of concern. The communication of risk should be open, interactive, and involve transparent exchange of information that may continue after the decision on translocation is made. The uncertainty in the model, model inputs, and the risk estimates in the risk assessment should be communicated between the involved parties. The entire risk assessment process should include an evaluation of uncertainty and data sources.

Section D: Instructions for NEFHCRA-1, NEFHCRA-2 and NEFHCRA-3 Forms

An Excel file is provided for the compilation of the NEFHCRA.

Each of the NEFHCRA forms should be scored as follows:

1. Choose the appropriate option for each situation and place its associated numerical value in the small box immediately to the right of that option.
2. Multiply the numerical value by the weighting factor (in parentheses) for the situational statement and place this value in the score column on the far right.
3. Total all of the scores and place this value in the **Total Risk Score** box at the bottom of the worksheet.

Section E: Final Scoring

Form NEFHCRA-1: For pathogen transfers into a fish culture station or facility, the following risk potential and general recommendations apply.

Risk Score	Risk Potential	Recommendation
359 and below	Low	Place fish into a standard facility; apply mitigation for pathogens as necessary. The transfer must not result in a reduction of the health status of the facility. If the transfer would result in a reduction of health status, the fish should be placed into isolation, quarantine or not allowed into the facility.
360 - 520	Moderate	Place fish into isolation/quarantine. The fish should be tested a minimum of 3 times in 2 years with at least 4 months between tests without the detection of listed pathogens before transfer or release. Sampling should be done at the 2% prevalence level (95% confidence).
521 and above	High	Place into quarantine or do not allow importation. Fish may only be transferred or released based on recommendations made by the NEFHC in the Risk Assessment Summary document.

Form NEFHCRA-2: For pathogen transfers out of a fish health station or facility, the following risk potential and general recommendations apply.

Risk Score	Risk Potential	Recommendation
455 and below	Low	Allow unrestricted transfer of fish.
456-684	Moderate	Allow fish to only be transferred to facilities or released into waters that are positive for the same pathogen(s) of concern.
685 and above	High	Stocking and transfers are not recommended. Potential exceptions would allow fish to only be stocked into the waters of origin or held in isolation/quarantine for further testing as suggested by the NEFHC.

Form NEFHCRA-3: For pathogen transfers from one wild fish population to another wild fish population, the following risk potential and general recommendations apply.

Risk Score	Risk Potential	Recommendation
224 and below	Low	Allow unrestricted transfer of fish.
225-330	Moderate	Allow fish to only be transferred to bodies of water or released into waters that are positive for the same pathogen(s) of concern.
331 and above	High	Stocking and transfers are not recommended. Potential exceptions would allow fish to only be stocked into the waters of origin or held in isolation/quarantine for further testing as suggested by the NEFHC.

Section F: Recommendations to Decision-Makers

A risk assessment can result in one of three outcomes:

- The request is recommended for approval without conditions.
- The request is recommended for approval with conditions such that specific preventive or mitigating measures are to be followed before the proposed translocation of a potential pathogen takes place.
- The request is not recommended for approval owing to a level of risk estimated to be unacceptable.

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Appendix VI

Disease Contingency Planning

Section A: Introduction

The purpose of a disease contingency plan is to quickly respond to and eliminate important disease agents from a fish culture facility. A disease contingency plan for specific pathogens should be prepared prior to a disease outbreak occurring. Responses will vary depending on the classification of pathogen encountered, and a corresponding contingency plan should be developed and followed. A critical aspect to developing a quick response to a disease outbreak is to have a task force organized and ready to develop a course of action for each diagnosed pathogen of concern. Encountered pathogens that require a contingency plan fit into one of four categories: 1) Emergency pathogens, 2) Limited A pathogens, and 3) Limited B pathogens, and 4) Restricted pathogens as listed in Appendix I.

Two critical aspects of an effective contingency plan are early pathogen detection and a timely response to contain the pathogen. For this to be achieved, an effective fish health surveillance program as well as effective communication between member agency staff and the fish health coordinator is necessary. Detecting the presence of pathogens or reporting mortality/suspicious disease signs as early as possible best facilitates effective resolution. When clinical disease signs or gross necropsies suggest the presence of a serious fish pathogen, stringent biosecurity and containment protocols should be followed for the affected population. This early containment helps reduce further spread of a potential disease while fish samples are tested in the laboratory; such tests may take several days to weeks for a definitive diagnosis.

There are varied responses to managing pathogens in fish culture facilities. In part, these varied responses are related to the status of the pathogen in local waters or intended stocking areas.

In general NEFHC recommends the following:

- No clinically diseased fish be stocked regardless of pathogen classification.
- Fish culture facilities where an Emergency pathogen has been detected should follow the emergency containment and eradication plan.
- Limited-A pathogens, which are not considered enzootic in the sub-basin of their isolation, should be handled as Emergency pathogens with containment and eradication procedures.

    - Efforts should be made to avoid spread of Limited-A pathogens in enzootic areas to reduce the potential impacts of disease, since these pathogens have been known to cause mortality in wild and hatchery fish.
- If Limited-B pathogens have been detected in a sub-basin known to be free of the specific pathogen, then a containment and eradication plan should be considered.
    - These pathogens are problematic since either no treatment is available or treated fish are known to remain infected with the pathogen at low levels (carrier fish), which may act to spread the disease agent.
    - Efforts should be made to avoid stocking the treated (carrier) fish into waters where fish could be negatively impacted by the presence of the pathogen. The member agency should identify high risk or sensitive waters in which stocking of carrier fish should be avoided; similarly low risk areas can be identified. For example, stocking fish that are potential carriers of Limited B pathogens specific to trout should not be stocked in waters that support natural reproduction to reduce the impacts to wild populations; an example of a lower risk stocking area would be a "put and take" fishery.
- If Restricted pathogens have been detected in a sub-basin known to be free of the specific pathogen, additional information regarding life history, etiology, and detection methods should be collected. Appropriate action established by the member agency should be taken to restrict and further reduce pathogen transmission. For additional guidance related to stocking of fish infected with a Restricted pathogen, a risk assessment (Appendix V) should be conducted.
- If high mortality in a fish stock is suspected to be caused by a Restricted pathogen, then the transfer/stocking of these fish should be avoided.
- Any OIE listed fish pathogens detected require notification of OIE authorities.

Other factors that will contribute to the design of a disease contingency plan include the fish culture facility design, water source, biosecurity, and treatment options for the pathogen. A disease contingency plan should be thought out for each of the pathogens listed in Appendix I.

Questions that should be considered for each individual facility include:

- Has the pathogen been previously identified within the member state or region?
    - *If no, then containment and eradication should be considered to avoid the introduction or further spread of the pathogen.*
- Is it feasible to disinfect the facility and eradicate the pathogen? For example, is the water source free of the pathogen in question?
    - *If yes, eradiation of pathogen by containment and disinfection should be done.*
- If eradication is possible, can reintroduction of the pathogen be prevented? For example, is the water source free of the pathogen in question?
    - *If yes, eradication of pathogen by containment and disinfection should be done.*

- If eradication is not feasible, can the pathogen be controlled to acceptable levels through treatments, vaccines, and other management approaches?
    - *If yes, then all practices should be used to minimize and reduce the levels of the pathogen within the facility.*
- Does transmission of the pathogen occur vertically (gametes are affected)?
    - *If yes, broodstock should be kept free of the pathogen and gametes from affected fish should not be used.*
- Do fish treated for a disease remain carriers of the pathogen?
    - *If yes, special consideration should be given to where these fish can be transferred/stocked.*
- If fish are believed to be carriers of a pathogen, is the pathogen resistant to the approved aquaculture drugs?
    - *If yes, then eradication by containment and disinfection should be considered. Antibiotic resistant bacterial strains should be eliminated from culture facilities and should not be stocked in the environment to reduce the prevalence of antibacterial resistant bacteria.*
- If carrier fish are to be stocked, has the pathogen been reported to cause mortality in local wild stocks or are other fish hatcheries located within the watershed of the intended stocking?
    - *If yes, avoid stocking those sensitive areas.*
- What are the economic costs associated with managing the pathogen?
    - *If managing pathogens to acceptable levels, adequate funding should be obtained for therapeutic treatments, vaccination programs, eradication of diseased fish, and disinfectants.*
- Are there any human health concerns with stocking carrier fish?
    - *If yes, fish should not be stocked and eradication should be considered.*

Section B: Containment and Eradication of Emergency Pathogens

Emergency pathogens are exotic to member states, and cause serious incurable diseases in finfish. For a listing of these pathogens refer to Appendix I. Control of these emergency pathogens depends upon prevention, early detection and eradication. Therefore, detections of these pathogens must be met with prompt containment and disease control of the entire facility involved. The following plan is recommended as a guideline.

Organization: Member agencies should have a contingency plan well in place before an emergency pathogen is detected.

This organizational plan should include the following:

- Delineation of the legal authority in order to act quickly if a pathogen is detected in a federal, state or private fish culture facility.
- Identification of the appropriate permits for use of the chemicals required to eradicate the pathogen.
- Establishment of emergency fishing restriction in the affected area.
- Establishment of funding to ensure that equipment, staffing, and supplies are available to conduct the eradication program.

The member agency should develop a task force to conduct the emergency disease eradication project.

The task force should include the following:

- An experienced fish health professional should be the project leader in charge of field operations.
- The manager of the affected facility and sufficient fishery personnel should assist the project leader for the duration of the efforts.
- A designated spokesperson assigned to media relations should be assigned to handle person relations during the eradication process. Clear communication to the public and neighboring hatcheries is critical throughout the process.
- Key tasks to be considered should at a minimum include the following:
    - Fish disposal plan
    - Facility disinfection plan. Surveillance of fish transferred to the facility following disinfection.

Quarantine and Epizootiological Investigations: Whenever an emergency pathogen is confirmed at any fish culture facility within a member state, an immediate quarantine of all fish at the facility should be imposed. If fish have been transferred from the affected facility to other fish culture facilities within the past year, similar quarantines should be issued to those receiving facilities until confirmatory inspection testing can be completed. The quarantine zone should apply to all waters within an area determined by the member agency. The member agency should closely examine the water flow and geographical makeup of the fish culture facility and nearby land to determine a logical quarantine zone. The zone can fall both within and outside that watershed as conditions warrant. All fish transfers within the quarantine zone shall be halted, including emergency fishing restrictions as necessary. The project leader should obtain

information on all shipments of fish from the facility during the previous year. All recipients of fish should be notified.

All fish populations from within the quarantine zone must be sampled at the earliest possible time. If other fish facilities are located within the quarantine zone, the task force leader in charge will call on each facility, explain the reason for his/her visit, the location of the infected facility, the nature of the disease, and how it is spread. The personnel should be advised of precautions necessary to prevent the spread of the disease and to whom they should report any suspicious disease signs among their own fish. These personnel should be informed of reliable current information. Strict biosecurity measures should be followed before entering or leaving fish facilities in the quarantine or buffer zones.

Strict biosecurity measures must be observed by all personnel working within the quarantine zone as pathogens can be spread by footwear, tires, and by other means. Protective, disposable plastic boots should be worn when working on the facility grounds or along streams where the viable disease agents may exist. Vehicles should not be driven into fish rearing areas. Each piece of equipment or clothing that may have become contaminated must be thoroughly cleaned and disinfected before it leaves the facility.

The quarantine zone should be determined by the task force. During the period in which initial survey information is being collected from within the quarantine zone, every effort must be made to observe all fish for signs of disease. Samples should be collected and tested in accordance with Appendix I. The specific location of all samples should be collected from each susceptible population along with other relevant observations.

Factors to be considered when establishing a quarantine zone and conducting an epizootiological investigation should include:

- Type and size of fish culture facility and species involved (i.e. small, self-contained recirculation facility vs. a large facility with a direct discharge of effluent)
- Proximity to other fish culture facilities
- Size and connectivity of the affected watershed
- Biosecurity practices at the facility prior to pathogen detection (i.e. chlorination/de-chlorination of effluent water)
- Known susceptibility of species in the affected watershed to the pathogen detected
- Other sources of pathogen spread (i.e. fomites, personnel, predators, etc.)
- Disease etiology

Note: Suspicious disease signs in fish must be reported immediately to the project leader.

Investigation and Confirmation: A fish health investigation should immediately be undertaken at the recipient station(s) to confirm the presence or absence of the causative agent of the suspect emergency disease. Positive samples will be sent to a second recognized fish health laboratory for confirmation. Surveys will be made on all lots of fish on the facility and within the quarantine zone (see Post Disinfection Quarantine Survey information below). Sampling should be done in accordance with Appendix I of these guidelines. The size and location of survey sites will be determined on the basis of natural fish barriers, type of terrain, and nature of the fish population and characteristics of the disease outbreak itself. In addition, spot-check surveys should be scheduled to include all susceptible fish populations located within the quarantine zone, including the watershed and no less than a five mile radius from the affected facility (see Post Disinfection Quarantine Survey information below). If confirmation of the pathogen occurs in the quarantine zone and not in the fish culture facility, then strict biosecurity practices must be used to avoid the introduction of the pathogen into the culture facility. Efforts should be made to eradicate the pathogen from the quarantine zone. While the pathogen is present in the quarantine zone, health surveillance for that pathogen in the fish culture facility should be elevated to three inspections per year at the 2% level of detection (95% confidence).

Disease Eradication and Fish Disposal: Upon confirmation of an emergency pathogen within the fish culture facility, immediate steps shall begin to assure the orderly decontamination of the facility. All gametes, fertilized eggs and fish will be promptly destroyed and disposed of by incineration, deep pit burying, or other biosecure manners. A firm commitment to prompt action is essential for effective containment and eradication of Emergency pathogens.

All stocks must be destroyed to avoid spread of the pathogen. They should be euthanized with rotenone, water saturated with carbon dioxide, or chlorine and buried in a deep pit or incinerated. A state agency official, such as a facility manager, should be in charge of stock disposal. He/she will secure the necessary equipment, materials and permits to conduct the disposal operation. He/she will assign qualified personnel to operate digging equipment and instruct them in the preparation of the burial pit or arrange for transportation to an incinerator.

A fish disposal operation would consist of the following events:

1. Determination that a disposal operation is necessary (project leader) and the method to be used.
2. Arrangement for the equipment and materials needed to carry out disposal (facility manager).
3. Preparation of the burial pit (facility manager and/or appropriate staff).
4. Disposal of infected or exposed fish (facility manager and/or appropriate staff).

These events should be carried out as soon as possible to limit further spread of the disease, further contamination of the facility or continued discharge of contaminated facility effluent. It is imperative that during the time between euthanasia and transportation of fish to burial or incineration, no wildlife, including birds and mammals, have access to the fish. Wildlife fish consumption can further spread the pathogen through the area.

The site chosen for a burial pit should be within the grounds of the facility with easy access from rearing units, but should also be a safe distance away from both areas subject to flooding and all water sources, such as streams, rivers, ponds or ground water. The burial trench should be at least seven feet wide and not less than seven feet deep with the length determined by allowing fourteen square feet of floor space for each 1,000 pounds of fish to be buried. As the fish are placed in the trench, they should be covered with unslaked lime. Lime is to be applied at the rate of 850 pounds for each 10,000 pounds of fish buried. This is to hasten decomposition and to discourage burrowing animals. The trench should be filled with earth without delay and the area should be included in the cleaning and disinfection procedures. If such a burial pit is not possible on the fish culture site, then the closest area adequate for a burial pit should be prepared and fish should be carefully transported to the pit. Careful attention to sanitary measures must be taken into account to prevent contaminating areas between the fish culture site and burial pit.

Cleaning and Disinfection: Cleaning and disinfection should start as soon as disposal is completed. The members of the task force working in affected areas must be supplied with personal protective equipment (rubberized rain gear including boots, coats, hats and gloves). These outer garments are to be removed and left in a secure location at the end of each day's work. These items should be thoroughly disinfected during the final phase of disinfection.

All fish rearing facilities should be brushed clean of moss, algae, dirt, and organic wastes. Rearing tanks, incubators, troughs, outdoor raceways, underground pipes between rearing units or water supply, and water supply head boxes and tail-race should all be thoroughly scrubbed. It may be possible to use a mechanical or water jet drain cleaning system to clean the underground pipes at the facility. Consideration should be given to the treatment of the effluent from these cleaning operations to minimize the potential spread of the pathogen to downstream locations. Earthen ponds should be drained and the entire bank area cleared of vegetation and debris.

Disinfection should begin as soon as the facilities are cleaned and readied. All buildings and the equipment within them should be disinfected with chlorine or other appropriate disinfectants.

Water supplies, pipeline systems and the facility effluent should be chlorinated. These are difficult to disinfect and success largely depends upon the length of time the disease organism is exposed to the disinfectant. Chlorine should be used at a minimum concentration of 200 parts per million for a period of at least one hour. If the chlorine disinfection can be left overnight in a safe manner, then this would be the most effective option. When using chlorine for disinfection it is important to neutralize the chlorine prior to release from the facility. Chlorine is highly toxic to fish and other aquatic life; after disinfection it should be neutralized by addition of sodium thiosulfate at 8 ppm for every 1 ppm of chlorine or the effluent can be filtered through activated carbon.

Clean hard-surface rearing units can be effectively disinfected by spraying them with a 1,000-ppm solution of Roccal or Hyamine 3500. There is a considerable residual effect with these compounds and all units treated with them should be thoroughly rinsed. Chlorine at 10,000 ppm or more may also be sprayed on hard surfaces where residual activity is not desired.

Earthen ponds, canals and the like present special problems for disinfection. Several treatments with unslaked lime (CaO) at the rate of two tons per acre may be required. Unslaked lime, the treatment compound of choice, should be applied to freshly-drained ponds prior to ponds drying out. The ponds should be left dried for a month or more. At that time, the remaining substrate should be removed and buried in a pit.

Fallowing Period: After disinfection of the facility, a predetermined fallowing period may be recommended prior to the introduction of new fish stock. This is particularly important in facilities where rearing units cannot be completely dried out or in situations where chlorine

disinfection cannot be effectively completed. A fallowing period will aid in ensuring that the facility has been completely eradicated of the pathogen. The length of the fallow period should be based on the time that the pathogen is able to remain viable outside of a fish host, along with environmental considerations in the geographic region of the facility. If rearing units are completely dried and exposed to sunlight then a fallow period may not be necessary. Instead the units may remain dry for a period of one month prior to restocking with fish. Following this, fish may be re-introduced into the system, starting with "sentinel" fish, which are selected based on the age and species that are most susceptible to the pathogen of concern. The "sentinel" fish should be kept in live-boxes near the outlet of each rearing unit. Ponds should be refilled and tested with "sentinel" fingerlings in live boxes for 120 days.

Post Disinfection Quarantine Surveys of the Fish Culture Station or Facility: The number of test fish should be determined by the size of the facility to be tested. Each rearing unit or lot with shared water supply should be tested by placing a minimum of 300 "sentinel" fingerlings of the species most susceptible to the pathogen in question, in a live-box near the outlet or directly into a pond. The water in the rearing units should be held at the normal operational level. Samples of fish from various locations will be collected after 60 days' exposure for laboratory testing. All fish will be sacrificed after 120 days' exposure for laboratory testing for the pathogen of concern. The test fish should be regularly fed and cared for during the exposure period. If relevant for the pathogen of interest, a stress test in the fish may be conducted to increase sensitivity of detection; this is done by manipulating temperature and using corticosteroid injections.

After the completion of a negative 120-day test period, all rearing units which are supplied with uninfected water may be restocked with pathogen-free fish or, preferably, eggs. Any mortality must be promptly investigated and these fish should be inspected for the causative emergency pathogen at intervals of 4 months or less for at least one year (three tests in the year). Testing should be done to detect the pathogen at a 2% prevalence level with 95% confidence. The quarantine may be released following three negative testing periods. Earthen ponds, ditches, and streams should be retested a second time. At the completion of two negative tests, these units may be restocked and the quarantine released. In instances where earthen pond and other fish rearing units adjoin, no production program will be initiated until the earthen ponds are determined to be free of the organism, as described above.

Appendix VII

USGS Regions of the Northeast States and the District of Columbia

State	Region					
	1	2	3	4	5	6
	New England	Mid-Atlantic	South Atlantic-Gulf	Great Lakes	Ohio	Tennessee
CT	X	X				
MA	X	X				
ME	X					
NH	X					
VT	X	X				
RI	X					
NY	X	X		X	X	
PA		X		X	X	
NJ		X				
DE		X				
MD		X			X	
DC		X				
VA		X	X		X	X
WV		X			X	

Region 01 New England

Subregion	HUC 8
Subregion 0101 St. John	01010001 -- Upper St John
	01010002 -- Allagash
	01010003 -- Fish
	01010004 -- Aroostook
	01010005 -- Meduxnekeag
Subregion 0102 Penobscot	01020001 -- West Branch Penobscot
	01020002 -- East Branch Penobscot
	01020003 -- Mattawamkeag
	01020004 -- Piscataquis
	01020005 -- Lower Penobscot
Subregion 0103 Kennebec	01030001 -- Upper Kennebec
	01030002 -- Dead
	01030003 -- Lower Kennebec
Subregion 0104 Androscoggin	01040001 -- Upper Androscoggin
	01040002 -- Lower Androscoggin
Subregion 0105 Maine Coastal	01050001 -- St. Croix
	01050002 -- Coastal
	01050003 -- St. George-Sheepscot
Subregion 0106 Saco	01060001 -- Presumpscot
	01060002 -- Saco
	01060003 -- Piscataqua-Salmon Falls
Subregion 0107 Merrimack	01070001 -- Pemigewasset
	01070002 -- Merrimack
	01070003 -- Contoocook
	01070004 -- Nashua
Subregion 0108 Connecticut	01080101 -- Upper Connecticut
	01080102 -- Passumpsic
	01080103 -- Waits
	01080104 -- Upper Connecticut-Mascoma
	01080105 -- White
	01080106 -- Black-Ottauquechee
	01080107 -- West
Subregion 0109 Massachusetts Rhode Island Coastal	01090001 -- Charles
	01090002 -- Cape Cod
	01090003 -- Blackstone
	01090004 -- Narragansett
	01090005 -- Pawcatuck-Wood

Region 01 New England (continued)

Subregion	HUC 8
Subregion 0110 Connecticut Coastal	01100001 -- Quinebaug
	01100002 -- Shetucket
	01100003 -- Thames
	01100004 -- Quinnipiac
	01100005 -- Housatonic
	01100006 -- Saugatuck
	01100007 -- Long Island Sound
Subregion 0111 St. Francois	01110000 -- St. Francois

Region 02 Mid-Atlantic

Subregion	HUC 8
Subregion 0201 Richelieu	02010001 -- Lake George
	02010002 -- Otter
	02010003 -- Winooski
	02010004 -- Ausable
	02010005 -- Lamoille
	02010006 -- Great Chazy-Saranac
	02010007 -- Missisquoi
Subregion 0202 Upper Hudson	02020001 -- Upper Hudson
	02020002 -- Sacandaga
	02020003 -- Hudson-Hoosic
	02020004 -- Mohawk
	02020005 -- Schoharie
	02020006 -- Middle Hudson
	02020007 -- Rondout
	02020008 -- Hudson-Wappinger
Subregion 0203 Lower Hudson-Long Island	02030101 -- Lower Hudson
	02030102 -- Bronx
	02030103 -- Hackensack-Passaic
	02030104 -- Sandy Hook-Staten Island.
	02030105 -- Raritan
	02030201 -- Northern Long Island
	02030202 -- Southern Long Island

Region 02 Mid-Atlantic (continued)

Subregion	HUC 8
Subregion 0204	02040101 -- Upper Delaware
	02040102 -- East Branch Delaware
	02040103 -- Lackawaxen
	02040104 -- Middle Delaware-Mongaup-Brodhead
	02040105 -- Middle Delaware-Musconetcong
	02040106 -- Lehigh
Delaware	02040201 -- Crosswicks-Neshaminy
	02040202 -- Lower Delaware
	02040203 -- Schuylkill
	02040204 -- Delaware Bay
	02040205 -- Brandywine-Christina
	02040206 -- Cohansey-Maurice
	02040207 -- Broadkill-Smyrna
	02040301 -- Mullica-Toms
	02040302 -- Great Egg Harbor
Subregion 0205	02050101 -- Upper Susquehanna
	02050102 -- Chenango
	02050103 -- Owego-Wappasening
	02050104 -- Tioga
	02050105 -- Chemung
	02050106 -- Upper Susquehanna-Tunkhannock
	02050107 -- Upper Susquehanna-Lackawanna
	02050201 -- Upper West Branch Susquehanna
	02050202 -- Sinnemahoning
	02050203 -- Middle West Branch Susquehanna
	02050204 -- Bald Eagle
	02050205 -- Pine
	02050206 -- Lower West Branch Susquehanna
Susquehanna	02050301 -- Lower Susquehanna-Penns
	02050302 -- Upper Juniata
	02050303 -- Raystown
	02050304 -- Lower Juniata
	02050305 -- Lower Susquehanna-Swatara
	02050306 -- Lower Susquehanna

Region 02 Mid-Atlantic (continued)

Subregion	HUC 8
Subregion 0206 Upper Chesapeake	02060001 -- Upper Chesapeake Bay
	02060002 -- Chester-Sassafras
	02060003 -- Gunpowder-Patapsco
	02060004 -- Severn
	02060005 -- Choptank
	02060006 -- Patuxent
	02060007 -- Blackwater-Wicomico
	02060008 -- Nanticoke
	02060009 -- Pocomoke
	02060010 -- Chincoteague
Subregion 0207 Potomac	02070001 -- South Branch Potomac
	02070002 -- North Branch Potomac
	02070003 -- Cacapon-Town
	02070004 -- Conococheague-Opequon
	02070005 -- South Fork Shenandoah
	02070006 -- North Fork Shenandoah
	02070007 -- Shenandoah
	02070008 -- Middle Potomac-Catoctin
	02070009 -- Monocacy
	02070010 -- Middle Potomac-Anacostia-Occoquan
	02070011 -- Lower Potomac
Subregion 0208 Lower Chesapeake	02080101 -- Lower Chesapeake Bay
	02080102 -- Great Wicomico-Piankatank
	02080103 -- Rapidan-Upper Rappahannock
	02080104 -- Lower Rappahannock
	02080105 -- Mattaponi
	02080106 -- Pamunkey
	02080107 -- York
	02080108 -- Lynnhaven-Poquoson
	02080109 -- Western Lower Delmarva
	02080110 -- Eastern Lower Delmarva
	02080201 -- Upper James
	02080202 -- Maury
	02080203 -- Middle James-Buffalo
	02080204 -- Rivanna
	02080205 -- Middle James-Willis
	02080206 -- Lower James
	02080207 -- Appomattox
	02080208 -- Hampton Roads

Region 03 South-Atlantic; Gulf

Subregion	HUC 8
Subregion 0301 Chowan-Roanoke	03010101 -- Upper Roanoke
	03010105 -- Banister
	03010201 -- Nottoway
	03010203 -- Chowan
Subregion 0304 Pee Dee	03040101 -- Upper Yadkin

Region 04 Great Lakes

Subregion	HUC 8
Subregion 0411 Southern Lake Erie	04110003 -- Ashtabula-Chagrin
Subregion 0412 Eastern Lake Erie	04120101 -- Chautauqua-Conneaut
	04120102 -- Cattaraugus.
	04120103 -- Buffalo-Eighteenmile
	04120104 -- Niagara
	04120200 -- Lake Erie
Subregion 0413 Southwestern Lake Ontario	04130001 -- Oak Orchard-Twelvemile
	04130002 -- Upper Genesee
	04130003 -- Lower Genesee
Subregion 0414 Southeastern Lake Ontario	04140101 -- Irondequoit-Ninemile
	04140201 -- Seneca.
	04140202 -- Oneida
	04140203 -- Oswego
Subregion 0415 Northeastern Lake Ontario Lake Ontario-St. Lawrence	04150101 -- Black.
	04150102 -- Chaumont-Perch
	04150200 -- Lake Ontario
	04150301 -- Upper St. Lawrence
	04150302 -- Oswegatchie
	04150303 -- Indian
	04150304 -- Grass
	04150305 -- Raquette
	04150306 -- St. Regis
	04150307 -- English-Salmon

Region 05 Ohio River

Subregion	HUC 8
Subregion 0501 Allegheny	05010001 -- Upper Allegheny
	05010002 -- Conewango
	05010003 -- Middle Allegheny-Tionesta
	05010004 -- French
	05010005 -- Clarion
	05010006 -- Middle Allegheny-Redbank
	05010007 -- Conemaugh
	05010008 -- Kiskiminetas
	05010009 -- Lower Allegheny
Subregion 0502 Monongahela	05020001 -- Tygart Valley
	05020002 -- West Fork
	05020003 -- Upper Monongahela
	05020004 -- Cheat
	05020005 -- Lower Monongahela
	05020006 -- Youghiogheny
Subregion 0503 Upper Ohio	05030101 -- Upper Ohio
	05030102 -- Shenango
	05030103 -- Mahoning
	05030104 -- Beaver
	05030105 -- Connoquenessing
	05030106 -- Upper Ohio-Wheeling
	05030201 ---Middle Island.
	05030202 -- Upper Ohio
	05030203 -- Little Kanawha
Subregion 0505 Kanawha	05050001 -- Upper New. North Carolina
	05050002 -- Middle New
	05050003 -- Greenbrier
	05050004 -- Lower New
	05050005 -- Gauley
	05050006 -- Upper Kanawha
	05050007 -- Elk
	05050008 -- Lower Kanawha
	05050009 -- Coal

Region 05 Ohio River: (continued)

Subregion 0507	05070101 -- Upper Guyandotte
	05070102 -- Lower Guyandotte
	05070201 -- Tug. Kentucky
Big Sandy-Guyandotte	05070202 -- Upper Levisa
	05070203 -- Lower Levisa
	05070204 -- Big Sandy
Subregion 0509 Middle Ohio	05090101 -- Raccoon-Symmes
	05090102 -- Twelvepole

Region 06 Tennessee River

Subregion	**HUC 8**
Subregion 0601 Upper Tennessee	06010101 -- North Fork Holston
	06010102 -- South Fork Holston
	06010205 -- Upper Clinch
	06010206 -- Powell