RECLAMATION

*Managing Water in the West*

# Patterns of genetic structure among southwestern populations of the invasive quagga mussel (*Dreissena bugensis*) in the United States

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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

Large populations of invasive quagga mussels (*Dreissena rostriformis bugensis*, Andrusov, 1897) are present in reservoirs along the lower Colorado River. These reservoirs have unique water quality characteristics which raised questions about the extent of gene flow and genetic divergence among those populations. In this study, we examined the neutral genetic structure among six populations from different reservoirs along the Colorado River in the southwestern United States. Individual quagga mussels were genotyped at 10 microsatellite DNA loci to analyze patterns of genetic diversity and population structure. Overall genetic divergence among the populations was negligible and populations at a single reservoir were not significantly genetically differentiated from the group. Some population pairings did exhibit significant, if slight, genetic differentiation, and there was a moderate pattern of isolation-by-distance. Observed morphological differences at some reservoirs are likely an environmental effect separate from heritable genetics. If significant environmental selective pressures are present they do not appear to have been strong enough to result in observable genetic bottlenecks over the relatively short time scale of the quagga mussel invasion of Colorado River

# Main Report

A manuscript containing pertinent data and results pertaining to patterns of genetic structure among southwestern populations of the invasive quagga mussel (*Dreissena bugensis*) in the United States has been finalized through Reclamation peer review and submitted to a refereed journal. The principal investigator of this work will update this section to include the submitted manuscript once the journal peer review process has been resolved and information is ready for public dissemination.

# Appendix

A manuscript containing pertinent data and results pertaining to patterns of genetic structure among southwestern populations of the invasive quagga mussel (*Dreissena bugensis*) in the United States has been finalized through Reclamation peer review and submitted to a refereed journal. The principal investigator of this work will update this section to include the submitted manuscript once the journal peer review process has been resolved and information is ready for public dissemination.

Patterns of genetic structure among invasive southwestern United States quagga mussel (*Dreissena rostriformis bugensis*) populations

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ABSTRACT—Over the last decade, large populations of invasive quagga mussels (*Dreissena rostriformis bugensis,* Andrusov, 1897) have become established in reservoirs along the lower Colorado River in the southwestern United States. We were interested in whether population genetic patterns among these populations have changed during continuing population expansion and establishment, and how current genetic patterns in reservoirs along the lower Colorado River would compare to other invasive *D. rostriformis bugensis* populations. We examined the neutral genetic structure among six populations from different reservoirs along the lower Colorado River in the southwestern United States. Individual quagga mussels were genotyped at ten microsatellite DNA loci to analyze patterns of genetic diversity and population structure. As in past studies of some of these same populations, overall genetic divergence among populations was negligible and no single reservoir population was significantly differentiated from the overall group. Some populations did exhibit significant, if slight, pairwise genetic differentiation, and there was a moderate pattern of isolation-by-distance. Studies of microsatellite DNA-based population genetic patterns in invasive populations of *D. rostriformis bugensis* in other parts of the world have been limited to recently established populations and likewise show similar absences of strong genetic structuring.

RESUMEN—Durante la última década se han establecido grandes poblaciones de los mejillones invasores quagga (*Dreissena rostriformis bugensis*, Andrusov, 1897) en embalses situados a lo largo del río Colorado, en el sudoeste de los Estados Unidos. En este estudio, proponemos analizar si los patrones genéticos de estas poblaciones han sufrido modificaciones durante su expansión y proceso de colonización. También analizamos si los patrones genéticos actuales en poblaciones a lo largo del curso bajo del río Colorado son comparables a otras poblaciones invasoras de *D. rostriformis bugensis*. Con tales objetivos, analizamos la estructura genética neutra de seis poblaciones procedentes de diferentes embalses de la parte baja del río Colorado en el sudoeste de los Estados Unidos. Los mejillones fueron genotipados indivualmente en 10 loci de microsatélites de ADN para poder analizar patrones de diversidad genética y la estructura de la población.  Confirmando previos estudios realizados en algunas

de las mismas poblaciones, la divergencia genética entre poblaciones resultó insignificante y ninguna de las poblaciones únicas de los embalses se identificó como significativamente diferente del resto. No obstante, algunas poblaciones presentaban leves diferencias genéticas y se detectó un patrón moderado de *aislamiento-por-distancia*. Otros estudios de patrones genéticos de poblaciones de *D. rostriformis bugensis* basados en microsatélites de ADN en otras zonas se limitan a poblaciones establecidas recientemente y asimismo muestran una ausencia de marcada estructuración genética.

The quagga mussel (*Dreissena rostriformis bugensis* Andrusov, 1897) was introduced to the United States (U.S.) via ballast water in the Great Lakes in 1989 (May and Marsden, 1992), which was the first documented expansion of this invasive Eurasian species. Unlike native North American freshwater bivalves, the dreissenid mussels disperse through their planktonic larval stage (veligers; Ackerman et al., 1994). This life trait has allowed the quagga mussel to become widely distributed throughout the U.S., from the Great Lakes and northeastern states, south along the Mississippi River and its tributaries, and into the western states. In the southwestern U.S., quagga mussels were first identified in Lake Mead in 2007 (McMahon, 2011) and have since become established in five states.

Since the detection of quagga mussels in Lake Mead, populations have been found in additional Colorado River reservoirs as far north as Lake Powell and as far south as Imperial Dam near the border of the U.S. and Mexico (Figure 1). The Colorado River is a critical water resource for the southwestern U.S. states of Colorado, Utah, Arizona, Nevada, and California, and the Mexican states of Baja California and Sonora. The Colorado River is one of the world's most controlled rivers with over 29 major hydroelectric and flood control dams and hundreds of miles of canals. Quagga mussels are aggressive biofoulers that can cause physical obstruction of flow in water conveyance systems. Intake structures such as pipes and screens can become clogged, reducing delivery capacities, pumping capabilities, and hydropower generation functions. Chemical degradation (corrosion) of infrastructure also results from mussel fouling of metallic structures and equipment. The Colorado River drainage is a highly stressed ecosystem with significant human impacts, including water diversion, agricultural runoff, and wastewater runoff. Quagga

mussels further threaten the ecosystem by filtering large amounts of water which can negatively impact zooplankton populations, leading to cascading effects in the local food web. These shifts in ecology can impact fish populations and cause a proliferation of weeds and algal blooms.

The quagga mussel has emerged as a widespread invasive species. According to the current U. S. Geological Survey records (USGS, 2017), quagga mussels in the southwestern U.S. have established in at least 11 locales, including one in the state of Baja California in Mexico. Quagga mussels in reservoirs south of Lake Mead most likely dispersed via water flow, whereas those in reservoirs north of Lake Mead were likely transported over land by infested boats. It is likely that these fairly recent populations are still adapting to the unique habitat characteristics of these systems, and have not yet achieved genetic drift-migration equilibrium. For example, water quality is variable between reservoirs along the lower Colorado River due to geology, agricultural runoff, and fluctuating flow regimes that may impact turbidity. Quagga mussels in the southwestern U.S., where populations have the potential to be small (or to have few effective breeders) and to receive very little (or no) interpopulation gene flow, are more susceptible to genetic drift, which could lead to genetic differentiation and adaptation in response to unique ecological stressors or other evolutionary forces. Information on population genetic structure is important when dealing with an invasive species, as it is useful to identify populations that may be experiencing genetic isolation, which could lead to adaptive processes that may impact the efficacy of control measures (Marsden et al., 1996).

Published population genetic studies conducted for *D. rostriformis bugensis* include the utilization of allozymes (Marsden et al., 1996), RAPDs (Stepien et al., 2002), and microsatellites (Wilson et al., 1999b; Therriault et al., 2005; Brown & Stepien, 2010; Imo et al., 2010). Microsatellites are a powerful genetic marker for population genetic investigations due to their rate of mutation and associated high levels of polymorphism (Slatkin, 1995). Multiple suites of microsatellite markers have been developed for the quagga mussel (Wilson et al., 1999a; Feldheim et al., 2011) and there have been several studies that have analyzed the population genetics of quagga mussels in North America and Europe using microsatellites (Marescaux et al., 2016). For these past studies, the number of loci used and the number of

individuals analyzed varies greatly (3-10 loci across 24-599 individuals). Observed patterns in pairwise $F_{ST}$ values and levels of heterozygosity, when reported, also vary greatly. In general, the previously reported genetic diversity of quagga mussels in the southwestern U.S. is reduced relative to the Great Lakes populations (observed heterozygosity is lower). While most Great Lakes populations, excluding Lake Ontario, are genetically similar to European populations, the southwestern U.S. populations are more genetically similar to Lake Ontario (Stepien et al., 2013), suggesting that the founder population of the quagga mussel to the southwestern U.S. came from Lake Ontario.

Having received anecdotal evidence that morphological differences might be emerging among different populations of quagga mussels in the lower Colorado River, it was determined that an investigation into current population genetic patterns could be of interest. Even though later analyses indicated a lack of significant morphological differences among populations (Pucherelli et al., 2016), periodic characterization of the population genetics in a newly established invasive population should be of interest to population biologists and those invested in invasive quagga mussel control. Therefore, in this study, our objective was to employ a larger suite of microsatellite DNA markers across a larger per population sample size than used in earlier published studies in order to provide a current overview of lower Colorado River quagga mussel population genetics. In doing so, we investigated interpopulation linkages, potential expansion pathways, and the degree to which different populations might be isolated from one another.

MATERIALS AND METHODS—*Sample Collection*—Our collection sites focused on quagga mussel populations that have become established in lower Colorado River reservoirs, which form part of the state borders of Arizona, Utah, Nevada, and California in the U.S. During 2015, we sampled 30 individual quagga mussels from populations in six different reservoirs, spread over approximately 640 river miles (Table 1; Fig. 1). Quagga mussels were collected from plastic plates deployed at each site for recruitment, preserved in alcohol, shipped to the laboratory on ice, and stored at -20°C until they were processed.

*DNA Extraction and Genotyping*—DNA extractions were completed in batches of 12 with samples from only one reservoir processed in each batch to reduce cross-contamination. Mantle tissue from each individual was excised using sterilized scissors and tweezers, after which tissue samples were placed into 1.5 ml Eppendorf tubes. DNA extractions were carried out using the DNeasy Blood and Tissue Kit (Qiagen) and eluted in 50 µl of deionized sterile water. Genomic DNA concentrations (ng/µl) for a subset of samples were determined with the NanoDrop® ND-1000 UV-Vis Spectrophotometer (Nanodrop Technologies). We used a subset of samples to initially assess the 12 microsatellite DNA markers developed by Wilson et al. (1999a; *Dbug* primers) and Feldheim et al. (2011; *Dbu* primers), and selected 10 (due to inconsistent results across replicate runs for the other two markers) for use with the entire data set, including markers *Dbug*1-5 and *Dbu*74, 75, 92, 93, and 110. The polymerase chain reaction (PCR) amplification protocol was adapted from Feldheim et al. (2011) and optimized to include M13 primers modified with 6FAM, HEX, or TET (Eurofins MWG Operon, Huntsville, Alabama), which were used as universal labels in conjunction with the M13-tagged *Dbug* and *Dbu* primers for allele detection during fragment analysis (Schuelke, 2000). PCR amplifications were performed in 10 µl reactions containing 0.5 U of GoTaq DNA polymerase (Promega Corporation, Madison, Wisconsin), 1× GoTaq Flexi Buffer (Promega), 2 mM MgCl2 (Promega), 0.2 µM BSA, 0.15 µM forward primer, 0.3 µM reverse primer, 0.45 µM labeled M13 primer (HEX, 6FAM, or TET), 0.2 mM each dNTP, and ~30 ng genomic DNA. PCR temperature cycling conditions consisted of: (1) an initial denaturation cycle of 94°C for 5 min, (2) 35 cycles of 94°C for 30 sec, annealing temperature for 1 min (50°C for *Dbu*74, *Dbu*75, and *Dbu*110, 52°C for *Dbu*92 and *Dbu*93, 54°C for *Dbug*1 and *Dbug*4, 56°C for *Dbug*2, and 58°C for *Dbug*3 and *Dbug*5), and elongation at 72°C for 30 sec, and (3) a final extension of 72°C for 10 min. Labeled PCR products were multiplexed on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) by loading 1 µl of each PCR product (2-3 loci per run), 0.5 µl of Genescan ROX 500 size standard (ABI), and 9-10 µl of HIDI formamide (ABI). Resulting electropherograms were analyzed using GeneMapper software (ABI) and manually scored. Failed PCRs were repeated at least once, with

ultimately unsuccessful amplifications treated as missing data. All homozygotes and a subset (25%) of heterozygotes were amplified three times to ensure accuracy of genotypes.

*Analysis of Genetic Diversity and Population Structure*—Standard diversity statistics for each locus in each population were calculated using Microsatellite Analyzer (MSA; Dieringer and Schlötterer, 2003), including number of alleles per locus ($A$), observed heterozygosity ($H_O$), and expected heterozyosity ($H_E$) to determine current population genetic statistics for comparison with previous studies. Hardy-Weinberg equilibrium (HWE) was calculated for each locus/population combination using Fisher's exact tests in GENEPOP, with a Bonferroni correction for multiple tests applied to the critical value. We tested for linkage disequilibrium (LD) between pairs of loci in each population to ensure each locus contributed independently to genotype determination. We used GENEPOP 4.2 (Raymond and Rousset, 1995; Rousset, 2008) to check for the presence of null alleles and genotyping error, utilizing both the EM algorithm (Dempster et al., 1977) and Brookfield's (1996) method, because null alleles are known to affect measures of genetic differentiation. MSA was also utilized to calculate global $F_{ST}$, $G_{ST}$, and $G_{ST}$' over all loci, as well as pairwise $F_{ST}$ values for each population pair, as standard measures of genetic differentiation for comparison to prior studies. We used R package Poppr 2.2.0 (Kamvar et al., 2014) to conduct an AMOVA of pairwise $F_{ST}$ values to investigate genetic differentiation among populations. Isolation by Distance (IBD) was explored using Isolation-by-Distance Web Service version 3.23 (Jensen et al., 2005) with a simple, straight-line pairwise geographic distance matrix for all populations and 1000 randomizations of the raw data under the standard settings for Genetic Distance $F_{ST}$ to determine whether genetic differences were correlated to geographic distances.

Patterns of genetic structure were investigated without *a priori* grouping of individuals into genetic clusters using STRUCUTRE v. 2.3 (Pritchard et al., 2000; Pritchard et al., 2010). Ten independent runs were conducted for $K$ = 1-10 clusters, with burn-ins of 500,000 iterations followed by 1,000,000 iterations for each run, using the admixture ancestry model, assumed correlated allele frequencies, and flat prior of $K$. The STRUCTURE runs were compiled and uploaded to Structure Harvester (Earl and vonHoldt, 2012) in order to compare likelihood values across $K$. Additionally, we

used $\Delta K$ (Evanno et al., 2005) as reported by Structure Harvester, to further assess the most likely number of clusters in our data. The outcome of this analysis was used to both determine the most likely number of clusters and to understand how population designation based on sampling location corresponded to emergent patterns of structure in the genetic data.

RESULTS—*Genetic Diversity*—While all 12 published *D. rostriformis bugensis* loci (Wilson et al., 1999a; Feldheim et al., 2011) were originally run across a subset of our samples during PCR optimization, we removed two (*Dbug*6 and *Dbu*141) that did not reliably amplify DNA (allele peaks were numerous and cluttered) prior to genotyping our 180 samples. The number of alleles (*A*) per locus per population ranged from 7.0 to 8.9, with the exception of *Dbu*92, which, as reported in Feldheim et al. (2011), exhibited a total of only 2 alleles. Excluding *Dbu*92, $H_E$ ranged from 0.73 to 0.75 across the six populations, while $H_O$ ranged from 0.48 to 0.54 (Table 1). Most loci demonstrated significant deviations from HWE, with 43 out of 60 population-by-locus comparisons out of HWE following Bonferroni correction. Three of the 53 loci pairs (*Dbu*92 excluded due to lack of polymorphism) exhibited significant LD, including the following pairings: *Dbug*5 and *Dbu*74, *Dbug*2 and *Dbu*93, and *Dbug*2 and *Dbu*110. Our dataset was free from genotyping failure (rate = 0.0000), but null alleles were detected for all populations (range: 0-0.4116) across all but one locus (*Dbug*3). We observed similar values across our six sampled populations ($N$ = 180) for number of alleles (mean $A$ = 7.80), allelic richness (mean $A_R$ = 7.47), observed heterozygosity (mean $H_O$ = 0.51), and inbreeding (mean $F_{IS}$ = 0.296). Mean expected heterozygosity ($H_E$) across our sampled populations was 0.74.

*Population Structure*—Pairwise genetic differentiation for our lower Colorado River quagga populations ranged between 0 and 0.0180, with overall $F_{ST}$ = 0.0037 ($P$ = 0.0810), $G_{ST}$ = 0.0258 ($P$ = 0.0222), and $G'_{ST}$ = 0.1093 ($P$ = 0.0222). An AMOVA found no significant differences between populations ($\phi_{ST}$ = 0.0010, $P$ = 0.315). The largest (significant) pairwise $F_{ST}$ values were between Lake Powell and Lake Mead ($F_{ST}$ = 0.0180, geographic distance = 310 km, Figure 1; Table 2) and between Lake Powell and Imperial Dam ($F_{ST}$ = 0.0164, geographic distance = 657 km, Figure 1; Table 2). Other

populations determined to be genetically distinct from each other include Lake Mead and Imperial Dam ($F_{ST}$ = 0.0087, geographic distance = 351 km, Figure 1; Table 2), and Lake Powell and Senator Wash ($F_{ST}$ = 0.0060, geographic distance = 525 km, Figure 1; Table 2). These observations correspond to a pattern of IBD, though not all populations separated by >300 km were significantly differentiated. However, none of the more proximate population pairs (Senator Wash and Imperial Dam = 3 km, Lake Mohave and Lake Mead = 95 km, and Lake Havasu and Lake Mohave = 108 km) were significantly differentiated. The observable relationship between geographic distance and genetic distance, despite little genetic differentiation among populations, was further supported by a significant statistical correlation between Rousset's genetic distance ($F_{ST}/(1-F_{ST})$) and geographic distance ($r$ = 0.6325, p = 0.0220).

The analytical results from STRUCTURE further support the scenario of a well-mixed, largely undifferentiated set of quagga mussel populations along the Colorado River. All individuals sampled appear to belong to a single genetic cluster ($K$ = 1). Based on the $\Delta K$ method, there was some indication that two genetic clusters might exist ($K$ = 2), but the $\Delta K$ method is insensitive to scenarios where there is only a single cluster ($K$ = 1; Evanno et al., 2005) and the posterior log likelihood ($L(K)$) for $K$ = 1 (mean LnProb = -5597.547) was higher than that for $K$ = 2 (mean LnProb = -5723.247) or any other $K$.

DISCUSSION—We explored patterns of genetic diversity and population structure among southwestern U.S. quagga mussel populations as compared to populations in the eastern U.S. and Europe. Deviation from HWE was not unexpected for our samples, as the southwestern U.S. populations are very recently founded (2007 and later), and thus migration-drift equilibrium has likely not been established yet. Methods for predicting the presence and frequencies of null alleles in microsatellite data sets are commonly based on the degree to which observed allelic homozygosity exceeds expected homozygosity under conditions of HWE (Pemberton et al., 1995; Brookfield, 1996). Deviations from HWE can also arise from conditions common to small, recently established populations, including undetected population structure and nonrandom mating, founder effect and genetic drift, and natural selection for particular genotypes. In such cases, absence of HWE and associated high levels of observed homozygosity might

lead to overestimation of null allele frequencies by common methods. However, since dreissenid mussels are known to exhibit high levels of null alleles (Marescaux et al., 2016), we do not discount the possibility of some null alleles in our dataset. While null alleles can cause small overestimations of $F_{ST}$, their bias on assignment tests (like STRUCTURE) leads to more conservative estimates of population structure (Carlsson, 2008). Likewise, overestimates of linkage disequilibrium are expected in populations that have not arrived at HWE (Slatkin, 2008). $F_{ST}$ is a preferred measure of genetic differentiation in the presence of null alleles and is also fairly robust to small levels of linkage disequilibrium (Whitlock, 2011), therefore providing a conservative estimate of population genetic patterns. Although we report multiple measures of genetic differentiation, we will focus our discussion on $F_{ST}$ due to its conservative basis, and because it is widely used in other quagga mussel genetics studies for comparison.

Our measures of genetic diversity for southwestern U.S. quagga mussel populations are higher than those reported by Jennett (2013), but lower than those reported by Marescaux et al. (2016). In terms of the populations sampled both by Jennett (2013) from 2010-2011 and for our study in 2015 (Table 3), and for those loci used by both studies (Table 3), our 2015 samples exhibited, in general, a 2X increase in allelic diversity across sites and, with the exception of two loci, a 1.2-3.5X increase in allelic diversity across loci. The general increase in allelic diversity may be an artifact of a larger overall (132 vs. 180) and per locale sample size (15-20 vs. 30) in our study. Allelic richness, which may account for differences in sample size, was not reported by Jennett (2013). A true increase in the number of alleles within these populations, which are the oldest established quagga mussel populations in the southwestern U.S., would have to occur via mutational processes, additional introductions (i.e., immigration) of quagga mussels from outside the region, or significant regional population mixing through gene flow. The time frame (4-5 years) between sampling events for the two studies is much too short to allow mutation to be a significant contributor to the increase in alleles.

Our results cannot rule out the potential for additional introduction events to have occurred, however, we observed that for both complete data sets (not just shared populations), there were, on average, two fewer alleles per locus in our study overall than in those sampled by Jennett (2013), despite

genotyping a larger number of samples. Jennett (2013) sampled a larger number of populations (8 vs. 6) than we did though, including two reservoirs and two pumping stations that were connected via aqueduct rather than the main body of the Colorado River, which could explain the higher overall number of alleles detected in that study. Southwestern U.S. populations likely continue to be fairly well isolated from eastern U.S. populations by a combination of geographic distance, unconnected watersheds, and state watercraft inspection and decontamination regulations (e.g., Colorado Parks and Wildlife, 2016). In terms of increasing allelic diversity resulting from migration and gene flow, our results would be supportive of that scenario. If significant gene flow was a result of continued introductions from the more genetically diverse eastern North America populations (Wilson et al., 1999b; Brown & Stepien, 2010; Feldheim et al, 2011), we would not expect genetic diversity in the southwestern populations to remain stable or decline. Information on allelic diversity in southwestern U.S. quagga mussel populations from Marescaux et al. (2016) and Brown and Stepien (2010) was reported for clumped geographic or allelic datasets in such a way as to not allow for comparative analysis with our findings.

Surprisingly, and somewhat in contrast to a scenario of well-connected subpopulations with high levels of gene flow, we observed an apparent trend towards increasing average $F_{IS}$ (Table 3) for some populations, which would indicate increasing levels of inbreeding. However, because samples were collected from plates placed in singular locales at each sampled reservoir, it's possible that reproduction occurred in a small area, causing multiple related individuals to colonize the plates (nonrandom sampling). Yet, while adult quagga mussels are mostly sedentary, the veligers are free-floating. During spawning, adults release gametes into the water column, where external fertilization takes place (Neumann et al., 1993). There is no recorded background information on how quagga mussel genotypes are distributed throughout the reservoirs they populate, so veliger genotypes could be highly mixed in any one area or not mixed much at all (e.g., if water flow is low). The interplay, therefore, between physical migration and subsequent genetic mixing may be complex in these newly established populations where human-mediated dispersal (recreational boat traffic) may be an important factor in population genetic structuring.

In terms of mean observed heterozygosity ($H_O$) for southwestern U.S. quagga mussel populations, we obtained generally low estimates (Table 1) of $H_O$ compared to Brown and Stepien (2010) and Marescaux et al. (2016), which were in the range of $H_O = 0.7$ for Lake Mead and Lake Mohave (Marescaux et al., 2016). It should be noted that our respective studies utilized somewhat different suites of microsatellite loci, including Brown and Stepien's use of *Dbu*141, which exhibits high allelic diversity and high average $H_O$, and our use of *Dbug*2, which exhibits high allelic diversity, but a relatively low $H_O$. Our estimates of $H_O$ are generally higher than those obtained by Jennett (2013), who did not utilize *Dbu*141 or *Dbu*110 (which also exhibits high allelic diversity and high average $H_O$) and who also used *Dbug*2. Based on these various data, a clear picture of trends in $H_O$ for southwestern U.S. quagga mussels is not apparent.

The overall lack of strong differentiation ($F_{ST} = 0.0037$, $P = 0.0810$) in our sampled southwestern U.S. populations is likely due to the substantial initial effective population sizes, which appears to be a common feature of quagga mussel invasions (Therriault et al., 2005; Stepien et al., 2013) and can suppress the influence of genetic drift, and/or adequate levels of gene flow during intervening years to prevent rapid differentiation among populations. However, because the introduction of the southwestern U.S. populations was less than a decade ago, even in the case of limited gene flow, genetic drift is unlikely to have had enough time to act upon the isolated populations. Our finding of only one genetic cluster ($K = 1$) and potentially increasing mean population allelic diversity align with this premise.

A few years ago, Jennett (2013) reported pairwise $F_{ST}$ values up to 0.3237, with $K = 2$ or 5. Jennett (2013) did not sample Lake Powell (collections were made prior to that infestation), but all four Colorado River reservoirs and Lake Pleasant (Peoria, AZ) made up one core population with mixed lineages, while the two pumping stations (in Pheonix, AZ and Tucson, AZ) and half of the samples from the Otay Reservoir (San Diego, CA) were starkly different. The pumping stations and Otay Reservoir are located near the terminuses of the Colorado River Aqueduct and are more geographically isolated from the Colorado River. In the more recent study by Marescaux et al. (2016), the western North America populations made up a single, separate genetic cluster from all other sampled populations ($K = 2$), with

pairwise $F_{ST}$ values between western North America and other regions ranging between 0.025-0.030. The other populations sampled (eastern North America, Eastern Europe, Western Europe, and the Pontic region) had pairwise $F_{ST}$ values of only 0.002-0.007 and demonstrated a lack of differentiation between the native and invasive ranges (excluding western North America) that suggest a history of repeated introductions between Europe and eastern North America. Even at $K = 2$, Marescaux et al. (2016) notes that the admixture present among the clusters points to weak differentiation. Likewise, while significant genetic structure was detected among some of our sampled populations (Lake Powell vs. Lake Mead, Senator Wash, and Imperial Dam, and Imperial Dam vs. Lake Mead), the level of differentiation was weak (Table 2), and the pattern of IBD detected was mild. The lack of significant differentiation between Lake Powell and both Lake Havasu and Lake Mohave suggests that one of those reservoirs are the source of the infestation of Lake Powell, unless Lake Powell was infested (just not detected) prior to the discovery of quagga mussels in Lake Mead.

While preliminary, we considered the possibility that quagga mussel genetic structure might be impacted by water quality differences between reservoirs. A population survey conducted by Pucherelli et al. (2016) found comparatively small quagga mussel populations in the southern-most reservoirs of the Colorado River (Senator Wash and Imperial Dam). Conductivity, temperature, and total suspended solids (turbidity) were all higher at these sites compared to upstream locations, although a complete water quality data set was not obtained for Lake Powell. While our results only support a single genetic cluster for the southwestern U.S., we note that either Senator Wash or Imperial Dam were included in three of the four significantly differentiated pairwise genetic comparisons. The combined observations of increased genetic diversity, potentially driven by migration between subpopulations, and increasing average inbreeding ($F_{IS}$) within populations, might support a scenario where currently undescribed environmental factors are influencing genetic mixing and individual reproductive fitness. We must keep in mind though that while there are some water quality differences between reservoirs, they are all ultimately connected and may not show significant differences.

While some genetic trends may be emerging, the population structure and genetic diversity of the quagga mussel in the southwestern U.S. and Mexico are still very dynamic. Continued monitoring of these populations over time could yield useful observations on the dynamics of freshwater invasive species in major river systems and may provide additional information that could contribute to future management strategies for invasive populations of this species. Future sampling should again compare quagga populations both along the main Colorado River and throughout the Colorado River Basin (including the Colorado River Aqueduct), as Jennett (2013) accomplished, but should include efforts to sample the large reservoirs at multiple sites to ensure all potential genotypes at the site are captured.

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TABLE 1—Summary of sampling location, sample size (*n*), and genetic diversity for six *Dreissena rostriformis bugensis* populations, including average number of alleles per locus (*A*), allelic richness ($A_R$), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), and inbreeding coefficient ($F_{IS}$), based on the average of 10 microsatellite loci.

| Location | Latitude | Longitude | *n* | *A* | $A_R$ | $H_E$ | $H_O$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|
| Lake Powell | 36° 56' 29" N | 111° 29' 06" W | 30 | 7.00 | 6.87 | 0.73 | 0.54 | 0.218 |
| Imperial Dam | 32° 53' 00" N | 114° 28' 03" W | 30 | 7.30 | 7.11 | 0.74 | 0.52 | 0.347 |
| Senator Wash | 32° 54' 16" N | 114° 28' 50" W | 30 | 8.10 | 7.72 | 0.74 | 0.51 | 0.317 |
| Lake Havasu | 34° 17' 47" N | 114° 08' 32" W | 30 | 8.90 | 8.24 | 0.75 | 0.54 | 0.269 |
| Lake Mohave | 35° 11' 50" N | 114° 34' 10" W | 30 | 7.90 | 7.56 | 0.75 | 0.50 | 0.318 |
| Lake Mead | 36° 01' 58" N | 114° 46' 16" W | 30 | 7.60 | 7.32 | 0.75 | 0.48 | 0.309 |
| | *Mean* | | | *7.80* | *7.47* | *0.74* | *0.51* | *0.296* |

TABLE 2—Genetic differentiation among six *Dreissena rostriformis bugensis* populations from microsatellite data. Pairwise $F_{ST}$ values are presented below the diagonal and associated *P*-values are presented above the diagonal. Italicized values are significantly differentiated at $\alpha < 0.05$ following a sequential Bonferroni correction. Overall $F_{ST} = 0.0037$ ($P = 0.0810$), $G_{ST} = 0.0258$ ($P = 0.0222$), and $G_{ST}' = 0.1093$ ($P = 0.0222$) were determined across 10 microsatellite loci and 30 individuals from each population.

| | Powell | Imperial | Senator | Havasu | Mohave | Mead |
|---|---|---|---|---|---|---|
| Powell | | 0.0012 | 0.0350 | 0.1277 | 0.1281 | 0.0001 |
| Imperial | *0.0164* | | 0.8413 | 0.8549 | 0.2118 | 0.0416 |
| Senator | *0.0060* | 0.0000 | | 0.8187 | 0.3353 | 0.5126 |
| Havasu | 0.0108 | 0.0000 | 0.0006 | | 0.8167 | 0.1821 |
| Mohave | 0.0019 | 0.0000 | 0.0000 | 0.0000 | | 0.2665 |
| Mead | *0.0180* | *0.0087* | 0.0000 | 0.0103 | 0.0034 | |

TABLE 3—Comparison of the change in number of alleles ($N_A$) detected by locus for three *Dreissena rostriformis bugensis* populations and eight loci that both Jennett (2013) and Lindsay et al. (2017) sampled (in 2010-2011 and 2015, respectively) and analyzed.

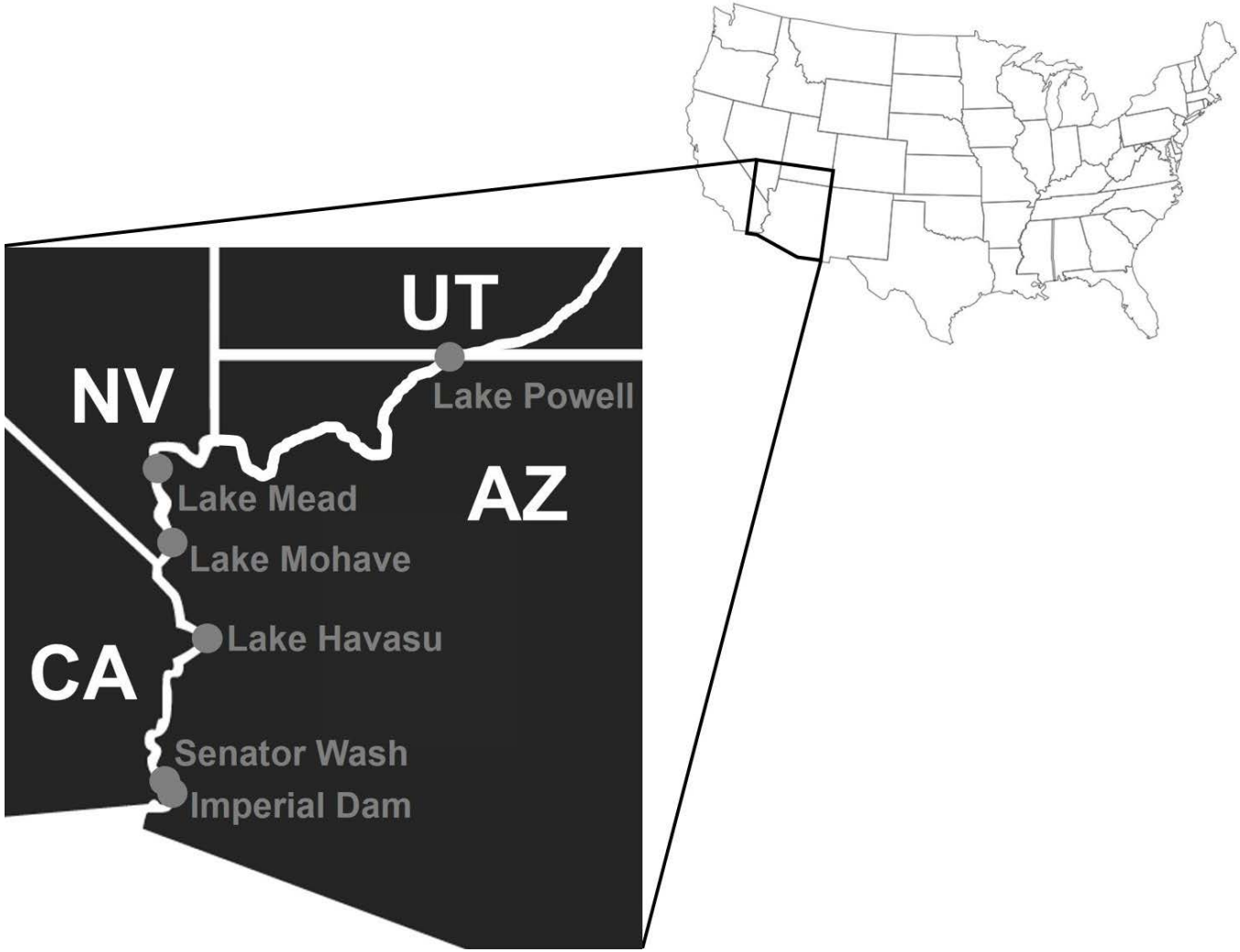
| Locus | Jennett (2013) | | | Lindsay et al. (2017) | | | Average Difference by Locus |
|---|---|---|---|---|---|---|---|
| | Lake Mead | Lake Mohave | Lake Havasu | Lake Mead | Lake Mohave | Lake Havasu | |
| *Dbu*74 | 12 | 8 | 12 | 12 | 11 | 16 | 1.24 |
| *Dbu*75 | 4 | 3 | 6 | 7 | 7 | 6 | 1.69 |
| *Dbu*92 | 4 | 3 | 2 | 2 | 2 | 2 | 0.72 |
| *Dbu*93 | 3 | 3 | 3 | 7 | 6 | 7 | 2.22 |
| *Dbug*1 | 3 | 3 | 2 | 8 | 7 | 11 | 3.50 |
| *Dbug*2 | 3 | 3 | 5 | 10 | 11 | 11 | 3.07 |
| *Dbug*3 | 6 | 5 | 7 | 11 | 13 | 13 | 2.10 |
| *Dbug*4 | 3 | 6 | 5 | 5 | 3 | 5 | 1.06 |
| Average Difference by Population | | | | 2.03 | 1.89 | 1.93 | |

TABLE 4—Comparison of the changes in observed heterozygosity ($H_O$) and inbreeding coefficient ($F_{IS}$) for the three populations that have been sampled and analyzed multiple times over the years since *Dreissena rostriformis bugensis* was discovered in Lake Mead, Arizona.

| | | Population | | | |
|---|---|---|---|---|---|
| | Author | Lake Mead | Lake Mohave | Lake Havasu | Year Sampled |
| *n* | Lindsay et al. (2017) | 30 | 30 | 30 | 2015 |
| | Marescaux et al. (2016) | 20 | 20 | – | 2007 [a] and 2012 |
| | Jennett (2013) | 20 | 15 | 20 | 2010-2011 |
| | Feldheim et al. (2011) | 24 | – | – | 2007 [a] |
| | Brown and Stepien (2010) | 24 | – | – | 2007 |
| $H_O$ | Lindsay et al. (2017) | 0.48 | 0.50 | 0.54 | 2015 |
| | Marescaux et al. (2016) | 0.67 | 0.68 | – | 2007 [a] and 2012 |
| | Jennett (2013) | 0.46 | 0.40 | 0.37 | 2010-2011 |
| | Feldheim et al. (2011) | 0.40 | – | – | 2007 [a] |
| | Brown and Stepien (2010) | 0.70 | – | – | 2007 |
| $F_{IS}$ | Lindsay et al. (2017) | 0.309 | 0.318 | 0.269 | 2015 |
| | Marescaux et al. (2016) | 0.182 | 0.164 | – | 2007 [a] and 2012 |
| | Jennett (2013) | – | – | – | 2010-2011 |
| | Feldheim et al. (2011) | – | – | – | 2007 [a] |
| | Brown and Stepien (2010) | 0.043 | – | – | 2007 |

[a] Samples collected by Brown and Stepien (2010) in 2007 were reanalyzed by both Feldheim et al. (2011) and Marescaux et al. (2016) with slightly different suites of loci.

FIG. 1—Map depicting the location of six *Dreissena rostriformis bugensis* sampling sites along the Colorado River and Arizona border with Utah, Nevada, and California in the southwestern United States.



# 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: Team (//bor/do) (T:), ENGRLAB, HYDLAB, RDLES, MUSSEL SAMPLES, 2015, 2015 Prop C, Microsatellite project

- Point of Contact name, email and phone: Sherri Pucherelli, spucherelli@usbr.gov, 303-445-2015

- Keywords: *Dreissena bugensis*, invasive mussel, genetic structure, microsatellites

- Approximate total size of all files:  36.1 MB