

Drought in an evolutionary context: molecular variability in Flannelmouth Sucker (*Catostomus latipinnis*) from the Colorado River Basin of western North America

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SUMMARY

1. Fishes can often rebound numerically and distributionally from short-term (i.e. seasonal) drought, yet their capacity to recover from decades or centuries of drought is less apparent. An exceedingly warm and dry period swept the intermontane west of North America ca. 7500 years BP, concomitant with an abrupt extinction of >35 mammal species. Were larger fishes in mainstem rivers also impacted by this drought?

2. The Colorado River Basin encompasses seven states in western North America and drains 600 000 km². Its endemic mainstem fish community is ancient (i.e. Miocene) but depauperate.

3. We evaluated one widely distributed candidate species (flannelmouth sucker, *Catostomus latipinnis*) for basin-wide genetic and geographic structure at three fast-evolving mitochondrial (mt) DNA genes, ND2 with 589 bp and ATPase 8 and 6 with 642 bp. It is hypothesized that a concomitant signature would be present in the mtDNA of this species, if indeed it had been seriously bottlenecked by post-Pleistocene drought. A total of 352 individuals were sequenced from 24 populations (4–40 individuals/population; average of 14.7).

4. Only 49 unique haplotypes were found, 53% of which represented single individuals. Haplotype diversity was high (0.905 ± 0.007) whereas nucleotide diversity was low (0.002 ± 0.000).

5. A significant and positive geographical cline ($P < 0.001$) in nucleotide diversity was observed as sampling locations progressed upstream from southwest to northeast. These results divided the Colorado River Basin into three reaches: the lower reach with six populations and 83 individuals; the upper reach with seven populations and 83 individuals; and the middle reach with 11 populations and 186 individuals. An analysis of molecular variance (AMOVA) revealed that 81.5% of the total genetic variation was within populations, 16% among populations within reaches and 2.5% among reaches. Only the last was significant. Populations from the three reaches diverged from one another by 3400–11 000 years BP. Haplotype distribution suggested populations in the upper Colorado River are expanding.

6. The lack of genetic variation and recent coalescence of lineages in *C. latipinnis* are unusual given its fossil history, broad geographical sampling, the rapid rate of mtDNA evolution and the number (and evolutionary rate) of the genes examined. The most parsimonious explanation for these data is a rapid expansion following a recent period of

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Dedication: This paper is dedicated to F. James Rohlf on the occasion of his 65th birthday.

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low effective population size at the end of the Pleistocene.

7. The intense drought is suggested at the end of the Pleistocene (late-to-mid-Holocene), severely impacted not only large mammals but also larger fishes in western North American rivers. These perspectives have important implications for management of endangered and threatened species in this region.

Keywords: *Catostomus latipinnis*, Colorado River, genetic bottleneck, mitochondrial DNA, post-Pleistocene drought

Introduction

Effects of drought on fishes are well established in both brackish (Mol *et al.*, 2000) and freshwater systems (Freeman *et al.*, 1988; Kelsch, 1994; Closs & Lake, 1996; Matthews & Marsh-Matthews, 2003). Estuaries, for example, often suffer serious declines in productivity concomitant with a reduction in nutrient loading from drought-impacted rivers (Livingston *et al.*, 1997). This in turn has manifold effects on the fish community (Bennett, Ostrach & Hinton, 1995; Livingston, 1997a). While death is the most obvious result of drought in freshwater systems (Tramer, 1977), other effects are more surreptitious and may pass unnoticed. For instance, drought can induce a marked breakdown in the behavioural hierarchies of fishes (Sloman, Taylor & Metcalfe, 2001), increase their parasite loads (Medeiros & Maltchik, 1999) and sever gene flow among populations to the extent that genetic variability is reduced (Rutledge, Zimmerman & Beitingger, 1990; Faber & White, 2000).

Although populations typically rebound numerically and distributionally from short-term (i.e., seasonal) drought (Canton *et al.*, 1984; Ross, Matthews & Echelle, 1985; Meffe, 1990; Boulton, 2003), the capacity to recover from a genetic bottleneck induced by prolonged drought (i.e. extending over years or decades) is less apparent. Such droughts must have played major roles in the evolution of aquatic species. To understand this phenomenon better, one could ask how prevalent in the modern era were such devastating droughts? To answer this question, Tarboton (1995) employed tree ring data to extrapolate 442 years of discharge for the Colorado River in western North America. He detected severe drought from 1943–1964, and 1579–1601 (the latter a compendium of three sequential droughts). Furthermore, the best estimate of a recovery time for the

1579 drought was 400–700 years. However, when Tarboton (1995) rearranged these 22 drought years to reflect a continuous (rather than episodic) record of declining flows, recovery time ballooned over 5-fold (i.e. 2000–10 000 years). This prolonged duration of recovery provoked Matthews (1998) to reason that disastrous prehistoric droughts must have destroyed riverine fish faunas on a massive scale and furthermore, recolonization during later and more pluvial periods must have taken decades (if not centuries). The erosion of genetic variability in these fishes following such a drought is but a source of speculation.

The effects of this period on the evolution of freshwater fishes remains largely unexamined. This is understandable in that the relative recentness of these historic events can often render them opaque to molecular scrutiny. Furthermore, Pleistocene glaciation itself had a major (and often overshadowing) impact on this fauna (Avisé, Walker & Johns, 1998; Douglas, Brunner & Bernatchez, 1999; Brunner *et al.*, 2001). In fact, survival of many Palaeartic fishes often depended upon the presence of glacial refugia (Bernatchez & Wilson, 1998). In this paper, a broad temporal and spatial perspective is employed to approach the questions posed by Matthews (1998). It was hypothesized that relentless, long-term and prehistoric drought severely impacted the genetic diversity of fishes in western North America. Furthermore, it is suggested that the effects of this drought have reverberated within the DNA of fish species for thousands of years. These effects are thus detectable in the present time and are suitable for quantitative analysis. These effects were then examined by means of modern molecular techniques and computational methodologies that allow genetic data to be coalesced through time.

It is recognized that estimation of coalescence or divergence times from molecular data (as above) is

often a challenging proposition. This is because numerous factors can confound such estimates and often render their derivation more difficult. In this paper, three aspects recognized by Arbogast and Slowinski (1998) as problematic are addressed. These are: nucleotide saturation effects (i.e. superimposed substitutions), error rates associated with estimations and derivation of implicit tests to determine if a molecular clock holds for the data.

Methods

The study species

Catostomus latipinnis Baird and Girard is part of an ancient, endemic and depauperate Colorado River ichthyofauna that extends to the Miocene (Miller, 1959; Minckley, Hendrickson & Bond, 1986; Smith *et al.*, 2002). Fossil remains have been found in early Pleistocene beds of the Little Colorado River Basin (Uyeno & Miller, 1963, 1965), and in Stanton's cave (Grand Canyon National Park; Miller & Smith, 1984). Historically, the species was distributed in all moderate-to-large rivers throughout the Colorado River Basin (Minckley & Holden, 1980). By the 1970s, it was extirpated in the lower basin below Hoover dam as a result of water control projects and introductions of non-native fishes. It has only been reintroduced in the last 15 years (C.O. Minckley, personal communication). Adults usually reached a total length (TL) of 300–400 mm (Minckley & Holden, 1980), while the maximum TL recorded by Douglas and Marsh (1998) was 661 mm. *Catostomus latipinnis* and other large-river endemic fishes in western North America are believed to achieve great age (reviewed by Douglas & Marsh, 1998) and this clearly has implications for their management.

Knowledge of the life history of this species is sketchy. It typically inhabits pools and deeper runs of rivers and often enters mouths of smaller tributaries (Minckley, 1973, 1991). In the Yampa River, ripe adults congregated at upstream ends of cobble bars to spawn (McAda & Wydowski, 1985). Ripe *C. latipinnis* were caught in Grand Canyon from March–May at the confluence of the Paria River and other low gradient streams (cited in Douglas & Marsh, 1998). Postreproductive fish remained in tributary habitats throughout the summer and returned to the mainstem in winter, when temperature difference between

tributary and mainstem equilibrated. Reproductive behaviour was reported by Weiss, Otis and Maughan (1998) and summarized by Douglas and Douglas (2000). Adults feed upon aquatic invertebrates (primarily dipterans), organic debris and ingest sand as an apparent by-product of benthic feeding. Individuals in northern Grand Canyon also ingest an introduced filamentous alga [*Cladopora glomerata* (L.) Kütz.] that serves as substrate for amphipods, chironomids and diatoms (Shannon, Blinn & Stevens, 1994; Stevens, Shannon & Blinn, 1997; McKinney, Ayers & Rogers, 1999).

Although *C. latipinnis* was once widely distributed in the Colorado River Basin (see above; also Smith, 1992), its apparent selection of wider and slower moving areas as favoured habitat (Minckley, 1973) may have encouraged local isolation at tributaries. Additionally, these slackwater areas would be interspersed among longer and tighter canyon-bound areas, thus enhancing the potential for endemism. Chart and Bergersen (1992) argued that adult movements are size-related, with larger individuals being more sedentary. They also indicated that adult *C. latipinnis* occupy a definable home range. Tyus and Karp (1990) found that the related razorback sucker (*Xyrauchen texanus* Abbott) demonstrated spawning site fidelity in the Green River.

Study sites and sampling

The Colorado River Compact of 1922 (Martin, 1989a) divided the Colorado River into upper and lower basins. The point of demarcation is Lees Ferry, 25 km downstream from Glen Canyon Dam (Fig. 1), in a region of northern Grand Canyon termed Marble Canyon. Sampling was primarily accomplished during 1997–1999, first within lower basin Colorado River through Grand Canyon, then within the upper basin Colorado River, often with the assistance of agency personnel. Fishes from the Virgin River (i.e. downstream from Grand Canyon) were sampled in 1993 during a multi-agency effort, to remove introduced red shiner [*Cyprinella lutrensis* (Baird & Girard)] (Timmons, 1998). These three areas (i.e. upper and lower Colorado River and Virgin River) are termed 'basins' in this paper as opposed to 'reaches' that are defined statistically in the Results. The 24 sampling sites are depicted in Fig. 1 and their locations are described in Appendix 1.



Fig. 1 Map depicting sampling locations (closed circles) for *Catostomus latipinnis* in the Colorado River Basin of western North America.

Molecular approaches

Tissue samples were primarily pelvic fin clips, taken either directly from individuals released alive, or from frozen specimens. Total genomic DNA was isolated using the PureGene DNA Isolation Kit (D-70KB; Gentra Systems, Inc., Minneapolis MN, U.S.A.) and stored in DNA hydrating solution (same kit). Amplifications of the mitochondrial ND2 gene sequences were accomplished with standard PCR conditions using primers designed as a part of this study. These are: C-Gln (5'-AAC CCA TAC TCA RGA GAT CA-3') and C-Trp (5'-ACT TCT ACT TAR AGC TTT GAA GG-3'). Mitochondrial ATPase 8 and 6 genes were amplified using primers specified in Bermingham and Martin (1998); (see also Perdices & Doadrio, 2001).

Single-stranded sequencing reactions were conducted with fluorescently-labelled dideoxy terminators according to manufacturer's recommendations [Applied Biosystems Inc. (ABI), Forest City, CA, U.S.A.]. Labelled extension products were gel-separated and analysed with an automated DNA sequencer (ABI model 377) located in the sequencing facility at Arizona State University. All samples were

sequenced in the forward direction and problematic sequences were re-sequenced in a forward manner. Sequences were aligned manually using SeqPup (Gilbert, 1999). Although evolutionary rates of ATPase 8, 6 and ND2 appear similar (Kumar, 1996), the effectiveness of combining these sequences for analysis was tested by the partition homogeneity test of Farris *et al.* (1994), as implemented in PAUP* (Swofford, 1998). Tajima's (1989) D-statistic was also computed to determine if sequence evolution was consistent with neutral expectations (using DNAsp: Rozas & Rozas, 1999).

Molecular analyses and genetic diversity

Haplotypes were compiled from raw sequence data using the software MacClade (Maddison & Maddison, 1998). These served as unweighted input to the maximum parsimony (MP) algorithm of PAUP*. Shortest trees were sought by using heuristic searches that employed accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, retention of minimal trees (MULPARS), and collapse of zero-length branches to yield polytomies. Support for individual nodes was evaluated by non-parametric bootstrapping, using 1000 pseudoreplicates per analysis with 100 random addition sequences per pseudoreplicate. A node with a bootstrap value >70% was considered strongly supported (Hillis & Bull, 1993). Trees were rooted using *Xyrauchen texanus* (Smith, 1992). An unrooted minimum spanning tree was also derived using Kimura-2 distances and the program MEGA2 (Kumar *et al.*, 2001). Nucleotide diversity (π : average weighted sequence divergence among haplotypes, varying between 0% for no divergence to over 10% for deep divergence) was calculated for each locality and among reaches. Values provided an estimate of the probability that two randomly chosen homologous nucleotides are identical. Haplotype diversity (h : a measure of the frequencies and numbers of haplotypes among individuals) was calculated for each locality and among reaches. Both diversity indices were derived using ARLEQUIN (Schneider, Roessli & Excoffier, 2000). Clinal variation in nucleotide diversity was examined using isotonic regression (Gaines & Rice, 1990), as implemented in BIOMstat (Rohlf, 1998). Here, sample sizes of populations, the variance of π and 1000 permutations of the dataset were used to test for the

presence of a consistent increase in the dependent variable (i.e. π) as a function of the rank order of location, an ordinal designation of populations from southwest (i.e. Virgin River) to northeast (Green River above Flaming Gorge Dam) (Sokal & Rohlf, 1995). This test defines significant reaches within- and among-basins, if indeed they exist. The distribution of genetic diversity among samples was estimated by AMOVA (Excoffier, Smouse & Quattro, 1992) using ARLEQUIN. Here, sequence variation was partitioned within- and among-populations and reaches (as in traditional F_{st}), with the exception that AMOVA takes into account the number of base differences among haplotypes and their frequencies. When genetic interchange among populations is elevated, most of the variations will be found within populations. When genetic interchange is reduced, populations will diverge and variation will be distributed among populations or reaches or both.

Molecular analyses and population dynamics

The mismatch distribution (Rogers & Harpending, 1992), defined as the number of nucleotide differences between all pairs of individuals, was also computed. The mean of these differences was calculated, fitted against an expected Poisson distribution (as per Slatkin & Hudson, 1991) and tested using a Kolmogorov-Smirnov one-sample test. In a second analysis, we apportioned individuals into reaches, computed the mismatch distributions and tested as above. DNAsp was also used to calculate the expected mismatch distribution for a population in expansion versus one of constant size. In the former instance, the expectation is that a population now expanding after a reduction in numbers will show a rapid increase in the proportion of alleles that are identical, or nearly so, because most alleles are descendants of one or a few ancestral types. As a result, a plot of the pair-wise differences among alleles will produce a Poisson distribution (Rogers & Harpending, 1992). In contrast, a stable or declining population is expected to reflect a geometric (or multimodal) distribution of allelic differences (see, for example, Lavery, Moritz & Fielder, 1996). Observed and expected distributions were again compared using a Kolmogorov-Smirnov test.

Methods for estimating population sizes using the mismatch distribution have been criticized as less efficient than using coalescent events within the genealogical tree (Felsenstein, 1992). Thus the struc-

ture of the phylogenetic tree (as per Nee *et al.*, 1995) was employed to make inferences about numbers of lineages versus time, using the programme END-EPI (Rambaut, 1998). When numbers of lineages are plotted logarithmically over time, an exponentially growing population will reflect a convex graph (i.e. one with decreasing slope). However, the convex graph will linearize when given an 'epidemic' transformation (Nee *et al.*, 1995). In contrast, a population of constant size will demonstrate a concave graph (i.e. increasing slope) over time that again becomes linear with an epidemic transformation. Observed and expected frequency distributions are compared using the Kolmogorov-Smirnov statistic. The programme MIGRATE (Beerli & Felsenstein, 1999) was also used to estimate the past migration rates between reaches, using the Kimura 2-parameter model and a migration matrix model with asymmetric rates and different subpopulation sizes. The analysis employed a maximum likelihood estimator based upon the coalescent.

Molecular analyses and temporal patterns of population divergence

Sequence divergence (p) values were generated across the three statistically defined stream reaches, based on 1000 bootstrapped sequences and employing MEGA2. The p -distance is merely the proportion (p) of nucleotide sites at which the two sequences compared are different. It is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared. Values were corrected for within-group variation and converted to provisional estimates of genealogical separation times (Avise *et al.*, 1998). This was accomplished using a molecular clock, considered typical of the study markers [i.e. 1.3% divergence per Ma calibrated for fishes separated by the rise of the isthmus of Panama (Bermingham, McCafferty & Martin, 1997)]. Before employing a molecular clock, Tajima's test (1993) was first employed to compare the representative sequences from three reaches of the Colorado River (defined below) with the outgroup. Tajima's test is based on the expectation that, under a uniform (i.e. clock-like) rate of substitution, the number of sites shared by the outgroup and one of the two ingroups should be the same for both ingroups. Sequences were selected randomly from different drainages within each reach and comparisons involved upper versus middle,

upper versus lower, and middle versus lower tests. Five such evaluations were performed, each involving the above three comparisons.

Results

Genetic diversity and spatial patterns

The PCR amplifications and automated sequencing of ND2 resulted in 589 base pairs (bp) of unambiguously readable sequence that contained no insertions or deletions. Similarly, 642 bp of sequence were obtained for ATPase 8 and 6, again with no insertions or deletions. Combining of the partial sequences of these three genes was supported by a non-significant partition homogeneity test (PAUP*: $P > 0.34$). Congruence among genes further suggested that sequences obtained were indeed mitochondrial rather than nuclear paralogs. Additionally, the large number of closely related haplotypes (Appendix 2) supported this conclusion as nuclear copies would be much less variable because of mutation repair mechanisms.

On all 352 individuals, the combined 1231 bp were 96.3% monomorphic and 3.7% polymorphic. Only 52% of the latter were parsimony informative (i.e. 2% of total). There were 49 total haplotypes, 71% of which occurred solely in the upper basin. Grand Canyon possessed but 6% of the unique haplotypes and Virgin River none. Grand Canyon and the upper basin shared 18% of their haplotypes while the Virgin River, Grand Canyon, and upper basin shared 4%. Overall, the 11 haplotypes shared among the three areas accounted for 83% of all the individuals. The most common haplotype (17.6% of individuals) was found in 21 of 24 populations (Appendix 3). None of the haplotypes linked Virgin River solely with either Grand Canyon or upper basin. All haplotypes were closely related with a maximum (uncorrected) sequence divergence of 0.6% (average divergence = 0.3%).

Phylogenetic analysis confirmed a lack of genetic structure among *C. latipinnis* haplotypes. A total of 9980 most parsimonious trees were produced, each with length = 127, consistency index = 0.858 and retention index = 0.723. However, the MP analysis did not yield clusters that conformed to geographic areas, but instead arrayed haplotypes in a starburst pattern (not presented). An unrooted minimum spanning tree (Fig. 2) underscored the disparity in numbers of haplotypes between upper and lower basins.

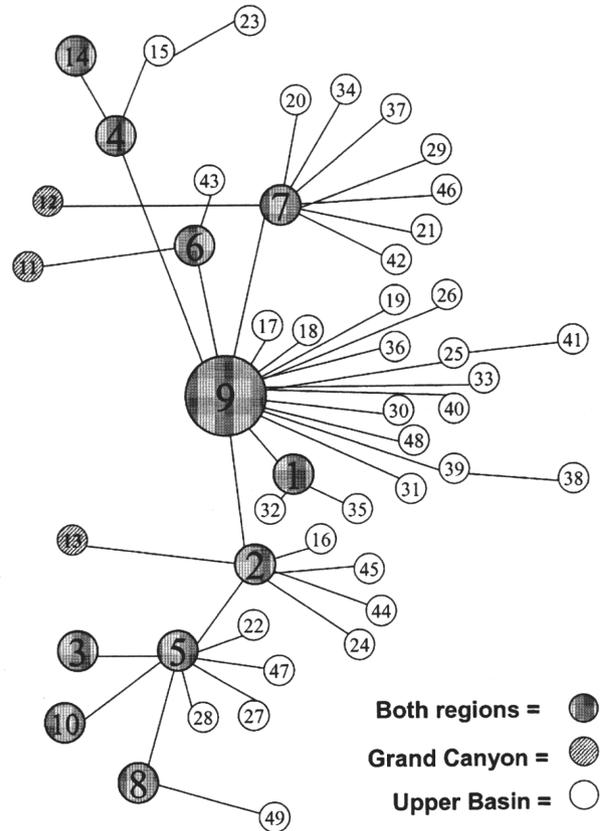


Fig. 2 Unrooted minimum spanning tree based on the Kimura 2-parameter model, depicting 49 haplotypes of *Catostomus latipinnis*. Open circles to the right of the figure are upper basin haplotypes exclusively. Grey circles in the centre are haplotypes shared between the two basins, while slashed circles to the left are strictly Grand Canyon haplotypes. Numbers within circles refer to haplotypes listed in Appendices 2 and 3. Size of circles is proportional to the number of haplotypes within each.

Overall, haplotype diversity was high ($\mu = 0.905 \pm 0.007$ SD) whereas nucleotide diversity was low (0.002 ± 0.000). However, a significant and positive geographic cline was observed in nucleotide diversity ($E^2 = 0.07919$; $P < 0.001$) as sampling locations moved from southwest to northeast. These results were then used to divide the Colorado River Basin into three reaches (Fig. 3): a lower reach (comprising six populations and 83 individuals from the Virgin River and lower Grand Canyon); an upper reach (comprising seven populations and 83 individuals from the Yampa and upper Green rivers) and a topographically longer middle reach that extended from mid-Grand Canyon through lower Green and upper Colorado rivers (11 populations and 186 individuals). Haplotype diversity was quite high in all three reaches (0.81–0.90),

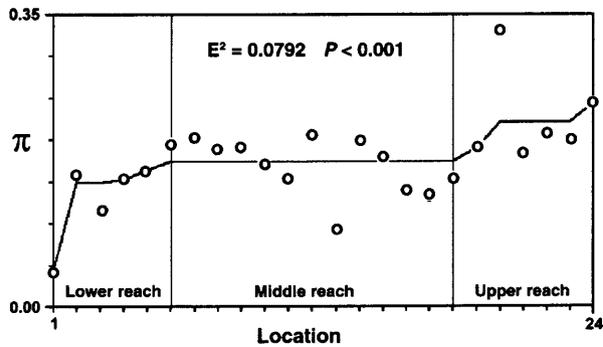


Fig. 3 Isotonic regression of nucleotide diversity (π) versus rank order of *Catostomus latipinnis* populations from southwest (Virgin River) to northeast (Yampa and upper Green rivers). Regression is significant at $P < 0.0011$. Regression plot is divided into three river reaches: lower, middle, and upper (defined in Results). The sixth and 18th samples were grouped into lower and upper reaches, respectively as a means of balancing samples among regions. Their placement did not affect significance.

whereas nucleotide diversity was low (0.0019–0.0023). Analysis of molecular variance indicated a lack of population subdivision, with 81.5% of genetic variation common to populations and 16% apportioned among populations within reaches. Although variances were smallest among the three reaches (at 2.5%), they were significantly different ($P < 0.05$). Tajima's D-statistic was non-significant for all three.

Genetic diversity and temporal patterns of population dynamics

The mismatch distribution for both upper and middle reaches (Fig. 4a, b) were not statistically different from that of an expanding population ($P = 0.7$ and $P = 0.19$, respectively) but each differed significantly from the distribution displayed by a stable population (both $P < 0.001$). In contrast, the mismatch distribution for the lower reach (Fig. 4c) differed significantly from the distribution displayed by an expanding population ($P < 0.01$). When data were reflected in a semilogarithmic 'lineages through time' plot, the curve (Fig. 5a) deviated significantly from a linear relationship ($P < 0.01$). It instead rose sharply at the beginning (after a low time period) and became less steep towards the latter time period, as it emulated an exponentially growing population. This interpretation was sustained under the epidemic transformation, where the data approximated a linear relationship (Fig. 5b). The sharp rise at the end of the latter plot

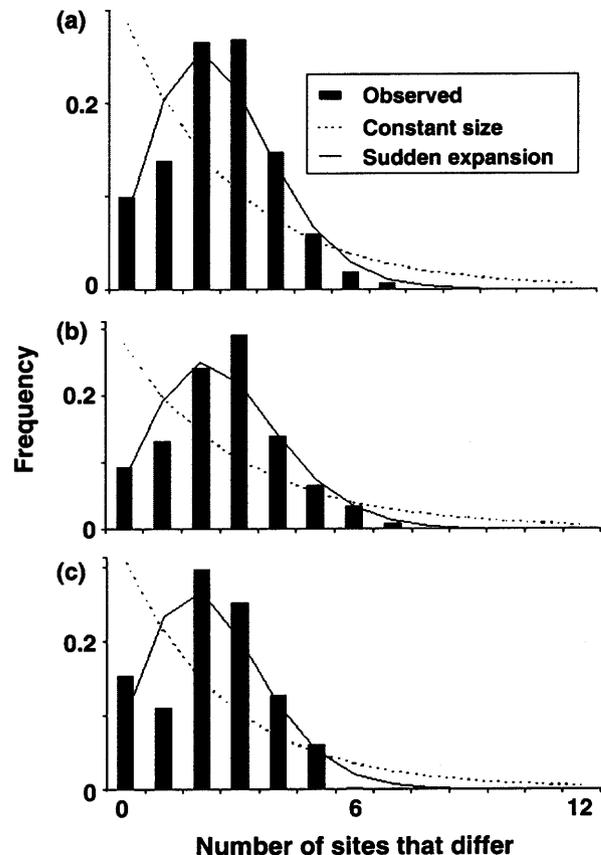


Fig. 4 Mismatch distributions of *Catostomus latipinnis* populations located in (a) upper reach, (b) middle reach and (c) lower reach of the Colorado River (See Results for definition of reach).

implies that growth was potentially greater than exponential during the most recent period (as per Lavery *et al.*, 1996). Results from migration analyses (Fig. 6) confirm that the greatest amount of movement was from lower and middle reaches into the upper reach. Migrations from upper to middle, from upper to lower, and from middle to lower reaches were clearly insubstantial.

Results from Tajima's test (i.e. the triplet clock test) were consistently non-significant for each of the three comparisons within all five evaluations, suggesting that sequences in the three reaches can be treated as effectively rate-uniform and thus suitable for estimating dates of divergence. The p -distances and standard errors (in parentheses) for *C. latipinnis* grouped into lower, middle and upper reaches are as follows: Lower–Middle = 0.0071% \pm 0.0071; Middle–Upper = 0.0102% \pm 0.0045; and Lower–Upper = 0.0183% \pm 0.0095. Using the molecular clock, middle and upper

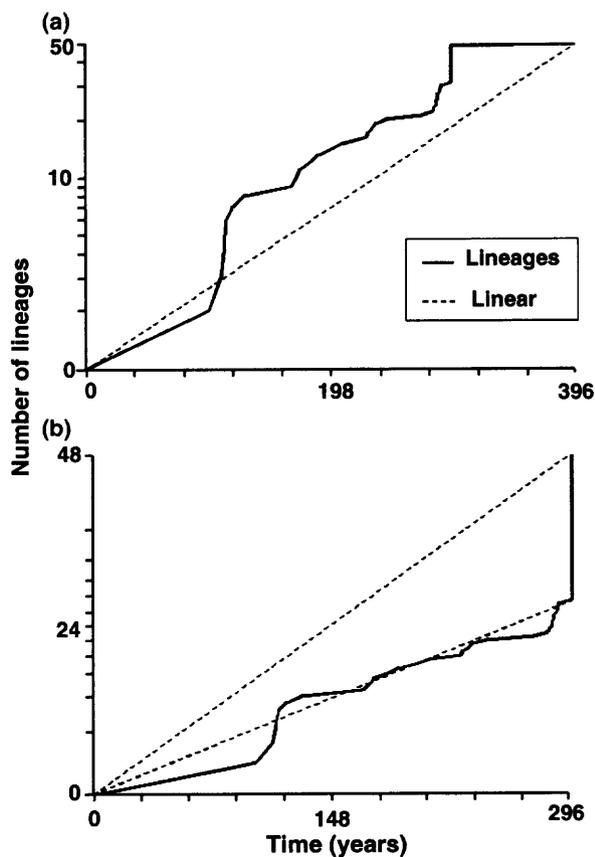


Fig. 5 (a) Lineage-through-time plot for *Catostomus latipinnis* in the Colorado River Basin and (b) same plot with an epidemic transformation.

reach *C. latipinnis* diverged from one another 11 400–4400 years BP, while the lower and middle reaches diverged 11 000–30 years BP.

Discussion

The native ichthyofauna of western North America is clearly ancient. Yet the very low levels of genetic diversity we found basin-wide in *C. latipinnis* were quite puzzling, in spite of the fact that we sampled widely and copiously, used three fast-evolving mtDNA genes, and applied contemporary methodology in a search for evolutionarily significant units (ESUs; Moritz, 1994a). Instead, our molecular data pointed to a more recent evolution for this species, one congruent with the hypothesis of a basin-wide crash during late Pleistocene/early Holocene. In addition, a 'starburst' phylogeny of alleles, Poisson mismatch distributions and a pattern of increasing numbers of lineages through time strongly indicated exponential population growth following the bottleneck (as per Zink *et al.*, 2000). Migration analyses and enumeration of singleton haplotypes also suggested that *C. latipinnis* expanded its distribution from the lower to the upper reach following this bottleneck. Below, we discuss specific aspects of our findings in the context of these general results.

Haplotypic diversity and *Catostomus latipinnis*

Grant and Bowen (1998) classified marine fishes into four groups based upon different combinations of haplotype (h) and nucleotide diversity (π). Their categories are defined according to demographic events that would alter the likelihood of mtDNA lineage survival and the time to ancestral coalescence of lineages. *Catostomus latipinnis* of the Colorado River Basin clearly falls within category 2 of Grant and Bowen (1998), which is a species with high h but quite low π . This situation is represented in a phylogenetic sense by several prevalent haplotypes embedded within a cluster of 'twigs' that are one or a few mutations removed from the central haplotypes. This is because new mutations are retained as a result of relaxed selection promoted by rapid population growth (Avice, Neigel & Arnold, 1984; Rogers & Harpending, 1992). Shields and Gust (1995) also reported such a pattern when they reviewed mtDNA

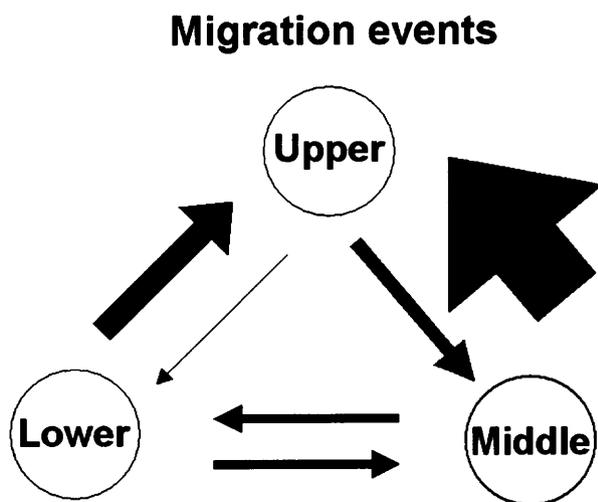


Fig. 6 Propensity for migration between the three study reaches depicted in Fig. 3 and defined in Results.

diversity in marine fishes. The demographical events that would most likely give rise to this condition are a rapid expansion following a period of low effective population size (Grant & Bowen, 1998).

The most obvious question is, 'what could have caused the shallow genetic architecture found in *C. latipinnis* from the Colorado River Basin?' Given that this species (and others) are known to be ancient inhabitants of western North America (Minckley, 1991; Smith *et al.*, 2002), the premise of relatively recent speciation can be ruled out. However, while *C. latipinnis* is of ancient origin, tremendous fluctuations in population numbers during recent times could have effectively reset its evolutionary trajectory. In other words, a post-Pleistocene bottleneck event could have reduced *C. latipinnis* to extremely small numbers and its evolutionary history would essentially start anew from that point. In a similar example, Baker, Piersma and Rosenmeier (1994) found that mtDNA lineage variation in Arctic-breeding shorebirds (red knot, *Calidris canutus* L.) was minimal, thus precluding determination of relationships among populations and geographical regions. These birds were apparently bottlenecked through a small population in late Pleistocene and only expanded into their current broad distribution within the last 10 000 years (Baker & Marshall, 1997). Similarly, Walker *et al.* (1998) found a single mtDNA control region haplotype among 66 snapping turtles (*Chelydra serpentina* L.) collected across 10 different states in the southeastern United States. These researchers noted how unusual it was to recognize only a single ESU within an otherwise phylogeographically rich region that had previously demonstrated considerable genetic diversity in other turtle species (Walker & Avise, 1998). As with red knot, the most parsimonious explanation for this situation is a severe late Pleistocene bottleneck followed by a relatively recent range expansion.

Molecular clock

Can we utilize the mtDNA data herein to provide insight with regard to time scales and thus determine from which period the shallow genetic architecture originated? The concept of a molecular clock (i.e. regularity of nucleotide substitutions with respect to time) is a disputed topic (see Hillis, Mable & Moritz, 1996, and references therein). Not every codon position will experience the same number of substitu-

tions, primarily because of differences in mutation rate, selection and fixation. Therefore during a given time interval, some codons acquire more than one substitution whereas others experience none (see Douglas *et al.*, 2002). Substitution rates will also fluctuate among different DNA regions and lineages of organisms (Avise, 1994). In the present study, an attempt was made to compensate for these inequities by choosing regions that are well researched, with known substitution patterns and constraints (ATPase: Fagen & Saier, 1994; ND2: Bielawski & Gold, 1996) and by testing these regions for similar rates of evolution. As our evaluations were within a single species and sequence divergence among study reaches was less than 1%, saturation effects were negligible (as per Klicka & Zink, 1998; Douglas *et al.*, 2002). Additionally, we used net percent sequence divergence (i.e. divergence between reaches adjusted for divergence within reaches) and bracketed the estimates using standard errors of the calculations. We also failed to reject the hypothesis that sequences from individuals in each region evolved in a clockwise fashion. The estimates suggest that the upper and middle reaches of the basin diverged from one another 11 400–4400 years BP, and thus within a late Pleistocene/mid-Holocene timeframe.

Occurrence of a post-Pleistocene bottleneck

The documented record of modern drought in the Colorado River Basin has stimulated an assessment of past riverine conditions and provoked causal hypotheses. Can a similar perspective be utilized to evaluate prehistoric environmental conditions, particularly in light of the timeframe provided above? The answer is 'yes'. During the transition from the Wisconsin glaciation (24 000–10 000 years BP) to the interglacial period (termed the hypsithermal; Pielou, 1991), catastrophic drought swept North America from west to east. The extreme warmth and dryness of this period caused forests and grasslands across the western half of the continent to shift dramatically northward and also upward in altitude, wherever there were mountains. Effective moisture on the Kaibab and other high plateaus in northern Arizona and southern Utah (Fig. 1) decreased significantly, with woodland lakes either drying completely or decreasing markedly in depth (Weng & Jackson, 1999). In southcentral California, a region of close

geographical proximity, maximum temperature and drought occurred between 7000–4000 years BP (Davis, 1999), while in the Great Basin, harsh conditions and regional drought similarly held sway from 7000–5000 years BP (Wigland & Rhode, 2002). These warmer and drier conditions also left indelible imprints upon coral reefs in New Guinea (Tudhope *et al.*, 2001). The rapidity by which climatic changes occurred during the transition period was determined using high-resolution ice cores from central Greenland (Alley, 2000). In less than 4 years during this period, average temperatures in Greenland rose by 9.4 °C.

The massive habitat alteration evoked by aridity was concomitant with a striking series of extinctions. Approximately, 35–40 species of large herbivorous and carnivorous mammals (Webb, 1984) as well as numerous larger predatory and carrion-feeding birds (Steadman & Martin, 1984) disappeared during this period. Simultaneous and sudden extinction episodes occurred in South America (Horton, 1984) as well. Yet climatically induced extinction episodes are not rare in North America. At least six have occurred over the past 10 million years BP (Webb, 1984), sparked primarily by a wobbling periodicity in the earth's orbit that allows varying amounts of solar radiation to be received at the earth's surface (Webb & Bartlein, 1992). However, the drought that occurred in late Pleistocene/early Holocene is rated as one of the more dramatic short-term events in Earth's evolutionary history (Frakes, Francis & Syktus, 1992). It ranked second in severity amongst the six recorded extinction episodes and was considered the worst if only large-bodied mammals are considered (Webb, 1984). While many species were completely extirpated during this period (Martin, 1989b), others went through quite severe population crashes. Interestingly, these reductions are recognized only through examination of mitochondrial and nuclear DNAs in extant populations. For example, both grey wolf (*Canis lupus* L.) and coyote (*C. latrans* Say) showed recent coalescence of mtDNA haplotypes that reflect severe post-Pleistocene population reductions (Vilà *et al.*, 1999). Similarly, North American puma [*Puma concolor* (L.)] crashed during the hypsithermal but was founded again by small number of migrants from eastern South America that survived a similar fate on that continent (Culver *et al.*, 2000). Mitochondrial DNA haplotypes in Central and South American jaguar

[*Panthera onca* (L.)] also reflect a severe population bottleneck in the recent past, followed by a subsequent population expansion (Eizirik *et al.*, 2001). These effects were not limited to mammals alone. Along the Pacific coast of North America, both birds (Zink *et al.*, 2000) and marine gastropods (Hellberg, Balch & Roy, 2001) show similar responses. Although the late Pleistocene/Holocene drought was indeed severe (as above), some researchers (Phillips, 1994; Phillips, Suau & Templeton, 2000) have argued that its impacts have been largely overlooked in most taxa.

Management units and Catostomus latipinnis

How are these perspectives translated into management recommendations? First, we gained an evolutionary overview of the species as a whole (as advocated in Brunner *et al.*, 2001), because the analyses were conducted distribution-wise. We conclude that *C. latipinnis* represents a single ESU. Its division into potential management units (MUs; Moritz, 1994b) will require the employment of faster-evolving nuclear markers (as per Brunner, Douglas & Bernatchez, 1998; Douglas *et al.*, 1999). Second, although *C. latipinnis* re-colonized the entire basin during the past 10 000 years and might have done so repeatedly in its evolutionary history, it could no longer do so today. Glen Canyon Dam has severed the connection between upper and lower basin populations, while the downstream Lake Mead reservoir separates the Virgin River drainage from the Grand Canyon (Fig. 1). To the north, Flaming Gorge Dam also sundered populations in the upper Green River from those in the remainder of the basin. While *C. latipinnis* clearly had the historic potential to recover from a severe crash and subsequent bottleneck, its basin-wide recovery would today be inhibited because of the fragmented nature of the riverine system. Interestingly, genetic diversity in the related (and endangered) razorback sucker (*X. texanus*) declines from the lower to the upper basin and the majority of genetic variation in this species is within, rather than among populations (Dowling, Minckley & Marsh, 1996). The management strategy for this species was also to consider it a single, basin-wide population.

Catostomus latipinnis was once a candidate species for listing but is now considered as a 'species of concern' (U. S. Fish and Wildlife Service, 1994). It remains widely distributed in the basin and is

abundant in some parts of the system (Douglas & Marsh, 1998; Douglas & Douglas, 2000). It thus may appear less affected by activities of mankind (as per Minckley & Douglas, 1991; Douglas, Marsh & Minckley, 1994) than those species now formally listed. If however, the low genetic diversity observed in this study were found within an endangered species, it would be interpreted from the standpoint of small population sizes, demographic instability, and the inbreeding/bottleneck effects that stem from habitat reduction, fragmentation and concomitant loss of gene flow. Thus, the identification of a similar pattern in a widespread species forces us to reconsider our perspectives on the endangered fishes of the Colorado River system. While the *prima facie* reason for their endangerment may still be mankind-related (see below), an alternative hypothesis to account for the genetic effects we see today must accommodate historic drought during the late Pleistocene/Holocene. This aspect, for example, would loom large when researchers assessed genetic variability in endangered species (such as *X. texanus*) and used these data to define such parameters as ESU, MU, minimum viable population size and other management-oriented parameters (as per Matocq & Villablanca, 2001). Finney *et al.* (2000) offered a similar perspective when they demonstrated that population numbers of sockeye salmon [*Oncorhynchus nerka* (Walbaum)] were strongly affected by climatic fluctuations during the past 300 years. Because sockeye salmon is commercially important, harvest limits must now accommodate the fact that salmon dynamics are naturally mediated by climate.

There are other management-related difficulties with native Colorado River fishes that pertain less to climatic variability and more to activities of mankind. Hypolimnetic releases from Lake Powell reservoir that resulted from closure of Glen Canyon Dam in 1963 were a sudden and artificial climate change imposed upon endemic fishes in Grand Canyon (reviewed by Douglas & Marsh, 1996). Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were also stocked in Grand Canyon at Lees Ferry so as to utilize the cold tailwater releases. This fishery has subsequently developed into world-class stature. At present, the Lees Ferry area is managed solely for trout, because the endangered humpback chub (*Gila cypha* Miller) does not occur until 75 km downstream (Valdez & Masslich, 1999) and native fishes in the Lees Ferry

area, to include *C. latipinnis*, are believed stable (McKinney, Persons & Rogers, 1999b). However, the conservation status of native fishes in the Colorado River Basin is still an unresolved question. Natural and mankind-induced climatic effects will clearly affect population dynamics of these species and the number and distribution of management units is still unresolved for all species. Thus, a determination of population stability may indeed be premature. We have demonstrated that a much faster-evolving genetic marker must be used to test each species for presence of MUs. Results from this study are insufficient for that purpose.

Finally, managers must realize that even long-lived and widely distributed species such as *C. latipinnis* are still relatively fragile when evaluated at an evolutionary time scale. Greater numbers and broader distributions in a particular study species (as per Douglas & Marsh, 1998) are often assumed to juxtapose with the existence of adequate levels of genetic diversity. This study demonstrates the fallacy of uncritically accepting such a premise. Given this, agencies must steadfastly avoid the acceptance of attractive but untested assumptions (as above) and instead employ as a basis for adaptive management, a broad geographic template with historic and extant perspectives (Douglas & Brunner, 2001). Our findings also provide a benchmark against which other mainstem Colorado River species can be compared. Many of the latter are either endangered or 'of concern', and this study provides a metric by which their long-term conservation and management can be measured. Given the past and recent histories of native fishes in southwestern North America (Minckley & Douglas, 1991), benchmarks such as ours are clearly necessary.

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References

- Alley R.B. (2000) *The Two-mile Time Machine. Ice Cores, Abrupt Climate Change, and Our Future*. Princeton University Press, Princeton, NJ.
- Arbogast B.S. & Slowinski J.B. (1998) Pleistocene speciation and the mitochondrial DNA clock. *Science*, **282**, 1955a.
- Avise J.C. (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York, NY.
- Avise J.C., Neigel J.E. & Arnold J. (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution*, **20**, 99–105.
- Avise J.C., Walker D. & Johns G.C. (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London Series B*, **265**, 1707–1712.
- Baker A.J. & Marshall D. (1997) Mitochondrial control region sequences as tools for understanding evolution. In: *Avian Molecular Systematics and Evolution* (Ed. D.P. Mindell), pp. 51–82. Academic Press, New York, NY.
- Baker A.J., Piersma T. & Rosenmeier R. (1994) Unraveling the intraspecific phylogeography of knots *Calidris canutus*: a progress report on the search for genetic markers. *Journal of Ornithology*, **135**, 599–608.
- Beerli P. & Felsenstein J. (1999) Maximum likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, **152**, 763–773.
- Bennett W.A., Ostrach, D.J. & Hinton, D.E. (1995) Larval striped bass condition in a drought-stricken estuary: Evaluating pelagic food-web limitation. *Ecological Applications*, **5**, 680–692.
- Bermingham E. & Martin A.P. (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, **7**, 499–518.
- Bermingham E., McCafferty S.S. & Martin A.P. (1997) Fish biogeography and molecular clocks: perspectives from the Panamanian isthmus. In: *Molecular Systematics of Fishes* (Eds T.D. Kocher & C.A. Stepien), pp. 113–128. Academic Press, New York, NY.
- Bernatchez, L. & Wilson C.C. (1998) Comparative phylogeography of nearctic and palearctic freshwater fishes. *Molecular Ecology*, **7**, 431–452.
- Bielawski J.P. & Gold J.R. (1996) Unequal synonymous substitution rates within and between two protein coding mitochondrial genes. *Molecular Biology and Evolution*, **13**, 889–892.
- Boulton A.J. (2003) Parallels and contrasts in effects of drought on stream macroinvertebrate assemblages. *Freshwater Biology*, **48**, 1173–1185.
- Brunner P.C., Douglas M.R. & Bernatchez L. (1998) Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from Central Alpine lakes. *Molecular Ecology*, **7**, 209–223.
- Brunner P.C., Douglas M.R., Osinov A., Wilson C.C. & Bernatchez L. (2001) Holarctic phylogeography of Arctic Charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **55**, 573–586.
- Canton S.P., Cline L.D., Short R.A. & Ward J.V. (1984) The macroinvertebrates and fish of a Colorado stream during a period of fluctuating discharge. *Freshwater Biology*, **14**, 311–316.
- Chart T.E. & Bergersen E.P. (1992) Impact of mainstream impoundment on the distribution and movements of the resident flannelmouth sucker (*Catostomidae: Catostomus latipinnis*) populations in the White river, Colorado. *The Southwestern Naturalist*, **37**, 9–15.
- Closs G.P. & Lake P.S. (1996) Drought, differential mortality and the coexistence of a native and introduced fish species in a southeast Australian intermittent stream. *Environmental Biology of Fishes*, **47**, 17–26.
- Culver M., Johnson W.E., Pecon-Slattery J. & O'Brien S.J. (2000) Genomic ancestry of the American Puma (*Puma concolor*). *Journal of Heredity*, **91**, 186–197.
- Davis O.K. (1999) Pollen analysis of Tulare lake, California: great basin-like vegetation in Central California during the full glacial and early Holocene. *Review of Palaeobotany and Palynology*, **107**, 249–257.

- Douglas M.E. & Marsh P.C. (1996) Population estimates/population movements of *Gila cypha*, an endangered cyprinid fish in the Grand Canyon region of Arizona. *Copeia*, **1996**, 15–28.
- Douglas M.E. & Marsh P.C. (1998) Population and survival estimates of *Catostomus latipinnis* in northern Grand Canyon with distribution and abundance of hybrids with *Xyrauchen texanus*. *Copeia*, **1998**, 915–925.
- Douglas M.E., Marsh P.C. & Minckley W.L. (1994) Indigenous fishes of western North America and the hypothesis of competitive displacement: *Meda fulgida* (Cyprinidae) as a case study. *Copeia*, **1994**, 9–19.
- Douglas M.E., Douglas M.R., Schuett G.W., Porras L.W. & Holycross A.T. (2002) Phylogeography of the western Rattlesnake (*Crotalus viridis*) complex, with emphasis on the Colorado plateau. In: *Biology of the Vipers* (Eds G.W. Schuett, M. Höggren, M.E. Douglas & H.W. Greene), pp. 11–50. Eagle Mountain Publishing, LC, Eagle Mountain, UT.
- Douglas M.R. & Brunner P.C. (2001) Biodiversity of Central Alpine *Coregonus* (Salmoniformes): impact of one hundred years of management. *Ecological Applications*, **12**, 154–172.
- Douglas M.R. & Douglas M.E. (2000) Late season reproduction by big river Catostomidae in Grand Canyon (Arizona). *Copeia*, **2000**, 238–244.
- Douglas M.R., Brunner P.C. & Bernatchez L. (1999) Do assemblages of *Coregonus* (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? *Molecular Ecology*, **8**, 589–603.
- Dowling T.E., Minckley W.L. & Marsh P.C. (1996) Mitochondrial DNA diversity within and among populations of razorback sucker (*Xyrauchen texanus*) as determined by restriction endonuclease analysis. *Copeia*, **1996**, 542–550.
- Eizirik E., Kim J.-H., Menotti-Raymond M., Crawshaw P.G. Jr, O'Brien S.J. & Johnson W.E. (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). *Molecular Ecology*, **10**, 65–79.
- Excoffier L., Smouse P.E. & Quattro J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics*, **131**, 470–491.
- Faber J.E. & White M.M. (2000) Comparison of gene flow estimates between species of darters in different streams. *Journal of Fish Biology*, **57**, 1465–1473.
- Fagen M.J. & Saier M.H. (1994) P-type ATPase of eukaryotes and bacteria: sequence analyses and construction of phylogenetic trees. *Journal of Molecular Evolution*, **38**, 57–99.
- Farris J.S., Källersjö M., Kluge A.G. & Bult C. (1994) Testing significance of incongruence. *Cladistics*, **10**, 315–319.
- Felsenstein J. (1992) Estimating effective population size from samples of sequences: a bootstrap Monte Carlo integration method. *Genetic Research Cambridge*, **60**, 209–220.
- Finney B.P., Gregory-Eaves I., Sweetman J., Douglas M.S.V. & Smol J.P. (2000) Impacts of climatic change on Pacific Salmon abundance over the past 300 years. *Science*, **290**, 795–799.
- Frakes L.A., Francis J.E. & Syktus J.I. (1992) *Climate Modes of the Phanerozoic. The History of the Earth's Climate Over the Past 600 Million Years*. Cambridge University Press, Cambridge, U.K.
- Freeman M.C., Crawford M.K., Barrett J.C., Facey D.E., Flood M.G., Hill J., Stouder D.J. & Grossman G.D. (1988) Fish assemblage stability in a southern Appalachian stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1949–1958.
- Gaines S.D. & Rice W.R. (1990) Analysis of biological data when there are ordered expectations. *American Naturalist*, **135**, 310–317.
- Gilbert D.G. (1999) SeqPup, a Biosequence Editor, Version 0.9f. University of Indiana, Bloomington, IN.
- Grant W.S. & Bowen B.W. (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.
- Hellberg M.E., Balch D.P. & Roy K. (2001) Climate-driven range expansion and morphological evolution in a marine gastropod. *Science*, **292**, 1707–1710.
- Hillis D.M. & Bull J.J. (1993) An empirical test of bootstrapping as a method of assessing confidence in phylogenetic analysis. *Systematic Biology*, **42**, 182–192.
- Hillis D.M., Mable B.K. & Moritz C. (1996) Applications of molecular systematics: the state of the field and a look to the future. In: *Molecular Systematics*, 2nd edn (Eds D.M. Hillis, C. Moritz & B.K. Mable), pp. 515–543. Sinauer, Sunderland, MA.
- Horton D.R. (1984) Red kangaroos: last of the Australian megafauna. In: *Quaternary Extinctions* (Eds P.S. Martin & R.G. Klein), pp. 639–680. University of Arizona, Tucson, AZ.
- Kelsch S.W. (1994) Lotic fish-community structure following transition from severe drought to high discharge. *Journal of Freshwater Ecology*, **9**, 331–342.
- Klicka J. & Zink R.M. (1998) Pleistocene speciation and the mitochondrial DNA clock. *Science*, **282**, 1955a.
- Kumar S. (1996) Patterns of nucleotide substitution in mitochondrial protein coding genes of vertebrates. *Genetics*, **143**, 537–548.

- Kumar S., Tamura K., Jakobsen I.B. & Nei M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics*, **17**, 1244–1245.
- Lavery S., Moritz C. & Fielder D.R. (1996) Genetic patterns suggest exponential population growth in a declining species. *Molecular Biology and Evolution*, **13**, 1106–1113.
- Livingston R.J. (1997) Trophic responses of estuarine fishes to long-term changes of river runoff. *Bulletin of Marine Science*, **60**, 984–1004.
- Livingston R.J., Niu X., Lewis F.G. III & Woodsum G.C. (1997) Freshwater input to a Gulf estuary: long-term control of trophic organization. *Ecological Applications*, **7**, 277–299.
- McAda C.W. & Wydowski R.W. (1985) Growth and reproduction of the flannelmouth sucker, *Catostomus latipinnis*, in the upper Colorado river basin, 1975–76. *The Great Basin Naturalist*, **45**, 281–286.
- McKinney T., Persons W.R. & Rogers R.S. (1999a) Ecology of flannelmouth sucker in the Lees Ferry tailwater, Colorado river, Arizona. *Great Basin Naturalist*, **59**, 259–265.
- McKinney T., Ayers A.D. & Rogers R.S. (1999b) Macroinvertebrate drift in the tailwaters of a regulated river below Glen Canyon dam, Arizona. *The Southwestern Naturalist*, **44**, 205–210.
- Maddison W.P. & Maddison D.R. (1998) *MacClade, analysis of phylogeny and character evolution*. Version 3.07. University of Arizona, Tucson, AZ.
- Martin R. (1989a) *A Story that Stands Like a Dam: Glen Canyon and the Struggle for the Soul of the West*. Henry Holt and Company, New York, NY.
- Martin L.D. (1989b) Fossil history of terrestrial carnivora. In: *Carnivore Behavior, Ecology, and Evolution* (Ed. J.L. Gittleman), pp. 536–568. Cornell University Press, Ithaca, NY.
- Matocq M.D. & Villablanca F.X. (2001) Low genetic diversity in an endangered species: recent or historical pattern? *Biological Conservation*, **98**, 61–68.
- Matthews W.L. (1998) *Patterns in Freshwater Fish Ecology*. Chapman & Hall, New York, NY.
- Matthews W.J. & Marsh-Matthews E. (2003) Effects of drought on fish across axes of space, time and ecological complexity. *Freshwater Biology*, **48**, 1233–1255.
- Medeiros S.F. & Maltchik L. (1999) The effects of flood and drought on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. *Journal of Arid Environments*, **43**, 351–356.
- Meffe G.K. (1990) Post-defaunation recovery of fish assemblages in southeastern blackwater streams. *Ecology*, **71**, 657–667.
- Miller R.R. (1959) Origin and affinities of the freshwater fish fauna of western North America. In: *Zoogeography* (Ed. C.L. Hubbs), pp. 187–222. American Society for the Advancement of Science, Washington, D.C.
- Miller R.R. & Smith G.R. (1984) Fish remains from Stanton's Cave, Grand Canyon of the Colorado, AZ, with notes on the taxonomy of *Gila cypha*. In: *Archaeology, Geology, and Paleobiology of Stanton's Cave* (Ed. R. Euler), pp. 61–65. Grand Canyon Natural History Association Monograph 6, Grand Canyon, AZ.
- Minckley W.L. (1973) *Fishes of Arizona*. Arizona Game and Fish Department, Phoenix, AZ.
- Minckley W.L. (1991) Native fishes of the Grand Canyon region: an obituary? In: *Colorado River Ecology and Dam Management* (Ed. Committee to Review the Glen Canyon Environmental Studies), pp. 124–177. National Academy, Washington, D.C.
- Minckley W.L. & Douglas M.E. (1991) Discovery and extinction of western fishes: a blink of the eye in geologic time. In: *Battle Against Extinction: Native Fish Management in the American West* (Eds W.L. Minckley & J.E. Deacon), pp. 7–17. University of Arizona Press, Tucson, AZ.
- Minckley W.L. & Holden P.B. (1980) *Catostomus latipinnis* Baird and Girard, flannelmouth sucker. In: *Atlas of North American Freshwater Fishes* (Eds D.S. Lee, C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister & J.R. Stauffer), p. 381. North Carolina State Museum of Natural History, Raleigh, NC.
- Minckley W.L., Hendrickson D.L. & Bond C.E. (1986) Geography of western North American freshwater fishes: description and relationships to intracontinental tectonism. In: *Zoogeography of North American Freshwater Fishes* (Eds C.H. Hocutt & E.O. Wiley), pp. 519–613. John Wiley & Sons, New York, NY.
- Mol J.H., Resida D., Ramlal J.S. & Becker C.R. (2000) Effects of El Niño-related drought on freshwater and brackish water fishes in Suriname, South America. *Environmental Biology of Fishes*, **59**, 429–440.
- Moritz C. (1994a) Application of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.
- Moritz C. (1994b) Defining 'Evolutionary Significant Units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.
- Nee S., Holmes E.C., Rambaut A. & Harvey P.H. (1995) Inferring population history from molecular phylogenies. *Philosophical Transactions of the Royal Society of London, Series B*, **349**, 25–31.
- Perdices A. & Doadrio I. (2001) The molecular systematics and biogeography of the European cobitids based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **19**, 468–478.
- Phillips C.A. (1994) Geographical distribution of mitochondrial DNA variants and the historical biogeography

- of the spotted salamander, *Ambystoma maculatum*. *Evolution*, **48**, 597–607.
- Phillips C.A., Suau G. & Templeton A.R. (2000) Effects of Holocene climatic fluctuation on mitochondrial DNA variation in the ringed salamander, *Ambystoma annulatum*. *Copeia*, **2000**, 542–545.
- Pielou E.C. (1991) *After the Ice Age. The Return of Life to Glaciated North America*. University of Chicago Press, Chicago, IL.
- Rambaut A. (1998) *END-EPI, a computer program for inferring phylogenetic process*. Version 1.0. Evolutionary Biology Group, University of Oxford, Oxford, U.K.
- Rogers A.R. & Harpending H. (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.
- Rohlf, F.J. (1998) *BIOMstat: statistical software for biologists*. Version 3.3. Applied Biostatistics Inc., Seatauket, NY.
- Ross S.T., Matthews W.J. & Echelle A.A. (1985) Persistence of stream fish assemblages: effects of environmental change. *American Naturalist*, **126**, 24–40.
- Rozas J. & Rozas R. (1999) DNAsp version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics*, **15**, 174–175.
- Rutledge C.J., Zimmerman E.G. & Beiting T.L. (1990) Population genetic responses of two minnow species (Cyprinidae) to seasonal stream intermittency. *Genetica*, **80**, 209–219.
- Schneider S., Roessli D. & Excoffier L. (2000) *Arlequin, a software for population genetic analysis*. Version 2. University of Geneva, Switzerland.
- Shannon J.P., Blinn D.W. & Stevens L.E. (1994) Trophic interactions and benthic animal community structure in the Colorado river, Arizona, U.S.A. *Freshwater Biology*, **31**, 213–220.
- Shields G.F. & Gust J.R. (1995) Lack of geographic structure in mitochondrial DNA sequences of Bering sea walleye pollock, *Theragra chalcogramma*. *Molecular Marine Biology and Biotechnology*, **4**, 69–82.
- Slatkin M. & Hudson R.R. (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.
- Slovan K.A., Taylor A.C. & Metcalfe N.B. (2001) Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. *Animal Behaviour*, **61**, 325–333.
- Smith G.R. (1992) Phylogeny and biogeography of the Catostomidae, freshwater fishes of North America and Asia. In: *Systematics, Historical Ecology, and North American Freshwater Fishes* (Ed. R.L. Mayden), pp. 778–826. Stanford University Press, Stanford, CA.
- Smith G.R., Dowling T.E., Gobalet K.W., Lugaski T., Shiozawa D.K. & Evans R.P. (2002) Biogeography and timing of evolutionary events among Great Basin fishes. In: *Great Basin Aquatic Systems History* (Eds R. Hershler, D.B. Madsen & D.R. Currey), pp. 175–234. Smithsonian Contributions to the Earth Sciences No. 33, Smithsonian Institution Press, Washington D.C.
- Sokal R.R. & Rohlf F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman, New York, NY.
- Steadman D.W. & Martin P.S. (1984) Extinction of birds in the late Pleistocene of North America. In: *Quaternary Extinctions* (Eds P.S. Martin & R.G. Klein), pp. 466–477. University of Arizona, Tucson, AZ.
- Stevens L.E., Shannon J.P. & Blinn D.W. (1997) Colorado river benthic ecology in Grand Canyon, Arizona, USA: dam, tributary, and geomorphological influences. *Regulated Rivers: Research and Management*, **13**, 129–149.
- Swofford D.L. (1998) *PAUP*, Phylogenetic Analysis Using Parsimony (and other methods)*. Version 4.04b. Sinauer Publishers, Sunderland, MA.
- Tajima F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.
- Tajima F. (1993) Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics*, **135**, 599–607.
- Tarboton D.G. (1995) Hydrologic scenarios for severe sustained drought in the southwestern United States. *Water Research Bulletin*, **31**, 803–813.
- Timmons R.M. (1998) *Post-perturbation genetics of *Catostomus latipinnis* and *Pantosteus clarki* (Teleostei: Catostomidae)*. MS Thesis. Arizona State University, Tempe, AZ.
- Tramer E.J. (1977) Catastrophic mortality of stream fishes trapped in shrinking pools. *American Midland Naturalist*, **97**, 469–478.
- Tudhope, A.W., Chilcott C.P., McCulloch M.T., Cook E.R., Chappel J., Ellam R.M., Lea D.W., Lough J.M. & Shimmield G.B. (2001) Variability in the El Niño – southern oscillation through a Glacial – Interglacial cycle. *Science*, **291**, 1511–1517.
- Tyus H.M. & Karp C.A. (1990) Spawning and movement of razorback sucker, *Xyrauchen texanus*, in the Green River basin of Colorado and Utah. *The Southwestern Naturalist*, **35**, 427–433.
- U. S. Fish and Wildlife Service (1994) Endangered and threatened wildlife and plants; animal candidate review for listing as endangered or threatened species; proposed rule (November, 1994). *Federal Register*, **50** CFR, part 17.
- Uyeno T. & Miller R.R. (1963) Summary of late Cenozoic freshwater fish records for North America. *Occasional*

- Papers of the Museum of Zoology University of Michigan*, **631**, 1–34.
- Uyeno T. & Miller R.R. (1965) Middle Pliocene cyprinid fishes from the Bidahochi Formation, Arizona. *Copeia*, **1965**, 28–41.
- Valdez R.A. & Masslich W.J. (1999) Evidence of reproduction by humpback chub in a warm spring of the Colorado river in Grand Canyon, Arizona. *The Southwestern Naturalist*, **44**, 384–387.
- Vilà C., Amorim I.R., Leonard J.A., Posada D., Castroviejo J., Petrucci-Fonseca F., Crandall K.A., Ellegren H. & Wayne R.K. (1999) Mitochondrial DNA phylogeography and population history of the grey wolf (*Canis lupus*). *Molecular Ecology*, **8**, 2089–2103.
- Walker D. & Avise J.C. (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annual Review of Ecology and Systematics*, **29**, 23–58.
- Walker D., Moler P.E., Buhlmann K.A. & Avise J.C. (1998) Phylogeographic uniformity in mitochondrial DNA of the snapping turtle (*Chelydra serpentina*). *Animal Conservation*, **1998**, 55–60.
- Webb S.D. (1984) Ten million years of mammal extinctions in North America. In: *Quaternary Extinctions* (Eds P.S. Martin & R.G. Klein), pp. 189–210. University of Arizona, Tucson, AZ.
- Webb T. III & Bartlein P.J. (1992) Global changes during the past 3 million years: climatic controls and biotic responses. *Annual Review of Ecology and Systematics*, **23**, 141–173.
- Weiss S.J., Otis E.O. & Maughan O.E. (1998) Spawning ecology of flannelmouth sucker, *Catostomus latipinnis* (Catostomidae), in two small tributaries of the lower Colorado river. *Environmental Biology of Fishes*, **52**, 419–433.
- Weng C. & Jackson S.T. (1999) Late glacial and Holocene vegetation history and paleoclimate on the Kaibab plateau, Arizona. *Palaeography, Palaeoclimatology, Palaeoecology*, **153**, 179–201.
- Wigland P.E. & Rhode D. (2002) Great Basin vegetation history and aquatic systems: the last 150 000 years. In: *Great Basin Aquatic Systems History* (Eds R. Hershler, D.B. Madsen & D.R. Currey), pp. 309–367. Smithsonian Contributions to the Earth Sciences No. 33, Smithsonian Institution Press, Washington D.C.
- Zink R.M., Barrowclough G.F., Atwood J.L. & Blackwood-Rago R.C. (2000) Genetics, taxonomy, and conservation of the threatened California gnatcatcher. *Conservation Biology*, **14**, 1394–1405.

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Appendix 1 Locations for the 24 populations of *Catostomus latipinnis* evaluated in this study, where AB = population abbreviation; N = sample size; ST = state; CO = county; and LOC = general location

AB	N	ST	CO	LOCATION
166/202	6	AZ	Coconino	River mile 166–202 in Grand Canyon
BDL	15	UT	Washington	Beaver dam Wash
BWL	6	WY	Sublette	Burnt and Willow lakes
C15	20	CO	Mesa	River mile 177
DDI/Q	20	UT	Emery	Ivy and Quitcupah Cks./Dirty Devil River
DES	9	UT	Uintah	Desolation CN of Green River (River mile 65)
ESC	10	UT	Garfield	Escalante River at Rt. 12 bridge
FRE	7	UT	Wayne	Fremont River below Rt. 24 bridge
FGD	17	WY	Sweetwater	Flaming Gorge Reservoir (big sandy confl.)
GUN	18	CO	Delta	Gunnison River at Redlands passage
HAV	20	AZ	Coconino	Havasus Creek
KAN	21	AZ	Coconino	Kanab Creek
LCR	40	AZ	Coconino	Little Colorado River
M2M	15	AZ	Coconino	Lees Ferry reach
MEC	11	AZ	Apache	McElmo Creek (Navajo nation)
PAR	15	AZ	Coconino	Paria River
SHN	20	AZ	Coconino	Shinumo Creek
SJR	10	UT	San Juan	San Juan River (river mile 11–157)
SPN/Q	4	AZ	Mohave	Spencer Creek (river mile 246)
SRR	20	UT	Emery	San Raphael River above confl. Green river
VWF	17	UT	Washington	Washington fields diversion
YAM	12	CO	Moffat	Yampa River (River mile 38–43)
YDT	9	CO	Moffat	Yampa River at Duffy tunnel (river mile 109)
YXM	10	CO	Moffat	Yampa River at Cross Mt. (river mile 63)

Appendix 2 Sequence divergence in haplotypes of *Catostomus latipinnis* at three mitochondrial DNA loci: ATPase 8, ATPase 6, and ND2. Only variable sites are listed by sequence location, where A = Adenine; G = Guanine; T = Thymine; C = Cytosine; and N = number of individuals per haplotype

Nucleotide position	ATP8					ATP6					ND2																							
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5																			
Haplotype 1	A	C	A	A	G	A	T	A	C	T	A	A	G	A	G	T	A	A	A	A	A	G	G	G	G	A	G	A	T	G	A	25		
2	T	56
3	T	7
4	T	5
5	T	45
6	T	31
7	T	62
8	T	19
9	T	10
10	T	29
11	T	1
12	T	2
13	T	1
14	G	T	2
15	T	5
16	T	1
17	T	G	1
18	T	1
19	T	2
20	T	1
21	T	1
22	T	2
23	T	1
24	T	1
25	T	2
26	T	1
27	T	1
28	T	1
29	T	1
30	T	1
31	T	2
32	T	1
33	T	5
34	T	1
35	T	1

