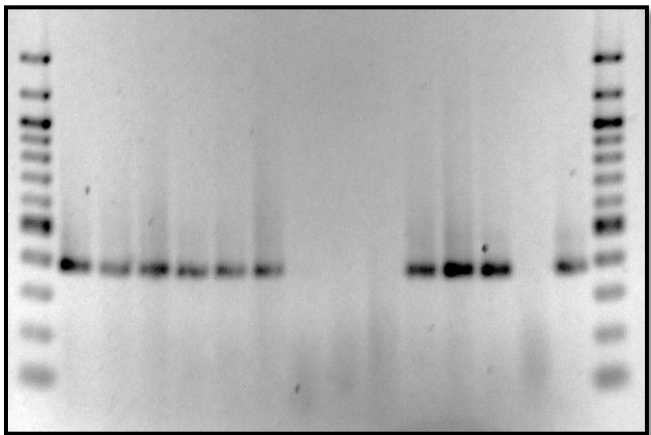


RECLAMATION

Managing Water in the West

Using Microsatellite Analysis to Track Genetic Changes in Quagga Mussel Populations in the Western United States

Final Report ST-2015-5173-01



U.S. Department of the Interior
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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 mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Executive Summary

Invasive dreissenid mussels threaten the health and function of Reclamation waters and facilities, causing physical obstruction of flow in water conveyance systems. Dreissenid mussels are known to inhabit a range of suitable habitat, which helps to predict their spread. It is possible that this habitat suitability will shift overtime as the mussels adapt to new conditions. Understanding the genetic variability within quagga mussel populations throughout the Western United States can help Reclamation predict mussel distribution patterns and provide insight into genetic weaknesses or genes that can be targeted for control purposes. Microsatellite analysis is a method of analyzing genetic variability that is particularly useful to ecologists attempting to learn more about invasive species population history and structure. Microsatellite analysis can reveal the source population and help determine if there were multiple introductions, while also tracking population flow, migration, and relatedness of individuals. This information would be particularly helpful to better understand the spread of invasive species like dreissenid mussels, potentially uncovering inoculation sources and population change as a result of habitat variability. The goal of this paper is to investigate the current literature to gain a better understanding of how microsatellite analysis has been used for dreissenid mussel population analysis and how it can be used to answer questions about Western quagga mussel populations.

Introduction

The quality of Reclamation waters will continue to shift in the presence of drought and climate change. When faced with these alterations, aquatic organisms such as invasive mussels must either have a wide range of tolerances or be able to adapt to maintain or expand populations. Several studies have investigated the limits of water quality parameters associated with the quagga mussels' (*Dreissena bugensis*) ability to sustain and reproduce. However, the ability of quagga mussel population genetics to change may alter their suitable habitat parameters, allowing for continued range expansion or modification. Understanding the genetic variability within quagga mussel populations throughout the Western United States can provide Reclamation with insights into genetic weaknesses or genes that can be targeted for control purposes. This valuable information may also help predict future mussel distribution patterns and impacts to Reclamation waters. Moreover, without a clear understanding of a population's genetic diversity, control measures may fail to target the entire population.

Preliminary analyses of quagga mussels in the West, conducted by the University of Arizona have found a significant degree of genetic diversity, even within the same system. Analyzing microsatellites (repeated DNA segments) can provide information about genetic diversity within a species, including gene flow, genetic variants, and population structure. More information regarding the genetic diversity and distribution of invasive mussels will provide better estimates of habitat suitability, which will be highly useful to Reclamation managers in preparing for—and preventing or minimizing—the impacts of infestation. Enhanced confidence regarding suitability levels would allow prioritization of budget allocation and decision processes for water bodies that are at high risk for problematic infestations.

The long-term goal of this research project is to develop a greater understanding of quagga mussel genetic variability within the Lower Colorado River system. Each reservoir along the Lower Colorado River has unique water quality characteristics and established mussel populations. Microsatellite analysis may be able to uncover variability within the system and within a single reservoir. This literature review will help to clarify the goal and direction of this research.

Literature Review

Invasive dreissenid mussels threaten the health and function of Reclamation waters and facilities. Invasive mussel populations cause physical obstruction of flow in water conveyance systems, ranging from roughening to complete blockage. Intake structures, such as pipes and screens can become clogged, reducing delivery capacities, pumping capabilities, and hydropower generation functions. Invasive mussels affect all submerged components, conduits and other structures such as trash racks, fish screens, raw water distribution systems for turbine cooling, fire suppression systems, water intakes (service, domestic, and irrigation), irrigation canals, gauging stations, weirs, gates, diffuser gratings, drains, and virtually all types of instrumentation in contact with raw water. Chemical degradation (corrosion) of infrastructure is also resulting from mussel fouling of metallic structures and equipment.

Based on population explosions at Hoover, Davis and Parker Dams, it is likely that mussels will continue to spread and colonize additional Western waters. This range expansion could seriously impact additional Reclamation operations, potentially resulting in the interruption of hydropower and water delivery at significant economic costs. Virtually none of Reclamation's structures were built with design considerations necessary to contend with an aquatic organism having the bio-fouling potential of these mussels. In infested systems, it is often necessary to continuously replace or clean plugged equipment to avoid lengthy interruptions in operations. Budget planning for increased maintenance or retrofitting systems to deal with invasive mussels can be problematic for Reclamation managers, as costs can be considerable.

Development of control measures for hydropower facilities has proven to be difficult and it is possible that control may be influenced by the amount of genetic homogeneity in the population (Marsden et al. 1996). A greater understanding of quagga mussel genetic variation may provide insight into genetic vulnerability or genes that can be targeted for control purposes. When considering reproductive strategy, dreissenid mussels are characterized as r-strategists. They reproduce rapidly and have a high fecundity. Production of large amounts of offspring allows at least some proportion of the population to survive regardless of predation or mortality. This strategy allows for genetic flexibility and rapid adaptation (Mills et al. 1996; Ram et al. 2012).

Quagga mussels are the dominant dreissenid species in the Western United States, and the majority of genetic research on North American quagga mussels has been limited to the Great Lakes region (Baldwin et al. 1996; Claxton et al. 1998; Wilson et al. 1999b; and Grigorovich et al. 2008). However, two publications examining quagga mussel genetic diversity in the Lower Colorado River have been produced (Brown and Stepien 2010; Jennett 2013).

Genetic Analysis of a Population

Genetic analysis of a population can provide information on the genetic variation within and among geographically distinct populations of a species. This information is particularly important when dealing with invasive populations, because eradication and control efforts will often fail if a population is treated as a homogenous introduction (Lee 2002). Studies have shown that understanding the genetic diversity within a population of an invasive species helps define “eradication units” (Collins et al. 2002) and will help to determine if efforts to exterminate a local population will be successful.

Landscape genetics is a rapidly growing field describing the spatial distribution of genetic variation by integrating data and analysis methods from landscape ecology, spatial statistics, geography, and population genetics (Storfer et al. 2010). Genetic analysis can also uncover information about how a species expands its range and develops new variation. Analysis can uncover founder effects, which are random genetic changes seen in newly formed populations because the population founders only carried a fraction of the total variance seen in the whole population. Variation in a population can also result from micro-geographical level effects that are caused by adaptation to localized habitat variability.

Genetic analysis of invasive species provides insight into the genetic variance that allows for quick adaptations in response to environmental change, helping to predict range expansion (Lee 2002). It also can identify cases of hybridization, where two populations mate (Roman and Darling 2007), increasing genetic variance and masking of unfavorable alleles (Lee 2002).

Mills et al. (1996), Claxton et al. (1997), and Ram et al. (2012) have indicated that dreissenid mussels have a high level of genetic variance and phenotypic plasticity. These observations are supported by the fact that dreissenid mussels have a wide range of environmental tolerances (Higgins and Vander Zanden 2010). This genetic variance is likely a result of multiple dreissenid introductions to the United States from the source population in Europe (Therriault et al 2005), and/or the large number of larvae (veligers) transported (Brown and Stepien 2010).

Molecular Methods

There are several molecular methods available to analyze alleles. Allozyme variation is a protein based method that identifies protein changes resulting from DNA nucleotide base changes. This method has more recently been replaced with polymerase chain reaction (PCR) based methods. Restriction fragment length polymorphism (RFLP) is used to identify a species and assess genetic diversity by cutting DNA with restriction enzymes and comparing the resulting fragments. Genetic differences can be identified at the population level using single nucleotide polymorphism (SNP). A SNP is where a single base variation is

identified at a specific position in the DNA. The proportion of each base at the specific location in a population can be used to identify genetic differences between populations.

Microsatellites are genetic markers that are particularly useful to ecologists attempting to learn more about a population's history and structure. Microsatellite analysis can reveal the source population and help determine if there were multiple introductions (Wilson et al. 1999b, Collins et al. 2002). The analysis also helps track population flow, migration, and relatedness of individuals (Selkoe and Toonen 2006). This information would be particularly helpful to better understand the spread of invasive species like quagga and zebra mussels, potentially uncovering inoculation sources and population change as a result of habitat variability. Other applications for microsatellite analysis are gene tagging and quantitative trait locus (QTL) analysis, hybridization and breeding, functional genomics, forensic science, genome mapping, diversity and cultivar analysis, diagnostics and human diseases, taxonomic and phylogenetic studies, and pedigree and gender identification (Selkoe and Toonen 2006).

Microsatellite analysis is also referred to as DNA fingerprinting, and microsatellites are also called simple sequence repeats (SSRs), variable tandem repeats (VNTR), or short tandem repeats (STR). Microsatellite markers, or loci, are two to five base pair repeats on a highly variable region of the DNA segment. The number of microsatellite repeats is variable (5-40); therefore the number of repeats at each locus can be used to distinguish genetically diverse individuals (Selkoe and Toonen 2006). These segments have a high mutation rate and high diversity within a population, making them useful for revealing small shifts in genetic diversity (Eisen 1999). The length of each locus can be easily distinguished by high resolution gel electrophoresis, which allows many individuals and many loci to be rapidly genotyped for a significantly lower price than DNA sequencing (Selkoe and Toonen 2006). Microsatellites are inherited from both the maternal and the paternal lineages, which allows for analysis of hybridization and paternity.

Statistical Analysis of Genetic Data

Analysis of microsatellite data is often completed using bioinformatics programs such as: BOTTLENECK, STRUCTURE, GENECLASS2, etc. The following tests have been used for the analysis of genetic diversity (Jennett 2013).

1. Hardy Weinberg expectations - a mathematical model that predicts that gene (and allele) frequencies will remain the same over many generations, but only under ideal conditions with the absence of any evolutionary influences.

2. Analysis of molecular variance (AMOVA) - a statistical method of analysis that allows the researcher to detect measurable genetic differences by comparing how mean molecular variance.
3. Nei's genetic distance- an estimator of the separation time among populations based upon measurable genetic differences between and within populations. Nei's specific mathematical formula assumes that variation comes from genetic drift and mutation.
4. Fst- the F statistic is a measure of inbreeding based on calculations using different heterozygosity's. The range of F is 0-1. Fst compares a sub-population to the whole population.
5. Analysis of Wahlund effect- refers to reduction of heterozygosity (when an organism has two different alleles at a locus) as populations diverge, resulting from geographic barriers
6. Linkage disequilibrium- is the presence of statistical associations between alleles at different loci that are different from what would be expected if alleles were independently, randomly sampled.
7. Bonferroni correction- is a method used to counteract the problem of multiple comparisons and control the family-wise error rate.
8. Mantel test-statistical test to evaluate the correlation between genetic and geographic distance.
9. Fishers exact test for genotypic differentiation- is a statistical significance test used in the analysis of contingency tables.
10. Other commonly used tests- Bayesian model-based methods, pairwise tests, and M or migration value.

Previous Studies using Microsatellite Markers for Quagga Mussel Genetic Diversity

Wilson et al. (1999a) developed six microsatellite primer sets (Dbug1-Dbug6) specific to quagga mussels. The primers failed to amplify zebra mussel samples. The goal of developing these primers was to clarify patterns of colonization and diffusion that characterized the rapid spread of the mussels in North America. Wilson et al. found high levels of intraspecific variability (heterozygosity ranging between 0.389-0.811). The high variability and species specificity indicated that these microsatellites would be useful markers to identify gene flow between introduced populations of quagga mussels.

The same authors used these original six microsatellite markers to analyze quagga mussel populations in the Great Lakes region (Wilson et al. 1999b). They were attempting to determine how quagga mussels disperse in North America, and clarify how humans contribute to this spread. The results of this research produced F_{st} values that differed between populations. The values did not clearly correlate with geographical relationships. F_{st} values are a measure of inbreeding based on calculations using different heterozygosity's. These results suggested that there was high gene flow between geographically distant populations. These findings suggested that quagga mussels were primarily being transported to new locations via human mediated activities, such as boating. Isolation-by-Distance modeling and Analysis of Molecular Variance (AMOVA) calculations indicated that quagga mussel populations in North America were experiencing a complex pattern of inter-population gene flow as a result of rare long-distance jump dispersal events.

Therriault et al. (2005) also used the Dbug1-Dbug6 microsatellite primer sets designed by Wilson et al. (1999a) to measure dispersal and population relatedness in the mussel's native range in the Black Sea basin in Ukraine and an invaded range in the Volga River system in Russia. The objective of this study was to identify the level of genetic diversity between endemic and invading populations in the Proto-Caspian region. Samples were collected from source and invaded populations, making it possible to identify founder effects, repeated introductions, or genetic bottlenecks. Microsatellite diversity at the six loci was found to be similar between source and invaded populations. These results suggest that the inoculation size was large or there were multiple introductions that enhanced the genetic diversity. The similar genetic diversity indicates that the invasive populations did not experience bottleneck or founder effects. The diversity observed likely facilitated successful establishment of quagga mussel populations in the Volga River system, indicating that high genetic diversity within a source population may be important for invasion success.

Brown and Stepien (2010) were the first to analyze Western quagga mussel populations with microsatellite markers. They analyzed and compared 269 quagga mussels from 12 Eurasian and North American sites, including Lake Mead NV, Lake Matthews CA, 8 Great Lakes sites, 2 Black Sea sites, and 1 Caspian Sea site. They recovered 228 quagga mussel alleles using 9 loci (Dbug1, Dbug4, Dbug5, Dbu74, Dbu75, Dbu92, Dby93, Dbu110, and Dbu141). This study also included zebra mussel population analysis and it was found that neither species experienced a genetic bottleneck when it was introduced into the Great Lakes. Therefore, it is likely that a large number of each species was introduced at once, which likely contributed to their successful establishment. Results indicate that

North American zebra mussels originate from multiple non-native European populations and North American quagga mussels originate from native estuaries in Southern Bug and Dnieper Rivers. Quagga mussel populations in the Colorado River and in California showed founder effects. Structural analysis of each population found that Lake Mead and Lake Matthews populations are genetically similar to Lake Ontario populations. Therefore Lake Ontario is likely the source population for Western quagga mussels.

Brown and Stepien (2010) used six new microsatellite markers (Dbu74, Dbu75, Dbu92, Dby93, Dbu110, and Dbu141) in their study that were developed by Feldheim et al. (2011). Feldheim et al. (2011) also developed 8 markers for zebra mussels. Using these new markers, they conducted an indepth analysis of two zebra mussel populations (Lake Erie, OH and San Justo, CA) and two quagga mussel populations (Lake Erie, OH and Lake Mead, NV) and found that the markers were effective. Additional dreissenid microsatellite research was conducted by Peñarrubia et al. (2015). This research describes the methods used to identify, test, and validate new polymorphic microsatellite loci using a massive parallel sequencing platform.

Jennett (2013) conducted a quagga mussel microsatellite study primarily focused on the Lower Colorado River System. The goals of this study were to determine population structure of quagga mussels in the Colorado River, determine if there was a single or multiple invasion events, and compare the genetic diversity of each population. Jennett collected 134 mussels from Lake Mead, Lake Mohave, Lake Havasu, Lake Pleasant, Otay Reservoir, Yuma area, Salt Gila Pumping Station, and Sandario Pumping Station. She used eight microsatellite markers (Dbug1, Dbug2, Dbug3, Dbug4, Dbu74, Dbu75, Dbu92, and Dbu93) for her analysis. Originally, descriptive statistics indicated that the quagga mussel population in the main corridor of the Colorado River was genetically homogenous, but closer look at the population structure shows three mixed-lineages with high gene flow among locations.

Mussels from Lake Mohave, Otay Reservoir and Sandario Pumping Station all departed from the Hardy-Weinberg Equilibrium suggesting that gene frequencies are not maintained over several generations. This finding was most surprising at Lake Mohave since it is in the main river channel and indicates that there may be more inbreeding occurring at this site. Of all the sites tested, Otay Reservoir and Sandario Pumping Station appear to be distinct unique populations. Greater genetic diversity was indicated because of the high number of alleles found per locus and the presence of private alleles. Jennett's research also exposed the occurrence of multiple invasions as Otay Reservoir was found to contain two

distinct populations. Secondary dispersal was also detected in Yuma, where a mussel with unique lineage from Otay was found.

Status of Reclamation's Research and Future Directions

In 2015, adult mussels from six Reclamation water bodies with established mussel populations were collected, preserved, and sent to the Reclamation Detection Laboratory for Invasive Species (RDLES). Mussel samples were collected from: Lake Mead, Lake Mojave, Lake Havasu, Imperial Dam, Senators Wash, and Lake Powell. Phenotypic descriptions, photographs, and measurements were taken of each adult mussel. DNA was extracted from thirty adults from each location. The DNA was isolated from each mussel using the Food and Drug Administration's (FDA) standard operating procedure (SOP) for generating DNA barcodes suitable for species identification. Tissue was taken from each mussel and placed into a 1.5-mL sterile micro-centrifuge tube. A Qiagen DNeasy extraction kit was used to isolate the DNA. Following the DNA extraction, each sample was analyzed by polymerase chain reaction (PCR) for the quagga mussel cytochrome oxidase 1 (COI) gene to ensure that the DNA extraction had worked. PCR was also completed to confirm that the sample was from a quagga mussel. All mussels used for this analysis produce a positive COI band and were confirmed to be quagga mussels.

The extracted DNA was sent to the US Army Corp of Engineers (USACE) Engineer Research and Development Center (ERDC) genetics laboratory for microsatellite analysis. The Army Corps of Engineers is interested in this project, and in 2015 contributed \$40,000 of in-kind services to analyze these samples. In 2016 we plan to continue this partnership, completing the analysis and comparing the results to those published for other mussel populations in the United States.

If funds are awarded for 2016, additional analysis will be conducted on the cytochrome oxidase 1 gene and at least eight microsatellite markers to determine the historical lineage of the mussels collected in 2015. The literature does not fully address temporal genetic change in quagga mussel populations. Brown and Stepien (2010) analyzed quagga mussel populations in Lake Erie during two sampling years and found significantly different genetic structure with a large gain in alleles. Comparing the results of our data to those obtained by Jennett (2013) would help determine if temporal variation is occurring in the lower Colorado River. It may be interesting to monitor Western quagga mussel

populations over the course of several years to track shifts in population structure and new introductions and adaptations. The inclusion of Lake Powell in this study will be of particular interest because it is a relatively new invasion. We may be able to determine the source of the inoculation and if there were multiple inoculation events.

As these data are collected, a database will be developed to record the phenotypic and genetic information gathered from the different populations. Existing water quality data for each location will be included in the database to determine if genetic variability can be tied to water quality attributes. If warranted, additional sites will be included in the analysis for comparison. This database will enable researchers to examine existing diversity and potential for adaptations in quagga mussel populations over time.

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Point of Contact name, email and phone:

Sherri Pucherelli: spucherelli@usbr.gov (303-445-2015)

Jacque Keele: jkeele@usbr.gov (303-445-2187)

Denise Hosler: dhosler@usbr.gov (303-445-2195)