

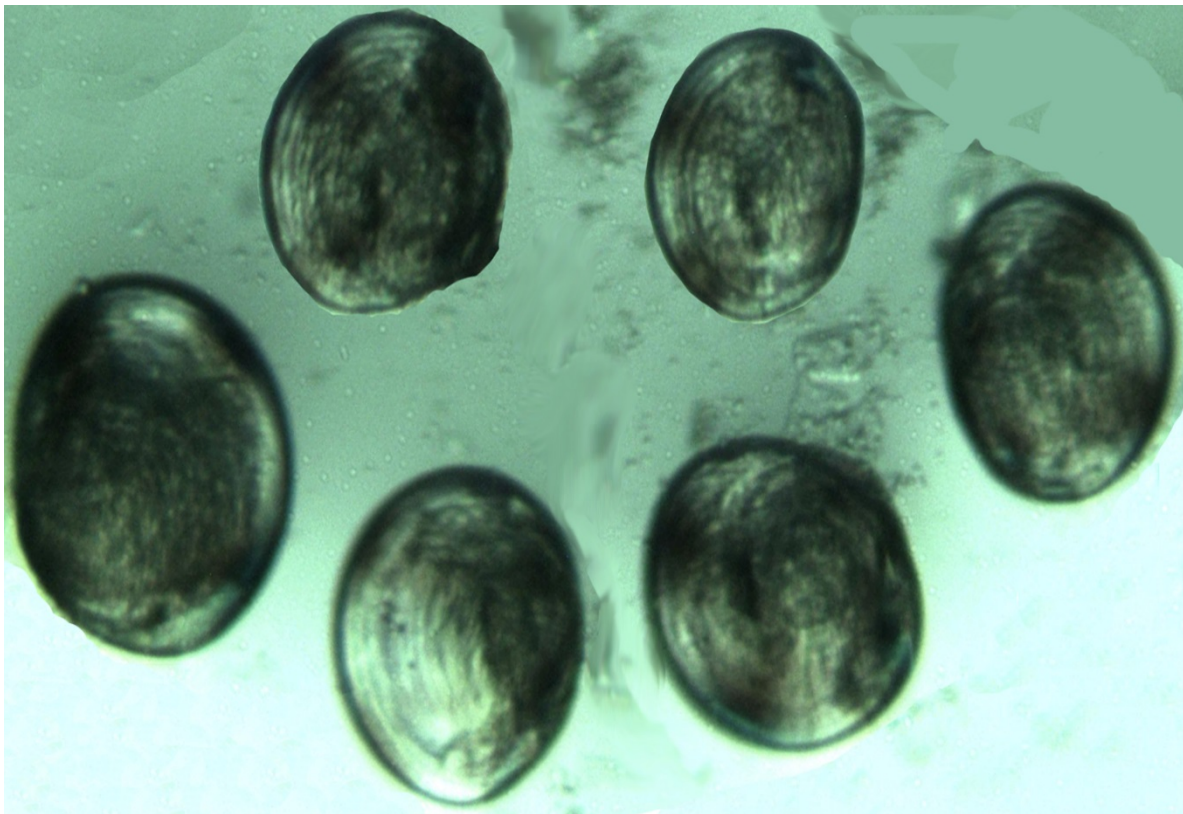
# RECLAMATION

*Managing Water in the West*

## Reclamation Detection Laboratory for Exotic Species (RDLES)

### Invasive Mussel Detection and Research Related to Spread, Control, and Management

Research and Development Office  
Science and Technology Program  
(Final Report) ST-2018-2617-01



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

September 2018



## **Mission Statements**

Protecting America's Great Outdoors and Powering Our Future

The Department of the Interior protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.

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RDLES provides monitoring of sites where mussels are established to assess population dynamics (e.g. Canyon Lake Reservoir, AZ).

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**Final Report ST-2018-2617-01**

## **Reclamation Detection Laboratory for Exotic Species (RDLES)**

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## Acronyms and Abbreviations

AIS	Aquatic Invasive Species
ANSTF	Aquatic Nuisance Species Task Force
ASDSO	Association of State Dam Safety Officials
BIA	Bureau of Indian Affairs
BLM	Bureau of Land Management
CPLM	Cross-Polarized Light Microscopy
eDNA	Environmental DNA
FWS	Fish and Wildlife Service
GESTEM	Girls Exploring Science, Technology, Engineering and Math
GPS	Global Positioning System
ICAIS	International Conference on Aquatic Invasive Species
NALMS	North American Lake Management Society
NIST	National Institute of Standards and Technology
NPS	National Park Service
PCR	Polymerase Chain Reaction
QA/QC	Quality Assurance/Quality Control
RDLES	Reclamation Detection Laboratory for Exotic Species
SNWA	Southern Nevada Water Authority
SOP	Standard Operating Procedure
USACE	United States Army Corps of Engineers
USACE-ERDC	USACE-Engineer Research and Development Center
USGS	United States Geological Survey
UT DWR	Utah Division of Wildlife Resources
WRP	Western Regional Panel

# Executive Summary

The Reclamation Detection Laboratory for Exotic Species (RDLES) provides expertise in field sampling, analytical microscopic, and molecular methods for the detection and identification of threatened and invasive species. RDLES provides data that is utilized for environmental compliance and to manage invasive, threatened, and endangered species that impact Reclamation. The laboratory is currently focused on invasive dreissenid mussels and has developed expertise in multiple disciplines of this topic. The RDLES lab is involved in national and international groups that collaborate to conduct research and develop standards for invasive mussel early detection and control methodology. RDLES provides services to Reclamation regional and area offices, state aquatic invasive species (AIS) programs, water districts, and other agencies with a variety of monitoring and research projects.

Over the last three years the RDLES laboratory has analyzed 4,414 samples. Of these, 230 samples were positive, including those from sites of known infestations, such as Lake Mead, Nevada and Canyon Lake Reservoir, AZ. Positive samples from eight waterbodies were first-time findings, resulting in notification to Regional Mussel Task Force Leads, the Reclamation Science Advisor, and state AIS coordinators regarding detection of invasive mussels in the waterbody. In addition, RDLES provides monitoring of sites where mussels are established to assess population dynamics (e.g. Canyon Lake Reservoir, Arizona). RDLES staff also undertake public outreach by participating in water festivals for students, and training college and high school students to work in the laboratory. Other laboratories consult with RDLES for training and use the standard operating procedures developed here for their own detection programs.



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# Background

Invasive dreissenid mussels (*Dreissena bugensis* (quagga) and *Dreissena polymorpha* (zebra)) pose serious risks to Reclamation managed habitat and infrastructure in the United States. The arrival of quagga mussels in Lake Mead in 2007 triggered Reclamation to invest in developing and maintaining the Reclamation Detection Laboratory for Exotic Species (RDLES). Based upon experience with zebra mussels in the Eastern U.S., if mussels are detected early facility operators may have three to five years to plan, budget, and implement protective measures before mussel populations are large enough to impair generation of hydropower and delivery of water by clogging critical structures such as pipes, water intakes, drains, gates, and trash racks. One of the central goals of the early detection and monitoring effort is to provide Reclamation facility managers the early warning they need to plan for the arrival of invasive mussels. Early actions may also be taken to prevent the spread of mussels to other water bodies.

The advantages of an in-house early detection laboratory include customized support for the agency with improved quality control, tailored testing, and cost efficiency. RDLES provides quality sampling and analytical work for the detection of mussels with shorter turn-around times and high Quality Assurance/Quality Control (QA/QC) standards. Reclamation experts can detect sample anomalies that may require additional attention or research. Many state and federal entities are dependent on RDLES for invasive mussel sample analysis and expert guidance in sample analysis.

## Sampling Methods

Water samples are received from various locations across the western United States, from both Reclamation waterbodies and facilities, and state and local agencies. Sampling methods are generally standardized across all agencies. Plankton tow nets (Figure 1) are used to collect several samples, either vertically or horizontally, at specified locations, usually launches and marinas where there is recreational activity as these areas are often where inoculation occurs.



**Figure 1: Water sampling showing the plankton tow net**

Once the samples are collected the water is placed into clean bottles and buffered with baking soda to prevent degradation of mussel shells. Alcohol is also added as a preservative and to kill all living organisms in the sample.

At a minimum, the bottle is labeled with the collection date, reservoir name, and the location on the waterbody from which the sample was collected. Other information may also be provided such as sampler names, water volume, water temperature, and Global Positioning System (GPS) points, though this varies widely between locations and/or individuals/agencies. Water quality data is also collected concurrently with plankton tow net sampling, although the amount of information provided again varies. RDLES recommends the use of dedicated nets for each sampled waterbody to minimize the risk of cross-contamination. A chain of custody form was developed in an effort to standardize the labeling and data collection (Appendix A).

All nets are decontaminated in 5% acetic acid (vinegar) between sample locations, including multiple locations on the same reservoir. Samples are kept cool after collection and during shipment to the laboratory. The Field Sampling Standard Operating Procedure (SOP) detailing collection methods can be found on the RDLES webpage, <https://www.usbr.gov/mussels>.

## Sample Processing

All samples arriving at the laboratory are logged into a central database (Figure 2).



**Figure 2: Samples after being logged in at RDLES**

During login, all available data is entered, and a unique identifier is created for every sample. Data is encoded into a barcode that is attached directly to the sample bottle. Raw water samples are prepared using established protocols, detailed in the Laboratory SOP which can be found on the RDLES webpage, <https://www.usbr.gov/mussels>, and microscopic analysis is completed

utilizing cross polarized and regular light microscopy. Briefly, water samples are settled overnight in Imhoff cones (Figure 3).



**Figure 3: Samples are set up overnight prior to analysis**

Previous research has shown that due to the density of the veliger's hard shell they will fall to the bottom of the settling cone. From the cone, the bottom 15 mL of the sample is collected and then analyzed by microscopy (Figure 4).



**Figure 4: Samples awaiting analysis**

Water samples from non-infested waters in which suspect veligers have been found within the last five years are considered priority samples. In addition to microscopy, they are also analyzed for the presence of quagga and zebra mussel environmental DNA (eDNA) by polymerase chain

reaction (PCR) methods. The data and results that are collected are used to further optimize sample collection and handling methods.

## Notifications of Findings

Between 2016 and 2018 RDLES detected eight first-time dreissenid mussel findings. A first-time finding is a highly sensitive and politically charged topic for Reclamation and its partners and stakeholders. These results are communicated directly to Reclamation area offices and stakeholders following a strict notification protocol (Appendix B). Negative findings and veliger counts from waterbodies known to have mussel infestations are communicated through an access-controlled SharePoint site. Currently, dreissenid mussel data is maintained in a large database that can be utilized for environmental and population data analysis relevant to control activities. This data has been utilized by the United States Army Corps of Engineers (USACE) and the United States Geological Survey (USGS) for various research purposes. Information about Reclamation’s early detection program is regularly presented at professional meetings with collaborators and at international conferences. RDLES will continue to modify and update Reclamation’s mussel website and produce content such as instructional videos and interactive maps.

## Results

RDLES received an average of 1,475 raw water samples per year between 2016 and 2018. Appendix C lists the waterbodies sampled during that timeframe. Samples were collected from every region in Reclamation by RDLES staff, other Reclamation employees, and state employees. Roughly half of the received samples were analyzed by microscopy only. In 2016 and 2017 samples from Lake Mead, a waterbody with a known mussel infestation, were analyzed utilizing FlowCam technology. After 2017, FlowCam use was discontinued for samples with veligers and samples were analyzed by counting by microscopy. Beginning in 2018, samples from Canyon Lake Reservoir, AZ, were also limited to veliger counts by microscopy due to a growing infestation. Details regarding regional sample numbers and analysis performed are available in Tables 1-3. In all three tables, “Veliger Count” refers to the number of samples where veligers were counted in known positive samples.

**Table 1: Number of Samples Received in 2016 by Region and Analysis Type**

Region	Total # Samples Rcvd	Microscopy Only	Microscopy & PCR	Veliger Count	FlowCam
GP	285	191	94	0	0
LC	192	0	140	0	52
MP	315	174	141	0	0
PN	2	2	0	0	0
UC	648	415	233	0	0
Non-BOR	6	3	3	0	0

Totals	1448	785	611	0	52
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**Table 2: Number of Samples Received in 2017 by Region and Analysis Type**

Region	Total # Samples Rcvd	Microscopy Only	Microscopy & PCR	Veliger Count	FlowCam
GP	323	182	141	0	0
LC	236	0	109	82	45
MP	338	206	132	0	0
PN	7	7	0	0	0
UC	690	390	300	0	0
Non-BOR	30	26	4	0	0
Totals	1624	811	686	82	45

**Table 3: Number of Samples Received in 2018 by Region and Analysis Type**

Region	Total # Samples Rcvd	Microscopy Only	Microscopy & PCR	Veliger Count	FlowCam
GP	312	230	82	0	0
LC	306	125	75	106	0
MP	148	83	65	0	0
PN	0	0	0	0	0
UC	568	367	201	0	0
Non-BOR	8	8	0	0	0
Totals	1342	813	423	106	0

All results generated by RDLES are uploaded to a SharePoint site and shared with Federal and State partners. In addition, an internal password protected database of sample results is maintained. All positive findings are reported according to RDLES protocols to the appropriate partner. Any follow up tests that the partner requests are carried out to ensure that the findings are validated. Data collection and reporting methods will continue to be optimized. Data from each field season is available to Reclamation researchers for use in research projects such as modeling mussel spread based on habitat suitability, the impacts of sample preservation and holding time on results.

# **Collaboration**

RDLES staff participates in a number of collaborative efforts with partners to develop, share, and modify standard operating procedures for all stages of analysis, as well as various research projects utilizing invasive mussel population data. Subject matter includes field sampling methods, sample handling, and DNA analysis techniques.

In 2018, RDLES staff provided inputs to the Western Regional Panel (WRP) in their efforts to standardized laboratory and field sample protocols. Topics discussed included hygiene practices aimed at preventing cross-contamination of samples during sample collection, laboratory processing, and analysis, as well as buffering and preservation techniques to ensure samples arrive at the laboratory in a viable state. Staff continue to develop and modify SOPs and QA/QC practices based on input from collaborators.

RDLES provides training, follow-up advice, and technical expertise for partners in order to maintain sample QA/QC, as well as for other laboratories establishing their own early detection programs. Both the Field Sampling SOP and the Laboratory SOP have been widely shared with regional and area offices conducting their own sampling efforts. In December 2017, RDLES hosted two employees from the PN Regional Laboratory in Boise, ID. During their three-day visit, training was conducted on eDNA methods and veliger identification using cross-polarized light microscopy (CPLM). Visitors were provided with training samples containing ostracods, corbicula (clams), and mussels for side-by-side comparison of similar organisms. Also, provided were training manuals with high-resolution photographs and a veliger stock solution for further training of interns in the PN laboratory.

RDLES routinely answers email and telephone requests for research assistance, and regularly provides regional and area offices with veliger samples and clean sample bottles, as well as information and advice on the purchase of equipment such as water quality measurement devices, plankton tow nets, and sample bottles.

Protocols for innovative technologies such as quantitative PCR and next generation DNA sequencing will be developed to improve detection methods for invasive mussels. Additionally, RDLES staff provide expertise in the identification of other aquatic organisms of concern. In 2018 the RDLES laboratory worked with the Roy Water Conservancy District in Utah to identify clam shrimp which are causing clogging in delivery pipes. The Roy Water Conservancy water distribution system is downstream of Rockport Lake and Echo Reservoir, both of which contain Reclamation facilities (Wanship Dam and Echo Dam, respectively). The laboratory subsequently worked with the state of Utah to determine whether these waterbodies are the source of the clam shrimp. No evidence of clam shrimp was detected in the samples obtained from these waters.

## **DOI Safeguarding the West from Invasive Species Initiative**

RDLES participates in the Department of the Interior (DOI) Safeguarding the West from Invasive Species Initiative, a multi-agency initiative whose goal is to contain the spread of

invasive mussels. In collaboration with USGS, National Park Service (NPS), Bureau of Indian Affairs (BIA), Fish and Wildlife Service (FWS), the Columbia River Inter-Tribal Fish Commission, Utah Division of Wildlife Resources (UT DWR), AZ Department of Fish and Game, Bureau of Land Management (BLM), Aquatic Nuisance Species Task Force (ANSTF), DOI Policy, Management and Budget, and other Reclamation offices, RDLES committed to providing resources in the areas of Prevention, Early Detection Monitoring and Research. Table 4 outlines the specific commitments of RDLES staff.

**Table 4: RDLES Commitments to the DOI Safeguarding the West from Invasive Species Initiative**

Category	Commitment Details	Collaborating Agencies
Prevention	Improve predictive models and methodologies for identifying waterbodies at risk of mussel infestation in the West, with a focus on the Columbia and Snake River reservoir systems	USGS/Reclamation/NPS
Early-Detection Monitoring	Assess various eDNA methodologies and develop criteria, guidelines, and decision-support tools for using eDNA in concert with other tools to increase the probability of detecting mussel populations	USGS/Reclamation
	Develop, apply, and evaluate eDNA and microscopy technologies to support sampling and detection efforts	Reclamation
	Conduct eDNA sampling efforts in high risk waters on the Blackfeet Indian Reservation, through BIA project funding	Reclamation
	Continue to analyze water samples from across the western United States and provide results to staff and stakeholders	Reclamation
	Continue monitoring for mussels in Bureau of Reclamation waters and facilities, as well as connected waters	Reclamation
Research	Continue research on effective technologies for the detection,	Reclamation



	prevention, control, and management of invasive mussels in lab and field settings	
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RDLES also collaborates with USACE on Predictive Modeling commitments. RDLES Research Coordinator Sherri Pucherelli was tasked to organize a prize-challenge to explore mussel avenues of research related to the containment and control of mussel spread. As part of this ongoing process, RDLES staff set guidelines for the competition, evaluated proposals for merit, and along with a team of outside judges, ranked proposals for feasibility.

## Outreach

RDLES participates in a variety of outreach activities aimed at sharing knowledge among partners, the research community, and other interested entities. Staff provided over a dozen professional presentations to USACE Engineer Research and Development Center (USACE-ERDC), North American Lake Management Society (NALMS), Southern Nevada Water Authority (SNWA), National Institute of Standards and Technology (NIST), Association of State Dam Safety Officials (ASDSO), WRP, and State Agencies and Stakeholder Task Forces in AZ, CO, ID, MT, NE, NM, NV, UT, and WY. Appendix D contains a publication based on an International Conference on Aquatic Invasive Species (ICAIS) presentation given by Denise Hosler.

Additionally, the RDLES laboratory is included in most TSC facility tours provided to visiting VIPs, school groups, leadership classes, and other interested parties, allowing for widespread education and informative opportunities. Laboratory staff assist in the organization of Reclamation's annual Take-Your-Child-to-Work event, providing input into the format of the event, and giving presentations and hands-on demonstrations of field equipment, sample collection and processing, and microscopy. Each spring, RDLES staff participates in several local water festivals for grade school students including the Aurora and Westminster, CO, water festivals, as well as Girls Exploring Science, Technology, Engineering & Math (GESTEM), a day of hands-on workshops presented by area volunteers for 7<sup>th</sup> grade girls from the Denver Metro Area. Each summer RDLES supports 4-6 college interns, with several high school students interning at various times throughout the year (Figure 5).



**Figure 5: RDLES staff with 2018 summer interns**

Interns are fully trained in laboratory and field methods and are provided individual assignments meant to enhance their knowledge of Reclamation and RDLES, while providing practical application of education. An informational website is maintained, and plans are in place to produce instructional videos and interactive maps. A flyer has been created (Appendix E) with reminders and tips related to collecting, preserving, and shipping samples.

## Next Steps

In the coming years RDLES staff will continue to receive and analyze samples from a wide range of waterbodies across the western United States. This work will continue to provide an early warning of mussel activity that will support the decisions that water managers must make. In 2018, two new staff were added to the RDLES team, providing additional microscopy technicians, as well as support for other projects the current staff is involved in. We will continue to provide support and training to our partners in regional and area offices and expect to continue outreach efforts by participating in working groups, presenting at various meetings, sponsoring college and high school interns, and participating in water festivals. Plans are in place to optimize the methods used for sample collection, analysis, and data management. Our SOPs are updated and shared with our partners as they are refined. Data will be collected for use in mussel management, modeling projects, population trend analysis, economic studies, and others. New products such as videos, flyers, posters, and presentations will be created to increase standardization, as well as to provide information and education.

# **Appendix A – Chain of Custody and Instructions**

In an effort to collect standardized information from all agencies submitting water samples for analysis, RDLES staff created the Chain of Custody form shown here.

**RECLAMATION DETECTION LABORATORY FOR EXOTIC SPECIES  
 INVASIVE MUSSELS FIELD SAMPLING LOG**

Sample Collector and Agency: \_\_\_\_\_

Net Diameter: \_\_\_\_\_

Phone Number: \_\_\_\_\_

Type and % Alcohol Added: \_\_\_\_\_

Email: \_\_\_\_\_

Baking Soda Added (circle): Y / N

Date Collected	Water Body	Sample Location	Tow Type (V or H)	Number of Tows	Length of Tows (M)	Total Water Depth (M)	Secchi Depth (M)	Coordinates (decimal degrees preferred)

Special Instructions: \_\_\_\_\_

Address to Return Cooler: \_\_\_\_\_

Page \_\_\_\_\_ of \_\_\_\_\_

## FIELD SAMPLING INFORMATION

### Secchi Depth Reading & Total Water Depth:

- Lower secchi disk until black and white marking undistinguishable and record depth
- Lower disk to bottom of sample site and record total depth

### Sampling:

- Record diameter of plankton tow net opening
- Vertical Tow: best for deep water, plankton tow net is lowered vertically off dock or boat to just above maximum depth or 30 m, and slowly pulled up
- Horizontal Tow: best for shore sampling or flowing water, throw net as far as possible and estimate distance, slowly reel in making sure entire opening of net is submerged
- Collect 5 tows per site and put in one bottle

### Preservation:

- Add 0.2 mL baking soda per 100mL sample
- Add 20% volume of alcohol

### Bottle Labeling:

- Label bottle with: date collected, water body name, sample location at reservoir, tow type (vertical or horizontal), number of tows, and length of tows
- Use meters for all measurements

### Net Hygiene:

- Dedicated nets should be used for any positive water body, and sprayed with bleach after
- Ideally each water body (positive or not) should have its own net
- Soak net in vinegar between each site and then rinse before taking next sample

### Water Quality Data:

- Collect as much water quality data as possible using a multiprobe
- Email water quality data to: **spucherelli@usbr.gov**

### Shipping:

- Ship samples immediately after collection, SHIP OVERNIGHT
- Seal bottle with electrical tape, place diaper over sample bottle, place in plastic bag
- Pack sample bottles in a cooler with blue ice packs, NO WET ICE

Address: **US Bureau of Reclamation Attn: RDLES**  
**6<sup>th</sup> Ave & Kipling St, Bldg 56 Room 1200**  
**(Mailcode 86-68560)**  
**Denver CO 80225**

### Contact Information:

- If you have questions or would like confirmation of cooler arrival at RDLES, email tracking number to: **dmench@usbr.gov**

For complete Standard Operating Procedure go to <https://www.usbr.gov/mussels/>

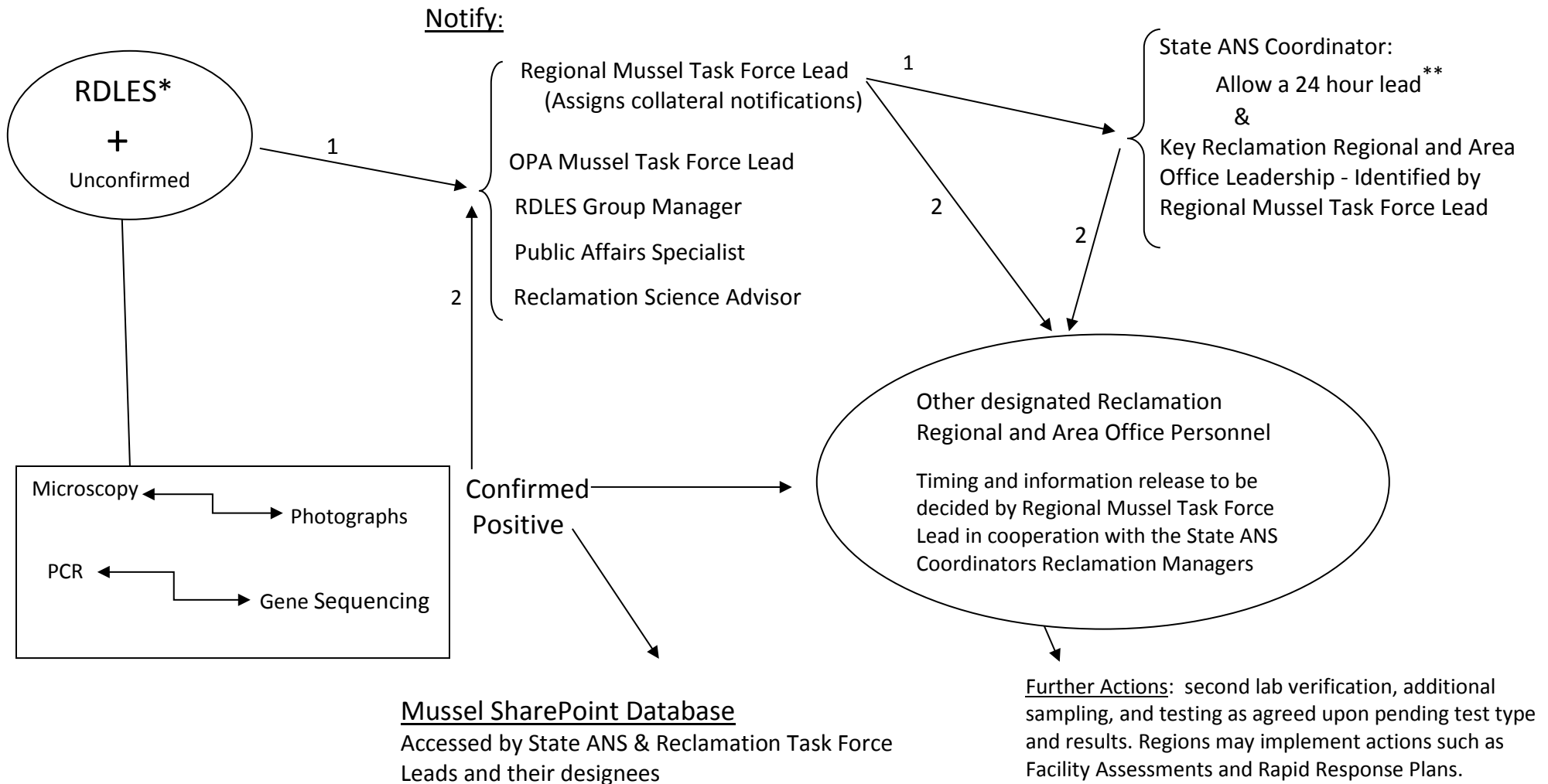


## **Appendix B – Notification Protocols**

Because first-time findings are highly sensitive and politically charged, the protocols shown here are strictly adhered to in order to ensure stakeholders and partners have accurate and timely notification.

# Notification Protocol for First Time Mussel Findings

To protect dissemination of sensitive information, and assure appropriate notification following the confirmation of results



\* Acronyms: RDLES =Reclamation Detection Laboratory for Exotic Species, PCR=Polymerase Chain Reaction, ANS=Aquatic Nuisance Species

\*\*All public releases are delegated to the State ANS Coordinator, as they generally manage the recreation on waters where Reclamation has facility operations. A minimum of 24 hours to 48 hours is granted to the State ANS to arrange for public release of information. No test results are to be written and sent in an email until after the State ANS Coordinator has released the information to the public or given permission for information dissemination.



## Additional RDLES information:

1. Reclamation performs microscopy routinely on all water samples.
2. If a water body has tested positive by microscopy at any time in the past, PCR testing will be performed on samples from that water body regardless of microscope result on that sample.
3. All positive PCR results will be sent for gene sequencing to verify organism's DNA.
4. Reclamation will make remaining bulk water sample available (up to six months) for independent lab testing, which most States require prior to water body classification.
5. RDLES positive results may be confirmed by:
  - a. Microscopic photos verified by dreissenid mussel expert.
  - b. Positive microscopic results verified with positive PCR results.
  - c. Positive microscopic results with positive PCR results verified with gene sequencing.
6. State ANS Coordinators routinely request independent lab verification of RDLES test results which may or may not confirm Reclamation test results:
  - a. Microscopy results agreed upon/not agreed upon
  - b. PCR results replicated/not replicated
  - c. If PCR results replicated, then gene sequencing replicated/not replicated.
7. Each State has its own definition of what constitutes a positive water body and the action it takes to manage the water body is dependent upon its definition.
8. Reclamation does not make water body designations; however, it does make notifications of all positive test results for a water body.
9. All of RDLES confirmed test results are posted to the Reclamation Mussel SharePoint Database and that data is available to designated State and Reclamation employees.
10. Reclamation follows standard operating procedures and quality control and assurance practices which are documented and available on the Reclamation Mussel Internet site at <http://www.usbr.gov/mussels/index.html>



## **Appendix C – Waterbodies Sampled from 2016 through 2018**

AZ	Apache Lake (Horse Mesa Dam)
AZ	Bartlett Lake
AZ	Canyon Lake (Mormon Flat Dam)
AZ	Granite Reef Diversion Dam
AZ	Roosevelt Lake
AZ	Saguaro Lake (Stewart Mountain Dam)
AZ-NV	Lake Mead (Hoover Dam)
CA	Boca Reservoir
CA	Cachuma Lake (Bradbury Dam)
CA	Casitas Lake
CA	Eastern Municipal Water District
CA	Folsom Lake
CA	Lake Arrowhead
CA	Lake Berryessa (Monticello Dam)
CA	Millerton Lake (Friant Dam)
CA	New Melones Lake
CA	O'Neill Forebay
CA	Prosser Creek Reservoir
CA	San Luis Reservoir (BF Sisk Dam)
CA	Shasta Reservoir
CA	Stampede Reservoir
CA	Stony Gorge Reservoir
CA	Trinity Lake
CA	Whiskeytown Lake
CO	Blue Mesa Reservoir
CO	Carter Reservoir
CO	Crystal Reservoir
CO	Estes Lake (Olympus Dam)
CO	Granby Reservoir
CO	Grand Lake
CO	Green Mountain Reservoir
CO	Gunnison River
CO	Horsetooth Reservoir
CO	McPhee Reservoir
CO	Pinewood Reservoir
CO	Pueblo Reservoir
CO	Ruedi Reservoir
CO	Shadow Mountain Reservoir
CO-NM	Navajo Reservoir
KS	Cedar Bluff Reservoir
KS	Keith Sebelius Reservoir
KS	Kirwin Reservoir
KS	Lovewell Reservoir
KS	Norton Lake
KS	Webster Reservoir
Manitoba, Canada	Whirlpool Lake, Riding Mountain National Park
MT	Canyon Ferry Reservoir

MT	Clark Canyon Reservoir
MT	Fresno Reservoir
MT	Gibson Reservoir
MT	Helena Valley Reservoir
MT	Nelson Reservoir
MT	Tiber Reservoir (Lake Elwell)
ND	Edward Arthur Patterson Lake
ND	Heart Butte Reservoir (Lake Tschida)
ND	Jamestown Reservoir
ND	McClusky Canal
NE	Calamus Reservoir (Virginia Smith Dam)
NE	Davis Creek Reservoir
NE	Enders Reservoir
NE	Medicine Creek Reservoir
NE	Red Willow Reservoir
NE	Swanson Reservoir (Trenton Dam)
NM	Brantley Reservoir
NM	Caballo Reservoir
NM	El Vado Reservoir
NM	Elephant Butte Reservoir
NM	Farmington Lake
NM	Heron Reservoir
NM	Sumner Lake
NV	Lahontan Reservoir
NV	Pyramid Lake
NV	Rye Patch Reservoir
OK	Atoka Lake
OK	Broken Bow Lake
OK	Fort Cobb Lake
OK	Foss Reservoir
OK	Hefner
OK	Holdenville City Lake
OK	Hugo Lake
OK	Konawa Lake
OK	Lake Lawtonka
OK	Lake Of The Arbuckles
OK	Lake Thunderbird (Norman Dam)
OK	McGee Creek Reservoir
OK	Pine Creek
OK	Tenkiller
OK	Tom Steed Reservoir
OR	Agency Lake
OR	Clear Lake
OR	Gerber Reservoir
OR	Klamath Lake Upper
OR	Klamath River
OR	Lake Ewauna

OR	Tule Lake
OR	Wilson Reservoir (Lost River Diverson Dam)
SD	Angostura Reservoir
SD	Belle Fourche (Orman) Reservoir
SD	Pactola Reservoir
SD	Shadehill Reservoir
UT	Baker Reservoir
UT	Big Sandwash Reservoir
UT	Big Springs Ute Tribe Hatchery
UT	Bishop Springs
UT	Bottle Hollow Reservoir
UT	Bullock Reservoir
UT	Causey Reservoir
UT	Cleveland
UT	Cleveland Reservoir
UT	Cottonwood Reservoir
UT	Currant Creek Reservoir
UT	Cutler Reservoir
UT	Deep Creek
UT	Deer Creek Reservoir
UT	Dougherty Basin Reservoir
UT	East Canyon Reservoir
UT	Echo Reservoir
UT	Electric Lake
UT	Fairview Lake
UT	Fish Lake
UT	Fisheries Experiment Station Hatchery
UT	Forsyth Reservoir
UT	Fountain Green Fish Hatchery
UT	Glenwood State Fish Hatchery
UT	Grantsville Reservoir
UT	Gunlock Reservoir
UT	Gunnison Bend Reservoir
UT	Hams Fork
UT	Huntington North Reservoir
UT	Huntington Power Plant Pond
UT	Hyrum Reservoir
UT	Jackson Flat
UT	Joes Valley Reservoir
UT	Johnson Valley Reservoir
UT	Jones Hole Fish Hatchery
UT	Jordanelle Reservoir
UT	Kamas Hatchery
UT	Kolob Reservoir
UT	Koosharem Reservoir
UT	Last Chance Lakes
UT	Leland Harris

UT	Little Cottonwood Canyon Creek
UT	Little Dell Reservoir
UT	Long Park Reservoir
UT	Lost Creek Reservoir
UT	Mammoth (Huntington) Reservoir
UT	Mammoth (Huntington) Reservoir Hatchery
UT	Mammoth Creek State Fish Hatchery
UT	Manning Meadow Reservoir
UT	Mantua Fish Hatchery
UT	Mantua Reservoir
UT	Matt Warner Reservoir
UT	Midview Reservoir (Lake Boreham)
UT	Midview Reservoir (Ute Tribe)
UT	Mill Meadow Reservoir
UT	Millsite Reservoir
UT	Minersville Reservoir
UT	Moon Lake
UT	Navajo Lake
UT	Newcastle Reservoir
UT	Newton Reservoir
UT	Nine Mile Reservoir
UT	Otter Creek Reservoir
UT	Ouray Fish Hatchery
UT	Palisades Reservoir
UT	Panguitch Lake
UT	Pelican Lake
UT	Pineview Reservoir
UT	Piute Reservoir
UT	Pole Creek Lake
UT	Porcupine Reservoir
UT	Provo River
UT	Quail Creek Reservoir (Quail Lake)
UT	Recapture Reservoir
UT	Red Butte
UT	Red Fleet Reservoir
UT	Rockport Lake (Wanship Dam)
UT	Rosebud
UT	Sand Hollow Reservoir
UT	Santa Clara River
UT	Scofield Reservoir
UT	Sheep Creek Lake
UT	Smith and Morehouse Reservoir
UT	Snow Lake
UT	Snow Lake Hunter Power Plant
UT	Springville fish hatchery
UT	Starvation Reservoir
UT	Steinaker Reservoir

UT	Strawberry Reservoir
UT	Tropic Reservoir
UT	Upper Enterprise Reservoir
UT	Utah FES
UT	Utah Lake
UT	Virgin River
UT	Wahweap State Fish Hatchery
UT	White Rocks Fish Hatchery
UT	Wide Hollow Reservoir
UT	Willard Bay Reservoir (Arthur Watkins Dam)
UT	Yankee Meadow Reservoir
UT	Yuba Reservoir
UT-ID	Bear Lake
UT-WY	Avintaquin
UT-WY	Flaming Gorge Reservoir
UT-WY	Lake Canyon
WY	Alcova Reservoir
WY	Boysen Reservoir
WY	Buffalo Bill Reservoir
WY	Fontenelle Reservoir
WY	Glendo Reservoir
WY	Goldeneye Reservoir
WY	Gray Reef Reservoir
WY	Grey Reef Reservoir
WY	Healy Reservoir
WY	Jackson Lake
WY	Keyhole Reservoir
WY	LAK Reservoir
WY	Lake Desmet
WY	Pathfinder Reservoir
WY	Seminole Reservoir
WY	Sibley Lake
WY	Tie Hack Reservoir
WY-MT	Bighorn Lake (Yellowtail Dam)



## **Appendix D – ICAIS Publication**

This document is a publication based on an International Conference on Aquatic Invasive Species (ICAIS) presentation given by Denise Hosler.

## Management in Practice

## Where is the body? Dreissenid mussels, raw water testing, and the real value of environmental DNA

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### Editor's note:

This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (<http://www.icaiss.org/html/previous19.html>). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

### Abstract

The Bureau of Reclamation has been monitoring the waters in the western U.S. since 2006 for the presence of dreissenid mussels. Currently, Reclamation has evaluated over 17,000 raw water samples representing over 400 western water bodies. This data includes water bodies where mussels had invaded and control methods were being tested. Primarily however, the program tested western waters for the purposes of tracking the dreissenid mussel invasion. Utilizing the United States Army Corps of Engineers (USACE) program for zebra mussel detection, Reclamation developed a protocol for raw water testing for determination of dreissenid mussel presence in western waters that included microscopy and DNA testing. The results of testing clashed with definitions, and triggered concerns for costly false positives that round robin testing did not substantiate. During that time, a clear understanding of the conflicting test results was not available for the stakeholders and partners participating in the mussel detection program. The large body of data revealed some unique information on the invasion of mussels in the western US; from the way samples were collected and preserved, to the slower than anticipated spread. The Reclamation Detection Laboratory for Exotic Species (RDLES) conducted research looking more closely at the science involved in the detection of invasive mussels in raw water plankton tow net samples. As research revealed information about the lack of microscopic findings, the value of environmental DNA (eDNA) findings for invasive species and mission essential projects became apparent. This article will present an overview of the Reclamation invasive mussel program detection, monitoring, and briefing on some control research activities. RDLES research developments have far-reaching applications for future management activities and decisions with many lessons learned about planktonic sampling from this large body of data and the related discovery of benefits of eDNA testing for numerous species of concern.

**Key words:** dreissenid mussels, environmental DNA, eDNA, water testing

### Introduction to Reclamation Project background and goal

In 2007, adult quagga mussels (*Dreissena rostriformis bugensis* Andrusov, 1897) were discovered in the Colorado River Basin at Lake Mead, the first significant population in a Reclamation reservoir. The core mission of the Bureau of Reclamation is to operate

and maintain projects to ensure continued delivery of water and power benefits to the western United States (U.S). Reclamation delivers 10 trillion gallons of water to more than 31 million people each year and Reclamation is the second largest producer of hydro-electric power in the western U.S. Dreissenid mussel populations in the lower Colorado River Basin dramatically increased in the months after the

initial discovery and the concerns for potential threats to water delivery and the hydropower generation facilities at Hoover, Davis, and Parker Dams generated the need for detection testing. In 2008, larval mussels were found in Pueblo Reservoir, Colorado, and zebra mussel (*Dreissena polymorpha* Pallas, 1771) adults were found in San Justo Reservoir in California. Increasing the concern for mussel spread and impacts to facility operations in the western US.

In April 2009, the Reclamation Research and Development Office (R&D) requested and received \$4.5M of American Recovery and Reinvestment Act (ARRA) funds to undertake a mussel detection project for Reclamation reservoirs and facilities. The Project goal was to provide the earliest possible detection of mussel larvae in Reclamation reservoirs in order to obtain 3–5 years of lead time before an infestation becomes large enough to seriously impact water and hydropower operations. The lead time can be used to plan for, budget, and install technologies which prevent mussel settlement on and inside critical infrastructure, or which facilitate rapid removal of adult colonies. The Reclamation Detection Laboratory for Exotic Species (RDLES), of the Technical Services Center (TSC) worked cooperatively with the Western Regional Panel and the 100<sup>th</sup> Meridian Initiative in 2007 and 2008, to develop a protocol for field sampling and laboratory testing that would provide the greatest confidence in the analytical results (low rates of false positives and negatives). Testing and adoption of this protocol occurred just prior to the start of the Mussel Detection Project. Initially, each of the five Reclamation Regions identified its top 15 water bodies of concern, based upon the potential for a mussel infestation to complicate, impair, or significantly increase the cost to maintain critical operations. From the list of 75, 60 water bodies were selected as the priority water bodies to be monitored by the Project. Working through the Western Panel and the State's Aquatic Nuisance Species (ANS) Coordinators, the project manager was able to enlist participation from four Western States. Contributions from the States, combined with Reclamation efforts, expanded the sampling from 60 to 136 water bodies in the first year. At the end of the ARRA funding period in 2011, the R&D Office recognized that RDLES had developed sensitive preparation methods for water samples and expanded analytical capabilities to include microscopy and polymerase chain reaction (PCR). Due to the increase in detections and the improvement in analytical capabilities, R&D continued funding the RDLES mussel detection program. To date, RDLES has analyzed over 17,000 samples representing over 425 western water bodies (Figure 1).

## Lessons learned in mussel detection

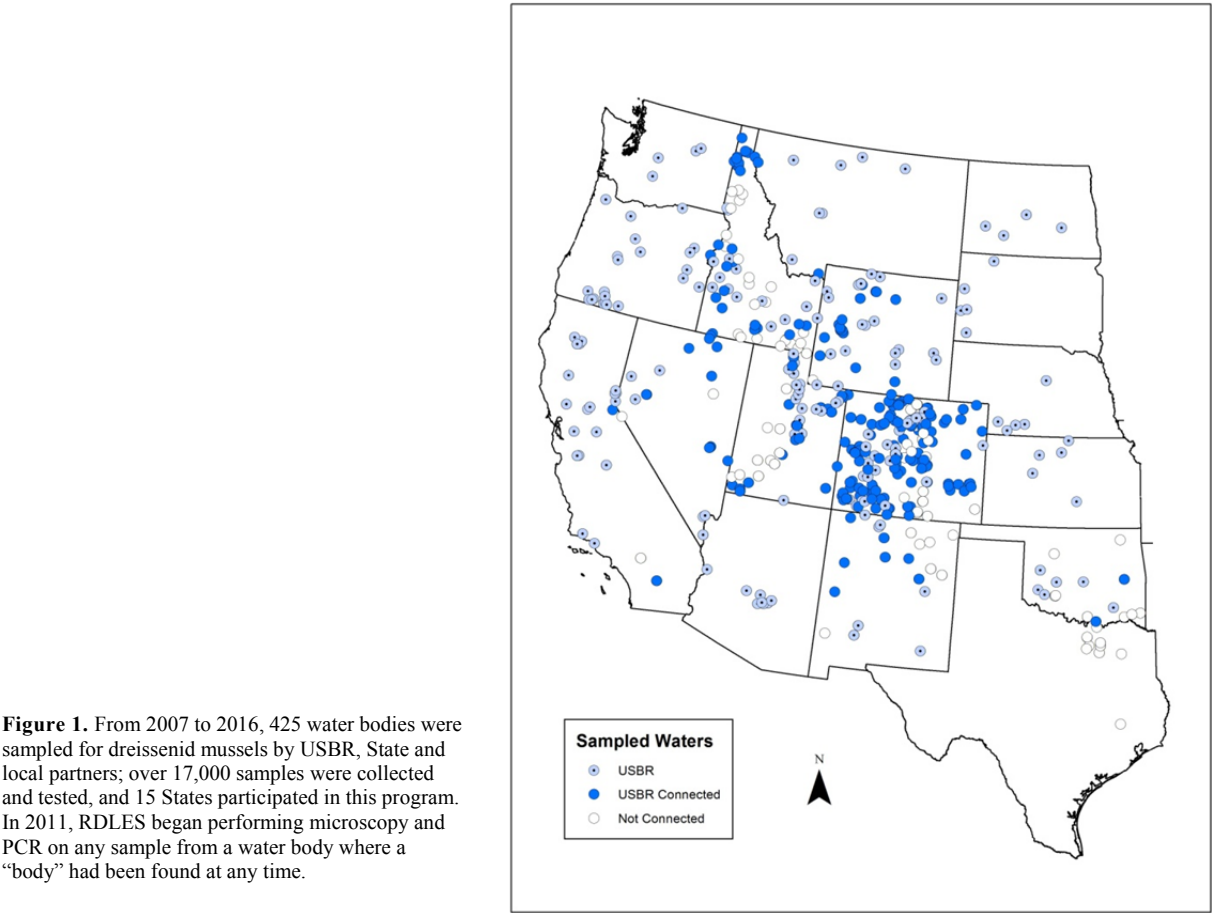
Initially, from 2007–2011, detection protocol was a linear, stepwise process. Samples were prepared and analyzed by cross-polarized light microscopy (XLM), and when a larval mussel (veliger) was detected the sample was analyzed by PCR to verify the presence of dreissenid mussel DNA. Early in 2011, RDLES began testing samples with PCR if a veliger had ever been detected in that samples water body. This change in protocol presented some challenges for the stakeholders and RDLES staff as well. Due to the level of effort required to prevent the spread of the mussels, managers needed to be sure that the water body was indeed positive with reproducing mussels. A large concern was that environmental DNA (eDNA) in the absence of microscopic veliger detection in the same water sample did not reveal the DNA source. It was vital to confirm mussel colonization for reservoir management and mussel spread mitigation (Hosler 2011; Reclamation 2013).

In 2009, Zehfuss determined statistically that of 327 water samples, 59.3 % of positives occurred at a marina or boat launch. A reevaluation of the samples analyzed from 2009 to 2012 confirmed this finding (Table 1). Mussels are spread primarily through boating and other human activities that move mussels from an infested water body to an uninfested one.

In 2009 and 2011 Reclamation participated in 2 double-blind round robin tests sponsored by Fish and Wildlife Service (Frischer et al. 2011). The results of these tests found that cross polarized light microscopy was the most sensitive and accurate method for mussel detection. PCR results were less sensitive and reliable by 75.8 versus 96.3 percent. The study indicated that PCR was seven times more likely to produce an incorrect result. Interestingly, false negatives were the most common error for all test methods.

In 2011, the RDLES staff was having good success with PCR and the quality assurance and quality controls (QA/QC) on PCR was becoming consistent and more reliable. In 2012, gene sequencing by an outside laboratory began to consistently confirm all but the weak signal PCR detections and the identification of the species DNA present in the water sample. The dilemma of positive results without the microscopic detection of the veliger remained a large concern for all involved in the management of dreissenid mussels in the western US.

The concern in 2011 and 2012 was that RDLES PCR results were due to false positives, yet in the laboratory the QA/QC passed and the gene sequencing was reliable, leading to the belief that the test results were valid. The data indicated that there were many one-time positives, yet fewer repeat positives



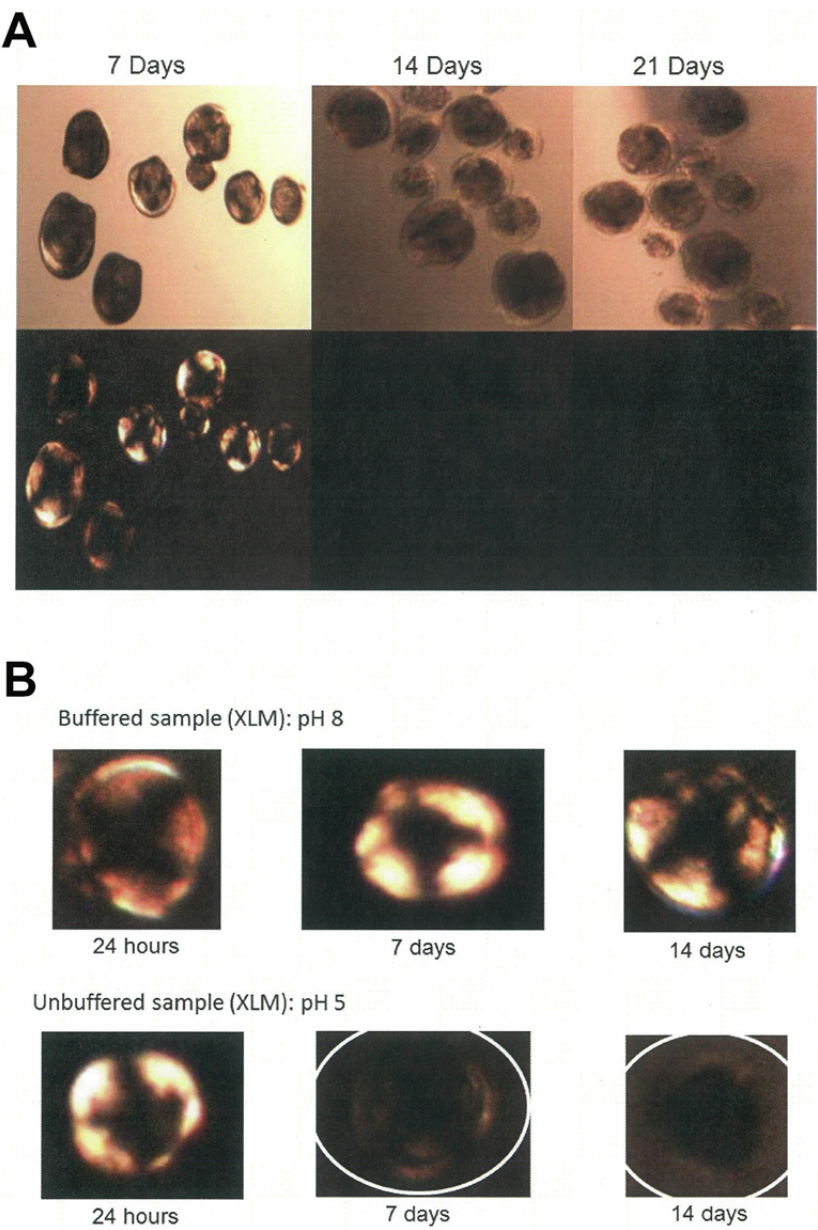
**Table 1.** Microscopic Veliger Findings: 11,683 Samples Analyzed and 419 Positive samples or 4%.

First Time Positives (52 water bodies)		All Positive Findings (85 water bodies)	
Location	Percent	Location	Percent
31 at marina/boat launch	60%	41 at marina/boat launch	48%
8 at dam	15%	14 at dam	17%
12 at midlake	23%	13 at midlake	15%
1 at hatchery	2%	2 at no boating reservoirs	2%
		2 at hatchery	2%
		4 at a canal	5%
		9 in a river	11%
TOTAL	100%	TOTAL	100%

(Table 2). The lab staff began to keep a list of the locations where a body or positive microscopic finding had occurred, and at that time, the decision was made to utilize all detection test methods available on sample locations where a microscopic finding had ever occurred.

There had been some early studies at RDLES looking at pH and veliger sample degradation, however, the concerns for false positives triggered additional intensive studies to further understand the

laboratory results (O’Meara et al. 2013). It turned out that sample preservation and handing was a significant factor for the mussel monitoring program. The larger study to determine optimum preservation and sample handling, revealed that pH shifts in water samples had a great deal to do with microscopic detection (Figure 2). Even samples collected at a pH of 8.5 could arrive at the lab 2–3 days later with a pH of 4 after preservation with alcohol (Carmon et al. 2014a; Pucherelli et al. 2014; Reclamation 2013).



**Figure 2.** Cross-polarized microscopic (CPLM) unbuffered veliger a pH 5: (A) Veligers in the unbuffered samples lost birefringence after 7 days would no longer be detected through RDLES microscopy methods. Buffered samples were found to remain consistently birefringent through time. (B) Veliger degradation studies found veligers lost birefringence by day 14 and were no longer detectable by CPLM microscopy. The veliger bodies can be seen under regular light microscopy and still be detected by PCR. Reclamation photos by Jamie Carmon, 2013.

**Table 2.** Positive Results 2008–2016: Detection ≠ Infestation

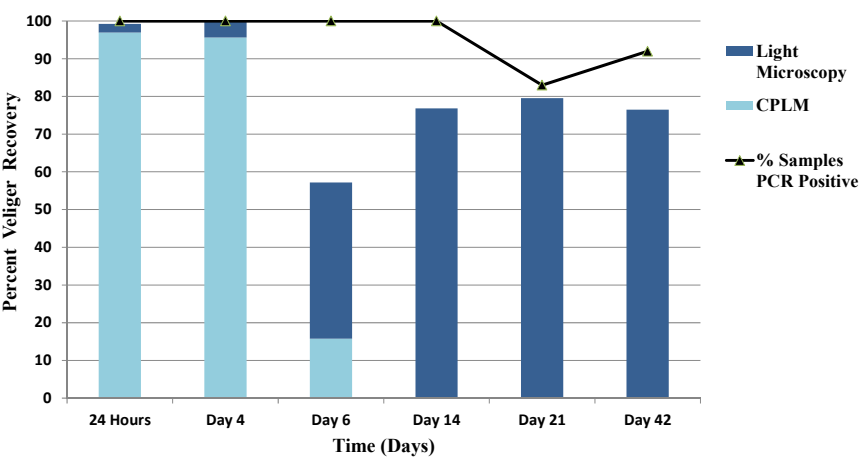
Total Samples	Total Positives by microscopy	Number of Positives at each water body:		
		One	Two	>Three
15,945	790 samples or 67 water bodies in 11 States	46	17	20

Each water body has 3–4 sample locations.

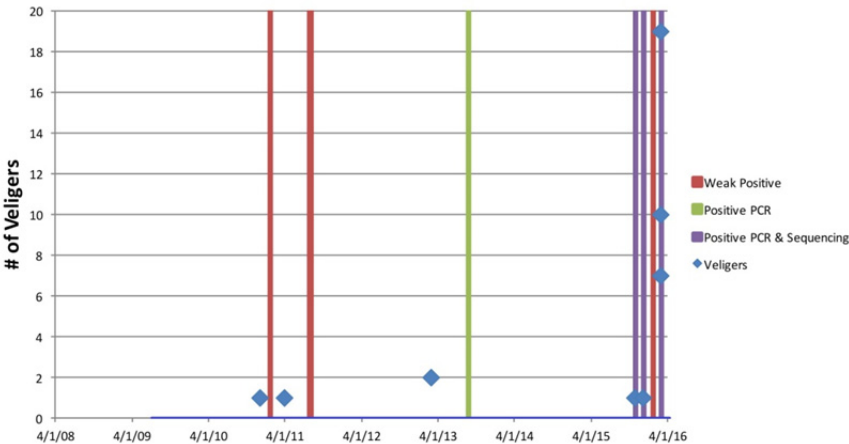
RDLES staff did a great deal of sampling and testing to determine if laboratory contamination was interfering with accurate test results (Carmon and Hosler 2015). The results of the extensive testing of microscopes, lab equipment, glassware, countertops,

and walls revealed no measurable lab contamination. However, the laboratory does continue to utilize Good Laboratory Practices (GLP) with weekly lab decontamination activities to reduce the likelihood of lab contamination.

**Figure 3.** Veligers in non-buffered samples lost their birefringence by day 14, but the tissue present continued to yield a positive PCR signal for 42 days of testing.



**Figure 4.** Microscopy and PCR test results for an Arizona reservoir, between 2009 and 2016.



While the staff was monitoring the diminishment of birefringence character, PCR or DNA testing was occurring simultaneously during the study. The results of these tests demonstrated that if the pH dropped below 7.0, the veliger would not be detected after 14 days, yet the mussel DNA could be detected reliably for 42 days (Figure 3).

Overall, RDLES modified and improved sample handling and preservation procedures, so that the microscopic detection of an organism occurred along with a positive PCR and gene sequencing result. The molecular methods modified existing PCR and increased sensitivity 100 times what it had been previously and extended the utilization of eDNA for the detection of dreissenid mussels (Keele et al. 2014; Carmon et al. 2014b). These methods have been expanded for the successful DNA analysis of other invasive, endangered, and rare aquatic organisms. While the question of eDNA source remains, some the patterns in mussel detection were becoming apparent to the lab staff.

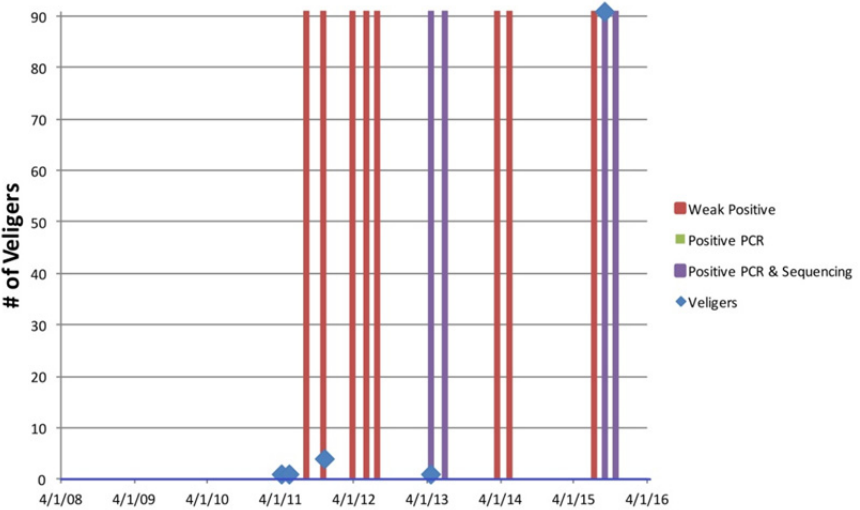
**Data findings and lessons learned**

Prior to 2011 when the protocol for testing was linear (XPL followed by PCR), the PCR testing was not refined enough to give a consistent one veliger, one positive PCR result. At that time, it was common to get a veliger detection and a weak positive PCR result. These weak positives were not necessarily reproducible and gene sequencing frequently failed.

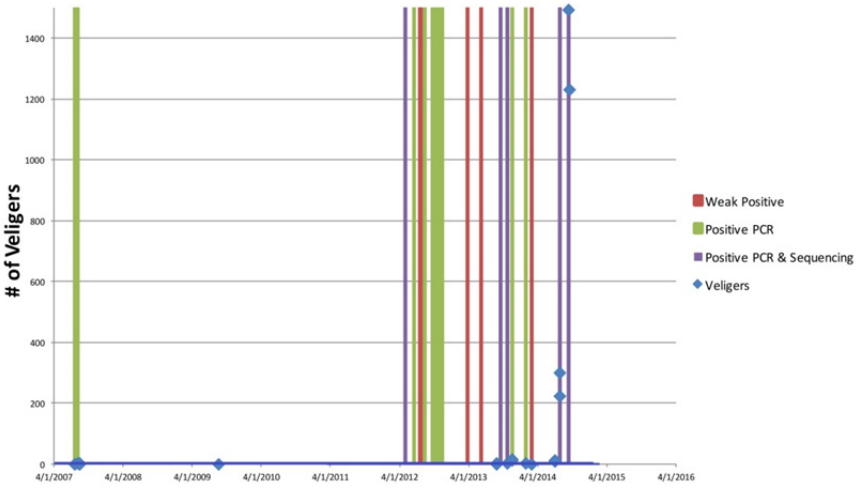
However, it became apparent that as the PCR methodology improved, the positive PCR signal became stronger in the bulk plankton tow sample when the veliger numbers seemed to increase. Additionally, as the PCR product produced successful gene sequencing, positive results by all methods of detection appeared to increase (Figures 4–7). Interestingly, the opposite pattern appeared in a reservoir where the microscopic detection of veligers decreased (Figure 7).

In Figures 4–7, the year in the title indicates when mussel monitoring on the water body began. The heavy blue line on the X axis indicates the duration of

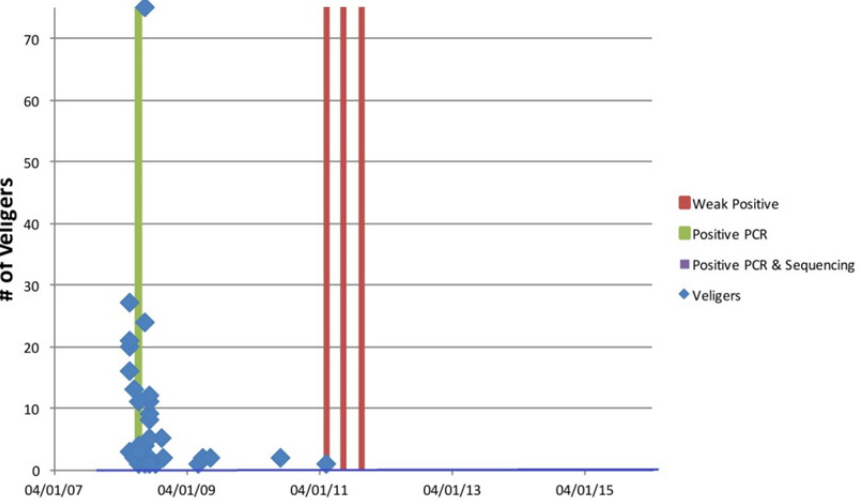
**Figure 5.** Microscopy and PCR test results for a New Mexico reservoir between 2007 and 2016.



**Figure 6.** Microscopy and PCR test results for a Utah reservoir, between 2007 and 2016. After 2010 RDLES samples were collected from penstocks, not the reservoir.



**Figure 7.** Microscopy and PCR test results from a Colorado reservoir, between 2008 and 2015. Dreissenid mussels have not been detected by molecular methods since 2012.





sampling, which is generally performed on a monthly basis. Prior to 2012, PCR for dreissenid mussels was in the early development and gene sequencing was randomly successful. The 2012 optimization increased the signal detection and yielded consistent gene sequencing results.

At RDLES, the detection results seemed to follow a general trend in that positives by microscopy appeared sporadic and the veliger numbers remain low. Interestingly, eDNA testing seemed to have a similar weak response, yet as PCR methods improved, positive PCR results paired with a confirmatory gene sequence more consistently. The exception to this was the Colorado reservoir which seems to indicate a declining mussel population both by the disappearance of veligers and negative PCR results.

## Discussion

It is a reasonable concern for managers that eDNA detections in the absence of a microscopic veliger detection in a water sample lacks the DNA source. It is vital to confirm mussel colonization for reservoir management and mussel spread mitigation. That being said, the eDNA data does indicate there may be evidence of potential colonization where there have been veliger detections. Additionally, it appears that the lack of eDNA may also be helpful in determining a lack of successful establishment or presence. The limits of knowing the source of eDNA must be emphasized when using eDNA, however, its value as an assessment tool should not be dismissed. Beyond invasive species, eDNA may be a helpful tool in biologic assessments where invasive, endangered, native species, and/or critical habitat may be at risk.

## Acknowledgements

A special thanks to the Reclamation Research and Development Office, Lower Colorado Dams Office, RDLES Staff: Kevin Bloom, Suzanne Brenimer, Jamie Carmon, Tanna George, Andrew Humes, Jacque Keele, Kevin Kelly, Susan McGrath, Rachael Lieberman, Diane Mench, Scott O'Meara, Yale Passamaneck, Sherri Pucherelli, Jeremiah Root, Ben Roske, Kyle Rulli, Kevin Scofield, Francesca Tordonato, Scott Thullen, Anne Williamson, Dan S. Williamson, and the numerous RDLES interns and temporary staff not mentioned above. Additional Reclamation staff: Curtis Brown, Chris Holdren, Michael J. Horn, Joe Kubitschek, Davine Lieberman, S. Mark Nelson, Michael Simonavice, and Richard Wydowski.

## References

- Carmon J, Hosler DM (2015) Understanding Dreissenid Veliger Detection in the Western United States. In: Wong WH, Gerstenberger SL (eds), *Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States*, Chapter 10. CRCPress/Taylor & Francis Group Boca Raton, pp 123–139, <https://doi.org/10.1201/b18447-14>
- Carmon J, Keele J, Pucherelli SF, Hosler DM (2014a) Effects of buffer and isopropanol alcohol concentration on detection of quagga mussel (*Dreissena bugensis*) birefringence and DNA. *Management of Biological Invasions* 5: 151–157, <https://doi.org/10.3391/mbi.2014.5.2.08>
- Carmon J, Keele J, Pucherelli S, Hosler D (2014b) PCR Detection of quagga mussels, intracellular DNA and dissolved DNA. Research and Development Office, Invasive Mussels Final Report 2014-01
- Frischer ME, Nierzwicki-Baur SA, Kelly KL (2011) Reliability of Early Detection of *Dreissena* spp. Larvae by Cross Polarized Light Microscopy, Image Flow Cytometry, and Polymerase Chain Reaction Assays, Results of a Community Double-Blind Round Robin Study. Reclamation, Skidway Institute of Oceanography, and Darrin Fresh Water Institute. [http://www.musselfmonitoring.org/Reports/RR11%20Final%20Report%20\(2010\).pdf](http://www.musselfmonitoring.org/Reports/RR11%20Final%20Report%20(2010).pdf)
- Hosler DM (2011) Early detection of dreissenid species: Zebra/Quagga mussels in water systems. *Aquatic Invasions* 6: 217–222, <https://doi.org/10.3391/ai.2011.6.2.10>
- O'Meara S, Hosler DM, Brenimer S, Pucherelli SF (2013) Effect of pH, ethanol concentration, and temperature on detection of quagga mussel (*Dreissena bugensis*) birefringence. *Management of Biological Invasions* 4: 135–138, <https://doi.org/10.3391/mbi.2013.4.2.06>
- Keele J, Carmon J, Hosler DM (2014) Optimization of Early Detection of Invasive Mussels with Polymerase Chain Reaction Technical Memorandum No. 86-68220-14-13, Research and Development Office, Invasive Mussels Final Report 2014-01
- Pucherelli S, Carmon J, Keele J, Hosler, DM (2014) Impact of Sample Preservation on the Detection of Invasive Mussel Larvae by Microscopy and PCR, Research and Development Office, Invasive Mussels Final Report 2014-01, September 2014
- Reclamation (2013) Improving Accuracy in the Detection of Dreissenid Mussel Larvae Technical Memorandum No. 86-68220-13-10, Reclamation Detection Laboratory. <http://www.usbr.gov/mussels/docs/MussellLarvaeDetectionReport.pdf>
- Zehfuss KP (2009) Probability of Veliger Detection: Estimation of Sampling Error and Confidence Interval. Paper submitted to Reclamation Research and Development Office (August 2009) (copies available upon request to DM Hosler).



## **Appendix E – Sample Flyer**

The sample flyer was created by RDLES staff as a means of passing on critical information to those partners collecting and shipping water samples.

# MUSSEL SAMPLING PROTOCOLS

As we head into the 2018 Dreissenid mussel sampling season, the RDLES staff would like to send a brief reminder about sample handling to ensure that everything runs smoothly. Following these recommendations will help to ensure the integrity of the results.

## Sample Collection

Please make every effort to eliminate sediment in your samples. If the mouth of the net drags the bottom during collection, please dump it and re-collect. Sediment interferes with settling procedures in the lab and is extremely difficult to look through. The cleaner the sample, the faster we will be able to process.

## Net Hygiene

**Dedicated nets** – dedicated nets should be used for any water body known or suspected to have mussels; wherever possible, each water body should have its own net, whether infested or not

**Vinegar treatment** – soak net in vinegar between sampling sites; rinse thoroughly before sampling

**Bleach treatment** – rinse nets quickly in bleach at end of day; rinse thoroughly; hang to dry

## Sample Preservation

**Buffer with baking soda** – 2 x 0.1 gram scoops per 100 mL of sample (please contact RDLES to request scoops)

**Preserve with alcohol** – add alcohol equivalent to 20% of sample volume (ethanol or isopropanol/rubbing alcohol; NOT denatured alcohol)

**Keep samples cool** – keep samples on ice during sampling; refrigerate after sampling

## Bottle Handling

**Label with the following information** - lake/reservoir name, specific location on the reservoir, collection date, tow type (horizontal or vertical), number and length/depth of tows

**Electrical tape** – securely seal the lid to the bottle with electrical tape to ensure samples do not leak

**Priority Samples** – clearly state on the bottle if the sample needs immediate analysis (i.e. fish hatchery transfer)

**Additional information** – please provide (either in the cooler or by email) any additional information collected with sample (GPS coordinates, YSI data, etc). This information is entered into our database (GPS coordinates are especially helpful as some locations are referred to by multiple names).

## Shipping

**Ship samples overnight** – samples should arrive in our lab within TWO WEEKS of the sample date to be viable.

**Ship samples with cold packs** - DO NOT USE WET ICE. Most shippers will discard wet or leaking packages.

**Include correct return address** – do not use the address of the shipping location as we cannot return packages to those addresses. We will return coolers as soon as possible.

**Ship samples to this address:** US Bureau of Reclamation Attn: RDLES  
6<sup>th</sup> Ave & Kipling St, Bldg 56 Room 1200  
(Mailcode 86-68560)  
Denver CO 80225

## **Data Sets that Support the Final Report**

If there are any data sets with your research, please note:

- Share Drive folder name and path where data are stored:  
\\bor\do\tsc\Jobs\DO\\_NonFeature\Science and Technology\2016-PRG-Invasive Mussel Detection, Spread, Control, and Management
- Point of Contact name, email, and phone: Diane Mench, [dmench@usbr.gov](mailto:dmench@usbr.gov), 303-445-2050
- Short description of the data: Pictures, Excel Spreadsheets, Word Documents
- Keywords: Early Detection, RDLES, Quagga/Zebra Mussels
- Approximate total size of all files:



