

# RECLAMATION

*Managing Water in the West*

## **Feasibility of Establishing a Quarantine Facility for Invasive Species Research**

**Research and Development Office  
Science and Technology Program  
(Final Report) ST-2019-19010-01**



**U.S. Department of the Interior  
Bureau of Reclamation  
Research and Development Office**

**September 2019**



## Mission Statements

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## **Feasibility of Establishing a Quarantine Facility for Invasive Species Research**



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Prepared by: Scott O'Meara  
Botanist, BOR TSC, Hydraulic Investigations and Lab Services Ecological Research Lab



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Peer Review: Rheannan Quattlebaum  
Biologist, BOR TSC, Hydraulic Investigations and Lab Services Ecological Research Lab

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# Acronyms and Abbreviations

ANS – Aquatic Nuisance Species

ASSP - Approved Species-Specific Protocol

CDC – Centers for Disease Control and Prevention

DI – De-Ionized

DNA – Deoxyribonucleic acid

HVAC – Heating Ventilation and Air Conditioning

SOP – Standard Operating Procedures

USDA – United States Department of Agriculture

# Executive Summary

The most fruitful overall approach to research is typically a combination of both field and laboratory settings, where controlled laboratory experiments may be further investigated in field environments, and conversely field observations may produce ideas that benefit from testing in controlled laboratory experiments. Reclamation currently lacks the ability to research live invasive organisms in a laboratory setting. Various State and Federal regulations regulate the transport of live invasive species, although permits may be necessary for specific purposes such as scientific research. Culturing live exotic organisms is also regulated but may be permitted in facilities where measures are taken to prevent potential escapes, or “quarantine” facilities.

This project is a preliminary investigation into the potential to obtain permits and develop a Reclamation quarantine facility to keep live invasive organisms on-site for laboratory-based studies. The ability to conduct lab research on live organisms would greatly enhance the capability of Reclamation to develop our understanding of various aspects of invasive species and effectively mitigate their impacts.

Permitting and regulatory concerns would involve conversations with state and local agencies to demonstrate the level of safety and security that would be in place; there is no official approval process for establishing a federal quarantine lab. Similarly, transport of live organisms would involve several layers of both physical barriers and techniques to prevent potential escapes, but there is no formal permitting process required.

There are several locations in the Denver Federal Center Building 56 that are currently vacant and would be amenable to a quarantine facility. Modifications to the HVAC system, electrical fixtures, windows, doors, floor drains, and other features would be required. Lab equipment needed would depend on specific project requirements, but many recirculating culture tanks are commercially available. Operation of the quarantine facility would include biosecurity training, limited personnel access, and containment or decontamination procedures for all materials entering and exiting the facility.

A quarantine facility would provide the capability to conduct a variety of controlled studies on live organisms with multiple benefits to Reclamation in combatting invasive species and carrying out its mission. Future steps in regards to construction of a quarantine facility are not entirely clear. Nevertheless, this study will provide an informative foundation should Reclamation move forward with this idea.



# Contents

Executive Summary .....	vi
Background and Introduction .....	1
Permitting.....	1
Quarantine Facility .....	1
Transport of Invasive Organisms.....	2
Other Potential Regulatory Concerns .....	2
Quarantine Facility Establishment.....	2
Location .....	2
Equipment.....	3
Quarantine Facility Operations .....	3
Security and Confinement .....	3
Training .....	3
Transportation and Shipping .....	3
Facility Containment .....	4
Artificial freshwater .....	4
Facility Capabilities .....	5
References.....	7
Appendix A.....	8

## Tables

Table 1. Potential projects conducted in a quarantine lab and impact to Reclamation’s mission .....	6
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# Background and Introduction

The Reclamation Ecological Research Lab is heavily involved in multiple aspects of invasive species that adversely affect Reclamation facilities. Developing state-of-the-art methods for assessment and mitigation of invasive species impacts, as well as enhancing the scientific body of knowledge regarding invasive species and their interactions with the environment are core functions of the lab and the services they provide.

The majority of current Reclamation invasive species research is conducted in the field, generally at Reclamation facilities where invasive species populations are established. Field research is highly valuable for providing real world context and can represent a more accurate environment for applied studies. However, the inability to control certain variables, as well as the impossibility of exactly characterizing the environment in which the study is conducted may limit the extrapolation of some field research, and/or provide inconsistent results.

Laboratory-based studies allow greater control over influencing variables, can better represent a true experimental design, and provide for more accurate and reproducible observations, but may suffer somewhat from the effects of an artificial environment.

The most fruitful overall approach to research is typically a combination of both field and laboratory settings, where controlled laboratory experiments may be further investigated in field environments, and conversely field observations may produce ideas that benefit from testing in controlled experiments.

Reclamation currently lacks the ability to research live organisms in the laboratory. Various State and Federal regulations regulate the transport of live invasive species, although permits may be necessary for specific purposes such as scientific research. Culturing live exotic organisms is also regulated, but may be permitted in facilities where measures are taken to prevent potential escapes, or “quarantine” facilities.

This project is a preliminary investigation into the potential to obtain permits and develop a Reclamation quarantine facility to keep live invasive organisms on-site for laboratory-based studies. The ability to conduct lab research on live organisms would greatly enhance the capability of Reclamation to develop our understanding of various aspects of invasive species and effectively mitigate their impacts.

## Permitting

### Quarantine Facility

Our investigation did not discover a formal organization tasked with approving quarantine facilities for aquatic organisms. The USDA has authority for plants and insects, while the CDC appears to have authority for medical research. Since the Reclamation quarantine facility would be located in Colorado, consultation and/or approval from the state Department of Agriculture or

## Feasibility of Establishing a Quarantine Facility for Invasive Species Research

Department of Natural Resources would likely be required. See Appendix A for applicable Colorado regulations and potential routes of approval.

### Transport of Invasive Organisms

The transportation of live organisms is governed by Federal and State law. Two of the most pertinent statutes are Title 16 and Title 18 of the Lacey Act:

*Under Title 16 of the Lacey Act (16 U.S.C. 3372) it is unlawful to import, export, transport, sell, receive, acquire, or purchase in interstate or foreign commerce any fish, or wildlife taken, possessed, transported, or sold in violation of any law or regulation of any state.*

*Under Title 18 of the Lacey Act (18 U.S.C. 42) "The importation into the United States, any territory of the United States, the District of Columbia, the Commonwealth of Puerto Rico, or any possession of the United States, or any shipment between the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, or any possession of the United States . . . is hereby prohibited."*

A 2017 court ruling, *United States Association of Reptile Keepers v. Zinke*, decided that Title 18 does not prohibit transport of injurious organisms between states, and no federal permit would be required. However, the court ruling did not impact Title 16. Therefore, even though no federal permit is required to transport species, state laws are still in force, so permission from Colorado, and the source state, would be required.

### Other Potential Regulatory Concerns

The Bureau of Reclamation Office of Policy and Administration, Environmental Compliance Division, was contacted to determine any potential regulatory concerns for establishing a quarantine laboratory. A response was not received prior to the submission of this report; any information acquired in the future will be amended to future reports or briefings.

## Quarantine Facility Establishment

### Location

Two viable options currently exist in Building 56 for locating a quarantine facility: Room 1775 which currently houses the Aquatic Research Facility, operated by the Fisheries Group, and the 1085 suite, which was the previous location of the Aquatic Research Facility.

The 1085 Suite is not currently leased by Reclamation but would be the most appropriate location for a quarantine facility. The space encompasses Rooms 1085, 1090, and 1095, and consists of 1,447 square feet of usable space. These three rooms have not been occupied since the Fisheries group (8290) vacated it in 2013, and the room remains largely in the condition it was in at the time of their move. Entry to the suite is through a single door in an isolated hallway providing very controlled access of personnel, thereby reducing the risk of accidental escape of organisms. An additional double door in room 1090 is egress only and offers easy access outside and directly to the parking lot. Flow within the suite is one room at a time so there is ample room to implement physical and chemical hygiene barriers.

The Room 1775 facility is a shared space with the water treatment group and has multiple entrances. Modifying this space would require the construction of a dividing wall and closure of access points. The resulting area would consist of ~1750 square feet of usable space. The room is located in the center of Building 56 and the HVAC system is currently linked to other spaces. Significant modification would be required to establish a quarantine laboratory in this space.

## Equipment

Necessary equipment will depend entirely on what projects are approved for use in the quarantine facility. Potential equipment includes self-contained rack systems, such as those designed to study zebrafish, that have been developed to hold, monitor, and filter multiple tanks of water simultaneously. Racks are available in a variety of sizes, and the number of tanks per shelf can be adjusted based on the size needed. Rack systems can incorporate several filtration measures, including: particulate filters, fluidized bed biological filters, carbon filters, and ultraviolet sterilizers.

Other equipment could include large holding tanks for growing study organisms and the food necessary for their survival, dedicated DNA analysis equipment, and computers. A water filtration system requiring plumbing modifications, a dedicated HVAC system, and at least one fume hood will likely be required.

# Quarantine Facility Operations

## Security and Confinement

This section is largely taken from the ANS Task Force ASSP for *Dreissena polymorpha* (zebra mussel) research.

## Training

All personnel working in the laboratory will understand:

1. Life history, biology, and basic environmental needs of the species present
2. Potential environmental problems if species are released
3. Correct procedures for handling and disposing of specimens
4. Decontamination procedures
5. Laboratory security and mishap response procedures

## Transportation and Shipping

Live aquatic animals or plants transported to the facility require both primary and secondary containment. Primary containment must be a heavy-walled container, preferably plastic, with a water-tight top. Additional layers of containment will be necessary to assure protection against potential escape of organisms; the primary container will be bagged in a sealable plastic bag such as Ziploc, and transported in a waterproof plastic cooler. Additional details regarding transport of exotic organisms can be found in Reclamation's dreissenid sampling SOP (Reclamation 2013).

## **Feasibility of Establishing a Quarantine Facility for Invasive Species Research**

### **Facility Containment**

Facility entrances will be access controlled; the lab will remain locked when not in use. Only personnel with the required training will be allowed entry without an escort. Work spaces will be clearly marked as restricted areas containing live aquatic organisms.

Doors will be self-closing steel, sealed on the top and sides by a magnetized rubber gasket, door sweeps on the bottom to provide a complete seal. Windows should be triple glazed with an outer shatterproof layer, sealed in frames, and permanently closed. All electrical outlet covers and light fixtures will be water tight and sealed against insect intrusion. A backup generator will be installed to maintain constant power during outages.

Walls and ceilings will be painted with a high-gloss white epoxy paint for easy detection of insects or other organisms. Wall, ceiling, and window seams will be sealed with a silicone-based sealant. The floor will be seamless and easily cleaned/disinfected.

The containment facility will require a dedicated HVAC system. Vents and HVAC ducts into the laboratory will be covered with mesh screens small enough to prevent the escape of any organism being studied. A potential setup may include air drawn into the room by a large fan through a bank of standard paper filters, passed through a chamber where steam is added from a humidifier, then passed through another  $\frac{3}{4}$ " polyester filter before entering the lab. In the exhaust system, air can be filtered by the  $\frac{3}{4}$ " polyester filter and 100-mesh nylon screen before entering the exhaust ducts and pumped through a dedicated squirrel cage fan and another series of paper filters before exiting the building.

All sink and floor drains will be plugged when not in use to prevent loss of contaminated water and other material. Signs should be posted over all sinks warning against disposal of untreated water.

Specimen housing systems will be static, closed-loop systems. Containers housing live organisms will be kept in trays or liners capable of holding all the water in the container in the event of a leak. Before water is discharged into the sewer it will be treated with a combination of micro-screen filtration, UV, and chlorination.

### **Artificial freshwater**

Maintaining healthy, sustainable populations of aquatic animals in the lab will require the creation of artificial freshwater to replicate natural habitats. The specific chemical composition of freshwaters needed will vary depending the species cultured and their natural habitats. Common ions include calcium, magnesium, sodium, and potassium (Smith et al. 2002). Other components may be needed based on the organism and the nature of the study being conducted.

After the necessary components of the artificial water are identified, concentrated stock solutions can be created. Most of these stocks can be stored for at least 30 days without changes in concentration (Sugiyama et al 2016). The stock solutions are then combined with ultrapure water to match the composition desired. Recipes for creating synthetic freshwater have been developed for select waterbodies, and can likely be modified to meet Reclamation's needs (Smith et al 2002, Sugiyama et al 2016).

It is possible that a simplified version of artificial freshwater would be sufficient to keep organisms alive for a limited length of time. Stoeckel et al. (2004) cultured and successfully induces spawning of dreissenid mussels in a laboratory over a 24 week period in an artificial water consisting of 10 mL artificial seawater (28-30%), 990 mL DI water, 200 mg CaCl, and 100 mg Na(CO<sub>3</sub>)<sub>2</sub>, per 1,000 mL.

Since living organisms would be kept in the lab, the water chemistry is expected to change as components are taken up, metabolized and excreted. Chemical components of the water could also be removed by the filters associated with a recirculating water system. Off-the-shelf systems are available that monitor and adjust some characteristics of aquatic laboratory systems, including pH and calcium. It is likely that these systems could be modified to control all desired components of the synthetic water. The published methods for creating artificial freshwater do not address the inclusion of organic material, which would be necessary for sustaining organisms over long periods of time.

## Facility Capabilities

Laboratory-based studies allow greater control over influencing variables, can better represent a true experimental design, and provide for more accurate and reproducible observations. Developing state-of-the-art methods for assessment and mitigation of invasive species impacts, as well as enhancing the scientific body of knowledge regarding invasive species and their interactions with the environment will allow Reclamation to continue leading the field of invasive species research.

Examples of specific projects and their benefits are presented in Table 1 below.

## Feasibility of Establishing a Quarantine Facility for Invasive Species Research

**Table 1. Potential projects conducted in a quarantine lab and impact to Reclamation’s mission**

<u>Project Description</u>	<u>Impact on Mission</u>
Expose invasive mussels to a variety of chemicals, pesticides, and reagents	Evaluate potential compounds to eradicate or control mussels at Reclamation facilities
Culture algae and test canal linings designed to inhibit algae attachment	Facilitate the adoption of innovative linings to improve the flow of water in distribution systems
Study the basic biology and life cycle of invasive quagga and zebra mussels	Similar studies are currently conducted offsite, increasing costs and limiting the ability to perform long-term analysis. Also, no location in the Western US currently supports both zebra and quagga mussels, so studies assessing their interactions are not possible.
Study the basic biology of other invasive organisms and organisms of concern	New threats are constantly emerging, and Reclamation needs the ability to rapidly assess treatment methods in a controlled environment. This facility would establish the Ecological Research Lab as a Reclamation-wide resource for lab-based studies of organisms of concern.
Evaluate the impact of various treatments at the genetic level of invasive species	The quagga and zebra mussel genomes have been sequenced. This allows researchers to examine impacts at the molecular level.
Evaluate aspects of environmental DNA (eDNA)	Interest in eDNA has grown rapidly in recent years and has the potential to provide very early detection of threats. Basic questions about how long eDNA persists under various environmental conditions remain unanswered.
Assess vector threat posed by ballast tanks and other containers	Determining the conditions under which mussels survive in ballast tanks and other areas of personal watercraft will allow states to base inspection procedures on the best science available. These studies will be more effective when performed in a controlled environment.
Evaluate biocontrol organisms prior to release in the environment	There is a need at the Federal level for a space where transgenic organisms and other biocontrol options can be evaluated before release.



## References

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# Appendix A

## Colorado Regulations

- Colorado prohibits the possession and transport of nuisance or pest species. Colorado Parks and Wildlife or Department of Agriculture staff can provide exemptions. A Scientific Collecting license could be required. Additional regulations could apply based on the source state of the organism.
- Applicable regulations follow:
  - Code of Colorado Regulations 2 CCR 405-8, Chapter P-8 – Aquatic Nuisance Species (see #801, Possession of Aquatic Nuisance Species)
    - (<https://www.sos.state.co.us/CCR/GenerateRulePdf.do?ruleVersionId=7487&fileName=2%20CCR%20405-8>)
    - # 801 - POSSESSION OF AQUATIC NUISANCE SPECIES. Except as provided in these regulations or authorized by the Division or under Title 33 or Title 35 C.R.S., it shall be unlawful for any person to possess, import, export, ship, transport, release, place, plant, or cause to be released, placed, or planted into the waters of the state any aquatic nuisance species.
  - Colorado Revised Statutes 2016, Title 33, Parks and Wildlife (see Article 10.5 ANS)
    - (<https://leg.colorado.gov/sites/default/files/images/olls/crs2016-title-33.pdf>)
    - 33-10.5-105. Prohibition of aquatic nuisance species - penalties. (1) No person shall: (a) Possess, import, export, ship, or transport an aquatic nuisance species; (b) Release, place, plant, or cause to be released, placed, or planted into the waters of the state, an aquatic nuisance species
  - Colorado Revised Statutes 2016, Title 35, Agriculture (see Article 4 Pest Control)
    - (<https://leg.colorado.gov/sites/default/files/images/olls/crs2016-title-35.pdf>)
    - 35-4-108. Unlawful to transport pests. (1) It is unlawful for any person, by any means whatsoever, to knowingly transport, into or in Colorado, live pests or host material which may be injurious to agriculture or horticulture in this state, without permission from the commissioner. (2) The commissioner may issue and enforce a hold order against any person who owns or controls any nursery stock, agricultural crop, or other plant material that is suspected of harboring a plant pest or disease, for the purpose of isolating the material, restricting its movement, and specifying appropriate sanitary measures pending completion of testing to confirm the presence of such pest or disease.

- Code of Colorado Regulations 2 CCR 406-13, Chapter W-13 – Possession of Wildlife, Scientific Collecting, and Special Licenses
  - (<https://www.sos.state.co.us/CCR/GenerateRulePdf.do?ruleVersionId=6912&fileName=2%20CCR%20406-13>)
  - #1301 – POSSESSION. No person shall, at any time, have in possession or under control any wildlife caught, taken or killed outside of this state which were caught, taken or killed at a time, in a manner, or for a purpose, or in any other respect which is prohibited by the laws of the state, territory or country in which the same were caught, taken or killed; or which were shipped out of said state, territory or country in violation of the laws thereof.
  - #1315 - PURPOSE. A Scientific Collecting License may be issued for the purpose of importing, marking or banding or temporary or permanent possession of wildlife and collection of wildlife specimens for the purpose of scientific collections or bona fide scientific research.

### **Colorado Approvals (Department of Agriculture or Department of Natural Resources)**

- There are no existing forms for soliciting approval to import or possess invasive, nuisance, or pest species. It appears approval will need to be granted by individual agencies or departments, based on the type of organism. Maybe a blanket Scientific Collecting license could cover all species we want to possess, after initial approval of the facility and program is given by State staff.
  - For aquaculture:
    - CPW Aquatics Section (303-291-7358)
    - Probably CPW Invasive Species Program (Elizabeth Brown - 303-291-7295)
  - For plants:
    - Approval from CDA Plant Import Program (Laura Pottorff - 303-869-9071)
  - For circus, exotic, and zoo animals: (not likely to apply to Reclamation research)
    - Approval and license from CPW (303-291-7227)
    - Current USDA Exhibitors license for circus and exotic animals
    - Possibly a veterinary inspection or permit
  - For Scientific Collecting License
    - See 2 CCR 406-13, Chapter W-13, #1316 – License Requirements
    - Online form (<https://cpw.state.co.us/aboutus/Pages/SWL-CollectionMammalBird.aspx>)
    - Need detailed project plans, approval by appropriate section manager
    - There is no fee associated with this license for federal entities



