

Genetic structure of an expanding population of Humpback Chub in Grand Canyon

M. C. Dzul^{1*}, R. Massatti², C. B. Yackulic¹, E. Omana Smith³, and K. Young^{4†}

¹U.S. Geological Survey, Southwest Biological Science Center, Grand Canyon Monitoring and Research Center, Flagstaff, Arizona, USA

²U.S. Geological Survey, Southwest Biological Science Center, Flagstaff, Arizona, USA

³National Park Service, Grand Canyon National Park, Flagstaff, Arizona, USA

⁴U.S. Fish and Wildlife Service, Arizona Fish and Wildlife Conservation Office, Flagstaff, Arizona, USA

*Corresponding author: M. C. Dzul. Email: mdzul@usgs.gov.

†Kirk Young has retired.

ABSTRACT

Objective: Humpback Chub (HBC) *Gila cypha* in Grand Canyon declined in abundance and distribution over the latter part of the 20th century but have substantially increased in abundance and distribution over the past two decades. Although previous genetic work suggested that HBC in Grand Canyon belong to one genetic group, here we evaluate the genetic structure of HBC in Grand Canyon to determine whether relic populations in western Grand Canyon may have contributed unique variation to the recent population expansion or whether differences in behavior linked to migration in eastern Grand Canyon may promote assortative mating and heretofore unrecognized population structure.

Methods: Using fin clips collected from 167 individual HBC representing four sampling sites within Grand Canyon, we extracted DNA and developed data sets consisting of approximately 20,000 anonymous genomic loci. We quantified patterns of genetic diversity, and we accounted for outlier single-nucleotide polymorphisms to ensure that our interpretations of genetic patterns were not misled by adaptive processes and did not mask adaptively important genetic variation.

Results: Despite behavioral variation and the possibility that individuals with unique genetic variation survived in isolated warmwater sites within western Grand Canyon, HBC were not differentiated by sampling site or by differences in migratory behavior. Heterozygosity and nucleotide diversity were consistently high among sampling sites, and inbreeding coefficients were close to zero.

Conclusions: The HBC in Grand Canyon constitute a single genetic population. Our results do not preclude a genetic basis to migratory behavior, but our data suggest that this trait does not lead to assortative mating. Furthermore, while HBC may have survived in discontinuous warmwater refugia in western Grand Canyon during decades when the main stem was too cold for spawning, our data did not reveal any noticeable spatial variability in HBC genetics in the main stem after the recent HBC population expansion.

KEYWORDS: Colorado River, genetics, *Gila* spp., Grand Canyon, Havasu Creek, Humpback Chub, Little Colorado River, partial migration, population expansion, translocations

LAY SUMMARY

Despite differences in behaviors and a history of population expansions and contractions both before and after human management of the Colorado River, Humpback Chub in Grand Canyon are genetically homogenous.

INTRODUCTION

Preserving genetic diversity and maintaining spatial genetic structure are important components of conservation planning for imperiled species (Moritz, 1994; Paz-Vinas et al., 2018). Genetic diversity is often considered a proxy for the ability of a population to adapt to environmental change (Crawford

& Whitney, 2010; DeWoody et al., 2021; García-Dorado & Caballero, 2021); populations with higher genetic diversity have more adaptive potential because they have more alleles available in their gene pool, which may be advantageous in response to various selective pressures. Additionally, spatial structure in genetic diversity can be indicative of adaptation to local site characteristics, reproductive barriers, or both

(Ackerman et al., 2013; Flanagan et al., 2018). In particular, genetics can be used to develop conservation units, which can then be used to develop management plans that inform endangered species listing or delisting; translocation, stocking, or captive breeding programs; and threat identification (Barbosa et al., 2018; Fraser & Bernatchez, 2001; Moritz, 1994).

Before the construction of large dams and introductions of nonnative species in the 20th century, the Colorado River was home to a highly endemic assemblage of fishes that evolved in a dynamic riverine environment (Miller, 1958; Minckley, 1991), with large floods followed by periods of low base flow, high suspended sediment concentrations, and seasonally variable water temperatures (Schmidt et al., 2022; Wright et al., 2009). Like other native fishes of the Colorado River, the Humpback Chub (HBC) *Gila cypha* experienced decreases in population sizes and constrictions in geographic range after nonnative species introductions and dam construction throughout the Colorado River and its major tributaries (Minckley, 1991; U.S. Fish and Wildlife Service [USFWS], 2018). Currently, there are five recognized extant HBC populations that occur across Utah, Colorado, and Arizona; the Grand Canyon population is estimated to be the largest (USFWS, 2002a, 2018). Moreover, genetic analyses have shown that Grand Canyon HBC have experienced less hybridization with Roundtail Chub (RTC) *G. robusta* and may therefore represent the most genetically “pure” population within the species (Bohn et al., 2019; Chafin et al., 2019; Douglas & Douglas, 2007).

Water temperatures in Grand Canyon have varied substantially over decadal scales and are believed to be a primary driver of both the decline and the more recent growth of the HBC population in Grand Canyon, with unknown impacts on genetic diversity. The water temperature of the Colorado River is determined primarily by the temperature of the water released from Lake Powell via Glen Canyon Dam (GCD), with the volume of releases playing a more important role in determining water temperatures in further downriver segments (Mihalevich et al., 2020). Lake Powell began filling in 1963, and by the early 1970s, water drawn from the hypolimnion of Lake Powell was released from GCD, resulting in water temperatures that were generally too cold for HBC reproduction and growth (Gorman & Van Hoosen, 2000; Robinson & Childs, 2001); we refer to this period (1970s–1990s) as the “coldwater period.” By the early 2000s, water temperatures started warming as the water level in Lake Powell slowly declined such that water was released from the warmer metalimnion or epilimnion (hereafter, the “warming period”; Dibble et al., 2021). As water flows downstream from GCD, it slowly warms due to solar irradiation, causing western Grand Canyon (WGC) to be generally warmer than eastern Grand Canyon, particularly in summer and fall (Mihalevich et al., 2020).

During the coldwater period, HBC that spawned in the Little Colorado River (LCR), a tributary to the Colorado River located about 124 km downstream of GCD (hereafter, “LCR-spawning HBC”) were considered the stronghold of HBC within Grand Canyon. Cold water temperatures generally precluded HBC from spawning and rearing in the main-stem Colorado River (with some exceptions; see Andersen et al., 2010), whereas LCR spawners persisted by spending part or all of their lives in the warmwater LCR (Kaeding & Zimmerman, 1983). Abundances of LCR-spawning adults (>200 mm total

length [TL]) declined to lower than 5,000 in the early 2000s (Coggins & Walters, 2009), likely due to cold water temperatures in the main stem limiting growth and forcing individuals to spend extended periods in vulnerable early life stages (Clarkson & Childs, 2000; Coggins et al., 2011; Yackulic et al., 2018). Adult abundances of LCR spawners increased during the warming period and have exceeded 9,000 since 2009 (Dzul et al., 2022; Yackulic et al., 2014). More recently, it has been shown that the LCR-spawning group of HBC exhibits partial migration and includes two life history types: LCR residents and LCR migrants (Yackulic et al., 2014). This partial migratory behavior may lead to divergent selection if it facilitates assortative mating due to differences in spawning habitat preferences or spawning phenology (Buchinger et al., 2023), but genetic divergence is less likely if migratory behavior is dependent on environmental conditions (Chapman et al., 2012).

Compared to the LCR-spawning group, HBC in other parts of Grand Canyon were rare during the coldwater period and tended to occur throughout the Colorado River in “aggregations,” a term coined by Valdez and Ryel (1995) to describe “a consistent and disjunct group of fish with no significant exchange of individuals with other aggregations, as indicated by recapture of passive integrated transponder (PIT) tagged juveniles and adults and movement of radio-tagged adults.” Some aggregation sites occurred in proximity to warmwater springs or tributaries, which led biologists to hypothesize that aggregations were a result of a once-contiguous population becoming fragmented due to small pockets of warmwater refugia within a cold river. In WGC (here defined as downstream of the confluence with Havasu Creek [HAV], ~277–477 km downstream of GCD), HBC were extremely rare during the coldwater period and occurred at only one aggregation site (Colorado River near Pumpkin Spring), which was estimated to have five adult HBC (Valdez & Ryel, 1995).

During the coldwater period, Douglas and Douglas (2007) collected tissues from about 10 HBC individuals in WGC for genetic analysis and observed very slight differentiation from LCR-spawning HBC; however, those authors noted that their analysis of WGC was limited by low sample size. During the warming period and after the formation of Pearce Ferry Rapid (a large rapid that impedes upstream movement from Lake Mead), increases in the catch of HBC were observed at aggregation sites starting as early as 2006 (Persons et al., 2017); by 2017, HBC became widespread throughout the western portion of Grand Canyon downstream of the confluence with HAV, both inside and outside of aggregation sites (Rogowski et al., 2018; Van Haverbeke et al., 2017). Currently, our observations indicate that adult HBC in WGC likely outnumber LCR spawners. The history of HBC decline, the possibility of isolated refugia in warmwater areas of WGC, and the subsequent population expansion since warming of the river merit genetic investigation to ensure that HBC conservation units are properly assigned. To our knowledge, this is the first study to assess the genetics of HBC in WGC since the population expansion.

Beginning in 2011, HBC were translocated into HAV (located ~277 km downstream of GCD). Translocated fish were originally collected from the LCR beginning in 2010, taken to a hatchery for 1 year, and then stocked into HAV as part of a conservation plan to expand the HBC population in Grand Canyon outside of the LCR. Since the time translocations started, wild

reproduction has been documented in HAV (Healy et al., 2020) and some translocated individuals have moved from HAV back into the LCR (Healy et al., 2022). A genetic analysis of wild-spawned HBC in HAV may provide insight as to whether these fish are the progeny of the translocated HBC or some other HBC group (e.g., WGC group), which could help to inform assessments of the success of this conservation action.

Here, we quantify patterns of HBC genetic differentiation across fish sampled from four Grand Canyon sites to address three study questions: (1) “Do the HBC experiencing rapid population growth in WGC harbor unique genetic variation?”; (2) “Do differences in migratory behavior identified in LCR-spawning HBC lead to assortative mating and, hence, heretofore unrecognized population structure?”; and (3) “If WGC individuals differ genetically from LCR spawners, to which group do nontranslocated HAV HBC belong?” The level of genetic distinctiveness among fish has important implications for species conservation, as the number of genetically defined populations within Grand Canyon relates to the identification of conservation units and, hence, representation and redundancy criteria used in recovery planning (USFWS, 2002b, 2018). Additionally, genetic differentiation across groups can be used to guide translocation efforts that bolster breeding populations across the distribution of HBC (e.g., Yackulic et al., 2021).

METHODS

Species description

Humpback Chub were listed as endangered in 1967 (USFWS, 2002b); however, the species was downlisted to threatened in 2021 (USFWS, 2021). After the initial listing, six HBC populations were identified, of which five are currently extant (Westwater, Black Rocks, Desolation/Gray Canyon, Cataract Canyon, and Grand Canyon) and one is functionally extirpated (Dinosaur National Monument; USFWS, 2018). Genetic studies of HBC suggest that the five extant populations fall into three genetic clusters: (1) Black Rocks, Westwater, and Cataract Canyon; (2) Desolation/Gray Canyon; and (3) Grand Canyon (Bohn et al., 2019; Douglas & Douglas, 2007). There is some evidence of both recent (Chafin et al., 2019) and historic (Douglas & Douglas, 2007) hybridization of HBC with two related species: the RTC and the Bonytail (BTC) *G. elegans*.

Within the Grand Canyon population, partial migration of LCR-spawning HBC has led to two different life history types: (1) LCR residents, which spend their whole life cycle in the LCR and typically exhibit faster growth and a shorter lifespan; and (2) LCR migrants, which are born and rear in the LCR but typically move into the Colorado River within their first year of life and exhibit slower growth and a longer lifespan (Dzul et al., 2021, 2023; Yackulic et al., 2014). Upon reaching adulthood (~200 mm TL), LCR migrants begin migrating into the LCR during spring months, presumably to spawn (Douglas & Marsh, 1996; Kaeding & Zimmerman, 1983). Humpback Chub exhibit variability in growth and survival according to spatial location and their size (Dzul et al., 2023; Yackulic et al., 2014), and this affects age at maturity and lifespan across sites. For LCR residents, HBC typically take approximately 3 years to reach adult size (200 mm TL), and once this size is attained,

the lifespan is short (1–2 years; Dzul et al., 2023; Yackulic et al., 2014). For LCR migrants, there is substantial variability in growth and age at maturation based on water temperatures, and the size of juvenile HBC when they out-migrate from the LCR is also highly variable. Generally, the attainment of adult size can occur within 5–8 years (but may occur in 3–4 years if warmer water temperatures like those observed in 2022 become more common), and the lifespan as adults is typically 6–10 years (Dzul et al., 2023), although some individuals can live much longer. Growth and survival of WGC HBC are intermediate to those of LCR migrants and LCR residents (Dzul et al., 2023).

It should be noted that 227 HBC from the LCR were released into WGC (~420 km downstream of GCD) during 2018, including 23 fish that were sonic-tagged to evaluate movement (K. Young, personal observation). This action occurred well after the documented increase in WGC HBC (Van Haverbeke et al., 2017), suggesting that the population expansion in WGC was almost exclusively through natural reproduction and minimally aided by translocation efforts.

Field methods

To investigate HBC genetic patterns within Grand Canyon, we sampled tissue from 167 fish representing four focal groups in October 2020. We captured fish spread throughout sampling sites (see Figure 1); we sampled in October because at that time, LCR migrants were in the Colorado River whereas LCR residents were in the LCR (Yackulic et al., 2014). All tissues from all HBC groups were pelvic fin clips sampled with ethanol-sterilized scissors and preserved in nondenatured 95% ethanol. Resident HBC in the LCR (60 tissues; hereafter, “LCR-RES”) were collected during long-term USFWS monitoring of the LCR. The LCR-RES fin clips were all collected about 10.0–13.5 km upstream from the confluence with the main stem to minimize the probability of including migrant individuals as part of the sample. Additionally, fin clips from fish that migrated into and out of the LCR (40 tissues; hereafter, “LCR-MIG”) were collected from a fixed monitoring site in the Colorado River (~1.9–7.2 km downstream of the LCR confluence). Fin clips from HBC in WGC (40 tissues) were collected from a fixed monitoring site (located ~239.8–245.4 km downstream of the LCR confluence), and fin clips from HBC in HAV (27 tissues) were collected in the lowest 6 km of the creek. Fin clips collected from HAV included five HBC that were translocated from the LCR into HAV in 2014. Sampling methods varied across groups. Humpback Chub in Grand Canyon were captured using either baited hoop nets (for HAV; Healy et al., 2020) or unbaited hoop nets (for LCR-MIG, LCR-RES, and WGC; Dzul et al., 2022; Van Haverbeke et al., 2013); in the main stem, electrofishing was also used (for LCR-MIG and WGC; Dzul et al., 2022).

In addition, we obtained 25 fin clip tissues representing three out-groups, including 15 HBC from the upper Colorado River basin (the Desolation/Gray Canyon population [DGP]) and five fish from each of two closely related species, BTC and RTC. Out-groups allowed us to compare our results with those of previous studies (e.g., Douglas & Douglas, 2007) and to ensure that we were not mistaking genetic variation resulting from hybridization (e.g., Chafin et al., 2019) as HBC

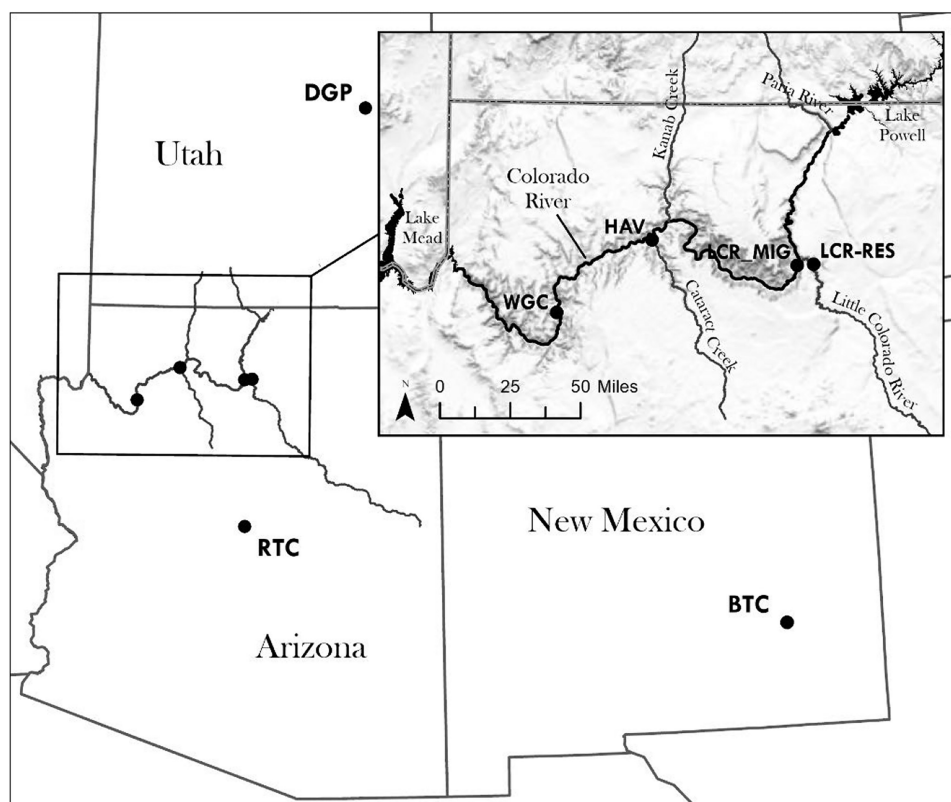


Figure 1. Map of locations of *Gila* spp. used in genetic analyses. Genetic groups included Bonytail (BTC) raised at the Southwestern Native Aquatic Resources and Recovery Center (Dexter, New Mexico); Roundtail Chub (RTC) obtained from Bubbling Ponds Hatchery (Cornville, Arizona) and originally from the Verde River, Arizona; and Humpback Chub (HBC) from the Desolation/Gray Canyon population (DGP), which were obtained from the Southwestern Native Aquatic Resources and Recovery Center but originally collected in the Green River, Utah. Within Grand Canyon, HBC groups corresponded to sampling sites in or near the Little Colorado River (LCR) and included LCR residents (LCR-RES; obtained 10.0–13.5 km upstream of the Colorado River confluence), LCR migrants (LCR-MIG; obtained from the Colorado River, ~1.9–7.2 km downstream from the LCR confluence), Havasu Creek (HAV; obtained from the lower ~6 km of the creek), and western Grand Canyon (WGC; obtained ~239.8–245.4 km downstream of the LCR confluence). (Figure credit: Erica Byerley, U.S. Geological Survey.)

genetic variation. Fin clips from BTC and DGP were frozen and provided by the Southwestern Native Aquatic Resources and Recovery Center (Dexter, New Mexico). There are no self-sustaining populations of BTC in the wild (USFWS, 2002b). The DGP tissues originated from sampling efforts conducted in the Green River during 2015. Fin clips from RTC were collected from Bubbling Ponds Hatchery (Cornville, Arizona) in October 2020, representing fish that were originally collected from the Verde River in Arizona. The RTC fin clips were preserved in 95% nondenatured ethanol.

Extraction of DNA, library preparation, and data processing

Genomic DNA from the 192 fin clip samples was extracted using DNeasy 96 Blood and Tissue Kits (Qiagen, Germantown, Maryland) and individually barcoded and processed into one library using a modified restriction fragment-based procedure (Peterson et al., 2012). Briefly, DNA was doubly digested with the restriction enzymes *EcoRI* and *MspI*, followed by ligation of Illumina adaptor sequences and combinatorial barcodes using forward and reverse indexing reads; barcodes were included in indices instead of in line with DNA fragments, resulting in reads that had more data. Ligation products were pooled and amplified using 18 cycles of PCR. A Pippin Prep system

(Sage Science, Beverly, Massachusetts) was used to size select amplicons from 400 to 600 bp. The library was sequenced on a NovaSeq 6000 (Illumina, San Diego, California) at the University of Oregon's Genomics and Cell Characterization Core Facility (Eugene, Oregon) to generate single-end 118-bp reads.

Raw data were demultiplexed and cleaned (i.e., to exclude raw reads containing more than four low-quality sites and/or adapter contamination) using “fastq-multx” in the package ea-utils (Aronesty, 2011) and “process_radtags” in STACKS version 2.6 (Catchen et al., 2013). The remaining processing was accomplished using STACKS and followed the *r80* protocol detailed by Rochette and Catchen (2017). Specifically, parameters affecting assembly were assessed based on how parameter combinations affected *r80* loci (i.e., those found in 80% of samples or more); the optimal parameter set associated with the plateau of the number of *r80* loci was selected (Paris et al., 2017; Rochette & Catchen, 2017). Values used in the final de novo assembly were as follows: The minimum depth of coverage to initiate a new stack ($-m$ in *ustacks*) was 3, the number of mismatches allowed between stacks ($-M$ in *ustacks*) was 2, and the distance allowed between catalog loci ($-n$ in *cstacks*) was 2.

The “populations” program in STACKS was executed twice to generate the single-nucleotide polymorphism (SNP) data sets used in analyses. The first run included all individuals and resulted in a data set used to calculate sampling site summary statistics, including observed heterozygosity (H_{OBS}), expected heterozygosity (H_{EXP}), nucleotide diversity (π), and inbreeding coefficient (F_{IS}) and pairwise genetic differentiation index (F_{ST}) values between sampling sites. Parameters included the minimum percentage of individuals across populations required to process a locus ($-R=0.8$), minimum minor allele frequency required to process a nucleotide site at a locus ($-\text{min_maf}=0.02$), maximum observed heterozygosity required to process a nucleotide site at a locus ($-\text{max_obs_het}=0.7$), minimum number of populations in which a locus must be present to process the locus ($-p=2$), and the correction applied to F_{ST} values ($-\text{fst_correction}=p_value$). STACKS calculates pairwise population F_{ST} by averaging analysis of molecular variance-corrected SNP F_{ST} values across all SNPs; SNPs that were significantly different from an F_{ST} of zero are identified using a Fisher’s exact test (Catchen et al., 2013; see also Excoffier et al., 1992, and Weir, 1990). We further assessed whether pairwise population F_{ST} values significantly deviated from zero by calculating 95% CIs using “boot.ppfst()” ($nboot=1,000$) from the R package Hierfstat (Goudet, 2005). However, we note that pairwise population F_{ST} values that do not overlap with zero do not necessarily imply significant population differentiation (Frankham et al., 2002). In the second run, we included only Grand Canyon HBC to create a data set tailored for identifying outlier SNPs (see below). Parameters were the same as above except that we also required a minimum percentage of individuals in a sampling site for a locus to be processed ($-r=0.7$), which increased the probability that allelic variation was accurately captured within sites. The STACKS program minimizes paralogs through the $-\text{max_obs_het}$ parameter and by removing highly repetitive stacks in the ustacks program (Rochette & Catchen, 2017). Using the second data set containing only Grand Canyon HBC, we calculated pairwise relatedness (ϕ) values for all individuals using the relatedness2 estimator in VCFtools (Danecek et al., 2011; Manichaikul et al., 2010). Pairwise ϕ values $0.354 > \phi > 0.177$ indicate first-degree relatives, such as full siblings and parent–offspring pairs, while values $0.177 > \phi > 0.0884$ suggest second-degree relatives, such as half-siblings, grandparent–grandchild, and uncle/aunt–niece/nephew relationships.

Population structure among Grand Canyon HBC sampling sites

To address (1) whether WGC HBC maintain unique genetic variation and (2) whether differences among LCR behavioral traits may be leading to assortative mating and the generation of population structure, we used two approaches, including Bayesian clustering implemented in STRUCTURE version 2.3.4 (Falush et al., 2003) and principal components analysis (PCA) in R version 4.0.5 (R Core Team, 2021). Each method relies on different assumptions to quantify differentiation among individuals that may result from divergence in isolation (question 1) or nonrandom mating (question 2). We executed 20 independent runs in STRUCTURE for each set of genetic clusters (hereafter, “K-values”) ranging from 1 to 9; each run

utilized an admixture model with correlated allele frequencies, where population membership was not assigned a priori, and consisted of 100,000 burn-in iterations and 250,000 Markov chain–Monte Carlo iterations. We used DISTRUCT (Rosenberg, 2004) to visualize results. Principal components analysis was conducted in R using “dudi.pca()” ($\text{center}=T$, $\text{scale}=T$) from the package ade4 (Dray & Dufour, 2007) on a scaled genind object created using “read.structure()” and “scaleGen()” from the package Adegenet (Jombart, 2008). We assessed the results of these analyses in tandem along with relatedness as indicated by pairwise F_{ST} to determine biologically relevant, genetically defined groups instead of using model selection methods that can be misleading (i.e., Janes et al., 2017).

We repeated STRUCTURE and PCA on data sets including only neutral or outlier SNPs to understand whether individuals were differentiating in genetic variation that may be attributed to adaptive processes. We identified outlier SNPs in a data set that only included Grand Canyon HBC sampling sites by using pcadapt version 4.3.3 (Privé et al., 2020), a method that makes no a priori assumptions about selection pressure. The program pcadapt uses principal component (PC) loadings for identifying outlier SNPs, where SNPs are weighted for each PC axis. As such, the method is meant to be less reliant on assumptions regarding the underlying demography, which is appropriate for our data set given the low genetic variation across Grand Canyon HBC individuals (see Results). We ran pcadapt without correction for stratification, as preliminary tests indicated that setting K equal to 1 allowed the most inclusive (i.e., largest) set of SNPs to be identified. To classify outliers, we used thresholds at which outlier SNPs are expected to be falsely identified as adaptive (q -values) of 0.05 and 0.15 to construct stringent and relaxed outlier data sets. We utilized the same methods for executing STRUCTURE and PCA on each narrow and broad outlier (and corresponding neutral) data set.

RESULTS

Next-generation sequencing data quality, processing, and data set construction

Illumina sequencing produced over 3.68×10^8 reads across 192 individuals (average of 1.92×10^6 reads/individual). Eleven individuals were removed due to low coverage of usable processed reads (Table 1). After the removal of low-quality reads and adapter contamination, over 3.08×10^8 reads remained to identify homologous loci and SNPs in STACKS (Table 1). The data set including HBC and out-groups contained 20,360 loci composed of 2,245,473 sites. After filtering criteria were applied, 11,691 variant sites (i.e., SNPs) remained for calculating sampling site summary statistics and pairwise F_{ST} values and performing PCA. The data set including only Grand Canyon HBC contained 20,566 loci composed of 2,268,173 sites. After application of filtering criteria to that data set, 12,140 variant sites remained for PCA and STRUCTURE analyses and for identifying outlier SNPs. Heterozygosity (H_{OBS} and H_{EXP}) and nucleotide diversity (π) were consistent across HBC sampling sites and lower within BTC and RTC. Values of F_{IS} indicated that H_{EXP} did not deviate from H_{OBS} (values $\pm$ SE were close to or overlapping with zero; Table 2). Pairwise population

Table 1. Details of sampling sites and processing. Columns indicate the number of individuals per sampling site (#ind), sampling site coordinates (Latitude/Longitude), average number of reads per individual per sampling site after data processing (Processed reads), and average number of reads per individual per sampling site used to construct loci (Utilized reads). Values outside of parentheses in the #ind column indicate the number of individuals used for analyses if fewer than the total (see Results). Spatial locations correspond to either the hatchery location (for Bonytail and Roundtail Chub) or the Humpback Chub field collection site (all other groups).

Source and abbreviation	#ind	Latitude	Longitude	Processed reads ($\pm$ SD)	Utilized reads ($\pm$ SD)
Western Grand Canyon (WGC)	35 (40)	35.916233	113.334649	1,840,060 $\pm$ 397,700	1,510,076 $\pm$ 88,200
Havasas Creek (HAV)	25 (27)	36.296067	112.736870	2,166,800 $\pm$ 143,743	1,898,254 $\pm$ 151,542
Little Colorado River migrants (LCR-MIG)	38 (40)	36.169014	111.811668	2,080,051 $\pm$ 531,248	1,866,404 $\pm$ 78,332
Little Colorado River residents (LCR-RES)	60	36.173867	111.708984	1,948,324 $\pm$ 208,067	1,761,002 $\pm$ 103,035
Desolation/Gray Canyon population (DGP)	15	39.296017	110.062061	1,486,205 $\pm$ 237,062	1,389,995 $\pm$ 93,655
Roundtail Chub (RTC)	3 (5)	34.474674	111.800951	1,262,591 $\pm$ 339,094	1,183,592 $\pm$ 78,540
Bonytail (BTC)	5	33.194344	104.350733	1,511,205 $\pm$ 211,355	1,390,165 $\pm$ 58,518

Table 2. Genetic summary statistics calculated across single-nucleotide polymorphisms (i.e., variant sites only) for *Gila* spp. sampling sites, including average sample size (number of individuals [#ind]) used to calculate statistics and associated variation ($\pm$ SE) across loci, observed heterozygosity (H_{OBS}), expected heterozygosity (H_{EXP}), nucleotide diversity (π), and the inbreeding coefficient (F_{IS}). Humpback Chub groups include western Grand Canyon (WGC), Havasas Creek (HAV), Little Colorado River migrants (LCR-MIG), Little Colorado River residents (LCR-RES), and the Desolation/Gray Canyon population (DGP). The F_{IS} is not reported for Roundtail Chub (RTC) or Bonytail (BTC) because low sample sizes can generate spurious results.

Source	#ind	H_{OBS}	H_{EXP}	π	F_{IS}
WGC	31.1	0.230 $\pm$ 0.002	0.235 $\pm$ 0.001	0.239 $\pm$ 0.002	0.03 $\pm$ 0.02
HAV	22.9	0.224 $\pm$ 0.002	0.228 $\pm$ 0.002	0.233 $\pm$ 0.002	0.03 $\pm$ 0.02
LCR-MIG	35.2	0.236 $\pm$ 0.002	0.239 $\pm$ 0.001	0.242 $\pm$ 0.001	0.02 $\pm$ 0.02
LCR-RES	55.7	0.239 $\pm$ 0.002	0.244 $\pm$ 0.001	0.246 $\pm$ 0.001	0.03 $\pm$ 0.03
DGP	12.9	0.216 $\pm$ 0.002	0.216 $\pm$ 0.002	0.225 $\pm$ 0.002	0.03 $\pm$ 0.02
RTC	2.5	0.065 $\pm$ 0.002	0.049 $\pm$ 0.001	0.065 $\pm$ 0.002	NA
BTC	4.1	0.118 $\pm$ 0.002	0.098 $\pm$ 0.002	0.114 $\pm$ 0.002	NA

F_{ST} values calculated across loci were consistently low among Grand Canyon HBC comparisons (average = 0.008), indicating few allelic differences among these sampling sites; F_{ST} values were higher in comparisons with DGP (average = 0.038) and highest in comparisons with RTC (average = 0.113) and BTC (average = 0.205; Table 3). All pairwise F_{ST} values were significantly different from zero ($p < 0.05$), as their 95% CIs calculated by bootstrapping allelic variation within population pairs did not overlap with zero. Our relatedness analysis suggested that of the sampled fish, seven pairs had first-degree relationships (five pairs from HAV and two pairs from LCR-RES) and 22 pairs had second-degree relationships (14 pairs from HAV, six pairs from LCR-RES, and two pairs from WGC; Figure 2).

Population structure and history

Population genetic analyses provided consistent support for little genetic structure within Grand Canyon HBC groups (LCR-MIG, LCR-RES, HAV, and WGC). Analyses with STRUCTURE (excluding RTC and BTC) illustrated that the primary axis of genetic differentiation (i.e., $K=2$) was between DGP and the Grand Canyon groups (blue and red, respectively, in Figure 3A). Analysis of $K=3$ differentiated several individuals from HAV from the remaining Grand Canyon HBC (brown in Figure 3A), while analysis of $K=4$ identified a consistent genetic axis throughout Grand Canyon

sampling sites (purple in Figure 3A). Principal components analysis on all individuals differentiated RTC, BTC, and HBC (i.e., DGP, LCR-RES, LCR-MIG, HAV, and WGC) on PC1 and PC2, respectively; a subsequent PCA on HBC excluding the out-groups reiterated the STRUCTURE patterns in that DGP was separate from Grand Canyon sites (i.e., LCR-RES, LCR-MIG, HAV, and WGC) on PC1 (similar to $K=2$), while 16 HAV individuals were separate from the remaining Grand Canyon individuals on PC2 (similar to $K=3$; Figure 3B). Five of the 16 HAV individuals segregating on PC2 were translocated from LCR (see Methods), and the remaining 11 segregants shared either first- or second-degree relationships with the translocated individuals (Figure 2). These data, interpreted alongside pairwise F_{ST} values, support one genetically defined group when considering Grand Canyon HBC and two genetically defined groups when considering Grand Canyon HBC and the DGP sampling site (similar to the findings of Douglas & Douglas, 2007).

We utilized pcadapt to identify outlier SNPs within our Grand Canyon HBC genotype data set and to investigate whether adaptive variation may be masked by patterns across neutral SNPs. We identified 32 outlier SNPs when using a stringent q -value threshold ($\alpha=0.05$) and 66 outlier SNPs when using a relaxed q -value threshold ($\alpha=0.15$). We performed STRUCTURE analyses and PCA on outlier and neutral data sets; across all analyses, we found that outlier SNPs did not

Table 3. Pairwise genetic differentiation index F_{ST} values calculated using the program STACKS. Comparisons within Humpback Chub groups include western Grand Canyon (WGC), Havasu Creek (HAV), Little Colorado River migrants (LCR-MIG), Little Colorado River residents (LCR-RES), and the Desolation/Gray Canyon population (DGP). All F_{ST} values are significantly different from zero ($p < 0.05$). Additional abbreviations are as follows: BTC = Bonytail and RTC = Roundtail Chub.

Group	WGC	HAV	LCR-MIG	LCR-RES	DGP	RTC
BTC	0.212	0.251	0.197	0.158	0.273	0.335
WGC		0.014	0.005	0.005	0.037	0.116
HAV			0.011	0.009	0.050	0.149
LCR-MIG				0.002	0.034	0.106
LCR-RES					0.031	0.082
DGP						0.168

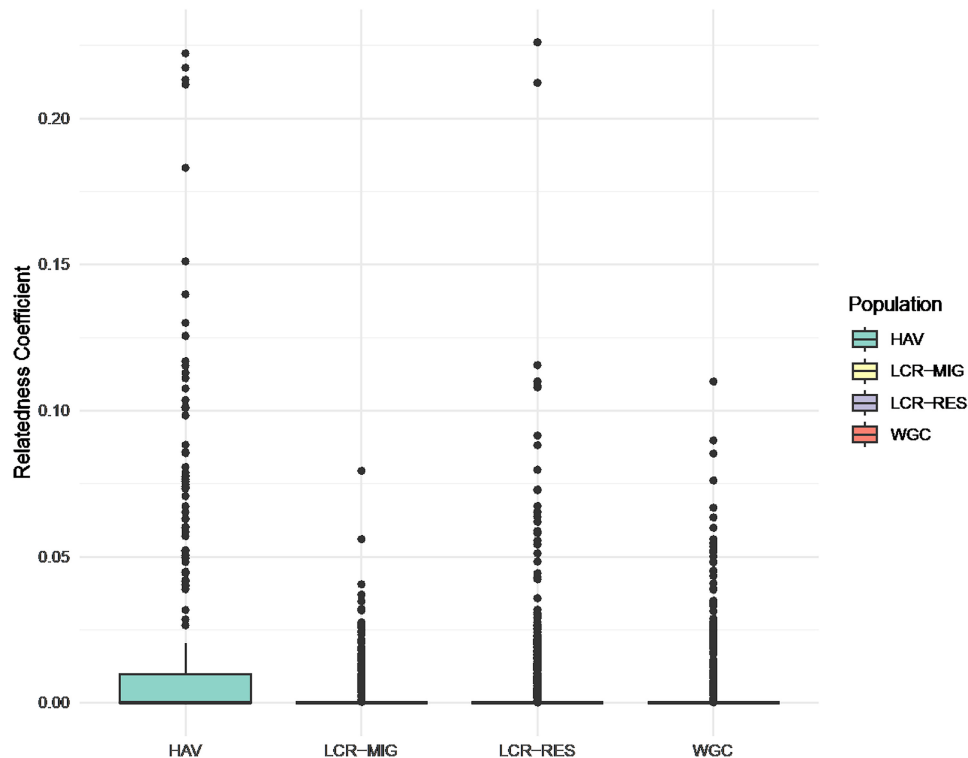


Figure 2. Box-and-whisker plots showing the distribution of pairwise relatedness (ϕ) values for Humpback Chub. Mean values are zero for each sampling site, indicating that most individuals were not related. Pairwise ϕ values $0.354 > \phi > 0.177$ indicate first-degree relationships, while values $0.177 > \phi > 0.0884$ suggest second-degree relationships. All pairwise ϕ values greater than 0.098 for Havasu Creek (HAV) represent individuals that segregated at a high principal component 2 value in Figure 3B (white triangles), including five individuals that were translocated from the Little Colorado River (LCR) to HAV. Abbreviations are as follows: LCR-MIG = LCR migrants, LCR-RES = LCR residents, and WGC = western Grand Canyon.

segregate between sampling sites inconsistently with neutral SNPs, as would be predicted if rare adaptive variation influenced behavioral traits (i.e., in LCR-MIG versus LCR-RES). In fact, patterns of differentiation across outlier SNPs mirrored the patterns across neutral SNPs (Figures S1–S4 [see online Supplementary Material]). Because 5 of the 16 fish segregating along PC1 were translocated from LCR into HAV and the remaining 11 fish were related to the translocated individuals (Figure 2), we hypothesize that the outlier SNPs represented unique neutral genetic variation carried into HAV when the fish were translocated. Similar analyses including only LCR-RES and LCR-MIG individuals did not produce different results (data not shown).

DISCUSSION

Humpback Chub in Grand Canyon demonstrate spatial variability in vital rates (e.g., survival, growth, and movement; Dzul et al., 2023; Yackulic et al., 2014) and have experienced rapid changes in population size over the past century (Coggins & Walters, 2009; Dzul et al., 2022; Valdez & Ryel, 1995; Van Haverbeke et al., 2017). Although there is some uncertainty in the historic range of HBC, most records suggest that a contiguous population occurred throughout Grand Canyon before the construction of GCD (USFWS, 2018; Valdez & Ryel, 1995), and this is supported by the results of our genetic analysis. We found no evidence of substantive genetic differentiation across Grand Canyon HBC, as evidenced by low values of F_{ST} in all

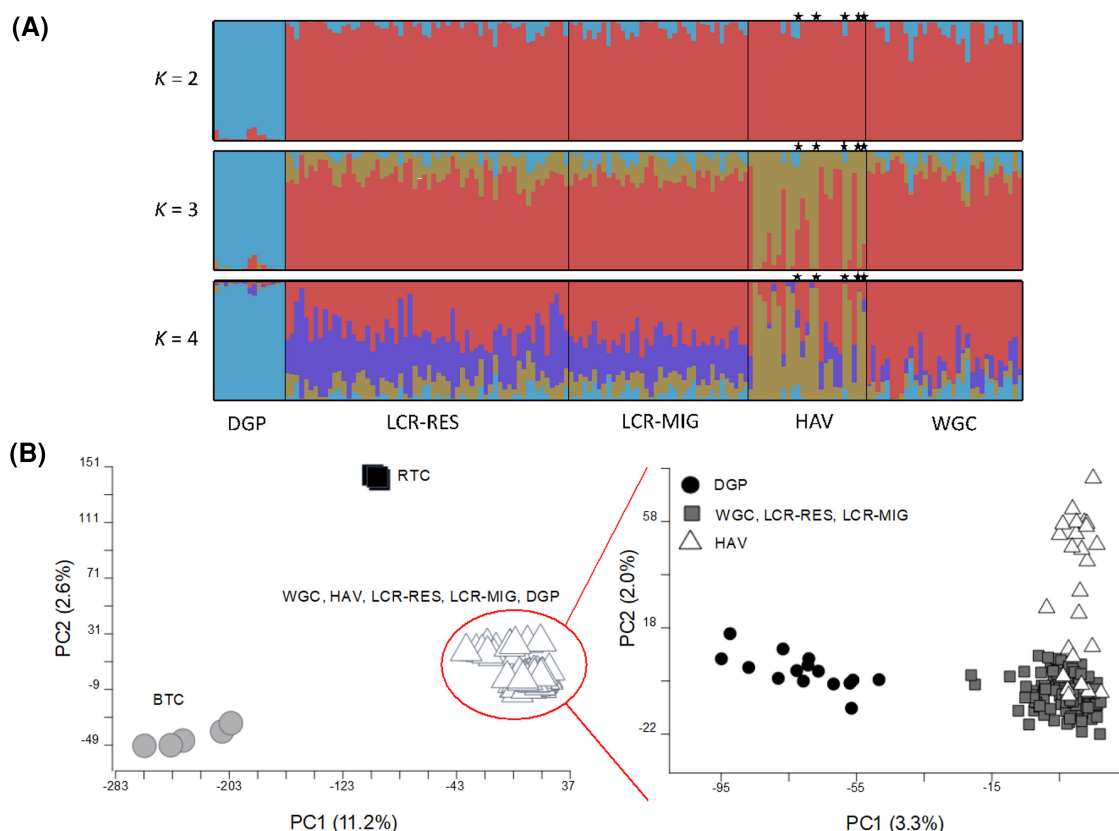


Figure 3. Genetic structure (A) within Humpback Chub (HBC) and (B) among HBC, Roundtail Chub (RTC), and Bonytail (BTC). Plots for K -values of 2–4 (panel A) illustrate the hierarchical partitioning of genetic structure. Humpback Chub that were translocated into Havasu Creek (HAV) are denoted with stars above their respective bars. Principal components analysis (PCA) plots (panel B) include all species (left) and only HBC (right). Abbreviations are as follows: DGP = Desolation/Gray Canyon population, LCR-RES = Little Colorado River residents, LCR-MIG = Little Colorado River migrants, and WGC = western Grand Canyon.

pairwise comparisons of sampled groups. The cohesive gene pool of Grand Canyon HBC suggests high rates of gene flow throughout habitat and the lack of assortative mating within or among groups of fish. Furthermore, we find no evidence for unique genetic variation that may have resulted from hypothetical warmwater refugia in WGC during the coldwater period.

Overall genetic differentiation was low across Grand Canyon HBC, but we observed slight differentiation in some HAV individuals (Figure 3). Interestingly, differentiated individuals included five LCR-spawned HBC that were collected in 2013 as age-0 fish and stocked into HAV the following year (i.e., stocked in 2014 as age-1 fish). In addition, these fish shared close relationships with conspecifics at HAV (Figure 2), suggesting that they successfully reproduced after translocation. Although we cannot pinpoint the mechanism driving this differentiation, we note that (1) the location from which fish were sourced might have had slight genetic differences compared to the LCR fish sampled herein; and (2) differentiation is very low, with some genetic methods able to detect it (i.e., PCA and STRUCTURE) and others unable to do so (F_{ST}). Additional sampling within the LCR may provide clues as to where this variation originated.

Like HBC, numerous fishes and other vertebrates exhibit partial migration and alternative life history strategies (Chapman et al., 2011). An individual's decision to migrate (or not) is often condition-dependent (e.g., trade-off between

individual growth rate and food availability; Brodersen et al., 2008; Chapman et al., 2012) and sometimes indirectly influenced by the individual's genotype (Dodson et al., 2013). In the case of LCR-spawning HBC, resident and migrant individuals likely spawn sympatrically but could potentially segregate based on characteristics such as body size (Dzul et al., 2023) or behavior, which could have a genetic basis. There is some evidence that meristic traits of HBC vary longitudinally within the LCR (Kaeding & Zimmerman, 1983), which could be indicative of genetic differences. Numerous studies acknowledge that genetic variability, phenotypic plasticity, and environmental conditions/thresholds all interact to influence the migration decisions of individuals (Pulido, 2011; Sandlund & Jonsson, 2016; Skov et al., 2010) so that the genetic basis of migration decisions can be cryptic (Pulido, 2011). Despite these observed phenotypic differences among residents and migrants, they were similar genetically, which supports a panmictic population. However, the lack of genetic distinctiveness of LCR migrants and residents does not preclude the possibility of a genetic basis to life history type.

The genetic similarity across LCR migrants and residents suggests that the observed life history differences (Dzul et al., 2023) may be due to other factors, such as physiological responses to differing temperatures and food availabilities (Lee et al., 2013; Sandström et al., 1995) or phenotypically plastic responses to

environmental thresholds (Pulido, 2011; Skov et al., 2010). In particular, warmer water temperatures lead to faster growth of HBC (Dzul et al., 2016; Gorman & Van Hoosen, 2000), and numerous studies have documented reductions in adult life-span associated with fast growth (Lee et al., 2013; Metcalfe & Monaghan, 2003; Weinstein et al., 2019). This temperature-induced variability in life histories can increase population resilience: The combination of short-lived fast growers and long-lived slow growers results in a population that is able to recover rapidly after disturbances and to withstand long periods of poor reproduction or recruitment (Polačik et al., 2014; Taylor et al., 2019). Physiological plasticity, or the ability of animals to shift physiological processes in response to temperature, may also be an important factor that allows for HBC to successfully inhabit diverse thermal habitats within river networks (Kingsolver & Huey 2008; Seebacher et al., 2014).

Our sampling design and analyses reinforce the genetic similarity among Grand Canyon HBC and may inform management in response to emerging threats. Although HBC have thrived in Grand Canyon over the past decade due to warming water temperatures, continued increases in water temperatures due to drought (Dibble et al., 2021; Vernieu et al., 2005) will produce new threats. While warmer water temperatures may be beneficial for HBC (Robinson & Childs, 2001; Van Haverbeke et al., 2017), they may also facilitate the spread of nonnative piscivorous warmwater fishes (Dibble et al., 2021), particularly if water temperatures increase above 16°C in the Colorado River near the LCR confluence (Eppheimer et al., 2024). Nonnative warmwater piscivores have decimated native fishes, including HBC, in the upper Colorado River basin (Johnson et al., 2008; Tyus & Saunders 2000). In 2022, the water temperature of releases from Lake Powell were 4°C warmer than at any other point in the past 50 years, as reservoir elevations declined to their lowest level in 50 years (U.S. Geological Survey [USGS], 2024). Many warmwater species found in Lake Powell were entrained and appeared to spawn in the Colorado River below GCD, including Smallmouth Bass *Micropterus dolomieu* (Eppheimer et al., 2024). Grand Canyon tributaries, such as the LCR and HAV, provide physical barriers (e.g., waterfalls) and environmental deterrents (e.g., floods and high levels of dissolved CO₂; Stone et al., 2018) that may help to shelter HBC and other native fishes from piscivory by nonnative fishes and may provide genetic refugia if nonnative warmwater fishes spread and impact the main-stem HBC population. If HBC need to be augmented due to threats, our data suggest that sources of fish are interchangeable within Grand Canyon, providing robust management options to maintain healthy and viable populations.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *North American Journal of Fisheries Management* online.

DATA AVAILABILITY

Data and data analysis code generated during this study are available from the USGS ScienceBase Catalog (Massatti & Dzul, 2025).

ETHICS STATEMENT

Data collection followed the protocols developed by Persons et al. (2017) and the American Fisheries Society guidelines described by Jenkins et al. (2014). Fieldwork by the USGS since October 2024 has been conducted under Institutional Animal Care and Use Committee Permit USGS-SBSC-2024-05; USGS fieldwork was also conducted under USFWS Permit TE821356-0.

FUNDING

Funding was provided by the U.S. Bureau of Reclamation.

CONFLICTS OF INTEREST

Authors do not perceive any conflicts of interest. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

ACKNOWLEDGMENTS

We thank Steve Mussmann (USFWS) for providing BTC samples as well as HBC samples from the DGP. Additionally, we thank Tiffany Love-Chezem (USFWS), Mariah Giardina (USFWS), Michael Yard (USGS), and David Ward (USFWS) for help with sample collection. We appreciate Erica Sukovich (USGS) for helping to prepare samples for genetic analysis and Erica Byerley (USGS) for GIS support.

REFERENCES

- Ackerman, M. W., Templin, W. D., Seeb, J. E., & Seeb, L. W. (2013). Landscape heterogeneity and local adaptation define the spatial genetic structure of Pacific salmon in a pristine environment. *Conservation Genetics*, 14, 483–498. <https://doi.org/10.1007/s10592-012-0401-7>
- Andersen, M. E., Ackerman, M. W., Hilwig, K. D., Fuller, A., & Alley, P. D. (2010). Evidence of young Humpback Chub overwintering in the mainstem Colorado River, Marble Canyon, Arizona, USA. *Open Fish Science Journal*, 3, 42–50. <https://doi.org/10.2174/1874401X01003010042>
- Aronesty, E. (2011). *ea-utils: Command-line tools for processing biological sequencing data*. <https://expressionanalysis.github.io/ea-utils/>.
- Barbosa, S., Mestre, F., White, T. A., Paupério, J., Alves, P. C., & Searle, J. B. (2018). Integrative approaches to guide conservation decisions: Using genomics to define conservation units and functional corridors. *Molecular Ecology*, 27, 3452–3465. <https://doi.org/10.1111/mec.14806>
- Bohn, S., Mussman, S., & Wilson, W. (2019). *Genetic evaluation of Upper Basin Colorado River Gila cypha*. U.S. Fish and Wildlife Service, Southwestern Native Aquatic Resources and Recovery Center.
- Brodersen, J., Nilsson, P. A., Hansson, L.-A., Skov, C., & Brönmark, C. (2008). Condition-dependent individual decision-making determines cyprinid partial migration. *Ecology*, 89, 1195–1200. <https://doi.org/10.1890/07-1318.1>
- Buchinger, T. J., Hondorp, D. W., & Krueger, C. C. (2023). Spatiotemporal segregation by migratory phenotype indicates potential for assortative mating in Lake Sturgeon. *Oecologia*, 201, 953–964. <https://doi.org/10.1007/s00442-022-05280-y>
- Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, 22, 3124–3140. <https://doi.org/10.1111/mec.12354>

- Chafin, T. K., Douglas, M. R., Martin, B. T., & Douglas, M. E. (2019). Hybridization drives genetic erosion in sympatric desert fishes of western North America. *Heredity*, 123, 759–773. <https://doi.org/10.1038/s41437-019-0259-2>
- Chapman, B. B., Brönmark, C., Nilsson, J. Å., & Hansson, L. A. (2011). The ecology and evolution of partial migration. *Oikos*, 120, 1764–1775. <https://doi.org/10.1111/j.1600-0706.2011.20131.x>
- Chapman, B. B., Hulthén, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L.-A., & Brönmark, C. (2012). Partial migration in fishes: Causes and consequences. *Journal of Fish Biology*, 81, 456–478. <https://doi.org/10.1111/j.1095-8649.2012.03342.x>
- Clarkson, R. W., & Childs, M. R. (2000). Temperature effects of hypolimnial-release dams on early life stages of Colorado River basin big-river fishes. *Copeia*, 2000, 402–412. [https://doi.org/10.1643/0045-8511\(2000\)000\[0402:TEOHRD\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2000)000[0402:TEOHRD]2.0.CO;2)
- Coggins, L. G., Jr., & Walters, C. J. (2009). *Abundance trends and status of the Little Colorado River population of Humpback Chub: An update considering data from 1989–2008* (Open File Report 2009-1075). U.S. Geological Survey.
- Coggins, L. G., Jr., Yard, M. D., & Pine, W. E., III. (2011). Nonnative fish control in the Colorado River in Grand Canyon, Arizona: An effective program or serendipitous timing? *Transactions of the American Fisheries Society*, 140, 456–470. <https://doi.org/10.1080/00028487.2011.572009>
- Crawford, K., & Whitney, K. (2010). Population genetic diversity influences colonization success. *Molecular Ecology*, 19, 1253–1263. <https://doi.org/10.1111/j.1365-294X.2010.04550.x>
- Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R.; 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
- DeWoody, J. A., Harder, A. M., Mathur, S., & Willoughby, J. R. (2021). The long-standing significance of genetic diversity in conservation. *Molecular Ecology*, 30, 4147–4154. <https://doi.org/10.1111/mec.16051>
- Dibble, K. L., Yackulic, C. B., Kennedy, T. A., Bestgen, K. R., & Schmidt, J. C. (2021). Water storage decisions will determine the distribution and persistence of imperiled river fishes. *Ecological Applications*, 31, Article e02279. <https://doi.org/10.1002/eap.2279>
- Dodson, J. J., Aubin-Horth, N., Thériault, V., & Páez, D. J. (2013). The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews*, 88, 602–625. <https://doi.org/10.1111/brv.12019>
- Douglas, M. R., & Douglas, M. E. (2007). *Genetic structure of Humpback Chub Gila cypha and Roundtail Chub G. robusta in the Colorado River ecosystem—Final report*. Department of Fish, Wildlife, and Conservation Biology, Colorado State University.
- 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. <https://doi.org/10.2307/1446938>
- Dray, S., & Dufour, A. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20. <https://doi.org/10.18637/jss.v022.i04>
- Dzul, M. C., Kendall, W. L., Yackulic, C. B., Winkelman, D. L., Van Haverbeke, D. R., & Yard, M. (2021). Partial migration and spawning movements of Humpback Chub in the Little Colorado River are better understood using data from autonomous PIT tag antennas. *Canadian Journal of Fisheries and Aquatic Sciences*, 78, 1057–1072. <https://doi.org/10.1139/cjfas-2020-0291>
- Dzul, M. C., Yackulic, C. B., Giardina, M., Van Haverbeke, D. R., & Yard, M. (2023). Vital rates of a burgeoning population of Humpback Chub in western Grand Canyon. *Transactions of the American Fisheries Society*, 152, 443–459. <https://doi.org/10.1002/tafs.10415>
- Dzul, M. C., Yackulic, C. B., Kendall, W. L., Winkelman, D. L., Conner, M. M., & Yard, M. (2022). Incorporating antenna detections into abundance estimates of fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 79, 436–447. <https://doi.org/10.1139/cjfas-2021-0003>
- Dzul, M. C., Yackulic, C. B., Korman, J., Yard, M. D., & Muehlbauer, J. D. (2016). Incorporating temporal heterogeneity in environmental conditions into a somatic growth model. *Canadian Journal of Fisheries and Aquatic Sciences*, 74, 316–326. <https://doi.org/10.1139/cjfas-2016-0056>
- Eppheimer, D. E., Yackulic, C. B., Bruckerhoff, L. A., Wang, J., Young, K. L., Bestgen, K. R., Mihalevich, B. A., & Schmidt, J. C. (2024). Declining reservoir elevations following a two-decade drought increase water temperatures and non-native fish passage facilitating a downstream invasion. *bioRxiv* 576966. <https://doi.org/10.1101/2024.01.23.576966>
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491. <https://doi.org/10.1093/genetics/131.2.479>
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164, 1567–1587. <https://doi.org/10.1093/genetics/164.4.1567>
- Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications*, 11, 1035–1052. <https://doi.org/10.1111/eva.12569>
- Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). *Introduction to conservation genetics*. Cambridge University Press.
- Fraser, D. J., & Bernatchez, L. (2001). Adaptive evolutionary conservation: Towards a unified concept for defining conservation units. *Molecular Ecology*, 10, 2741–2752. <https://doi.org/10.1046/j.1365-294X.2001.t01-i-01411.x>
- García-Dorado, A., & Caballero, A. (2021). Neutral genetic diversity as a useful tool for conservation biology. *Conservation Genetics*, 22, 541–545. <https://doi.org/10.1007/s10592-021-01384-9>
- Gorman, O. T., & Van Hoosen, R. R. (2000). *Experimental growth of four native Colorado River fishes at temperatures of 12, 18, and 24°C*. Grand Canyon Monitoring and Research Center.
- Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, 5, 184–186. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
- Healy, B. D., Budy, P., Conner, M. M., & Omana Smith, E. C. (2022). Life and death in a dynamic environment: Invasive trout, floods, and intraspecific drivers of translocated populations. *Ecological Applications*, 32, Article e2635. <https://doi.org/10.1002/eap.2635>
- Healy, B. D., Omana Smith, E. C., Schelly, R. C., Trammell, M. A., & Nelson, C. B. (2020). Establishment of a reproducing population of endangered Humpback Chub through translocations to a Colorado River tributary in Grand Canyon, Arizona. *North American Journal of Fisheries Management*, 40, 278–292. <https://doi.org/10.1002/nafm.10408>
- Janes, J. K., Miller, J. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., Cullingham, C. I., & Andrew, R. L. (2017). The K = 2 conundrum. *Molecular Ecology*, 26, 3594–3602. <https://doi.org/10.1111/mec.14187>
- Jenkins, J. A., Bart, H. L., Jr., Bowker, J. D., Bowser, P. R., MacMillan, J. R., Nickum, J. G., Rose, J. D., Sorensen, P. W., Whitledge, G. W., Rachlin, J. W., & Warkentine, B. E. (2014). *Guidelines for the use of fishes in research*. American Fisheries Society.
- Johnson, B. M., Martinez, P. J., Hawkins, J. A., & Bestgen, K. R. (2008). Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. *North American Journal of Fisheries Management*, 28, 1941–1953. <https://doi.org/10.1577/M07-199.1>
- Jombart, T. (2008). Adegnet: An R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Kaeding, L. R., & Zimmerman, M. A. (1983). Life history and ecology of the Humpback Chub in the Little Colorado and Colorado rivers of the Grand Canyon. *Transactions of the American*

- Fisheries Society, 112, 577–594. [https://doi.org/10.1577/1548-8659\(1983\)112<577:LHAET>2.0.CO;2](https://doi.org/10.1577/1548-8659(1983)112<577:LHAET>2.0.CO;2)
- Kingsolver, J., & Huey, R. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research*, 10, 251–268. <https://doi.org/10.17615/jtzc-v174>
- Lee, W.-S., Monaghan, P., & Metcalfe, N. B. (2013). Experimental demonstration of the growth rate–lifespan trade-off. *Proceedings of the Royal Society B: Biological Sciences*, 280, Article 20122370. <https://doi.org/10.1098/rspb.2012.2370>
- Manichaikul, A., Mychaleckyj, J. C., Rich, S. S., Daly, K., Sale, M., & Chen, W.-M. (2010). Robust relationship inference in genome-wide association studies. *Bioinformatics*, 26, 2867–2873. <https://doi.org/10.1093/bioinformatics/btq559>
- Massatti, R., & Dzul, M. C. (2025). *Gila spp. (Bonytail Chub, Humpback Chub, and Roundtail Chub) genomic data from various locations including the Colorado River in the Grand Canyon, AZ (2020–2024)* [Data release]. U.S. Geological Survey. <https://doi.org/10.5066/F725IGFZ>
- Metcalfe, N. B., & Monaghan, P. (2003). Growth versus lifespan: Perspectives from evolutionary ecology. *Experimental Gerontology*, 38, 935–940. [https://doi.org/10.1016/S0531-5565\(03\)00159-1](https://doi.org/10.1016/S0531-5565(03)00159-1)
- Mihalevich, B. A., Neilson, B. T., Buahin, C. A., Yackulic, C. B., & Schmidt, J. C. (2020). Water temperature controls for regulated canyon-bound rivers. *Water Resources Research*, 56, Article e2020WR027566. <https://doi.org/10.1029/2020WR027566>
- Miller, R. R. (1958). Origin and affinities of the freshwater fish fauna of western North America. In C. L. Hubbs (Ed.), *Zoogeography* (Publication 51, pp. 187–222). American Association for the Advancement of Science.
- Minckley, W. (1991). Native fishes of the Grand Canyon region: An obituary? In *Colorado River ecology and dam management: Proceedings of a Symposium May 24–25, 1990, Santa Fe, New Mexico*, (pp. 124–177). National Academies Press. <https://doi.org/10.17226/1832>
- Moritz, C. (1994). Defining ‘evolutionarily significant units’ for conservation. *Trends in Ecology & Evolution*, 9, 373–375. [https://doi.org/10.1016/0169-5347\(94\)90057-4](https://doi.org/10.1016/0169-5347(94)90057-4)
- Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space: A road map for STACKS. *Methods in Ecology and Evolution*, 8, 1360–1373. <https://doi.org/10.1111/2041-210X.12775>
- Paz-Vinas, I., Loot, G., Hermoso, V., Veyssiere, C., Poulet, N., Grenouillet, G., & Blanchet, S. (2018). Systematic conservation planning for intraspecific genetic diversity. *Proceedings of the Royal Society B: Biological Sciences*, Article 285, 20172746. <https://doi.org/10.1098/rspb.2017.2746>
- Persons, W. R., Van Haverbeke, D. R., & Dodrill, M. J. (2017). *Colorado River fish monitoring in Grand Canyon, Arizona; 2002–14 Humpback Chub aggregations* (Open-File Report 2016-1177). U.S. Geological Survey.
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, 7, Article e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Polačik, M., Blažek, R., Řežucha, R., Vrtilek, M., Terzibasi Tozzini, E., & Reichard, M. (2014). Alternative intrapopulation life-history strategies and their trade-offs in an African annual fish. *Journal of Evolutionary Biology*, 27, 854–865. <https://doi.org/10.1111/jeb.12359>
- Privé, F., Luu, K., Vilhjálmsson, B. J., & Blum, M. G. (2020). Performing highly efficient genome scans for local adaptation with R package pcadapt version 4. *Molecular Biology and Evolution*, 37, 2153–2154. <https://doi.org/10.1093/molbev/msaa053>
- Pulido, F. (2011). Evolutionary genetics of partial migration—The threshold model of migration revisited. *Oikos*, 120, 1776–1783. <https://doi.org/10.1111/j.1600-0706.2011.19844.x>
- R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Robinson, A. T., & Childs, M. R. (2001). Juvenile growth of native fishes in the Little Colorado River and in a thermally modified portion of the Colorado River. *North American Journal of Fisheries Management*, 21, 809–815. [https://doi.org/10.1577/1548-8675\(2001\)021<0809:JGONFI>2.0.CO;2](https://doi.org/10.1577/1548-8675(2001)021<0809:JGONFI>2.0.CO;2)
- Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes from RAD-seq short-read data using Stacks. *Nature Protocols*, 12, 2640–2659. <https://doi.org/10.1038/nprot.2017.123>
- Rogowski, D. L., Osterhoudt, R. J., Mohn, H. E., & Boyer, J. K. (2018). Humpback Chub (*Gila cypha*) range expansion in the western Grand Canyon. *Western North American Naturalist*, 78, 26–38. <https://doi.org/10.3398/064.078.0105>
- Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, 4, 137–138. <https://doi.org/10.1046/j.1471-8286.2003.00566.x>
- Sandlund, O. T., & Jonsson, B. (2016). Life history plasticity: Migration ceased in response to environmental change? *Ecology of Freshwater Fish*, 25, 225–233. <https://doi.org/10.1111/eff.12204>
- Sandström, O., Neuman, E., & Thoreson, G. (1995). Effects of temperature on life history variables in perch. *Journal of Fish Biology*, 47, 652–670. <https://doi.org/10.1111/j.1095-8649.1995.tb01932.x>
- Schmidt, J. C., Bruckerhoff, L., Salehabadi, H., & Wang, J. (2022). The Colorado River. In A. Gupta (Ed.), *Large rivers: Geomorphology and management* (2nd ed., pp. 253–319). Wiley.
- Seebacher, F., Beaman, J., & Little, A. G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Functional Ecology*, 28, 137–148. <https://doi.org/10.1111/1365-2435.12156>
- Skov, C., Aarestrup, K., Baktoft, H., Brodersen, J., Brönmark, C., Hansson, L. A., Nielsen, E. E., Nielsen, T., & Nilsson, P. A. (2010). Influences of environmental cues, migration history, and habitat familiarity on partial migration. *Behavioral Ecology*, 21, 1140–1146. <https://doi.org/10.1093/beheco/arq121>
- Stone, D. M., Young, K. L., Mattes, W. P., & Cantrell, M. A. (2018). Abiotic controls of invasive nonnative fishes in the Little Colorado River, Arizona. *The American Midland Naturalist*, 180, 119–142. <https://doi.org/10.1674/0003-0031-180.1.119>
- Taylor, B. M., Choat, J. H., DeMartini, E. E., Hoey, A. S., Marshall, A., Priest, M. A., Rhodes, K. L., & Meekan, M. G. (2019). Demographic plasticity facilitates ecological and economic resilience in a commercially important reef fish. *The Journal of Animal Ecology*, 88, 1888–1900. <https://doi.org/10.1111/1365-2656.13095>
- Tyus, H. M., & Saunders, J. F., III. (2000). Nonnative fish control and endangered fish recovery: Lessons from the Colorado River. *Fisheries*, 25, 17–24. [https://doi.org/10.1577/1548-8446\(2000\)025<0017:NFCAEF>2.0.CO;2](https://doi.org/10.1577/1548-8446(2000)025<0017:NFCAEF>2.0.CO;2)
- U.S. Fish and Wildlife Service. (2021). *Endangered and threatened wildlife and plants; reclassification of the Humpback Chub from endangered to threatened with a Section 4(d) rule*. Federal Register 86:198(18 October 2021):57588–57610.
- U.S. Fish and Wildlife Service. (2002a). *Bonytail (Gila elegans) recovery goals: Amendment and supplement to the Bonytail Chub Recovery Plan*. U.S. Fish and Wildlife Service, Mountain–Prairie Region.
- U.S. Fish and Wildlife Service. (2002b). *Humpback Chub (Gila cypha) recovery goals: Amendment and supplement to the Humpback Chub Recovery Plan*. U.S. Fish and Wildlife Service, Mountain–Prairie Region.
- U.S. Fish and Wildlife Service. (2018). *Species status assessment for the Humpback Chub (Gila cypha)*. U.S. Fish and Wildlife Service, Mountain–Prairie Region.
- U.S. Geological Survey. (2024). *Colorado River at Lees Ferry, AZ - 09380000*. *Water Data for the Nation* [Online database]. Retrieved October 2024 from <https://waterdata.usgs.gov/monitoring-location/09380000>
- Valdez, R. A., & Ryel, R. J. (1995). *Characterization of the life history and ecology of the Humpback Chub (Gila cypha) in the Colorado River, Grand Canyon, Arizona—Final report* (Contract 0-CS-40-09110, Technical Report TR-250-08). BIO/WEST, Inc.
- Van Haverbeke, D. R., Stone, D. M., Coggins, L. G., Jr., & Pillow, M. J. (2013). Long-term monitoring of an endangered

- desert fish and factors influencing population dynamics. *Journal of Fish and Wildlife Management*, 4, 163–177. <https://doi.org/10.3996/082012-JFWM-071>
- Van Haverbeke, D. R., Stone, D. M., Dodrill, M. J., Young, K. L., & Pillow, M. J. (2017). Population expansion of Humpback Chub in western Grand Canyon and hypothesized mechanisms. *The Southwestern Naturalist*, 62, 285–292. <https://doi.org/10.1894/0038-4909-62.4.285>
- Vernieu, W. S., Hueftle, S. J., & Gloss, S. P. (2005). Water quality in Lake Powell and the Colorado River. In S. P. Gloss, J. E. Lovich, & T. S. Melis (Eds.), *The state of the Colorado River ecosystem in Grand Canyon* (Circular 1282, pp. 69–85). U.S. Geological Survey.
- Weinstein, M., Liotta, M. N., Solitt, A., Hunt, A., Abbott, J. K., Rios-Cardenas, O., & Morri, M. R. (2019). Selection on growth rates via a trade-off between survival to sexual maturity and longevity in the swordtail fish *Xiphophorus multilineatus*. *Evolutionary Ecology*, 33, 549–566. <https://doi.org/10.1007/s10682-019-09989-w>
- Weir, B. S. (1990). *Genetic data analysis: Methods for discrete population genetic data*. Sinauer.
- Wright, S. A., Anderson, C. R., & Voichick, N. (2009). A simplified water temperature model for the Colorado River below Glen Canyon Dam. *River Research and Applications*, 25, 675–686. <https://doi.org/10.1002/rra.1179>
- Yackulic, C. B., Korman, J., Yard, M. D., & Dzul, M. (2018). Inferring species interactions through joint mark–recapture analysis. *Ecology*, 99, 812–821. <https://doi.org/10.1002/ecy.2166>
- Yackulic, C. B., Van Haverbeke, D. R., Dzul, M., Bair, L., & Young, K. L. (2021). Assessing the population impacts and cost-effectiveness of a conservation translocation. *Journal of Applied Ecology*, 58, 1602–1612. <https://doi.org/10.1111/1365-2664.13908>
- Yackulic, C. B., Yard, M. D., Korman, J., & Van Haverbeke, D. R. (2014). A quantitative life history of endangered Humpback Chub that spawn in the Little Colorado River: Variation in movement, growth, and survival. *Ecology and Evolution*, 4, 1006–1018. <https://doi.org/10.1002/ece3.990>