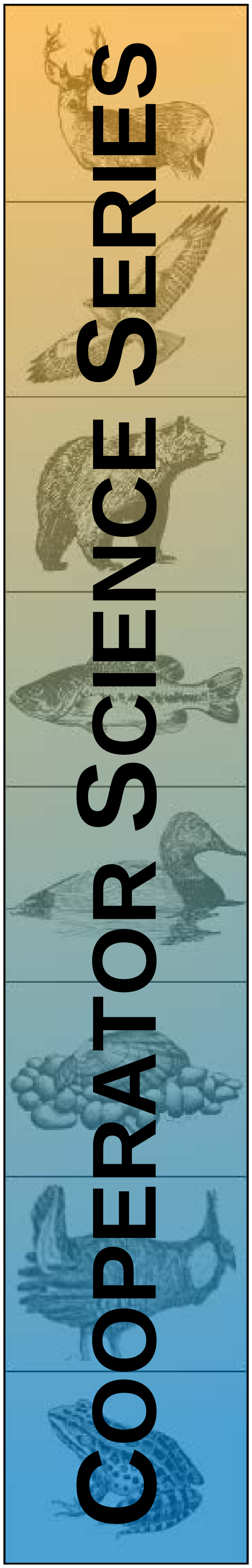

Guidance Documents: Continued Support to Improve Operations of Fish Hatcheries and Field Sites to Reduce the Impact or Prevent Establishment of New Zealand Mudsnails and Other Invasive Mollusks

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Final Report

Guidance Documents: Continued Support to Improve Operations of Fish Hatcheries and Field Sites to Reduce the Impact or Prevent Establishment of New Zealand Mudsnails and Other Invasive Mollusks

U. S. Fish and Wildlife Service, Region 6 Aquatic Invasive Species
Catalog of Federal Domestic Assistance (CFDA) Number: 15.608

by

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Project Summary

This project tested and revised a risk assessment/management tool authored by Moffitt and Stockton designed to provide hatchery biologists and others a structure to measure risk and provide tools to control, prevent or eliminate invasive New Zealand mudsnails (NZMS) and other invasive mollusks in fish hatcheries and hatchery operations. The document has two parts: the risk assessment tool, and an appendix that summarizes options for control or management.

The framework of the guidance document for risk assessment/hatchery tool combines approaches used by the Hazard Analysis and Critical Control Points (HACCP) process with those developed by the Commission for Environmental Cooperation (CEC), of Canada, Mexico, and the United States, in the Tri-National Risk Assessment Guidelines for Aquatic Alien Invasive Species. The framework approach for this attached first document assesses risk potential with two activities: probability of infestation and consequences of infestation. Each activity is treated equally to determine the risk potential. These two activities are divided into seven basic elements that utilize scientific, technical, and other relevant information in the process of the risk assessment. To determine the probability of infestation four steps are used that have scores reported or determined and averaged. This assessment follows a familiar HACCP process to assess pathways of entry, entry potential, colonization potential, spread potential. The economic, environmental and social consequences are considered as economic impact, environmental impact, and social and cultural influences.

To test this document, the Principal Investigator worked to identify interested hatchery managers through contacts at regional aquaculture meetings, fish health meetings, and through the network of invasive species managers and scientists participating in the Western Regional Panel on Aquatic Nuisance Species and the 100th Meridian Initiative's Columbia River Basin Team, and the Western New Zealand Mudsnail Conference in Seattle. Targeted hatchery workshops were conducted with staff at Dworshak National Fish Hatchery Complex (ID), Similkameen Pond, Oroville WA, and Ringold Springs State Hatchery (WA).

As a result of communications with hatchery staff, invasive species managers, and on site assessments of hatchery facilities, the document was modified and enhanced. Additional resources were added to keep it up to date. The result is a more simplified tool that can lead hatchery or management personnel through the process of risk assessment and provide an introduction to the risk management and communication process.

In addition to the typical HACCP processes, this tool adds steps to rate and consider uncertainty and the weight of evidence regarding options and monitoring results . Uncertainty of outcome exists in most tools that can be used to control or prevent NZMS or other invasive mollusks from infesting an area. In additional this document emphasizes that specific control tools and plans must be tailored to each specific setting to consider the economic, environmental and social influences. From the testing and evaluation process, there was a strong recognition that a number of control and prevention tools previously suggested and reported in the literature from laboratory and small scale trials may not be compatible with regional and national regulations, economic constraints, social or cultural constraints, engineering or water chemistry characteristics of each facility.

The options for control are summarized in the second document, Review of Control Measures for Hatcheries Infested with NZMS (Appendix A) that provides sources for additional resources and specific tools, and guidance regarding the feasibility and success of each approach. This tool also emphasizes that management plans need to be adaptive and incorporate oversight from professionals familiar with measuring risks of fish diseases, and treatments (e.g. the fish health practitioners and water quality and effluent management teams). Finally, with such a team, the adaptive management approach must be ongoing, and become a regular component of hatchery operations.

Although it was the intent that this two part document would be included as part of the revised National Management and Control Plan for the NZMS proposed by the U.S. Fish and Wildlife Service (USFWS) and others, it is provided as a stand-alone document.

Guidance Document for Fish Hatcheries to Assess Risk, and Prevent, Control or Eradicate Infestations of the New Zealand Mudsnail and Other Invasive Mollusks

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Idaho Cooperative Fish and Wildlife Research Unit

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I. Introduction and Basic Biology

Fish hatcheries provide ideal environments for aquatic species, often maintained at constant temperatures and utilize constant flows throughout the year, and therefore are at risk to infestation by aquatic nuisance or invasive species. Nutrients from fish wastes can provide enhanced habitat and sources of nutrients. Hatchery stocking, and fish relocations can introduce unwanted organisms to new locations. In many anadromous hatcheries, broodstock entering fish holding facilities carry an abundant flora of organisms from prior migratory and rearing locations. Public outreach activities are generally incorporated into management plans, because hatcheries are often located near recreational sites such as fishing areas, wildlife preserves, and other public access points Although important, each activity has a risk associated with it.

This document will focus on assessment and control tools for NZMS *Potamopyrgus antipodarium*, and in addition provide supplemental information and tools that are useful to measuring risks of infestation of zebra mussels, *Dreissena polymorpha;* quagga mussels, *Dreissena rostriformis bugensis*, and Asian clams *Corbicula fluminea.*

New Zealand Mudsnails - The NZMS has been transported to many locations outside of its native environment including Australia, Japan, Europe, and the United States. The first known infestation in the United States was in southern Idaho, at The Nature Conservancy's Thousand Springs Preserve in 1987. Populations of NZMS are now found in many other western states including Washington, Oregon, California, Nevada, Arizona, Utah, Colorado, Wyoming and Montana. In 1991, populations were found in the Great Lakes area; first Lake Ontario and later in Lake Superior and Lake Erie. They have since spread from these areas through natural movements of fish, birds and mammals, aquarium trade, recreationalists, and other vectors to additional locations. Because they are very small, ranging in size from 80 µm to 6 mm and they can live in a variety of habitats, such as estuaries, lakes and rivers, their transport often is successful. A freshwater prosobranch, NZMS have an operculum that closes tightly allowing them to resist desiccation and chemical treatment. NZMS are parthenogenic females so that one NZMS can populate an area. Females brood their young, with up to 50 neonates at a time and can reproduce one to six times throughout the year. NZMS can reach densities of over 500,000 snails per square meter in certain locations, and then they can compete with native snails and macroinvertebrates. NZMS have been reported to consume up to 75 percent of the primary productivity. Gravel, sand and mud are typical substrates and snails are also found on aquatic

vegetation. Fish can consume NZMS, and the snails can survive passage through the digestive tract. Several parasites are known to infect NZMS in their native range, but few infections are reported outside their native range.

Zebra/Quagga Mussels – The zebra mussel and quagga mussel are both native to Eastern Europe. Zebra mussels are native to the Caspian and Black Seas (Eurasia); quagga mussels are native to the Dneiper River drainage in Ukraine. Zebra mussels were first found in North America in 1988 in the Great Lakes in Lake St. Clair that connects Lake Huron and Erie, and have since been identified throughout the Illinois, Mississippi, and Hudson Rivers, and into many states of the Eastern and Midwest. In 2008, they were discovered in Central California. Quagga mussels were observed in 1991 in Lake Erie, and since populations are now identified in many other parts of the country. The quagga mussel appears to have the widest range of tolerable conditions for feeding, and reproduction. However, they are not tolerant of salinity, with reported limits of 5 ppt. The mussels have caused serious economic losses due to fouling of infrastructure, and can alter the trophic dynamics and ecology of infested waters, and outcompete native mollusks and other native species. A calcium-based risk assessment for zebra and quagga mussels ranked the Columbia River and most of California's water bodies as high to medium risk of infestation based on calcium concentrations >28 mg/L. The mussels have an annual cycle of gonadal growth and gamete maturation, and spawn in single or multiple events from late spring through summer. Temperature and rates of temperature change, food, and conspecifics are variables that determine reproductive responses. Both species are dioecious, but synchronous spawners. Temperature of reproduction is more limited for zebra mussels (> 12°C) than with quagga mussels (>7°). For this reason, the quagga mussel is likely a more serious threat to aquatic systems, and can survive at lower water depths in lake and reservoir systems. The fertilized eggs develop as planktonic veligers that disperse large distances with currents, are carried in water including fish hauling tanks, and bilge, ballast, and other vessels.

Asian Clam - The Asian clam was first detected in US waters in 1938 in the Columbia River, Washington.The clams have spread to least 44 states. In running waters, they provide a re-occurring problem with biofouling. In lake environments, they can become a serious problem because of their capacity of filtration, and their wide range of conditions in which they live. They are hermaphrodites, and synchronous spawners, and will spawn when temperatures warm above 15 to 18°C. The larval stage can attach to vegetation and floating debris for long distance

dispersal. Juvenile *Corbicula* are more likely to be carried in bilge and live well water in boats, in fish hauling tanks, or on vegetation attached to anchors and trailers or in sediments left on anchors. Another significant dispersal agent is thought to be passive movement via water currents. There remains some question regarding transport by waterfowl. Unlike zebra and quagga mussels, *Corbicula* can use its pedal foot to feed on organic material and tiny organisms (microbes, protists, meiofauna) in the sediment as well as filter feed, creating competition with native species. Bivalves, particularly when in dense populations, excrete significant amounts of inorganic nutrients, particularly nitrogen that, in turn, can stimulate the growth of algae and macrophytes. In cases of large infestations sometimes mass mortality can release large amounts of nutrients. Furthermore, the shells can provide a hard substrate and calcium source on soft sediments, creating new habitat for zebra or quagga mussels.

II. Regulatory Environment

This manual does not provide a summary of regulations but provides several web resources that are up to date references for regulations. In the US, the Lacey Act, the Clean Water Act, Executive Order 13112, the National Management and Control Plan for NZMS, and state Aquatic Nuisance Species plans provide a summary of National and state rules. For the western region the most up to date resources are available on http://www.westernais.org/regulations. This website is an outcome of the Columbia Basin Team of the 100th Meridian Initiative. This initiative was developed by the U.S. Fish and Wildlife Service (USFWS) in the 1990s to include federal, state, and tribal agencies and stakeholders with the goal to stop the spread of aquatic nuisance species (ANS) via recreational pathways. The educational emphasis has been on watercraft assessments, protocols for disinfection, and prioritizing pathways of introduction. The same web (westernais.org) provides a summary of all relevant state and several Canadian provincial regulations.

The Nonindigenous Aquatic Nuisance Prevention and Control Act (NANCPA) of 1990, Section 1204(b) authorized funding for the implementation of State ANS Management Plans. The Aquatic Nuisance Species Task Force (ANSTF) was established as an intergovernmental organization dedicated to prevent and control aquatic nuisance species, and implement the act. The ANS Task Force consists of 13 federal agency representatives and 12 ex-officio members,

and is co-chaired by representatives of the USFWS and National Oceanic and Atmospheric Administration (NOAA). Amendments to the NANCPA, and the National Invasive Species Act, the National Invasive Species Act of 1996 (P.L. 101-636) provided the creation of regional panels of public and private entities, such as the Western Regional Panel on Aquatic Nuisance Species that was created in 1997 to focus on Western North America. Voting members in this panel include U.S. federal agencies, state governments, U.S. territorial governments, Canadian Provinces, Canadian federal agencies, Mexican federal agencies, Tribal governments or interests, and other interests (including but not limited to trade, scientific or professional associations and societies, non-governmental organizations and academic institutes).

Within the USFWS, fish hatcheries have been directed to use the Hazard Analysis and Critical Control Point (HACCP) plans to assess their activities to control unwanted or invasive species. Although importation and interstate transport of zebra mussels is prohibited by the Lacey Act (18 USC 42), the U.S. government's role has been to encourage and foster coordinated state efforts to prevent further invasions by providing technical assistance, grants, tools and forums for exchange of information among all stakeholders. Training in HACCP can be obtained through special workshops and through the National Conservation Training Center (NCTC) of the USFWS, and also Sea Grant within NOAA. A web link http://www.haccp-nrm.org/listplans.asp, provides a compilation of plans developed for hatcheries in US. However, many of these plans were developed some time ago and need to be updated with the most recent information.

State Programs – Most of the states have a collaborative approach with authority not within a single agency, and separation of terrestrial and aquatic species is common. The website (www.westernais.org) provides pertinent information on regulations for individual states, and many provinces. For example, in Washington State, RCW 77.60 defines the term ANS as a "nonnative aquatic plant or animal species that threatens the diversity or abundance of native species, the ecological stability of infested waters, or commercial, agricultural, or recreational activities dependent on such waters." The state categorizes organisms into several classes, depending on if they are in the state, and the existing distribution. For example, Class 1 groups are of highest concern and have none or limited distribution in the state. Goals are to prevent spread or introduction and the list includes the mollusks: zebra/quagga (Dreissena spp), and the Asiatic clam (*Potamocorbula amurensis).* Class 2 species are those to be controlled, and

prevented from dispersal, and include NZMS and the Japanese oyster drill (*Ceratostoma inornatum).* Class 3 species are those which have become established, and have impact, but there are no available or appropriate management techniques to mitigate their effects. Asian clam (*Corbicuila fluminea*) would be an example.

III. The Risk Assessment Process

This manual provides tools and summaries of information to assist hatchery managers and biologists that are not experts in invasive and nuisance species or control strategies. Because there are conflicting reports regarding control or prevention strategies, this manual is designed to provide guidance and empower the local biologists and hatchery personnel with information that is useful but should be verified for efficacy at each facility keeping in mind that each facility is unique and a different environment. A risk assessment process involves a series of steps (Figure 1) that detail an adaptive process. By following the suggested steps, biosecurity awareness and protocols can be developed. The goal of the risk assessment process is to keep a hatchery facility at a low risk of infestation. This document contains control information and strategies that can be used if an infestation occurs. However, the implementation of specific tools must consider the local water quality/chemistry characteristics, and the availability of resources, as well as regulatory and management objectives.

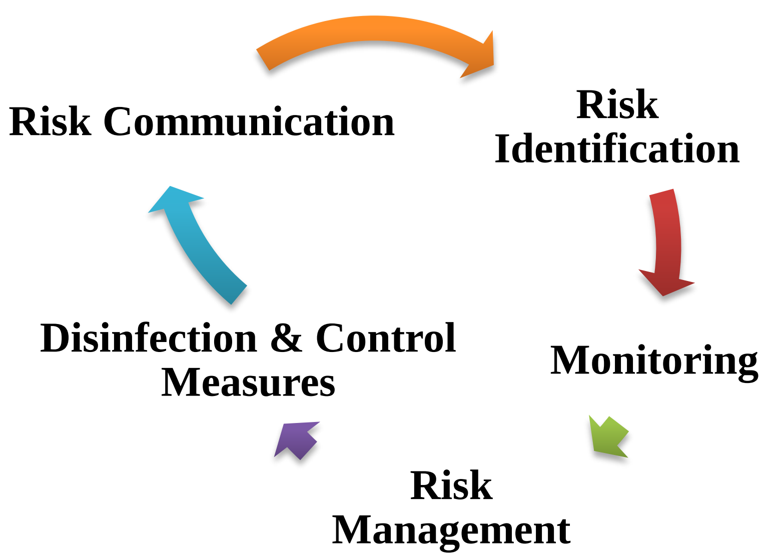

Figure 1. Risk analysis, assessment, management and communication process for aquaculture biosecurity.

Four steps are important components used to develop an effective prevention, management, and control plan. We provide a step –by–step process of developing an assessment of the risk potential of an infestation, monitoring to determine status of the facility, consideration of management options, and tools for disinfection and control. Using a framework allows all aspects to consider for managers or administrators to choose to prevent, control, manage or ignore infestations. (Figure 2)

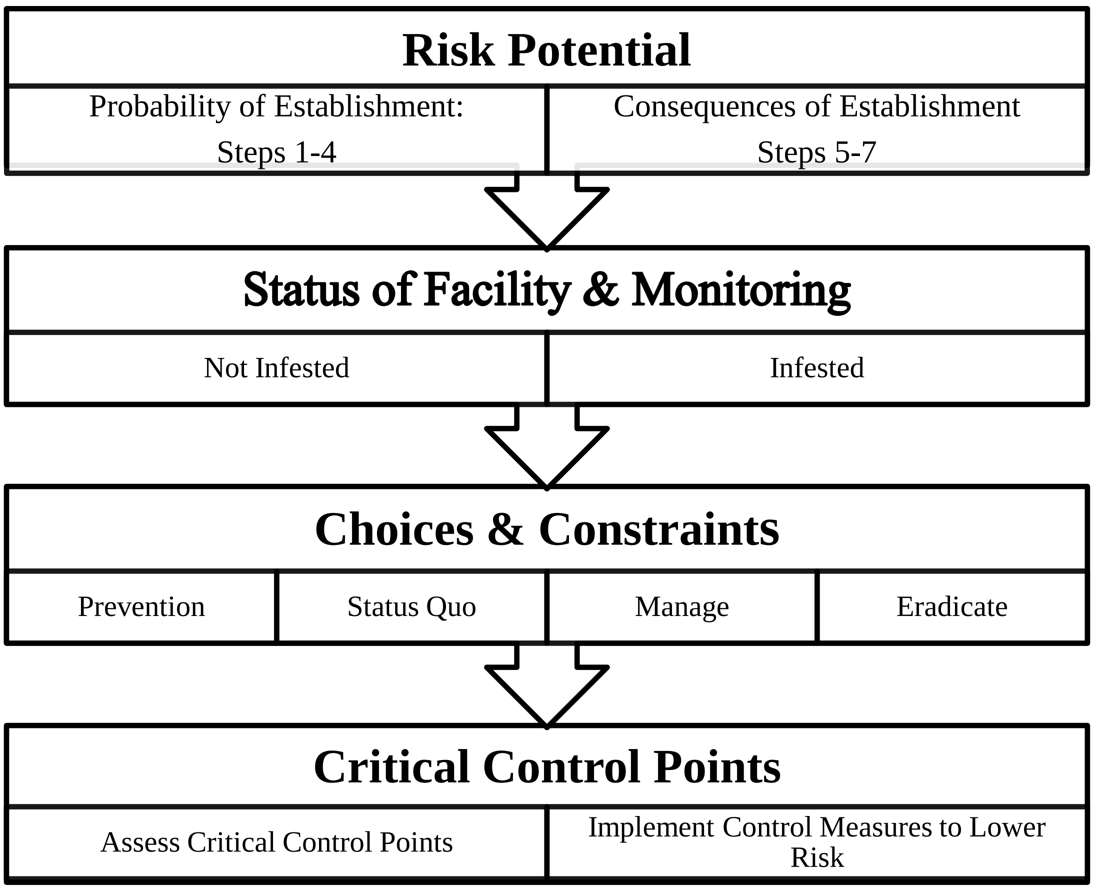

Figure 2. Four components of the adaptive management risk analysis process. Each component has steps and decision points that include feedback loops needed for risk assessment, risk management and communication process.

The framework starts with identification of risk potential with four steps assist to determine the probability of establishment, which when combined with the three steps of determining the consequences of establishment, will provide an assessment of risk and help to

define the appropriate preparation. The document provides guidance on methods for monitoring a facility and disinfecting tools. The assessment of facility status, and choices and constraints inform the resulting actions that managers will take. These decisions are further constrained and guided by local, state, and national regulations.

With the best understanding of the laws and regulations, a manager can determine the action that is appropriate for the facility. The HACCP process helps to determine where in a particular aquaculture pathway there are critical points to control entry of invasive species. The tools to reduce the risk of infestation or entry are summarized. Implementation of appropriate tools should help prevent or control invasive mollusks from infesting a hatchery, however regular monitoring and review of the risk assessment process is advisable to determine the certainty that control measures implemented are reducing the risks of infestation. The process uses adaptive management through iterations to lower the uncertainty, lower risk potential, and decrease the likelihood of infestation.

IV. Determining Risk Potential

Risk potential is divided into two components: probability of establishment and consequences of establishment. In this document, each component is treated equally to determine the risk potential; however, if warranted the parameters associated with determining the risk potential can be weighted before averaging to incorporate dynamic relationships in the environment. These steps are recorded and evaluated using Forms 1 – 4, pages 35-37.

$$Risk\ Potential = Probability\ of\ Establishment\ \&\ Consequences\ of\ Establishment$$

These two components are divided into seven basic elements that utilize scientific, technical, and other relevant information to form the risk assessment. These elements may be determined using quantitative, qualitative, or a combination of both methods. Strength of the risk assessment is determined by the information gathered by the assessor.

Steps to Estimating Probability of Establishment

The “probability of establishment” component of the risk assessment has four steps that are analyzed, ranked, and reported. Biological information on the target species is integrated in these steps. The strength of the assessment is only as good as the information collected and used in this analysis. The transparent nature of this document helps managers add more information or

change existing information based on their expertise or new information that is available.

Step 1: Assessment of Establishment

Determine the pathways that the NZMS, and other mollusks can be established. The forms should be completed for each species, separately. Considerable information has been published that details the survival of each species and life stage. Potential vectors to be considered include: fishing gear, boats, and transport pathways from infested water bodies. The following list applies:

- Anglers, recreationalists and associated gear
- Aquarium trade
- Boats, ballast or live wells on boats
- Aquatic birds and mammals
- Effluent ponds
- Field crews and associated gear
- Fish collection, fish transportation
- Open water sources, canals, culverts
- Visitors

Documenting these pathways will demonstrate due-diligence in taking the first steps to proactively prevent infestations. The more pathways that are realized for possible infestation, the more certainty exists in the risk assessment. These are listed on Form 1, page 35.

Step 2: Entry Potential

Each pathway has a qualitative probability that NZMS or other mollusks can survive transit into the facility. Elements to consider are the organism’s ability to “hitchhike,” survive during transit due to environmental conditions, the lifecycle stage of the organism in transit, the number of potential organisms associated with the pathway, or even some intentional introductions. The likelihood of the target mollusk surviving the pathway can be ranked using 6 categories; uncertain, very high, high, medium, low and very low. “Uncertain” can be used if not much information is known. An example of a “very high” likelihood is NZMS surviving transport on wet muddy gear from an infested area where field crews were working. A “low likelihood” rating could apply to a clean, drained, dry boat being transported from a non-infested area. The likelihood associated with each pathway is specific to each facility based on environmental factors such as humidity and temperature, water source and regulatory actions.

Use of the recommended tools and procedures can lower the entry potential. Each facility assessment should rank each pathways using one of the 6 categories. This process can be done qualitatively or quantitatively on Form 1. The total risk can be calculated by averaging the scores together.

Step 3: Colonization Potential

The "colonization potential" considers many of the specific environmental characteristics around the hatchery facility to determine the likelihood of the area supporting the establishment of NZMS, zebra/quagga mussels and Asian clams. Factors that are considered are stream/water body, water quality, water chemistry and human mediated characteristics. These are listed on Form 2, page 36

Stream/water body characteristics:

- Flow rate - NZMS dislodgement probability was higher on bedrock and cobbles than on gravel and pebbles, staying in place at velocities greater than 160 cm/s. Transport of veligers can occur in nearly any flow rate if infested areas are above the facility. The Asian clam has a mucous assisted flotation stage of the veligers, and even smaller sized juveniles (<1mm).
- Expansion rate - NZMS will expand out to other areas downstream and upstream from source populations at flows below 30 cm/s. The can move upstream as well as downstream, but more likely movements are downstream. Expansion of Asian clams, or zebra/quagga is dependent on established populations, mostly in a downstream direction, but movement upstream is possible. They can drift on byssal threads, particularly at small juvenile sizes. Asian clams are capable of moving in the substrate using their foot.
- Spread rate - The rate of upstream colonization from original sites by NZMS decreases as flow increases with 30 cm/s as the maximum flow tested. The zebra and quagga mussels are capable of detaching and moving, but rates are not well quantified. Veliger stages can be transported with water. The validated highest probability of zebra and quagga mussel transport is via attachment to boats and vessels. Asian clams are capable of considerable movement in adult and veliger stages.
- Depth - NZMS have been found at various depths in the Great Lakes; however, as the depth increases the density of NZMS decreases. Quagga mussels are capable of living at

lower depths than Zebra mussels. Asian clams can survive in fairly deep water, and in a variety of substrates, and can burrow in substrate to as depth as 16 cm.

- Stream order - Higher order streams are more likely to support NZMS colonization. In addition, higher order streams are more likely to have Asian clams, as the thermal regime would be more benign.
- Elevation - NZMS have not been reported in streams of high elevation, and are more frequently reported at elevations of 1000 meters or less. We have little information about toleration of zebra/quagga mussels or Asian clam distribution, but Asian clams are abundant in parts of Lake Tahoe, more than 1,800 m elevation with an abundant population of Asian clams.
- Light intensity - There is no phototactic responses in NZMS, but they graze at night. The Zebra mussel is reported strongly photophobic in some experiments, but not in others. Little is known about Asian clams.
- Habitat type - NZMS can be found in a variety of habitats, runs, riffles, eddies, edges and vegetation. Zebra mussels are capable of surviving in a variety of habitats and high flow rates, providing there are surfaces to attach, but quagga mussels have recently infested soft bottom substrates of variable flow rates.
- Substrate type - NZMS are most likely found in gravel and vegetation and less likely in sediment within the habitat. Hard substrate is generally needed for zebra mussels but quagga mussels will use soft substrates. Asian clams prefer soft sand substrate, but can burrow into embedded substrate with considerable cobble and reach depths of up to 16 cm - although most are around 4 cm.
- Connectivity-Since - NZMS will survive the digestive tract of trout, fish can spread the snails to other connected waters. Also documented are transfers of snails by human or animal movements within or across watersheds. Clams and mussels can be transferred with fish or bird movements within and across watersheds, as they can survive in the digestive tracts.

Water Quality Characteristics

- Season - Densities of NZMS are the greatest in spring, summer, and fall months and related to the production of algae. Zebra/quagga mussels reproduce depending on water

temperature. Since the adults are rather immobile, the veligers are seasonal. Asian clams can survive temperatures as low as 1.5°C to upwards of 30°C, but generally reproduce only when water temperature rises above about 15°C. There are some reports of Asian clam survival for short times in even colder temperatures.

- Temperature - Optimal temperature for NZMS is 15 to 18ºC. Maximum survival temperatures for NZMS were reported at > 96 h at 32.4±2.5ºC. Minimum temperatures for survival in the lab or field were 0 to -4°C for a 3 to 7 day exposure. According to most sources, the optimal temperature for zebra mussel reproduction is 14 to 16°C, and that for quagga is lower- down to 9°C.
- Specific conductivity - Significant reductions in survival and growth occur at low levels of specific conductivity. Recent studies of quagga and zebra mussels found that the conductivity of source water affected the response of potassium as a toxicant.
- Calcium concentration - NZMS growth was inhibited in calcium-free water. Literature reviews report calcium levels of quagga and zebra mussel populations to range from 28 mg/L in European lakes, to concentrations of 12 to15 mg/L or lower from studies of North American distributions. A survey and mapping project illustrated areas with high, medium and low calcium and within the Columbia River, many regions were determined highly suitable.
- pH - NZMS grew fastest and had the highest fecundity at mid-range pH levels (8-9). For zebra and quagga mussels, a range of pH of 7.5 to 9.3 is reported. Exposure to elevated pH >11 can be a control tool for all life stages the mollusks.
- Phosphorus ratios - Size and reproduction of the NZMS is dependent on the amount of phosphorous present in the system to promote primary production. Since the quagga/zebra mussels and Asian clams use filter feeding, they are dependent on productivity in the open and littoral zones of lakes and rivers. The Asian clam can also pedal feed, adding more methods for survival.
- Salinity - NZMS can survive a wide range of salinities, from 0 to 15 parts per thousand (ppt). The optimum salinity for NZMS is 5 ppt. Zebra/quagga are not able to survive in salinity >5 ppt. Asian clams can survive up to 13 ppt and even up to 24 ppt for shorter exposures.

Human and Other Animal Mediated Characteristics

- Disturbance/pollution - NZMS have been found in highly disturbed areas, as NZMS grow and survive in sediment and nutrient rich waters. NZMS can survive higher concentration of ammonia levels over a long-term and short-term duration compared to other aquatic invertebrates. Estrogen pollution stimulated embryo production at low doses and caused inhibitory effects at higher doses in the laboratory. Populations of quagga and zebra mussels and Asian clams are found in highly disturbed and human altered environments.
- Angling/stocking - Angling and fish stocking practices are indicators of potential vectors and vehicles for NZMS populations. NZMS can be transported on the gear as adults and subadults, and with fish and hauling water. Transport of fish and water are all potentially contaminated with dispersal stage juveniles and should be considered a high risk with control measures used and validated for each location.
- Birds - NZMS are carried from one river system to another by animal vectors. The early life history stages of Asian clams can be easily carried on birds and within the GI tract of birds and fish.
- Boats, ballast and live wells - The NZMS and other mollusks can be carried in boats or attached to boats that are not disinfected. The exposure time and conditions affect relative survival. The planktonic or early life history forms cannot survive for long periods, but adult stage survival highly dependent on humidity and temperature. If there is boat traffic near the hatchery facility, potential transport must be considered in all assessments.
- Construction and firefighting equipment – The caissons, floating cofferdams and pontoon barges used for construction are high risk transport mechanisms. Construction at and near hatcheries can be a potential vector for transport. In addition, fire equipment that enters hatcheries could bring residue of contamination.

Assessment of Robustness of Data

This step requires an assessment of the strength of all data used for assessments of risk. Studies of the life history and habitat characteristics of these targeted invasive mollusks are used in understanding the likelihood of colonization and establishment. Characteristics or parameters need to be examined and assigned a ranking of high, medium of low to estimate how that specific characteristic can be attributed to the colonization and establishment. Evaluations of the

reliability of each source of information for each characteristic should be made to determine the likelihood that these data are applicable. Certainty can be ranked using the following deterministic parameters:

- Lab/field – Examination of where the trials or studies were conducted to relate to the effectiveness of applying the same procedures to a field setting at a hatchery.
- Source type – Determination of information source such as peer reviewed literature, thesis or dissertations, unpublished reports, or personal communications.
- Temperature and time of year relationship – Was temperature reported in study? Temperature can affect the outcome of all life history characteristics and understanding that role in the data is important. Time of the year affects the growth and nutritional phase of organisms studied.
- Source population - Genetic variations could account for uncertainty in the likelihood of colonization and establishment.
- Calculated/provided - The determination if the characteristic ranking was "provided" as part of the literature (provided) or gleaned from the data provided by the source (calculated).
- Other - Some of the characteristics that are important in determining the likelihood and establishment of the mollusks are not yet available. Surrogates can be used however, there is a high uncertainty associated with using this data.

In examining each characteristic for a specific fish hatchery, assign a likelihood ranking and fill out Form 2 (page 36). If using quantitative measures use the following to incorporate a number rank of each likelihood ranking; 4=high, 3=medium, 2=low. The next step is to assign a weighted certainty to each characteristic as it applies to a facility. If using quantitative measures the following case-specific weights are recommended to determine the weighted certainty of the data reported applying to the specific hatchery. The following are proposed weighting factors can be considered but these should be developed independently.

- Lab/field: field=0.90, lab=0.75 and 0.5 theoretical
- Source type: peer reviewed=0.9, thesis=0.8, report=0.7, and personal communication=0.6
- Temperature: 1-(|tested temp-facility temp|*0.1)
- Source population: if source population is different, estuary vs. stream, or US vs. Europe,

etc.=0.5

- Calculated/provided: provided=0.9 and calculated=0.8
- Surrogate use 0.5 otherwise use 1

Certainty=1-((0.9 field, 0.75 lab or 0.5 theoretical)*(source: 0.9 peer reviewed, 0.8 thesis, 0.7 report, or 0.6 personal communications)* 1-(|tested temp-facility temp|*0.1)*(0.5 if source population is different)*(0.9 for provided or 0.8calculated)*(0.5 if surrogate))

Add the weighted certainty from the characteristic ranking to determine the resulting risk for each characteristic. The characteristics can then be averaged to determine the colonization potential.

Step 4: Spread Potential

The spread potential estimates the probability of the organism spreading beyond the colonized area. Natural dispersal and density are characteristics that enhance spread potential. Ranking of potential for spread is determined by how close and dense the nearest known population of NZMS, quagga/zebra mussel, or Asian clam population is from the facility. These are listed on Form 3, page 37.

To determine the closest population, contact the state Invasive Species Coordinator, look at the U.S. Geological Survey (USGS) database (http://nas.er.usgs.gov/), or monitor surrounding areas from the hatchery to determine the distance from the facility of a known population. Determine the density of the population closest using monitoring techniques that estimate density. Once the characteristics are ranked in Form 3, the rankings can be averaged to determine the spread potential to the facility.

Summarizing Risks of Establishment

Using the templates and forms 1-3, the average risks from steps 1-4 can be averaged together to calculate the risk of establishment (Figure 3).

Figure 3: Combining 4 steps to determine risk of establishment at a hatchery facility.

Table 1. Suggested resulting risk ranking for weighted calculations for average of steps in risk of establishment determination.

Calculated Risk of Establishment	**Risk Ranking**
>6.0	Uncertain
5.0-6.0	Very high
4.0-5.0	High
3.0-4.0	Medium
2.0-3.0	Low
<1.0-2.0	Very Low

Steps to Consider Regarding the Consequences of Establishment

This section has the remaining three steps to complete the risk potential assessment. The consequences of establishment include an understanding of how an infestation would affect the hatchery operations, and also the consequences to the fish rearing program, such as stocking, mitigation programs, or species recovery measures. . These consequences are going to differ based on the rules, regulations, and culture of each state in managing infestations. The assessments can also lead to positive effects under each category, not just negative effects. These should be summarized on Form 4 page 37.

Step 5: Economic Impact Potential

Assess the economic impact to the hatchery if any of the target species become established. Elements to consider are the costs of direct control and management, loss in production, fines, or specialized personnel costs. These costs can also include lost fisheries, and even loss of ESA listed species associated with the program and other factors related to hatchery production at that facility. Determine or estimate and explain the economic cost. Fill in Form 4 with the appropriate ranking: uncertain, very high, high, medium, low, or very low.

Step 6: Environmental Impact Potential

Assesses the environmental impact that NZMS or other invasive mollusks will cause if established at or near the facility. Characteristics to consider are ecosystem degradation, modification, or destabilization, reduction of biodiversity, reduction, or elimination of endangered/threatened species, loss, or reduction in quality of preferred habitat conditions for native species, or consequences of control actions. Determine the environmental impact of infestations and the impact of control measures. Fill in Form 4 with the appropriate ranking: uncertain, very high, high, medium, low, or very low.

Step 7: Social and Cultural Influences

This step prompts the assessment to estimate the social and cultural practices if NZMS, or other mollusks are introduced to the facility. Attributes to consider include effects on cultures of national and region importance and social effects not easily captured under the economics step. This section should consider any tribal trust relationships associated with the facility and with the species being reared. These consequences should be explored to consider other tribal treaties and agreements especially with regard to facilities rearing anadromous fish. Fill in Form 4 with the appropriate ranking: uncertain, very high, high, medium, low, or very low. Average of step 5 through 7 ranking level will be the risk ranking of the consequences of establishment.

Summarizing the Risk Potential

The risk potential of a facility for a NZMS infestation is the average of the risk of

establishment, with ranking and the consequences of establishment, as shown in Figure 4.

Figure 4: Average risk of establishment ranking with consequences of establishment to obtain the risk potential. This combines the assessment completed in all four forms

V. Determining the Status of the Facility

It is essential to determine if a facility is already infested with NZMS or other target mollusks. Strategic monitoring is essential to determine if the facility is infested with an unwanted organism, and determine management options.

Monitoring

Monitoring is an essential tool/methodology as part of facility biosecurity protocols. By having biosecurity protocols in place, rapid response and other management strategies can be implemented effectively. A monitoring protocol must identify the target organisms, occur on a regular schedule, and be performed consistently. Documentation and record keeping of all aspects of monitoring is important. The frequency of monitoring should depend on the perceived risk associated with the facility and the colonization potential of the invasive species. If there are low perceived risks of infestation, monitoring can be structured around appropriate times and be less frequent. However, if the facility has a medium risk of being infested, then more frequent monitoring should occur, such at scheduled intervals. If the risk of infestation is high, then extensive monitoring must be conducted. Extensive monitoring should include additional sampling sites, use of several methods, and frequent sampling. In this section we present some general considerations for tools used to collect and analyze samples for invasive mollusk species. We discuss sample size and effort, sample labeling, retention, and oversight and review.

<u>Locations of Monitoring</u>

Upstream, downstream and within target areas such as intakes, headboxes of rearing units, and effluent of the facility (Figure 5). Multiple locations on station may need to be monitored depending on the layout. Considerations such as incoming water flow, nutrient availability, access by people or animals, and areas for the target organisms to inhabit are considerations in choosing a good monitoring site. Consider all areas surrounding the aquaculture facility. Areas around the aquaculture facility that are near popular fishing and recreating sites, irrigation diversion structures, migratory waterfowl use areas and where other people frequent the water are recommended. Examine areas at least 60 m (200 feet) from the hatchery, especially if the water supply is from an open source such as springs, open wells, or streams. NZMS have been found in brackish and freshwater, specifically estuaries, lakes, rivers, and streams. For all mollusks, monitor areas with high and low calcium content, hard and soft substrates, and the following habitat types: sand, leaf litter, organic detritus, silt, algae, aquatic macrophytes, gravel, cobble, and boulders.

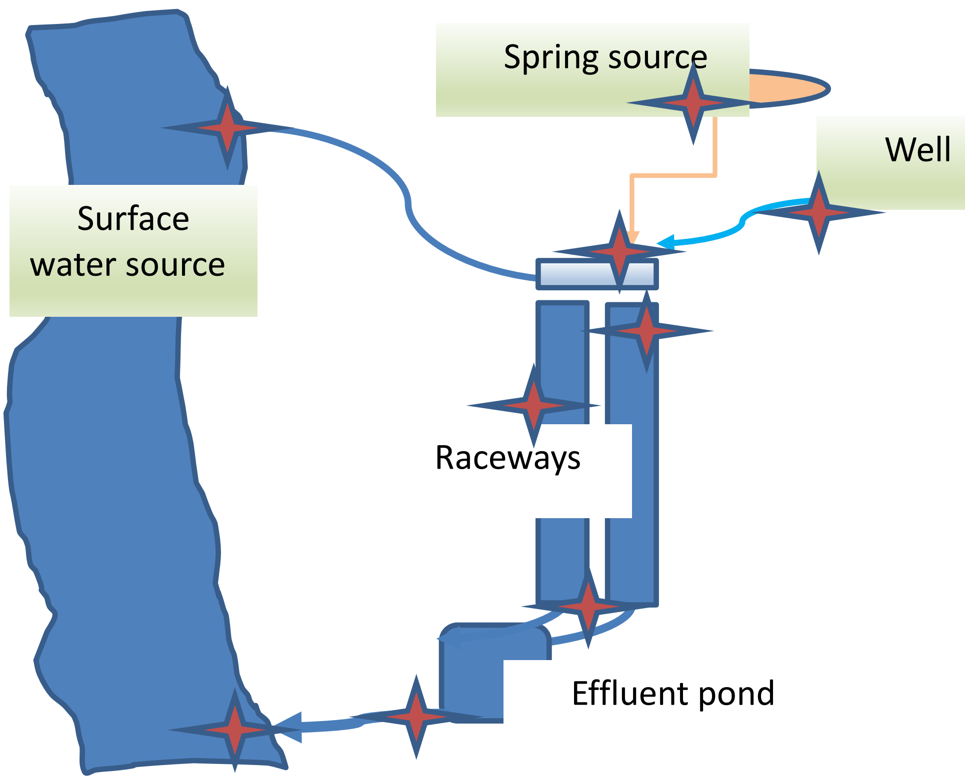

Figure 5: A generalized layout of a fish hatchery with suggested monitoring sites as stars.

Tools for collection

Once sites are selected, the appropriate sampling tool is needed. Tool selection will consider the habitat being monitored and time of year. Multiple tools are necessary to monitor a facility. Disinfection and record keeping of all disinfection of monitoring gear is important.

Surber & Hess Samplers- Samplers can be used offsite or even in settling ponds with directional flows in gravel to silt substrate. They can be used to estimate a measureable collection area and can be used to collect adult stages of NZMS s, zebra/quagga mussel adults, and Asian clams. Sampling is especially efficient when populations are large in late spring, summer and fall. Effective uses of the samplers include choosing an accurate mesh size, 250-500 micron mesh nets. Disturb the area within the square frame and scrub individual substrate particles within sampling area to use these samplers to effectively monitor.

Kicknet- Kicknets are useful in flowing water, and can collect a variety of invertebrates including NZMS adults, zebra/quagga mussel adults, and Asian clams. Kicknets can be used year-round to sample multiple areas with little effort, although spring, summer and fall are the best times to sample. Multiple collections should be used, with 1-2 minute timed sampling periods, across the width of a stream or canal area.

Plankton Tow Net- Plankton nets are useful for collecting NZMS adults and neonates along with zebra/quagga mussel veligers. This tool can be used year-round, except when water is frozen. It is best used when veligers, neonates, and NZMS adults are in water column, such as spring, summer, and fall when timed to specific temperatures. When determining which plankton tow net to use consider the cod end, the mesh size of the net. Measuring the length of time the net is towed and flow rates of water and water depth will allow calculation of area or volume sampled.

Ponar Grab – Substrate samplers are used to provide quantitative samples of the sediments such as small cobble, gravel to silt on site or off site. They are useful to locate NZMS adults, zebra/quagga mussel adults, and Asian clams that are buried or in softer or smaller substrates. This tool can be used year-round when water is not frozen; however a boat may be needed for access to deep areas. It is important to sieve and rinse the sediment away to find the organisms, and this activity should include disinfection step.

Snorkeling- This tool can be used in shallow waters to efficiently monitor structures and shorelines. The best time to sample with this method is early spring to late fall, when populations

are numerous and not buried. Effective protocols include turning over rocks, pools, and examining areas that cannot be monitored with shoreline surveys.

SCUBA- Underwater divers can check submerged structures; however this method can be very costly because it requires a licensed professional. SCUBA divers can look for NZMS adults, zebra/quagga mussel adults, and Asian clams especially on vertical underwater structures. There is the potential to monitor year-round with this method; however, later spring, summer or fall are the best time for finding populations.

Shoreline Survey- This survey methodology is easy to use by walking in a zig-zag pattern in the water, looking under rocks, stumps, on vegetation, in mud 2-3 meters from shoreline in each direction. It is useful for NZMS adults, zebra/quagga mussel adults, and Asian clams, and can be used year-round, and is the best option in the winter. For large infestations, shells may be visible on the shore. Visit area after a storm, high boat traffic, or when water levels are the lowest to be the most successful.

Suction Dredges- Dredges can move a slurry of water and sediment, and are efficient at locating adult mollusks in shallow water in the late spring, summer and fall. Because of potential water quality impacts, there may be local regulations limiting use of this sampling method. This tool has been used for removal of Asian clams.

Samplers or Traps- Samplers or traps are devices that can be hung below the water line on existing structure and are easily monitored for colonization. Samplers and traps work best in the early spring and late fall, during colonization times. There are a variety of commercial or home-designs that are available.

Small mesh Aquarium Nets- These tools are commonly found at hatchery facilities. They are likely to find NZMS adults, zebra/quagga mussel adults, and Asian clams in shallower head boxes, tanks and raceways on a facility. This tool can be used year round. Use by scraping the sides of surfaces in an upward motion from bottom of unit to top to collect algae and organisms attached to walls.

Sample Size/Effort

Large number of a population are easier to detect than smaller population sizes, and the smaller organisms are also an important consideration. Sampling gear, timing, repetition, and analyzing processes can help maximize the likelihood of detecting an invasive aquatic mollusk

infestation. Sampling during peak times of reproduction, high colonization times, and where the organisms are most likely to be are essential to finding an invasive mollusk. With parthenogenic and hermaphrodites, only one individual can start a population. Sampling effort should be recorded, evaluated, and in testing of efficiency of effort can be accomplished in known infested areas to assist with assessment of appropriate effort.

Tools for Analysis

Sample collection and retention in individual sterile or disinfected containers is absolutely necessary. Containers must be clearly marked with date, sample location, methodology and name of collector. Other parameters such as water temperature, type of substrate, and other environmental information should also be collected. Labels should be well designed to assure that samples are not confused. When analyzing samples, it is important to keep everything clean and separate as to avoid cross-contamination. If sending samples off to a lab, then a chain-of-custody should be implemented. The chain-of-custody provides documentation who sent or released the sample, where a sample travels, time a sample is received, who received the sample and who is responsible for the sample. This documentation needs to be precise enough to potentially hold up in court or be scrutinized by agency or political policy makers. To be safe, document everything. Some samples can be analyzed in the field, while others can only be done in a laboratory environment. Reference specimens and photos can provide valuable field and laboratory sampling information. When looking for adults, samples can be sorted in the field, and only what is of interest can be retained. As a matter of practice, you want a representative sample of all organisms collected retained for future analysis.

Sieves can be used to accelerate the sorting process. Sieve sizes can range from 10 um to 4 mm openings. Dissecting microscopes and magnifying glasses can aide in identification of the organism. When looking for neonates or veligers, lab analysis is necessary with dissecting or compound microscope. Samples with veligers should be allowed to settle for up to 2 hours. The bottom portion of the sample would yield the likely location of neonates and veligers.

Sample Retention

Sample retention is important for providing proof as well as maintaining a library that can be used for future comparison of genetic information and sample comparison. Preserved samples can be stored for long periods of time, preferably in 70% ETOH. A depository reference site can be established to maintain and store the samples.

Oversight and Review

After protocols are established, a review process should be conducted. Validation of techniques will identify gaps in process and mistakes by testing known positives and negatives for consistency. The review process should have accountability steps to confirm protocols were followed or to evaluate the effectiveness of a management action. Additional review processes would include a quality assurance unit to inspect all procedures. Having an independent entity examine the protocols and records that the facility is adhering to lends additional credibility. Lastly, there is the option of a peer review that inspects the protocols and makes suggestions to keep up with current research and apply the most up to date management actions at the facility. By going through such a review process, there is more awareness and transparency of protocols for the public and other facilities.

Disinfection of Monitoring Equipment

A decontamination-disinfection process, when implemented correctly, can be a cost effective method of reducing unwanted organisms and are an important step in any risk management program. Thorough cleaning and washing to remove organic debris prior to the application of any disinfectant is essential. The following flow diagram, illustrates how the options for treating gear can be implemented (Figure 6).

Decontamination should be implemented at the sampling site to reduce the risk of cross-contamination of samples from other locations and prevent potential movement of viable mollusks. If it is not feasible to decontaminate on site, measures should be in place to contain and isolate contaminated equipment. Gear can be placed into a bag and disinfected later when more convenient. Established protocols and disinfection areas need to be in place to ensure new locations are not contaminated.

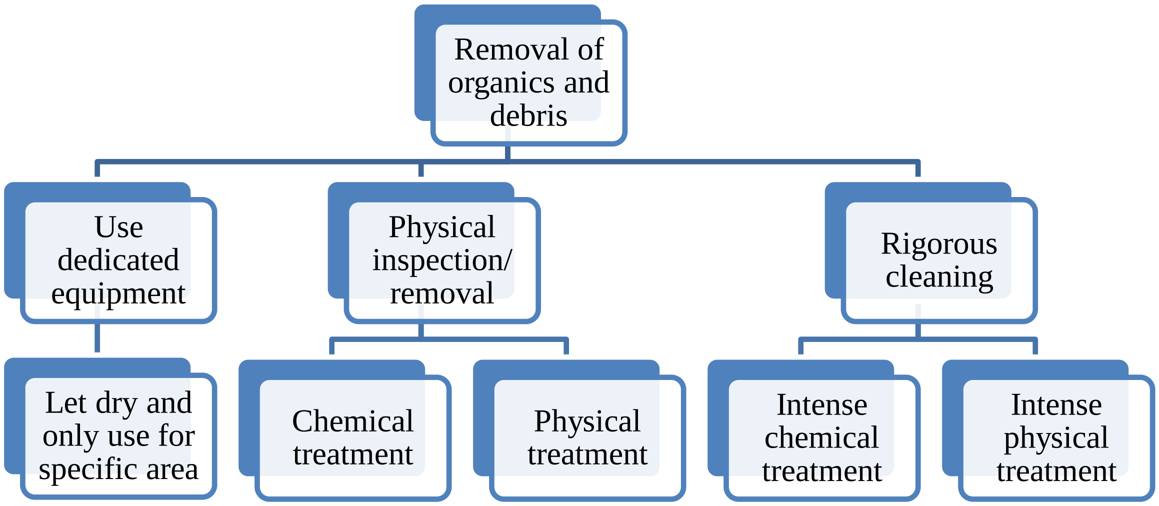

Figure 6. Flow diagram of options for treating gear to remove invasive mollusks.

Disinfectant Labels

All chemicals must be used in accordance with label instructions. It is a violation of federal law to use a product in a manner inconsistent with its labeling. Be sure to read and incorporate the labels, Safety Data Sheets (SDS) or Material Safety Data Sheet (MSDS), and spill response into protocols.

Physical Inspection and Removal

Physical inspection and removal processes are fairly simple. Inspecting gear to remove organic matter and sediments will eliminate much of the potential for contamination however, inspection and removal for large adult mollusks will decrease decontamination times and increase effectiveness. Even though invasive mollusks such as NZMS are small, most adults can be seen and removed, but they will cling and crawl onto many surfaces. Zebra/quagga mussels are less of a concern to foul equipment due to their difficulty attaching. However, early life history stages (dispersal stages) can attach to gear, and Asian clams have a more resilient veliger stage with considerable shell armor. The inspection and removal process will not remove dispersal forms of invasive mollusks; therefore, chemical or physical treatment is necessary. If an area of sampling gear cannot be inspected, disinfection treatment is advised.

Two broad methods for decontamination are chemical and physical treatment. Chemical treatment options include a plethora of chemicals that have been tested to kill invasive mollusks.

However, caution is advised because understanding of each chemical action, its effect on objects being decontaminated, and the environmental regulations are important. Physical treatment options need consideration of clean space for drying, time for treatments, and availability of adequate space and conditions.

Chemical Treatments

Each chemical has its unique characteristics, hazards, toxicities, and efficacy against organisms. Environmental conditions such as organic matter, pH, or water hardness can impact the action of each chemical disinfectant. Appendix A provides more details on specific approaches, recommendations, including sources of peer reviewed documents supporting methodology. A general suite of chemical types is reviewed here.

Alcohols: Activity - denature proteins, causing membrane damage and cell lysis.

Corrosive Properties: can cause damage to rubber and plastic (remove waxes and other coatings) and can be very irritating to injured skin,

Environment: evaporate quickly, flammable, Organic matter: Activity is very limited in the presence of organic matter.

Pros: fast acting, easily obtainable, preservative.

Cons: limited against viruses and spores, flammable, damages equipment, evaporates quickly, relatively long contact time.

Surfaces: good on hard surfaces, and metal instruments

Alkalis

Examples: Sodium hydroxide, sodium carbonate, calcium oxide.

Activity: soaponifies liquids within envelopes.

Corrosive properties: can be corrosive, personal protection precautions.

Environment: toxic to humans, Organic matter: not easily deactivated.

Pros: effective, Cons: corrosive, toxic.

Surfaces: not good for aluminum, but works on stainless steel.

Halogens

Examples: Chlorine or iodine compounds

Activity: chlorines use electronegative nature to denature proteins and iodine functions by denaturing proteins to interfere with enzymatic systems

Corrosive properties: Chlorine is very corrosive, iodine is considered corrosive to metals

and rubber. relatively safe

Environment: chlorines make other chlorine toxins, and iodine, depending on concentration, irritate skin, stain clothes, Organic matter: Both are easily deactivated at low levels of organic debris.

Pros: Low toxicity, low cost, easy to use

Cons: lose activity quickly by organics, deactivated by sunlight, stains surfaces. Iodine is not labeled for use on hard surfaces but is labeled for use as antiseptic on living tissues. .

Oxidizing Agents

Example: hydrogen peroxide, peracetic acid, Virkon Aquatic

Activity: denatures proteins and lipids

Corrosive properties: in high concentration irritating and damage clothing, safe in water.

Environment: relatively safe, Organic matter: deactivated by organic matter

Pros: obtainable, effective, environmentally safe

Cons: Irritating and damaging in high concentrations, higher cost.

Phenols- hydroxyl on aromatic ring

Examples: Lysol, Pine Sol

Activity: denatures proteins and inactivates membrane-bound enzymes to alter cell wall permeability

Corrosive properties: may cause skin irritation and has residual properties

Environment: concentrations greater than 2% are highly toxic to all animals. Organic matter: active in the presence of organic matter

Pros: easily obtained and effective in some studies.

Cons: irritating to skin, and must rinse off

Quaternary Ammonium Compounds

Examples: Sparquat, Bardac

Activity: cationic detergents attracted to the negatively charged surfaces of organisms, where they irreversibly bond phospholipids in the cell membrane and denature proteins impairing permeability.

Corrosive: non-corrosive

Environment: highly toxic to fish.. Organic matter: some easily deactivated, others not, Considered hazardous waste

Pros: easily obtained, non-corrosive and effective

Cons: highly toxic, disposal issues, inactivated by soaps and detergents. Can cause genetic alterations.

Other Chemicals: Toxic metals

Examples: Copper sulfate solutions

Corrosive Properties: will corrode metals, non-corrosive to other materials,

Environment: Small concentrations are toxic to aquatic invertebrates and vertebrates

Organic matter: not deactivated by organic material

Pros: effective, non-corrosive

Cons: disposal complications and labeling agreements. The toxicity is related to pH, organic dissolved fraction, and calcium hardness of the receiving water. Copper sulfate is reported to disrupt surface epithelia function and peroxidase enzymes in mollusks.

<u>Freezing, Heating and Drying Treatments</u>

Non-chemical options to disinfect gear include freezing, heating, and desiccations. Time, temperature, and feasibility need to be considered in each of these methods. After the removal of organic material and debris, gear can be treated without chemicals. Exposure times should be higher when incomplete removal of organic matter is possible or if the facility is higher risk for adult mollusks. Areas on equipment that cannot be physically examined but are potential hiding spots for invasive mollusks should also go through a rigorous cleaning regiment. Rinsing equipment is recommended after treatment to remove dead organisms and ensure equipment is decontaminated from treatment options.

Freezing and Heating

Studies have shown that subzero temperatures will kill all life history stages of mollusks. Freezing temperatures should be maintained for a suitable period of time to penetrate treated gear and materials. The efficacy will depend on the density of any infestation, and inspection should occur before returned to use.

A hot water exposure can be effective, but the exposure time and temperature is critical to successful decontamination. Domestic water heater temperatures range from 37 – 60°C (100 to 140ºF), and most protocols for wash systems specify an exposure to 60 – 71°C (140 – 160°F) and thus domestic water sources may not be sufficient. Normal car washes generally do not reach

or exceed the recommended 140-160°F, however, there are specialized truck washes across the nation used by animal transport haulers. For a list of approved high heat truck washes nearby, visit www.biosecurity.org. When using wash systems, collection of debris to prevent further infestation from the decontamination site is recommended. We have observed expulsion of neonate NZMS from highly stressed organisms, and reports for the other species, especially Asian clams, include release of brooded veligers after stress.

Desiccation

Leaving gear to dry out completely will be effective, but the time needed depends on the relative humidity and ambient temperatures. Recommended drying time at temperatures greater than 27°C (80°F) for infested gear is three days depending on humidity levels. All gear must be completely dry; and not contain any organic matter.

Evaluation of the certainty of the physical/chemical treatments

Sources of information regarding treatments with or without chemicals should be evaluated and a ranking assigned. Probability values should be assigned to estimate and rank each disinfectant. Some approaches such as those following can be used to assess certainty.

Factors that are important are number of replicate trials and temperatures tested, the source of information, and if the presence of neonates or veligers was included in evaluations. Failure to consider dispersal state veligers and neonates can elevate the risks that incomplete disinfection occurred. Suggested weighting factors for quantifying the tools can be based on assessment that consider the source and depth of information provided. For example peer reviewed should receive higher ranking e.g. =0.9, thesis=0.8, report=0.7, and personal communication=0.6; Consideration of neonates, Asian clam brooded veligers releases: assessed=1, not assessed=0.5

VI. Choices and Constraints

Sampling results will determine the hatchery status, and management pathway. If the facility is found infested, the choices are to follow status quo or prevention pathways in the decision tree (Figure 7). Development of a strong biosecurity plan can help to keep the facility clean and uninfested. If the facility is infested, the choices are to follow status quo, manage to

minimize spread outside the infested facility, or select an eradication/disinfection option (Figures 2 and 7). If eradication is accomplished, the monitoring and response protocols should remain in place to determine an acceptable time period to consider the facility clean. Some local regulations may apply in this process and could be similar to those for reportable pathogens.

Consideration of a strong biosecurity plan include that disinfection is a key attribute to help keep the facility free of invasive mollusks. When designing the disinfection plan the following should be considered and assessed to make a strong biosecurity program.

- Target organisms-determine the organisms and life stage that is the most difficult to kill or address in the location.
- Chemical or physical methods-determine which is appropriate for the gear, time, and cost.
- Characteristics of disinfectant-determine what the chemical is safe to use on, i.e. metals, dyes, etc. and light sensitivity, other chemical reactions, etc.
- Activity of the chemical-some chemicals can be used repeatedly for long periods of time, but protocols for each chemical must include validation of concentration and activity.
- Deactivation of chemical-determine how the chemical is deactivated to mitigate spills, or ensure that it is still active and effective.
- Environmental concerns- determine the proper disposal procedures for each chemical and ensure compliance through record keeping.
- Effects on gear and workers-determine the effect on gears and health concerns as some chemicals are more caustic than others.
- Organics and other debris must be removed as they will deactivate many of the chemical disinfectants. These include leaf litter, organisms, fine particulate organic matter and soil. Use a scrub brush, water rinse, scraper, or other physical removal actions to remove organics and debris.
- Individual equipment used- buy separate nets, brushes, and gear for each individual area. This is the best option, but storage and drying areas are also important considerations.

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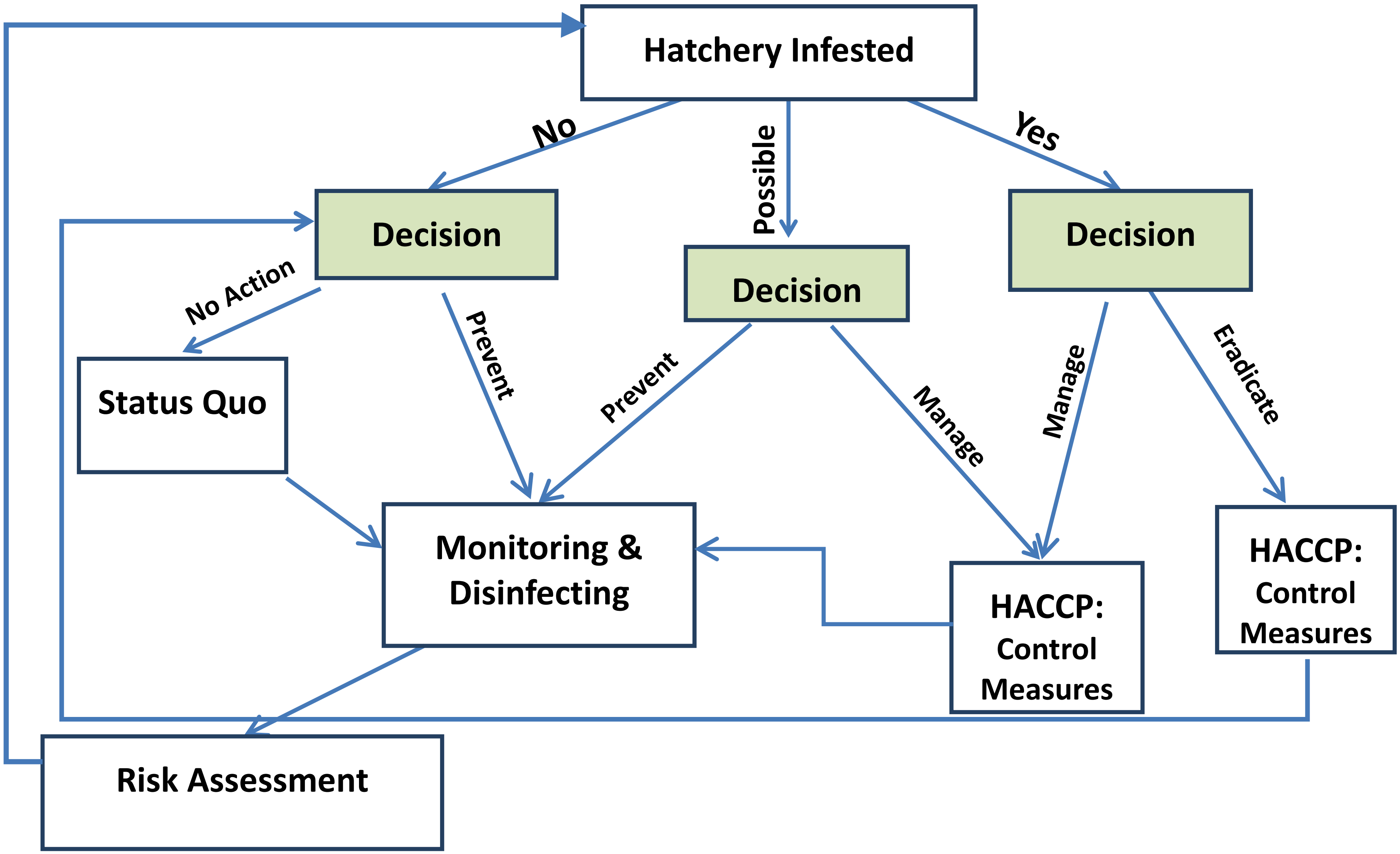

Figure 7. Decision tree to evaluate infestation and the choices that lead to actions such as monitoring and disinfection, HACCP and control measures, and risk assessment.

VII. Critical Control Points (HACCP & Certainty Rankings)

The hazard analysis and critical control point process (HACCP) can be used as a comprehensive planning tool to identify critical control points throughout a pathway utilized by invasive species to infest a facility. HACCP takes into account other non-target species including plants, animals, diseases, pathogens, and parasites. Hatchery staff with the US Fish and Wildlife Service are trained in the processes of HACCP, and they are instructed to use the process to assess their daily routines for potential risks associated with infestation of non-target species. http://www.haccp-nrm.org/listplans.asp

The same process is used in this document, but the pathways that the NZMS or other invasive mollusks utilizes to infest the facility are also evaluated. By evaluating the pathway instead of an activity, the hatchery manager ensures that the pathway of NZMS or other invasive mollusks is controlled and monitored. The result of the HACCP process is supplemented with additional guidance to combine the ranking from Forms 1 – 4 with the HACCP. These supplemental forms are provided as a summary exercise document after the HACCP documents (pages 44-45).

The HACCP Planning Process

This process is described in detail at *www.**haccp-nrm**.org/*. It has 5 steps which correspond to a specific form. The HACCP steps and forms are provided on pages 38-43.

Step 1 (HACCP 1) – Pathway Description. Choose the highest ranked pathways from Risk Potential form 1 that affect the facility. Describe how, when, where, why, what and who the pathway effects the facility. The description should offer a historical working reference to facilitate communication.

Step 2 (HACCP 2) - Identify Potential Hazards. Even though this document is focused

on NZMS, there are other unwanted species that can infest the facility through many of the same pathways. By incorporating a broad range of organisms into the HACCP planning process, the document is strengthened. The control measures in this document are evaluated for NZMS, Asian clams and quagga/zebra mussels; however, the same process can be used for other control measures focused on the specific unwanted species. Risk potential similar to the method described in this document can help focus this section to determine the likelihood of each organism infesting the facility. This step will ensure that the manager has working knowledge of all potential hazards.

Step 3 (HACCP 3) - Flow Diagram. The flow diagram is an important visual tool to describe and determine the flow of the pathway into and through the facility. This process will show where, when and how the pathway affects the operations of the facility. By determining the flow of the pathway in steps, management and control actions can be utilized to reduce risks of potential infestations.

Step 4 (HACCP 4) - Hazard Analysis Worksheet. This worksheet incorporates each step of the pathway listed in step 3, incorporates the potential hazards identified in step 2. The worksheet steps the user through a risk assessment process, and the responses are recorded and justified. Control measures are evaluated at each step as well, description and evaluation of control measures of NZMS and other invasive mollusks can be found in this document. Critical control points are identified in this step as well. Management, use of control measures, and monitoring are most effective at critical control points. *Repeat steps 1-4 for all pathways of concern.*

Step 5 (HACCP 5) - HACCP Plan. The critical control points are identified and recorded on this form. Complete this form by designing techniques, methods, and treatments

to deal with each of the significant hazards that you identified. The steps to complete this form involve setting the controls, establishing monitoring procedures, and establishing a verification and record keeping system as documentation. Monitoring is essential in determining if the control measures were effective.

Pathway Control Measure Evaluation

This supplemental document identifies each pathway analyzed, and control measures, but uses the risk potential assessment to assist in determining the effectiveness and the amount of risk reduction provided for a facility by the implemented control measure. The colored matrix combines the pathway ranking with control measures rankings for effectiveness. The documentation is documented on page 41.

Monitoring Evaluation Record Keeping

This form assures that the compliance for monitoring the facility and pathways is accomplished. The form is a template to assure the critical control points were assessed with worker initials and dates monitoring was performed. Corrective actions and evaluations can be attached and documented to keep record of failures, noncompliance, maintenance issues, or other activities and evaluations.

VIII. Forms for Determining Risk Potential

Form 1. Assessment of Entry and Establishment

This form includes potential of surviving and establishing

Pathway	Is the facility affected by this pathway (yes=1, no=0)	Likelihood of organism surviving transit in pathway to area*	Multiply column B*C
Anglers, recreationists, & gear			
Aquarium trade			
Boats, ballast, live wells			
Aquatic birds and mammals			
Effluent pond			
Field crews and associated gear			
Fish collection, transportation			
Open water, canals, culverts			
Visitors			
Other			
		Average risk for 1&2:	

* Determine likelihood based on areas climate or regulations. Ranking: 6= uncertain, 5=very high, 4=high, 3=medium, 2=low, 1= very low

Form 2: Colonization Potential with Attributes of Habitat

More can be provided in assessments.

Characteristic	Ranking	Rank #	Certainty *	Resulting Risk (C+D)
Stream/Water Body				
Flow rate				
Flow rate- expansion rate				
Flow-upstream spread rate				
Depth				
Stream order				
Elevation				
Light intensity				
Habitat type				
Substrate type				
Connectivity				
			Average of Stream:	
Water Quality				
Season				
Temperature (max.)				
Optimal temperature				
Temperature (min)				
Specific conductivity				
Calcium concentration				
pH				
Phosphorus ratios				
Salinity				
			Average of Water Quality:	
Human Mediated				
Disturbance/pollution				
Angling/stocking				
Birds/flyway				
Boats, ballast, live wells				
Construction, other				
			Average of Human Mediated:	
			3. Average of risks:	

Ranking: 6=uncertain, 4=high, 3=medium, 2=low
* Suggested certainty =1-((0.9 field, 0.75 lab or 0.5 theoretical)*(source: 0.9 peer reviewed, 0.8 thesis, 0.7 report, or 0.6 personal communications)* 1-(|tested temp-facility temp|*0.1)*(0.5 if source population is different)*(0.9 for provided or 0.8calculated)*(0.5 if not NZMS))

Form 3: Spread Potential

	6-uncertain	5-very high	4-high	3-medium	2-low	1-very low	Facility ranking
Distance	not known	<10 miles	<50 miles	within state	neighboring state	not in country	
Density	not known	>100,000	>10,000	>1,000	>100	>1	
					4. Average of risks:		

Form 4: Consequences of Establishment

	Example risks	Ranking level
5. Estimate of economic impact	Based on federal and state regulations: cost of control or management	
6. Environmental effects potential	Reduction or elimination of endangered/threatened species Loss of quality habitat Increase disease in fish and bird populations Reduction in native biodiversity from less or elimination of quality preferred habitat	
7. Social and cultural influences	Can have positive and negative connotations (+) increased amount of decomposition (-) loss of anglers in region Tribal consultation and mitigation effects and outcomes from lost fishery	
	Average risk:	

IX. HACCP Forms

HACCP Step 1 – Pathway Description

Pathway Description	
Facility:	Site:
Project Coordinator:	Pathway: e.g. Visitors, field crews, open water sources
Site Manager:	
Address:	
Phone:	

Pathway Description-Effects on Facility i.e. Who; What; Where; When; How; Why

HACCP Step 2 – Identify Potential Hazards

(to be transferred to column 2 of HACCP Step 4 – Hazard Analysis Worksheet)

Hazards: Species Which May Potentially Be Moved/Introduced
Vertebrates:
Invertebrates: New Zealand mudsnails
Plants:
Other Biologics (e.g. disease, pathogen, parasite):
Others (e.g. construction materials, etc.):

HACCP Step 3 – Flow Diagram

Flow Diagram Outlining Pathway into and through Facility
Described in HACCP Step 1 – Pathway Description
(to be transferred to column 1 of the HACCP Step 4 – Hazard Analysis Worksheet)

Step 1	
	⇓
Step 2	
	⇓
Step 3	
	⇓
Step 4	
	⇓
Step 5	
	⇓
Step 6	
	⇓
Step 7	
	⇓
Step 8	
	⇓
Step 9	
	⇓
Step 10	

HACCP Step 4 - Hazard Analysis Worksheet

1 Steps (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards probable? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this Step a critical control point? (yes/no)
Step 1	Vertebrates				
	Invertebrates				
	Plants				
	Others				
Step 2	Vertebrates				
	Invertebrates				
	Plants				
	Others				

Hazard Analysis Worksheet (continued)

1 Steps (from HACCP Step 3 - Flow Diagram)	2 Potential hazards identified in HACCP Step 2	3 Are any potential hazards probable? (yes/no)	4 Justify evaluation for column 3	5 What control measures can be applied to prevent undesirable results?	6 Is this Step a critical control point? (yes/no)
Step #	Vertebrates				
	Invertebrates				
	Plants				
	Others				
Step #	Vertebrates				
	Invertebrates				
	Plants				
	Others				

For additional pages, select entire page and copy to a new page

HACCP Step 5 – HACCP Plan Form

HACCP Plan Form (all CCP's or "yes's" from column 6 of HACCP Step 4 – Hazard Analysis Worksheet)								
			Monitoring					
Critical Control Point (CCP)	**Significant Hazard(s)**	**Limits for each Control Measure**	**What**	**How**	**Frequency**	**Who**	**Evaluation & Corrective Action(s) (if needed)**	**Supporting Documentation (if any)**

Facility:	**Pathway:**
Address:	
Signature:	**Date:**

X. Pathway Control Measure Evaluation Forms

Combines HACCP with Uncertainty Measures

Supplemental Document 1: Pathway Control Measure Evaluation

Supplemental Document 1			
Pathway	**Original Pathways Ranking (Risk Potential form 1)**	**Critical Control Point control measure certainty ranking**	**New risk potential [a]**

[a] determined by table 1 matrix of evaluation

This colored matrix can assist the staff in evaluation of control measures used (their ranking from experience and or reports of use), and used against in context of the ranking of various pathways or risk potential for an infestation. The goal would be to keep the risk as acceptable or lower the risk significantly.

Control measure ranking

Control measure ranking					
Very High	LS	LS	LS	LS	OK
High	LS	LS	LS	OK	UR
Medium	LS	LS	OK	UR	UR
Low	LS	OK	UR	UR	UR
Very Low	OK	UR	UR	UR	UR
	Very Low	Low	Medium	High	Very High

Pathway ranking

LS= lower risk significantly
OK= lower risk
UR= undetermined risk or no effect

Note: Monitoring is the only way to determine effectiveness of control measure at the facility and it is the only way to determine the amount the measure reduced the infestation risk

Supporting Document 2: Monitoring Evaluation Record Keeping

This form can be used to track HACCP Plan implementation over the year

		Initial and date when monitoring has been completed										
Critical Control Point (form 5)	**Frequency (form 5)**	**Jan.**	**Feb.**	**March**	**April**	**May**	**June**	**July**	**Aug.**	**Sep.**	**Nov.**	**Dec.**

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The authors are grateful for critical feedback of the document and processes obtained from several in person interactions and workshops. Reviews were conducted with staff from Washington Department of Fish and Wildlife, Washington Invasive Species Council, the Columbia Basin Team, and Kooskia and Dworshak National Fish Hatchery staff. We are especially thankful for critical reviews of the final draft by L. Sprague, Idaho Fish Health Center, USFWS; and D. Parrish, Idaho Department of Fish and Game. This project was funded by the US Fish and Wildlife Service, Region 6, contract officer, Joanne Grady.

Appendix A

Review of Control Measures for Hatchery Facilities Infested with NZMS

Control measures especially targeted at NZMS are summarized in this document. Evaluations of the feasibility and efficacy of each measure should be conducted for each facility to determine the specific risks, and options available within the constraints of hatchery design and purpose. We suggest that fish health professionals familiar with biosecurity and quantification of disease risks should be engaged in evaluations of efficacy, record keeping, testing, and all procedures should be used in the context of safety and legal applications. Many tools are provided in published literature that may not be applicable within the framework of the regional and national goals. All applications should be discussed and evaluated for regulatory, environmental and human safety measures. There are a number of resources available that summarize tools used to mitigate invasive and nuisance species. The US Fish and Wildlife Service and American Fisheries Society have partnered to provide a table of disinfecting reagents useful in aquaculture (Bowker and Trushenski 2016). An extracted table from this source is provided in this document. In addition, the US Army Corps of Engineers has a large data base of tools targeted at invasive and aquatic nuisance species control available detailing many chemical and mechanical tools available on line http://glmris.anl.gov/documents/docs/anscontrol/All27ANSControlFactSheets.pdf.

This review provides some details from published and on line resources. As a precaution, we suggest all control measures must be evaluated for local efficacy to consider:

- The degree to which the control measure was found to be effective.
- If the recommendations were based on laboratory, small scale or field or typical hatchery conditions.
- Monitoring of presence and survival of neonates from adult NZMS
- Quality or presence of information that has had peer review.
- Source population, water quality, temperature, and time of year trials were conducted.

Source Water Filtration

Rotary microscreen drum filters - There has been little research reported on the effectiveness of drum filters with NZMS. With the correct screen size, wastes such as NZMS and neonates can be filtered out of water sources. Utah Division of Wildlife Resources (Miles 2008) recommended mesh size of ~ 100 µm. Drum filters only remove snails from inflow, so barriers must be created to prevent snails from crawling around filters, or remaining attached to the filter and dropping into receiving water. Collaborating with the supplying company regarding installation and sizing will be essential to lower the risk of failure. Summerfelt and Penne (2005) demonstrated drum filter removals were effective on small to large particles. The Washington Department of Fish and Wildlife has planned a placement of a drum filter at Ringold Springs Hatchery to remove snails from source water for a raceway system, but no reports on efficacy are available.

Hydrocyclone - Tests of a hydrocyclone filtration system (Nielson et al. 2012a) at Hagerman National Fish Hatchery showed promise as a tool to remove NZMS, but has not been put into production use. The hydrocyclone was 100% effective at filtering all sizes of snails from an infested spring water source, and was designed for 100 gpm (3.785 L/min) flow. The hydrocyclone has no moving parts, but has baffles and pore sizes to assure that the target particle sizes are removed. Nielson et al. (2012a) used the head pressure of a gravity drop from the water source to the filtration site to supply the pressure needed for the filter, and no pumping was necessary. Having a system that operates on gravity is more reliable for long term operations

where power or pump failure could be experienced. The filtrate of snails removed by the hydrocyclone must be sent to some container, or off site location away from the filtered source water. Each hydrocyclone unit must be designed for specific flow rates and particle size removal, and sizing should be discussed in concert with engineers at hydrocyclone manufacturing companies to obtain the correct head and pressure for the flow of water needed. The hydrocyclone can be linked with a depuration strategy (page 53) to provide clean water sources during purging.

Ozone- Ozone induces NZMS to rapidly retract into their shells as shown in unpublished studies at the University of Idaho (Capual 2006). No mortality was observed following treatment with ozone >0.75 mg/L, over intervals up to 30 minutes. However, ozone was proposed to be useful to precipitate and remove snails from intakes, provided a collection and treatment operation could follow their exposure.

Screens - Mesh screens have been used in hatcheries in Montana to filter their NZMS infested spring water (Eileen Ryce, personal communications). Mesh sizes of 80 µm or less are recommended to remove all sizes of drifting neonates (Nielson et al. 2012a). However, the filters can foul and should be carefully monitored and constructed in redundancy.

Ultra Violet Light- UV will eventually kill snails but it may take up to several weeks (Wong and Wagner 1956). There has been no research conducted on NZMS to test the effectiveness of UV.

Disinfection Treatments – thermal

Freezing - Freezing temperatures, below -2ºC, are required over a 2 to 3 day period to kill NZMS (Moffitt and James (2012a, b). Field observations of freezing to remove infested populations in Capitol Lake in Washington State were made as the water was drained from the lake and the littoral areas were frozen for several days with air temperatures below -2ºC (Cheng and LeClair 2011). The event was only 98% successful, as some NZMS were found in treatment sites 4 days later. Hatchery wastes and equipment can be frozen and successfully decontaminated with freezing. Richards et al. (2003) reported 100% efficacy of freezing for 2 h at -3°C and recommended this tool for gear and equipment.

Hot Water and Drying - Studies by Dwyer et al. (2003) show there are effective ways to eradicate NZMS using hot water. NZMS were exposed to three different temperatures for three intervals of time. Results indicate that all NZMS exposed to 45°C (113°F) for 60 seconds died.

However, using 50°C (122°F) water killed 100% of the NZMS in 15 seconds. Hot water washing may be difficult to achieve in an outdoor setting. Hot water treatments are used for boat wash stations to decontaminate quagga and zebra infested vessels. These have not been well tested on NZMS except that the pressure washing would likely remove any attached snails. Pressure washing recommended in many standard hatchery operations to clean raceways and ponds, and would enhance removal of infestation of NZMS or any mollusks. Richards et al. (2003) reported heating to 40 °C for 2 h was efficacious in treatments of snails in dry substrates. And recommended treatment of gear at no less than 29–30°C and a low humidity level for a minimum of 24 h.

Propane Torch - A propane flame torch was used by Ken Cline to remove NZMS from his facility on Boulder Creek, Colorado, in 2007, and others have used similar tools to remove unwanted material from raceways walls. The concrete walls were not damaged and the snails exploded when they were heated to such high temperatures (Ken Cline, personal communications).

Barriers

Aeration Barriers -A wall of bubbles produced by an aeration pump is proposed to serve as a velocity barrier deterring the upstream migration of NZMS. Pumped air around the pipes opening forming a thick wall of bubbles was proposed by Larry Dalton (Utah Division Wildlife Resources) but not validated.

Copper Barriers - Analysis by Myrick and Conlin (2011) showed that copper and copper based materials served as NZMS deterrents by reducing their crawling distance under static conditions. Copper strips (roof flashing) were used in Oplinger and Wagner's study (2009b) in 1, 2, 3, and 4 inch strips. They found that snails crossed the strip with no significant difference from the control. Copper concentrations in the water samples were below 0.008 mg/L. Further studies found that copper sheeting or copper mesh were the most effective surfaces for deterring upstream migration (Hoyer and Myrick 2012). The copper based barriers need to be constructed of either material containing 99.9% pure copper with a minimum distance of 250 cm. Copper based painting was also investigated however, the paints were not as effective as the copper sheeting and mesh. All levels of copper that were leached into the water were less than the Environmental Protection Agencies minimum requirements. Water chemistry of the surrounding water did make a difference in the crawling distances observed on the copper barriers (Hoyer and

Myrick 2012). The copper barrier must be maintained and cleaned to reduce fouling and maintain effectiveness.

Electrical Barriers- Oplinger (2008) investigated using electrical barriers to inhibit upstream movement of NZMS. Water temperature, conductivity, level of electrodes in the water, and spacing of the electrodes affects the electrical current flow through the water. Stainless steel is the best metal to use, because it corrodes slower than other metals. Oplinger and Wagner (2008) reported problems with some materials used. The use of electrical barriers can result in safety risks, and additionally, the areas around the barriers could allow for passage.

Velocity Barriers - Holomuzki and Biggs (1999) determined that most NZMS were dislodged from gravel, cobble, and pebble substrates at near bed measured velocities of 150 cm/s. NZMS were still found in crevices of the rocks at the high velocities. Near bed velocities of 150 cm/s were achieved with flow rates of 50 cm/s; the shear stress created by the gravel, cobble and pebbles created higher velocities. During testing with copper substrates Hoyer and Myrick (2011) determined that velocities greater than 33 cm/s were needed to dislodge NZMS from smooth surfaces. The velocity needed to remove NZMS from pipes has not been researched for an array of pipe sizes, and surface roughness is an important factor. Upstream dispersal is documented and was modeled by Sepulveda and Marczak (2012) in field tests studies.

Terrestrial Barriers: Transfer overland was considered low probability for transport around filtration sites or structures. Oplinger and Wagner (2009b) found that terrestrial travel was very unlikely for the NZMS, so minimal efforts were needed in preventing terrestrial movement of NZMS. Recent studies by Alonso et al. (2016) revealed that NZMS survived translocation in sediment and clay substrates for short durations (<5 d). Therefore the transfer with animal transits over land is possible. Volitional upstream migrations in watered substrates have been reported and observed as a factor in population expansions (Haynes et al. 1985; Sepulveda and Marczak 2012; McKenzie et al. 2013).

Physical Removal Measures

Disturbance cleaning- The tools that are used to clean the raceways, specifically a pond scrubber, are effective in removing algae from the bottoms of the raceways. Hagerman National Fish Hatchery has used a large scrubber that is very effective in cleaning raceways. Power washing is also effective to remove debris in dewatered raceways or ponds. These tools are

supported by several sources in the literature that report flow fluctuation can enhance downstream drift and movements (Holomuzki and Biggs1999; Schreiber et al. 2003).

Sieves- Richards (2008) and Oplinger and Wagner (2009a) describe a method to sieve eggs from contaminated sources to remove NZMS. This method can be simple to use by gently sieving the rainbow trout eggs with a <2 mm sieve and then rinsing using the circular motion. The snails would fall away from the eggs when agitated and then washed through the sieve. Richards (2008) also suggested transporting the eggs, promoting agitation to ensure snails are removed. This method does not remove neonates, and additional treatment may be needed to remove neonates.

Suction Dredge - Venturi suction dredges are used in mollusk studies for collecting organisms (e.g. Gates et al. 2013). These tools are combined with divers, and have been used to remove NZMS from areas in soft substrates to reduce populations of NZMS, but clearly they are not 100% effective. A similar tool was used in removal operations of Asian clams in Lake Tahoe with mixed success (Wittmann et al. 2012).

Chemical Removals- Drips into systems with or without fish

All drip tools used with or without fish in rearing systems are subject to regulatory discharge and safe use with fish either through EPA or FDA permitting. A summary of some of the tools and the proposed uses are provided but they are not endorsed without full exploration of regulatory requirements. Refer to Bowker and Trushenski (2016) for the most up to date details on recommended and approved use of aquaculture chemicals. All use must be through an approved labeled product for aquaculture, and use of equivalent chemicals without labels are not allowed. It is not legal to use or discharge a pesticide product in aquaculture unless it is labeled for such use or there is an experimental exemption from FDA or EPA.

Potassium Chloride – The application of KCl is considered by FDA as low regulatory compound 10-2000 ppm. The use of 750 mg/L KCl in concert with low dose of formalin has been recommended as a control for zebra mussel veligers in transport vessels (Edwards et al. 2000; 2002). Current studies have shown a wide response related to conductivity and sodium levels in the source water (see Moffitt et al. 2016). It is known that potassium will relax muscular activity in mollusks (Fisher et al. 1991; Medler et al. 1999). Studies to assess the effect of KCl on NZMS have not shown efficacy (Moffitt, unpublished).

Potassium Permanganate - Using potassium permanganate as a drip system into

raceways is an example of chemical treatment. Oplinger and Wagner (2010) determined that a 2 mg/L concentration of KMnO4, was not effective in controlling or eliminating NZMS from the raceway. This tool is labeled for pond treatments of warm water aquaculture at 1 - 10 mg/L for 1 hour. Oplinger and Wagner (2010) found a 2 mg/L concentration of potassium permanganate used to help suppress disease outbreaks caused some reduction but not complete removals. At the University of Idaho, potassium permanganate at 1 mg/L could cause snails to relax, but was not efficacious in 1 h (Stockton 2011).

Bayluscide - Bayluscide, acts as a molluscicide (Andrew et al. 1982) with nicolosamide as its active ingredient, has been used as a drip treatment within raceways to remove NZMS. McMillin and Trumbo (2009) reported that a 1 mg/L nicolosamide concentration over eight hours resulted in a 98% mortality of NZMS in studies conducted in a concrete-lined irrigation canal. Bayluscide half-life in water was 1.8 days and 2.6 days in sediment. Bayluscide is also toxic to fish at the same concentrations for mollusk species, and is approved in sea lamprey control and is currently labeled by the U.S. Environmental Protection Agency as a molluscicide for control of snail populations in aquaculture ponds (U.S.EPA 1999).

Hydrogen Peroxide - Hydrogen peroxide was found to be an effective chemical in day to multiple day exposure to eradicate NZMS. They proposed this for use in a continuous low level exposure (Oplinger and Wagner 2015). The lowest concentrations of hydrogen peroxide that produced 100% mortality of NZMS in their lab tests were 750 mg/L for a 24-h exposure and 75 mg/L for a 96-h exposure. These exposures exceed those in the FDA approved uses of this compound most of which are 30 – 60 min (Bowker and Trushenski 2016), and likely would need permitting.

Sodium Chloride - When exposed to concentrated solutions of NaCl, NZMS will contract into their shell. Oplinger et al. (2009) showed that 60% of NZMS would contract into their shells and roll off a surface when exposed to a sodium chloride solution of 11.0 g/L. Lower concentrations produced a lower percentage of snail contraction. Oplinger and Wagner (2015) suggested this tool could be used with flushing flows to remove snails that had closed up. LeClair and Yuk (2011) report the efficacy of salt treatments with Capital Lake, Washington, using seawater inflows. However, NZMS are considerably tolerant of elevated salinity, and are reported able to survive in brackish water environments, as opposed to the zebra and quagga mussels (Duncan and Klekowski 1967; Bersine at al. 2008; Davidson et al. 2008) and

populations show ability to adapt (Hoy et al. 2012).

Carbon Dioxide - Pressurized CO_2 treatment is effective at decontaminating NZMS collected in filtered water. Studies conducted by Nielson et al. (2010) showed that pressurized CO_2 is more effective than non-pressurized CO_2. Non-pressurized CO_2 is effective at killing NZMS; however it takes considerable time and uses larger quantities of CO_2.

Copper Sulfate - Copper is toxic to mollusks (Watton and Hawkes 1984). Copper products (copper sulfate and copper carbonates or chelates) can be used to control mollusks in open water systems, but require a Special Local Need Label (also known as a Section 24-c) issued by the USEPA (USACE 2012) Copper compounds have a long life and are highly toxic to salmonids. The cheapest and most commonly used form is copper sulfate, which is available either as a crystal or a powder known as "Bluestone" or "Blue powder". Water quality, especially alkalinity and pH are critical in treatment efficacy. Copper toxicity increases at low pH and especially low alkalinity. Copper sulfate is labeled by FDA for use for treatment of ichthyophthiriasis on Ictalurid catfish cultured in earthen ponds. Dosages are 0.4 to 1 mg/L per 100 mg/L total alkalinity (as $CaCO_3$) as an indefinite exposure once daily for 5 to 11 consecutive days. Copper is registered for use by EPA to control freshwater snails that may be a vector for schistosomes and other trematode cercariae that may affect exposed swimmers or farm-raised fish. Many considerations are relevant to use of copper, and toxicity to fish and other aquatic biota are important considerations.

Other Management Strategies:

Depuration - Several studies have documented the consumption of NZMS by fish. Haynes et al. (1985) found that rainbow trout fed willingly on NZMS and that the snails survived the passage through the digestive tract and produced neonates within 24 hours. Oplinger et al. (2009) found that NZMS would pass through a rainbow trout's digestive tract live after 48 hours after consumption. The rate of passage of NZMS in rainbow trout was modeled by Bruce et al. (2008). Using these models and feeding studies, Bruce and Moffitt (2010) recommend feeding fish heavily for 96 hours and then depurating for at least 48 hours in a NZMS clean water source before transportation. Bruce and Moffitt (2010) found that both rainbow trout and steelhead consumed NZMS, with rainbow trout consuming almost twice as many NZMS as steelhead. They also determined that a maintenance diet increased the total consumption of snails, even

though starved fish consumed snails as well. No live NZMS were found in the digestive tract or feces after 48 hours (Bruce et al. 2008). However, for a depuration strategy to be successful, a facility must have a clean water source. Unpublished studies by Sealey et al. (2009) showed that tilapia when fed NZMS are likely to grind and digest them, therefore not allowing for survival out of the digestive tract. There was 100% survival of NZMS regurgitated.

Mixed Cell - Studies conducted in a mixed cell raceway (Stockton and Moffitt 2015), illustrated velocities and particle removal rate of particles in test systems with and without fish. The testing used plastic beads to mimic NZMS, which were removed from the raceway in 20 minutes. The velocities, an average of 23 cm/s, were sufficient to remove NZMS based on theoretical calculations. Further testing with NZMS is needed to increase the certainty of this control measure, especially if combined with depuration strategies.

Water Quality - The effect of different water quality should be considered in the risks of invasion and survival. Vazquez et al. (2016) speculated that the low conductivity of water in Northern California likely limited the expansion potential of populations. Other considerations have been reviewed to consider nutrients that affect food availability are important parameters. The presence of treatment lagoons used for waste waters of aquaculture facilities provide high nutrient environments. McKenzie et al. (2013) considered a number of abiotic factors affecting the reproduction and survival of populations in Boulder Creek, Colorado, and found the linear model with the highest level of support for predicting variability in snail fecundity included water temperature, snail shell length, water hardness (calcium carbonate), and nutrient levels (total phosphate).

Decontamination Station and Disinfection Tools

Disinfection stations are only as good as the disinfectant used, and the retention and frequency of replacement of bath systems. A decontamination station should be place at key locations for gear and equipment. The disinfectant must be safe to gear and equipment, and personnel. The current guidance for aquaculture chemicals (Bowker and Trushenski 2016) provides a chart (attached to this document) summarizing various disinfecting tools recommended in aquaculture operations. Selected and additional tools are reported in more detail here.

Quaternary Ammonium Compounds (QACs) - Schisler et al. (2008) reported efficacy of

over the counter chemicals such as 409. They found industrial strength disinfectants such as Sparquat 256 were recommended over these household chemicals. Hosea and Finlayson (2005) also reported efficacy and often different results were found. The influence of water quality parameters are important modifiers in the responses to different treatments. Moreover, some compounds such as Sparquat 256 are no longer available (Stout et al 2016). Stout et al (2016) tested Quat 4; Green Solution High Dilution Disinfectant, and super HDQ Neutral. The strongest performer was HDQ, but all were effective as bath disinfectants at 0.4% and sprays at 0.8% for 10 min.

Sodium Hydroxide – Studies using quantities of lye as NaOH to elevate the pH of water to 11 to 12 pH have shown this tool efficacious as a disinfecting agent for contained vessels or surfaces with mollusks and other invertebrates (Moffitt et al 2015a, b). Neutralization of pH can be accomplished with addition or CO_2 or simple aeration. Estimated LT 99 for adult sized NZMS at elevated pH of 11.5 to 12 was estimated as 39 h, 95% CI = 31.9-60.7 h.

Virkon® Aquatic - Stockton and Moffitt (2013) tested the efficacy of Virkon® Aquatic as a disinfectant for gear, and also evaluated the colonization of different wading gear by NZMS. They found a 2% solution of Virkon® for 15-20 min bath was 100% effective and evaluated the effect of the compound on the condition of the gear to report little harm. Additional studies to document the efficacy of Virkon® on quagga mussels, and the safety of Virkon to fish have been prepared (Stockton-Fiti and Moffitt, in review, Aquaculture).

Disclaimer: Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government

Table 5. Disinfectants and their use for field gear and hard surfaces. See bottom of table for definitions of abbreviations. Click here to return to text.

Disinfectant Concentration Contact Time	Surfaces	Deactivated by organic matter	Corrosive	NZMS	ZQM	MC Spores	MC Tams	IHNV	VHS	SVCV	KHV	ISA	IPN	LMBV	WSIV	RANA	BKD	FUR, ERM	CWD, COL	Chytrid	Disposal	Pros and Cons
Low Level Disinfectants: Kill most vegetative bacteria, some fungus, some enveloped viruses, do not kill mycobacteria or bacterial spores.																						
Benzalkonium chloride (QAC) 500ppm Contact 10 min (except as noted)	Plastics, floors, counter tops	Y	N	Y	Y	Y 10 min	Y 10 min	Y	Y 5 min	Y	Y		N	Y	Y		1000 ppm	Y		Y 5 min	unknown	**Pros:** easily accessible, non-corrosive **Cons:** highly toxic to fish, disposal issues, not labeled for aquatic use, bath type use
Didecyl dimethyl ammonium chloride (QAC) 400ppm Contact 5 min (except as noted)	Plastics, floors, counter tops	Y	N	Y	Y	Y 5 min	Y 1 min	Y 5 min	Y 5 min	Y 5 min	Y 5 min		N	Y 5 min	Y 5 min	Y 10 min	1000 ppm	Y 5 min		Y 1 min	unknown	**Pros:** non-corrosive, no rinse spray on **Cons:** disposal issues, hard to find, not labeled for aquatic use
Phenols (Lysol, Pinesol) Contact 15 min	Hard surfaces	N	N	Y	Y				Y												unknown	**Pros:** common household products **Cons:** not labeled for use of field gear, irritating to skin, must rinse
Intermediate Level Disinfectants: Kill vegetative bacteria, most viruses and most fungi, but not resistant bacterial spores																						
Chlorine 200-500ppm 10-60 min	All surfaces except plastics	Y	Y	N	Y 60 min	Y 15 min	Y 10 min	Y 5 min	Y 5 min	Y 10 min		Y 15 min	Y 30 min	Y 10 min		Y 15 min	Y 5 min	Y 10 min		Y 10 min	neutralize with sodium thiosulfate	**Pros:** works well, inexpensive, readily available **Cons:** highly corrosive, odors, human toxicity?
Virkon Aquatic 0.5%-1% 5-30 min (except as noted)	Waders, boots boats nets, all field gear	Y	N	Y 60 min	Y*		Y 5 min	Y 5 min	Y 10 min	Y 10 min		Y 10 min	Y 10 min	Y 20 min		Y 1 min	Y 10 min	Y 5 min		Y 1 min	dilute, pour on ground away from surface waters	**Pros:** non-corrosive, considered environmentally safe, biodegradable, can use as a no-rinse spray on **Cons:** cost, efficacy not determined for some pathogens
Ethyl Alcohol 70-80%	Hands, tools, counter tops	N	N	N	Y				Y				Y	N						Y	unknown	**Pros:** readily available **Cons:** evaporates quickly and may not get proper contact time, expensive, not good for field equipment, fixes organics to hard surfaces, inactivated by sunlight, flammable
Isopropyl Alcohol 60-80% 10-30 min (except as noted)					30				2				10							1	unknown	
Iodine 100-250ppm 20-30 min (except as noted)	Better as antiseptic on tissues	Y	Y	N	N	N	N	Y 10 min	Y 10 min	Y 20 min		Y 5 min		Y 10 min				500 30 min		Y 1 min	neutralize with sodium thiosulfate	**Pros:** antiseptic, inexpensive, **Cons:** corrosive to metals, stains, long contact time, cannot over concentrate, highly toxic to aquatic animals

Table 5. Disinfectants and their use for field gear and hard surfaces (continued). Click here to return to text.

Disinfectant Concentration Contact Time	Surfaces	Deactivated by organic matter	Corrosive	NZMS	ZQM	MC Spores	MC Tams	IHNV	VHS	SVCV	KHV	ISA	IPN	LMBV	WSIV	RANA	BKD	FUR, ERM	CWD, COL	Chytrid	Disposal	Pros and Cons
High Level Disinfectants: Destroy vegetative bacteria, mycobacteria(TB), fungi, enveloped (lipid or hydrophilic) and non enveloped virus (non lipid), but not necessarily bacterial spores. Must be capable of sterilization when contact time is extended.																						
Hydrogen Peroxide 3-5% 5 min (except as noted)		N	Y	15 min			N	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y			unknown	Pros: can add to QACs & iodine to make them more effective Cons: destroys soft tissues when over exposed
Peroxigard 1:16			N					Y	Y	Y				Y							unknown	Pros: no-rinse spray on
Formaldehyde 1%-3%	better when mixed w alcohol	N	N	N				?	16 h	5 h		16 h	5 min							5 min	titrations	Pros: easily accessible Cons: highly toxic, odors, personal protective gear required to protect applicator
Other Disinfection Options																						
Heat				5 m	15 m		5 m 75°C (167°F)	2 m 50°C (122°F)	5 m 55°C(131°F)	30 m 60°C (140°F)		2 m 50°C (122°F)	30 m 60°C (140°F)			15 m 60°C (140°F)	10 m 80°C (176°F)			5 m 60°C (140°F)	NA	
Ozone 8 ppm 3 min									Y				Y					Y	Y			
pH >12 or <4 > 4 hr									Y	Y	Y pH <4											
Complete Drying >20°C					7 d	60 d	1 h		14 d	5 d				5 d		4 d				6 h	NA	

Recommended active ingredient concentrations appear in red under the chemical along with the general minimum contact time. In columns where a Y appears, the contact time is listed below in minutes (min), hours(h), or days (d). If there is a blank, it is unknown at this time. If a contact time for a chemical was longer than feasible recommended time (generally longer than 1 hour) or the compound is known to not be effective, an N appears in the column. For example, a 10% formalin solution will only kill 20% of NZMS in a 1 hour exposure, therefore, it is listed as a N. Please remember that it is in violation of federal law to use a disinfectant other than how it is labeled.

Pathogen and/or invasive/nuisance species abbreviations are as follows: NZMS= New Zealand mud snail, ZQM = zebra/quagga mussels, MC Spores = Myxobolus cerebralis (whirling disease) myxospores, MC Tams = Myxobolus cerebralis (whirling disease) triactinomycon spores, IHNV =infectious hematopoietic necrosis virus , VHS = viral hemorrhagic septicemia virus, SVCV = spring viremia of carp virus , KHV = koi herpes virus, ISA = infectious salmon anemia virus, IPN = infectious pancreatic necrosis virus, LMBV = largemouth bass virus, WSIV = white sturgeon iridovirus, RANA = ranavirus, BKD = bacterial kidney disease, FUR = furunculosis, ERM =enteric redmouth, CWD = coldwater disease, COL = columnaris disease, Chytrid = chytrid fungus

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