RECLAMATION

Managing Water in the West

Use of Novel Hypervirulent Parasites to Control Naïve North American Dreissenid Populations

Research and Development Office
Science and Technology Program
(Final Report) ST-2018-1625-01

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

September 2018

Mission Statements

The U.S. Department of the Interior protects America’s natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power 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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14. ABSTRACT *(Maximum 200 words)* Reclamation collaborated with Molloy & Associates to identify novel hypervirulent parasite for the control of invasive dreissenid mussels in North America. The research focused on identification of populations of multiple dreissenid species in lakes in their native Balkans range. Mussels from these populations were dissected and parasites were collected and identified. Molloy & Associates final report (Appendix 1) details the scope, results, and future directions of the project. Reclamation has worked closely with Molloy & Associates, participating in field trips and performing the molecular analysis to identify parasites. The identification and adaption of a naturally occurring parasite into a biological control agent will be a multi-year effort and the work accomplished by this research effort has provided promising results.		
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BUREAU OF RECLAMATION

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Science and Technology Program

Hydraulic Investigations and Laboratory Services, 86-68560

(Final Report) ST-2018-1625-01

Use of Novel Hypervirulent Parasites to Control Naïve North American Dreissenid Populations

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Executive Summary

The Bureau of Reclamation is collaborating with Molloy & Associates to identify a biological control agent for the control of invasive dreissenid mussels. This is a long-term research project that requires the identification of novel hypervirulent parasites from the mussel's native range. There is currently no practical large-scale method for the eradication of invasive quagga and zebra mussels (*Dreissena bugensis* and *Dreissena polymorpha*). There have only been two successful open-water eradication efforts, both of which used the chemical treatment potash. This method would be too expensive for use at large scale. There is a need for the development of a cost-effective treatment method that does not rely on the traditional resource-intensive approach of applying non-specific chemicals throughout entire water bodies. Using a hypervirulent parasite would be an ideal alternative, not only because it would be specific to the invasive mussels, but also because it would be self-replicating and self-spreading – eliminating the cost of treating an entire water body.

This two-year project has laid the groundwork for the identification and future testing of possible novel parasites for the biological control of quagga and zebra mussels in the United States. The first step of this process was to identify isolated populations of a variety of dreissenid mussel species in the mussel's native range of the Balkans. Mussels from these populations were dissected for parasites and the parasites were identified by molecular methods. The results of this two-year effort were promising, indicating additional work should be pursued. Therefore, a new proposal for a three-year study was submitted to the Research and Development Office in order to continue the project. The report submitted by Molloy & Associates (Appendix 1) provides background information on the problem, project scope, international collaborators, details on the trips that were made to Eurasia, summarizes the research goals and progress, includes images of some of the parasites that have been identified, and presents future research directions.

Staff from the Reclamation Detection Laboratory for Exotic Species (RDLES) traveled to Montenegro in April 2017 to participate in a sampling trip and assisted with the collection and dissection of mussels to find parasites. RDLES was primarily responsible for the identification of parasites using molecular techniques which included determining the best extraction methods for the single celled organisms, optimizing primers and polymerase chain reaction (PCR) methods, and sequencing PCR products. The collaboration between RDLES and Molloy & Associates has been successful thus far and provides a solid foundation for future work that will hopefully include the identification of candidate parasites that can be used to control invasive mussels in North America.

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Background

Two species of invasive dreissenid mussels, *Dreissena polymorpha* (zebra mussel) and *Dreissena rostriformis "bugensis"* (quagga mussel), arrived in North America in the late 1980's [1]. In the thirty years since their arrival, these invasive mussels have been able to spread and establish populations in fresh water lakes, reservoirs, and rivers. In 2007, quagga mussels were discovered in Lake Mead, Nevada and their impact to Reclamation facilities became an ongoing issue that had to be addressed. Dreissenid mussels are prolific breeders, so their populations can grow quickly. In addition, these mussels settle on hard surfaces such as water intakes, gates, diversion screens, hydropower equipment, pumps, pipelines, and boats. This settlement behavior can lead to operational issues such as overheating and unplanned outages. Finally, dreissenid mussels are filter feeders and can change the natural ecology of a waterbody. Finding ways of controlling and mitigating the impact of dreissenid mussels on Reclamation facilities has been an ongoing challenge.

Currently, there is no viable ways of controlling the quagga and zebra mussels once they become established in a waterbody. Boat inspection programs across the Western United States focus on stopping the introduction of the mussels. But once the population is established the options for management are few. There have only been two successful eradications of dreissenid mussels in North America using potassium chloride (potash). Scaling a chemical treatment to a large body of water is not financially feasible, so new novel ways of eradicating dreissenid mussels must be found.

In 2017, a collaboration with Molloy & Associates was started to investigate the use of hypervirulent parasites as a biocontrol method for controlling quagga and zebra mussels. Biological control is the use of a natural enemy to suppress or eradicate a pest species population [2]. This process relies on research identifying an organism (insect, parasite, virus, etc..) that can specifically target the invasive species without causing harm to other species or the environment. There are many examples in the literature of where an insect is introduced to control an invasive plant [3]. In addition, viruses have been investigated for their use in controlling pest insect species [4].

Identification of a hypervirulent parasite offers a feasible way of controlling the dreissenid mussels in North America. Infectious disease caused by hypervirulent parasites can have long-term, devastating impacts on animal and plant populations. This is especially true when "naive" animal or plant populations are exposed to "novel" parasites that they have been geographically separated from for millions of years. Such naive host populations can be ravaged by the virulence of these novel parasites since they have not co-evolved and have little to no resistance to infection. An accidental introduction can have unknown impacts on a population. For example, the native Eastern oyster population was devastated by the introduction of the parasite *Haplosporidium nelson* in the 1950's [5]. Organisms that are approved for biocontrol release must undergo regulatory review and testing to prevent unintended consequences to the environment.

For this research project, Balkans populations of a variety of dreissenid mussel species are being evaluated for parasites that do not occur in quagga and zebra mussel populations in the United States. There are several dreissenid species (*D. caputlacus*, *D. anatolica*, *D. blanci*, and *D. carinata*) that are present in Eurasia. These isolated populations have diverged millions of years ago from *D. bugensis* and *D. polymorpha* and could have parasites that the invasive dreissenid mussels in North America have never encountered. Since North American populations of zebra and quagga mussels have not encountered the parasites of their European/Asian cousins, infection may prove lethal. The goal is to eventually develop these parasites into biological control organisms to be used against dreissenid populations in North America.

Methods

This research effort is a collaboration between Molloy & Associates and the RDLES Laboratory. Close coordination occurs by monthly phone calls and annual in-person meetings where research findings and updates are presented, and next steps are discussed.

A detailed summary of Molloy & Associates research and progress can be found in Appendix 1. This collaborator has been responsible for five research goals. First, to communicate with researchers across Europe and establish collaborations. Second, finding parasites in the cousin mussel *D. carinata* in the Balkans by visiting different waterbodies, collecting and dissecting mussels (Figure 1). Third, using morphology to identify the parasites and sending the samples to the RDLES Laboratory for molecular analysis. Fourth, to explore the federal regulatory steps involved in importing a parasite for biological control. Finally, to communicate progress of activities to Reclamation and to present at conferences and meetings and give presentations to the public about this research. As part of this research, three trips to the Balkans were completed for the collection of parasites.

In April 2017, two RDLES staff, Dr. Jacque Keele and Dr. Yale Passamaneck traveled to Podgorica, Montenegro with Dan Molloy for 8 days. Work was completed at a laboratory in the University of Montenegro. During this trip, the RDLES staff assisted with mussel collection and dissections for parasites (Figure 2) and were able to see the conditions and obstacles that will have to be overcome to make this a viable long-term project in the Balkans. Samples were collected from four different waterbodies: Skadar Lake (this Report's cover photo), Bojana River, Sasko Lake, and Malo Blato Lake.

Dr. Dan Molloy taught the RDLES staff how to perform dissections and identify ciliate parasites of the genus *Ophryoglena*. Hundreds of mussels were dissected. The digestive gland was removed, place in a petri dish, gently cut up and observed for parasites (Figure 3). There were many dissections where parasites were not observed. Whenever a *Ophryoglena* was observed it was pipetted into an Eppendorf tube and preserved in alcohol. When unknown parasites were observed, Molloy was consulted to give an identification, and the parasite was then preserved in alcohol for molecular analysis (Figure 4). Tissue samples from the mussel's foot or gill was also taken for molecular analysis to confirm the morphological identification. All samples were mailed to the RDLES laboratory in Denver, Colorado for molecular analysis.

To maximize the molecular information gathered, DNA extraction was performed individually on single ciliates, allowing one-to-one assignment of morphology and DNA sequence. This approach is challenging as each ciliate is a single-celled organism, meaning a very small initial amount of DNA is available for recovery. After testing commercial DNA extraction kits (including REDExtract-N-Amp Tissue PCR Kit; Sigma-Aldrich), it was found that the optimal technique is to use a lab-made buffer containing surfactants and the enzyme Proteinase K to lyse cells and degrade proteins. Because of the small amount of starting material, the resultant lysed material was used directly in downstream applications to eliminate potential loss for additional sample handling and cleaning. Extracted DNA from these reactions was then amplified using a polymerase chain reaction (PCR). PCR reactions targeted the *cytochrome oxidase I* (*COI*) gene, using degenerate or taxon-specific oligonucleotide primers. Amplified DNA fragments were then provided to a commercial laboratory for DNA sequencing.

DNA was also extracted from six adult mussels using the DNeasy Blood and Tissue Kit (Qiagen). PCR was carried out using barcoding primers to the COI gene [6]. The PCR product was then sent to a commercial laboratory for DNA sequencing.

Figure 1: Dan Molloy dissecting mussels for parasites (Montenegro 2017).

Figure 2: Collecting mussel samples from deeper water (Montenegro 2017).

Figure 3: Mussels dissected and ready to be analyzed for parasites (Montenegro 2017).

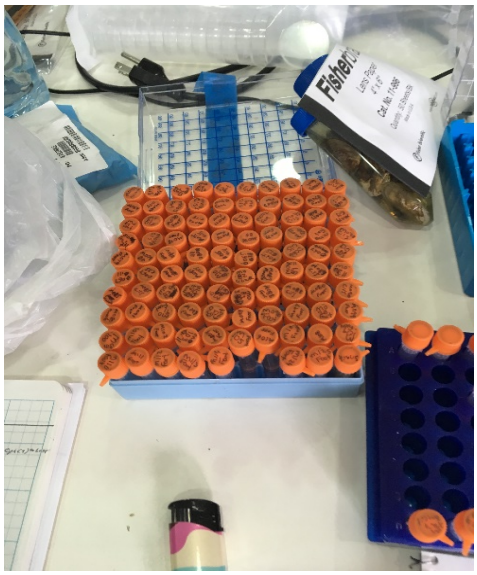

Figure 4: Samples prepared and ready to be sent back to the RDLES laboratory for molecular analysis (Montenegro 2017).

Results

Several hundred mussels were dissected in Montenegro by RDLES staff. Not all of them contained parasites. Over eighty samples were collected by RDLES staff and sent back to Denver, CO for analysis. Dr. Molloy sent additional mussel tissue samples for analysis from his other trips. DNA analysis of the mussel tissue collected by RDLES staff confirmed they were *D. carinata.*

Molecular analysis has been conducted to identify species-specific DNA sequences from isolated ciliates, and their dreissenid hosts. These DNA sequences provide a diagnostic tool which complements morphological analysis. Computational analysis of theses DNA sequences also provides information on the evolutionary relationships between the different ciliates identified. Using this approach, a success rate of over 95 percent from isolated ciliate to DNA sequence has been achieved. To date, DNA extraction, amplification, and sequencing has been performed successfully from 58 ciliates collected in Montenegro in 2017. In the future, additional ciliate samples will be analyzed to better understand the diversity of ciliate parasites present in dreissenids. These techniques will be a key tool for identification in future tests for parasite virulence.

The sampling trip to Montenegro also showed RDLES staff the importance of having local contacts to facilitate the work. This was particularly important and enabled the researchers to accomplish a great deal in brief period.

The initial years of this research provided a strong starting point for this complex research project. In addition to the identification of several mussel populations and parasites, Dr. Molloy was able to establish a network of researchers throughout the Europe and Asia that are willing to

provide technical expertise and resources which will greatly benefit the project. During his last trip in 2018, Dr. Molloy also established a field lab trailer on Krupac Lake, Montenegro, that will now serve as a dedicated facility for the project's team of collaborators and technical staff to conduct extensive mussel dissections and experimentation.

Next Steps

A research proposal was submitted to the Research Office to continue this work into 2021. The proposed research will build upon the research outlined in this report and the major tasks include the following:

-Travel to New York in March 2019 to start experiments that will define the life cycle of the ciliate *Ophryoglena hemophaga*. This parasite is the only known dreissenid host specific parasite present in North America. Understanding its life cycle will be applied to mapping the life cycles of other *Ophryoglena* species in Eurasia.

-Additional trips to Europe and Asia to collect isolated dreissenid mussels and parasites. Obtaining samples from Turkey will be a goal for the next three years, as little is known about the parasites present in the dreissenid mussels in the region. RDLES staff will travel as needed to assist in these collection trips.

-RDLES staff will continue to molecularly analyze the parasites and their host mussels to better understand the diversity of parasites in the Balkans.

-Continue to have regular communication with Molloy & Associates about the progress of the research.

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Appendix A – Final Report from Molloy & Associates

Below is the Final Report for this project prepared by Molloy & Associates. This report covers the work and research done from April 2017 to September 2018.

Dreissena carinata – A common species in the Balkans whose parasites are under study

FINAL REPORT

TITLE

Use of Novel Hypervirulent Parasites to Control Naïve North American Dreissenid Populations

PERIOD OF PERFORMANCE

April 2017 – September 2018

INTRODUCTION

The Problem

Dreissena polymorpha (zebra mussels) and *Dreissena rostriformis bugensis* (quagga mussels) pose a significant challenge to Reclamation facilities. One key element contributing to this challenge is the lack of a practical method for large-scale, open-water control of dreissenid populations once they become established throughout a water body. As a result, Reclamation facilities located on such infested water bodies are subjected to constant reinfestation.

Although concerns exist about the potential environmental impact of virtually all currently available molluscicides, it is clearly the prohibitive total cost of open-water dreissenid control programs that currently eliminates them as a mussel mitigation option, and this is unlikely to change anytime soon. To date, there have been only two well documented, successful, open-water dreissenid eradication treatment programs. One in the United States (Fernald and Watson 2013) and one in Canada (Manitoba Department of Conservation 2014). Both used potassium chloride (potash) as the control agent. In each program, total budget costs were remarkably similar, i.e., approximately $1,400 (USD) per acre-foot of water treated (D. P. Molloy, personal communication). Total budget cost in both these programs included the potash application as well as the myriad of other costs related to the control programs, such as fund raising, planning, organization, regulatory permitting, and posttreatment mussel mortality monitoring. The high cost of these control programs was not due to the cost of potash, as it represented only a fraction of total program cost. Based on this latter cost rate, treating Lake Mead (whose volume is >25,000,000 acre-feet) would have a total project control program cost of ~36 billion dollars.

Obviously, total project control costs would have to be drastically reduced from ~$1,400 per acre-foot before any control method would ever see widespread use in Western water bodies. Even if the total program cost for applying a control agent throughout an entire water body were reduced more than a thousand-fold to ~$1 per acre-foot (i.e., a cost reduction many would consider highly improbable to achieve), the estimated total Lake Mead treatment program cost would still be prohibitively high at ~25 million dollars. Furthermore, why would anyone ever consider recommending such a one-time costly multimillion-dollar Lake Mead treatment program when not only would the odds of truly eradicating every quagga mussels from that water body be highly improbable, but the reinfestation of that water body would soon occur via the incoming waters from untreated upper reaches of the Colorado River. Yes, control/eradication programs treating isolated, small water bodies (e.g., approximately <100 surface acres or <1,000 acre-feet) might still currently be considered affordable in some situations, but the prohibitively high cost of control programs designed to treat the entire volume of large water bodies will essentially rule them out for the foreseeable future.

A Potential Solution

The Reclamation research project reported herein, however, offers a potential solution to this seemingly intractable problem of prohibitively high control program costs. The key to the low cost of this proposed control approach is that it does not require treatment of the entire water body. In contrast to traditional control programs: 1) only a minuscule portion of the water body's volume will be treated ("seeded") with the control agent; 2) the control agent will subsequently amplify itself and begin to self-

spread throughout the water body. There is only one type of control agent capable of doing that – a live one, a biological control agent.

This report describes the research progress made in the first year of a project to find such a live control agent. The project is specifically designed to find – to "hunt down" – a hypervirulent (i.e., extremely lethal), highly-specific dreissenid parasite that one day (following years of comprehensive environmental safety studies) would be introduced into Western water bodies where it will leave a path of dead dreissenids in the path of its spread. Moreover, the self-spreading of the live control agent will occur not only within the treated water body, but also from treated to untreated water bodies.

The Science Behind the Proposed Solution

Infectious disease caused by hypervirulent parasites can have long-term, devastating impacts on animal and plant populations. This is especially true when "naïve" populations are exposed to "novel" parasites that they have been geographically separated from for millennia. Naïve host populations can be ravaged by the virulence – the extreme lethality – of such novel parasites since the naïve hosts have not co-evolved with them and thus have limited, if any, resistance to infection. Well known examples of this devastating naïve/novel species phenomenon are the loss of naïve chestnut and naïve elm populations in North America following the introduction of novel fungal parasites that normally parasitize their cousin chestnut and elm species in Asia. Bivalves can also be naïve to novel parasites. Eastern oyster (*Crassostrea virginica*) populations on the Atlantic coast, for example, were decimated when a parasite from a cousin, the Pacific coast oyster (*Crassostrea gigas*), was inadvertently introduced into east coast waters (Burreson et al. 2000).

North American zebra and quagga mussel populations have geographically-isolated, cousin *Dreissena* species in Eurasia (e.g., *D. caputlacus*, *D. anatolica*, *D. blanci,* and *D. carinata*), and these cousins are infected with parasites (Molloy et al. 1997, Molloy et al. 2010). The geographical isolation of these cousin *Dreissena* species suggests that zebra and quagga mussels likely have not been exposed to their cousins' parasites and thus may find them to be hypervirulent/deadly. The research project reported herein was thus launched in 2017 to begin a comprehensive examination of the parasites of these Eurasian cousin *Dreissena* species, starting with cataloging/identifying what parasites are present and ultimately evaluating if any might prove to be hypervirulent to zebra and quagga mussels and thus possible candidates as future biocontrol agents in North America.

CORE PROJECT TEAM MEMBERS

- Prior Project Manager: Denise Hosler, Reclamation (April 2017 – March 2018)
- Current Project Manager: Jacque Keele, Reclamation (April 2018 – September 2018)
- Contractor: Daniel Molloy, Molloy & Associates, LLC
- Team Scientist: Yale Passamaneck, Reclamation
- Team Scientist: Sherri Pucherelli, Reclamation

COLLABORATING SCIENTISTS

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	Dr. Ryan Carnegie Expertise: Bivalve parasites Institution: Virginia Institute of Marine Science, Gloucester Point, Virginia, USA Link: http://www.vims.edu/people/carnegie_rb/

THREE EURASIAN RESEARCH TRIPS DURING THE PROJECT PERIOD

April 1-20, 2017
Sampling in Albania, Macedonia & Montenegro

- During this 3-week trip, Dan Molloy did lab and fieldwork with collaborating scientists Pesic, Jovicevic, Shumka and Trajanovski.
- Jacque Keele and Yale Passamaneck visited April 11-19 to do lab and fieldwork with Dan in Montenegro; their focus was collecting parasites for subsequent molecular identification at their Reclamation RDLES lab in Denver. (PHOTO TO RIGHT: THEY REMOVE *D. CARINATA* FROM A FISH NET AT LAKE MALO BLATO.)

October 1 – November 12, 2017

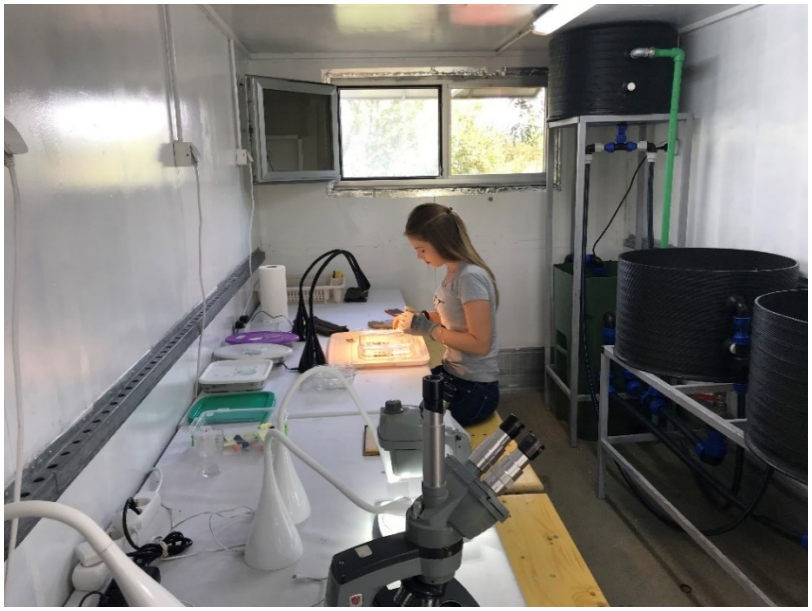
Conference & Sampling in Greece, Montenegro, and Macedonia

- During this six-week trip, Dan Molloy gave a keynote talk describing this project at the International Symposium of Ecologists in Sutomore, Montenegro.
- Dan also did lab and fieldwork with collaborating scientists Jovicevic, Shumka, and Trajanovski. (PHOTO TO RIGHT: DAN EXAMINING MUSSELS ON A ROCK IN MONTENEGRO.).

May 28 – July 13, 2018

Sampling in Montenegro, Albania, and Macedonia

- During this seven-week trip, Dan Molloy did lab and fieldwork with collaborating scientists Fokin, Jovicevic, Shumka, and Trajanovski.
- Dan also used Molloy & Associates' newly established field lab trailer located on Krupac Lake, Montenegro, for extensive mussel dissections (PHOTO TO RIGHT: MONTENEGRIN UNIVERSITY STUDENT NIKOLINA DRASKOVIC PREPARES MUSSELS FOR DISSECTION IN THE KRUPAC LAKE FIELD LAB TRAILER.)



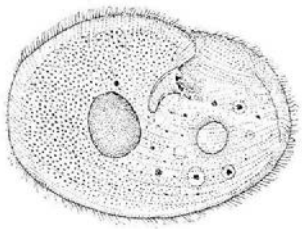
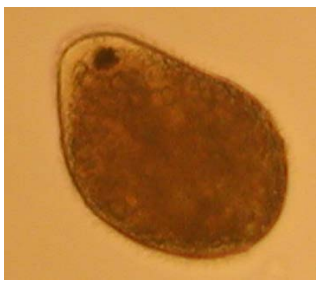
SUMMARY OF PROJECT RESEARCH GOALS AND PROGRESS

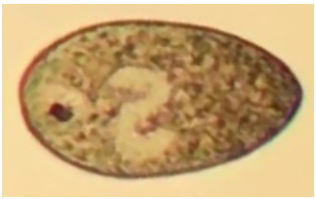
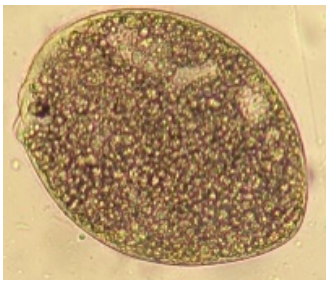
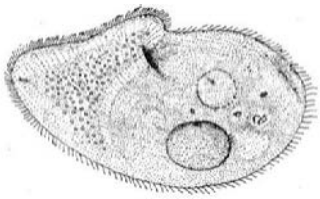
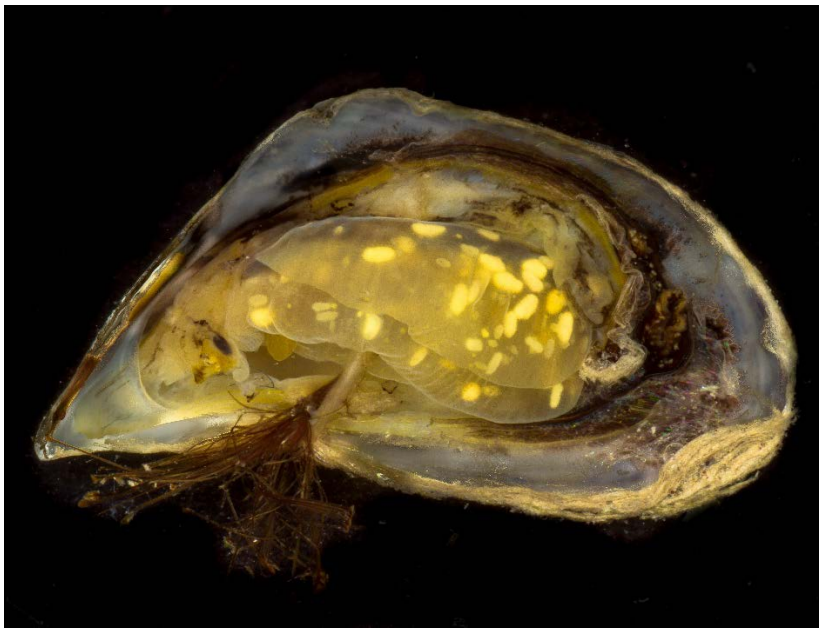
#	GOAL	WHY HAVE THIS GOAL?	PROGRESS
1	Attract collaborating scientists with diverse expertise and train local supporting technical staff to accelerate research progress.	• It is essential to have at least one foreign scientist from each Eurasian country in which sampling is occurring to facilitate project logistics in their home country. • Success of the project requires: o the participation of team members with diverse scientific expertise. o the successful training/hiring of locals as technical staff.	Solid progress achieved on this goal: • Collaborations established with 9 scientists from 8 countries (Italy, Macedonia, Albania, Russia, Montenegro, United States, Turkey, Finland). • Seven local technical staff members hired/trained to provide field/lab support to the project in 5 countries (Macedonia (2 techs), Albania (1 tech), Montenegro (2 techs), Italy (1 tech), United States (1 tech).
2	Focus on finding parasites in the "cousin" mussel *Dreissena carinata* in Albania, Macedonia, and Montenegro using dissection and histological tissue sectioning as detection methods.	Among the four "cousin" *Dreissena* species in Eurasia, it was decided to focus almost entirely on the parasites of *D. carinata* in three Balkan countries as it would likely maximize project productivity due to: • the extensive network of collaborating scientists and technical support in these three Balkan countries. • the access to a lake-side field lab trailer owned by Molloy & Associates at Krupac Lake, Montenegro – a water body with a variety of *D. carinata* parasites.	Solid progress achieved on this goal: • Decision to focus on sampling *D. carinata* in Balkans proved prudent as multiple samples were collected from Macedonia, Albania and especially Montenegro. • Thousands of *D. carinata* were examined and a wide variety of parasites observed by dissection (see next section). • Hundreds of *D. carinata* were processed for detection of microparasites using histological examination. • Selected *D. carinata* tissues that exhibited signs of disease have been sent off for histological examination for possible parasite presence.
3	Identify the parasites observed in *D. carinata* by using molecular and morphological criteria.	It is critically important to be able to recognize (to confidently identify) each parasite that is found in *D. carinata because:* • this will eventually help define the host specificity of each parasite. • parasite species recognition is critically important as this project will consider using parasite species as future biocontrol agents only if they are host-specific to dreissenid mussels (i.e., they are never found infecting other organisms).	Solid progress achieved on this goal: • Many of the parasites encountered, especially ciliates in the genus *Ophryoglena,* have been determined by molecular and/or morphological analysis to be new species. • The morphological and molecular information on these new species is now being organized for inclusion in manuscripts that will formally name these new species.
4	Explore the federal regulatory steps for the importation of a biological control agent.	This project is being very proactive in learning what steps will be required to import a parasite from Eurasia as a candidate biocontrol agent.	Solid progress achieved on this goal: • USFWS staff have informed us that that they (not USDA-APHIS) are the lead federal agency for approval of importation of parasites as biocontrol agents. • They have also outlined the process of applying for approval of the necessary importation permits.

5	Communicate project activities and progress not only among team members, but also to the public and to professional scientific groups.	Technology transfer is important.	Solid progress achieved on this goal with four talks describing this project presented: • Molloy, D. P. 2017. Biological Control of Zebra and Quagga Mussels. Invited Seminar Speaker at Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana-Champaign, Illinois. • Molloy, D. P. 2017. An International Collaborative Project Documenting the Parasites of *Dreissena* spp. Mussels throughout Eurasia. Keynote Speaker at International Symposium of Ecologists. Sutomore, Montenegro. • Molloy, D. P. 2018. Progress in Reclamation's Biocontrol Research Project: An Update Briefing to RDLES Team Members and Other Reclamation Staff. Denver, Colorado. • Molloy. D. P. 2018. Needle in a Haystack Research Projects for Environmental Protection: Two Down and One To Go. Keynote Speaker at University of Minnesota Natural Resources Conference, Brainerd, Minnesota.

SOME OF THE PARASITES THUS FAR DISCOVERED IN *DREISSENA CARINATA*:

MIGHT ONE OF THEM SOMEDAY PROVE TO BE HYPERVIRULENT TO ZEBRA AND/OR QUAGGA MUSSELS?

Conchophthirus klimentinus – a ciliate that parasitizes the gills of *D. carinata*.	
A new species of *Ophryoglena* ciliate that parasitizes the digestive gland ducts of *D. carinata*.	

A new species of *Ophryoglena* ciliate that parasitizes the digestive gland tubules of *D. carinata*.	
A new species of *Ophryoglena* ciliate that parasites the gills of *D. carinata*.	
A new species of *Conchophthirus* ciliate that parasitizes the gills of *D. carinata*.	
Phyllodistomum trematode worms (the tiny bright yellow ovals) that parasitize the gills of *D. carinata*.	

FUTURE RESEARCH DIRECTIONS

The following are priority research activities in Eurasia:

- Sample during all four seasons; some parasites have a seasonal preference for their development in host mussels.
- Sample more mussel locations; the more geographically isolated and ancient these locations are, the higher the probability of finding parasites that our North American zebra and quagga mussels will be naïve to since they have never encountered them before.
- Sample more dreissenid cousin species; to date, the project has focused exclusively on the parasites in *D. carinata* in samples from Albania, Macedonia, and Montenegro, but the project should also include sampling of the cousin species *D. blanci* (in Greece), *D. anatolica* (in Turkey), *D. caputlacus* (in Turkey), and also ancient Eurasian isolated populations of *D. polymorpha* and *D. rostriformis*.
- Sample more mussel life stages; adults are the stage traditionally examined in parasite studies and that is the path the project has followed thus far; sampling should include examination of younger mussel stages, including juveniles and veligers; a search of the scientific literature indicates that dreissenid veligers have apparently never ever been investigated for parasites; they would make a highly desirable mussel life stage to kill with a biocontrol agent, as that would prevent fouling from ever getting started.
- Continue to foster the expansion of the project's network of collaborating Eurasian scientists and local lab/field technical support staff – both of which have proven key to project productivity thus far.
- Continue to rely on the RDLES lab to provide molecular biology capabilities for the genetic verification of the species both of the parasite as well as the host dreissenid mussel.
- Initiate experiments to define the life cycle of selected Eurasian dreissenid parasites; in particular conduct experiments to demonstrate what the infective stage of a particular parasite species is; that knowledge is essential to designing cross-infection trails to assess whether a *D. carinata* parasite can infect zebra and quagga mussels (e.g., wouldn't it be a tragedy to claim that zebra and quagga mussels are non-susceptible to infection by a *D. carinata* parasite, when in fact the infection trial used a stage of the *D. carinata* parasite which was not the infective stage).

The following are priority research activities in North America:

- Initiate experiments to define the life cycle of the only dreissenid-host-specific parasite known to have made the trans-Atlantic crossing from Europe: the ciliate *Ophryoglena hemophaga*; knowing its life cycle will likely be invaluable in deciphering the life cycles of other *Ophryoglena* spp. that this project is working with in Eurasia.
- Dissect dreissenids (both zebra and quagga mussels) in North America to see what parasites are present.

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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: Z:\DO\TSC\Jobs\DO_NonFeature\Science and Technology\2017-PRG-Using Hypervirulent Parasites to Control North American Dreissenid Populations
- Point of Contact name, email, and phone: Jacque Keele, jkeele@usbr.gov, 303-4452187
- Short description of the data: (types of information, principal locations collected, general time period of collection, predominant files types, and/or unusual file types.) Images of the collection trip in the Balkans, spreadsheet of samples, and background literature related to mussel parasites.
- Keywords: Quagga and Zebra mussels, invasive species, hypervirulent parasite
- Approximate total size of all files: 126 MB (folder size)