Population Genetic Analysis of Bluehead Sucker [*Catostomus* (*Pantosteus*) *discobolus*] Across the Species' Range

Draft Final Report

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EXECUTIVE SUMMARY

Quantifying intraspecific variation in widespread and long-lived species is crucial for an understanding of their ecological and evolutionary processes, and how these have juxtaposed with human impacts to create negative situations at the ecosystem level. Analyses of neutral molecular markers is often of assistance here, simply because they provide a ready source of interpretable data on spatial population structure and gene flow within and among study populations, and how these have been impacted by habitat reduction and deterioration. A good example of widespread and long-lived species in need of critical adaptive management would be the big river fishes of the American West. Many of these fall under protection of the Endangered Species Act and/or have been listed as 'endangered' by state agencies. The Bluehead Sucker (*Catostomus discobolus*; Catostomidae), is one such species. Native to the Colorado, Bonneville and Snake River drainages, it has steadily declined in abundance and distribution throughout its range, thereby attracting conservation attention. State agencies in the Southwest have initiated a joint, multidisciplinary approach to develop a range-wide management plan for this declining species.

The current study, a component of the Bluehead Sucker management plan, quantifies range-wide population structure and identifies regions with high vs low gene flow for the purpose of allocating populations of this species to management units. To this end, 1,092 fish from 39 locations throughout the range of *C. discobolus* were genotyped at 16 microsatellite (msat) loci.

Genetic diversity was high in most regions, and population structure manifested itself into 11 genetic clusters. Bluehead Sucker in Bonneville Basin and Upper Snake River are clearly distinct from their conspecifics in the Colorado River Basin. Populations sampled in Arizona from the Grand Canyon, Canyon de Chelly and upper Little Colorado River watershed formed three unique groups. Within the Upper Colorado River five genetic clusters were identified: two in tributary drainages in Utah (Fremont and Muddy rivers, San Rafael River), three in mainstem Upper Basin rivers and major tributaries,

straddling Utah and Wyoming (Green and Upper Colorado River drainages), one in Ringdahl Reservoir in Wyoming, and one in the Zuni River drainage in New Mexico. Mainstem riverine populations in the Upper Colorado River Basin showed high levels of admixture, whereas tributary populations appeared largely isolated from the mainstem, yet occasionally contribute migrants into it.

Gene flow is clearly an important factor for maintaining genetic diversity in this species, with small and isolated populations reflecting genetic erosion. The maintenance of distinct management units with open migration corridors to allow natural levels of gene flow is clearly a prerequisite for the long-term success of this species. Below we offer seven management considerations.

Consideration 1: Our analyses revealed that Bluehead Sucker is partitioned into 10 distinct genetic clusters. Most of these should be managed as a distinct entity, albeit with some caveats (explained in more detail below). The 10 management units roughly correspond to:

I. Upper Snake River and Bonneville Basin
II. Ringdahl Reservoir
III. “North-Utah:” Upper Green River drainage in Wyoming and Yampa River in Colorado
IV. “Central-Utah:” Upper Green River in Utah, Duchesne River, White River, and Upper Colorado River in Colorado
V. “South-Utah:” Green River below confluence with Price River, Price River drainage, Colorado River above confluence with Green River in Colorado and Utah, Dolores River and San Juan River in Utah
VI. San Rafael River drainage
VII. Fremont and Muddy river drainages
VIII. San Juan River drainage in Canyon de Chelly, Arizona
IX. Colorado River drainage in Grand Canyon, Arizona
X. Zuni River in New Mexico
XI. Upper Little Colorado River

Consideration 2: Bluehead Sucker in Weber, Bear and Upper Snake rivers are clearly distinct from their conspecifics in the Colorado River Basin. Given the unique genetic diversity represented by these populations, management actions should be directed towards their long-term persistence. Further, to prevent reduction of genetic diversity and loss of unique alleles, efforts should be made to maintain actively reproducing populations that exhibit a natural age structure and are of sufficient size.

Consideration 3: Bluehead Sucker inhabiting the Green and Upper Colorado rivers are allocated to three genetic clusters that show high levels of admixture. These populations could be treated as a single management unit. However, two aspects should be considered.

Current genetic diversity within populations is high and reflects extensive gene flow throughout the region, but habitat alterations have now truncated migration of individuals among certain tributaries and reaches. In the long term, populations may diverge through genetic drift (loss of rare alleles, change in allele frequencies). To counteract this process, connectivity amongst populations within specific areas should be maintained to facilitate natural gene flow, albeit at a reduced scale.

Focusing on subsets of populations within the Green and Upper Colorado river basins would also maintain the observed north-to-south gradient in assignment probabilities found within the three genetic clusters. Implementing similar management plans for separate areas within the region would effectively result in generating replicate sets of populations. This in turn would be additional assurance that the genetic diversity of the Green and Upper Colorado river region is maintained.

Consideration 4: In addition to the larger Green and Upper Colorado river management group, two separate management units should be established for populations from western tributaries in Utah. One encompasses populations in the San Rafael drainage, whereas the other includes the Muddy and Fremont river drainages.

Bluehead Sucker in these areas represents two distinct genetic clusters, but the processes responsible for this subdivision are not clear. Divergence of these populations could echo ancient basin evolution, or simply reflect isolation and lack of gene flow. Distinct gene pools could also indicate local adaptation or, alternatively, introgression by another species, such as Mountain Sucker.. Comparing ecology of Bluehead Sucker in these tributaries vs those from other areas might also provide additional data, if indeed these populations exhibit unique life history characteristics, habitat requirements or physiological preferences.

Consideration 5: Similarly to the situation above, Bluehead Sucker in Grand Canyon, Canyon de Chelly and the upper Little Colorado River also represent three distinct genetic groups and should be managed as separate units. Distinctness of Grand Canyon populations vs those from the Upper Colorado River Basin is mirrored by other species, such as Humpback Chub and Speckled Dace, and likely reflects ancient basin evolution, potentially combined with local adaptation.

In the current study, Bluehead Sucker in Canyon de Chelly did not show any affinity to the single population from the Zuni River, suggesting the former do not represent the *C. d. yarrowi* subspecies. However, status of Zuni Bluehead Sucker and populations in adjacent drainages in the Upper Little Colorado River drainage is currently being examined in an ongoing study by researchers at Arizona State University.

Consideration 6: The remaining two genetic clusters are represented by Bluehead Sucker in Ringdahl Reservoir and the population of Agua Remora in the Zuni River drainage. Each contains a single population and both are characterized by low genetic diversity, suggesting their distinctiveness is caused by isolation and genetic drift.

Due to its isolation, the population in Ringdahl Reservoir is sheltered from introgressive hybridization by introduced White and Longnose sucker, and thus could potentially represent a pure source of Bluehead Sucker genes. However, the reduced genetic diversity in this population is a roadblock against using these fish for translocation, supplementation or broodstock establishment. Likely, any of these actions would involve small numbers of individuals, thus inducing an additional population bottleneck that would further reduce genetic diversity in subsequent generations. It also appears that this population represent a subset of alleles found in the other populations in the Upper Green River drainage, and forms a distinct genetic cluster simply due to differences in allele frequencies, and not because unique alleles are present.

INTRODUCTION

Conservation biology forged an alliance between basic research and “real-world” pragmatism, and by so doing expanded our perspectives on conserving biological resources (Soulé 1986). Consequently, knowledge of ecosystems and their components has grown exponentially in the last 20 years, leading to conservation practices that are not only more successful but also more collaborative and adaptive. Despite these accomplishments, our abilities to quantify natural patterns and processes, the most important components for protecting biodiversity, have lagged somewhat, and have proven to be more daunting than anticipated. Several aspects confound this issue and promote differences amongst researchers and policy makers. Some of these are: The recognition of vast (and cryptic) biodiversity imbedded within an already-overwhelming number of described species (Bickford et al. 2006); the dilemma of how best to recognize and measure this biodiversity (e.g., ecological exchangeability; Crandall et al. 2000); and the growing disagreements about what to conserve (e.g., “genes, species or ecosystems”; Bowen 1999).

Noss (1990) suggested that the most comprehensive research and conservation plans should measure all levels of biodiversity, in that details found at one level may be absent from others. This holistic approach would allow scientists to quantify then combine lower-level biodiversity so as to better understand the higher tiers. One traditional measure has been to quantify richness at the species-level, yet this fails to account for those evolutionary processes that have shaped both biogeographic patterns and intraspecific variation (Purvis and Hector 2000). For example, widespread polytypic species often display more intraspecific variation than is found among closely-related sister species (Douglas et al. 2006a). Quantifying this variation should be vital to conservation in the broad sense, for it provides an understanding not only of extinction risk (Spielman et al. 2004) but also of evolutionary potential (Reed and Frankham 2001). While ‘extinction risk’ is an important (and recognizable) metric, ‘evolutionary potential’ is less so, probably due to it being a composite of short-term microevolutionary events that lead to macroevolutionary change (Carroll et al. 2007). Nevertheless, quantifying

intraspecific biodiversity (a microevolutionary phenomenon) has now become an integral aspect of conservation planning at all levels, primarily because cost and effort are decreasing concomitant with an increasing information content (Avise 2003).

Despite the controversy surrounding a species-centric approach to conservation and management (Franklin 1993; Lambeck 1997; Simberloff 1998), the importance of species-specific life histories cannot be overstated. Proper management must recognize ecological and evolutionary processes in general, and particularly the role of individual species as ecosystem components. These are the first steps in developing region-wide, cross-taxon conservation strategies (Manley et al. 2004). Consequently, quantifying intraspecific variation (or lack thereof) will clarify both local and range-wide processes in widespread species (Oakey et al. 2004; Whitley et al. 2006), and underscore those that shape the biodiversity of entire geographic regions (Petit et al. 2003; Douglas et al. 2002).

There is a dearth of knowledge regarding intraspecific variation in many widespread species (Whitley et al. 2006), primarily because these entities are often neither commercially important nor charismatic. Additionally, the expanded perspective required by these studies is often problematic in itself (in spite of the benefits alluded to above). This is because they often fall beyond the scope of available time, funds, or even interest (Slatkin 1987), or because they deal with non-'T&E' (threatened and endangered) species (Rice and Emery 2003). The failure to engage conservation on this broader geographic scale hampers management of all species within each community.

The vast watersheds of the American West provide habitat for numerous long-lived and wide-ranging endemic fish species. The origins of these basins predate the Miocene, possibly extending to the Oligocene (reviewed by Minckley et al. 1986). The biodiversity contained within this region is likewise ancient and has been shaped by orogeny, tectonism and historic climate change (Minckley et al. 1986; Steig 1999). Unfortunately, these ancient drainages and their resident biodiversity are being impacted by a variety of anthropogenic disturbances. Riverine impoundments (Benke 1990; Poff et

al. 2007) now block ancient migration routes, which in turn, constrain movements and life histories of native fishes (Hampe and Petit 2005). Dams also fragment suitable habitat available to many species, resulting in smaller and often more homogeneous habitat patches that remain so because the availability of optimal conditions is reduced (Poff et al. 2007). Native fishes must either adapt or perish. Impoundments have also facilitated establishment of non-native fish species by desiccating long reaches of river and decreasing temperatures and sediment load overall (Minckley et al. 2003). In fact, southwestern streams have the largest proportion of non-native fishes in the United States, with >50% of species introduced (Rahel 2000). This is even more alarming when one considers that native species decline precipitously as non-natives increase and expand (Olden and Poff 2005). As a result, 80% of native fish species from the lower Colorado River Basin are now extinct, extirpated, critically imperiled or currently declining (Minckley 1991). Nevertheless, western watersheds provide conservation opportunities as well, beyond the simple proposition of halting immediate extinctions. For example, intraspecific variation within native fishes can be examined in the context of their basin-wide evolution, as a means of understanding those historic factors promulgating endemic fish biodiversity, while simultaneously offering tangible arguments for preservation of stream reaches and mitigation of anthropogenic impacts.

Genetic diversity, microevolutionary processes and demographic parameters should be spatially and temporally quantified as part of a comprehensive management plan to conserve endemic Western North American fishes. One way to accomplish this at the population level is through the application of microsatellite (msat) DNA. This molecular marker has broad applications to ecological, population genetic and conservation-related applications, and its use has increased steadily since its inception (Goldstein and Schlötterer 1999). The application of msat loci to infer population structure and genetic diversity provides a conservation basis by defining management units and detecting cryptic breaks in gene flow within the range of each native species. In this study, we apply these tools to a single species, the Bluehead Sucker (*Catostomus discobolus*) so as to produce a range-wide genetic blueprint for this species that will allow agency biologists and managers to effectively partition time, efforts and dollars (per

Manel al. 2003; Palsbøll et al 2007) in the conservation of native fishes across western North America.

The purpose of this research was to examine msat variation as a mechanism to: (i) quantify genetic diversity in a wide-ranging western fish species; (ii) identify population genetic structure across its range; (iii) detect regions that contain significant genetic variability, with the ultimate goal of identifying management units (MUs); and (iv) highlight factors that may potentially impact other native fish species within the same watersheds.

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 1872). It is native to western North America and has a broad distribution spanning multiple river basins in the southwestern United States. 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 also 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,* is restricted to headwater tributaries of the Zuni River in New Mexico.

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 high-gradient streams with cobble substrate, but can also exist in low elevation mainstem rivers (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). This species matures at 2-4 years and can live up to 20 years in larger rivers (Minckley 1991). Conversely, individuals in small tributaries 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).

DNA Extraction

A total of 1,131 Bluehead Suckers were collected from 35 locations across the range of the species (Figure 1, Table 1). A total of 30 individuals, including 26 from the Little Sandy River, WY, nine from Henry's Fork WY and four individuals from the Weber River, UT were removed from the analyses because of msat scoring complications, leaving 1,092 specimens for analyses with an average 28 individuals per location (range = 10-62, mode = 28). Either fin clips or whole fish (if juveniles) were collected and all samples were preserved in 100% ethanol. Genomic DNA was extracted using the Puregene DNA Purification Tissue kit (Fish fin clip protocol Gentra-CAT# D-7010A) or Qiagen DNeasy Tissue kit (Qiagen-CAT# 69506). Sampling locations are referred to as 'populations', based on the null hypothesis that each location represents a distinct gene pool reproductively isolated from all other.

Microsatellite loci

To quantify population structure and genetic diversity, we assessed variation across 16 fast-evolving msat loci (Tranah et al. 2001; Table 2) partitioned into four multiplex sets. 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 3100 Genetic Analyzer with standard electrophoretic parameters. An internal size standard (Liz500 or Liz 600, ABI) was run with each sample. Alleles were sized with GENESCAN v3.7 and scored with GENOTYPER v3.7 (PE Biosystems). Data were evaluated for the presence of null alleles and scoring errors using MICROCHECKER v2.2.3 (van Oosterhout et al. 2004).

Genetic diversity

Exact tests for deviations from Hardy-Weinberg and linkage equilibria were computed in GENEPOP v4.0 (Raymond and Rousset 2004). *P*-values were estimated with the Markov Chain method with 10,000 dememorization, 200 batches and 5,000 iterations. All tests were assessed for significance at $\alpha = 0.05$ Bonferroni adjusted for 16 comparisons to $\alpha = 0.0031$. Expected (H_E) and observed (H_O) heterozygosity, numbers of alleles per population and per locus and number of private alleles per population were calculated in GENALEX v6.1 (Peakall and Smouse 2006). Allelic richness was calculated using rarefaction based on the smallest diploid sample size of 20 and was implemented in HP-RARE (Kalinowski 2005). Rarefaction generates allelic richness values that account for differences in sample size and thus are more appropriate for comparison amongst sampling locations.

Population structure and divergence

To estimate genetic divergence and gene flow among sampling locations, pair-wise estimates of F_{ST} and associated bootstrap 95% confidence intervals were calculated in FSTAT v2.9.3.2 (Goudet 2002). The significance of divergence was assessed with the genotypic G-based exact test in GENEPOP with identical parameters set as previously mentioned and significance assessed at $\alpha = 0.05$.

The hierarchical structure of Bluehead Sucker genetic diversity was evaluated by the variance in allele frequencies using locus-by-locus Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992; Michalakis and Excoffier 1996) as implemented in ARLEQUIN v3.1 (Excoffier et al. 2005). The three major river basins, Snake River, Bonneville and Colorado River, were used as major partitions of variation.

An “ad hoc” clustering algorithm in the program STRUCTURE (Pritchard et al. 2000) was used to identify regions of admixture between two or more gene pools. This program

uses a Markov Chain Monte Carlo approach to cluster individuals based on minimized linkage disequilibrium and the highest posterior probability. The benefit of the STRUCTURE algorithm is the lack of *a priori* assumptions regarding physical sampling locations. 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 200,000 and these determined that k = 9 through 13 had the highest posterior probabilities. The subsequent runs focused on k = 9 through 13 with each k replicated 10 times and burn-in and chain lengths extended to 200,000 and 500,000, respectively. This repetitive testing format allowed for evaluation of consistent MCMC convergence as large complicated data sets can cause the chain to converge on multiple values (Waples and Gaggioti 2006).

To further examine gene flow among Bluehead Sucker populations in the upper Colorado River Basin we used a Mantel test with 1,000 permutations implemented in GENETIX v4.05 (Belkhir et al. 2004) to test for Isolation-by-distance among 16 sampling locations that STRUCTURE defined as areas of admixture (see results; Fig. 3). The matrices consisted of genetic distance F_{ST}/ 1- F_{ST} (per Rousset 1997) and river distance in kilometers (km). Additionally, the program ISOLDE in GENEPOP was used to generate data for a scatter plot of genetic distance vs geographic distance. Significance was assessed at Bonferroni α = 0.0031.

Historical population dynamics

Understanding historical population dynamics is an important component of conservation genetics and necessary to interpret patterns of genetic diversity. The Wilcoxson signed rank test, implemented in BOTTLENECK (Piry et al. 1999), was used to test for shifts in excess of measured heterozygosity compared with expected heterozygosity at equilibrium (Cornuet and Luikart 1996; Luikart and Cornuet 1998). This test is able to detect recent genetic bottlenecks [within $4N_E$ generations]. Both the infinite alleles model (IAM) and the two-phase model (TPM) were selected and run for 1,000

iterations. An option for step-wise mutation model was also available, but msat loci rarely conform to this model. Consequently, the two-phase model is more appropriate as it allows for multi-repeat jumps in allele size. Also, as populations near equilibrium, loci tend to approach the infinite allele model. Tests run under both models account for differences in time to equilibrium among loci. Parameters for the TPM were set at 90% of loci conform to the step-wise mutation model and default variance of 30. Significance for two-tailed tests (heterozygote excess and deficiency) were assessed across all loci at α = 0.05 Bonferroni adjusted for 16 comparisons to α = 0.0031. Only significant deviations under both models were considered true signals of a population bottleneck.

RESULTS

Microsatellite loci

All 16 loci were polymorphic averaging 32.6 alleles per locus (range = 10 to 57; Table 1). Significant deviations from HWE occurred in only 2.85% of the tests (i.e., 12 of 420), with 21 expected by chance alone at the 5% level. This in turn suggests that deviations from HWE were minimal and would not impact further analyses. Significant LD tests occurred in 11.66% of the comparisons (49 of 420), with 33 of these attributable to the Havasu Creek population. Once the latter was removed from the analysis, the 16 remaining significant tests were less than the 21 expected by chance alone. Seven of these 16 exhibited consistent patterns at two loci: Dlu 4235 showed deviations in a total of 14 tests across four populations, while Dlu 4300 deviated in eight tests across three populations. Again, neither exceeded the 21 significant tests one would expect by chance alone. Furthermore, no pattern was apparent in LD for any locus-pair or for geographically proximate populations. One potentially problematic issue was the interpretation of pair-wise calculations involving Havasu Creek due to violations of linkage equilibrium assumptions of many statistical analyses. Additionally, MICROCHECKER revealed potential null alleles in nine populations (Table 2), while differences in heterozygote deficiencies occurred at six loci, possibly due to the inclusion in the analysis of genetically divergent groups from the Zuni River and Bonneville Basin.

Genetic diversity across range

Overall, genetic diversity for *C. discobolus* was high, with mean population H_E varying from 0.36 (SD = 0.26) to 0.85 (SD = 0.09; Table 3). Lowest values were found in the population from the Zuni River (C30), followed by three populations in the Bonneville Basin (B1-B3). Mean number of alleles per population ranged from 2.73 to 15.7, with fewest alleles found in the Zuni River drainage. Mean numbers of alleles ranged from 2.6 (SD = 1.3) in the population from the Zuni River (C30) to 15.4 (SD = 6.5) at one location

in the Yampa River (C6). Taking sample size into account, mean allelic richness was again lowest in C30 at 2.3 (SD = 1.), but highest at 7.2 (SD = 1.7) in the population from the Price River (C12). Among the three basins, populations from the Colorado River Basin showed highest allelic richness, with the single population from the Snake River and the three populations from the Bonneville basin exhibiting lower levels.

Genetic diversity in Utah

Utah populations exhibited varying degrees of genetic diversity (Table 3) with the Bonneville Basin characterized by distinctly low diversity values. Within the Colorado River Basin, Utah populations (C5-C16, C20-C23) generally showed higher genetic diversity than did populations in Wyoming (C1-C4), Colorado (C17-C19), or Arizona (C25-C29). Allelic richness in Utah ranged from 5.6 - 7.2 and mean H_E from 0.75 - 0.85. Generally, Utah populations in (or proximate to) mainstem Green or Colorado rivers revealed higher genetic diversity than did those in tributaries, albeit with variation. This trend is also reflected in number of private alleles, with the highest (A_{PR} = 18) occurring in Price River (C12). Interestingly, populations within reservoirs lacked private alleles, with the exception of Strawberry Reservoir (C8).

Population divergence across range

Genetic divergence was variable among populations with F_{ST} values ranging from 0.00 – 0.45 (95% confidence interval 0.10 – 0.17; Table 4). Greatest divergence was between the Zuni River population (C30) and any other population (F_{ST} = 0.32 – 0.45). As expected, comparisons between populations from different basins also exhibited elevated F_{ST} values, with a clear distinction between those from the Snake River (S1) and Bonneville basins (B1-B3) vs Grand Canyon (C25-C29) and Canyon de Chelly (C24). A similar trend was observed among populations within the Colorado River Basin, with Canyon de Chelly and Grand Canyon isolated from populations in the rest of the

basin. Interestingly, populations in the upper Little Colorado River drainage (C31-35) showed elevated F_{ST} values in pairwise comparisons among drainages, including Grand Canyon and Canyon de Chelly. Surprisingly, there appears to be little divergence between the Snake River and Bonneville basin (F_{ST} = 0.07 to 0.08).

AMOVA revealed that 14.3% of genetic variation in Bluehead Sucker was distributed among basins, 9.0% among populations within a basin, and 76.7% within populations (Table 5). However, it is important to note that AMOVA results reflect patterns across the entire dataset and are highly dependent on how populations are partitioned into basins and drainages.

Population divergence in Utah

Populations within Utah appear quite similar and F_{ST} values rarely exceeded a 0.1 threshold, yet only a single pair-wise test was non-significant [i.e., that involving Fremont (C20) and Dirty Devil (C21) rivers]. The four pair-wise comparisons resulting in highest F_{ST} = 0.10 were: Range Creek (C11) vs Millsite Reservoir (C15), Green River at Split Mountain (C7) vs Dirty Devil (C21), Green River at Split Mountain (C7) vs Muddy River (C22), and San Rafael River (C13) vs Muddy River (C22). These divergences reflect either large river distances between populations (e.g., C7 and C22) or partial isolation of tributary populations (e.g., C13 and C22).

Overall Population structure based on cluster analysis

Exploratory runs in STRUCTURE revealed that a statistically valid number of genetic clusters occurred between k = 9 and 12. After increasing the burn-in and chain lengths, the posterior probability values (Ln[Pr (X|K)]) struck a plateau at K = 11, remained stable through K = 12, then decreased through K = 14 (Figure 2). Major clusters (Figure 3) as defined by STRUCTURE at K = 10 were: (I) Snake River/Bonneville Basin (S1, B1-B3), (II)

Ringdahl Reservoir, WY (C4), (III) Upper Green River, WY, (IV) Upper Colorado River, UT, (V) Colorado River, UT, (VI) San Rafael River drainage, UT (C13-C15), (VII) Fremont and Muddy rivers, UT (C20-C22), (VIII) Canyon de Chelly, AZ (C24), (IX) Grand Canyon, AZ (C25-C29), (X) Zuni River, NM (C30), and (XI) upper Little Colorado River, AZ (C31-C35). Three clusters exhibited high levels of admixture and were geographically ill-defined. They encompass drainages in the upper Green and Colorado rivers and can be loosely defined as: (III) Upper Green River, WY (C1-3) and Yampa River, CO (C5-C6), (IV) Upper Green and Colorado rivers, CO and UT, including major tributaries such as White and Duchesne rivers (C7-9, C18), and (V) Upper Green and Colorado rivers, CO and UT, including Price and Dolores rivers (C10-12, C16-17, C19, C23). Seven locations were detected as regions of admixture between the two Upper Green and Colorado River Clusters (IV) and (V), with less than 50% of the individuals assigned to either cluster. These locations were: Green River at Split Mountain, UT (C7), Strawberry Reservoir, UT (C8), White River, UT (C9), Green River at Desolation Canyon (C10), Price River, UT (C12), Colorado River at Westwater Canyon, UT (C19), and San Juan River, UT (C23).

Population structure in Utah based on cluster analysis

Out of the 11 clusters detected across the range of *C. discobolus,* five were mostly within the borders of Utah. The two Weber River populations (B2, B3) from the Bonneville Basin were assigned to cluster (I) and formed a well-defined group together with the population from the Snake (S1) and Bear rivers (B1) in Wyoming. The seven locations identified as admixture zones (C7-C10, C12, C19, C23) were also in Utah and were assigned to clusters (IV) and (V). Clusters (VI) and (VII) were well-defined and contained within Utah. They consisted of locations in the San Rafael (C13-C15), Fremont and Muddy river drainages (C20-C22), respectively.

After STRUCTURE defined the region of admixture and the cline in assignment of individuals between the Upper Green River (III) and Colorado River (IV and V) clusters,

we performed a Mantel test to determine if isolation-by-distance contributes to the population structure in this area. Pair-wise genetic and geographic distances were generated between sixteen locations that comprised the two clusters. A Mantel test implemented in GENETIX was non-significant after Bonferroni correction (Z = 4131.92; P = 0.055). Additionally, the plot of genetic vs geographic distance displayed a broad scatter around the midpoint with little increasing slope, as evident in the Pearson correlation coefficient (r) = 0.29 (Figure 4). Thus, isolation-by-distance was not significant and did not influence the assignment of individuals to clusters III through V.

Genetic population bottlenecks

The software BOTTLENECK was used to detect recent genetic bottlenecks (within $4N_E$ generations) as an explanation for decreased genetic diversity in some locations. Across all 39 sampling locations, only seven were significant under either the infinite alleles model (IAM) or Two-phase model (TPM). However, only one population, Ringdahl Reservoir, WY (C4) was significant under two-tailed tests for both models (IAM, *P* = 0.00002; TPM, *P* = 0.00168).Low genetic diversity in other populations may be due to more ancient bottlenecks but with sufficient passage of time to allow heterozygosity and allele numbers to equilibrate.

DISCUSSION

Within the last decade, molecular and evolutionary genetics have contributed substantially to conservation and management by providing critical data previously unattainable from traditional ecological studies. For instance, researchers have been able to infer dispersal patterns (Chapuisat et al. 1997), gene flow (Reichow and Smith 2001) and current and historical population dynamics (Roman and Palumbi 2003) for many species of conservation concern. Additionally, molecular methods have been used to delineate taxonomic uncertainties and to identify conservation units (i.e. ESUs and MUs; Moritz 1994). The combination of molecular, morphological and ecological data provides the most comprehensive approach to guide management decisions (Bowen 1999, Bowen and Roman 2005). However, limited funding and lack of information about the basic ecology for many threatened and endangered species often constrains conservation efforts. Here, molecular tools are useful because they can provide insight into both evolutionary history (i.e., patterns) as well as their underlying ecological processes.

Msat analyses have gravitated to the forefront of conservation genetics, molecular ecology and evolutionary biology, due largely to their inherent yet powerful characteristics. Msats are codominant (Sunnucks 2000), which facilitates precise identification of alleles and prevents the loss of information typically found with dominant markers. Msats are selectively neutral (Tachida and Iizuka 1992), which means that observed patterns reflect demographic history rather than selection. In addition, msats are quite prevalent in the genomes of most organisms, including fishes (Christiakov et al. 2006; Zane et al. 2002), thus reducing the effort required for their isolation while concomitantly providing multiple operational loci with ample statistical power. Because msats exhibit high mutation rates, they yield abundant polymorphisms at the population level (Ellegren 2004) an aspect essential for inferring population divergence, bottlenecks, founder events and hybridization (Beaumont and Bruford 1999). Furthermore, generation of msat data is relatively easy and straightforward compared to other molecular genetic data (e.g., sequence data), while their PCR-based amplification is

easily derived from non-invasive sampling regimes (Beaumont and Bruford 1999). Finally, msat primers can be multiplexed (i.e., several loci amplified in a single PCR reaction) thus reducing effort and funds required to generate the molecular data.

Plight of Western Fishes

The Colorado River Basin is the core watershed in the arid Southwest, and is an essential ecosystem for the maintenance of desert biodiversity. However, millions of people also rely on the river for consumptive use and hydropower generation. Consequently, the Colorado River is “one of the most controlled rivers on Earth” (Fradkin 1981), a fact that severely tests the long-term persistence of its endemic fishes. Unfortunately, many of the latter have declined or are declining to the point that restoration has now become a much greater challenge.

Movement patterns and gene flow among populations of indigenous fishes in the Colorado River Basin are poorly understood, and Bluehead Sucker is no exception. The primary focus of this study was to quantify intraspecific genetic variation of Bluehead Sucker as a means of describing population structure and identifying areas in the Colorado River and adjacent basins where molecular divergence has occurred. An understanding of genetic variability inherent in Bluehead Sucker, as well as the underlying processes that shaped this diversity, are necessary for the development of a range-wide management plan. This in turn will allow limited funding to be more effectively allocated, while also identifying populations that can be grouped into practical, cohesive units for purposes of adaptive management.

Large scale patterns of genetic diversity

Broad patterns of genetic structure in Bluehead Sucker uncovered by our msat data were consistent with previous findings based on mitochondrial DNA sequence analysis (Douglas et al. 2006b). Overall, populations from the major basins differed considerably and formed clearly discernable gene pools. In addition, regional patterns were congruent with morphological and taxonomic diversity recognized in Bluehead Sucker (Smith 1966). However, msat data also revealed some unexpected results. All are described in more detail below.

Our analyses uncovered 11 distinct genetic clusters in Bluehead Sucker, seven of which were clearly defined, whereas the remaining three were characterized by high levels of admixture. Genetic clusters and populations contained within each are:

I. Upper Snake River (S1) and Bonneville Basin (B1-3)
II. Ringdahl Reservoir (C4)
III. “North-Utah:” Upper Green River drainage in Wyoming (C1-3) and Yampa River in Colorado (C5-6)
IV. “Central-Utah:” Upper Green River in Utah (C7), Duchesne River (C8), White River (C9), and Upper Colorado River in Colorado (C18)
V. “South-Utah:”Green River below confluence with Price River (C10), Price River drainage (C11-12), Colorado River above confluence with Green River in Colorado (C17) and Utah (C19), Dolores River (C16) and San Juan River in Utah (C23)
VI. San Rafael River drainage (C13-15)
VII. Fremont and Muddy river drainages (C20-22)
VIII. San Juan River drainage in Canyon de Chelly, Arizona (C24)
IX. Colorado River drainage in Grand Canyon, Arizona (C25-29)
X. Zuni River in New Mexico (C30)
XI. Upper Little Colorado River, Arizona (C31-35)

Snake River and Bonneville Basin

Populations from the Bear and Weber rivers (B1-B3) in the Bonneville Basin formed a distinct genetic cluster which included the population from the Upper Snake River (S1). Close similarity among populations in the Bonneville Basin and Snake River was surprising, given the independent history of the two basins since early Pleistocene and divergence of other desert fishes, such as Utah Chub (*Gila atraria*, Johnson 2002) and Leatherside Chub (*Lepidomida copei* and *L. alicia*, Johnson et al. 2004). Based on F_{ST} analysis, the Snake River population (S1) was slightly more divergent from the other three than was the Bear River population (B1) from the two Weber River samples (B2 and B3).

All four populations exhibited moderate levels of genetic diversity and descriptive statistics such as allelic richness and heterozygosity clearly reflected lower numbers when compared to populations in the Colorado River Basin. This can be attributed at one level to the occurrence of these populations within small and isolated streams. Private alleles (those unique to a population) were found in each of the three drainages. One sample from the Weber River revealed none (B2) whereas the other (B3) had a remarkable 11 private alleles. Anecdotal information suggests the latter population consists of mostly senescent individuals with little recruitment (pers. com. P. Aaron Webber, Utah Department of Natural Resources). Together, the four populations revealed 21 private alleles not shared with other Bluehead Sucker in the Colorado River Basin. Given the unique genetic diversity harbored in the Weber and Bear rivers, it would be important to maintain this gene pool and actively manage these populations to prevent their decline and the potential loss of unique alleles.

Colorado River Basin

Bluehead Sucker in the Colorado River Basin was partitioned into 10 genetic clusters, most of which were geographically well-defined and clearly reflected population

structure shaped by drainage isolation. Smith (1966) recognized extensive morphological variation in Bluehead Sucker, another indication that population isolation may be a plausible explanation for divergence.

A distinct regional pattern was also apparent, with elevated genetic diversity in populations from the Green and Upper Colorado river drainages and reduced diversity in downstream Grand Canyon populations (C25-C29) or isolated tributaries such as the Zuni River in New Mexico (C30), Canyon de Chelly in Arizona (C24) or upper Little Colorado River drainage (C31-C35).

A similar pattern of declining downstream genetic diversity has been reported for Flannelmouth Sucker, *Catostomus latipinnis* (Douglas et al. 2003), another native Colorado River catostomid sympatric with *C. discobolus* throughout much of its range. Populations from the Upper Colorado River Basin showed distinctly higher haplotype diversity than did populations from Grand Canyon. Douglas et al. (2003) attributed this to a climate-induced population bottleneck during the early Holocene. They argued that a massive drought forced large-river fishes into Lower Colorado River Basin refugia. As the climate stabilized and the Upper Colorado River Basin became again hospitable, Flannelmouth Sucker expanded and recolonized upstream, leaving a genetic trace that reflects evidence of founder events and population expansion in the different drainages.

In contrast, Dowling et al. (1996) analyzed restriction fragment length polymorphisms (RFLPs) in Razorback Sucker, *Xyrauchen texanus*, another Colorado River endemic and found an opposite pattern (i.e., lower genetic diversity in the Upper Basin, and higher diversity in the Lower Basin). They argued this pattern was inconsistent with an expected hypothesis of downstream larval drift providing unidirectional gene flow with the cumulative effect of generating higher genetic diversity in downstream populations. Instead, Dowling et al. (1996) argued the Lower Colorado River Basin (i.e., drainages below Grand Canyon) represented Pleistocene refugia for Razorback Sucker. Reproduction in this species is severely limited by cold-water temperatures and the Upper Basin during the Pleistocene may have been uninhabitable

due to glacial runoff. Reduced genetic diversity in the Upper Basin may thus reflect multiple extinction-recolonization events. Dowling et al. (1996) further suggested that impoundments have also reduced the genetic diversity through thermally induced population bottlenecks.

Our analyses of mtDNA variation in Bluehead Sucker (Douglas et al. 2006b, 2008) are congruent with regional patterns in msat DNA diversity described above, and suggests that historic, rather than contemporary factors were the driving forces that shaped genetic diversity in Bluehead and Flannelmouth suckers. Similarly to our study, Costello et al. (2003) evaluated msat DNA variation in Bull Charr (*Salvelinus confluentus*) and concluded that patterns of genetic diversity were due to repeated historic extinctions and subsequent founder events associated with Pleistocene glacial advances and retreats. They also argued that historic factors are more important than contemporary population dynamics in shaping patterns of genetic diversity within populations. However, unlike the extensive physical glaciation of Northern North America, the Pleistocene generated climate fluctuations in Southwestern North America, which in turn induced expansion and contraction of populations (Douglas et al. 2006a).

Bluehead Sucker is able to tolerate lower temperatures and steeper gradients than other Colorado River catostomids, allowing it to persist in high-elevation streams typically inhabited by salmonids. If inferences are correct about an early Holocene bottleneck due to river desiccation, then this species may have been one of only a few native fishes that were able to survive in multiple, high elevation refugia in the Upper Colorado River Basin.

Another noteworthy difference in genetic diversity between Upper Colorado Basin and Grand Canyon populations is number of private alleles. However, distribution of private alleles among populations in the Upper Colorado River Basin suggests that *de novo* mutations, not solely historic factors, may have contributed to the observed higher genetic diversity. It is interesting that these populations are able to maintain private alleles despite the indication of extensive gene flow among the tributaries (see below).

Bluehead Sucker in Wyoming

Bluehead Sucker in Wyoming represented the northernmost populations of this species in the Colorado River Basin. They cluster into two different groups. One population, sampled from Ringdahl Reservoir (C4), formed its own cluster and is discussed in more detail below. The other three (C1-C3) grouped with individuals from the Green and Upper Colorado rivers, suggesting high levels of gene flow in upper and lower Green River, at least until recently. Flaming Gorge Dam was completed in 1964, which in turn would have isolated populations in Wyoming. Thus, genetic similarity implies relictual genetic diversity.

In mainstem rivers, Bluehead Sucker can live to 20+ years of age and thus will have overlapping generations. Longevity of this fish may have obscured the recent fragmentation of the river. Lippé et al. (2006) had similar findings when investigating a highly endangered catostomid from eastern North America (i.e., *Moxostoma hubbsi*). This species had higher levels of genetic diversity than expected, and the authors suggested that long generation times might have provided a buffer sufficient for this fish to withstand genetic erosion and to prevent msat DNA divergence.

Clustering of Bluehead Sucker from Ringdahl Reservoir as a divergent group is somewhat surprising. It represents one of few known populations of Bluehead Sucker existing in an impoundment. This location lacks any potential for immigration which makes the population valuable from a management stance, in that barriers prevent invasion of introduced non-native suckers (e.g. *Catostomus commersoni*). Introgressive hybridization between introduced and indigenous catostomids has recently been identified as a major problem in the Upper Green River watershed (Gill et al. 2004, 3005; Kern et al. 2006, 2007; Keith et al. 2003). Due to its isolation, the Ringdahl Reservoir population might represent genetically pure Bluehead Sucker in Wyoming.

However, the population is characterized by very low genetic diversity. Analyses detected a population bottleneck that seemingly occurred within the last 0.5 to 4 effective

population size (N_e) generations. Most dams in the Western North America were built in the early to mid 20th century indicating that the Ringdahl Reservoir population of Bluehead Suckers has been isolated for less than 100 years. Founder events and genetic drift are likely responsible for the genetic characteristics of this population and its low genetic diversity renders it undesirable as a donor population for broodstock establishment or for translocation efforts.

A few additional Bluehead Sucker were collected from the Henrys Fork drainage in Wyoming (within which Ringdahl Reservoir is located). However, scoring difficulties prevented detailed analyses of these individuals or their comparison with Bluehead Sucker from other drainages. Therefore, it was not possible to determine if genetic divergence of Bluehead Sucker in Ringdahl Reservoir is due to ancestry or population demographics.

Bluehead Sucker in Utah

Population structure of Bluehead Sucker in Utah is defined by three main features: (a) high levels of admixture among most populations, (b) a north-to-south gradient of group membership in three clusters, and (c) divergence of populations in western vs eastern tributaries. Each is discussed in more detail below.

The majority of individuals from the Green and Upper Colorado rivers was assigned to three loosely defined genetic clusters, each with high levels of admixture, thus implying extensive gene flow among populations (exceptions discussed below). However, a North-to-South geographic trend in assignment probabilities was apparent. Individuals from the Yampa River (C5-6) exhibited higher (>50%) probability of being assigned to a cluster containing the northern-most populations in the Colorado River Basin [those from Wyoming (C1-3)]. The population just downstream from the confluence of the Yampa and Green rivers reflected a clear transition with ~equal assignment probabilities to the North- and Central-clusters, respectively. Individuals

sampled in downstream drainages, such as Duchesne and White rivers, also showed highest affinity with this Central cluster, as did individuals from the 15-mile reach of the Colorado River in Colorado (C18). The latter is surprising, since populations sampled just downstream (C17 and C19) showed highest affinities to a Southern cluster, which also encompassed individuals from the Price River drainage (C11-12), Desolation Canyon of the Green River (C10), Dolores River (C16), and San Juan River in Utah (C23).

Despite this apparent north-to-south trend, we did not detect an isolation-by-distance effect when genetic vs geographic or river distances were compared among the 16 populations. Our sampling might not be fine-grained enough to allow detection of subtle patterns, which may be further reduced by extensive gene flow among neighboring populations. Nevertheless, detection of this geographic trend suggests large-scale processes as an underlying mechanism and this north-to-south gradient may be a faint signal of ancient basin evolution.

Populations in western tributaries (downstream of Desolation Canyon) represented two divergent groups. Three populations from the San Rafael drainage (C13-15) formed a genetic cluster with little admixture. A similar pattern was found in populations from the Fremont (C20), Dirty Devil (C21) and Muddy (C22) rivers, which also formed a well defined genetic cluster. Smith (1966) noted that predorsal scale counts in Bluehead Sucker from San Rafael, Dirty Devil (Fremont River) and Price River drainages differed from those found in the remainder of the range. This seemingly implies very little gene flow between these tributary populations and the mainstem, and our msat data are congruent with this hypothesis, particularly for San Rafael and Fremont River drainages but not for the Price River drainage.

Individuals from the Price River (C12) exhibited ambiguous assignment, with most showing membership in both the Central- and South-Utah clusters. In contrast, the population from Range Creek, a tributary to the Price River, reflected clear alliance with the South-Utah cluster. Smith (1966) documented a specimen of *Pantosteus* collected

from the Price River that displayed characteristics intermediate between Bluehead and Mountain Sucker (*C. platyrhynchus*), and suggested hybrids may be present in the Price River. Our STRUCTURE analysis indicated that some individuals from the Price River showed elevated affinities with the Grand Canyon cluster. This may in fact be due to differences among individuals within the Price River, rather than their genetic similarity to Grand Canyon Bluehead Sucker. STRUCTURE can allocate individuals to a particular cluster if indeed they are quite divergent from all other clusters. In other words, the program recognizes these individuals as different, and assigns them to a cluster that is sufficiently divergent from all others to accommodate their unusual genotypes, even if they are a poor match to the other genotypes in this cluster. If Smith (1966) is indeed correct in his assessment, then historic introgression and contemporary gene flow may work against one another in the Price River population, preventing substantial divergence while maintaining the introgressed alleles. A range-wide molecular investigation of *C. platyrhynchus* would help to clarify this situation.

Divergence of Bluehead Sucker from western tributaries is interesting, and the processes that have facilitated it are currently unknown. However, certain hypotheses could be considered and tested. For example, are there unique selective regimes in these tributaries that would facilitate their divergence? Differences in morphology, as noted by Smith (1966), would support this notion. Have historic (and undocumented) stream captures transferred fish from the Bonneville Basin? However, the fact that individuals in these populations show no alliance with those from the Bonneville Basin and Snake River would argue against this. Did the western tributaries serve as refugia from adverse climatic events? Slightly reduced genetic diversity in these populations when compared to others in the Colorado River Basin would suggest that isolation may indeed be a factor, with low occurrence of private alleles indicating unidirectional gene flow from the tributaries into the mainstem. These and other potential scenarios warrant further investigation.

Bluehead Sucker in Grand Canyon

Bluehead Sucker in Grand Canyon formed a distinct genetic cluster clearly separated from conspecifics in other parts of the basin, and exhibited little mixed ancestry. Perhaps, the deep canyon-bound reaches inherent to this region have selected for certain genotypes and morphologies, thus allowing populations to diverge while providing selective pressures that act against the immigration of Upper Basin fish. The majority of Grand Canyon fish have pencil-thin caudal peduncles, which differ from the intermediate-to-deep peduncles found in the Upper Basin and its tributaries (Smith 1966). This divergence extends beyond that mediated by Lake Powell and Glen Canyon Dam.

Within Grand Canyon, populations sampled from numerous tributaries differed little, as was evident from low (and non-significant) F_{ST} values. These patterns were mirrored in our msat DNA analysis of Humpback Chub (*Gila cypha*) in the Colorado River Basin (Douglas and Douglas 2007). Humpback Chub from Grand Canyon clustered as a unique gene pool clearly separated from populations in the Upper Colorado River Basin. High levels of gene flow (mostly downstream drift of larvae and juveniles) maintained similarity amongst populations across different reaches of the Grand Canyon.

Oakey et al. (2004) analyzed mtDNA genome variation in Speckled Dace (*Rhinichthys osculus*) and also detected distinct clades representing Upper, Middle and Lower Colorado River Basins. They concluded that these patterns juxtapose with the hypothesis proposed by Minckley et al. (1986) of an ancient origin for the modern ichthyofauna in Western North America, one that reflects drainage evolution since the Oligocene to mid-Miocene. Congruence among data sets derived for three different species inhabiting the Colorado River Basin strongly suggests that common factors might be responsible for distinctness of Grand Canyon populations.

Noteworthy are observations of Bluehead Sucker in Havasu Creek. This population displayed significant linkage disequilibria, which could be indicative of sampling across divergent groups. A potential explanation is that populations above

recognized barriers in Havasu Creek have been isolated for an extended time, and genetic divergence among fishes at the confluence (where specimens were sampled for this study) stems from downstream migration.

Alternatively, genetically divergent alleles could reflect introgression from past hybridization with a closely related species, the Desert Sucker [*C.* (*Pantosteus*) *clarkii*; Smith 1966]. *Catostomus clarkii* is not known to occur upstream of Hoover Dam (Smith 1992), but instead is considered an ecological equivalent species in the Gila River basin that fills a niche similar to that of the Bluehead Sucker of the Colorado River Basin. However, Douglas et al. (2006b, 2008) discovered Desert Sucker mtDNA haplotypes in Bluehead Sucker populations from Grand Canyon. One scenario to explain this is hybridization between the two species. Since they are not known to co-occur, an historic contact zone must be invoked to explain the exchange of genetic material between the two. A level of validity for this hypothesis is the fact that mtDNA haplotypes characteristic of Sonora Sucker, *C. insignis*, were detected among Flannelmouth Sucker populations in Grand Canyon (Douglas et al. 2003). Sonora Sucker similarly inhabits the Gila River basin and tributaries, and is considered an ecological equivalent to the Flannelmouth Sucker.

Bluehead Sucker in Canyon de Chelly

Our analysis also included 56 Bluehead Sucker from four locations in and around Canyon de Chelly National Monument in Arizona. Due to small sample sizes in three of the four locations, all individuals were pooled into a single population (C24) for analyses. The Canyon de Chelly sample exhibited low genetic diversity, comparable to levels found in other isolated populations, such as Bluehead Sucker in the Snake River and Bonneville Basin or the Zuni River drainage. Similar to these populations, they also formed a distinct genetic group and showed little affinity to any of the other nine clusters. Noteworthy is distinctness of Bluehead Sucker in Canyon de Chelly from populations downstream in the San Juan River (C23), further underscoring their isolation.

Bluehead Sucker from the Zuni River

The Zuni River drainage was represented by a single population, Agua Remora (C30), characterized by very low genetic diversity, and which formed a distinct cluster exclusive from other Bluehead Sucker..Genetic distinctness of Bluehead Sucker from the Zuni River is congruent with its designation as a distinct subspecies, *C. d. yarrowi*. The latter is recognized as a candidate for federal listing as endangered and exists solely as isolated populations in headwater tributaries (Carman 2004). Its low genetic diversity suggests small population sizes and high levels of isolation, both of which are recognized as detrimental to long term persistence of this unique gene pool. A recovery plan for the Zuni Bluehead Sucker (Carman 2004) has been drafted to guide management actions geared towards preservation of this unique gene pool.

Bluehead Sucker from the Upper Little Colorado River

The upper Little Colorado River drainage was represented by a five populations, (C31-C35), characterized by moderate genetic diversity. Individuals from these locations formed a distinct cluster exclusive from other populations. Genetic distinctness of Bluehead Sucker from in the Upper Little Colorado River is congruent with Minckley's (1973) observations about its distinct morphology (thickened body shapes, short fins, small sizes). According to Smith (1966), an ancient stream capture between the Little Colorado River and the Rio Grande basins could have facilitated hybridization between *C. discobolus* and *C. plebeius*, and resulting introgression of characters would explain its morphological distinctiveness. Our analyses did not show closer affinity of populations in the upper Little Colorado River with those from drainages in geographic proximity, including Canyon de Chelly and Zuni River.

Conservation and Management Implications

Bluehead Sucker is a highly vagile species with a potential for gene flow over large areas. In this sense, its life history is congruent with that of other stream fishes that in fact disperse much farther than expected (Gowan and Fausch 1996). Little is known about the range-wide population structure of this species, and this combined with insufficient knowledge of its ecology and basic life history attributes, have limited management options.

Our study examined population structure in Bluehead Sucker across its range by assessing msat DNA diversity across 1,092 individuals sampled from 39 locations. Our results suggest 11 distinct gene pools, most of which are geographically well defined. Some are congruent with known biogeography barriers (Bonneville Basin vs Colorado River) and others with recognized morphological diversity (Zuni Bluehead Sucker, Little Colorado River). Populations in isolated tributaries are generally characterized by reduced genetic diversity.

Our findings reinforce the argument that gene flow among tributaries is important and must be considered as a significant factor in sustaining current levels of genetic diversity displayed by the species. Mortiz (1994) argued that management units should be identified based on significant divergence of nuclear allele frequencies. If indeed we based our designation of management units on this definition, then Bluehead Sucker could be partitioned into 10 management units in the Colorado River Basin plus one in the Bonneville Basin/ Snake River drainage. Specific considerations are provided below.

Consideration 1: Our analyses revealed that Bluehead Sucker is partitioned into 10 distinct genetic clusters. Most of these should be managed as a distinct entity, albeit with some caveats (explained in more detail below). The 11 management units roughly correspond to:

I. Upper Snake River and Bonneville Basin
II. Ringdahl Reservoir
III. “North-Utah:” Upper Green River drainage in Wyoming and Yampa River in Colorado
IV. “Central-Utah:” Upper Green River in Utah, Duchesne River, White River, and Upper Colorado River in Colorado
V. “South-Utah:” Green River below confluence with Price River, Price River drainage, Colorado River above confluence with Green River in Colorado and Utah, Dolores River and San Juan River in Utah
VI. San Rafael River drainage
VII. Fremont and Muddy river drainages
VIII. San Juan River drainage in Canyon de Chelly, Arizona
IX. Colorado River drainage in Grand Canyon, Arizona
X. Zuni River in New Mexico
XI. Upper Little Colorado River, Arizona

Consideration 2: Bluehead Sucker in Weber, Bear and Upper Snake rivers are clearly distinct from their conspecifics in the Colorado River Basin. Given the unique genetic diversity represented by these populations, management actions should be directed towards their long-term persistence. Further, to prevent reduction of genetic diversity and loss of unique alleles, efforts should be made to maintain actively reproducing populations that exhibit a natural age structure and are of sufficient size.

Consideration 3: Bluehead Sucker inhabiting the Green and Upper Colorado rivers are allocated to three genetic clusters that show high levels of admixture. These populations could be treated as a single management unit. However, two aspects should be considered.

Current genetic diversity within populations is high and reflects extensive gene flow throughout the region, but habitat alterations have now truncated migration of individuals among certain tributaries and reaches. In the long term, populations may diverge through genetic drift (loss of rare alleles, change in allele frequencies). To counteract this process, connectivity amongst populations within specific areas should be maintained to facilitate natural gene flow, albeit at a reduced scale.

Focusing on subsets of populations within the Green and Upper Colorado river basins would also maintain the observed north-to-south gradient in assignment probabilities found within the three genetic clusters. Implementing similar management plans for separate areas within the region would effectively result in generating replicate sets of populations. This in turn would be additional assurance that the genetic diversity of the Green and Upper Colorado river region is maintained.

Consideration 4: In addition to the larger Green and Upper Colorado river management group, two separate management units should be established for populations from western tributaries in Utah. One encompasses populations in the San Rafael drainage, whereas the other includes the Muddy and Fremont river drainages.

Bluehead Sucker in these areas represents two distinct genetic clusters, but the processes responsible for this subdivision are not clear. Divergence of these populations could echo ancient basin evolution, or simply reflect isolation and lack of gene flow. Distinct gene pools could also indicate local adaptation or, alternatively, introgression by another species, such as Mountain Sucker.. Comparing ecology of Bluehead Sucker in these tributaries vs those from other areas might also provide additional data, if indeed these populations exhibit unique life history characteristics, habitat requirements or physiological preferences.

Consideration 5: Similarly to the situation above, Bluehead Sucker in Grand Canyon, Canyon de Chelly and the upper Little Colorado River also represent three distinct genetic groups and should be managed as separate units. Distinctness of Grand Canyon populations vs those from the Upper Colorado River Basin is mirrored by other species, such as Humpback Chub and Speckled Dace, and likely reflects ancient basin evolution, potentially combined with local adaptation.

In the current study, Bluehead Sucker in Canyon de Chelly did not show any affinity to the single population from the Zuni River, suggesting the former do not represent the *C. d. yarrowi* subspecies. However, status of Zuni Bluehead Sucker and populations in adjacent drainages in the Upper Little Colorado River drainage is currently being examined in an ongoing study by researchers at Arizona State University.

Consideration 6: The remaining two genetic clusters are represented by Bluehead Sucker in Ringdahl Reservoir and the population of Agua Remora in the Zuni River drainage. Each contains a single population and both are characterized by low genetic diversity, suggesting their distinctiveness is caused by isolation and genetic drift.

Due to its isolation, the population in Ringdahl Reservoir is sheltered from introgressive hybridization by introduced White and Longnose sucker, and thus could potentially represent a pure source of Bluehead Sucker genes. However, the reduced genetic diversity in this population is a roadblock against using these fish for translocation, supplementation or broodstock establishment. Likely, any of these actions would involve small numbers of individuals, thus inducing an additional population bottleneck that would further reduce genetic diversity in subsequent generations. It also appears that this population represent a subset of alleles found in the other populations in the Upper Green River drainage, and forms a distinct genetic cluster simply due to differences in allele frequencies, and not because unique alleles are present.

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TABLES

Table 1. Summary of 16 microsatellite loci used to genotype 1,092 *Catostomus discobolus.* Loci were developed by Tranah et al. (2001). Listed are for each locus: repeat motif (Di = two nucleotides; Tetra = four nucleotides), total number of alleles (#A), allele range (in base pairs) and expected heterozygosity (H_E) across all populations.

Locus	Motif	#A	Range (bp)	H_E
Dlu27	Di	25	187-261	0.66
Dlu209	Di	41	132-248	0.67
Dlu229	Di	26	120-180	0.56
Dlu230	Di	28	100-176	0.60
Dlu233	Di	17	117-191	0.69
Dlu245	Di	10	185-229	0.51
Dlu257	Di	57	147-515	0.81
Dlu276	Di	27	109-175	0.68
Dlu409	Tetra	30	144-258	0.89
Dlu434	Tetra	28	178-310	0.73
Dlu456	Tetra	40	142-310	0.87
Dlu482	Tetra	28	140-280	0.83
Dlu4153	Tetra	42	167-335	0.90
Dlu4184	Tetra	31	154-282	0.86
Dlu4235	Tetra	53	171-463	0.88
Dlu4300	Tetra	39	186-338	0.91

Table 2. Results from MICROCHECKER analysis. Data were based on 16 microsatellite loci genotyped across 1,092 *Catostomus discobolus* collected from 39 locations. Each locus was evaluated for potential null alleles, scoring errors or large allele drop-out in each population. Listed are loci for which a potential problem was detected in a particular population.

Locus	Population	Potential problem
Dlu 27	C26	null allele
Dlu 233	C12	null allele
Dlu 233	C27	null allele
Dlu 233	C29	null allele
Dlu 245	C11	null allele
Dlu 276	C30	scoring error or null allele
Dlu 434	C28	null allele
Dlu 4153	C12	null allele
Dlu 4153	C18	null allele

Table 3. Overview of *Catostomus discobolus* sampled from 39 locations in three major river basins. Listed are: Basin (SR = Snake River; BB = Bonneville Basin; CR = Colorado River); drainage within basin; sampling locality, map ID number, and numbers of individuals assessed from each location. Detailed sampling information is provided in Appendix 1 and sampling localities are depicted in Figure 1.

Basin	Major Drainage	Location	ID	*N*
SR	Snake R	Snake River, WY	S1	30
BB	Bear R	Bear River, WY	B1	21
BB	Weber R	Weber River, UT	B2	34
BB	Weber R	Weber River 2, UT	B3	21
CR	Big Sandy R	Big Sandy River, WY	C1	28
CR	Big Sandy R	Little Sandy River, WY	C2	17
CR	Little Snake R	Muddy Creek, WY	C3	23
CR	Henry's Fork	Ringdahl Reservoir, WY	C4	33
CR	Yampa R	Yampa River, CO	C5	28
CR	Yampa R	Yampa River - Lily Park, CO	C6	37
CR	Upper Green R	Green River - Split Mountain, UT	C7	16
CR	Duchesne R	Strawberry Reservoir, UT	C8	28
CR	White R	White River, UT	C9	10
CR	Green R	Desolation Canyon, UT	C10	46
CR	Green R	Range Creek, UT	C11	24
CR	Price R	Price River, UT	C12	28
CR	San Rafael R	San Rafael River, UT	C13	30
CR	San Rafael R	Joe's Valley Reservoir, UT	C14	25
CR	San Rafael R	Millsite Reservoir, UT	C15	28
CR	Dolores R	Dolores River, UT	C16	30
CR	Upper Colorado R	Black Rocks, CO	C17	18
CR	Upper Colorado R	15-mile reach, CO	C18	26
CR	Upper Colorado R	West Water Canyon, CO	C19	22
CR	Dirty Devil	Fremont River, UT	C20	40
CR	Dirty Devil	Dirty Devil, UT	C21	46
CR	Dirty Devil	Muddy River, UT	C22	25
CR	San Juan R	San Juan River, UT	C23	30
CR	San Juan R	Canyon de Chelly, AZ	C24	56
CR	Colorado R-Grand Cn	Little Colorado R - Grand Cn, AZ	C25	38
CR	Colorado R-Grand Cn	Shinumo Creek, AZ	C26	62
CR	Colorado R-Grand Cn	Kanab Creek, AZ	C27	21
CR	Colorado R-Grand Cn	Matkatamiba Canyon, AZ	C28	18
CR	Colorado R-Grand Cn	Havasu Creek, AZ	C29	52
CR	Zuni R	Agua Remora, NM	C30	21
CR	Willow Creek, AZ	Little Colorado River, AZ	C31	16
CR	Silver Creek, AZ	Little Colorado River, AZ	C32	19
CR	LCR - Wenima, AZ	Little Colorado River, AZ	C33	18
CR	Nutrioso Creek, AZ	Little Colorado River, AZ	C34	10
CR	East Fork Little Colorado	Little Colorado River, AZ	C35	17

Table 4. Genetic diversity among 39 populations of *Catostomus discobolus* assessed over 16 microsatellite loci. Listed are population ID number, sample size (*N*), mean number of alleles (A_M), allelic richness (A_R), number of private alleles (A_{PR}) and mean expected (H_E) and observed heterozygosity (H_O) with associated standard deviations (SD) for each statistic. Population ID numbers are explained in Table 3 and detailed sampling information is provided in Appendix 1.

ID	*N*	A_M	SD	A_R	SD	A_{PR}	H_E	SD	H_O	SD
S1	30	10.4	7.2	5.5	3.0	3	0.67	0.32	0.67	0.32
B1	21	8.4	5.1	5.1	2.7	3	0.64	0.30	0.62	0.34
B2	34	7.1	4.9	4.3	2.2	-	0.59	0.31	0.59	0.32
B3	21	8.9	4.6	5.1	2.2	11	0.64	0.26	0.64	0.27
C1	28	11.8	5.0	6.4	1.9	3	0.80	0.12	0.81	0.15
C2	17	9.7	3.5	6.3	1.7	1	0.80	0.10	0.79	0.13
C3	23	10.3	3.9	5.9	2.0	3	0.74	0.19	0.74	0.17
C4	33	6.1	2.2	4.6	1.2	-	0.72	0.12	0.74	0.11
C5	28	14.1	5.3	7.0	1.9	7	0.82	0.12	0.80	0.14
C6	37	15.4	6.5	7.1	2.0	6	0.83	0.12	0.82	0.13
C7	16	11.3	4.8	6.8	2.1	2	0.79	0.15	0.78	0.15
C8	28	12.9	6.0	6.7	2.1	3	0.82	0.12	0.83	0.12
C9	10	9.2	3.3	6.9	2.2	4	0.83	0.17	0.79	0.16
C10	46	15.6	6.3	6.9	1.9	2	0.84	0.10	0.82	0.11
C11	24	10.7	4.9	6.1	2.0	1	0.77	0.15	0.78	0.18
C12	28	15.0	5.2	7.2	1.7	15	0.85	0.09	0.84	0.08
C13	30	11.4	5.2	5.9	1.9	3	0.76	0.16	0.75	0.17
C14	25	9.9	4.9	5.7	2.1	1	0.76	0.15	0.75	0.19
C15	28	10.0	4.8	5.6	1.9	-	0.75	0.16	0.77	0.17
C16	30	14.4	6.4	6.9	2.1	3	0.81	0.14	0.84	0.15
C17	18	11.4	4.9	6.7	2.1	-	0.80	0.13	0.81	0.12
C18	26	15.2	7.0	7.2	2.1	8	0.83	0.13	0.81	0.15
C19	22	13.2	5.3	7.1	2.0	5	0.83	0.12	0.79	0.16
C20	40	12.6	6.6	6.3	2.1	2	0.78	0.16	0.79	0.16
C21	46	12.2	6.4	6.0	2.2	-	0.76	0.17	0.76	0.17
C22	25	10.9	5.3	6.1	2.1	-	0.77	0.14	0.79	0.15
C23	30	12.8	6.0	6.6	2.2	2	0.81	0.13	0.84	0.15
C24	56	7.9	3.9	4.2	1.4	5	0.66	0.15	0.61	0.16
C25	38	9.6	4.9	5.4	1.9	2	0.75	0.15	0.78	0.15
C26	62	9.6	4.4	4.9	1.5	-	0.73	0.14	0.70	0.15
C27	21	8.6	3.5	5.4	1.8	-	0.74	0.16	0.75	0.19
C28	18	7.6	3.5	5.1	1.8	-	0.72	0.14	0.72	0.16
C29	52	10.1	3.9	4.8	1.4	1	0.67	0.17	0.62	0.19
C30	21	2.6	1.3	2.3	1.0	-	0.36	0.26	0.34	0.25
C31	16	6.4	3.3	4.7	2.0	15	0.68	0.18	0.63	0.26
C32	19	5.8	2.0	4.4	1.4	7	0.68	0.18	0.70	0.20
C33	18	9.1	3.8	5.7	1.9	3	0.74	0.16	0.72	0.21
C34	10	6.1	2.4	5.0	1.8		0.68	0.20	0.67	0.22
C35	17	6.9	2.8	4.8	1.7		0.66	0.21	0.64	0.25

Table 5. Pair-wise F_{ST} values for 39 populations of *Catostomus discobolus*. Statistics were calculated in FSTAT and 95% bootstrap confidence interval for each value was 0.10 - 0.167. Shaded cells indicate non-significant values and were based on G Exact Test for population differentiation computed in GENEPOP. Significance was assessed at α = 0.05.

	S1	B1	B2	B3	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35
S1																																							
B1	0.08																																						
B2	0.08	0.06																																					
B3	0.07	0.05	0.01																																				
C1	0.19	0.20	0.24	0.20																																			
C2	0.20	0.21	0.25	0.21	0.01																																		
C3	0.21	0.22	0.26	0.23	0.04	0.05																																	
C4	0.25	0.25	0.29	0.26	0.08	0.09	0.14																																
C5	0.19	0.19	0.23	0.19	0.02	0.03	0.02	0.10																															
C6	0.17	0.17	0.21	0.18	0.03	0.03	0.03	0.08	0.00																														
C7	0.20	0.21	0.25	0.21	0.05	0.05	0.09	0.12	0.05	0.04																													
C8	0.17	0.17	0.21	0.17	0.04	0.04	0.06	0.09	0.03	0.02	0.04																												
C9	0.20	0.21	0.26	0.21	0.04	0.04	0.06	0.10	0.03	0.02	0.03	0.02																											
C10	0.17	0.17	0.21	0.18	0.03	0.03	0.04	0.08	0.01	0.01	0.05	0.02	0.02																										
C11	0.20	0.21	0.26	0.22	0.06	0.07	0.07	0.12	0.06	0.05	0.09	0.05	0.05	0.03																									
C12	0.18	0.18	0.23	0.19	0.04	0.03	0.06	0.08	0.03	0.02	0.05	0.03	0.03	0.01	0.02																								
C13	0.24	0.25	0.29	0.25	0.09	0.07	0.11	0.12	0.06	0.06	0.07	0.05	0.06	0.06	0.09	0.06																							
C14	0.24	0.24	0.28	0.25	0.09	0.08	0.11	0.12	0.07	0.07	0.08	0.06	0.06	0.06	0.09	0.06	0.01																						
C15	0.24	0.25	0.29	0.25	0.09	0.07	0.11	0.12	0.07	0.06	0.07	0.06	0.06	0.06	0.10	0.06	0.01	0.01																					
C16	0.18	0.18	0.23	0.19	0.04	0.04	0.06	0.10	0.02	0.02	0.05	0.02	0.02	0.01	0.04	0.02	0.06	0.07	0.06																				
C17	0.19	0.19	0.24	0.20	0.06	0.06	0.08	0.11	0.04	0.03	0.07	0.02	0.04	0.02	0.04	0.02	0.07	0.07	0.07	0.01																			
C18	0.18	0.18	0.22	0.18	0.03	0.03	0.06	0.08	0.02	0.02	0.05	0.02	0.03	0.01	0.05	0.01	0.06	0.06	0.05	0.01	0.01																		
C19	0.18	0.19	0.23	0.19	0.03	0.03	0.06	0.10	0.02	0.02	0.05	0.02	0.02	0.01	0.05	0.02	0.07	0.07	0.06	0.00	0.00	0.00																	
C20	0.20	0.21	0.25	0.21	0.07	0.07	0.08	0.12	0.06	0.04	0.09	0.04	0.05	0.04	0.05	0.04	0.09	0.08	0.09	0.04	0.04	0.03	0.04																
C21	0.21	0.21	0.25	0.22	0.07	0.07	0.08	0.12	0.06	0.05	0.10	0.04	0.06	0.04	0.05	0.04	0.09	0.09	0.09	0.04	0.04	0.03	0.04	0.00															
C22	0.20	0.21	0.25	0.21	0.08	0.08	0.09	0.12	0.07	0.05	0.10	0.05	0.07	0.05	0.05	0.04	0.10	0.09	0.09	0.05	0.04	0.03	0.04	0.01	0.01														
C23	0.19	0.19	0.23	0.20	0.04	0.04	0.06	0.09	0.02	0.02	0.06	0.02	0.04	0.01	0.04	0.02	0.07	0.07	0.07	0.02	0.02	0.02	0.01	0.04	0.04	0.05													
C24	0.27	0.28	0.30	0.28	0.17	0.16	0.17	0.20	0.13	0.12	0.18	0.15	0.15	0.12	0.14	0.12	0.17	0.17	0.17	0.13	0.14	0.12	0.13	0.13	0.13	0.13	0.12												
C25	0.22	0.22	0.27	0.23	0.09	0.09	0.12	0.14	0.08	0.07	0.09	0.06	0.07	0.05	0.06	0.04	0.10	0.10	0.10	0.05	0.05	0.06	0.05	0.08	0.08	0.08	0.06	0.15											
C26	0.23	0.23	0.27	0.24	0.11	0.11	0.13	0.15	0.10	0.09	0.12	0.08	0.10	0.07	0.09	0.06	0.12	0.12	0.12	0.07	0.06	0.07	0.07	0.09	0.09	0.10	0.07	0.16	0.03										
C27	0.22	0.23	0.27	0.23	0.09	0.08	0.11	0.14	0.08	0.07	0.08	0.05	0.07	0.05	0.06	0.04	0.09	0.10	0.10	0.05	0.04	0.05	0.05	0.07	0.08	0.08	0.05	0.15	0.00	0.02									
C28	0.24	0.24	0.29	0.25	0.10	0.10	0.13	0.14	0.09	0.08	0.10	0.07	0.08	0.06	0.07	0.05	0.10	0.10	0.10	0.06	0.05	0.07	0.05	0.08	0.09	0.09	0.06	0.15	0.00	0.00	0.00								
C29	0.27	0.27	0.31	0.28	0.13	0.12	0.15	0.17	0.11	0.10	0.13	0.10	0.10	0.09	0.08	0.08	0.12	0.13	0.13	0.08	0.08	0.10	0.09	0.11	0.11	0.12	0.08	0.18	0.03	0.05	0.03	0.02							
C30	0.41	0.45	0.44	0.44	0.35	0.36	0.39	0.40	0.33	0.32	0.36	0.33	0.39	0.32	0.39	0.33	0.36	0.37	0.37	0.34	0.38	0.34	0.34	0.36	0.37	0.38	0.35	0.40	0.36	0.36	0.39	0.40	0.37						
C31	0.27	0.29	0.32	0.29	0.19	0.19	0.22	0.24	0.17	0.16	0.18	0.16	0.19	0.16	0.20	0.16	0.19	0.19	0.20	0.17	0.17	0.16	0.16	0.20	0.21	0.20	0.17	0.27	0.19	0.22	0.20	0.21	0.24	0.43					
C32	0.26	0.29	0.32	0.29	0.18	0.17	0.21	0.23	0.17	0.16	0.18	0.16	0.18	0.17	0.20	0.16	0.20	0.21	0.21	0.18	0.19	0.17	0.16	0.21	0.21	0.21	0.18	0.26	0.19	0.21	0.20	0.20	0.23	0.36	0.20				
C33	0.22	0.23	0.25	0.22	0.14	0.12	0.16	0.16	0.12	0.11	0.13	0.12	0.13	0.12	0.15	0.12	0.15	0.15	0.15	0.13	0.13	0.11	0.12	0.14	0.15	0.14	0.13	0.20	0.15	0.17	0.16	0.16	0.19	0.37	0.18	0.16			
C34	0.25	0.26	0.29	0.25	0.15	0.14	0.17	0.18	0.13	0.12	0.14	0.12	0.14	0.13	0.15	0.12	0.15	0.14	0.15	0.14	0.14	0.12	0.13	0.15	0.15	0.14	0.13	0.20	0.15	0.17	0.15	0.15	0.18	0.43	0.20	0.17	0.03		
C35	0.26	0.28	0.30	0.27	0.19	0.18	0.22	0.23	0.17	0.16	0.18	0.17	0.19	0.17	0.20	0.16	0.20	0.20	0.20	0.18	0.19	0.16	0.17	0.20	0.20	0.20	0.17	0.26	0.20	0.22	0.20	0.21	0.23	0.39	0.21	0.18	0.06	0.09	

Table 6. Hierarchical analysis of molecular variance (AMOVA) based on 16 microsatellite loci genotyped across 39 populations of *Catostomus discobolus*. Variance components were significant with P < 0.0000 based on 16,000 permutations.

Source of Variation	Sum of Squares	Variance Component	Percentage Variation
Among Drainages	530.99	1.12	14.25
Among Populations / within Drainages	1620.75	0.71	9.01
Within Populations	12779.07	6.04	76.74
Total	14930.81	7.87	

Table 7. Proportion of membership of each pre-defined population (= Pop) in each of the designated clusters using program Structure at K=11. Populations are defined in Table 3 and clusters are depicted in Figure 3. Membership of populations within clusters is indicated by shading, with multiple cells marked indicating ambiguous assignment of a populations to a particular cluster.

Pop	Cluster										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
S1	0.881	0.004	0.009	0.018	0.018	0.010	0.015	0.009	0.007	0.006	0.022
B1	0.874	0.004	0.005	0.061	0.011	0.005	0.010	0.004	0.014	0.005	0.007
B2	0.976	0.002	0.003	0.004	0.002	0.003	0.002	0.002	0.002	0.003	0.002
B3	0.863	0.002	0.004	0.108	0.004	0.004	0.003	0.002	0.002	0.004	0.003
C1	0.003	0.031	0.862	0.030	0.045	0.006	0.007	0.003	0.005	0.005	0.003
C2	0.005	0.025	0.880	0.027	0.017	0.011	0.006	0.006	0.010	0.009	0.005
C3	0.003	0.005	0.826	0.097	0.022	0.012	0.011	0.006	0.012	0.002	0.004
C4	0.002	0.968	0.004	0.003	0.004	0.003	0.005	0.005	0.004	0.001	0.002
C5	0.004	0.015	0.677	0.137	0.099	0.018	0.010	0.010	0.020	0.005	0.006
C6	0.007	0.019	0.510	0.240	0.098	0.038	0.037	0.012	0.023	0.010	0.006
C7	0.008	0.014	0.335	0.393	0.170	0.029	0.007	0.004	0.025	0.006	0.008
C8	0.012	0.014	0.138	0.499	0.173	0.051	0.052	0.006	0.027	0.007	0.020
C9	0.004	0.014	0.194	0.416	0.321	0.021	0.009	0.005	0.009	0.003	0.004
C10	0.006	0.020	0.228	0.181	0.420	0.037	0.022	0.009	0.065	0.004	0.006
C11	0.004	0.008	0.061	0.025	0.819	0.006	0.041	0.010	0.019	0.002	0.005
C12	0.004	0.012	0.035	0.310	0.408	0.033	0.040	0.013	0.132	0.003	0.010
C13	0.002	0.005	0.030	0.039	0.013	0.876	0.014	0.005	0.011	0.003	0.003
C14	0.008	0.006	0.009	0.015	0.008	0.927	0.008	0.005	0.007	0.002	0.004
C15	0.002	0.005	0.013	0.009	0.011	0.922	0.017	0.004	0.011	0.002	0.004
C16	0.005	0.011	0.098	0.242	0.533	0.020	0.034	0.006	0.038	0.006	0.008
C17	0.004	0.019	0.063	0.152	0.562	0.015	0.108	0.004	0.063	0.003	0.007
C18	0.004	0.022	0.172	0.506	0.179	0.042	0.042	0.014	0.010	0.007	0.004
C19	0.004	0.005	0.143	0.300	0.448	0.007	0.033	0.009	0.043	0.005	0.004
C20	0.004	0.020	0.013	0.017	0.024	0.010	0.870	0.010	0.024	0.004	0.005
C21	0.003	0.013	0.006	0.011	0.011	0.013	0.911	0.010	0.014	0.003	0.004
C22	0.007	0.007	0.007	0.007	0.014	0.010	0.928	0.007	0.008	0.002	0.003
C23	0.006	0.011	0.117	0.355	0.381	0.012	0.028	0.037	0.027	0.004	0.021
C24	0.003	0.004	0.015	0.014	0.006	0.007	0.006	0.936	0.005	0.002	0.002
C25	0.004	0.009	0.013	0.014	0.034	0.014	0.019	0.006	0.876	0.003	0.009
C26	0.003	0.003	0.006	0.010	0.012	0.006	0.008	0.003	0.944	0.003	0.003
C27	0.006	0.006	0.018	0.037	0.046	0.011	0.008	0.004	0.852	0.002	0.011
C28	0.005	0.005	0.006	0.008	0.016	0.003	0.005	0.003	0.943	0.002	0.004
C29	0.003	0.007	0.017	0.019	0.026	0.006	0.009	0.004	0.899	0.004	0.006
C30	0.002	0.001	0.002	0.002	0.002	0.002	0.001	0.001	0.002	0.983	0.003
C31	0.003	0.008	0.004	0.002	0.004	0.004	0.009	0.013	0.002	0.004	0.946
C32	0.003	0.003	0.003	0.009	0.002	0.004	0.004	0.003	0.008	0.002	0.960
C33	0.009	0.008	0.007	0.005	0.007	0.011	0.066	0.006	0.012	0.004	0.864
C34	0.007	0.005	0.006	0.015	0.008	0.006	0.006	0.006	0.003	0.003	0.936
C35	0.003	0.004	0.004	0.005	0.008	0.007	0.006	0.008	0.006	0.004	0.945

Table 8. Pair-wise matrices generated to test for isolation by distance among 16 *Catostomus discobolus* populations sampled from the Upper Colorado River Basin using a Mantel test. Upper matrix shows pair-wise genetic distance FST/1-FST; lower matrix contains pair-wise river distances in kilometers. Populations are defined in Table 3.

Pop	C1	C2	C3	C5	C6	C7	C8	C9	C10	C11	C12	C16	C17	C18	C19
C2	0.01														
C3	0.04	0.05													
C5	0.02	0.03	0.03												
C6	0.03	0.03	0.03	0.00											
C7	0.06	0.05	0.09	0.05	0.04										
C8	0.04	0.04	0.06	0.03	0.02	0.04									
C9	0.04	0.04	0.06	0.03	0.02	0.03	0.02								
C10	0.03	0.03	0.04	0.01	0.01	0.05	0.02	0.02							
C11	0.07	0.07	0.08	0.06	0.05	0.10	0.05	0.05	0.03						
C12	0.04	0.04	0.06	0.03	0.02	0.05	0.03	0.03	0.01	0.02					
C16	0.04	0.04	0.06	0.02	0.02	0.05	0.02	0.02	0.01	0.04	0.02				
C17	0.06	0.06	0.08	0.04	0.03	0.07	0.02	0.04	0.02	0.04	0.02	0.01			
C18	0.04	0.03	0.07	0.02	0.02	0.05	0.02	0.03	0.01	0.05	0.01	0.01	0.01		
C19	0.03	0.04	0.06	0.02	0.02	0.05	0.02	0.03	0.01	0.05	0.02	0.00	0.00	0.00	
C23	0.04	0.04	0.06	0.02	0.02	0.06	0.02	0.04	0.01	0.04	0.02	0.02	0.02	0.02	0.01

Pop	C1	C2	C3	C5	C6	C7	C8	C9	C10	C11	C12	C16	C17	C18	C19
C2	53														
C3	329	329													
C5	292	295	281												
C6	318	322	308	27											
C7	321	324	310	29	56										
C8	538	541	527	246	273	217									
C9	420	424	410	128	155	100	176								
C10	604	607	593	312	339	283	256	151							
C11	591	594	580	299	326	270	249	140	35						
C12	699	703	689	407	434	379	320	219	102	130					
C16	993	997	983	702	728	673	645	541	389	425	492				
C17	1037	1041	1027	746	772	717	689	420	433	469	536	89			
C18	1104	1107	1093	812	839	783	755	486	500	535	602	155	66		
C19	1014	1018	1003	722	749	694	666	396	410	445	513	66	23	89	
C23	1276	1279	1265	984	1011	955	927	823	672	707	774	626	670	736	647

Table 9. Results BOTTLENECK analysis to detect recent (within $4N_E$ generations) genetic bottlenecks within 39 populations of *Catostomus discobolus* genotyped across 16 microsatellite loci. Provided are probability values of Wilcoxon signed rank tests (two tailed: Heterozygote excess and deficiency) for the Infinite Alleles Model (IAM) and the Two-Phase Model (TPM). Significance was determined at Bonferroni adjusted α = 0.0031. Populations are defined in Table 3.

Pop	IAM	TPM
S1	0.002	0.561
B1	0.252	0.597
B2	0.095	0.679
B3	0.495	0.011
C1	0.025	0.632
C2	0.021	0.899
C3	0.348	0.175
C4	**0.000**	**0.002**
C5	0.375	0.562
C6	0.025	0.860
C7	0.159	0.231
C8	**0.001**	0.562
C9	0.274	0.860
C10	0.021	0.706
C11	0.051	0.117
C12	0.033	0.175
C13	0.322	0.058
C14	0.016	0.706
C15	0.029	0.065
C16	0.073	0.323
C17	0.144	0.375
C18	0.044	0.274
C19	0.008	0.183
C20	0.083	0.979
C21	0.034	0.589
C22	0.011	0.768
C23	**0.001**	1.000
C24	0.011	0.025
C25	**0.000**	0.899
C26	0.009	0.404
C27	**0.001**	0.175
C28	0.013	0.433
C29	0.528	**0.000**
C30	0.027	0.339
C31	0.008	0.821
C32	0.001	0.159
C33	0.528	0.129
C34	0.044	0.821
C35	0.404	0.117

FIGURES

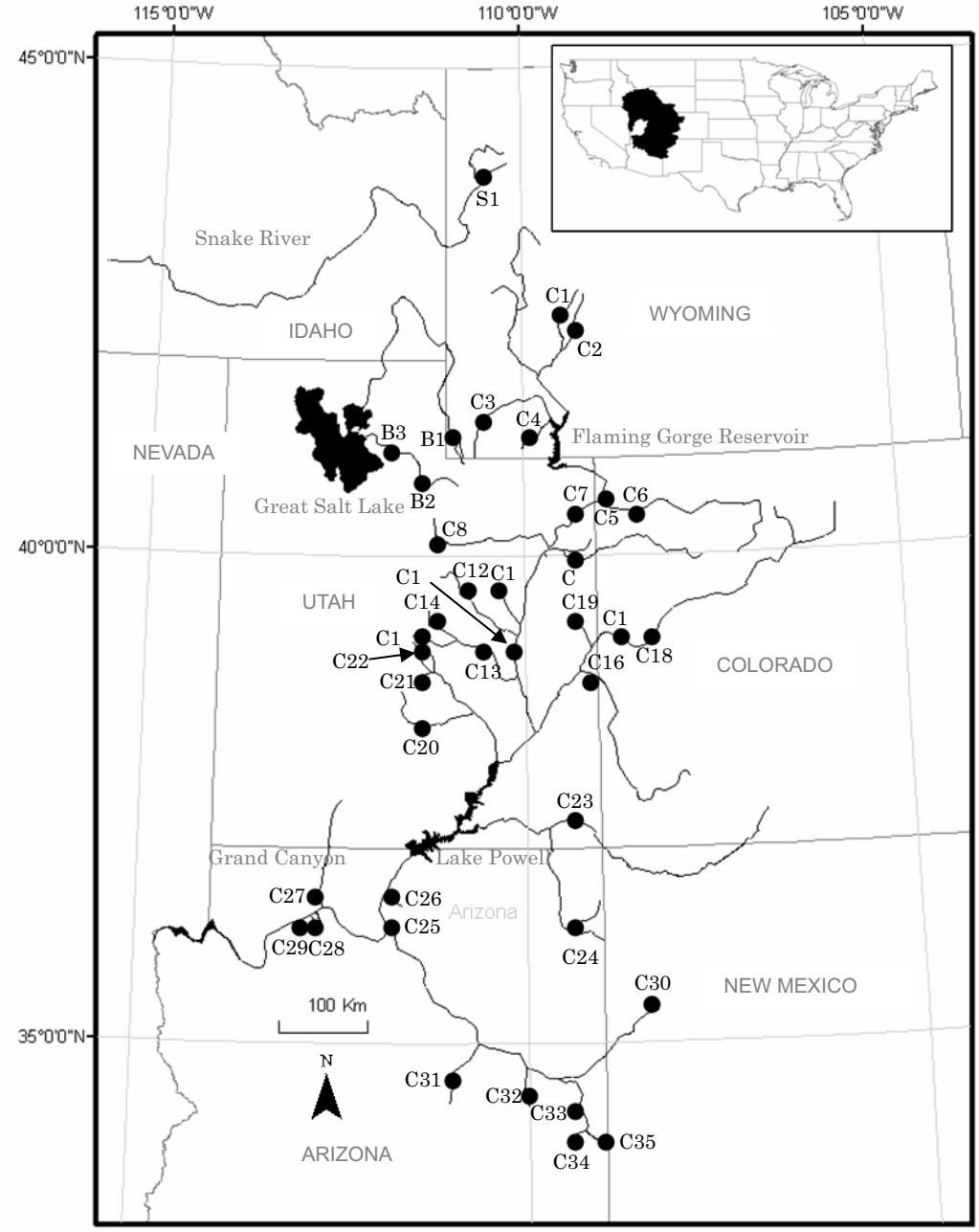

Figure 1. Sampling locations for 39 populations of *Catostomus discobolus*. Small insert shows species range. Sampling localities are defined in Table 3 and collection details provided in Appendix 1. Prefix designates major basin: S = Snake River, B = Bonneville Basin, and C = Colorado River Basin.

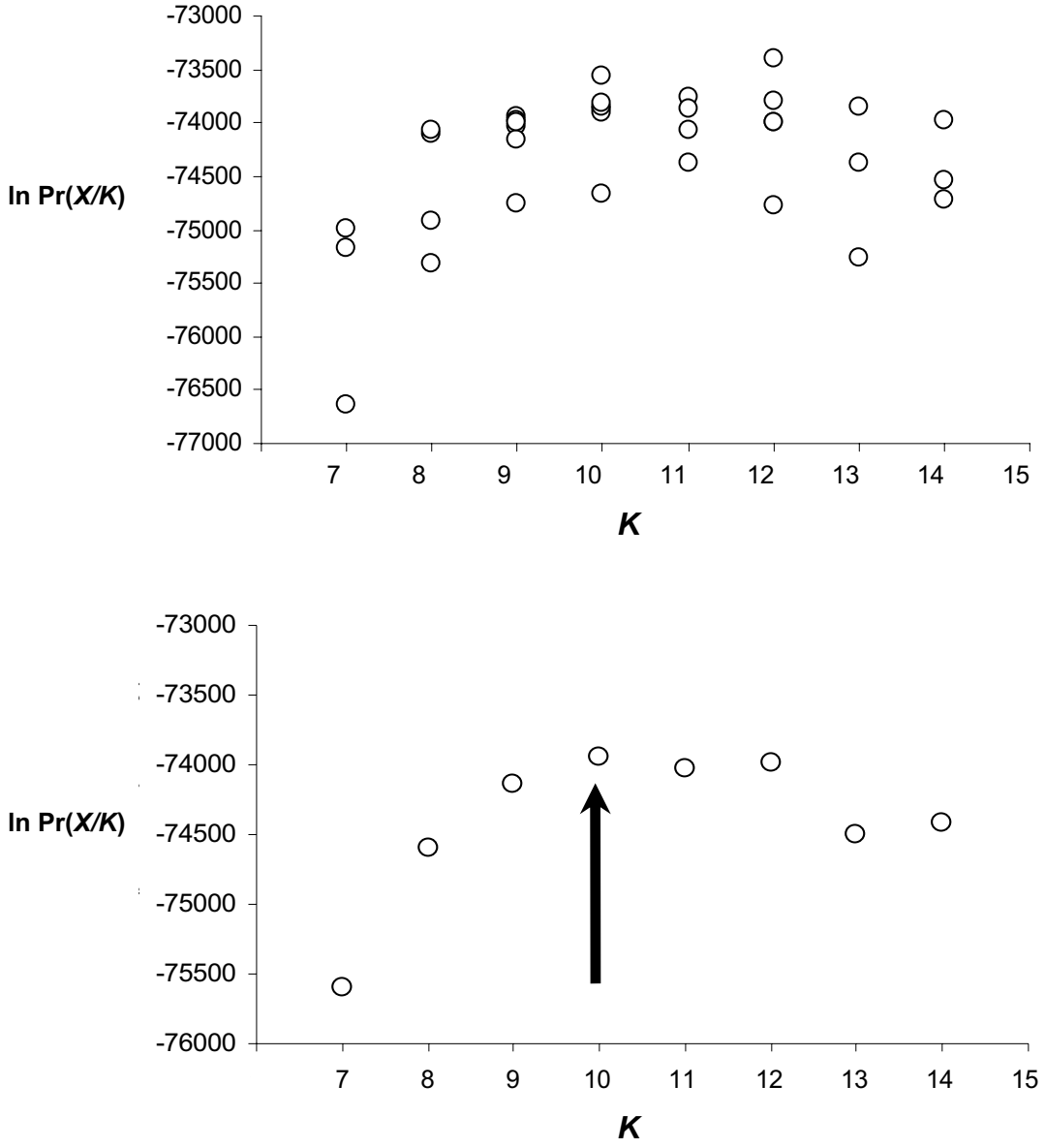

Figure 2. Plot of mean natural logarithm (ln) of posterior probabilities vs the predefined number of genetic clusters (*K*) computed in STRUCTURE. Data were based on 16 microsatellite loci screened across 1,092 *Catostomus discobolus* samples. Posterior probabilities were derived from Bayesian Markov Chain Monte Carlo runs of 200,000 burn-in and chain length of 500,000. Upper panel shows results of four batch runs each for *K* = 7 through 14. Lower Panel shows average of four batch runs. Error marks highest average posterior probability on which assignment plot was based.

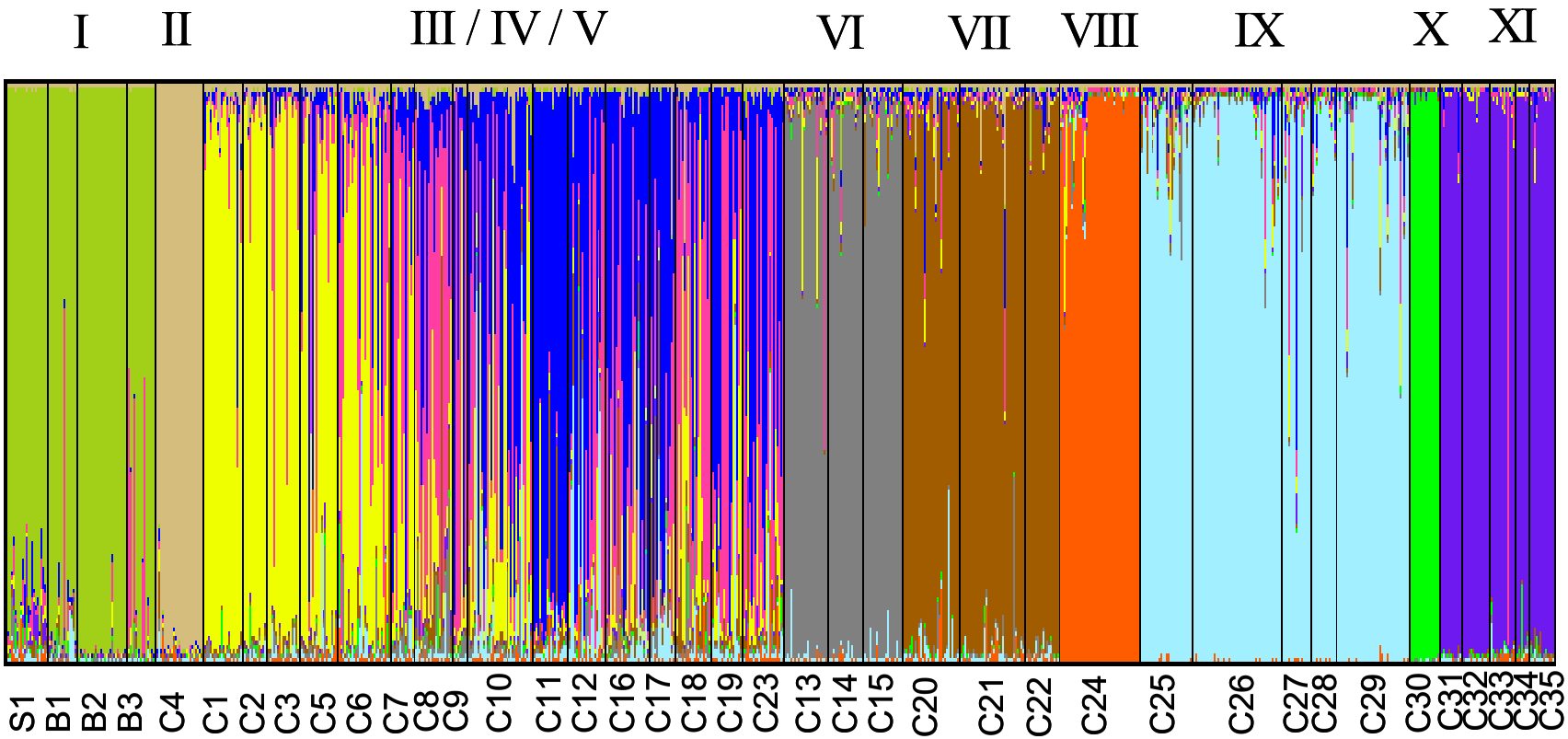

Figure 3. Admixture analysis from program STRUCTURE that diagrammatically displays the posterior probabilities of group membership for each of the 1,092 *Catostomus discobolus* assessed over 16 microsatellite loci. Analysis was based on a Bayesian Markov Chain Monte Carlo run of 200,000 burn-in and chain length of 500,000 assuming 11 genetic clusters (K = 11).

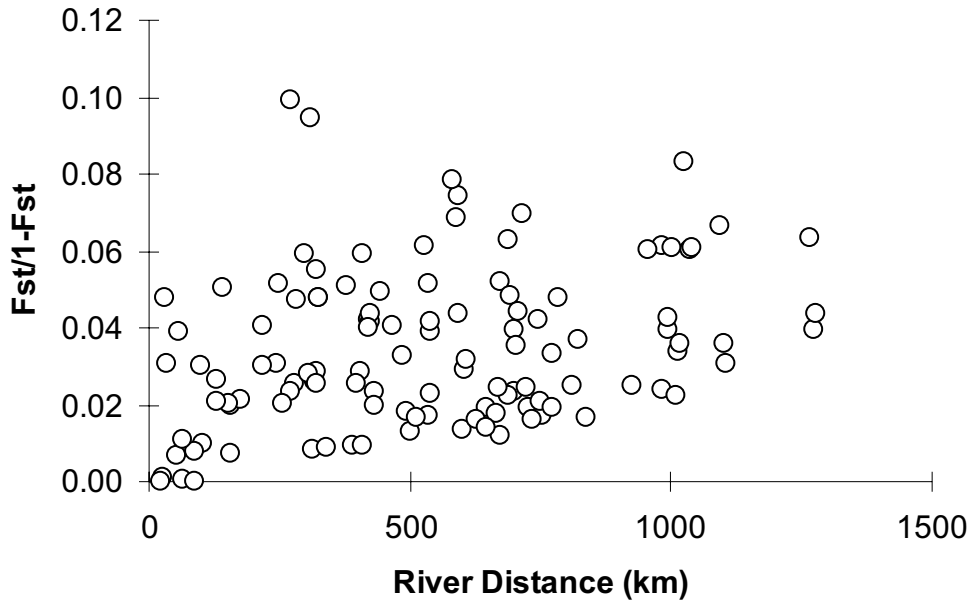

Figure 4. Isolation by distance (ISOLDE) analysis among 16 populations of *Catostomus discobolus* sampled from the Upper Colorado River Basin. Genetic distances were derived from 16 microsatellite loci and geographic distances reflect river distance between collection sites.

APPENDIX

Appendix 1. Overview of Bluehead Sucker [*Catostomus* (*Pantosteus*) *discobolus*] samples provided to the Douglas lab by associated agencies and/or collected by M. R. and M. E. Douglas from 1996—2005. Listed are drainages (numbers correspond to cartographic localities in Figure 1), within drainage sample locality, date of collection, and collectors. Cooperators: AGFD = Arizona Game and Fish Department; CDOW = Colorado Division of Wildlife; CSU = Colorado State University; UDWR = Utah Division of Wildlife Resources; WGFD = Wyoming Game and Fish Department.

Snake River Drainage (Snake River Basin)

Population: **Snake River (S1)**
Location: WY: Teton Co.; Snake River below Pacific Creek confluence
Date: 22-October-2007
Collectors: Kevin Gelwicks (WDGF)

Bear River Drainage (Bonneville Basin)

Population: **Bear River – Smiths Fork (B1)**
Location: WY: Lincoln Co.; Smiths Fork of Bear River
Date: 23 July 2005
Collectors: Pete Cavalli (WGFD)

Population: **Bear River – October (B1)**
Location: WY: Lincoln Co.; Bear River
Date: 14 October 2005
Collectors: Pete Cavalli (WGFD)

Population: **Bear River – August (B1)**
Location: WY: Lincoln Co.; Bear River
Date: 4 August 2005
Collectors: Pete Cavalli (WGFD)

Weber River Drainage (Bonneville Basin)

Population: **Chalk Creek (B2)**
Location: UT: Summit Co.; Chalk Ck section 01
Date: 31-March-2004
Collectors: Matt Anderson (UDWR)

Population: **Weber River (B2)**
Location: UT: Morgan Co., Weber River, section 07
Date: 8-Apr-2003 / 31-March-2004
Collectors: Matt Anderson, Craig Walker (UDWR)

Population: **Weber River (B3)**
Location: UT: Summit Co., Weber River
Date: 17-18-July-2007
Collectors: Aaron Webber (UDWR)

Big Sandy River Drainage – WY (Colorado River Basin)

Population: **Big Sandy River – BLM (C1)**
Location: WY: Sublette Co.; State land upstream of BLM enclosure
Date: 20 August 2003 / 30 September 2004
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – BSAR4 / BSAR4A

Population: **Big Sandy River – Reservoir (C1)**
Location: WY: Sublette Co.; BOR land above Big Sandy Reservoir, between Big Sandy Reservoir and the USGS gauging station near Farson (Station 09213500; T28N, S30, SW1/4)
Date: 19 August 2003
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – BSAR1

Population: **Big Sandy River - Sculpin Creek (C1)**
Location: WY: Sublette Co.; Sculpin Creek, ~1 mi upstream of Big Sandy R confluence
Date: 20 August 2003
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – SCCK1

Population: **Big Sandy River - Tabernacle Butte (C1)**
Location: WY: Sublette Co.; Big Sandy River, state land NW of Tabernacle Butte
Date: 21 August 2003
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – BSAR3

Population: **Big Sandy River – upstream (C1)**
Location: WY: Sublette Co.; Big Sandy River, upstream of Big Sandy Reservoir (R106W, T28N, S36, SW1/4)
Date: 8 October 2002
Collectors: Robb Keith (WGFD) – BSAR1

Little Sandy Creek Drainage – WY (Colorado River Basin)

Population: **Little Sandy Creek - BLM (C2)**
Location: WY: Sublette Co.; Little Sandy Creek, on BLM land upstream of diversion
Date: 9 July 2003
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – LSC3

Population: **Little Sandy Creek – Squaw Teat (C2)**
Location: WY: Sublette Co.; Little Sandy Creek, on state land near Squaw Teat
Date: 10 July 2003
Collectors: Kevin Gelwicks and Curtis Gill (WGFD) – LSC4

Population: **Little Sandy Creek – BLM upstream county line (C2)**
Location: WY: Sweetwater Co.; Little Sandy Creek, on BLM land upstream of Sweetwater/Sublette county line
Date: 11 August 2005
Collectors: Kevin Gelwicks (WGFD) – LSC3-2005

Muddy Creek Drainage (Carbon Co.) – WY (Colorado River Basin)

Population: **Upper Muddy Ck – McKinney Ck up beaver dam (C3)**
Location: WY: Carbon Co.; Upper Muddy Creek, McKinney Creek, just upstream of large beaver dam complex
Date: 11 August 2006
Collectors: Aaron Kern (WGFD) – GROV1

Population: **Upper Muddy Ck – down Hwy 789 Xing (C3)**
Location: WY: Carbon Co.; Upper Muddy Creek, 1.5 miles downstream of most northerly Hwy 789 crossing
Date: 23 August 2006
Collectors: Aaron Kern (WGFD) – MCLS9

Population: **Upper Muddy Ck – McKinney Ck up confluence Muddy Ck (C3)**
Location: WY: Carbon Co.; Upper Muddy Creek, McKinney Creek, 1.5 miles upstream of confluence with Muddy Creek
Date: 13 August 2006
Collectors: Aaron Kern (WGFD) – MKIN1

Henrys Fork Drainage – WY (Colorado River Basin)

Population: **Ringdahl Reservoir (C4)**
Location: WY: Sweetwater Co.; Henry's Fork, Ringdahl Reservoir, Little Dry Creek (UTM 12T E587450 N4556200)
Date: 13 May 2002
Collectors: Robb Keith and Kevin Gelwicks (WGFD)

Yampa River Drainage – UT/CO (Colorado River Basin)

Population: **Yampa River – Dinosaur NM (C5)**
Location: CO: Moffat Co.; Yampa River, Rm. 0 – 43, Dinosaur National Monument
Date: 1999/ June 2001
Collectors: T. Modde and S. Ross (USFWS)

Population: **Yampa River - Lily Park (C6)**
Location: CO: Moffat Co.; Yampa River at Lily Park
Date: August 2001
Collectors: R. Anderson (CDOW)

Duchesne River Drainage – UT (Colorado River Basin)

Population: **Strawberry River (C8)**
Location: UT: Duchesne Co.; Duchesne River, New Petross Ranch-First Ridge
Date: Fall 2004
Collectors: Ron Brunson (UDWR)

White River Drainage – UT (Colorado River Basin)

Population: **White River (C9)**
Location: UT: Mintah Co.; Cowboy Canyon
Date: Fall 2004
Collectors: Ron Brunson (UDWR)

Green River Drainage – UT (Colorado River Basin)

Population: **Split Mountain (C7)**
Location: UT: Uinta Co.; Green River, Split Mountain Area
Date: December 2007
Collectors: Trina Hedrick (UDNR)

Population: **Desolation Canyon (C10)**
Location: UT: Uintah Co.; Green River, Desolation Canyon (Rm. 26-83)
Date: June and July 2001/June 2002
Collectors: M. Hudson (UDWR)/ Julie Jackson (UDWR)

Population: **Range Creek (C11)**
Location: UT: Emery Co., Range Creek, near bunkhouse below barrier
Date: 15 August 2005
Collectors: Craig Walker (UDWR)

Price River Drainage – UT (Colorado River Basin)

Population: **Price River (C12)**
Location: UT: Carbon + Emery Co.; Price River, mainstem (three different sites)
Date: 5 July 2005, 20 June 2006
Collectors: Mike Ault (UDWR)

San Rafael River Drainage – UT (Colorado River Basin)

Population: **San Rafael R - mainstem (C13)**
Location: UT: Emery Co.; San Rafael River, ~ 2mi below to ~ 1mi above swinging bridge
Date: 11 May 2005, July through September 2006
Collectors: Craig Walker (UDWR)

Population: **Joe's Valley Reservoir (C14)**
Location: UT: Emery Co.; Joe's Valley Reservoir, near new boat ramp
Date: 03 June 2005
Collectors: Craig Walker (UDWR)

Population: **Millsite Reservoir (C15)**
Location: UT: Emery Co.; San Rafael River, Millsite Reservoir, southeast corner near dam and northwest shoreline near Ferron Ck
Date: 20 May 2005
Collectors: Craig Walker (UDWR)

Dolores River Drainage – UT (Colorado River Basin)

Population: **Dolores River - mainstem (C16)**
Location: UT: Emery Co.; Dolores River, mainstem, above and below station A
Date: 28/29 June 2006
Collectors: Craig Walker (UDWR)

Colorado River Upper Basin – CO, UT (Colorado River Basin)

Population: **Black Rocks Canyon (C17)**
Location: CO: Mesa Co., Colorado River, Black Rocks Canyon
Date: August 2000
Collectors: Pfeifer and McAda (USFWS)

Population: **Colorado River - 15 mile reach (C18)**
Location: CO: Mesa Co.; Colorado River at Grand Junction (Rm.175-177), below Corn Lake
Date: 18 September 2001
Collectors: R. Anderson (CDOW)

Population: **Westwater Canyon (C19)**
Location: UT: Grand Co.; Colorado River at Westwater Canyon
Date: Fall 2004
Collectors: Steve Meismer et al. (UDWR)/ Matt Anderson (UDWR)

Fremont River Drainage – UT (Colorado River Basin)

Population: **Fremont River (C20)**
Location: UT: Wayne Co.; Fremont River, just east of Capital Reef National Park boundary
Date: 5 October 2005
Collectors: M. Morvilius (UDWR)

Population: **Fremont River (C20)**
Location: UT: Wayne Co.; Fremont River, 0.5 – 1.5 miles east of Capital Reef National Park boundary
Date: 17 May 2005
Collectors: Matt Anderson (UDWR)

Population: **Fremont River (C20)**
Location: UT: Wayne Co.; Fremont River, near SR-24 bridge crossing
Date: 6 October 2005
Collectors: M. Morvilius (UDWR)

Muddy River Drainage – UT (Colorado River Basin)

Population: **Dirty Devil - Ivie Creek (C21)**
Location: UT: Emery Co.; Muddy Creek drainage, Ivie Creek, end of BLM road above ranch exit / I-70 bridge
Date: 7/27-October 2004/ July 2005
Collectors: Craig Walker (UDWR)

Population: **Dirty Devil - Quitchupah Creek (C21)**
Location: UT: Emery or Sevier Co.; Muddy Creek drainage, Quitchupah Creek
Date: 15/27-October 2004
Collectors: Craig Walker (UDWR)

Population: **Muddy Creek (C22)**
Location: UT: Emery Co.; Muddy Creek, mainstem, above and below I-70
Date: 22 August 2006
Collectors: Craig Walker (UDWR)

San Juan River Drainage – UT (Colorado River Basin)

Population: **San Juan River (C23)**
Location: UT: San Juan Co. San Juan River, RM 52.8-46
Date: 26-April 2004
Collectors: Matt Anderson (UDWR)

San Juan River Drainage – AZ (Colorado River Basin)

Population: **Coyote Wash (C24)**
Location: AZ: Navajo Co. Coyote Wash, Canyon de Chelly National Monument
Date: 14-15-May-2007
Collectors: Melissa Trammel (NPS)

Population: **Tsaile Creek (C24)**
Location: AZ: Apache Co.; Navajo Co. Tsaile Creek, Canyon de Chelly NM
Date: 22-July-1996/ 17-May-2007
Collectors: Michael and Marlis Douglas/Melissa Trammel (NPS)

Population: **Wheatfield Creek (C24)**
Location: AZ: Apache Co., Coconino Co.; Wheatfield Creek, 200 meters below Navajo Highway 12 Bridge/ Navajo Co.; at/below spring, 2 km up Canyon de Chelly confluence
Date: 22-July-1996/ 16-May-2007
Collectors: Michael and Marlis Douglas/Melissa Trammel (NPS)

Population: **Whiskey Creek (C24)**
Location: AZ: Apache Co.; Whiskey Creek, Navajo Reservation @ Highway 12
Date: 28-September-1997
Collectors: Michael and Marlis Douglas/ Melissa Trammel (NPS)

Colorado River in Grand Canyon – AZ (Colorado River Basin)

Population: **Little Colorado River (C25)**
Location: AZ: Coconino Co.; Little Colorado River, Grand Canyon, confluence to 12 km upstream (Rm. 61.5)
Date: June 2000
Collectors: Michael Douglas (CSU)

Population: **Shinumo Creek (C26)**
Location: AZ: Coconino Co.; Colorado River, Grand Canyon, Shinumo Creek above waterfall (Rm. 108.5)
Date: 1998/ 28 April 2000
Collectors: Michael and Marlis Douglas (CSU)

Population: **Kanab Creek (C27)**
Location: AZ: Coconino Co.; Colorado River, Grand Canyon, confluence of Kanab Creek (Rm. 143.5)
Date: April 1996/ April and May 1997/ May and June 2000
Collectors: Michael and Marlis Douglas (CSU)

Population: **Matkatamiba Canyon (C28)**
Location: AZ: Coconino Co.; Colorado River, Grand Canyon, Matkatamiba Canyon (Rm. 148)
Date: 28 June 1996
Collectors: Michael and Marlis Douglas (CSU)

Population: **Havasu Creek (C29)**
Location: AZ: Coconino Co.; Colorado River, Grand Canyon, confluence of Havasu Creek (Rm. 156)
Date: Fall 1998
Collectors: Michael and Marlis Douglas (CSU)

Zuni River Drainage – NM (Colorado River Basin)

Population: **Agua Remora (C30)**
Location: NM: McKinley Co.; Zuni River drainage, Agua Remora, middle and lower pools
Date: 24-25 May 2007
Collectors: S.M. Carman, T.L. Perez, C.W. Waller, P.L Hatch (NMGFD) – SMC07-001

Little Colorado River Drainage – AZ (Colorado River Basin)

Population: **Willow Creek (C31)**
Location: AZ: Coconino Co.; between Wiggins Crossing (FR225) and Hart Canyon
Date: 18-September 1999
Collectors: M. Lopez (AGFD)

Population: **Silver Creek (32)**
Location: AZ: Navajo Co.
Date: 17-November-1999
Collectors: M. Lopez

Population: **Little Colorado River (33)**
Location: AZ: Coconino Co.; Wenigma Wildlife Area
Date: 10-May-2000
Collectors: D. Dorum, M Sweetser (AGFD)

Population: **Nutrioso Creek (34)**
Location: AZ: Navajo Co.
Date: 3-March-2000
Collectors: M. Lopez, M. Sweetwater, L. Averietti

Population: **East Fork of the Little Colorado River (C35)**
Location: AZ: Apache Co.; East Fork Little Colorado River
Date: 14-August-2000
Collectors: M. Lopez and M. Rinker (AGFD)