U.S. Fish and Wildlife Service

HANDBOOK OF AQUATIC ANIMAL HEALTH PROCEDURES AND PROTOCOLS

Division of Fish and Aquatic Conservation
November 2022

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1 INTRODUCTION TO HANDBOOK

1.1 BACKGROUND

The U.S. Fish and Wildlife Service (Service) aquatic animal health policy (policy) in 713 FW 1 and 713 FW 2 serves as the basis for the agency's efforts to contain, control, and minimize the impacts of aquatic animal pathogens and diseases on the natural resources of the United States. The policy applies to all Service-managed facilities and field operations involving aquatic animals, including holding, translocation, and propagation.

1.2 PURPOSE

This Service's *Handbook of Aquatic Animal Health Procedures and Protocols* explains how to comply with the policy (713 FW 1 and 713 FW 2) by using good and acceptable practices and procedures.

2 AQUATIC ANIMAL HEALTH MANAGEMENT PLAN

2.1 OVERVIEW

Service fish health policy at 713 FW 2 directs that an Aquatic Animal Health Management Plan (Plan) be developed for all aquatic animals propagated, relocated, or released by Service-managed facilities or involving a Service-managed wild population. This chapter provides guidance for the development of the components that make up the overall Plan referenced in 713 FW 2.

The Plan is a collaborative effort of the Regional Fish Health Program (FHP) and either the facility manager or the manager responsible for a Service-managed aquatic animal activity. The Plan provides an annual overview of aquatic animal health activities for a Service-managed facility or for movements involving a Service-managed aquatic animal population in the wild. Although not required, input may also be provided by other Service offices with responsibilities aligned with the facility or activity, a Fish Technology Center, the Regional office, and formal partners involved in the daily operations of the facility or involved in the aquatic animal activity.

2.2 OBJECTIVES

This chapter provides guidance to Service personnel involved in the holding, translocation, and propagation of aquatic animals, to meet the requirements of 713 FW 1 and 713 FW 2. To achieve this goal, the chapter includes the following:

1) Annual inspection plan for each lot,
2) Lot-specific disease management plans, and
3) Facility biosecurity plan.

2.3 INTRODUCTORY COMPONENTS OF A PLAN

Each Plan must include the following information:

1) Timeline: Plans must be reviewed and updated on an annual basis or more often when the need arises, with the beginning of each covered period dependent on the production cycle of the facility.
2) Project Leader contact information, watershed, location (longitude, latitude).
3) Description of water source(s) and any treatment (UV, filtration, ozone).
4) Description of rearing units at facility (size and numbers).
5) Aerial photo of facility as an appendix.
6) Previous 5-year inspection history for facility.
7) Health regulations and agreements that apply to the facility.
8) Separate section for each lot under consideration at the facility to include:
    a. Common name and strain
    b. Genus and species
    c. Gamete source

d. Age(s) of lot at facility
e. Description of rearing and release schedule
f. Purpose for propagation at facility, i.e., recovery, sportfish, broodstock facility, refugia, research for potential propagation.

2.4 DEVELOPMENT OF THE FACILITY INSPECTION PLAN COMPONENT

The Regional FHP is responsible for the development of a list of pathogens and disease concerns that meets the criteria in 713 FW 2, section 2.6 for each Service-managed facility and aquatic animal activity. To complete this task, the FHP supervisor (also referred to as the Regional FHP supervisor throughout this handbook) should work in consultation with the facility manager, the manager responsible for a Service-managed aquatic animal facility, and the Fish and Aquatic Conservation Assistant Regional Director (FAC ARD). The Plan should include a pathogen list, testing methods, and a sampling plan.

1) The pathogen list must include pathogens that meet the following criteria:
   a. All endemic pathogens that have caused disease in a lot at the facility within the last 5 years and are deemed significant by the Regional FHP supervisor (see "significant pathogen" Appendix I, Glossary);
   b. All pathogens for which inspection is required by applicable Federal, State, Tribal, inter-jurisdictional, or foreign laws, regulations, or guidelines, including all inspections required for fish movements;
   c. Pathogens deemed significant by the Regional FHP supervisor using Appendix II (current U.S. National List of Reportable Animal Diseases and amphibian pathogens listed in chapter 1.3. of World Organization for Animal Health (OIE) Aquatic Animal Health Code) or Appendix III (Fish Pathogen List); and
   d. An emerging or exotic pathogen that is deemed significant.

2) Testing methods:
   a. When available, all test methods must include a screening and a confirmatory test; and
   b. For assays not found in American Fisheries Society (AFS) Fish Health Section guidance, a justification statement for using the assays selected must be included.

3) Sampling plans must include:
   a. All tests required by regulation; and
   b. For other pathogens on the pathogen list, the appropriate sample effort based on AFS Fish Health Section guidance.

   For special case aquatic animals, sampling must be based on a Disease Risk Assessment (DRA) in accordance with AFS Fish Health Section guidance and chapter 7 of this handbook.

Two example sampling plans (Tables 2.1 and Table 2.2) are presented below:

TABLE 2.1 EXAMPLE LOT INSPECTION TABLE:

Purple trout free-ranging brood stock from Red Creek - 2018 inspection plan (version 1).

Pathogen	Significant pathogen[1]	Sample type[2]	APPL[3]	Sample number	Frequency[4]
IHN virus	Yes	OF	2%	150	2 inspections during spawn season to reach APPL
IPN virus	Yes	KS	5%	60	2 inspections during spawn season to reach APPL
VHS virus	Yes	OF	2%	150	2 inspections during spawn season to reach APPL
R.salmoninarum	No	OF	5%	60	2 inspections during spawn season to reach APPL

1. Detection of a ***significant*** pathogen prompts a management action by the Regional Fish Health Program.

2. Sample Type: KS- kidney spleen, OF- ovarian fluid, KD – kidney, BL – blood, HD – cranial elements of head, IT – intestine.
3. APPL – Assumed Pathogen Prevalence Level in the population of 2%, 5%, and 10% (95% confidence level) as per AFS Fish Health Section guidance. Population size will dictate the sample number required to meet the APPL target.
4. Frequency of inspection for the year covered by the inspection plan.

TABLE 2.2. EXAMPLE LOT INSPECTION TABLE

Purple trout production at Blue Lake NFH - 2018 inspection plan (version 2).

Pathogen	Significant pathogen[1]	Sample type[2]	APPL[3]	Sample number	Frequency[4]
IHN virus	Yes	KS	5%	60	biannual
IPN virus	Yes	KS	5%	60	biannual
VHS virus	Yes	KS	5%	60	biannual
R.salmoninarum	No	KD	5%	60	biannual
M.cerebralis	yes	HD	5%	60	annual
N. salmonis	yes	Gill[5]	2%	120	annual – (July)
A.salmonicida and *Y. ruckeri*	no	KD	5%	60	biannual

1. Detection of a ***significant*** pathogen prompts a management action by the Regional Fish Health Program.
2. Sample Type: KS- kidney spleen, OF- ovarian fluid, KD – kidney, BL – blood, HD – cranial elements of head, IT – intestine.
3. APPL – Assumed Pathogen Prevalence Level in the population of 2%, 5%, and 10% (95% confidence level) as per AFS Fish Health Section guidance. Population size will dictate the sample number required to meet the APPL target.
4. Frequency of inspection for the year covered by the inspection plan.
5. Historic *Nucleospora salmonis* outbreak in watershed hatchery but not reported in last 5 years. Screening will be conducted from non-lethal gill clips/PCR assay (AFS Fish Health Section guidance method). If a detection occurs using the screening test, lethal kidney sampling for histology and PCR confirmation will be done.

2.5 DEVELOPMENT OF THE DISEASE MANAGEMENT PLAN COMPONENT

The disease management plan must include:

1) Management strategies to prevent or reduce losses from diseases that have occurred historically in the program, and
2) Response plans that outline responses to take if new pathogens are detected.

2.6 DEVELOPMENT OF THE BIOSECURITY PLAN COMPONENT

The purpose of the biosecurity plan is to prevent the introduction or release of significant pathogens from the facility. Appendix IV contains an example of such a plan. The plan needs to address all the following elements:

1) The specific purpose of the plan.
2) The pathogens included in the plan, including the significant pathogens identified on the facility.
3) Potential pathways of exposure such as:
    a. Water source
    b. Egg and fish source
    c. Fomites (vehicles)
    d. Non-cultured animal contacts
    e. Visitors
4) Any pathogen-related facility Hazard Analysis and Critical Control Points (HACCP) document(s).

a. A list of strategies for mitigation, such as carcass disposal
b. Fomite disinfection
c. Isolation actions
d. Water treatment
e. Egg and fish introductions
f. Aquatic Nuisance Species (ANS)

5) Checklist for actions to complete prior to animal movements.
6) A list of audit steps for mitigation measures:
    a. Who conducts the audit?
    b. What specific items and/or records are on the audit checklist?
    c. When is the audit conducted?
    d. How are audit results communicated to the appropriate hatchery personnel or team for review and corrective actions?
    e. Provision for audit records must be kept on file at the National Fish Hatchery or appropriate Service management office and by the Regional FHP staff for 7 years.

2.7 REPORTING PATHOGEN FINDINGS

Principles for reporting pathogen findings:

1) The 5-year disease history of a facility must be based on inspection results for all aquatic animal lots at the facility.
2) Any time that a pathogen is detected and confirmed by inspection, monitoring, or diagnostics on the facility's inspection plan for a lot, or a pathogen is detected that is an emerging or exotic pathogen deemed significant, the Service aquatic animal health professional must immediately report the detection for the facility on the Fish Health Inspection Report. If it is confirmed by inspection, monitoring, or diagnostics, the Service must notify the appropriate Regional Director, Assistant Regional Director, the Chief of the Branch of Hatchery Operations and Applied Sciences (BHOAS), the facility manager, and potentially affected Federal, State, and Tribal authorities.
3) Bacterial pathogens must be recorded by genus and species. Parasites and fungi must be recorded by genus, and species if known. Viruses must be recorded by their common names.
4) If a detected pathogen is on Appendix II and III and meets one or more of the following criteria, it must be recorded:
    a. New isolation for the geographic area in a new host species,
    b. New pathogen strain or disease manifestation,
    c. Potential for international spread, or
    d. Potential for zoonotic spread.

The Regional FHP supervisor, within 24 hours, must contact the office of the U.S. Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS) Area Veterinarian in Charge (AVIC) to report either the suspicion or confirmation of this pathogen occurrence. The Regional FHP supervisor should also contact the National Coordinator for Aquatic Animal Health, Aquaculture, and Technology for the Service, who will contact their appropriate counterparts at APHIS and the National Oceanic and Atmospheric Administration (NOAA) for national aquatic animal health coordination.

A Fish Health Inspection Report remains valid no longer than 1 year from the time the aquatic animal health professional signs it. If no pathogens on the respective inspection lists are detected at a facility within the inspection period, the facility disease status will be Listed Pathogen(s) Not Detected (LPND).

3 LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS)

3.1 INTRODUCTION TO LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS)

A Laboratory Information Management System (LIMS) is a software package that allows a Regional or national FHP to consistently manage samples and associated data across a network of laboratories. It improves access to data and allows for greater accountability and recall than paper-based approaches. A

LIMS supports the operation of laboratories by standardizing the collection, storage, analysis, and reporting of data. By using a LIMS, laboratories can automate workflows, integrate instruments, manage samples, and even track inventory.

3.2 KEY BENEFITS OF A LIMS

1) Enables workflow automation, reduces human error
2) Centralizes access and storage of data
3) Supports compliance efforts
4) Tracks supplies and inventory
5) Performs instrument run monitoring
6) Initiates data analysis
7) Integrates with instruments or other in-lab systems to improve lab efficiency
8) Standardizes and expands reporting

3.3 SERVICE LIMS PROGRAM

The Service will use a commercially available LIMS software package, which can be purchased, contracted, or developed in partnership with outside parties.

3.4 ADMINISTERING THE AQUATIC ANIMAL HEALTH LIMS WITHIN THE SERVICE

The LIMS software is nationally administered and managed by the BHOAS within the Division of Fish and Aquatic Conservation.

The BHOAS uses LIMS data to generate summary reports for distribution to the Fisheries Management Team (FMT), and for export to partners as needed.

Developing operational business rules for LIMS use in the laboratory is a BHOAS responsibility in collaboration with the LIMS Point Team (LPT).

The onsite operation of LIMS is the responsibility of the individual or individuals assigned to that duty by the FHP supervisor. LIMS data and records are managed under Service Data Management authority and procedures.

3.5 LIMS POINT TEAM

The LPT is a standing subcommittee of the FHC work group that supports the operations of LIMS and is facilitated by the BHOAS. The LPT plays a critical role in the management of LIMS.

Role of the LPT:

1) Point of information distribution and sharing
2) Troubleshooting
3) Promote the use of the software
4) Test and apply system changes and lead future development

Composition of the LPT:

1) One LIMS end user from each FHP facility employing LIMS. We recommend that each lab's primary LIMS user or administrator fill this role.
2) A member of the Headquarters BHOAS serves as facilitator of this team.
3) An alternate will be assigned for each FHP facility to ensure the proper function of the team and system.

LPT Meetings/Calls

1) Bi-weekly calls following system updates or periods of rapid development. Monthly calls at a minimum.
2) Calls are facilitated by the BHOAS.

3.6 LIMS USERS

LIMS is used exclusively by the FHCs to track all the work that the health biologists do in the laboratory. There are three types of users in the field, each with increasing authority. These roles may change as the LIMS develops over time. In some cases, one person may have to fill more than one role.

End user: The end user is the Health Biologist processing diagnostic samples, performing tests, and interpreting the results. A minimum of one person per facility is required, but additional trained staff are recommended. The end user enters cases and posts results.

Lab administrator: The lab administrator is the Project Leader or designee that ensures tests are performed properly and that a case or cases are properly entered and closed out. A lab administrator finalizes, approves, and signs reports.

Super users (Service data management policy calls these Data Stewards and Data Custodians): Super users are individuals granted full LIMS administrator status, and they function as direct liaisons with the software manufacturer for submission of diagnostic tickets and to troubleshoot operational issues. A minimum of two people manage the diagnostic ticket tool for the national system and function as the points of contact to submit diagnostic tickets to the software manufacturer. A super user has the ability to change LIMS permissions and is responsible for adhering to Service data management policy.

3.7 LIMS TROUBLESHOOTING

A user first should report an issue to a member of the LPT. If the LPT is unable to resolve the issue, the user reports the issue to the software manufacturer via a diagnostic ticket.

3.8 RULES FOR LIMS USE

1) All National Wild Fish Health Survey, routine inspections, diagnostic, and contract cases need to be entered into the Service's LIMS.
2) All data fields in LIMS must be entered and a determination needs to be made as to whether the case is funded with National Wild Fish Health Survey-dedicated funds. If so, this box must be checked.
3) Once all data is entered, a determination must be made as to whether this data is to be made public by checking the "public" box.
4) All data must be evaluated for the pervious checked boxes and if complete, the "completed" box is checked.
5) Data is uploaded to the Service server at the end of each month, so all samples need to be marked completed on a monthly basis.

4 PROCEDURES AND PROTOCOLS FOR THE USE OF DRUGS AND CHEMICALS ON AQUATIC ANIMALS

4.1 PURPOSE

The Service is committed to avoiding reliance on, and widespread use of, drugs and chemicals used in treatments to produce and/or maintain healthy aquatic animal populations, both in captive propagation programs and in the natural environment. However, the Service understands that there are circumstances when use cannot be avoided. Given this understanding, it is in the best interest of the animals being treated, the people caring for the animals, the environment, and the American people that only the safest and most effective compounds are used. It is in the best interest of the Service that drug use be appropriate to the level of regulatory oversight required (commercially available, Investigational New Animal Drug (INAD), Veterinary Feed Directive (VFD), prescribed by a licensed veterinarian). This chapter establishes procedures to ensure that the use of such drugs and chemicals by Service-managed facilities and staff are carried out in a safe, effective, judicious, and accountable manner.

The chapter includes standards and procedures concerning the acquisition, use, storage, and disposal of drugs and chemicals used by our Service facilities to capture, handle, propagate, rear, maintain, transport, or release aquatic animals under their care and/or charge. Further, it encompasses both managerial and technical aspects of such activities.

This chapter applies to all Service personnel directly or indirectly involved in such activities that take place in or on Service-managed facilities, as well as field-based activities related to the capture or release of aquatic animals and the transport or distribution of aquatic animals to and from our facilities. This chapter also applies to Service personnel on other facilities who participate in any of these activities. This chapter does not apply to the use of drugs and chemicals in a controlled laboratory setting where there is no contact with aquatic animals or the potential for effluent discharge to public surface waters.

4.2 SERVICE DRUG AND CHEMICAL USE POLICY – IMPLEMENTATION PROTOCOL

Relative to the use of drugs and chemicals by all Service-managed facilities and staff in association with the capture, handling, propagation, rearing, maintenance, transport, and/or release of aquatic animals, Service personnel must use the following protocols:

All drug and chemical use must have the approval of the appropriate Regional FHP supervisor, and when required by law, the servicing veterinarian through a Veterinary Client Patient Relationship (VCPR). If certain drugs or chemicals are used regularly or multiple times within a year and do not require a prescription or VFD (such as with recurring disinfectant or anesthetic use), then Service personnel only need to obtain and document approval for use annually. The Regional FHP supervisor must approve this use. For these specified drugs or chemicals used regularly with Regional FHP supervisor approval, Service personnel may submit necessary reports on an annual basis instead of for each daily or weekly use.

Drugs other than biologics may be used in/on aquatic species only if one of the following conditions applies:

1) A drug is approved by FDA specifically for the subject species and the intended use pattern (e.g., control mortality caused by bacterial coldwater disease, spawning, sedation, etc.).
2) A drug is approved by FDA for a species other than the subject aquatic species (e.g., dog, beef cattle, and swine) and the drug is prescribed by a licensed veterinarian under a valid VCPR (i.e., an extra-label prescription).
3) A drug is the subject of an FDA-authorized INAD exemption sponsored by the Service, the facility manager (Investigator) and attending aquatic animal health professional (Study Monitor) are actively enrolled (by calendar year) for a specific INAD drug use in the Aquatic Animal Drug Approval Partnership Program's (AADAP) National INAD Program, and the drug is used in

accordance with a study plan approved by AADAP. This would include the reporting requirements of use and conditions specified by the INAD process.

4) A drug is on FDA's list of Low Regulatory Priority (LRP) drugs, and its use is consistent with FDA's specified conditions for use.
5) A drug is on FDA's list of Deferred Regulatory Status drugs.
6) A drug is listed by FDA as an "Index drug," and the drug is purchased from the listed supplier and is used consistent with FDA's conditions for use as defined in the "Index List."

If the drug is a biologic, one of the following conditions must apply:

1) The biologic is a USDA-licensed vaccine, bacterin, antiserum, or other biological product.
2) The veterinary biologic is a legally imported foreign product for which the manufacturer has a legal representative residing in the United States who possesses a valid U.S. Veterinary Biological Product Permit to import the product for general distribution and sale.
3) The biologic is exempt from Federal regulation because it was:
    a. Manufactured by a veterinarian and is intended solely for use with their clients' animals under a VCPR;
    b. Manufactured by individuals or companies for use only in their own animals; or
    c. Manufactured in a State with a USDA-approved veterinary biologics regulatory program, and for sale only in the State in which it was manufactured.

For a condition or disease affecting a federally listed Threatened or Endangered (T&E) species, the Service may use unapproved drugs and chemicals for therapeutic use if:

1) An aquatic health professional determines that the health and welfare of the subject T&E species is in jeopardy;
2) Such use meets local, State, and Federal discharge permitting requirements; and
3) Prior to such use on a T&E species, the facility contacts AADAP and obtains permission to proceed, and completes and submits an AADAP Form TE-1 (Receipt of Drug) and a Form TE-2 (Drug Use Log).

Chemicals intended for use on aquatic species and aquatic species-associated rearing units and equipment, which have the potential for discharge to public surface waters, may be used only when all of the following conditions apply:

1) The user (applicator) has read the chemical's Safety Data Sheet (SDS) and all recommended safety precautions have been addressed (e.g., safety glasses, proper clothing, gloves, etc.).
2) It is a chemical deemed by EPA to be legally marketable and is used according to labeled specifications. This includes EPA-registered pesticides, pesticides that are the subject of an EPA-issued "Experimental Use Permit," and pesticides that are the subject of an "Emergency Exemption" provided to a Federal or State agency by the EPA Administrator who has determined that emergency conditions exist.
3) The chemical is used in accordance with the label, its use complies with applicable local and State laws, and in the case of a pesticide, the applicator holds a valid pesticide applicator certification or is under the direction of a certified pesticide applicator.

With respect to all chemical, pesticide, and drug use the facility must:

1) Comply with EPA's Aquaculture Effluent Guidelines (*For additional information on these guidelines, visit the EPA website)*.
2) Possess a valid National Pollutant Discharge Elimination System (NPDES) permit if required (in the form of an individual, blanket, or group permit) that permits the discharge of the subject compound.
3) Ensure the discharge of the subject drug or chemical does not exceed the NPDES limits either as the cumulative total or a single discharge limit.

4) Ensure use of the pesticide complies with the Department of the Interior's Integrated Pest Management Policy (517 DM 1) and the Service's policies at 242 FW 7, Pesticide Users Safety, and 569 FW 1, Integrated Pest Management.

Any other type (condition) of drug and chemical use by Service personnel that is not described above is NOT permitted.

All drug and chemical use (as defined within this handbook), no matter how it is used (i.e., approved, licensed, or registered products, or products used under emergency use, investigational use, extra-label use, low regulatory priority use, etc.), must be documented by the facility or entity using the drugs and/or chemicals as described below:

1) All facilities and entities must update the forms for Drug Receipt Records and Drug Use Records and/or Chemical Receipt Records and Chemical Use Records via the online database on at least a weekly basis. The total amount of each routinely used drug or chemical, such as disinfectants or anesthetics, may be reported at the end of each calendar year rather than at each use. This routine use information must be entered no later than 30 days following the end of each calendar year. Both field facilities and Regional FHCs should archive this information (originals and copies, respectively) for a period of 5 years.
2) If drugs are used under an INAD exemption, all other applicable National INAD Program forms must be completed and submitted to AADAP per national INAD Program (and FDA) requirements.
3) Additionally, facilities and entities must comply with all other reporting requirements as defined by the regulatory authority for drugs, chemicals, or biologics.

5 AQUATIC ANIMAL HUSBANDRY PRACTICES

5.1 IODOPHOR DISINFECTION OF FISH EGGS

5.1.1 PURPOSE

This section of the chapter describes the use of approved iodophor solutions (polyvinylpyrrolidone iodine) for the surface disinfection of certain fish eggs. Iodophor egg disinfection (IED) has been reported to increase survival in a variety of teleost eggs such as salmonids, sturgeon, grouper, and halibut. IED reduces the transmission of some pathogenic agents associated with the egg, coelomic fluid, or milt. However, the use of iodophor disinfection will not prevent vertical transmission. The range of recommended active iodine concentration for IED is reflective of the different sensitivities of various species and stocks. Hatchery personnel must first determine sensitivity of the eggs for a given fish species to water hardening and surface disinfection in iodophor prior to adoption of IED on a production level. In certain species, water hardening in iodophor results in poor eye-up of eggs or egg losses due to toxicity.

5.1.2 RECOMMENDED USAGE

5.1.2.1 Salmonid (trout, salmon and whitefish) egg disinfection

All salmonid eggs shipped from Service-managed facilities must be disinfected in 50-100 mg/L (ppm) iodine for 30-60 minutes during the water-hardening process. The ratio of eggs to iodophor solution should be a minimum of 1:4. Allowing an appropriate time for fertilization, eggs should then be washed in pathogen-free water (well water or UV filtered) to remove organic matter at a temperature similar to that experienced by the brood fish prior to disinfection. The objective of this step is to reduce the contaminant effects of semen, blood, and coelomic fluid on IED. It is essential that iodophor not come in contact with water used in the fertilization step. The pH of the iodophor solution should remain between six and eight. Testing with active iodine test strips for activity level of the IED should be done as appropriate to ensure the target level is achieved.

All salmonid eggs received at a Service-managed facility must be properly disinfected before they come into contact with fish cultural water, rearing units, or equipment at the receiving station, and preferably in an area separate from the incubation or rearing areas. Eggs being disinfected upon receipt must be rehydrated during temperature acclimation by placing them in pathogen-free water for 30-60 minutes. If temperature acclimation is not required, a similar time for rehydration is required before adding iodine compound to replenish water loss that occurs in the eggs during shipping. Eyed eggs must be disinfected in a solution providing 100 mg/L of active iodine for 10-30 minutes at an egg to iodophor solution ratio of 1:4, then held in pathogen-free water. Care must be taken to avoid treatment of hatched fry as they are extremely sensitive to iodophor exposure. Iodine may be buffered by the addition of sodium bicarbonate ($NaHCO_3$) if the pH is low.

Hatchery staff must monitor the color of the solution over the treatment period. Iodine concentrations below 20 mg/L (ppm) appear a light yellow and indicate that IED is not meeting minimum standards. The use of pathogen-free water at the appropriate temperature for egg fertilization, rinsing, and preparation of iodophor solution is an important factor in disease control. Additionally, the use of pathogen-free water for egg incubation will greatly enhance the effectiveness of IED.

The proper ratio of egg mass to iodophor solution, and gentle circulation, will improve IED efficacy. Iodine concentration within the egg mass will be significantly lower than the egg mass surface if the iodophor solution is not circulated. The primary objective is sufficient contact of all egg surfaces by the solution.

5.1.2.2 Non-salmonid egg disinfection

Before IED is incorporated as a standard practice in non-salmonid aquatic animal species, target animal safety and efficacy must be evaluated. Such factors as water quality parameters, water temperature, treatment methods (i.e., static bath versus flow through), and pre-treatment procedures to counteract egg adhesiveness or egg masses common to certain species must be considered. Peer-reviewed literature and the Regional FHP supervisor should be consulted prior to egg disinfection in any non-salmonid species.

5.1.3 HUMAN SAFETY

Several steps must be followed to ensure worker safety. When performing IED at the recommended concentrations, proper Personal Protective Equipment (PPE) such as rubber gloves, rain suits or aprons, rubber boots, and eye protection must be used. (See online Safety Data Sheet (SDS) for Ovadine®.) Eye protection can consist of either safety glasses or goggles to prevent eye injury from splash.

5.1.4 DISPOSAL

Disposal of iodophor solutions must comply with all local, State, and Federal policies and regulations.

5.1.5 REFERENCES CONSULTED

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5.2 FACILITY DISINFECTION AND SANITATION

5.2.1 PURPOSE

The following procedures are provided as recommendations for disinfecting a facility at which a significant, new, exotic, or emerging pathogen has been detected (see 713 FW 2). Since facilities often differ in operations, equipment used, and exposure to potential pathogens, the disinfection and sanitation procedures described here are subject to equivalency modification. Site-specific needs are often best captured in an HACCP plan. As an international standard (ASTM E2590 - 08), HACCP provides a structured method to identify risks and focus procedures to reduce or eliminate the spread of unwanted species or pathogens during specific processes.

Service policy at 750 FW 1 requires all relevant staff in field stations and offices to become familiar with the principles of HACCP as a quality control planning tool, identify how they will apply HACCP planning to their operations, and implement and document HACCP planning as standard operating procedure. For more information on the HACCP planning process, see the appendix to 750 FW 1.

The disinfection process may vary depending on whether the overall objective is disease prevention, control, or eradication. Procedures addressing eradication are considered as a last resort and generally involve destruction and removal of all aquatic animals as well as disinfection of aquaculture establishments and equipment. Disease control aims at limiting the spread of disease between or within aquaculture establishments. Although different approaches may be used to achieve the identified objective, the general principles described below should be applied in all cases.

5.2.2 DISINFECTION PROCESS

The disinfection process should include the following phases:

1) Cleaning and washing
    a. Cleaning and washing of surfaces and equipment should always precede the application of disinfectants. It is necessary to remove solid waste, organic matter (including biofouling), and chemical residues as these may reduce the efficacy of disinfectants. The detergent used should be compatible with the disinfectant and the surface being treated. After cleaning procedures, any excess water should be drained before application of disinfectants.
    b. Where treatment of water is required, the presence of suspended solids may also reduce the efficacy of some disinfectants. Removal of suspended solids through various processes such as filtration, sedimentation, coagulation, or flocculation should be performed.
    c. Biofilms, often referred to as slime, are a thin film of microorganisms and extracellular polymeric substances that adhere to surfaces and piping. Biofilms physically protect embedded microorganisms against disinfectants. In order to achieve effective disinfection, biofilms should be removed during the cleaning and washing stage prior to the application of disinfectants.
    d. All waste produced should be disposed of in a biosecure manner, such as burial with hydrated lime, because it may contain viable pathogenic agents that have the potential to spread infection if not controlled.

2) Application of appropriate disinfectants
    a. This phase involves the application of chemical compounds or physical processes that are appropriate to inactivate the pathogenic agent.
    b. Chemical disinfectants should be stored, used, and disposed of in accordance with local policies or regulations, permit requirements, and manufacturer's instructions. Disinfectants may present risks to the health of people, aquatic animals, and the environment. All relevant SDSs should be available for all chemicals used by staff, and all

chemicals should be properly labeled with hazard classification information. The proper PPE should be used in accordance with station-specific safety plans and procedures.

c. The application of disinfectants should take into account the type of material requiring disinfection and how disinfectants should be applied. Hard, non-permeable materials (e.g., polished metal surfaces, plastics, and painted concrete) can be cleaned thoroughly and allow contact with the disinfectant because there is little opportunity for infective material to lodge in crevices. Disinfection efficacy will decrease if the surface is corroded, pitted, or paint is flaking, so proper maintenance of equipment is essential. For permeable surfaces and materials (e.g., woven material, nets, and soil), a higher disinfectant concentration and a longer contact time is required because the surface area is greater, chemicals cannot penetrate easily, and residual organic matter may be present.
d. The choice of the application method should ensure that the agent contacts all surfaces for the required period of time. The application of disinfectants should be undertaken methodically (e.g., using a grid pattern) to ensure that complete coverage and adequate contact times are achieved. Each phase should start from the highest point and proceed downwards, commencing from the least contaminated areas. However, for some equipment, rinsing of surfaces with the disinfectant may be sufficient. When disinfectants are applied to vertical surfaces, care should be taken to ensure that the required contact time is maintained before the disinfectant drains away. Vertical surfaces may need retreatment or the addition of compatible foaming agents to prolong adherence to surfaces.
e. For pipes and biofilters, they should be filled completely with the disinfectant solution to ensure contact with all surfaces. Difficult to access and complex areas may require use of misting equipment or should be drained and allowed to fully dry.

3) Removal or inactivation of the disinfectant
   a. Removal or inactivation of chemical residues is important to avoid toxicity to aquatic animals, corrosion of equipment, and environmental impacts.
   b. Processes that may be employed for the removal or inactivation of chemical residues include rinsing of surfaces, dilution to acceptable levels, treatment to inactivate chemical agents, or time to allow deactivation or dissipation of the active compound. The need to remove or inactivate chemical disinfectants from treated effluent water should also be evaluated. These processes may be used in isolation or in combination.

5.2.3 MONITORING

Disinfection should be monitored to ensure the appropriate dose of disinfectant and disinfection efficacy. Depending on the application process and the pathogenic agent of concern, this may be done in different ways. Examples include measurement of the active agent (e.g., residual chlorine levels); indirect measurement of the active agent by an indicator process (e.g., monitoring oxygen reduction potential); use of indicator test strips such as for iodine, Virkon® Aquatic, and chlorine; and measurement of efficacy using indicator bacteria (e.g., heterotrophic bacteria plate counts).

5.2.4 RECORDKEEPING

Aquaculture establishments should keep records of the disinfection processes, including types of disinfectants and concentrations applied. The records should be sufficient to allow evaluation of the disinfection plan and to assist in future disinfection episodes.

5.2.5 SELECTION OF A DISINFECTANT

The disinfectant should be selected considering the following:

1) Efficacy against pathogenic agents,
2) Effective concentration and exposure time,
3) Ability to measure efficacy,
4) Nature of the items to be disinfected and the potential for them to be damaged during disinfection,
5) Compatibility with the available water type (e.g., fresh water, hard water, or seawater),

6) Availability of the disinfectant and equipment,
7) Ease of application,
8) The ability to remove organic matter,
9) Cost,
10) Impacts of residues on aquatic animals and the environment, and
11) User safety.

5.2.6 TYPES OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

Aquaculture facilities and equipment differ widely in their characteristics. Described below are some considerations for effective disinfection of different types of aquaculture rearing environments/vessels and equipment.

1) Ponds
    a. Ponds are generally large and may be earthen based or fitted with plastic liners. These characteristics together with the large volumes of water make cleaning prior to decontamination difficult, and high organic loads can affect many chemical disinfectants.
    b. Ponds should be drained of water and have as much organic matter as possible removed prior to disinfection. Preferably, they should be allowed to dry in between rearing cycles.
    c. Organic matter should be disinfected or disposed of in a bio-secure manner, such as burial with lime.
    d. Enough hydrated lime (calcium hydroxide) should be applied to cover the entire pond bottom and sides with a thin layer.
    e. Scraping, ploughing, or tilling of the base of unlined ponds also helps to incorporate liming compounds and with drying.
    f. Slowly add water to rapidly raise the pH (to 12 or higher) for 1 week.
    g. Allow adequate time for the lime to break down (dependent on rainfall, temperature, soil chemistry, and organic matter content).
    h. Check water pH before re-stocking.

2) Tanks
    a. Tank construction material (e.g., fiberglass, concrete, or plastic) will drive the type of disinfection method used. Bare concrete tanks are susceptible to corrosion by acids and potential damage by high pressure sprayers. They are also porous and require longer application of chemicals to ensure contact time of disinfectant. Plastic, painted, and fiberglass tanks are more easily disinfected because they have smooth, non-porous surfaces that facilitate thorough cleaning, and they are resistant to most chemicals. When disinfectants are applied to vertical surfaces, care should be taken to ensure that adequate contact time is maintained before the disinfectant is drained.
    b. Tanks should be drained of water and have as much organic matter as possible removed prior to disinfection.
    c. Tank surfaces should be cleaned thoroughly and washed using high-pressure sprayers or mechanical scrubbing with detergent to remove fouling such as algae and biofilms. Heated water may be used to enhance the cleaning process.
    d. Tank equipment should be removed for separate cleaning and disinfection, and all organic waste and debris removed and properly disposed.
    e. Any excess water should be drained before application of disinfectants.
    f. Following disinfection, tanks should be rinsed thoroughly to remove all residues and allowed to dry completely.

3) Pipes
    a. Disinfection of pipes may be difficult due to lack of access and formation of biofilms. Pipe construction material should be taken into consideration when selecting the disinfection method. All steps require pipes to be fully filled so that internal surfaces are treated.

b. Pipes can be cleaned effectively through the use of alkaline or acid solutions (see Table 5.1), or foam projectile pipe cleaning systems (projectile should be 20-30% larger than the internal diameter of the pipe) or pipeline pigging products or devices.
c. Effective disinfection in pipes requires the removal of biofilms, followed by flushing of the resulting particulate matter and thorough rinsing.
d. Once pipes are cleaned, chemical disinfectants or circulation of heated water can be used.

4) Cage nets, standard nets, and other fibrous materials
   a. Nets used in cage culture are often large, difficult to handle, have significant levels of biofouling, and are usually made from fibrous materials that trap organic matter and moisture. Nets should be dedicated to a single aquaculture establishment or area because they have a high likelihood of contamination and may be difficult to disinfect.
   b. To aid in the penetration of chemical disinfectants, nets should be thoroughly cleaned prior to disinfection by first removing organic matter and then washing with a detergent solution.
   c. Following cleaning, nets should be disinfected by complete immersion in chemical disinfectants or heated water (see Table 5.1 for temperature).
   d. Treatment duration should be sufficient to allow penetration into net material.
   e. The treatment method should be chosen considering the potential to weaken or damage nets, such as for dip nets, cage nets, and ropes.
   f. Following disinfection, nets should be completely dried before storage. Other fibrous materials such as wood, ropes, and dip nets have characteristics similar to cage nets, and they require special consideration, including using site-specific equipment whenever possible and discarding fibrous materials and replacing them with non-porous alternatives (e.g., replacing wooden dam boards or wooden handled nets with metal).

5) Vehicles
   a. A designated area, preferably off-site, should be established for disinfecting vehicles, including trailers.
   b. All potentially contaminated internal and external surfaces should be disinfected.
   c. Special consideration should be given to areas likely to be contaminated, such as the internal surface of containers, pipes, and transportation water.
   d. The application of corrosive disinfectants to vehicles should be avoided, but if used, all residues should be removed or rinsed following disinfection.
   e. Oxidative compounds such as chlorine (see Table 5.1) are the most commonly used disinfectants for vehicles.

6) Buildings
   a. Aquaculture establishments include buildings for culture, harvesting, and processing of aquatic animals, and other buildings associated with storing feed and equipment. The approach to disinfection may vary depending on the structure of the building and degree of contact with contaminated material and equipment.
   b. Buildings should be designed to allow effective cleaning and thorough application of disinfectants to all internal surfaces.
   c. Surfaces should be non-porous for more effective cleaning and disinfection.
   d. Wherever possible, buildings should be cleared of debris and emptied of equipment prior to disinfection.
   e. Misting or foaming agents are options for disinfection of complex areas and vertical surfaces.

7) Containers
   a. Containers range from simple plastic bins to complex tank systems used for the transport of gametes, harvested aquatic animal products, dead aquatic animals, and live aquatic animals.
   b. Containers are generally manufactured using smooth non-porous material (e.g., plastic, steel) which are typically disinfected easily. They should be considered high risk items

because they are in close contact with aquatic animals or their products (e.g., blood, ovarian fluid, milt, or diseased aquatic animals). The need to move them between locations makes them potential vectors for the spread of pathogens. For transporting live aquatic animals, containers may also have pipes and pumping systems and confined spaces that should also be disinfected.

c. All water should be drained from the container and any aquatic animals, fecal matter, and other organic material removed by scrubbing or flushing with clean water and disposed in a biosecure manner.
d. All pipes and associated pumps should also be inspected and flushed.
e. Containers should be washed using appropriate chemical detergents, combined with high-pressure water cleaners or mechanical scrubbing.
f. All internal and external surfaces of containers should be treated using an appropriate disinfection method.
g. All treated surfaces should be rinsed and inspected to ensure there are no organic residues, and they should be stored in a manner that allows them to drain and dry quickly.

8) Boats

As part of the disinfection planning process, an assessment should be made to identify areas likely to be contaminated, such as in and around machinery, holding tanks, bilges, and pipes. All loose equipment should be removed prior to disinfection. Additional procedures should be developed for well-boats because of their potential to transfer pathogenic agents through the discharge of contaminated water. Contaminated effluent water should be disinfected and neutralized if necessary, prior to discharge. In general, we recommend following the “Clean, Drain, Dry” procedure unless more in-depth disinfections and decontamination procedures are required to manage a specific pathogen or for field operations, such as collecting samples for eDNA analysis. Please refer to your field station’s HACCP plan for site- and activity-specific procedures.

a. CLEAN off visible aquatic plants, animals, and mud from all equipment before leaving water access: (Table 5.1).
b. Rinse equipment and boat hulls (with high pressure, hot water when possible).
c. Rinse interior compartments of boats with low pressure, hot water (120°F).
d. Flush motor with hot water (120°F) for 2 minutes (or according to owner’s manual).
e. Organic material should be regularly removed from decks and work areas.
f. Biofouling organisms that may act as vectors should be removed and disposed of properly.
g. DRAIN motor, bilge, live well, and other water containing devices before leaving water access.
h. DRY everything for at least 5 days OR wipe with a towel before reuse.
i. Boats and trailers should be allowed to dry, preferably in direct sunlight, to limit wastewater entering the aquatic environment.
j. Where boats cannot be removed to land, a disinfection method should be chosen that minimizes the discharge of toxic chemicals into the aquatic environment. It may be necessary to use divers to inspect and clean hulls.
k. When appropriate, mechanical methods such as high-pressure sprayers or steam cleaners should be considered as an alternative to chemical disinfection for cleaning above and below the waterline.

9) Biofilters

a. Biofilters associated with closed or semi-closed production systems are an important control point for pathogens. Biofilters are designed to maintain a colony of beneficial bacteria used to enhance water quality. The conditions that support these bacteria may also enhance survival of some aquatic animal pathogens. It is normally not possible to disinfect biofilters without also destroying beneficial bacteria. Therefore, potential water

quality issues should be taken into account when planning strategies for disinfection of biofilters.

b. The system should be drained, organic residues removed, and surfaces cleaned.
c. Disinfection of biofilter systems can be undertaken by modifying water pH levels (using either acid or alkaline solutions - Table 5.1).
d. The pH levels must be sufficient to inactivate the pathogenic agent (usually above pH 12), but should not be corrosive to pumps and equipment within the biofilter system.
e. Alternatively, the biofilter can be completely dismantled, including removal of biofilter substrate, and the components cleaned and disinfectants applied separately.
f. Some biofilter substrate can be disinfected by drying or fallowing and exposing it to direct sunlight.
g. For emergency disease response, the biofilter substrate should be replaced if it cannot be effectively disinfected.
h. Biofilter systems should be thoroughly rinsed before reusing.

10) Husbandry equipment
    a. Aquaculture establishments normally have a range of husbandry equipment items that come into close contact with aquatic animals and have potential to act as vectors. Examples include graders, automatic vaccinators, fish pumps, waders, rain gear, boots/shoes, gloves, nets, brushes, screens, dam boards, buckets, All Terrain Vehicles/ Utility Task Vehicles (ATVs/UTVs), carts, and other hatchery equipment.
    b. Each item should be examined to identify areas that come into close contact with aquatic animals and where organic material accumulates.
    c. If required, equipment should be dismantled to allow adequate cleaning and application of disinfectants.
    d. Multiple items can be simultaneously disinfected in a large tub if necessary.

11) Disinfection of intake water

    Aquaculture establishments may need to disinfect intake and effluent water to eliminate pathogenic agents. Ideally, a facility would choose a water source with low to no disease organisms, such as well water. However; when that is not possible, the most appropriate disinfection method will differ depending on the disinfection objective and the characteristics of the water to be disinfected. Potentially contaminated water (i.e., from a pond, tank, or effluent) should be disinfected with:

    a. Acid – lowering the pH such as with formic acid (HCOOH) or hydrochloric acid (HCl); mechanic separation (≤300um filter) and pH ≤3 for ≥8 hrs;
    b. Base – raising the pH such as with sodium hydroxide (NaOH); mechanic separation (≤300um filter) and pH ≥12 for ≥24 hrs;
    c. UV irradiation – mechanic separation (≤40 um filter) followed by UV irradiation using an UV-dose ≥25 mWs/cm^2 (254 nm) for VHSV, IHNV, ISAV, *Aeromonas hydrophila*, *A. salmonicida, Vibrio anguillarum*, and *Yersinia ruckeri*. A UV-dose of 1500 mWs/cm^2 is needed to inhibit IPNV;
    d. Chlorination – Amount needed depends on temperature, pH, organic contamination, and titer of the pathogen. Mechanic separation (≤300um filter) followed by an initial concentration of ≥50 mg/l residual chlorine and ≥2 mg/l residual chlorine after 25 mins of treatment;
    e. Heat – 65°C for 10 mins, 70°C for 5 mins, 75°C for 4 mins, 80°C for 3 mins, 85°C for 2 mins, 90°C for 1 min, 95°C for 45 sec, 100°C for 30 sec. Ensure proper stirring to avoid pockets of water not reaching desired temperature;
    f. Iodine - ≥150 ppm for 10 mins at pH <8; or
    g. Ozonation - ≥15mg/l residual ozone after 15 mins treatment.

TABLE 5.1 COMMON DISINFECTANTS IN AQUACULTURE

Agent/Method	**Useful Applications**	**Concentrations and Contact Times Conditions**	**Comments**
Alcohols	Skin antiseptic Surface disinfection	Ethanol: 70-80% Isopropanol: 60-80% 10-30 minutes	Evaporation can cause short/inadequate contact time Can dry/fix organic material onto hard surfaces
Chlorine	Clean equipment and surfaces Aqueous suspensions	General disinfectant: 1000 ppm for 10-30 minutes 500 ppm for 15 minutes to kill *Myxobolus cerebralis* (salmonid whirling disease) spores Equipment soak: 200 ppm for 10 minutes Water/pipelines: 50 ppm for 30 minutes Effluent water: 2 ppm for 1 hr	Corrosive Inactivated by organic material Monitor/maintain chlorine level during long exposures Must be neutralized prior to discharge into the environment
Iodine/Iodophor	Equipment Hands Foot bath Egg disinfection	Clean, dry surfaces/equipment: 100-200 ppm for 10 minutes General equipment, dry but not cleaned: 200-250 ppm for 10 minutes Cleaned ponds: 100 ppm for 1 hour Egg disinfection: 100 ppm for 10 minutes	Inactivated by organic material Monitor/maintain iodine level Slightly corrosive
Quaternary Ammonia Compounds (Quaternaries or “Quats”)	Non-critical surfaces Instrument soak	General: 500 ppm for 10 minutes *M. cerebralis* (salmonid whirling disease) spores -1500 ppm for 10 minutes	Inactivated by organic material
Virkon Aquatic®	Surfaces Equipment	General: 1 or 2% for 10 minutes	Inactivated by organic material and ultra-violet light Powder form is corrosive
Virkon Aquatic®	Boats	Not effective on Whirling Disease spores	Non-corrosive when mixed with water
Moist Heat	Heat resistant solids and liquids	65°C for 10 minutes. 70°C for 5 minutes. 75°C for 4 minutes. 80°C for 3 minutes. 85°C for 2 minutes.	Spores may be resistant Non-toxic/non-chemical option Risk of steam burns

Agent/Method	Useful Applications	Concentrations and Contact Times Conditions	Comments
		90°C for 1 minute. 95°C for 45 seconds. 100°C for 30 seconds.	
Dry Heat	Heat resistant solids	180°C for 2 hours	Spores may be resistant Non-toxic/non-chemical option Risk of burns
UV Light	Air Surfaces	Close range, high dose	Non-toxic/non-chemical option Lack of penetrating power Shadows reduce effectiveness Suspended solids need to be removed prior to use in water Caution must be taken to avoid over-exposure by staff
Hydrogen Peroxide (H2O2)	Surfaces Egg disinfection	General disinfection: 3% for 30 minutes or 30% for 3-5 minutes Egg fungicide: Up to 500 ppm for 10-60 minutes every other day	No toxic decomposition products Concentrated solutions may cause eye/skin irritation Inactivated by organic material If using 35% Perox-Aid, follow labeled instructions
Acids	Pipes Water Surfaces	Formic or hydrochloric acids at pH ≤3 for ≥8 hrs 5% acetic acid	Requires pre-treatment filtration Inactivated by organics Can be highly corrosive Can be caustic and cause chemical burns
Alkaline (base)	Pipes Water Ponds	Sodium or ammonium hydroxide, sodium carbonate (used in hot solution 180°F) or 4% solution; calcium oxide (1,000-2,000 kg/ha;181-363lb/surface acre)	May have limited antimicrobial spectrum Inactivated by organic materials Can be corrosive Can be caustic and cause chemical burns

Considerations for all methods:

1. Equipment and surfaces must be cleaned thoroughly prior to disinfection.
2. Contact time is critical for complete disinfection! Contact time reflects the exposure of equipment, a surface, water, etc. to a chemical or physical (UV light, heat, etc.) disinfection method, not the total amount of time spent disinfecting (for example, if you are using 65°C water to disinfect a raceway, you must apply 65°C water to each area of the raceway for a minimum of 10 minutes).
3. Read SDSs and product labels prior to disinfecting.
4. Wear all required PPE, keep separate from water.
5. Comply with all Federal, State, and local regulations.

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5.3 ISOLATION AND QUARANTINE

5.3.1 PURPOSE

The purpose of this section is to provide recommendations for the construction and general operation of facilities for the isolation and quarantine of live aquatic animals. This section may be used in risk management on a case-by-case basis to reduce the risk posed by movement of aquatic animal pathogens.

5.3.2 QUARANTINE AND BIOSECURITY PRINCIPLES

Quarantine can apply any time to any species and is often used to prevent movement of serious pathogens into a facility or into naive wild aquatic animal populations via infected broodstock, gametes, or juveniles. Basic requirements for effective quarantine must include following established protocols, which, at a minimum, address the following:

1) Proper quarantine facility location (protected against or not subject to flooding).
2) Adequate physical infrastructure or design appropriate for the level of containment needed.
3) Security and restriction of movement of quarantined subject or material in or out of the facility.
4) Proper disinfection of intake and effluent waters (see chapter 5.2).
5) An established set of operating requirements.
6) Prevention of accidental escapement or release of quarantined subject or material.
7) Appropriate and adequate holding period and testing requirements.
8) Documentation of staff movement into or out of the quarantine facility or unit.

5.3.3 GENERAL REQUIREMENTS FOR CONSTRUCTION OF A QUARANTINE FACILITY

1) The facility location is determined on a case-by-case basis and depends on individual facility layout and space. It should not be located in areas subject to flooding.
2) The facility should be structurally separated, such as with a separate building, from all other operations and used solely for holding designated aquatic animals in quarantine (see 713 FW 2).
3) The facility should not serve as access to other buildings or activities.
4) The facility should be weatherproof and properly maintained.
5) The facility should be a secure, lockable building potentially surrounded by security fence with self-closing doors.
6) The entrance to the facility should be through a separate outer change room for staff to wash hands and change outer clothing prior to entering or leaving the quarantine area.
7) The holding capacity of the facility should correspond with proposed quantities of the species to be held and provision must be made for growth and maturation of the parent stock and holding of all progeny and subsequent generations if necessary.
8) Drains leaving the facility should include appropriate screens or plugs to prevent accidental escape of animals and should go to a chlorine-resistant holding tank for containment and disinfection of possible uncontrolled water releases.
9) The facility should be equipped with all necessary supplies, equipment, tanks, and disinfectants, which never leave the facility.

10) The facility should be equipped with backup systems of essential components (e.g., water circulation, automatic shut-off water valves, electricity, temperature control, filtration, water treatment systems).

5.3.4 CRITERIA FOR MOVING ANIMALS INTO OR OUT OF QUARANTINE AND QUARANTINE DURATION

1) The FHP supervisor and the Service manager of the affected facility must perform a Disease Risk Assessment (DRA) (see chapter 6) prior to movement of aquatic animals into or out of a quarantine facility.

2) Specific criteria for movement and quarantine duration must be based on the DRA and all health data available for the quarantine population (or surrogates in the same waters of origin) and receiving facility or location.

3) Influent water should be from an approved groundwater source free of biological material or filtered and sterilized using an approved method such as ultraviolet (UV) irradiation or ozone disinfection.

4) All wastewater should be appropriately disinfected, and should not be discharged directly into natural waterways or areas that may drain into natural waterways (see section 5.2, agent/methods to disinfect water).

5) The quarantine facility should remain clean and tidy at all times.

6) No other animals or foods for aquatic animals should be permitted in the quarantine area.

7) All feeds used within the facility should have prior supervisor approval and be in a sanitary condition.

8) Live food should not be used unless no other alternative is sufficient for the dietary needs of the species and/or life stage in culture.

9) Animals should be monitored closely for signs of illness or abnormal behavior.

5.3.5 EQUIPMENT AND DISINFECTION GUIDELINES

1) Equipment used within the facility should not be shared between rearing units, and separate equipment should be kept for each unit.

2) All equipment within the quarantine facility should be disinfected after each use. See Table 5.1 for agents and methods to disinfect.

3) All footwear and protective clothing used in the quarantine area should be restricted to that facility.

4) A footbath containing an approved disinfectant should be maintained at the entrance of the quarantine area (see Table 5.1, Common Disinfectants in Aquaculture).

5.3.6 DISPOSAL OF DEAD ANIMALS

1) Aquatic animals that die in quarantine should be held in leak-proof bags or containers in an approved and labeled freezer kept within the quarantine unit and properly disposed (see chapter 7, section 7.3.4, Carcass Burial or Disposal Guidelines).

2) Any equipment that has been in contact with dead animals should be disinfected before reuse.

5.3.7 DISEASE OUTBREAK/PATHOGEN DETECTION PROCEDURES

1) If a significant pathogen is confirmed, a team of Service aquatic animal health professionals should follow the procedures in chapter 7.

2) Disease control measures within the quarantine unit may include the extension of quarantine, treatment of animals, and/or the destruction of stock.

3) A Service aquatic animal health professional should determine the appropriate procedures for disease or pathogen detection and any control measures.

5.3.8 RECORDKEEPING

A complete history of the quarantined aquatic animal stock should be maintained and, at a minimum, include the following information:

1) Supplier or country/area/facility of origin.

2) Date of arrival of parent stock.

3) Planned date of release.

4) Number of animals in original shipment and mortalities upon arrival.

5) Number of animals stocked in each rearing unit.

6) Details of any disease signs and number affected, by rearing unit.

7) Details of any diagnostic tests, examinations, and certifications.

8) Individual record sheets for each rearing unit with information relevant to each unit (i.e., identification, daily mortalities, treatments, feed type, and rates).

9) Quarantine facility operations and entry/exit logbooks.

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6 DISEASE RISK ASSESSMENT FOR SPECIAL CASE AQUATIC ANIMAL MOVEMENTS AND PROPAGATION PROGRAMS

6.1 PURPOSE

This chapter provides guidelines for the generation of Disease Risk Assessments (DRAs) for Service-managed facilities and personnel involved in the movement and rearing of special case aquatic animals.

6.2 OBJECTIVES

Our objective is to provide an understanding of what a DRA is and how to draft one. A DRA:

1) Must be limited to infectious pathogens of aquatic animals and should not address other risks associated with animal movements (genetic, non-infectious disease, water quality issues, or ecological). A DRA can include organisms that are not on any formal list that would be dangerous if introduced with animal movements. It has four main components:
    a. Hazard identification,
    b. Risk assessment,
    c. Risk management, and

d. Risk communication.

2) Identifies assumptions, areas of disagreement among the DRA team, potential mitigation steps to reduce risk, and qualitative risk estimates for specific hazards (pathogens).
3) Must include a communication plan to keep internal and external stakeholders informed of the process.

6.3 GENERAL INSTRUCTIONS FOR COMPLETING THE DRA

The DRA is prepared by a team consisting of the Regional FHP supervisor, the Service manager of the affected facility or aquatic animal program involved in movement and rearing of special care aquatic animals, and another Service member associated with the affected program (e.g., Fish and Wildlife Conservation Office biologist). The team can be expanded to include experts from other entities (e.g., academia, U.S. Geological Survey).

The FHP supervisor leads the team and is responsible for the narrative report. The DRA report must:

1) Define scope of the DRA:

    a. Genus and species of host animal (host),
    b. Source(s) of host: watershed and/or specific location (Latitude/Longitude),
    c. Life stage and/or age of host,
    d. Purpose of host movement,
    e. Likely number of hosts involved in movement,
    f. Estimated number and duration of movement, and
    g. Estimated frequency of movement.

2) Establish the timeline for the entire DRA process and include a DRA calendar. This information will drive the data assembly effort.

3) Include a communications plan to record all dates of DRA team meetings, written communications, and conference calls, and include all appropriate contacts. The DRA team will:

    a. Determine members on the contact list,
    b. Regularly update members on the contact list with the progress of the DRA (minutes of the team meetings/calls and status of the process, final report),
    c. Determine how each member is to be contacted (email, fax, phone, mail),
    d. Determine what team actions should be communicated, and
    e. Determine the communication schedule.

4) Identify an initial hazards (pathogen) list of all relevant pathogens (for examples, see chapter 2.4) that includes associated data for each pathogen to assist in the development of risk tables. Data needs may be assigned to DRA team members. Data for each pathogen should include:

    a. Detection within watershed or nearby watersheds,
    b. Host species specificity,
    c. Virulence of pathogen, and
    d. Local regulations on detection of given pathogen.

5) Rate the probability of release and infection for each pathogen on the initial hazards pathogen list using the terms in the “Risk Terms” that follows, and by displaying the results in a “Prioritized Pathogen” Table (see Table 6.1) (Vose 2001).

    Risk terms (probability of release & infection) (Vose 2001):

    a. High: Event would be expected to occur
    b. Moderate: Even chance of event occurring

c. Low: Unlikely to occur
d. Very Low: Rarely (< 10 %) occurs
e. Negligible: Chance of event is so rare as to ignore in practical terms

TABLE 6.1 PRIORITIZED PATHOGENS

Pathogen ID	Release and infection risk rating	Reference for risk rating	Presence of pathogen in proposed facility /watershed

6) Prepare hazard release scenarios for each of the hazards (pathogens) rated as moderate to high, by the team, for probability of release and infection.

Example of hazard release (transmission) scenarios:

a. Urine/feces release: High risk of transmission as it is a chronic release
b. Predation of infected fish: Low risk of transmission to population
c. Transport water contains pathogen: Moderate level of risk

7) Rate the risk of the consequence of unintentional pathogen release if the transmission of the hazard/pathogen were to occur (rate the consequence of this transmission accordingly) by using the terms that follow.

Consequence terms (modified from Nowak 2004):

a. High: High morbidity or mortality and serious biological effect on population, unlikely to control
b. Moderate: Some morbidity or mortality or biological effect on population, unlikely to control
c. Low: Low level morbidity or mortality, can be controlled in some situations
d. Very Low: Very minor morbidity or mortality, can be controlled in some situations
e. Negligible: No morbidity or mortality, easy to control

8) Since risk is the product of probability and consequences use the risk ratings for the Probability of Release & Infection determined in 5 and 6 (Table 6.1) and the Consequence of Infection & Disease terms determined in 7 to derive an Unmitigated Risk Estimate for each pathogen by using the matrix below in Table 6.2 to generate an Unmitigated Risk Estimate Report (Table 6.3).

TABLE 6.2. DETERMINATION OF RISK ESTIMATE

Probability of release & infection	Consequence of infection & disease				
	Negligible	**Very Low**	**Low**	**Moderate**	**High**
High	*negligible*	*very low*	*low*	*moderate*	*high*
Moderate	*negligible*	*negligible*	*very low*	*low*	*moderate*

Low	*negligible*	*negligible*	*negligible*	*very low*	*low*
Very Low	*negligible*	*negligible*	*negligible*	*negligible*	*very low*
Negligible	*negligible*	*negligible*	*negligible*	*negligible*	*negligible*

Note: The risk estimate is a product of the probability of release and infection (vertical axis) and the consequence of infection and disease (horizontal axis). While the descriptors for each axis appear to be the same, they have different meanings, making the matrix non-symmetrical (non-symmetrical means that a "negligible" probability of release and infection combined with "high" consequence of infection and disease is not the same as a "high" probability of release and infection combined with a "negligible" consequence).

TABLE 6.3. UNMITIGATED RISK ESTIMATE REPORT

Pathogen	Release and infection risk rating	Consequence risk rating	Risk estimate	Comments

9) Report the unmitigated risk estimate for each of the designated hazards (pathogens) in the DRA report.

10) Determine possible mitigation steps and their effect on the risk estimate in Table 6.3. For each pathogen that scored above negligible in Table 6.3, identify and select the most likely mitigation steps that could be taken to avoid or reduce exposure to the pathogen and repeat 5, 6, and 7 to produce a revised risk estimate (Table 6.4). Mitigation steps may include, but are not limited to:

    a. Movement of disinfected gametes instead of live fish,
    b. Isolation rearing and testing program prior to release,
    c. Isolation rearing through sexual maturity and release of tested progeny, and
    d. Multi-year survey of population or appropriate surrogate.

TABLE 6.4. MITIGATION STEPS AND REVISED RISK ESTIMATE FOR HAZARDS (PATHOGENS) WITH RISK ESTIMATES ABOVE "NEGLIGIBLE"

Pathogen list	Mitigation steps	Revise risk estimate after mitigation

Staff at the Regional FHP compile the DRA report using all relevant data and documentation. They also identify and include all data gaps and assumptions associated with the DRA in the final DRA report. The DRA is provided to the Service Assistant Regional Director - FAC who, in consultation with the Regional Director, uses it in their decision making. This report is kept on file for 7 years and stored digitally beyond 7 years by the representing FHP.

6.4 REFERENCES CONSULTED

Aquatic Animal Health Code. 2015. 18th edition. Office International des Epizooties' (OIE). Volume 2.2 Import Risk Analysis.

Aquatic Animal Health Code. 2017. 20th edition. Office International des Epizooties' (OIE). http://www.oie.int/en/standard-setting/aquatic-code/access-online/

Federal Register 65 (186):56916-56922. Joint Fish and Wildlife Service and National Marine Fisheries Service policy regarding controlled propagation of species listed under the Endangered Species Act (Joint Policy), published September 20, 2000.

Jakob-Hoff R.M., MacDiarmid S.C., Lees C., Miller P.S., Travis D. and Kick R. 2014. Manual of Procedures for Wildlife Risk Analysis. World Organization for Animal Health, Paris, 160 pp. Published in association with International Union for Conservation of Nature and the Species Survival Commission.

Nowak B.F. 2004. Assessment of health risks to southern Bluefin tuna under current culture conditions. Bulletin European Association of Fish Pathologists. 24(1):45 – 51.

Vose D. 2001. Qualitative verses quantitative risk analysis and modeling. In: Proceeding of the OIE International Conference on Risk Analysis in Aquatic Animal Health, Paris France 8-10 February 2000, pp19-26, editor C.J. Rodgers. Office International des Epizooties (OIE).

7 AQUATIC ANIMAL DISEASE ERADICATION PLAN

7.1 PURPOSE

Since pathogens may pose a threat to aquaculture and wild stocks worldwide, established pathogen response protocols should be in place to maximize the efficiency of response should a significant pathogen be detected in a Service-managed facility. The purpose of a disease eradication plan is to minimize losses and control the spread of the pathogen by quarantine and/or movement restrictions, husbandry changes, chemotherapeutic treatments, potential destruction of infected and exposed aquatic animals when appropriate, disposal of carcasses, decontamination of premises, and targeted surveillance of the Service-managed facility and any remaining susceptible animals.

Control of pathogens or diseases depends on careful biosecurity measures, evaluation, and containment procedures. When a significant pathogen is suspected and/or detected and confirmed at a Service-managed facility, the issue must be met with prompt action.

7.2 NOTIFICATION

When a significant pathogen is confirmed, the Regional FHP supervisor must immediately report this information to the facility manager, the Regional Director, the appropriate Assistant Regional Director or equivalent, the Chief of BHOAS, and potentially affected Federal, State, and Tribal partners. The Regional FHP supervisor or their designee must be designated as the sole point of contact for disseminating information to other partners. Any media contact must be through the Regional External Affairs office.

7.3 SUGGESTED STEPS TO FOLLOW AFTER DETECTION

7.3.1 QUARANTINE AND DISEASE ERADICATION PLAN

An immediate quarantine of all aquatic animals at the facility involved should be imposed. All fish and/or egg transfers or movements out and any importations should be suspended. This quarantine should be confirmed, in writing, to the manager of the affected facility from the Assistant Regional Director - FAC or equivalent and describe specific restrictions. Upon confirmation of a notifiable disease or pathogen (see APHIS website), the individual or individuals with aquatic animal health authority (in FAC) must notify the appropriate Federal authorities with jurisdiction (APHIS) 24 hours after confirmation and biannually, depending on the particular pathogen and its status in the United States. For more information on APHIS programs for notification, consult the following webpage: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/aquaculture/aquatic-animal-diseases/index

Based on the pathogen detected, the Regional FHP supervisor and facility manager should immediately begin to implement a containment plan and to develop a Disease Eradication Plan for the facility. A documented work plan of all necessary actions, strategies, and standard operating procedures should be developed by the team in order to quickly identify, contain, and eliminate infectious diseases or significant pathogens at a facility. The plan should provide necessary information to implement appropriate pathogen-specific control measures, personnel responsibilities, and instructions within the chain of command.

If suspect aquatic animals have been transferred from the affected facility to other Service-managed facilities within the past year, the facility manager must communicate findings with those facilities, and similar quarantines should be issued to the receiving facilities by the Regional Director of the affected Region until pathogen surveillance can be completed. The Service does not have authority to require quarantine of State, Tribal, or private facilities similarly affected, but the benefit and establishment of a voluntary quarantine should be discussed with partner agencies and private operators until the extent of the outbreak can be determined.

The FHP supervisor and any affected partners should jointly determine the appropriate quarantine zone. Specific actions to eliminate the movement of fish from the quarantine zone (i.e., halting fish distribution and instituting emergency angling regulations) should be discussed with the appropriate regulating authority, including State and Tribal authority, as appropriate. These conversations should occur at the Regional Director level.

7.3.2 SURVEILLANCE

Pathogen surveys should be made of all populations of aquatic animals on the facility and within the quarantine zone, as well as aquatic animal populations at facilities having received aquatic animals and/or eggs from the affected facility. The size and location of survey sites and specifics related to testing, such as species, sample number, and appropriate test protocols or procedures should be determined by the facility's aquatic animal health professional and affected partners. Strict sanitary measures must be observed by all personnel working within the quarantine zone as certain pathogens can be spread on shoes, boots, gloves, tires, and by other means.

7.3.3 PATHOGEN ERADICATION THROUGH AQUATIC ANIMAL DISPOSAL

Depending on the pathogen responsible, euthanasia of infected and/or diseased animals may or may not be the appropriate action. If euthanasia is the appropriate action, the following steps should be followed:

1) The Regional Director must approve euthanasia.

2) Once approved, immediate steps should begin to ensure the orderly decontamination of the affected facility(s).

3) The facility manager should determine the most appropriate method and/or site of disposal and the supplies, equipment, and materials needed to carry out disposal.

4) All gametes, fertilized eggs, and animals should be humanely euthanized in conjunction with current published American Veterinary Medical Association euthanasia and/or depopulation policy.

Affected aquatic animal carcasses or waste should not be transported without prior approval from the Regional Fish Health Program.

7.3.4 CARCASS BURIAL OR DISPOSAL GUIDELINES

These recommendations are general in nature. The choice of one or more of the recommended methods should comply with relevant local, State, and Federal regulation and policy. Disposal methods should take into consideration a range of factors, including the cause of mortality. For special case aquatic animals, it may be appropriate to carry out a Disease Risk Assessment.

1) Storage, Transport, and Labeling:
    a. Transport of any high-risk waste should be accompanied by appropriate documentation detailing origin, content, and destination to allow tracking, if required.
    b. Following collection, aquatic animal waste should be stored for the minimum time practical; however, where storage is necessary, there should be sufficient capacity for the expected waste.
    c. The storage area should be separated from aquaculture sites and bodies of water to minimize the risk of spread of pathogenic agents.
    d. The containers of stored aquatic animal waste should be durable, nonabsorbent, leak-proof, and secured to prevent contact with aquatic animals, other animals or birds, and unauthorized personnel or visitors.
    e. If low risk waste becomes contaminated with high risk waste, such waste should then be considered high risk waste.
    f. Containers, such as dumpsters, used for transport of aquatic animal waste should be leak-proof and labeled regarding content.

2) Disposal and Treatment of High Risk Waste

The recommended methods for disposal or treatment of high risk waste from aquatic animals are listed in priority order (from highest priority to lowest) below. However, if the following methods are unavailable or otherwise deemed inappropriate, high risk waste may be disposed of by other methods that ensure an equivalent reduction of risk if approved by the Regional FHP supervisor. Methods must comply with all Federal, State, and local disposal policies.

    a. Incineration: Incineration is a controlled burning process carried out in fixed or mobile air curtain incinerators and may only be capable of handling limited volumes of aquatic animal waste. Mobile air curtain incinerators are ideal since they allow the process to be carried out onsite, which means the aquatic animal waste does not have to be transported.
    b. Burial: Whenever possible, the aquatic animal waste should be subjected to a treatment that ensures inactivation of the pathogenic agents prior to burial. When selecting an acceptable burial site (pit), consider the following:
        1 Location: Should be within the grounds of the facility with easy access from rearing units but sufficiently removed from areas subject to flooding and from facility well fields;
        2 Comply with all Federal, State, and local health and environmental regulations and policies regarding parameters for burial (e.g., quantity of carcasses, depth to water table, and burial site liners' distances to wells, surface water, and property lines); and
        3 If burial within the facility grounds is impossible or impractical, the facility manager and Regional FHP supervisor must determine an alternate site.
    c. Access: Consideration should be given to ease of access for equipment and delivery of aquatic animal waste. Fencing and restricted admittance may be necessary.
    d. Construction and closure: Rocky areas should be avoided, and the bottom must be dry (not in a water table). Select soils with good stability, capable of withstanding the weight of equipment used to dig and fill the pits. Diversion banks should be constructed to prevent surface runoff entering the pit or to prevent any liquids escaping from the burial site. Pit dimensions depend on the volume of the aquatic animal waste to be buried and should be easy to fill.

e. Waste: Waste should be completely and thoroughly covered with unslaked lime (CaO) to hasten decomposition and prevent scavenging, and then covered with at least 4’ of soil mounded up to allow for settling.

3) Decontamination: All burial equipment should be decontaminated after use, including transport vehicles. See chapter 5, section 5.2.6.5, Vehicles, for decontamination protocols.

7.4 POST-DISPOSAL FACILITY DISINFECTION

See chapter 5.2.2 for guidelines.

7.5 REFERENCES CONSULTED

Gamroth, M., Loboy, Z., Moeggenberg, E., and D. Hansen. 2006. Disposal of animal mortality and byproducts. Oregon State University. Available: http://extension.oregonstate.edu/coos/agriculture/DeadAnimalDisposal. (June 2016).

United States Department of Agriculture (USDA). 2014. Foreign Animal Disease Preparedness & Response Plan; Standard Operating Procedures: Disposal. Available: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/sop/sop_disposal.pdf (Sept. 2017).

World Organization for Animal Health (OIE). 2017. International Aquatic Animal Health Code; Handling, Disposal and Treatment of Aquatic Animal Waste. OIE, Paris. (May-June 2016).

APPENDICES

APPENDIX I: GLOSSARY

The following terms are used in the aquatic animal health policy (713 FW 1, 713 FW 2, and this handbook):

Adults: Sexually mature aquatic animals, regardless of age.

Approved drugs: Drugs for which the U.S. Food and Drug Administration's (FDA) Center for Veterinary Medicine has assessed the safety and efficacy, and for which a definition in the Federal Register and Code of Federal Regulations has been provided as approved or conditionally approved for use as per the conditions stated on the drug label.

Aquatic animal: For 713 FW 1,713 FW 2, and the handbook, "aquatic animal" means any animal, excluding birds and mammals, which spends some portion of its life cycle in fresh, brackish, or salt water.

Aquatic animal activity: Any Service activity that involves the capture, holding, propagation, transfer, or release of aquatic animals or their gametes.

Aquatic Animal Drug Approval Partnership (AADAP) Program: A Fish and Aquatic Conservation/Headquarters (FAC/HQ) Branch located on the campus of the Bozeman Fish Technology Center; Bozeman, Montana. The mission of the AADAP Program is "Working with our partners to conserve, protect, and enhance the Nation's fishery resources by coordinating activities to obtain FDA approval for drugs, chemicals, and therapeutants needed in aquaculture and fisheries management programs." *For additional information on AADAP, as well as establishing a good source of general drug and chemical use guidance, visit the AADAP website.*

Aquatic animal health professional: A Service employee who meets the educational and professional standards equivalent to those of the Fish Health Section of the American Fisheries Society for Fish Health Inspector or Fish Pathologist.

Biologic: As defined by the U.S. Department of Agriculture Center for Veterinary Biologics (CVB), the term "veterinary biologic" describes a vaccine, bacterin, antiserum, diagnostic kit, or other product of biological origin that is used to prevent, treat, or diagnose an animal disease. These products generally work through some immunological mechanism or process. As it pertains to biologics, the CVB has jurisdictional authority for their licensing and all aspects of regulatory compliance.

(1) Antiserum: A blood serum that contains antibodies against specific pathogens and is used to stimulate immunity to specific diseases.
(2) Bacterin: A suspension of killed or weakened bacteria used as a vaccine.
(3) Vaccine: An antigenic preparation used to establish immunity to a disease. Vaccines can be prophylactic (e.g., to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen) or therapeutic (e.g., vaccines against some forms of cancer). Vaccines may be living, weakened strains of viruses or bacteria that intentionally give rise to asymptomatic-to-inconsequential infections. Vaccines may also contain killed or inactivated organisms or purified products derived from them. Vaccines fall into one of several groups, including, but not limited to, inactivated, live-attenuated, toxoids, subunits, conjugates, recombinant-vectors, and DNA vaccines.

Broodstock facility: A hatchery or other facility that maintains a population of aquatic animals onsite that will be used to provide fertilized eggs, gametes, and fingerlings for the site's use or for shipment to other facilities (also see primary broodstock facility).

Captive broodstock: Aquatic animals maintained or reared in captivity for the production of gametes.

Chemical: A compound or substance that has been purified or prepared.

Confirmatory test: A second and conclusive procedure for the identification of an isolated listed pathogen. Confirmatory tests are tests in the latest American Fisheries Society Fish Health Section guidance, World Organization for Animal Health (OIE) Aquatic Manual, or peer-reviewed literature.

Cooperative agreements: The legal instrument reflecting a relationship between the U.S. Government and a State, Tribe, local government, or other recipient when:

- The principal purpose of the relationship is to transfer a thing of value to the State, Tribe, local government, or other recipient to carry out a public purpose of support or stimulation authorized by a law instead of acquiring (by purchase, lease, or barter) property or services for the direct benefit of the U.S. Government; and
- Substantial involvement is expected between the executive agency and the State, Tribe, local government, or other recipient when carrying out the activity contemplated in the agreement.

Deferred regulatory status drug: Substance the FDA defines as being a drug, but which is not regulated at this time, and until further notice FDA chooses not to regulate. The only two such drugs as they apply to 713 FW 1 and 2 and the handbook are copper sulfate and potassium permanganate.

Diagnostic: Examination (targeting moribund animals) to determine disease etiology. The Service aquatic animal health official will note in the “Remarks” section of the Fish Health Inspection Report if diagnostic samples are used as part of an annual health inspection.

Drug: As defined by the Federal Food, Drug, and Cosmetic Act (FD&C), the term "drug" means the following:

- Articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them;
- Articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans or other animals;
- Articles (other than food) intended to affect the structure or any function of the body of humans or other animals; or
- Articles intended for use as a component of any articles specified in the three clauses above.

In addition to these FD&C definitions, and as they apply to 713 FW 1 and 2 and the handbook, biologics under the jurisdiction of U.S. Department of Agriculture’s CVB are classified as drugs.

Emerging disease or pathogen: An aquatic animal disease or pathogen not previously known to occur in a given population or which is increasing in incidence in a previously known population.

Enzootic: A disease or pathogen that is known to occur within well-defined geographic boundaries.

Epizootic: A rapid and persistent increase in morbidity or mortality, or both (significantly above normal levels) in a given population by a virulent disease agent during a specific period of time as determined by a Fish Health Program (FHP) supervisor. This definition does not specify the rate and period of persistence because these are population- and pathogen-specific.

Exotic disease or pathogen: An aquatic animal disease or pathogen strain not previously known to occur in a given geographical area in the United States. It requires a historical absence of the pathogen or disease using adequate detection methods as determined by the FHP supervisor.

Extra-label drug use (prescription): The prescription of a drug by a veterinarian as allowed by FDA and State regulations for a disease or species that is not currently on the label of the approved product. Extra-

label prescriptions may only be written for FDA-approved drugs and only within the context of a valid veterinary-client-patient relationship. *For additional information visit the Aquatic Animal Drug Approval Partnership Program website.*

Facility: Any location that is used for the specific purpose of holding aquatic animals or gametes, or both, and can be either indoors or outdoors, or both.

Fish Health Center (FHC): FHCs are at the core of the Service's Aquatic Animal Health Program and are located across the United States. FHCs are staffed with aquatic animal health professionals whose scientific and technical expertise is used to ensure the health, survival, restoration, and enhancement of fish and other aquatic species in support of national and Regional priorities. Among a broad array of other responsibilities, FHC staff detect and monitor pathogens, provide timely information and recommendations to fisheries managers so that they can make informed conservation and management decisions, and investigate emerging animal health issues that threaten the health and well-being of all aquatic species.

Fish Health Program (FHP): An FHP is a Regional Fish Health Center or equivalent staffed or supervised by Service aquatic animal health professionals.

Free ranging broodstock: Aquatic animals of any species captured from the wild for spawning.

Fungicide: A chemical developed to selectively kill fungi, molds, and other related non-photosynthesizing plants.

Herbicide: A chemical developed specifically to kill terrestrial or aquatic plants. Most herbicides selectively eradicate specific plant species or groups, while leaving all others unharmed. For example, algaecides can be effective for the eradication of undesirable aquatic plants.

Index drugs: Drugs that CVM has placed on the "Index List" of drugs for use in/on aquatic species as a result of data reviewed by both an outside expert panel and CVM, and which are determined to be safe and effective. Index drugs are not approved drugs as defined by the FD&C Act. CVM has defined Index drugs under the Minor Use and Minor Species Animal Health Act of 2004 as legally marketable by the manufacturer/distributor named within the "Index List" for that specific drug. To view the "Index List" visit the FDA website.

Inspection: A statistically based onsite sampling of all lots of aquatic animals on the facility that a Service aquatic animal health professional performs or supervises, with subsequent examination of the collected tissues and fluids for the detection of listed pathogens in accordance with procedures in 713 FW 1 and 2 and the handbook. Monitoring and diagnostic results may contribute to inspection sampling requirements. Service aquatic animal health officials must report inspection results on a Fish Health Inspection Report

Investigational New Animal Drug (INAD) exemption: An official exemption that the FDA issues to a drug company or other entity allowing for the use of an unapproved drug under strict conditions. The exemption permits the generation of data to support a New Animal Drug Application (NADA), the release or slaughter (i.e., "slaughter authorization") of food animals treated with the investigational or experimental drug, and the legal interstate transportation of the investigational drug. Any drug that is the subject of a valid INAD is an "investigational drug." *For additional information on available INADs and how to participate in Service's National INAD Program, visit the AADAP website on the topic.*

Isolation: Separating one group of aquatic animals from others. This could be isolation of sick aquatic animals, or those with unknown disease status, from others within or outside the same rearing area.

Laboratory: A building, part of a building, or other place that provides controlled conditions and is equipped to conduct scientific or technological tests, investigations, or experiments.

Listed pathogen: A pathogen of concern that is placed onto the lot's inspection plan and Fish Health Inspection Report.

Listed Pathogen Not Detected (LPND): When no pathogen on the lot's inspection pathogen list is detected.

Lot: A group of cultured aquatic animals of the same species, of the same year class, originating from the same spawning population, and sharing the same water supply.

Low Regulatory Priority (LRP) drugs: Compounds that FDA defines as drugs that are not approved and that FDA is not likely to approve, but (based on information provided to FDA) are drugs for which CVM will not likely take any regulatory action (i.e., prosecute the user of such an unapproved drug) if used under the conditions specified in FDA's published list of LRP drugs. *For additional information and a list of LRP, drugs visit the AADAP website on the topic.*

Monitoring: Periodic sampling and disease detection work that a Service aquatic animal health professional performs or supervises in accordance with procedures set forth or referred to in 713 FW 1-2. When monitoring is used as part of an annual health inspection, a Service aquatic animal health professional must document this in the "Remarks" section of the Fish Health Inspection Report.

Moribund: Severely ill and at the point of death.

Movements: Relocating aquatic animals, including on-station releases, transfers between facilities, and relocations of wild populations.

National Pollutant Discharge Elimination System (NPDES) permit: A permit that the U.S. Environmental Protection Agency (EPA) or a State permit program issues for discharge of any pollutant under section 402 of the Clean Water Act (33 U.S.C.1342.) For additional information on NPDES permitting, see the EPA website.

Pathogen: A bacterium, parasite, virus, or other microorganism that can cause disease.

Pathogen strain: A bacterium, parasite, virus, or other microorganism that differs phenotypically (e.g., host-specificity, virulence, etc.) and genotypically (i.e., unique genetic identity) from other pathogens of the same taxonomic group.

Pesticide: Under the Federal Insecticide, Fungicide, and Rodenticide Act: (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; (2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and (3) any nitrogen stabilizer. We do not use the term ‘‘pesticide’’ to include any article that is a ‘‘new animal drug’’ within the meaning of section 201(w) of the Federal Food, Drug, and Cosmetic Act, that has been determined by the Secretary of Health and Human Services not to be a new animal drug by a regulation establishing conditions of use for the article, or that is an animal feed within the meaning of section 201(x) of the Act (21 U.S.C. 321(x)) bearing or containing a new animal drug.

Piscicide: A type of pesticide that is lethal to fish and typically is used to eradicate non-native fish species.

Prevalence: The proportion of detectable cases of a disease or disease agent (i.e., pathogen) present in a population at a given time.

Primary broodstock facility: A hatchery or other facility whose primary role is to maintain onsite resident populations of salmonids that will be used to provide fertilized eggs and gametes for the National Broodstock program.

Quarantine: Maintaining a group of aquatic animals in isolation with no direct or indirect contact with other aquatic animals to allow observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent water and facility biosecurity measures in place.

Screening test(s): The survey for pathogen(s) in a population by means of a test, assay, or other laboratory procedure. Because the results of a screening test may have a high sensitivity but low specificity, they may provide presumptive identification of an agent, which will require a different, more sensitive test for confirmation. Screening tests are tests in the latest American Fisheries Society Fish Health Section guidance, World Organization for Animal Health (OIE) Aquatic Manual.

Sentinel animal: An animal that is placed in a given environment and then monitored to find out if an infectious disease or other harmful agent is present in that environment.

Service-managed facilities: Any land, water, or facilities the Service owns, leases, or otherwise controls. These properties include, but are not limited to, National Fish Hatcheries, Fish Technology Centers, Fish Health Centers, Fish and Wildlife Conservation Offices, National Wildlife Refuges, and Ecological Services Field Offices.

Significant pathogen: A pathogen, including an emerging disease or pathogen or an exotic disease or pathogen, that would likely adversely impact Service aquatic animal programs by producing high mortality or by triggering movement restrictions, or that, if detected, would change the status of a recognized disease zone or compartment in a manner that would restrict movements by other entities, including those of Service partners.

Special case aquatic animals: An aquatic animal for which standard testing procedures either do not exist or may not be appropriate due to the species involved or the limited number or condition of the population. For example, this may include a threatened or endangered species.

Statistically based sampling: The collection by a Service aquatic animal health professional, or their designated Service agent, of appropriate aquatic animal tissue or fluid samples, on a lot-specific basis, in sufficient numbers to meet sampling criteria for listed pathogens as required by 713 FW 1-2 and the handbook.

Stock: That portion of an aquatic animal population sharing a common gene pool and, for aquatic animal health purposes, a common environment.

Surveillance: The formal health examination (e.g., inspections, monitoring, diagnostics, and non-lethal techniques) of aquatic animals by Service aquatic animal health professionals.

Veterinarian/Client/Patient Relationship (VCPR): According to the FDA, a VCPR exists when:

1) The veterinarian has assumed responsibility for making clinical judgments regarding the health of the animal(s) and the need for medical treatment, and the client has agreed to follow the veterinarian's instructions;
2) The veterinarian has sufficient knowledge of the animal(s) to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of an examination of the animal(s), or by medically appropriate and timely visits to the premises where the animal(s) are kept; and
3) The veterinarian is readily available, or has arranged for emergency coverage, for followup evaluation if there are adverse reactions or a failure of the treatment regimen.

Note that the individual State Veterinary Boards may have their own requirements or definition of a VCPR. Individual State VCPR requirements may be more restrictive than what FDA provides, but not less restrictive. For more information and a listing of VCPR regulations and primacy by State, visit the FDA website.

Veterinary Feed Directive (VFD) drug: New animal drugs intended for use in or on animal feed that are limited to use under the professional supervision of a licensed veterinarian. Any animal feed containing a VFD drug can only be fed to animals based on an order, called a VFD, issued by a licensed veterinarian

under a valid VCPR. *For more information on VFD, drugs, visit the FDA website on VFD drugs. For more information on the extra-label use of VFD drugs, visit the FDA website on extra-label use.*

Watershed: A drainage system of connected rivers, lakes, or streams, or combination thereof, confined to a limited geographic basin.

APPENDIX II: 2021 NATIONAL ANIMAL HEALTH REPORTING SYSTEM (NAHRS) REPORTABLE DISEASES, INFECTIONS, AND INFESTATIONS

AQUACULTURE

Fish: Viral hemorrhagic septicemia (VHS)*

Fish: Infectious salmon anemia (ISA)(HPR-deleted)*

Fish: Infectious salmon anemia (ISA)(HPR0)*

Fish: Spring viremia of carp (SVC)*

Fish: Infectious hematopoietic necrosis (IHN)*

Fish: Epizootic hematopoietic necrosis disease*

Fish: Epizootic ulcerative syndrome (EUS) (Infection with *Aphanomyces invadans*)*

Fish: Gyrodactylosis (*Gyrodactylus salaris*)*

Fish: Red sea bream iridoviral disease*

Fish: Koi herpesvirus disease*

Fish: Infection with salmonid alphavirus*

Fish: Tilapia Lake Virus (TilV)

Mollusc: Infection with *Bonamia ostreae**

Mollusc: Infection with *Bonamia exitiosa**

Mollusc: Infection with *Marteilia refringens**

Mollusc: Infection with *Perkinsus marinus**

Mollusc: Infection with *Perkinsus olseni**

Mollusc: Infection with *Xenohaliotis californiensis**

Mollusc: Infection with abalone herpes virus*

Crustacean: Taura syndrome*

Crustacean: White spot disease (white spot syndrome virus)*

Crustacean: Necrotising hepatopancreatitis (*Candidatus Hepatobacter penaei*)(NHP, early mortality syndrome)*

Crustacean: Yellowhead (Infection with Yellowhead virus genotype 1)*

Crustacean: Infectious hypodermal and haematopoietic necrosis*

Crustacean: Crayfish plague (*Aphanomyces astaci*)*

Crustacean: Infectious myonecrosis*

Crustacean: White tail disease (*Macrobrachium rosenbergii nodavirus*)*

Crustacean: Acute hepatopancreatic necrosis disease (*V.parahemolyticus* pVA-1 plasmid)*

AMPHIBIAN

Infection with *Batrachochytrium dendrobatidis**

Infection with *Batrachochytrium salamandrivorans**

Infection with ranavirus (Ranavirus species)*

*2020 OIE-listed disease.

APPENDIX III: FISH PATHOGEN LIST

Virus

IHNV Infectious Hematopoietic Necrosis Virus

IPNV Infectious Pancreatic Necrosis Virus

VHSV Viral Hemorrhagic Septicemia Virus

OMV Oncorhynchus masou virus

LMBV Largemouth Bass virus

Bacteria

Aeromonas salmonicida

Yersinia ruckeri

Renibacterium salmoninarum

Piscirickettsai salmonis

Flavobacterium psychrophilum

Edwardsiella ictaluri

Parasites

Myxobolus cerebralis

Tetracapsula byrosalmonae

Nucleospora salmonis

APPENDIX IV. EXAMPLE OF BIOSECURITY PLAN

2018 Biosecurity Plan for Blue Lake National Fish Hatchery (NFH)

Prepared by:

Jerry Jones, Fish Health Center (FHC) Project Leader

Inclusive dates: October 1, 2017 – September 30, 2018

Revision dates:

Purpose: Prevent transmission of fish pathogens to production salmon and steelhead at Blue Lake NFH

Prioritized pathogen (hazard) threats:

IHNV (lower potential for VHSV IVa) – high prevalence of L-clade IHNV in adult fall and late fall brood stock

All viruses are to be avoided in production fish

Obligate salmonid bacteria:

Y.ruckeri – endemic especially in Fall-run juveniles, OTC feed treatment has been successful

A.salmonicida – low prevalence of isolation from brood stock only

F.psychrophilia (CWD) – isolated from adult salmon ovarian fluid; however, not considered a disease issue at hatchery

Potential parasite transmission:

Copepods – prevalent on steelhead adult “kelts”

Nucleospora salmonis – found at upstream hatchery in 1990s, no local detection since

Epitheliocystis – detected in steelhead juveniles, may be associated with low level mortality/leukemia symptoms

Facultative fish pathogens – endemic to facility:

F.columnaris – trigger > 18°C, handling or tagging events, associated with highest mortality in late fall Chinook and Steelhead, OTC feed treatment or option for 20 mg/L chloramine-T bath

Ichthyophthirius multifiliis – annual outbreaks in all production stocks beginning in June

Ichthyobodo spp. (Costia) – annual event for steelhead inside hatchery building that responds to formalin treatment – is source due to post-ozone contamination?

Hexamita spp. – low virulence, pond cleaning can help reduce transmission, 3% Epsom salt mixed with feed is a marginal treatment

Transmission pathways:

Egg-associated transmission (vertical and surface contamination)

Live fish movement

Juveniles from other hatchery or wild fish moved directly into hatchery

Water supply

Interruption of adequate ozone residual concentration

Aquatic animals within post-ozone water supply

Human movements

Bird and mammal introduction to raceways

Fomites (contaminated equipment) and external water – (e.g., adult broodstock transfer truck with river water, tagging trailer improperly disinfected)

Disposal of mortalities – during epizootics, pathogens are concentrated with mortalities

Mitigation steps:

Water supply

Adequate ozone residual concentration

46 in. pipe disinfection

P6 pipe disinfection

Raceway cleaning – removal of organics and exposure to sunlight for minimum 3 days

Weekly raceway sediment removal – reduce bacterial and parasite biofilm habitat

Gamete surface disinfection with 100 mg/L iodophor for 30 min as per chapter 5.

Avoidance of live fish transfer into facility without health inspection

Separation of spawning and ladder operations from production fish operations

Any in-pond activity associated with pre-release pond or other adult steelhead holding tanks that could transmit water borne copepod nauplii to production units

Separate (orange) outerwear and boots for spawning

Hand washing after working with adults

External surfaces of “egg buggy” are a potential transmission route into the hatchery building – regular disinfection during spawning operations will be difficult – 2018 pilot trial with Virkon® mat for wheel disinfection.

Forklift used for tank transport of adults – tank disinfection

Tagging operations

Trailer disinfection – verify that standard disinfection occurs prior to operation at hatchery

Temperature threshold for operation (< 65°F)

Avoidance of crowding for over a 6h period

Bird and mammal introduction

Fencing of raceways

Mortality disposal during elevated loss periods – daily collection in leak-proof container placed into burial pit (see eradiation portion of chapter 6).

Hatchery HACCP plan attachment:

Audit:

FHP supervisor and Fish and Wildlife Conservation Office Hatchery Evaluation lead (complete audit between September 15- 30 of each year), copies of audit form held at both NFH and by the Regional FHP facility for 7 years, deficiencies reported to Project Leader Blue Lake NFH with followup date [similar to safety walkthrough]

Action Items:

Record - iodophor in raceway broom barrels (#s) filled each week (bottles used?)

Separate spawn building outer wear for all staff and volunteers – count

Date of water system disinfection

Sign off sheet for trailer disinfection

Annual biosecurity plan meeting/sign off for NFH staff and FWCO Hatchery Evaluation staff