

A POPULATION GENETIC EVALUATION OF BLUEHEAD SUCKER (*CATOSTOMUS DISOBOLUS*) FROM CHUSKA MOUNTAIN AND DEFIANCE PLATEAU DRAINAGES (AZ/ NM) AND THE ZUNI RIVER (NM)

Final Report

Principal Investigators:

Dr. Marlis R. Douglas^{1,2}
PH: 479-575-4176
Email: mrd1@uark.edu

Dr. Michael E. Douglas^{1,2}
PH: 479-575-6343
Email: med1@uark.edu

Dr. Whitney J.B. Anthony²
PH: 217-333-6896
Email: wbannin@illinois.edu

Dr. Mark A. Davis²
PH: 217-333-6896
Email: davis63@illinois.edu

Mr. Max R. Bangs¹
PH: 479-575-7413
Email: mbangs@uark.edu

¹Department of Biological Sciences
Science-Engineering 601
University of Arkansas
Fayetteville AR 72701

²Illinois Natural History Survey
Prairie Research Institute
University of Illinois – Urbana/ Champaign
Champaign IL 61820

Contact Person: Dr. Marlis R. Douglas

TABLE OF CONTENTS

Executive summary	2
Introduction	4
Methods	7
Study organism	7
Sample collection	8
DNA extraction and data generation	9
Mitochondrial (mt)DNA diversity	9
Microsatellite (msat) DNA diversity.....	10
Assignment tests and derivation of population structure	10
Results	13
Mitochondrial (mt)DNA diversity and phylogeography	13
Key results from mtDNA analyses	14
Microsatellite (msat) DNA diversity and population isolation	14
Assignment tests and population structure	15
Key results from msat DNA analyses.....	16
Data from previous studies	17
Discussion	18
Results with management implications from mtDNA analyses	19
Results with management implications from msat DNA analyses	20
Comparison of our results with other studies	22
Overall summary and conclusions for management	23
Acknowledgments	24
Literature Cited	25
Tables	28
Figures	35

EXECUTIVE SUMMARY

The purpose of this study was to genetically evaluate Bluehead Sucker (*Catostomus discobolus*) from drainages on the Navajo Nation (Defiance Plateau and Chuska Mountains) and in the Zuni River drainage. Bluehead Sucker from the Zuni River had been recognized as a distinct subspecies (*C. d. yarrowi*), whereas populations on the Defiance Plateau and in the Chuska Mountains were considered the nominal subspecies (*C. d. discobolus*).

Our objectives in genetically evaluating Bluehead Sucker from the Navajo Nation and the Zuni River were:

- (1) To identify evolutionary lineages (ESUs) and management units (MUs);
- (2) To assess levels of gene flow and degree of isolation among populations;
- (3) To integrate our results within a range-wide perspective of Bluehead Sucker and to contrast them with other studies previously completed.

We obtained 340 samples representing Bluehead Sucker in the three study areas and employed mitochondrial DNA sequence analysis in combination with microsatellite DNA analysis to identify evolutionary lineages and management units. Results were then contrasted against our basin-wide data set of Bluehead Sucker.

Key Results from mtDNA analyses:

- (1) Zuni River, Defiance Plateau and Chuska Mountain share a common mtDNA haplotype, which falls within Bluehead Sucker ESU-2 (Colorado River lineage).
- (2) Chuska Mountain harbors a second haplotype that is not found elsewhere, but it also falls within Bluehead Sucker ESU-2.

(3) Introgression by Rio Grande Sucker was only detected at the Rio Nutria site, and was restricted to a single haplotype in a single individual. None of the sites on Navajo Nation land showed Rio Grande Sucker mtDNA haplotypes.

Key Results from Microsatellite DNA analyses:

(1) The Zuni River, Defiance Plateau and Chuska Mountains each represent a distinct gene pool, with no gene flow among them.

(2) Sites within the Zuni River and on the Defiance Plateau reflect similar patterns of genetic diversity. Sites within the Chuska Mountains exhibit among-site genetic differences, but appear to be connected via gene flow.

(3) Overall, genetic diversity in all three regions (1) is low-to-moderate, suggesting they are isolated.

(4) Genotypes from the Rio Nutria (Zuni River) show affinity with the Rio Grande Sucker, suggesting historic introgression and an admixed origin of this population. No admixture was detected in the other two sites in the Zuni River.

(5) In summary, all three regions described in (1) are unique, and each represents a management unit (MU) for Bluehead Sucker. The Zuni River and Chuska Mountains drainage were previously identified as MUs in Hopken et al. (2013) but samples representing the Defiance Plateau are new additions to the database and represent a previously unrecognized MU (i.e. MU10).

(6) Genotypes that define the Zuni Bluehead Sucker [*Catostomus (Pantosteus) discobolus yarrowi*] are not found in the Defiance Plateau and Chuska Mountains drainages and it would be scientifically incorrect to designate those areas on the Navajo Nation as being components of the Zuni Bluehead Sucker distribution.

INTRODUCTION

Threatened and endangered (T&E) species were recognized (Holycross and Douglas 2007) as a series of disjunct and demographically constrained populations with low numbers and without the gene flow that normally promotes homogenization (i.e., 'small population paradigm'; Caughley 1994). The impaired demographic status of T&E species can propel them into an inbreeding spiral that initially curtails individual survival and fecundity, and ultimately population sizes. Once this process (i.e., the 'extinction vortex'; Gilpin and Soule 1986) is initiated, it becomes self-sustaining and most often leads to extirpation. Here, one can clearly visualize the conservation importance of legally protecting these groups as state and/ or federally 'endangered' (see below).

For the purposes of adaptive management, federal, state, and tribal biologists work to group populations of T&E species into cohesive units (Sites and Crandall 1997; Haig et al. 2003). Implicit to this process is the identification of both historic and contemporary connectedness amongst these populations, in essence, a network that was maintained in the past but has subsequently dwindled as their habitats fragmented (Waser and Strobeck 1998). If prior connectedness for these populations can be established, their genealogical relationships can be more accurately determined and their conservation management plans specifically formulated. Yet these tasks are difficult to accomplish, and for a variety of reasons.

Direct measures of population connectedness, for example, are quite problematic to derive. Several traditional methods are available, but all can be faulted to varying degrees. For instance, mark/ recapture studies (particularly those conducted on fishes) are time-consuming, expensive, and extend over lengthy (multi-year) durations so as to garner appropriate data for accurate estimates (Douglas and Marsh 1996, 1998). A second direct measure would be to radio-track individuals so as to ascertain home ranges and patterns of dispersal by gender. But this technique is also problematic in that it is surgically invasive, hobbled by small sample sizes, with limited radio frequencies, and yields data that are both meager and statistically dependent.

In addition, none of the traditional measures actually reflect gene flow *per se* (i.e., the level of population connectedness we seek), but rather, depict the generalized process of dispersal. The major difficulty for this process is that migrant individuals must not only locate and reproduce in new populations (itself a difficult task), but their progeny must also survive as well to adulthood, and then be resampled. This often fails, even when 'best-case' scenarios are provided (Douglas and Brunner 2002).

Rather, a more modern and data-rich approach would be to evaluate gene flow and dispersal within and among populations by directly quantifying distributions of genetic 'alleles' (i.e., alternative forms of the same gene or genetic region) (Berry et al. 2004). Here, microsatellite (msat) DNA has shown great versatility for it has rapid mutation rates and, given this, often yield large numbers of alleles. Msats are thus well suited to examine the genetic or demographic structure of wild populations, or to differentiate (for example) among hatchery vs wild populations and their hybrids (Douglas and Douglas 2010). This methodology also promotes an enumeration of abundances by employing mark-recapture methods (as above), but utilizing alleles as 'genetic tags' (Palsbøll 1999), rather than the more cumbersome and laborious approach of artificially injecting into an individual a PIT tag (Douglas and Marsh 1996, 1998), or surgically inserting within it a radio-transmitter (Reed and Douglas 1998).

Newer approaches to data analysis are also being rapidly and efficiently invented with regard to genetic analyses, and this in turn promotes the development of relevant software. For example, Bayesian or coalescent-based analyses are now available that not only assign individuals to populations (Pritchard et al. 2000), but also quantify movements by first- or second-generation individuals among populations across landscapes or riverscapes (Wilson and Rannala 2003).

In this study, we applied coalescent and Bayesian analyses of mitochondrial (mt) and microsatellite (msat) DNA to infer contemporary vs historic gene flow and demographic parameters in Bluehead Sucker (*Catostomus discobolus discobolus*) from

two distinct biogeographic regions on the Navajo Nation (i.e., Defiance Plateau drainage of the upper Little Colorado River; Chuska Mountains drainage of the San Juan River). We then compared these data with those obtained from Bluehead Sucker (*Catostomus discobolus yarrowi*) in the Zuni River drainage (upper Little Colorado River), to test for presence of evolutionary significant units (ESUs) and management units (MUs) [where ESUs are recognized as populations with long histories of genetic separation, while MUs currently exchange so few individuals they are demographically independent regardless of past connectivity (Avice 2000)]. We were particularly interested in determining the genetic signatures of these populations and how congruent they are one to another. We also evaluated our data for evidence of hybridity with other suckers that are (or were) contemporaneous with Bluehead Sucker in our study basins.

This study is particularly germane given the Zuni Bluehead Sucker (*Catostomus discobolus yarrowi*) is currently listed as 'endangered' under the 1975 Wildlife Conservation Act of New Mexico (19 NMAC 33.1; Propst 1999), primarily because its capacity to persist and reproduce within the state is now impeded as a result of habitat modifications and predation by non-native fishes (Carman 2004). Furthermore, on 25 January 2013, the U.S. Fish and Wildlife Service (= Service) published a proposed notice that would elevate the Zuni Bluehead Sucker to 'endangered status' under the U.S. Endangered Species Act (ESA 1973), with an additional proposed designation of its critical habitat. The Service also contends that Bluehead Sucker found on the Navajo Nation are actually Zuni Bluehead Sucker (*C. discobolus yarrowi*) rather than the nominate form of Bluehead Sucker (*C. discobolus discobolus*) that is more broadly distributed within the Colorado River Ecosystem.

METHODS

Study organism: The Bluehead Sucker belongs to a clade of specialized suckers (Family Catostomidae; subgenus *Pantosteus*) distinguished morphologically by broad disc-shaped lips and cartilaginous scraping ridges on both jaws (Cope 1874). It is native to western North America and has a broad distribution spanning multiple river basins (Smith 1966, Smith et al. 2013). Two subspecies are recognized: *C. d. discobolus* and *C. d. yarrowi*. The former ranges from the headwaters of the Green and Colorado rivers to the Grand Canyon in Arizona (Figure 1). Disjunct populations exist in the Bear and Weber rivers of the Bonneville Basin and a few headwater tributaries in the Snake River Basin (Smith 1966). The second subspecies, *C. d. yarrowi* (Cope 1874), is restricted to headwater tributaries of the Zuni River in New Mexico. Baird and Girard (1854) also described the Rio Grande Sucker (*Catostomus plebeius*) from the Rio Grande in NM.

Catostomus discobolus is herbivorous, highly adaptable and able to thrive in a variety of habitats, which likely facilitates its wide distribution (Smith 1966). It generally prefers higher gradient, cobble substrate streams, but also can exist in low elevation mainstems (Minckley 1991). Like most indigenous fishes of the Colorado River, *C. discobolus* enters tributaries spring-through-summer and spawns April-through-May (Maddux and Kepner 1988). However, gravid females and newly hatched fry have been caught as early as February and as late as October (Douglas and Douglas 2000). Adults mature at 2-4 years and can live up to 20 years in larger rivers (Minckley 1991). Conversely, individuals in small tributaries, such as the Zuni Bluehead Sucker, mature within the first year and have a maximum age of five years (Propst et al. 2001).

Despite a widespread distribution, anthropogenic habitat alterations have provoked population declines and *C. d. discobolus* currently occupies only 45% of its historic range (Bezzerides and Bestgen 2002). Impoundments, habitat loss and introduction of non-native species are the most serious threats. Consequently, *C. d. discobolus* is considered a 'species of special concern' in Arizona, Colorado, Idaho, Utah, and Wyoming (Wyoming Game and Fish 2005). In New Mexico, *C. d. yarrowi* is listed as

endangered and occurs as small isolated populations in a few headwater tributaries comprising a fraction of its historic range (Propst et al. 2001).

Several studies (Smith 1966, Smith et al. 1983, 2013) have argued that the Zuni Bluehead differs from the Bluehead sucker in possessing a mosaic of traits reflecting introgression by the Rio Grande Sucker (*C. plebeius*). These authors concluded that Zuni Bluehead Sucker is also found further downstream in the upper Little Colorado River, including Kin Li Chee Creek. Crabtree and Buth (1987) argued that introgression in the Zuni Bluehead Sucker is isolated solely to the Rio Nutria, and other designated populations of Zuni Bluehead Sucker in the upper Little Colorado River are not introgressed but are indeed different from other Bluehead Sucker.

The taxonomic validity of the Zuni Bluehead Sucker remains in question, as does its distribution not only within the upper Little Colorado River, but across the drainages of the eastern Colorado Plateau.

Sample Collection: Fin clips were sampled from 368 Bluehead Sucker at 11 sites in the Zuni River (NM) and on the Navajo Nation (AZ/ NM) (Figure 2, Table 1). Zuni River samples represent the Zuni Bluehead Sucker (*C. discobolus yarrowi*) and were collected from 3 sites: Agua Remora, Rio Nutria and a refuge population maintained at the BioPark (NM) that was established from individuals captured at Tampico Springs and from the Rio Nutria. Bluehead Sucker were collected in two geomorphically distinct locations on the Navajo Nation: Defiance Plateau (three sites): Kin Li Chee Creek, Black Soil Wash and Bear Canyon; Chuska Mountain drainages (five sites): Tsaille Creek, Coyote Wash, Wheatfield Creek, Whiskey Creek, and Crystal Creek. Sample sizes and site acronyms are listed in Table 1 and mapped in Figure 2.

Some of the above samples were also available from previous studies (Douglas and Douglas 2012, Hopken et al. 2013), and included all 21 samples from Agua Remora and 65 samples from the Chuska Mountains (referenced as “Canyon de Chelly” in Hopken et al. 2013).

Data from previous studies were used where appropriate, so as to place the results of this study within a range-wide perspective. This included a large data base of mitochondrial (mt) DNA sequence data for Bluehead and Mountain sucker (see below), as well microsatellite (msat) DNA data from select populations of Bluehead Sucker and other *Pantosteus* species. The criteria for selecting populations to include in the microsatellite analysis was a combination of balancing representation (i.e., similar populations downstream from study drainages) versus the computing time required to analyze large data sets. The data sets are explained below.

DNA Extraction and Data Generation: Genomic DNA was extracted using Qiagen DNeasy kits. Mitochondrial DNA ATP8 and ATP6 genes were amplified (as per Hopken et al. 2013), sequenced using BigDye (ver.3.1) chemistry [Applied Biosystems Inc. (ABI), Forest City CA] and analyzed on an ABI Prism 3730 Genetic Analyzer. Sequences were aligned manually using Sequencher (Gene Codes, Ann Arbor MI).

To quantify population structure and genetic diversity, we assessed variation across 17 fast-evolving microsatellite loci (Tranah et al. 2001) that were partitioned into four multiplex sets. Details on methodology and evaluation of these loci are described in Hopken et al. (2013) and Douglas et al. (2008). Forward primers were dye-labeled with one of four fluorescent dyes (6FAM, VIC, NED and PET, dye set DS-33 by ABI) and loci amplified using standard protocols on a Geneamp PCR system 9700 [Applied Biosystems (ABI); California, USA]. Fragment analysis was executed on an ABI Prism 3730 Genetic Analyzer and with standard electrophoretic parameters. An internal size standard (Liz500 ABI) was run with each sample. Alleles were sized with GeneMapper 4.0 (Life Technologies).

Mitochondrial (mt)DNA diversity: To evaluate mtDNA diversity in Bluehead Sucker from the Zuni River and Navajo Nation drainages within a broad-scale regional context, individual sequence data were first collapsed into haplotypes and compared to a

range-wide data base containing 147 haplotypes of Bluehead Sucker (Fig. 3) and related species in the subgenus *Pantosteus* (Douglas and Douglas 2012). These included Rio Grande Sucker (*C. plebeius*); Desert Sucker (*C. clarki*); and Mountain Sucker (*C. platyrhynchus*). From this point, common names are used to designate species.

Microsatellite (msat) DNA diversity: To evaluate genetic diversity within and among populations, we grouped samples by site, and standard population genetic parameters were calculated in GenAlEx v6.4 (Peakall & Smouse 2012). For each site, we calculated mean numbers of alleles per locus, number of effective alleles (which takes frequency of alleles into consideration), number of private alleles per drainage (i.e., those only detected in that drainage), and unbiased expected heterozygosities (U_{HE}) (which again takes sample size into consideration). Pairwise F_{ST} estimates were generated (and significance calculated using 999 permutations) to serve as an estimate of gene flow among sites and to gauge the isolation of populations.

Assignment tests and derivation of population structure: An “ad hoc” clustering algorithm in the program Structure (Pritchard et al. 2000) was used to identify distinct gene pools, and potential admixture between/ among distinct populations. Structure uses a Bayesian approach (i.e., Markov Chain Monte Carlo) to cluster individuals with similar genotypes by evaluating all genotypes in the data set. In more technical terms, it minimizes linkage disequilibrium while optimizing posterior probabilities. The benefit of the Structure algorithm is the lack of *a priori* assumptions regarding sampling locations, population structure or interconnectedness, which in turn should improve the probability of detecting true genetic structure. Simulation parameters were set at “admixture” and “allele frequencies correlated among populations.” Exploratory analyses were run with a burn-in of 100,000 and a chain length of 500,000 so as to determine number of clusters with the highest posterior probabilities (i.e., K-value). Once an appropriate number for K was determined, analyses were repeated by increasing burn-in to 500,000 and a chain length of 1,000,000.

We then conducted analyses on several data sets to address the different objectives:

(1) The “**8-species**” data set contained all 340 Bluehead Sucker from the Zuni River/ Navajo Nation, respectively, as well as representatives of Bluehead Sucker populations and other *Pantosteus* species. This amounted to 84 Bluehead Sucker from the upper Little Colorado River (LCR) representing B-ESU-3 (Hopken et al. 2013); 81 Bluehead Sucker from the Colorado River representing B-ESU-2 (Hopken et al. 2013), 43 Desert Sucker, 35 Rio Grande Sucker, and 30 Mountain Sucker. These data were used to test gene pools, and to evaluate potential levels of introgression among them.

Intraspecific lineages with separate evolutionary trajectories are often designated as ‘evolutionarily significant units’ (ESUs), a distinction particularly important for declining species or those shrinking in distribution. ESUs were originally defined as being reciprocally monophyletic with regard to mtDNA and with significant divergence at nuclear loci (Moritz 1994). This definition (as followed herein) is objective and unambiguous, whereas others that invoke the necessity of ‘adaptive variation’ are deemed less so (Holycross and Douglas 2007). We previously identified three ESUs within Bluehead Sucker (Hopken et al. 2013, Fig. 4), based on mtDNA clades and microsatellite DNA analyses. That take-home message (also amplified with MUs below) is that molecular divergences among endemic fish populations often juxtapose quite well with drainage histories, and with their concomitant stream hierarchies.

(2) The “**11-sites**” data set contained only 340 study samples of Bluehead Sucker. These data were used to test for distinctiveness of gene pools, levels of gene flow among populations, as well as their degree of isolation and admixture (i.e., if any gene pools represented ‘management units’ or MUs).

Previously, MUs were characterized by significant differences in allele frequencies, as gauged by a comparison of pairwise F_{ST} values. This approach has recently been amended to include contemporary dispersal rates, such that MUs are now defined by demographic independence (i.e. genetic divergence approximating a

dispersal rate <10%, Palsbøll et al. 2006). We employed BAYESASS 3 (Wilson and Rannala 2003) to estimate migration rates for our STRUCTURE-derived groupings, using 10 million iterations (1 million discarded as burn-in) and 1000 iterations between MCMC sampling. Mixing parameter for allele frequencies, inbreeding coefficients and migration rates were iteratively adjusted so as to accrue acceptance rates of 35%, 38% and 40%, respectively (where values between 20% and 40% are deemed optimal). Hopken et al. (2013) employed a similar procedure in evaluating Bluehead Sucker MUs across its range.

RESULTS

Mitochondrial (mt)DNA diversity and phylogeography. Sequence analysis of ATPase 8 and ATPase 6 resulted in 852 base pairs (bp) coalesced into 4 haplotypes (Table 2); each of these had been previously identified in a range-wide study of Bluehead Sucker (Douglas and Douglas 2012; Hopken et al. 2013). These data have been incorporated (as Figure 3) into the Basin-wide haplotype network of Hopken et al. (2013).

All three areas (Zuni River, Defiance Plateau and Chuska Mountains) shared a common haplotype (BHS #108) found in the majority of samples (70%), and at all 11 sites. This haplotype is similar to others in Bluehead Sucker from the Colorado River drainage (Figure 1) and differs from the most common Bluehead Sucker haplotype by only a single point mutation (i.e., 1 nucleotide difference out of 852 = 0.12%) (Douglas and Douglas 2012). This haplotype was also detected as well in some populations from the Upper Little Colorado River (LCR) (Hopken et al. 2013).

Chuska Mountains samples revealed a second haplotype (BHS #109) that was found at all 5 sites, but is seemingly restricted to the Chuska Mountains and has not been detected in the remainder of the Colorado River Basin (Hopken et al. 2013). Overall, haplotype BHS #109 was detected in this area from 59 of 104 samples (57%), and was at high frequency at Tsaile Creek.

The two other haplotypes were restricted to single populations. The Tsaile Creek site was the only one that revealed a 3rd Bluehead Sucker haplotype (BHS #9) in 23% of its samples. This haplotype has been detected throughout the Colorado River Basin in both Bluehead and Mountain sucker populations. One sample from the Rio Nutria also revealed a haplotype found in Rio Grande Sucker.

All Bluehead Sucker haplotypes fall within BHS ESU-2 (Hopken et al. 2013), a lineage that is widespread throughout the Colorado River Basin. Another lineage, BHS

ESU-3, is restricted only to the Upper Little Colorado River, and was not detected in any samples from the Zuni River or from the Navajo Nation.

Key Results from Our mtDNA analyses:

(1) Zuni River, Defiance Plateau and Chuska Mountain drainages share a common mtDNA haplotype, which falls within Bluehead Sucker ESU-2 (Colorado River lineage).

(2) Chuska Mountain drainages harbor a second haplotype that is not found elsewhere, but it also falls within Bluehead Sucker ESU-2.

(3) Bluehead Sucker within the Defiance Plateau drainages of the Navajo Nation do not represent a new ESU, but rather, are components of a previously defined ESU (per Hopken et al. 2013) that also contains Chuska Mountain drainages, the Zuni River (NM), and the remainder of the Colorado River Basin, save the upper Little Colorado River.

(4) Introgression by Rio Grande Sucker was detected only at the Rio Nutria site (Zuni River, NM), and was restricted to a single mtDNA haplotype in a single individual. None of the Navajo Nation sites contained mtDNA haplotypes of Rio Grande Sucker.

Microsatellite DNA diversity and population isolation: Genetic diversity in 340 Bluehead Sucker from 11 sites in the Zuni River and the Navajo Nation was low to moderate across the 17 microsatellite loci, particularly when compared to populations throughout the Colorado River Basin (Hopken et al. 2013). The Zuni River exhibited lowest values, followed by slightly higher values for the Defiance Plateau, and higher still for the Chuska Mountain drainages, but still moderate when compared to range wide values (Table 3).

The Rio Nutria site (Zuni River) was an exception to this general pattern. However,

higher diversity in this population is simply the result of introgression by Rio Grande Sucker (Table 3). Two sites In the Chuska Mountains (i.e., Tsaile Creek and Coyote Wash) exhibited higher values for genetic diversity than did the other three in that area.

Genetic diversity also reflects population demography (i.e., its fluctuations over time), as well as the degree of isolation for individual sites. Pairwise F_{ST} estimates (Table 4) indicate strong separation among the three biogeographic areas, with substantial values ranging from 0.25-0.44 and suggesting considerable diversification. Within these areas, gene flow among sites was variable but generally low, indicating most populations are relatively isolated.

Several populations showed migration rates <10%, as calculated in BAYESASS 3. These were: Bio-Park (TAM) = 0.92 (a refugium population for the Zuni River); Bear Canyon Creek (BCN) = 0.92 (Defiance Plateau); Tsaile Creek (TSA) = 0.91; Coyote Wash (COY) = 0.89; and Crystal Creek (CYC) = 0.92 (all three within the Chuska Mountains).

The inbreeding coefficient (F_{IS}) was elevated in five populations, suggesting a genetic bottleneck at some point in the history of these populations. They were: Agua Remora (AGR) = 0.38 (Zuni River); Black Soil Wash (BKW) = 0.38 and Kin Li Chee Creek (KLC) = 0.37 (both in the Defiance Plateau); Wheatfields Creek (WHE) = 0.38 and Whiskey Creek (WHY) = 0-.25 (both in the Chuska Mountains).

Assignment tests and population structure: Cluster analysis was used to evaluate levels of introgression with other species of sucker known to hybridize with Bluehead Sucker (e.g., Rio Grande Sucker), and also to test distinctiveness of the Zuni River and Navajo Nation when compared with other drainages of the Colorado River Basin.

No introgression by either Desert or Mountain sucker was detected. However, all samples from the Rio Nutria exhibited genotypes of admixed origin, with roughly a third

of the gene pool showing affinity with Rio Grande Sucker (Figure 2; Table 5). The other two sites in the Zuni River did not show any signs of Rio Grande Sucker introgression. Also, no admixture with Rio Grande Sucker was detected in any of the sites on the Defiance Plateau or in the Chuska Mountains (Figure 4).

Populations in downstream areas of the Zuni and San Juan rivers were previously identified by Hopken et al. (2013) as unique MUs (management units) (Figures 4, 5). Assignment tests in this study also revealed that additional Bluehead Sucker in the Zuni River, on the Defiance Plateau and in the Chuska Mountains comprised unique gene pools that were distinct from the Upper LCR or the Colorado River.

Key Results from Our Microsatellite DNA analyses:

(1) The Zuni River, Defiance Plateau and Chuska Mountains each represent a distinct gene pool, with no gene flow among them.

(2) Sites within the Zuni River and on the Defiance Plateau reflect similar patterns of genetic diversity. Sites within the Chuska Mountains exhibit among-site genetic differences, but appear to be connected via gene flow.

(3) Genetic diversity in all three areas is low-to-moderate, suggesting they are isolated.

(4) Genotypes from the Rio Nutria (Zuni River) show affinity with the Rio Grande Sucker, suggesting historic introgression and an admixed origin of this population. No admixture was detected in the other two sites in the Zuni River.

(5) In summary, all three regions described in (1) are unique, and each represents a management unit (MU) for Bluehead Sucker. The Zuni River and Chuska Mountain drainages were previously identified as MUs in Hopken et al. (2013) but samples representing the Defiance Plateau are new additions to the database and represent a previously unrecognized MU (i.e. MU10).

(6) Genotypes that define the Zuni Bluehead Sucker [*Catostomus discobolus yarrowi*] are not found in the Defiance Plateau and Chuska Mountain drainages and it would be scientifically incorrect to designate those areas on the Navajo Nation as being components of the Zuni Bluehead Sucker distribution.

Data from previous studies: Smith et al. (1983) collected Bluehead Sucker from the Zuni River (NM) and from San Juan River drainages and evaluated them using starch-gel electrophoresis of allozymes. A map depicting their sampling sites is represented in Figure 6. The historic stream capture event that could have allowed Rio Grande Sucker to become part of the fish community in the Zuni River (upper Little Colorado River) is presented in Figure 7. Crabtree and Buth (1987) resampled these sites and generated new allozyme data that contradicted aspects of Smith et al. (1983). A phenogram (Crabtree and Buth 1987) depicting relationships of the study populations as well as their relationship with Desert and Rio Grande suckers is presented in Figure 8. The results of our microsatellite DNA study employing samples from the same populations as above (as well as additional ones) is presented in Figure 9, and is congruent with the results presented in Figure 8 (Crabtree and Buth 1987).

Our microsatellite analyses that assigned the 340 samples from the three study areas (data set “11 Sites”) revealed high affinity among sites within areas, and no admixture among areas (Figure 10, Table 5). Rio Nutria samples, consistent with the above analyses, showed an admixed origin, whereas the Agua Remora and BioPark populations were unambiguously assigned to the Zuni River gene pool. Similarly, samples from all three sites on the Defiance Plateau unambiguously assigned to a single area-specific cluster (Figure 10). Figure 11 depicts sample sites and geomorphic regions contrasted with microsatellite data for other Catostomid species. Again, Rio Nutria is depicted as admixed. Figure 12 depicts scatter plots comparing the three geographic regions (Zuni River, Defiance Plateau and Chuska Mountains) in pairwise comparisons. All three are quite distinct.

DISCUSSION

The Zuni Bluehead Sucker from the Zuni River in the upper Little Colorado River (LCR) Basin was recently proposed for Endangered Status under the Endangered Species Act (ESA 1973) in a notice published by the U.S. Fish and Wildlife Service (25 January 2013; FWS-R2-ES-2013-0002-0001). Drainages on the Defiance Plateau (also upper LCR) and the Chuska Mountains (San Juan River Basin) were included as “Areas Occupied at the Time of Listing.” The Navajo Nation expressed concern over the assertion that Bluehead Sucker in their drainages represented the *C. d. yarrowi* subspecies. Various studies had yielded inconclusive results regarding taxonomic affinity of Bluehead Sucker on the Navajo Nation. Discrepancies were likely due to locality differences in drainages examined, methodologies employed (e.g., phenotypic vs genetic, plus utilization of different molecular markers), as well as types of data analyses (e.g., phylogenetic vs population genetic), and their interpretations.

The purpose of this study was to genetically evaluate Bluehead Sucker (*Catostomus discobolus*) from three geographic areas in northeastern Arizona/northwestern New Mexico. Two are located on the Navajo Nation: Defiance Plateau and Chuska Mountains. The third represents the Zuni River drainage, upstream of its confluence with the upper Little Colorado River. Bluehead Sucker from the Zuni River had been recognized as a distinct subspecies (*C. d. yarrowi*), whereas populations on the Defiance Plateau and in the Chuska Mountains were considered the nominal subspecies (*C. d. discobolus*).

Given this, our objectives in genetically evaluating Bluehead Sucker from the Navajo Nation and the Zuni River were:

- (1) To identify evolutionary lineages (ESUs) and management units (MUs);
- (2) To assess levels of gene flow and degree of isolation among populations;
- (3) To integrate our results within a range-wide perspective of Bluehead Sucker and to contrast them with other studies previously completed.

We obtained 340 samples representing Bluehead Sucker in the three study areas and employed mitochondrial DNA sequence analysis in combination with microsatellite DNA analysis to identify evolutionary lineages and management units.

Results with Management Implications from mtDNA analyses: Our mtDNA analyses revealed that populations from all 3 areas are identical to the Bluehead Sucker lineage commonly found throughout the Colorado River Basin (BHS ESU-2 of Hopken et al. 2013). This is illustrated in Figures 3, 4 and 5 and is further supported by the following:

- Zuni River, Defiance Plateau and Chuska Mountain share a mtDNA haplotype common to the Colorado River lineage (B2 Hap #108; Figure 3).
- Chuska Mountain drainages harbor a second mtDNA haplotype that is unique to the area, but also a constituent of the Colorado River lineage (B2 Hap #109; Figure 3).
- Tsaile Creek exhibits a third mtDNA haplotype, also part of the Colorado River lineage (B2 Hap #9; Figure 3) that is, again, distributed throughout the Colorado River Basin.

The distinct Bluehead Sucker lineage (BHS ESU-3) identified by Hopken et al. (2013) in north-draining tributaries from the upper LCR basin (Figures 2 and 3) was not detected from the Defiance Plateau or the Zuni River (both part of the upper LCR basin). In other words, **Navajo Nation Bluehead Sucker (as well as the Zuni River populations) are components of the same evolutionary lineage recognized as *C. discobolus*, found throughout Green and upper Colorado River drainages, as well as Grand Canyon.**

One pertinent result from our mtDNA analysis is the relative low levels of genetic diversity in Zuni River and Defiance Plateau populations, and to a lesser extent, the

Chuska Mountains as well. This indicates the isolation of these areas from the mainstem Colorado River, a larger, genetically more diverse array of populations. It also identifies them as numerically smaller populations that likely experienced genetic bottlenecks over time, reducing their mtDNA diversity to a few common haplotypes.

Our mtDNA analysis identified a single individual from the Rio Nutria that maintained a Rio Grande Sucker haplotype (Table 2, Figure 3). None of the sites on the Defiance Plateau or the Chuska Mountains revealed haplotypes found in other sucker species. Thus, based on our analyses of 215 samples, **introgression by Rio Grande Sucker appears restricted to a single location in the Zuni River.**

Results with Management Implications from Microsatellite DNA analyses: Analysis of microsatellite loci allowed us to further examine potential introgression by Rio Grand Sucker as a defining characteristic of the Zuni Bluehead Sucker subspecies, as well as population connectivity and levels of isolation within and among the study areas.

- Consistent with our mtDNA analysis, the genetic **introgression of Rio Grande Sucker alleles was only detected in the Rio Nutria population** (Tan color of Figure 2 and 11; Green color in Figure 10; Table 5), and was recorded at approximately 36%, with most individuals showing admixed ancestry. Interestingly, neither Agua Remora nor the BioPark samples of Zuni Bluehead Sucker exhibited signs of admixture (Table 5).
- **Navajo Nation samples exhibited microsatellite genotypes that clearly represent Colorado River lineage Bluehead Sucker**, with no evidence of Mountain Sucker (*C. platyrhynchus*) or the Upper LCR lineage Bluehead Sucker (Figures 2 and 11).
- In addition, our results revealed that **Navajo Nation Bluehead Sucker is genetically distinct from the *C. d. yarrowi* subspecies** (Figures 9-12). **The**

Zuni River, Defiance Plateau and Chuska Mountain drainages each represent separate and distinct gene pools, with no gene flow among them (Tables 4, 5; Figures 10-12). Each geographic area is isolated and no population connectivity exists among them. Given the geomorphology of drainage relationships, this likely reflects an historic pattern rather than the result of contemporary (anthropogenic) alterations.

- However, because genetic diversity is low-to-moderate (Table 3) as compared to other Bluehead Sucker populations in the Colorado River Basin (Hopken et al. 2013, Douglas and Douglas 2012), bottlenecks or founder events likely occurred in each area and, consequently, genetic drift could have contributed to the population divergence we now find among geographic areas.
- **Populations within each area demonstrate various levels of connectivity** (Table 4 and 5; Figure 10). Sites within the Zuni River and on the Defiance Plateau reflect similar patterns of genetic diversity. High genetic similarity suggests potential **source-sink relationships**, with one population providing founders or migrants for another that, in turn, is smaller in size, declining in numbers, or had previously become locally extirpated.
- In contrast, sites within the **Chuska Mountains exhibit among-site genetic differences**, but appear to be connected via gene flow. Unique genetic diversity was detected in the Tsaille Creek and the Coyote Wash populations that is not replicated within the other 3 sites. Based on available data to date, we cannot determine if these patterns in Wheatfield, Whisky and Crystal creeks reflect traces of ancestral polymorphism that had been largely lost due to genetic drift (i.e., the random loss of alleles due to population fluctuations), or if the pattern indeed reflects a genetic divergence due to local adaptation.

Comparison of our Results with Other Studies: Our results are mostly consistent with findings by Turner and Wilson (2009), whose study was restricted to the Zuni River drainage in New Mexico. These researchers also reported Rio Grande mtDNA haplotypes as being restricted to the Rio Nutria, albeit at higher frequency (24.0%) than in our samples (6.6%). As with our findings, Turner and Wilson (2009) detected Bluehead Sucker mtDNA haplotypes only in the Agua Remora or Tampico Springs locations (represented in our study by samples from the BioPark). Similarly, their analyses of two nuclear loci (Xt4 and Rag) showed Rio Grande Sucker as being admixed at ~36% in the Rio Nutria, but not in the other two populations. Again, these data are consistent with our analyses of 17 nuclear microsatellite loci. The study by Schwemm and Dowling (cited in Turner and Wilson) employed the same two nuclear loci across a wider set of samples but this study is not published and thus was not available to us.

A comparison with earlier “genetic” studies that examined populations from Zuni River, Defiance Plateau and Chuska Mountains is a little more difficult, since the earlier studies employed protein-coding (allozyme) markers. Smith et al. (1983) examined morphometric and allozymic patterns in Bluehead Sucker from the Little Colorado River drainage and Chuska Mountains (Figures 6 and 7). They assayed three sites in the Zuni River (i.e., Nutria Creek, Rio Pescado and Radosevich) and detected introgression by Rio Grande Sucker in the Nutria Creek population (as did Crabtree and Buth 1983, Turner and Wilson 2009, and our study).

Their results were more ambiguous regarding the other two Zuni River populations and their assertion was that all Zuni River populations were introgressed. However, Buth and Crabtree (1986), Turner and Wilson (2009), and our study identified two of the three populations as lacking introgression by Rio Grande Sucker.

Furthermore, Smith et al. (1983) concluded that the Defiance Plateau population (i.e., Kin Li Chee Creek) was not biochemically different from the Chuska Mountain population (i.e., Whiskey Creek), a result that was contradicted by Buth and Crabtree (1986) and our microsatellite analysis. However, protein-coding loci (represented by

allozymes) are slower evolving than are microsatellite loci, and reflect evolutionary lineages (i.e., ESUs) rather than demographically independent populations (i.e., MUs). Thus, the conclusion by Smith et al. (1983) that Defiance Plateau and Chuska Mountain populations were genetically the same is consistent with results from our mtDNA analysis that indicate populations in Navajo Nation drainages and the Zuni River are all part of the Colorado River Bluehead Sucker ESU (Figure 5).

Overall Summary and Conclusions for Management: Bluehead Sucker from the three study regions is unique, and each area represents a management unit (MU) (Table 6; Figures 10, 11). The Zuni River and Chuska Mountain drainage were previously identified as such in Hopken et al. (2013) (i.e., MU9 and MU6, respectively; Figures 4, 5). However, samples representing the Defiance Plateau are new additions to the database and thus represent a previously unrecognized MU (i.e., MU10; Figures 10, 11).

Genotypes that define the Zuni Bluehead Sucker [*Catostomus discobolus yarrowi*] are not found in the Defiance Plateau and Chuska Mountain drainages and each geographic area harbors a genetically distinct group of Bluehead Sucker (Figures 10-12). Based on our data, it would be scientifically **incorrect to designate those areas on the Navajo Nation as new components of a distribution traditionally identified for the Zuni Bluehead Sucker.**

Bluehead Sucker in Northeastern Arizona/Northwestern New Mexico show a puzzling array of diversity that includes distinct gene pools in drainages of the Chuska Mountains, the Defiance Plateau, the Zuni River, as well as the upper Little Colorado River. This reflects a complex evolutionary history, with periods of isolation punctuated by drainage re-arrangements and potential stream captures, as already outlined ~50 years ago by Smith (1966, 1978), and again in Smith et al. (2013). While fascinating from an evolutionary stance, it is challenging to appropriately manage these fishes from a conservation stance, as numerous anthropogenic pressures threaten their unique and endemic aquatic habitats in the arid southwest. We hope our study will aid USFWS and the Navajo Nation in their endeavors to manage Bluehead Sucker.

ACKNOWLEDGMENTS

The Basin-wide perspective on Bluehead Sucker was initially supported by the *3-Species Conservation Agreement*, a multi-state consortium of agencies that coordinate efforts to manage native fishes range-wide. Navajo Game and Fish, specifically J. Cole and his field assistants, supported the extension of this research into drainages of the Navajo Nation. The U.S. Fish & Wildlife Service, particularly M. Mata and the Albuquerque (NM) office, facilitated acquisition of additional Zuni Bluehead Sucker samples and provided funding for generation of additional genotypes data.

The completion of earlier research provided the baseline for the current investigation and in that regard, numerous agencies contributed field expertise, specimens, technical assistance, collecting permits, funding or comments. Their guidance for project planning, coordination of logistics, confidence in our abilities and enthusiasm for the study organisms made studying Bluehead Sucker a very enjoyable collaboration. Particular thanks also go natural resource agencies in Arizona, Colorado, New Mexico, Utah and Wyoming as well as the Navajo Nation Department of Fish and Wildlife, National Park Service, U.S. Bureau of Reclamation and U.S. Fish and Wildlife Service. We specifically acknowledge: M. Anderson, R. Anderson, M. Ault, R. K. Beidinger, M. Breen, Brunson, S. Carman, P. Cavalli, K. Gelwicks, G. Gustina, T. Hedrick, M. Hudson, J. Jackson, J. Jimenez, S. Jones, R. Keith, J. Logan, M. Lopez, P. Martin, S. McKay, C. Melon, S. Meisner, D. Miller, M. T. Moody, Morvilius, B. Persons, S. Ross, G. Selby, D. Speas, K. Terry, P. Thompson, R. Timmons, M. Trammel, C. Walker, A. Webber, K. Wilson, J. Wood, and B. Zimmermann. We are also in debt to the students, postdoctorals and faculty who contributed to the development of our research: A. Albores, P. Brunner, T. Dowling, R. Cooper, J. Cotter, E. Fetherman, M. Hopken, K. Huyvaert, M. Kwiatkowski, S. Mussmann, A. Reynolds, J. Reynolds, C. Secor, and P. Unmack. Sampling procedures were approved under IACUC permit 98-456R (Arizona State University) and 01-036A-01 (Colorado State University). Funding was provided by grants from UDNR and WGFD to MRD and MED.

LITERATURE CITED

- Avice JC (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.
- Baird SF, Girard C (1854) Descriptions of some new fishes from the River Zuni. *Proceedings of the Academy of Natural Sciences, Philadelphia* **6**, 368—369.
- Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology* **13**, 551—561.
- Carman SM (2004) Zuni Bluehead Sucker (*Catostomus discobolus yarrowi*) Recovery Plan. Conservation Services Division, New Mexico Department of Game and Fish, Santa Fe, New Mexico, 40 pp.
- Caughley G (1994) Directions in conservation biology. *Journal of Animal Ecology* **63**, 215—244.
- Crabtree CB, Buth DG (1987) Biochemical systematics of the catostomid genus *Catostomus*: Assessment of *C. clarki*, *C. plebeius*, and *C. discobolus* including the Zuni sucker, *C. d. yarrowi*. *Copeia* **1987**, 843—854.
- Cope ED (1872) Recent reptiles and fishes: report on the reptiles and fishes obtained by the naturalists of the expedition. Pages 432—442 in Preliminary report of the U.S. Geological Survey of Wyoming and portions of contiguous territories in Part IV, Special Report (F.V. Hayden).
- Cope ED (1874) On the Plagopterinae and the ichthyology of Utah. *Transactions of the American Philosophical Society* **14**, 129—139.
- Douglas ME, Marsh PC (1996) Population estimates/ population movements of *Gila cypha*, an endangered cyprinid fish in the Grand Canyon region of Arizona. *Copeia* **1996**, 15—28.
- Douglas ME, Marsh PC (1998) Population and survival estimates for *Catostomus latipinnis* in northern Grand Canyon, with distribution and abundance of hybrids with *Xyrauchen texanus*. *Copeia* **1998**, 915—925.
- Douglas MR, Brunner PC (2002) Biodiversity of Central Alpine Coregonus (Salmoniformes): impact of one-hundred years of management. *Ecological Applications* **12**, 154—172.
- Douglas MR, Douglas ME (2010) Molecular approaches to stream fish ecology. *American Fisheries Society Symposium* **73**, 157—195.

- Douglas MR, Douglas ME, Hopken MW (2008) Genetic structure of Bluehead Sucker [*Catostomus (Pantosteus) discobolus*] across the Colorado River Basin, with emphasis on drainages in the state of Wyoming. Report to Wyoming Department of Game and Fish. Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins. 64 pp.
- Douglas MR, Douglas ME (2012) Mountain sucker (*Catostomus platyrhynchus*) population genetic analysis within and among river drainages in Utah. Report to Utah Division of Natural Resources. Illinois Natural History Survey, University of Illinois/ Urbana-Champaign IL. 77 pp.
- ESA (1973) Endangered Species Act; Title 16 United States Code, Sections 1531—1544.
- Gilpin ME, Soule ME (1986) Minimum viable populations: processes of species extinction. In: Soule ME (Ed), *Conservation Biology: The Science of Scarcity and Diversity*. Sinauer Press, Sunderland, MA, pp. 19—34.
- Haig SM, Beever EA, Chambers SM, Draheim HM, Dugger BD, Dunham S, Elliott-Smith E, Fontaine JB, Kesler DC, Knaus BJ, Lopes IF, Loschl P, Mullins TD, Sheffield LM (2003) Taxonomic considerations in listing subspecies under the US Endangered Species Act. *Conservation Biology* **20**, 1584—1594.
- Holycross AT, Douglas ME (2007) Geographic isolation, genetic divergence, and ecological non-exchangeability define ESUs in a threatened sky-island rattlesnake. *Biological Conservation* **134**, 142—154.
- Hopken MA, Douglas MR, Douglas ME (2013) Stream Hierarchy Defines Riverscape Genetics of a North American Desert Fish. *Molecular Ecology* **22**, 956—971.
- Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology and Evolution* **9**, 373—375.
- Palsbøll PJ (1999) Genetic tagging: contemporary molecular ecology. *Biological Journal of the Linnean Society* **68**, 3—22.
- Palsbøll PJ, Berube M, Allendorf FW (2006) Identification of management units using population genetic data. *Trends in Ecology and Evolution* **22**, 11—16.
- Peakall R, Smouse PE (2012) GenAIEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**, 2537—2539.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945—959.

- Propst DL (1999) *Threatened and Endangered Fishes of New Mexico*, Technical Report No.1. New Mexico Department of Game and Fish, Conservation Services Division, Santa Fe, New Mexico.
- Propst DL, Hobbes AL, Stroh TL (2001) Distribution and notes on the biology of Zuni bluehead sucker, *Catostomus discobolus yarrowi*, in New Mexico. *The Southwestern Naturalist* **46**, 158—170.
- Reed RN, Douglas ME (2002) Ecology of the Grand Canyon Rattlesnake (*Croalus viridis abyssus*) in the Little Colorado River Canyon, Arizona. *The Southwestern Naturalist* **47**, 130—139.
- Sites Jr JW, Crandall KA (1997) Testing species boundaries in biodiversity studies. *Conservation Biology* **11**, 1289—1297.
- Smith GR (1966) Distribution and Evolution of the North American Catostomid Fishes of the Subgenus *Pantosteus*, Genus *Catostomus*. *Miscellaneous Publications, Museum of Zoology, University of Michigan* **129**, 1—132.
- Smith GR (1978) Biogeography of Intermountain fishes, pp. 17—42. In: Intermountain Biogeography, a Symposium. (Harper KT, Reveal JL, eds.). *Great Basin Naturalist Memoirs* **2**.
- Smith GR, Stewart JD, Carpenter NE (2013) Fossil and recent Mountain Suckers, *Pantosteus*, and significance of introgression of Catostomin fishes of western United States. *Occasional Papers, Museum of Zoology, University of Michigan* **743**, 1—59.
- Smith GR, Hall JG, Koehn RK, Innes DJ (1983) Taxonomic relationships of the Zuni Mountain sucker, *Catostomus discobolus yarrowi*. *Copeia* **1983**, 37—48.
- Tranah GJ, Agresti JJ, May B (2001) New microsatellites for suckers (Catostomidae): primer homology in *Catostomus*, *Chasmistes*, and *Deltistes*. *Molecular Ecology Notes* **1**, 55—60.
- Turner TF, Wilson WD (2009) Conservation genetics of Zuni Bluehead Sucker (*Catostomus discobolus yarrowi*) in New Mexico. Conservation Services Division, New Mexico Department of Game and Fish, Santa Fe, New Mexico, 18 pp.
- Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution* **13**, 43—44.
- Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177—1191.

TABLES

Table 1. Overview of Bluehead Sucker (*Catostomus discobolus*) sampled from 11 sites in Northeastern Arizona/ Northwestern New Mexico. Sites listed under Zuni River represent the subspecies *C. d. yarrowi* (Zuni Bluehead Sucker), whereas sites from the Defiance Plateau and Chuska Mountains are on the Navajo Nation. Listed for each site are: Locality name and acronym (Code). Sample sizes (N) are provided for Total= all samples available, mtDNA= numbers of samples used for sequence analysis, msat= numbers of samples used for microsatellite analysis. Sampling localities are depicted in Figures 2 and 10.

Samples			N	N	N
Area	Site	Code	Total	mtDNA	msat
Zuni River	Agua Remora	AGR	21	16	21
	Rio Nutria	RNU	30	15	30
	Bio-Park Population	TAM	30	30	30
Defiance Plateau	Bear Canyon Creek	BCN	31	16	30
	Black Soil Wash	BKW	31	16	30
	Kin Li Chee Creek	KLC	31	16	31
Chuska Mountains	Tsaile Creek	TSA	40	26	39
	Coyote Wash	COY	42	25	41
	Wheatfields Creek	WHE	15	13	15
	Whiskey Creek	WHY	64	25	40
	Crystal Creek	CYC	33	16	33
Total			368	214	340

Table 2. Distribution of mtDNA haplotypes identified among 214 Bluehead Sucker (*Catostomus discobolus*) from 11 sites in Northeastern Arizona/ Northwestern New Mexico. Haplotypes were identified from sequence analyses of 852 base pairs of the ATPase 6 and 8 genes and compared against a basin-wide database derived from Bluehead Sucker and other *Catostomus* species (Douglas and Douglas 2012). Relationships of listed haplotypes to others identified in Bluehead Sucker are shown in Figure 3.

Area	Site	N	BHS #9	BHS #108	BHS #109	RGS
Zuni River	Agua Remora	16		16		
	Rio Nutria	15		14		1
	Bio-Park Population	30		30		
Defiance Plateau	Bear Canyon Creek	16		16		
	Black Soil Wash	16		16		
	Kin Li Chee Creek	16		16		
Chuska Mountains	Tsaile Creek	26	6	16	4	
	Coyote Wash	25		5	20	
	Wheatfields Creek	13		3	10	
	Whiskey Creek	25		13	12	
	Crystal Creek	16		3	13	
Total		214	6	148	59	1

Table 3. Genetic diversity assessed over 17 microsatellite loci in 340 Bluehead Sucker (*Catostomus discobolus*) from 11 sites in Northeastern Arizona/ Northwestern New Mexico. Listed for site are: N= sample size, Na= mean number of alleles, Ne= effective number of alleles, and UHe= unbiased heterozygosity (adjusted for sample size); se= standard error for each parameter. Sampling localities are depicted in Figures 2 and 10.

Area	Site	N	Na	Ne		UHe		
				se	se		se	
Zuni River	Agua Remora	21	2.5	0.3	1.9	0.2	0.37	0.06
	Rio Nutria	30	5.8	0.5	3.2	0.3	0.66	0.03
	Bio-Park Population	30	2.6	0.3	1.9	0.2	0.37	0.06
Defiance Plateau	Bear Canyon Creek	30	3.2	0.4	2.3	0.3	0.45	0.07
	Black Soil Wash	30	3.0	0.5	2.0	0.2	0.39	0.07
	Kin Li Chee Creek	31	3.2	0.5	2.3	0.3	0.46	0.07
Chuska Mountains	Tsaile Creek	39	6.9	0.9	3.9	0.5	0.68	0.03
	Coyote Wash	41	6.6	0.9	3.6	0.4	0.67	0.04
	Wheatfields Creek	15	3.8	0.3	2.6	0.2	0.59	0.04
	Whiskey Creek	40	4.9	0.6	2.9	0.3	0.59	0.04
	Crystal Creek	33	4.2	0.5	2.4	0.3	0.50	0.06
		30.5	4.3	0.2	2.6	0.1	0.52	0.02

Table 4. Levels of gene flow among Bluehead Sucker (*Catostomus discobolus*) from 11 sites in Northeastern Arizona/ Northwestern New Mexico. Listed are pairwise F_{ST} estimates derived from 17 microsatellite DNA loci screened across 340 samples. Site acronyms are listed in Table 1. Sampling localities are depicted in Figures 2 and 10.

	Pairwise F_{ST}										
	AGR	RNU	TAM	BCN	BKW	KLC	TSA	COY	WHE	WHY	CYC
AGR	0.00										
RNU	0.09	0.00									
TAM	0.02	0.10	0.00								
BCN	0.41	0.26	0.41	0.00							
BKW	0.44	0.28	0.43	0.06	0.00						
KLC	0.40	0.25	0.40	0.01	0.06	0.00					
TSA	0.30	0.19	0.30	0.21	0.24	0.21	0.00				
COY	0.32	0.20	0.32	0.25	0.27	0.25	0.06	0.00			
WHE	0.35	0.23	0.35	0.28	0.32	0.27	0.07	0.09	0.00		
WHY	0.35	0.23	0.35	0.29	0.32	0.28	0.05	0.07	0.05	0.00	
CYC	0.40	0.27	0.40	0.34	0.37	0.34	0.09	0.10	0.09	0.03	0.00

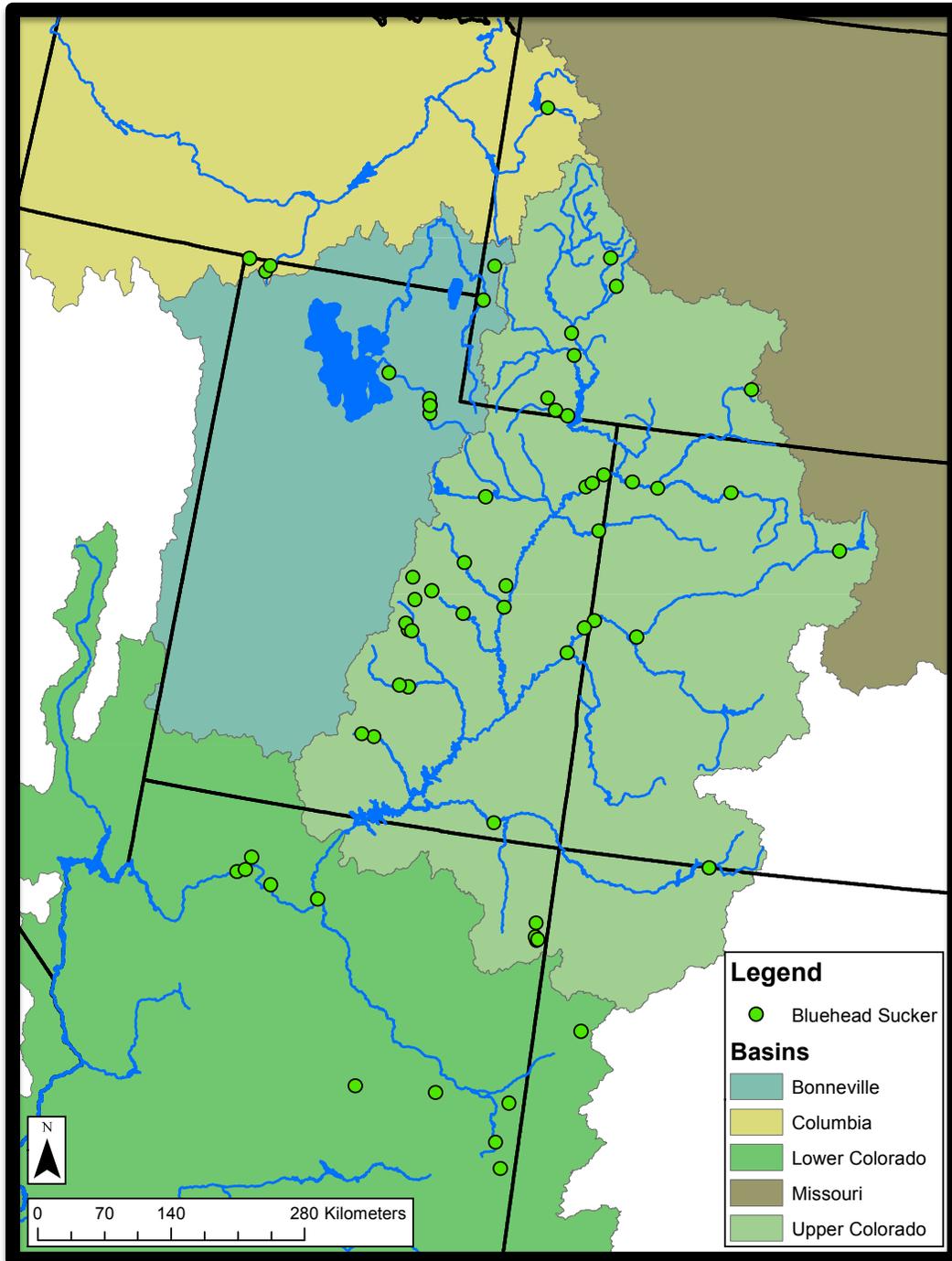
Table 5. Assignment of 340 Bluehead Sucker (*Catostomus discobolus*) sampled from 11 sites to gene pools as determined by program STRUCUTRE at K=6. Analysis was conducted on genotypes derived from 17 microsatellite DNA loci. Site acronyms are defined in Table 1 and clusters are depicted in Figures 2 and 10. Membership of populations within clusters is indicated by color, with multiple cells marked indicating ambiguous assignment of a populations to a particular cluster. Gene pool colors (selected arbitrarily by STRUCTURE) are consistent with color scheme in Figure 10, but not Figures 2 and 11.

Site	N	Inferred Cluster					
		1	2	3	4	5	6
AGR	21	0.001	0.000	0.998	0.000	0.000	0.000
RNU	30	0.000	0.386	0.606	0.000	0.007	0.001
TAM	30	0.000	0.001	0.998	0.000	0.000	0.001
BCN	30	0.000	0.001	0.000	0.001	0.997	0.000
BKW	30	0.001	0.001	0.001	0.001	0.996	0.000
KLC	31	0.001	0.001	0.000	0.000	0.998	0.000
TSA	39	0.222	0.000	0.000	0.748	0.000	0.029
COY	41	0.352	0.000	0.000	0.033	0.002	0.613
WHE	15	0.776	0.001	0.001	0.106	0.001	0.116
WHY	40	0.931	0.000	0.000	0.068	0.000	0.000
CYC	33	0.996	0.000	0.001	0.001	0.000	0.001

Table 6. Allocation of Bluehead Sucker sampled from the Zuni River and Navajo Nation to ESUs (= Evolutionarily Significant Units) and MUs (= Management Units) as identified in Douglas and Douglas (2012) and Hopken et al. (2013). Site acronyms are defined in Table 1 and sampling sites depicted in Figures 2 and 10. ESUs are depicted in Figure 5 and MUs in Figures 4, 5, and 10.

Area	Site	Code	ESU	MU
			mtDNA	msat
Zuni River	Agua Remora	AGR	B-ESU2	B-MU9
	Rio Nutria	RNU	B-ESU2	B-MU-9
	Bio-Park Population	TAM	B-ESU2	B-MU9
Defiance Plateau	Bear Canyon Creek	BCN	B-ESU2	B-MU10
	Black Soil Wash	BKW	B-ESU2	B-MU10
	Kin Li Chee Creek	KLC	B-ESU2	B-MU10
Chuska Mountains	Tsaile Creek	TSA	B-ESU2	B-MU8
	Coyote Wash	COY	B-ESU2	B-MU8
	Wheatfields Creek	WHE	B-ESU2	B-MU8
	Whiskey Creek	WHY	B-ESU2	B-MU8
	Crystal Creek	CYC	B-ESU2	B-MU8

FIGURES



3

Figure 1. Sampling locations in four drainage basins of western North America from which Bluehead Sucker (*Catostomus discobolus*) was sampled (Douglas and Douglas 2012). Geographic extent of basins is depicted as colored areas (see insert Box for basin designations) and collection sites are shown as green dots. Many of these samples were evaluated in Hopken et al. (2013) and are referenced in Figure 2.

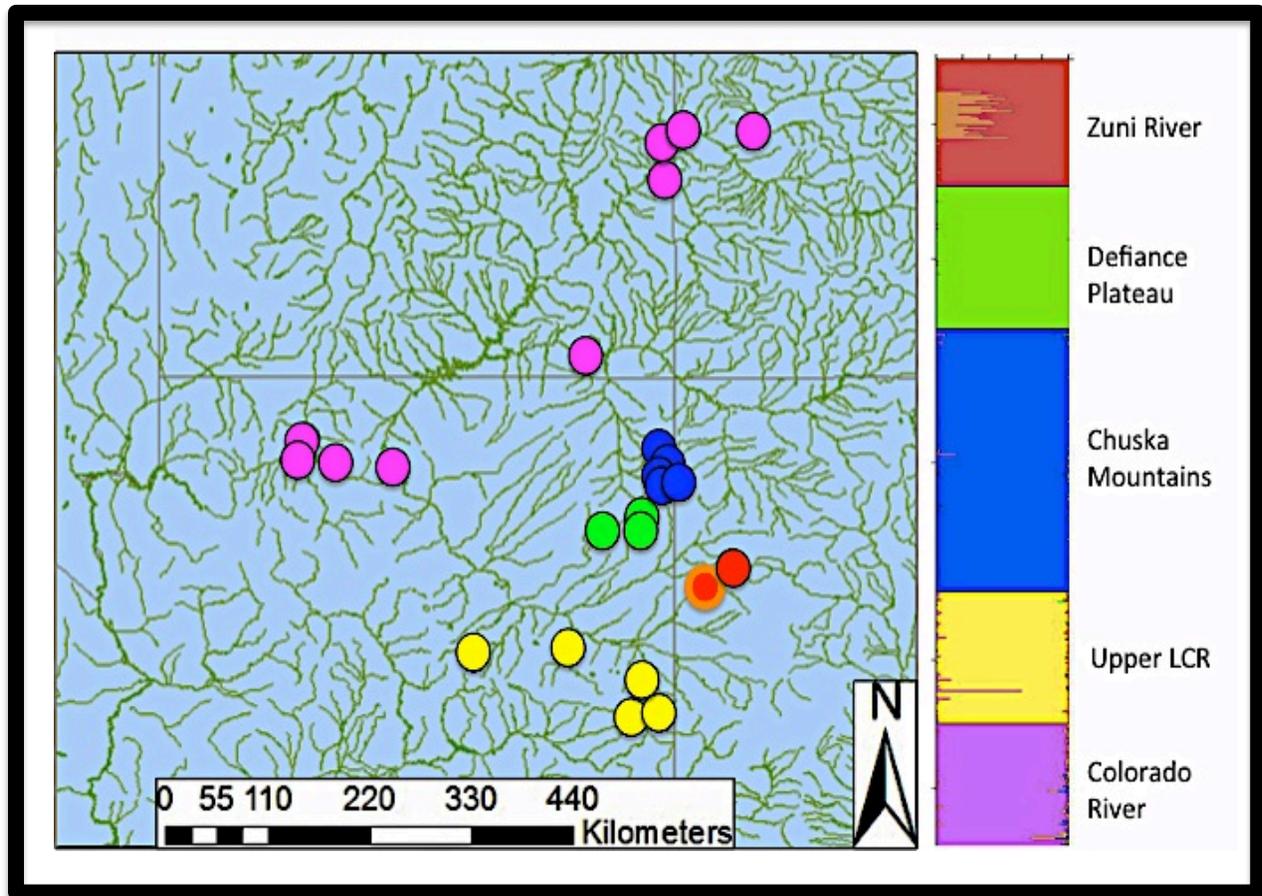


Figure 2. Sites in the Colorado River Basin of western North America from which Bluehead Sucker (*Catostomus discobolus*) was sampled for this study. Locations are indicated by dots: Red = Zuni River (orange halo = Rio Nutria); Green = Defiance Plateau; Blue = Chuska Mountains, with the latter two located on the Navajo Nation. Reference populations (Hopken et al. 2013) are: Yellow = Upper Little Colorado River (LCR); Pink = Colorado River in Grand Canyon + San Juan River + Upper Colorado River drainages. Details on sampling sites are provided in Table 1. Horizontal lines in bar plot = Individuals. Colors = Location dots = gene pools. Multiple colors (Zuni River, Upper LCR) = genotypes of admixed origin.

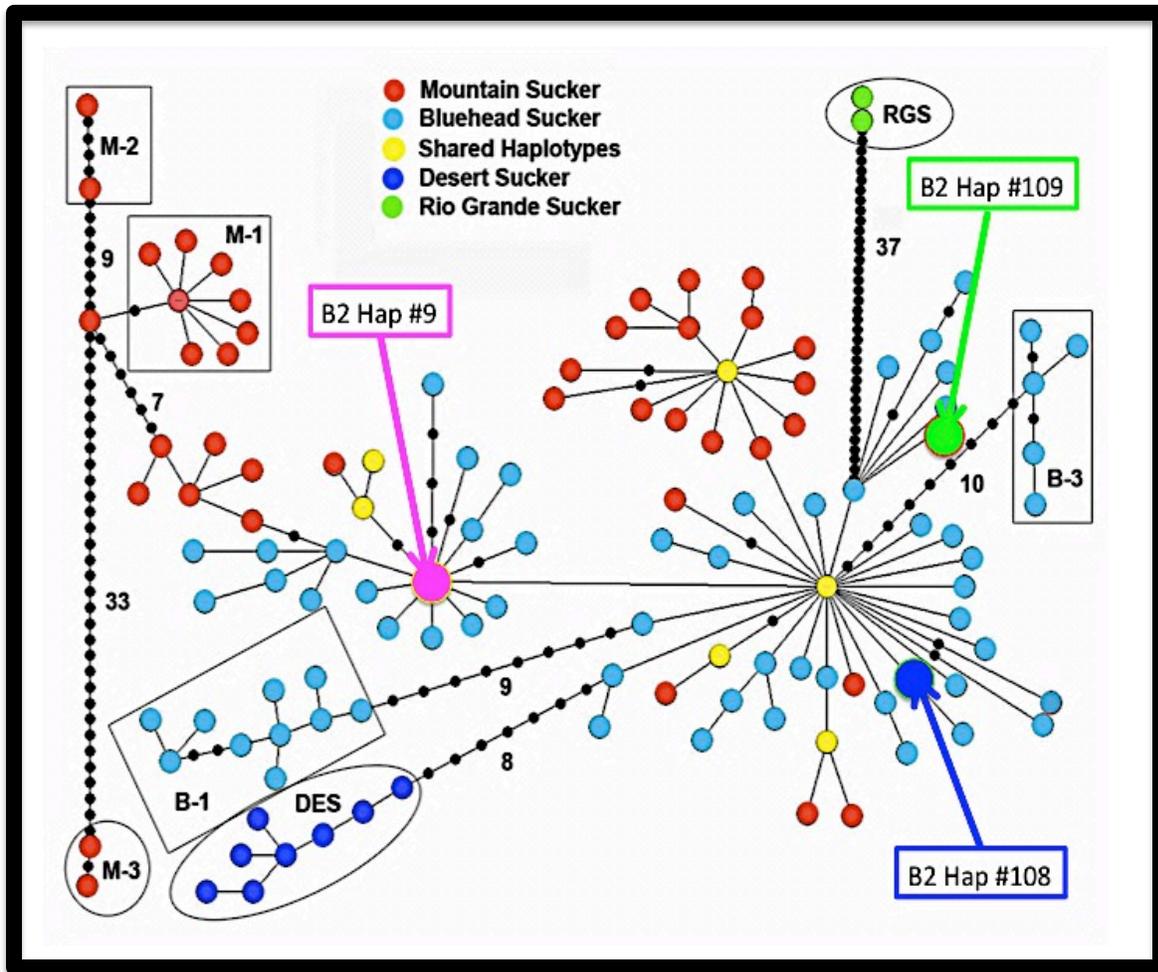


Figure 3. Relationship of haplotypes (B2 Hap#9, #108, #109) detected in 213 Bluehead Sucker from the Zuni River and on the Navajo Nation, as compared to other suckers found in the Colorado River Basin. Network depicts 135 haplotypes from analysis of 852 base pairs of mitochondrial DNA ATP8 and ATP6 genes assessed across 1,148 individuals. These were categorized as: 673 Bluehead Sucker (*Catostomus discobolus*) (= light blue circles) and 475 Mountain Sucker (*C. platyrhynchus*) (= red circles). Yellow = haplotypes shared between Bluehead and Mountain sucker; dark blue = Desert Sucker (*C. clarki*); and green = Rio Grande Sucker (*C. plebeius*). Lines between circles reflect single nucleotide substitutions and small black circles represent haplotypes not identified in the data set. Numbers on branches enumerate the number of nucleotide differences. Evolutionarily significant units (ESUs) in Mountain and Bluehead sucker are enclosed in circles or squares (i.e., M-1/ M-3, B-1, B-3). ESU B-2 and M-4 (Colorado River haplotypes) are represented by all remaining colored dots.

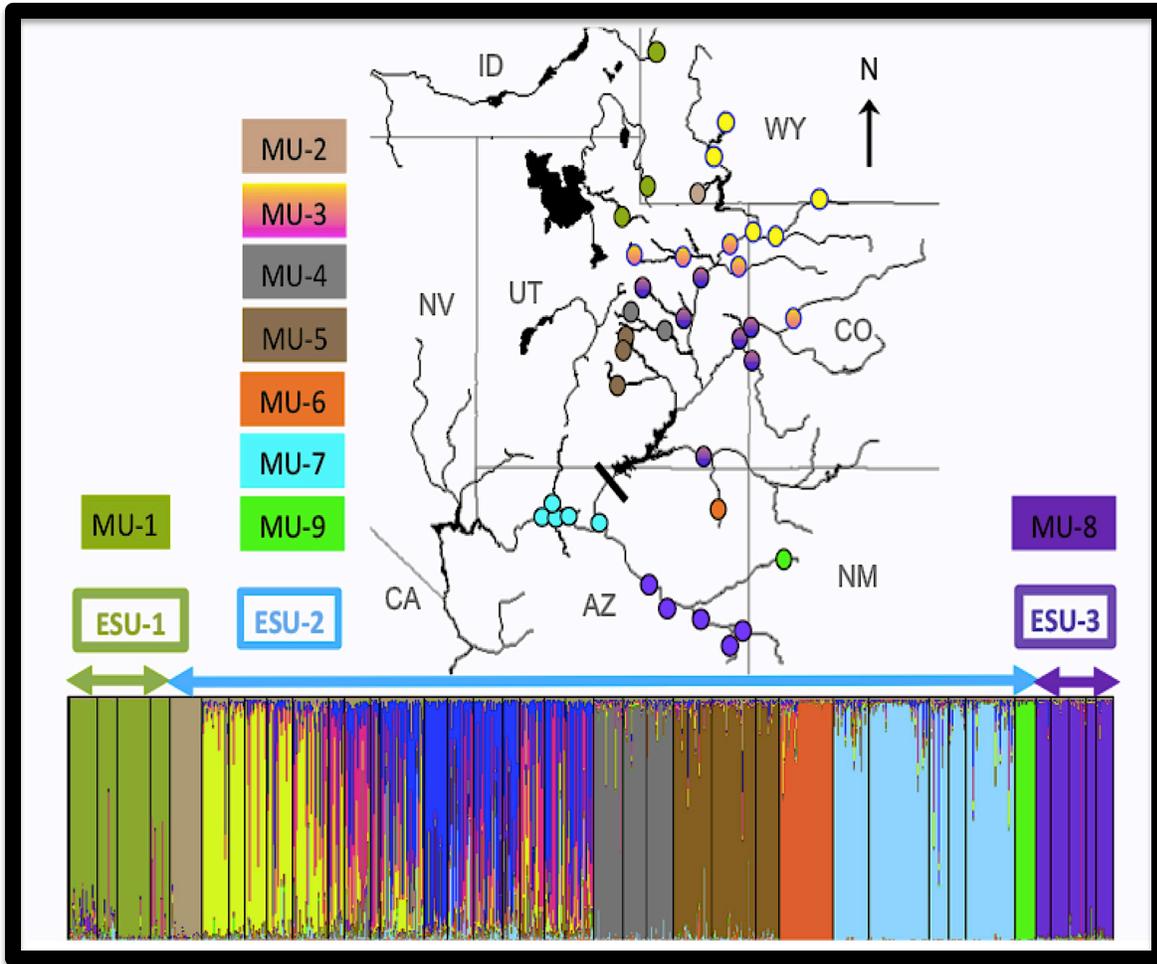


Figure 4. Management units (MUs) identified via assignment test of 1092 Bluehead Sucker (*Catostomus discobolus*) genotypes derived from 17 microsatellite DNA loci. Bar plot depicts assignment of individuals to gene pools (= colors) derived from STRUCTURE. Map shows geographic distribution of 9 MUs, with sampling site reflecting as color of the respective gene pool. MU-1 (green) = Bonneville Basin/ Snake River; MU-2 (light brown) = Ringdahl Reservoir; MU-3 (yellow-to-blue) = Green/Colorado River; MU-4 (grey) = San Rafael River; MU-5 (dark brown) = Dirty Devil River; MU-6 (orange) = Canyon de Chelly; MU-7 (light blue) = Grand Canyon; MU-9 (bright green) = Zuni River; MU-8 (purple) = Little Colorado River. Evolutionarily significant units (ESUs) that contain the MUs are also designated. See Hopken et al. (2013: table 1) for detailed information on sampling sites.

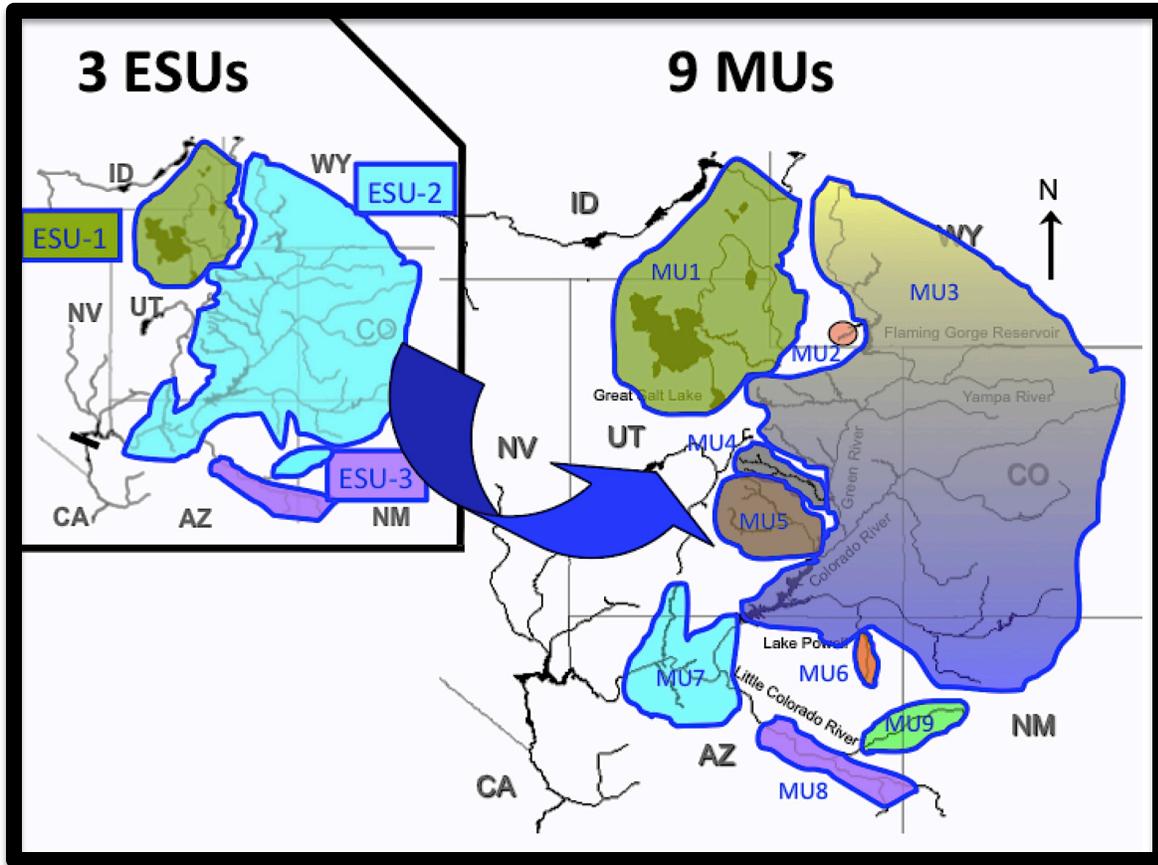


Figure 5. Geographic distribution of three evolutionarily significant units (ESUs) and nine management units (MUs) for Bluehead Sucker (*Catostomus discobolus*). ESUs (left figure) were derived from 836 bp of ATP-8 and ATP-6 mitochondrial DNA genes. ESU-1 (green) = Bonneville Basin/ Snake River; ESU-2 (blue) = Colorado River; ESU-3 (purple) = Upper Little Colorado River. Two MUs (right) correspond to ESUs (i.e., ESU-1 = MU-1, ESU-3 = MU-8), whereas ESU-2 subdivides into seven MUs (MU-2 through MU-7, and MU-9). See Hopken et al. (2013: table 1) for detailed information on ESUs and Figure 4 for MUs.

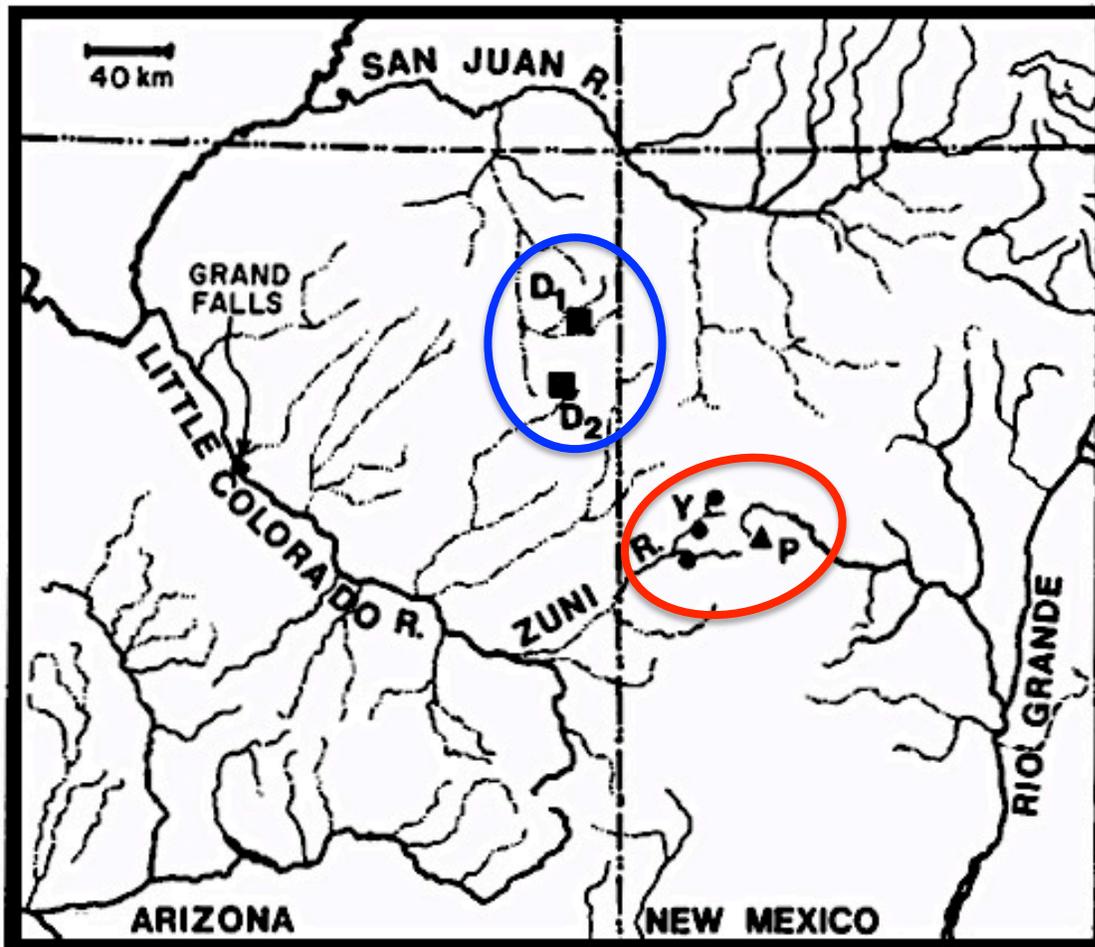


Figure 6. Sample locations (Smith et al. 1983) in the San Juan River (Blue Circle: D1 = Whiskey Creek; D2 = Kin Li Chee Creek); the Rio Grande (Red Circle: P = Wells Springs); and the Zuni River (upper Little Colorado River) [Red Circle: Rio Pescado, near confluence. lower left dot; Nutria Creek, middle dot; and Radosevich, upper dot). Only specimens from Kin Li Chee Creek, Nutria Creek, and the Rio Pescado were suitable for biochemical analyses. Modified after Smith et al. 1983: figure 2)

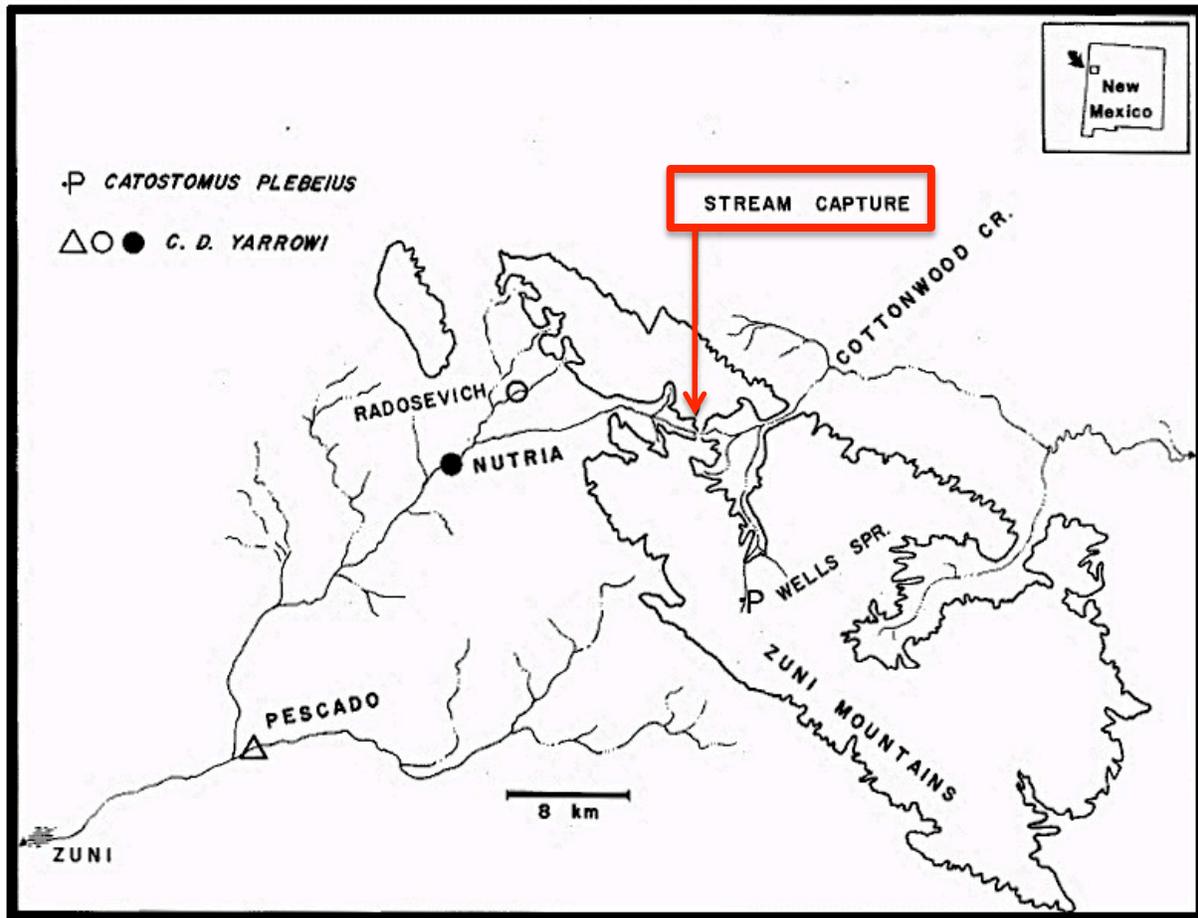


Figure 7. Details of upper Zuni River and Rio Grande River drainages (Smith et al. 1983) showing sample locations. The Rio Grande (P = Cottonwood Creek at Wells Springs); and the upper Zuni (open triangle = Rio Pescado, near confluence; closed circle = Nutria Creek; open circle = Radosevich). Only specimens from Nutria Creek, and the Rio Pescado were suitable for biochemical analyses. The proximity of upper Cottonwood Creek (Rio Grande) and upper Nutria Creek (Zuni River) suggests the probability of an historic stream capture event (red box). Modified after Smith et al. (1983: figure 3)

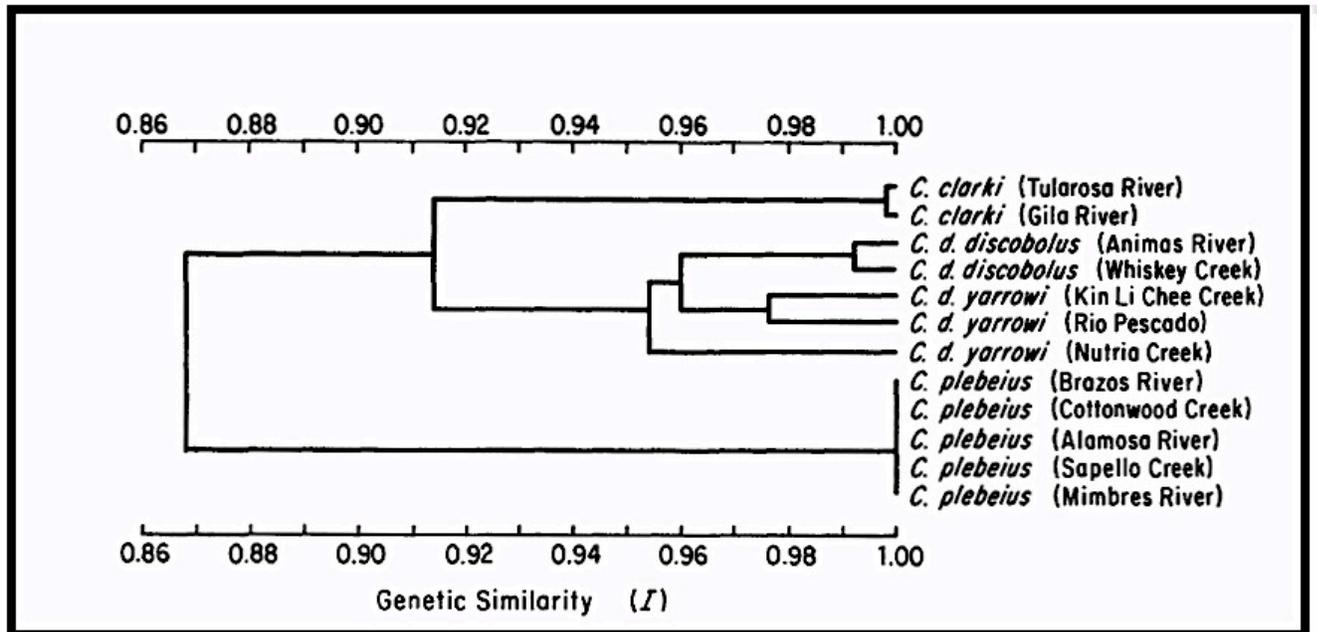


Figure 8. Phenogram (Crabtree and Buth 1987) depicting relationships among 12 geographic samples of *Catostomus*, as determined using Nei's genetic similarity measure. *C. d. yarrowi* (Nutria Creek and Rio Pescado) are Zuni River locations; *C. d. yarrowi* (Kin Li Chee Creek) is a San Juan River drainage; *C. d. discobolus* (Whiskey Creek and Animas River) also represent San Juan River drainages. Modified after Crabtree and Buth (1987: figure 2).

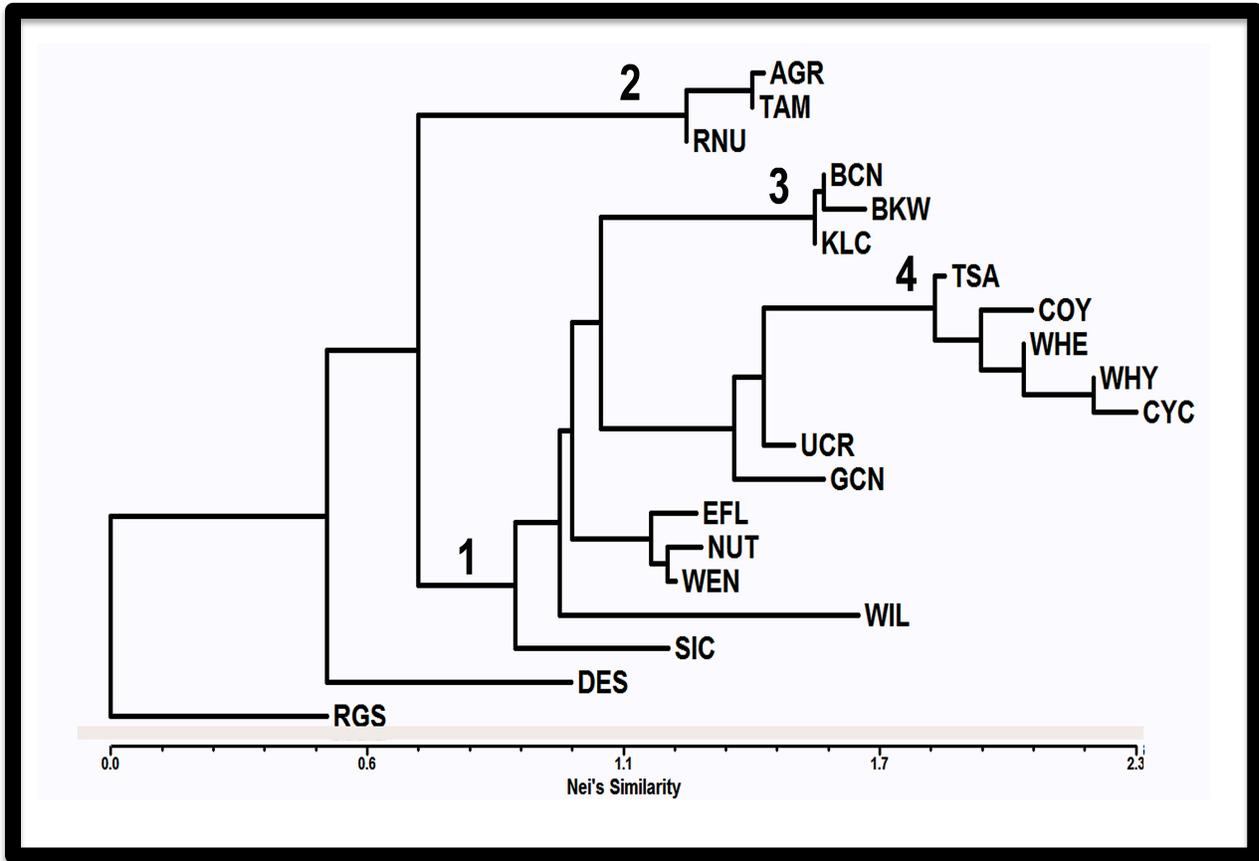


Figure 9. Neighbor-joining tree depicting relationships among 20 geographic samples of *Catostomus*, derived from 17 microsatellite DNA loci and summarized using Nei's genetic similarity measure (derived from our "8 Species" data set). RGS = Rio Grande Sucker; DES = Desert Sucker; 1 = ESU 2 & 3 (Hopken et al. 2013); 2 = Zuni River Bluehead Sucker; 3 = Defiance Plateau Bluehead Sucker; 4 = Chuska Mountain Bluehead Sucker. Sample abbreviations for representatives of other sucker species are: RGS = Rio Grande Sucker, and DES = Desert Sucker; for samples from the upper Little Colorado River drainage: EFL = East Fork, NUT = Nutrioso Creek, SIC = Silver Creek, WEN = Wenigma, and WIL = Willow Creek; for Colorado River mainstem samples: GCN = Grand Canyon, and UCR = Upper Colorado River; for Zuni River samples: AGR = Agua Remora, RNU = Rio Nutria, and TAM = BioPark population (includes Tampico Springs samples); for samples from the Defiance Plateau: BCN = Bear Canyon, BKW = Black Soils Wash, and KLC = Kin Li Chee Creek; for samples from the Chuska Mountains: COY = Coyote Canyon, CYC = Crystal Creek, TSA = Tsaile Creek, WHE = Wheatfields Creek, and WHY = Whiskey Creek.

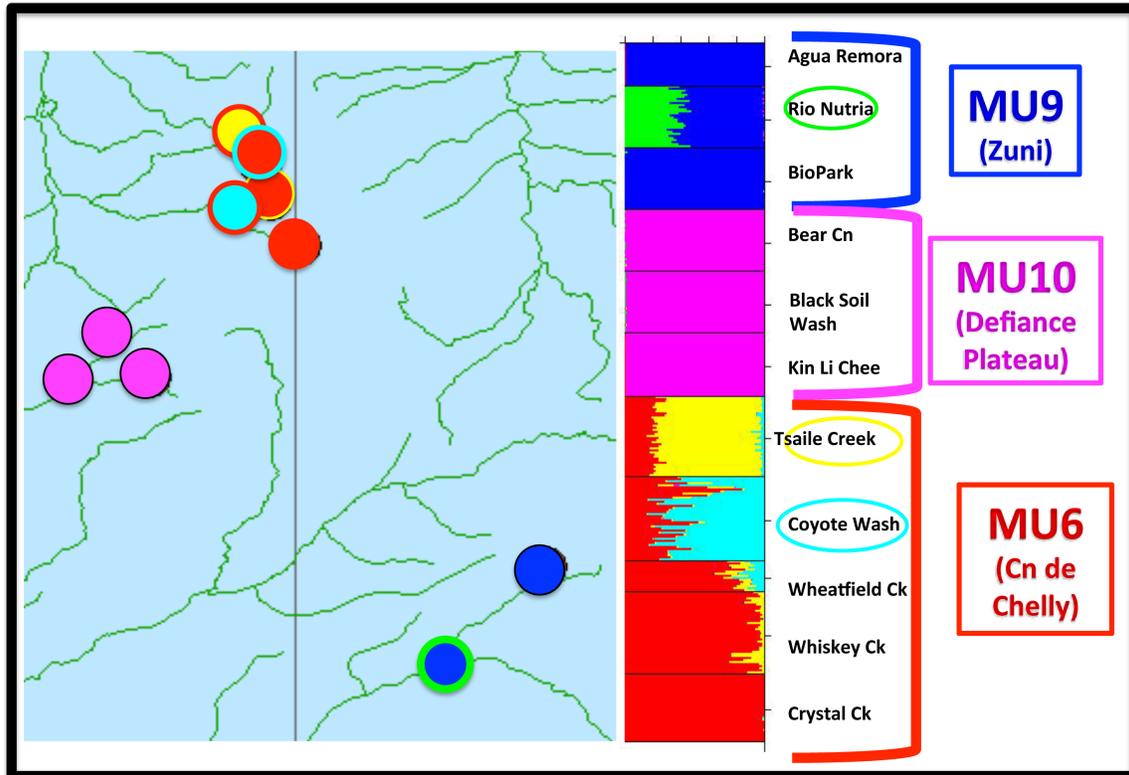


Figure 10. Distribution of distinct gene pools across 11 sampling sites in Northeastern Arizona/ Western New Mexico spanning the Zuni River drainage as well as the Defiance Plateau and Chuska Mountains on the Navajo Nation, and from which Bluehead Sucker (*Catostomus discobolus*) was sampled. Details on samples collected from each site are listed in Table 1. Colors reflect distinct gene pool as identified by Bayesian Assignment test of 17 microsatellite loci. Sites (to right of bar) are defined in Table 1 and Figure 2. Zuni River samples (MU9) = Zuni Bluehead Sucker, whereas Defiance Plateau (MU10) and Chuska Mountain samples (MU6) = Navajo Nation. Horizontal lines in bar plot = Individuals; colors = gene pools. Multiple colors (i.e., MU9, MU6) = genotypes of admixed origin. Boxes on right reflect designation to Management Units (MUs) as defined in Hopken et al. (2013), except for MU10 (Defiance Plateau), which was not included in that analysis. MU10 thus represents a new Management Unit for Bluehead Sucker.

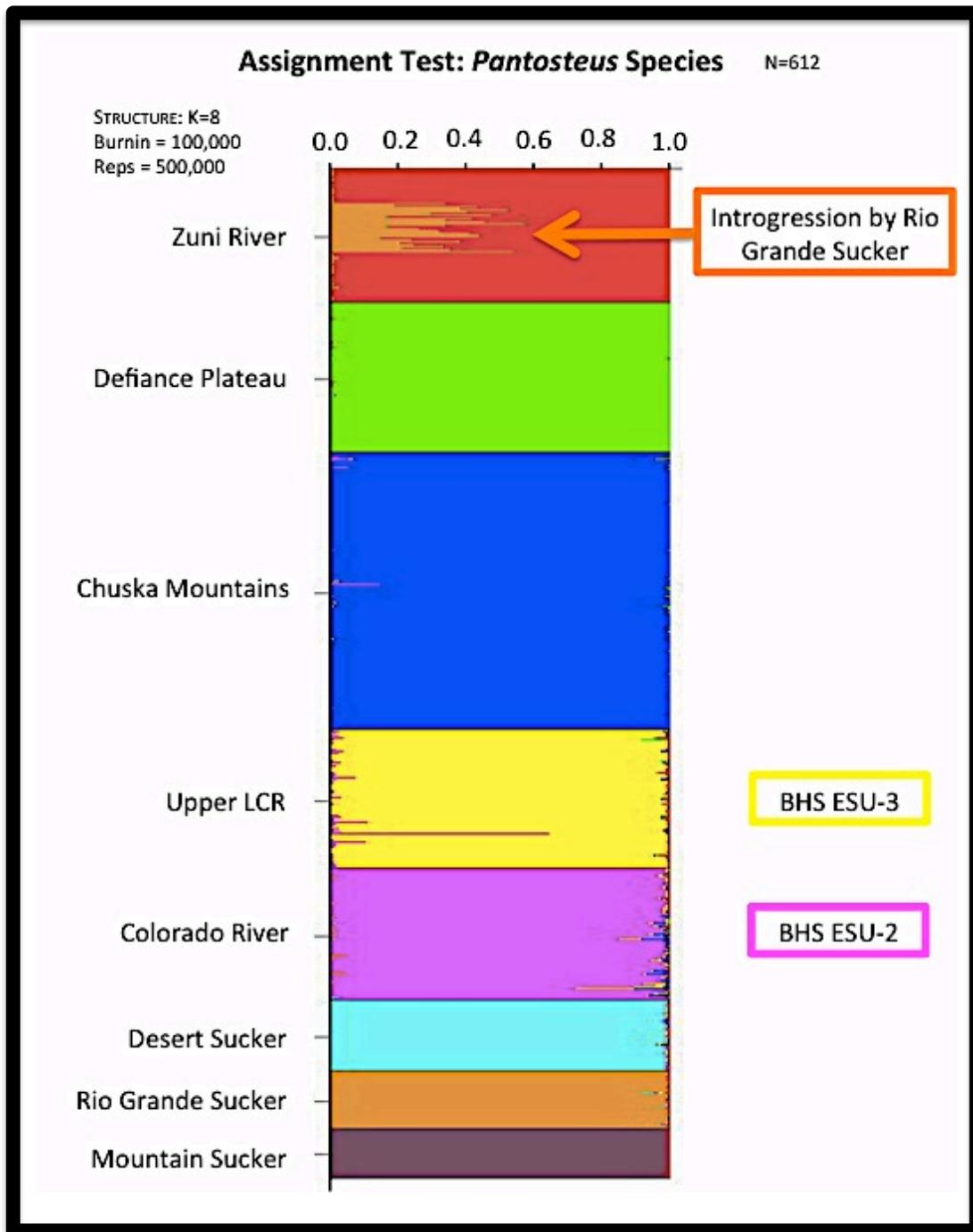


Figure 11. Allocation of 612 sucker [*Catostomus (Pantosteus)*] into distinct gene pools based on Bayesian Assignment test of genotypes derived from 17 microsatellite DNA loci. Sampling areas on left of bar plot are defined in Table 1 and Figure 10. Zuni River samples = Zuni Bluehead Sucker; samples from the Defiance Plateau and Chuska Mountains = Navajo Nation. Individuals = horizontal bars; colors = gene pools; multiple colors = introgression. Samples from the Upper LCR and Colorado River represent BHS ESU-3 and ESU-2, respectively (Hopken et al. (2013).

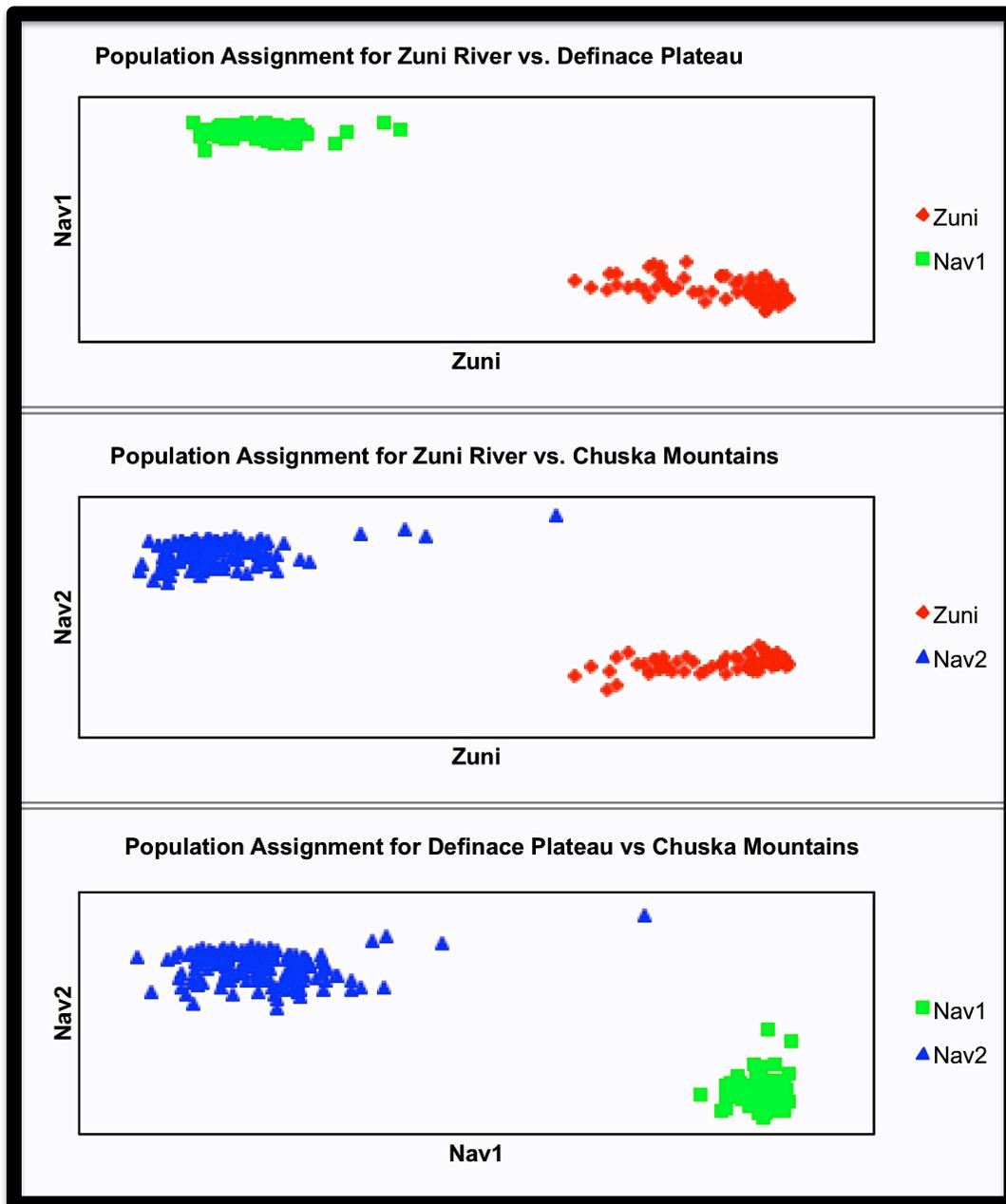


Figure 12. Pairwise population assignment of Bluehead Sucker (*Catostomus discobolus*) samples from the Zuni River (= Zuni) versus Defiance Plateau (= Nav1) and Chuska Mountains (= Nav2). Assignments are based on analyses using 17 microsatellite DNA loci as data. Sampling from each area is defined in Table 1 and depicted in Figures 2 and 10. Zuni River samples represent the Zuni Bluehead Sucker (NM), whereas samples from the Defiance Plateau and Chuska Mountains were collected on the Navajo Nation (AZ/ NM).