REABIC

Management of Biological Invasions (2017) Volume 8, Issue 3: 335–341
DOI: https://doi.org/10.3391/mbi.2017.8.3.07
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Open Access
Special Issue: Management of Invasive Species in Inland Waters

Management in Practice

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

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Received: 29 November 2016 / Accepted: 3 April 2017 / Published online: 23 June 2017

Handling editor: David Wong

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.icais.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 100th 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

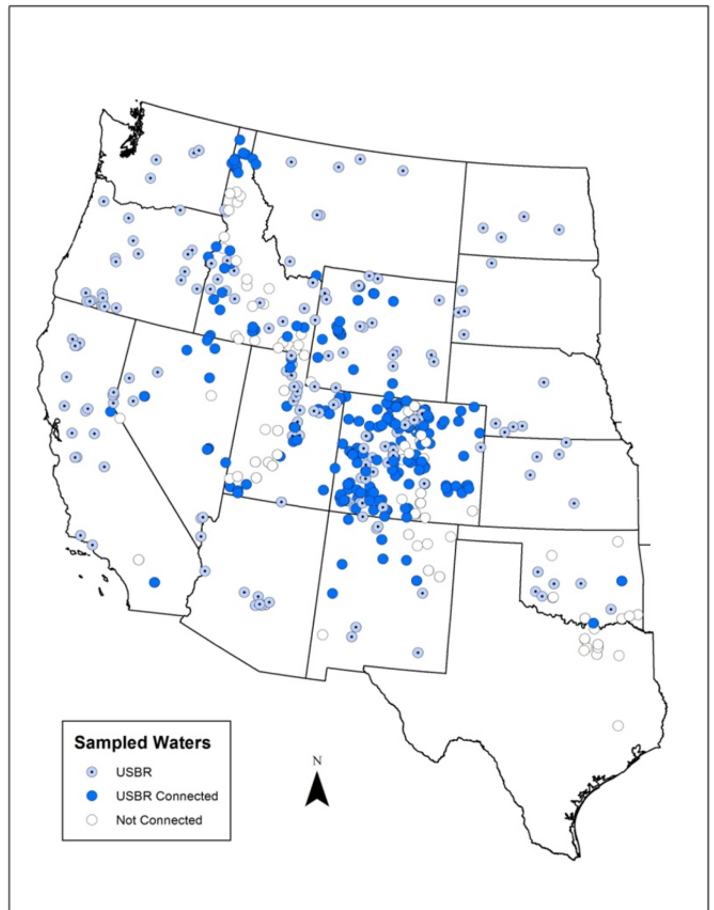


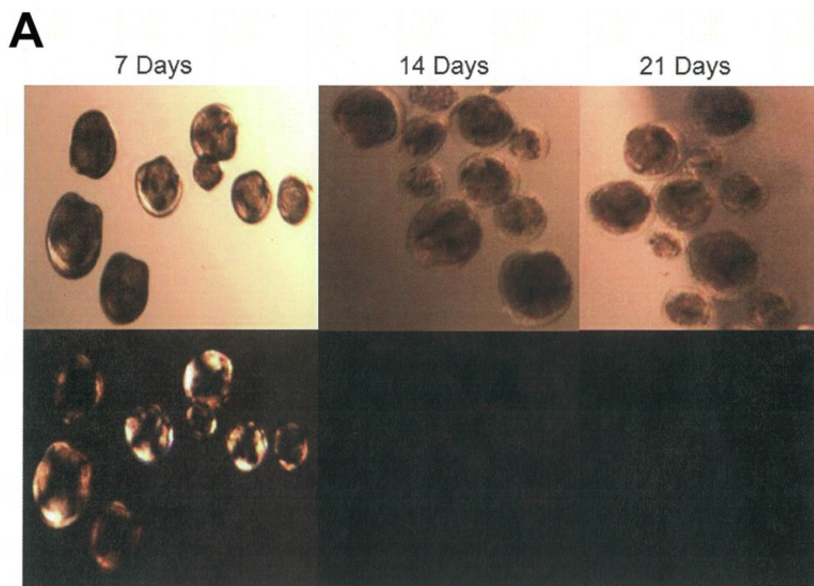
Figure 1. From 2007 to 2016, 425 water bodies were sampled for dreissenid mussels by USBR, State and local partners; over 17,000 samples were collected and tested, and 15 States participated in this program. In 2011, RDLES began performing microscopy and PCR on any sample from a water body where a "body" had been found at any time.

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
		41 at marina/boat launch	48%
31 at marina/boat launch	60%	14 at dam	17%
8 at dam	15%	13 at midlake	15%
12 at midlake	23%	2 at no boating reservoirs	2%
1 at hatchery	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).



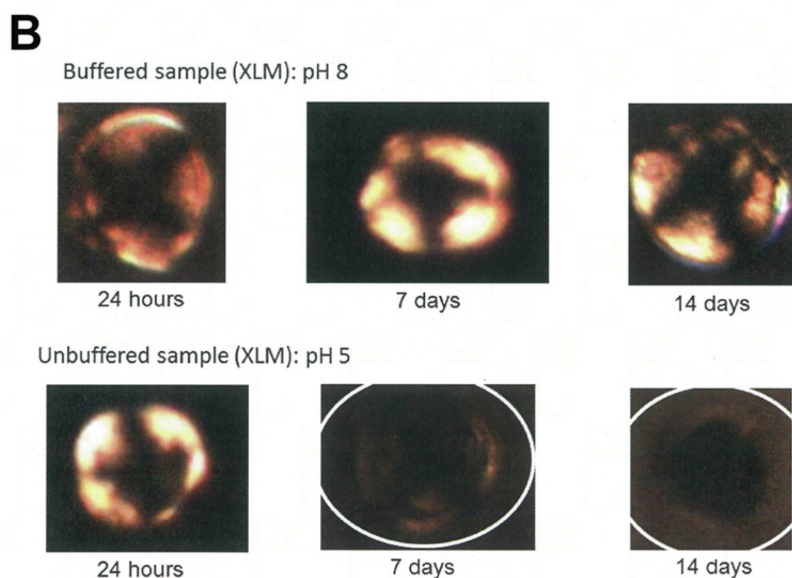


Figure 2. Cross-polarized microscopic (CPLM) unbuffered veliger a pH 5: (A) Veligers in the unbuffered samples lost birefringence after 7 days would no longer be detected through RDLES microscopy methods. Buffered samples were found to remain consistently birefringent through time. (B) Veliger degradation 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.

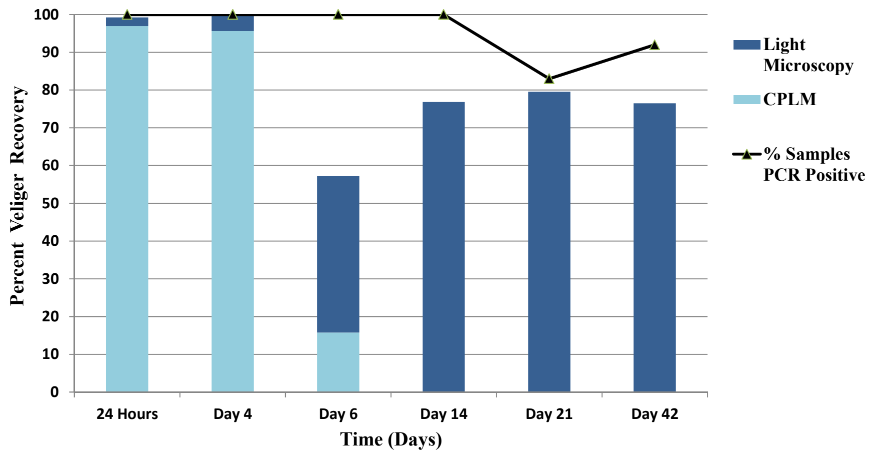


Figure 3. Veligers in non-buffered samples lost their bifringence by day 14, but the tissue present continued to yield a positive PCR signal for 42 days of testing.

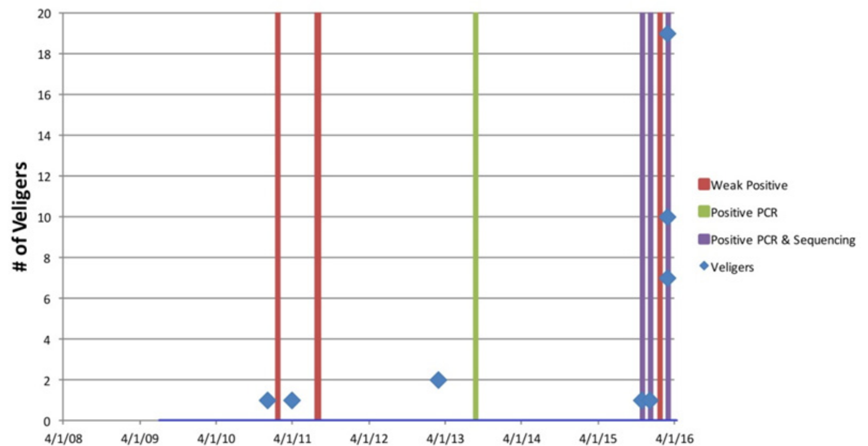


Figure 4. Microscopy and PCR test results for an Arizona reservoir, between 2009 and 2016.

While the staff was monitoring the diminishment of bifringence 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 sensitivity100 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

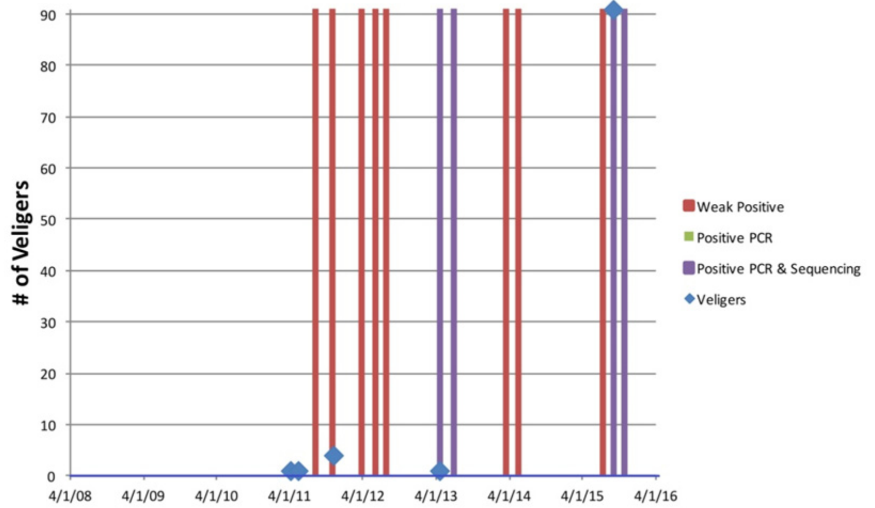


Figure 5. Microscopy and PCR test results for a New Mexico reservoir between 2007 and 2016.

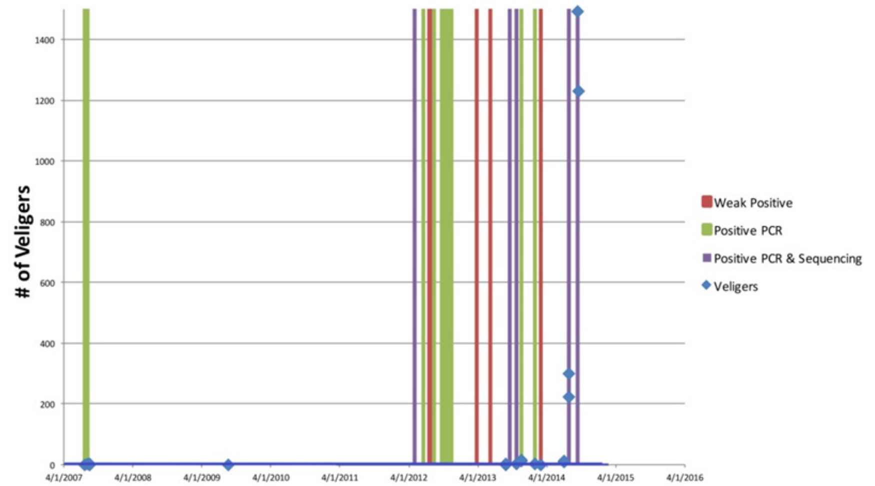


Figure 6. Microscopy and PCR test results for a Utah reservoir, between 2007 and 2016. After 2010 RDLES samples were collected from penstocks, not the reservoir.

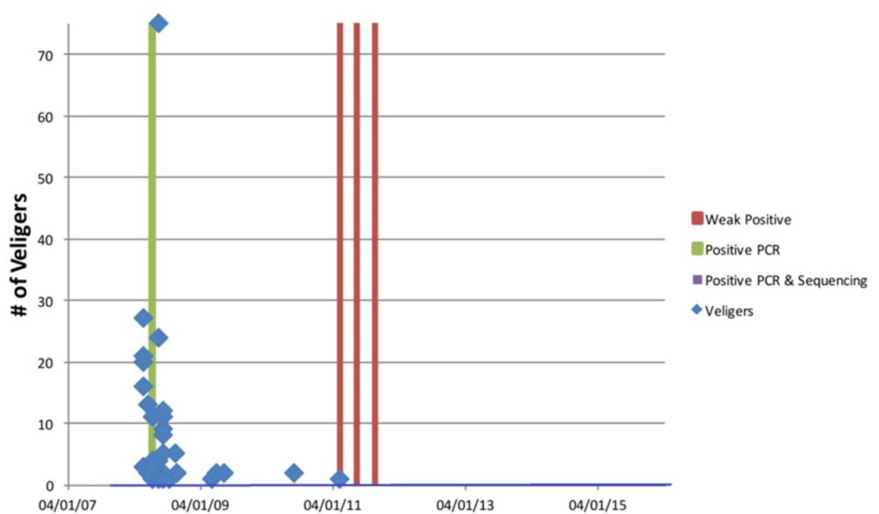


Figure 7. Microscopy and PCR test results from a Colorado reservoir, between 2008 and 2015. Dreissenid mussels have not been detected by molecular methods since 2012.

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.

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