

GENE FLOW OF COMMON SIDE-BLOTCHED LIZARDS ACROSS THE
COLORADO RIVER IN ARIZONA AND UTAH

By Tessa C. Corsetti

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Approved:

Carol L. Chambers, Ph.D., Co-Chair

Paul Beier, Ph.D., Co-Chair

Gerard Allan, Ph.D.

Andrew Holycross, Ph.D.

Brandon Holton, M.S.

ABSTRACT

GENE FLOW OF COMMON SIDE-BLOTCHED LIZARDS ALONG THE
COLORADO RIVER IN ARIZONA AND UTAH

TESSA C. CORSETTI

The 1963 completion of the Glen Canyon Dam in Arizona profoundly changed the Colorado River and altered habitat for many species. It created a relatively constant flow rate, much colder temperatures, and eliminated slow moving, warm periods that had historically characterized this system and gave wildlife opportunities to cross the river at certain times of year. The common side-blotched lizard (*Uta stansburiana*) provides an excellent opportunity to examine the effects of linear water barriers created by impoundments such as Glen Canyon Dam on migration and gene flow in wildlife. To assess the impact of these changes on an ectothermic reptile, we collected tissue samples from 241 common side-blotched lizards at three cross-river study areas in Arizona and Utah and used eight microsatellite loci to estimate gene flow as a function of genetic diversity and structure. One study area (Cataract Canyon) has annual periods of warm temperatures and low flows that most resembled pre-dam conditions, whereas the other two study areas (Badger/Jackass and Tanner/Basalt) have cold water temperature year-round and no low-flow episodes. We found genetic differentiation between pairs of sampling sites on opposite sides of the river in two out of three study areas. Surprisingly the greatest cross-river gene flow was in one of the two below-dam study areas characterized by cold temperatures and no periods of low flow. We speculate that the lack of a significant rapid in this study area, or the presence of two

pedestrian bridges ~27 km away, might facilitate cross-river movement in that study area. These results suggest that this small ectotherm can move across the post-dam Colorado River often enough to facilitate gene flow in under certain conditions, but the river presents a barrier to movement in most cases. Our study provides new genetic data for 241 common side-blotched lizards, a previously understudied species with minimal genetic information. These results provide a foundation for future studies on the effects of dams to animal movement and shed light on the genetic diversity and structure of the common side-blotched lizard in Arizona and Utah.

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Ethics Statement

Scientific collecting permits from Grand Canyon National Park and Canyonlands National Park were obtained for the 2020 and 2021 field seasons through the National Park Service Research Permit and Reporting System. Grand Canyon backcountry permits were obtained through the Grand Canyon National Park Backcountry Information Center. Canyonlands National Park backcountry permits were obtained through Recreation.gov.

Researchers on this project received approval from the Institutional Animal Care and Use Committee (IACUC) through the National Park Service and Northern Arizona University that clearly outlined researcher qualifications, proper handling, and contingency plans to ensure animal welfare. Protocol Number: 20-001.

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DEDICATION

To the Grand Canyon
for the best days of my life and the lessons I will never forget.

To Brandon Holton
for employing me at the Grand Canyon in 2017 and being my advocate and cheerleader
ever since. Thank you for teaching me what it means to be a great biologist and for
proving that it is possible to truly love what you do.

INTRODUCTION

Free flowing rivers, rivers that have not been significantly altered by dams or other human influences, make up only one third of global rivers (Grill et al. 2019; Nilsson and Berggren 2000). Only 25% of large rivers in the United States remain free flowing (Grill et al. 2019). Although dam effects to vegetation, sediment flow, aquatic invertebrates, and natural river integrity have been widely studied, few studies have explored the impacts of post-dam rivers as barriers to migration and gene flow in terrestrial vertebrates (Draut and Gillette 2010; Fagan et al. 2005; Holton 2018; Nilsson and Berggren 2000; Sankey et al. 2015; Webb et al. 2002). We hypothesize that the post-dam Colorado River may be limiting movement and gene flow of wildlife, specifically terrestrial ectotherms, in the Grand Canyon. Movement between populations creates large, demographically stable meta-populations, allows the animals to recolonize patches after local extirpation, and maintains the genetic diversity needed to adapt to changing conditions and stochastic events (Jones et al. 2016). The loss of habitat connectivity by fragmentation or degradation can create populations that are vulnerable to disease, severe weather events, invasive species, and loss of genetic diversity (Lacy 1987; Jones et al. 2016).

Human-induced fragmentation such as roads and urbanization, as well as geological barriers such as mountain ranges and large deep canyons, are known to fragment reptile populations (Travis and Keim 1995; Jones et al. 2016). Habitat loss and fragmentation can severely impact biodiversity at genetic and species levels, inevitably impacting ecosystem processes and health (Blair et al. 2012). However, little attention has been paid to the potential barrier effects of cold rivers created by impoundments

(Warren and Schwalbe 1986). The 1963 completion of Glen Canyon Dam produced a Colorado River with a relatively constant flow rate, much colder temperature, and no slow moving, warmer periods which until 1963 potentially made the river crossable for some animals at certain times of year (Figure 1) (Stevens et al. 2001; Webb et al. 2002). The Colorado River below Glen Canyon Dam is likely a barrier to cross-river movement by bighorn sheep in the Grand Canyon (Holton 2018) but the impact of this change - especially on ectothermic reptiles— has been largely understudied (Warren and Schwalbe 1986; Christoffel and Lepczyk 2012).

Studying dam impacts to riverine systems is complicated by the lack of pre-dam scientific data. Anecdotal information about the Colorado River pre-dam temperature, geomorphology, vegetation, and wildlife in the Grand Canyon is available in the form of interviews with pre-dam river runners, historical diaries, and historical scientific data and observations (Webb et al. 2002). Before the dam, highly variable flows historically carried an estimated 230,000 tons of sediment per day through the Grand Canyon; about 99% of this material is now trapped behind the dam, resulting in erosion of sandbars, eddies, and beaches below the dam through Grand Canyon (Topping et al. 2000). Historic pre-dam accounts regularly reported the Colorado River in Glen Canyon and at Lee's Ferry completely frozen over during low flow periods in the winter, often for several days at a time, allowing humans and animals to walk across (Webb et al. 2002).

Although temperature, sediment, and discharge data prior to dam completion is limited, during the past 10 years the Cataract Canyon section of the river upstream of Glen Canyon dam impoundment (Moab, UT) experiences median daily temperatures of 0°C during spring snowmelt rising to 28°C during low flow periods (Figure 2A). Water

temperature below the dam at Lee's Ferry now has a limited range of 8°C to 15 °C with the median remaining consistently around 10 to 11°C (Figure 2B).

In river reaches above the dam, such as in Cataract Canyon, sandbars occasionally emerge during low flow periods. Below the dam, the loss of these sandbars, combined with the loss of seasonal low flows, decreases opportunities for terrestrial wildlife to cross the river (Holton 2018). Once such animal, the common side-blotched lizard (*Uta stansburiana*), provides an excellent opportunity to examine the effects of linear water barriers that have experienced changes to hydrology and flow by impoundments such as Glen Canyon Dam. Those effects include potential loss of genetic diversity at the species level, coupled with fragmentation of previously connected populations, potentially leading to inbreeding depression within small, isolated populations on either side of the barrier.

Successful dispersal and migration through habitat connectivity are imperative to species abundance and fitness (Blair et al. 2012). Population genetics can be used to detect the presence of a landscape barrier (Blair et al. 2012). The potential impact of a linear barrier, such as a highway or river, can be quantified by measuring the genetic composition of individuals and populations on either side of the linear feature (Gregory and Beier 2014; Travis and Keim 1995). For example, a parent-offspring pair (first order relatives) observed on opposite sides of the barrier would indicate recent movement across said barrier. However, these individual movements are hard to detect and may not contribute to the long-term genetic and demographic vigor of the populations (e.g., if the immigrant dies before successful reproduction). Meaningful gene flow (or lack thereof) requires a measure of genetic distances between groups of individuals on both

sides of the barrier in question (Gregory and Beier 2014). The putative barrier must have existed long enough to have influenced genetic composition in the two populations.

Genetic divergence should be evident within 10–20 generations for populations consisting of 60-100 effective breeding individuals (Hare et al. 2011; Gregory & Beier 2014). Density of breeding common side-blotched lizards varies with the time of year, predator activity, and other factors, but mean effective population density is ~100 individuals per 2 ha site (Parker 1974). The mean generation time for common side-blotched lizards is ~1 year (Parker 1974) and the dam has existed for 55 years. If the cold, high-flow Colorado River is a barrier to gene flow, it should be evident today.

A cold river without annual periods of low flow would plausibly impede gene flow of non-flying small-bodied ectotherms. Identifying landscape characteristics, such as dam-altered rivers, that could be disrupting gene-flow and migration is a vital part of landscape and conservation management (Blair et al. 2012). Accordingly, we chose to assess the barrier effect of the altered river on the common side-blotched lizard, a widespread species in the American southwest (Brennan and Holycross 2009). The common side-blotched lizard is found in sandy desert scrub habitats or rocky habitats with substantial vegetative cover. They rarely occur in rocky areas with little vegetative cover (Tinkle 1967; Alloush 2013).

Our design included sampling at two study areas below the dam in Grand Canyon National Park, Arizona (Badger-Jackass study area and Tanner-Basalt study area) and one warm-water study area above the dam in Cataract Canyon, Canyonlands National Park, Utah that resembles pre-dam conditions (Spanish Bottom-Red Lake

study area). Cataract Canyon, compared to any other location on the Colorado River, is the farthest from human cities and agricultural and industrial land uses, and farthest from dams with impoundments that alter water temperature and flow regimes (U.S. Department of the Interior 2004, Draut and Gillette 2010). The nearest such impoundments to Cataract Canyon are Flaming Gorge Dam, 412 miles (663 km) upstream on the Green River, Granby Dam outside Granby, Colorado, Crystal Dam, Morrow Point Dam, and Blue Mesa Dam on the Gunnison River in Montrose County, Colorado, and McPhee Dam on the Dolores River in Montezuma County, Colorado.

Our primary interest was to compare cross-river gene flow in the above dam study area (Cataract Canyon) to cross-river gene flow in the two study areas below the dam. At each of these 3 study areas, we also compared genetic similarity between sampling sites (putative populations) on opposite sides of the river, and between sampling areas on one side of the river but about one river width apart. This comparison was to confirm that any genetic differentiation between cross river sites is due to the presence of the river and not simply the distance between the populations.

Our objectives were (1) to determine if the changes to river flow and temperature have created a barrier to gene flow in populations of common side-blotched lizards in the Grand Canyon and (2) to generate new data about the genetic diversity and structure of a common species with little available genetic information. This research will advance our understanding of how dams impact reptiles and their habitats, generate new genetic data for a common species, and support informed decisions regarding reptile management and conservation. Understanding how the Glen Canyon Dam has impacted the Colorado River and its biodiversity will provide a framework for future

studies on dam impacts to terrestrial ectotherms in the Southwest and in similar systems that have been altered by dams and other anthropogenic activities.

MATERIALS AND METHODS

Study areas and sampling sites

Each of our three study areas on the Colorado River included sampling sites on opposite banks of the river. River side (left or right) is in the downstream direction. The warm-water study area (most like pre-dam conditions) also referred to as Spanish Bottom-Red Lake Canyon study area (Figure 3A), was in Cataract Canyon, Canyonlands National Park. Cataract Canyon is a 27 km (17 mile) reach of the Colorado River mainstem. The two cross-river sampling sites in Cataract Canyon are Spanish Bottom (river right) and Red Lake Canyon (river left), five km (three miles) from the confluence of the Green and Colorado Rivers. The vegetation structure, climate, and geology are similar to the Grand Canyon (Draut and Gillette 2010). The river width is ~100 meters at its smallest point. This area is still characterized by episodic flooding and periods of slow, warm flow and sediment supply that more closely resembles natural conditions (Draut and Gillette 2010).

Spanish Bottom is an ancient sinkhole that is treeless except for tamarisk (*Tamarix spp.*), willow (*Salix spp.*), and cottonwoods (*Populus fremontii*) along the riverbank and prickly pear (*Opuntia basilaris*) and biocrust throughout the sinkhole. Red Lake Canyon, directly opposite Spanish Bottom (Figure 4A) is a large, bare canyon lined with cottonwoods and biocrust. It flows into the Colorado River in a large boulder field that is regularly covered by routine flooding. This area is 305 km (190 miles) above

Glen Canyon Dam. There are no sediment or flow gages below the confluence of the Green and Colorado Rivers in Cataract Canyon. Flow gages at Mineral Bottom on the Green River (Figure 5A) and Potash on the Colorado River (Figure 5B), 82 and 83 km (52 and 51 miles) from Spanish Bottom/Red Lake Canyon respectively, can provide a discharge estimate through Cataract Canyon.

The first of the two cold-water study areas below the dam (Figure 3B) was a pair of sampling sites at Badger Canyon (river right) and Jackass Canyon (river left), 37 km (23 miles) downriver from Glen Canyon Dam. These canyons are deeply incised in Kaibab limestone and Coconino sandstone. The vegetation is Great Basin desert scrub and dry wash riparian vegetation such as honey mesquite (*Prosopis glandulosa*) and catclaw acacia (*Senegalia greggii*) (Kearsley et al. 2015). Badger Creek Rapid separates Badger and Jackass Canyons (Figure 4B). The rapid separating the two canyons is class 5/6 rapid on a 1-10 scale with a 3.6-meter drop, measuring ~70 meters at its narrowest width (Martin and Whitis 2019). Badger and Jackass each contained two sampling sites on each side of the river, one along the river and the other > 70 meters (the approximate width of the river) away (Figure 6A).

The other cold-water study area was a pair of sampling sites at Tanner Canyon (river left) and Basalt Creek (river right) (Figure 4C), 135 km (85 miles) downriver from Glen Canyon Dam. Tanner Canyon supports Great Basin desert scrub and dry wash riparian honey mesquite and catclaw acacia (Kearsley et al. 2015). Tanner Canyon also contained two sampling areas – one at the mouth of Tanner Canyon, and one ~1km away at Below Tanner Rapid Camp (Figure 6B). Basalt Creek, a kilometer downriver, is a large prickly pear meadow. The beach is full of small to medium rocks and assorted

lowbush shrubs and biocrust. The riverbank is dominated by mesquite and invasive tamarisk. Tanner Rapid just upriver from 68 Mile Camp (Upper Tanner) above Tanner Canyon and Basalt Rapid just downriver from Basalt Camp (Martin and Whitis 2019) but there are no rapids between the two sites. The river between the two rapids is relatively calm and wide, ~75 m at its smallest width. USGS discharge data at these two sites is limited to gage data collected from Lee's Ferry, 13 and 113 km (8 and 70 miles) upriver from Badger/Jackass and Tanner/Basalt, respectively (Figure 5C).

Study Species

The common side-blotched lizard (*Uta stansburiana*, family Phrynosomatidae) is a small (<64 mm SVL) stocky lizard with gular folds and ear openings. It is easily identified by the prominent blue-black spot posterior to its front legs in both sexes (Figure 7) (Brennan and Holycross 2009). It is found in arid and semi-arid landscapes from northern Mexico to its northern limit in southern Washington, and east to western Colorado and Texas (Tinkle 1967). This lizard prefers open habitats with sufficient cover, primarily found in sandy, desert scrub habitats. It is also found in rocky desert shrub habitats with vegetative cover and open pinyon-juniper forests but are rarely found in rocky areas with little vegetation or heavily shaded, wooded areas (Brennan and Holycross 2009; Alloush 2013; Tinkle 1967). The common side-blotched lizard is often restricted to single microhabitats, likely due to competitive interactions with other species, high site fidelity, and ecological limitations (Tinkle 1967; Alloush 2013).

Both sexes exhibit high site fidelity and are highly territorial (Alloush 2013). Home range for males' averages ~446 m² (maximum 810 m²) and ~121 m² for females (maximum 202 m²) (Parker 1974). It is most often observed on the ground or sunning

themselves on rocks, rarely climbing into trees or shrubs unless they are being pursued or threatened (Alloush 2013). The common side-blotched lizard is currently a species of least concern on the IUCN Red List of Threatened Species (IUCN 2021).

Sample Collection

We sampled the common side-blotched lizard along their shoreline habitat next to the Colorado River and up canyons perpendicular to the river with suitable habitat until the minimum sample size had been collected. Our survey method included foot searches and hand-capture. We caught lizards with an unwaxed dental floss lasso made with a slip knot that tightened once the lasso had slipped over the lizard's head. Lizards were gently but firmly secured by hand as soon as possible to prevent injury, excessive stress, or possible tail ejection. The dental floss lasso was left around their necks in the event of escape attempts but was loosened immediately to ensure comfort and safety.

Lizards were held over a 1.5mL centrifuge tube containing Nucleic Acid Preservation (NAP) buffer solution (Camacho-Sanchez et al. 2013) with the tip of their tail dangling inside the centrifuge tube. Using clean and sharp surgical scissors, we cut 5-7mm of tissue from the tip of the tail, making sure that the tip fell cleanly into the NAP buffer solution in the centrifuge tube. Surgical scissors were retired upon first signs of dullness. The tail was immediately sprayed with a pain-relieving antiseptic solution (Bactine® Max Pain Relieving Cleansing Spray & Liquid, WellSpring Pharmaceutical Corporation) to ensure comfort and minimize the risks of infection following sample collection per IACUC protocol (see Ethics Statement).

Once antiseptic was applied, the lizards were marked with a non-toxic xylene-free paint marker in colors consistent with their natural coloration (Figure 8) (Richter et al. 2013). This was to avoid double sampling, prevent unnecessary disturbance to the animals in the form of repeated capture, and to decrease the chances of individuals dropping their tails. Lizards were released at their exact point of capture. Samples were kept on ice in the field when possible, then stored at -20°C prior to DNA extraction.

We recorded individual species, sex, approximate capture and release time, and tail condition. We aimed for a minimum of 30 individuals at each sampling site (Hale et al. 2012). We collected < 30 samples in two of the six sampling areas. Of the 241 individuals sampled, only one lizard lost its tail during processing. To the best of our knowledge, this was not due to excessive handling or researcher error. The lizard dropped its tail immediately upon being lassoed prior to being physically handled by researchers. The lizard was promptly marked, sprayed, and released. A sample was taken from the dropped tail once the lizard had been attended to.

DNA Extraction and Standardization

We performed DNA extractions using the Qiagen Blood and Tissue kit according to the manufacturer's protocol. Tissue samples were lysed overnight at 55°C in the provided lysis buffer. Extracts were quantified on a Nanodrop 8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and standardized to 5 ng/μL using molecular grade water. Samples with starting concentrations lower than 5 ng/μL were kept at their original concentrations.

Microsatellite Loci and PCR Conditions

Microsatellite loci are the most versatile of genetic markers. They are abundantly distributed throughout the genome of most eukaryotic organisms, can reveal differences among closely related populations, and are highly polymorphic, species specific, and co-dominant (Hale et al. 2012). They are also among the more affordable options for genetic analysis. We adapted nine microsatellite primers (10,000 M's, BRtt, IGs, MCC, NGff, PLkn, SMcL, SPhill, SVeg) (Table 2) from Zamudio & Sinervo (2000) with a universal tail labeling system (Walker et al. 2020). We PCR-amplified all loci in reactions containing 1X PCR buffer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), 2 mM of MgCl₂, 0.2 mM dNTPs, 0.1 mM labeled forward primer, 0.2 mM unlabeled reverse primer, 0.2 mM fluorescent label, 0.08 U/μL Platinum Taq DNA polymerase (Invitrogen), 0.02 μg/μL Ultrapure non-acetylated Bovine Serum Albumin (BSA), and 5 μL of DNA template in a 15 μL reaction (Walker et al. 2020). We performed all thermal cycling on an Applied Biosystems SimpliAmp Thermal Cycler. Each reaction followed modified conditions from Alloush (2013) beginning with 90°C for 2 min, followed by 45 cycles of 95°C for 1 min, 51°C for 1 min, and 72°C for 1 min, then concluded with a final extension of 72°C for 5 min.

Following amplification, PCR products were diluted as follows with molecular grade water prior to fragment analysis on an Applied Biosystems 3130 Genetic Analyzer: loci 10,000 M's, SMcL, and SVeg were diluted 1:100 and loci BRtt, IGs, MCC, NGff, PKIn, and SPhill were diluted 1:10. A 1 μL volume of each diluted PCR product was suspended in a reaction containing 10.92 μL HiDi formamide (Thermo Fisher

Scientific) and 0.08 μ L LIZ 1200 size standard ladder prior to fragment analysis. We scored alleles using GeneMarker software.

Quality Control

To control for genotyping errors, we re-amplified and re-sequenced any sample that produced an ambiguous genotype signal or suffered reaction failure. To enhance the performance of our reagents, we optimized the Platinum Taq polymerase concentration from 0.2 U/ μ L to 0.08 U/ μ L. We used Micro-Checker (Oosterhout et al. 2004) to check for scoring errors and null alleles. We used the program GenAlEx 6.5 (Peakall and Smouse 2012) to test for Hardy-Weinburg equilibrium (HWE) and linkage disequilibrium.

Genetic Analysis

Genetic diversity was determined by average observed and expected heterozygosity and average allelic richness. Due to the variability in sample size at each site and the sensitivity of allelic diversity to sample size, we performed rarefaction using the program HP-Rare (Kalinowski 2005).

To test whether sampling sites on the same side of the river comprised a single breeding population, we used Analysis of Molecular Variance (AMOVA) on each of three pairs of same-side sampling sites, Badger, Jackass and Tanner. Once random mating was confirmed within each of these three sampling sites, we used AMOVA to examine how genetic variation is partitioned between river right and river left in each of the three study areas. We used the fixation index (F_{ST}) generated from our AMOVA to

measure population differentiation due to genetic structure. AMOVA was performed using GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

We performed a Principal Coordinates Analysis (PCoA) using GenAlEx 6.5 to convert the genetic distance between populations into a map-based visualization. We used program STRUCTURE (Pritchard, Stephens, and Donnelly 2000) to investigate population genetic structure and determine distinct populations within our dataset. We used 20 runs per K (K=1-8), a burn-in of 100,000, and 500,000 Markov chain Monte Carlo (MCMC) iterations (Gilbert et al. 2012). Results from the runs for each value of K were averaged and graphed using CLUMPAK (Kopelman et al. 2015) and genetic clusters (K) were identified using Best K (Pritchard et al. 2000; Evanno et al. 2005).

RESULTS

Genotyping

Multilocus microsatellite data (n=8 loci) were obtained for 241 unique common side-blotched lizard individuals across six putative populations in Canyonlands and Grand Canyon National Parks from June 2020 to May 2021 (Table 1). All eight loci used were polymorphic. We excluded marker 5 (NGff) from the original nine loci dataset after scoring attempts failed to produce genotypes at the expected allele size for most samples. The eight remaining loci were used in all analyses, as there were no deviations from HWE nor evidence of linkage disequilibrium. In total, we successfully generated multilocus genotypes for 235 individuals for eight markers and failed to produce genotypes for 0.36% (7 of 1936) individual loci. Failures appeared in three populations (four from Jackass; two from Badger; one from Red Lake Canyon) and from

three different loci, indicating that failures were not systematic or specific to one population or locus. Although marker 6 (PLkn) accounted for four of seven total failures, this lack of information was negligible in the overall dataset. Micro-Checker (Oosterhout et al. 2004) confirmed that our dataset had no evidence of scoring errors, dropout of larger alleles, or null alleles.

Average observed heterozygosity (H_o) of eight loci across six populations was $H_o=0.45 (\pm 0.049)$ and average expected heterozygosity (H_e) was $H_e=0.44 (\pm 0.047)$, indicating a degree of genetic variability (Table 3). Badger and Jackass had greater raw allelic richness than other populations. Once rarified to account for differences in sample size, Tanner had the greatest allelic richness, followed by Badger, Red Lake Canyon, and Jackass. Spanish Bottom and Basalt had the lowest allelic richness, potentially due to the small sample sizes at both sites (20 and 16, respectively). Private alleles were detected in all populations (Table 3).

Gene flow between sampling sites on a single side of the river

Badger Canyon and Jackass Canyon

We found Badger1 and Badger2 showed 2% of variation due to differences among populations, 2% of variation was due to differences among individuals, and 96% of all variation between Badger1 and Badger2 was due to differences within individuals. The fixation index ($F_{ST}= 0.020$) for Badger1 and Badger2 supported our expectation that these two sampling areas consisted of a single population (Figure 9). We found Jackass1 and Jackass2 showed that variation among populations was 0, 8% of

variation was due to differences among individuals, and 92% of variation due to differences within individuals. The pairwise population value ($F_{ST}=0$) for Jackass1 and Jackass2 suggested high levels of breeding between the two sampling areas on the same side of the river, supporting our expectation that Jackass1 and Jackass2 are one breeding population (Figure 9).

These findings of high interbreeding within the Badger and Jackass sites indicated that these populations, identified a priori as separate populations, should be combined as single, panmictic populations. Any genetic differentiation between Badger and Jackass was likely due to the presence of the river and not simply the distance between the populations.

Tanner Canyon

We found Tanner1 and Tanner2 showed 2% of variation was due to differences among populations, 13% of variation was due to differences among individuals, and 85% of variation was due to differences within individuals. These sites are ~500 m apart, about 7x the distance between Badger1 and Badger2 and Jackass1 and Jackass2. While there was slightly more variation among individuals than was observed at Badger or Jackass, the fixation index ($F_{ST}=0.016$) maintains that these two populations have high levels of breeding and are a single effective population.

Cross-river Site Analysis

Spanish Bottom and Red Lake Canyon

Spanish Bottom and Red Lake Canyon indicated little overlap (Figure 10A) between the two populations and suggested genetic structure between the two cross-river sites. Fixation index showed genetic structure ($F_{ST}= 0.126$, $p= 0.001$) (Table 4) suggesting that breeding is limited between these two populations. $K=2$ confirmed Spanish Bottom and Red Lake Canyon are two populations (Figure 10B/C).

Badger Canyon and Jackass Canyon

Badger and Jackass also displayed little overlap and substantial structure (Figure 11). As at Spanish Bottom and Red Lake Canyon, there is limited intermixing between these sites on opposite sides of the river ($F_{ST}= 0.124$, $p=0.001$) (Table 4). $K=2$ confirmed that Badger and Jackass are two separate populations (Figure 11 B/C).

Tanner Canyon and Basalt Creek

Tanner and Basalt revealed more overlap than the other two cross-river pairs of sites (Figure 12A) and less genetic structure than the previous two sites ($F_{ST}= 0.053$, $p=0.001$) (Table 4). The lack of distinction between populations suggests less genetic differentiation than expected based on their relatively distant proximity from one another on either side of the Colorado River below the dam. $K=1$ confirmed that lizards can cross the river to access populations on the opposite side and successfully reproduce (Figure 12B/C).

Complete Site Analysis

We examined isolation by distance among all populations based on F_{ST} (Table 4) and found significant genetic structure among our cross-river research sites (Figure 13). Over a large geographic scale, populations that were geographically closer to each other were more genetically similar, as expected with isolation by distance. We found genetic similarity between pairs of sampling sites on opposite sides of the river – i.e., Spanish Bottom with Red Lake Canyon, Badger with Jackass, and Basalt with Tanner (Figure 13). Overall, the first three components of the principal coordinates analysis explained 35.76% of the total variation.

We found significant levels of genetic structure ($p=0.001$) among populations on river right and river left, with 23% variation due to differences among populations, and 77% of variation due to differences within individuals, with no significant partitioning of genetic variation due to differences among all individuals (Table 5). This suggests that there is distinct structure between the right and left sides of the river, specifically between Red Lake Canyon/Spanish Bottom and Badger/Jackass. Tanner and Basalt revealed more cross-river connectivity than at the other two study areas.

Best K analysis identified different values for K (assumed populations or genetic groups). When K was derived from one to eight potential populations/groups, Evanno et al. (2005) grouped 241 common side-blotched lizards into two populations (Figure 14A), while Pritchard et al. (2000) grouped 241 common side-blotched lizards into six populations (Figure 14B). Our K=2 findings by Evanno et al. (2005) should be considered carefully as there is evidence that STRUCTURE may be influenced by multiple factors that may result in an inaccurate K=2 genetic grouping (Blair et al. 2012;

Janes et al. 2016). In this case, it is possible that uneven sample size across multiple sample sites may be influencing Best K (Foster et al. 2018; Janes et al. 2017). The method by Evanno et al. (2005) should be considered in conjunction with another method of identifying K, in our case Pritchard et al. (2000) (Blair et al. 2012; Gilbert et al. 2012). These data suggest that the geographic distance between the sites (Blair et al. 2012) and potential uneven sample sizes are driving the K=2 grouping (Janes et al. 2017), but K=6 is consistent with our data ecologically. STRUCTURE analysis showed substantial genetic structure between almost all populations (Figure 15).

River Right

We found significant differentiation among populations on river right (Table 6A) (Spanish Bottom, Badger, and Basalt) ($F_{ST}=0.181$, $p=0.001$). There are three distinct populations on river right, even though they all exist on the same side of the Colorado River (Figure 16).

River Left

We found more genetic structure among populations on river left (Red Lake Canyon, Jackass, and Tanner) than any other sampling area ($F_{ST}=0.249$, $p=0.001$) (Table 6B). There are also three distinct populations on the left side of the Colorado River (Figure 17).

DISCUSSION

We found evidence for genetic differentiation between populations on opposite sides of the river in two of the three study areas and significant evidence for genetic differentiation among the three study areas. This supports the hypothesis that the Colorado River limits movement and gene flow of side-blotched lizards in some areas. However, the greatest cross-river gene flow occurred in one of the two study areas below the dam (Tanner and Basalt), suggesting that the fast, cold, post-dam river without low-flow periods can present less of a barrier under certain conditions than a site with regular periods of warmer water and lower flows (Spanish Bottom/Red Lake Canyon).

Each pair of sampling sites a river-width apart on one side of the river (Badger, Jackass, and Tanner) effectively constituted a single breeding population. These results support the idea that genetic differentiation across sampling sites within a study area is the result of the river acting as a barrier rather than distance. This is unsurprising due to this lizard's known home range (Parker 1974) and the lack of any putative barrier or observed fragmentation in these areas.

We found significant structure in two of the three research areas. Populations clustered into regional groupings, with overlap of certain populations. The presence of private alleles at all sites suggests limited gene flow between populations and/or high mutation rates of individual loci that exceed gene flow/migration. Analysis showed six populations with admixture (Figure 15) with STRUCTURE results showing clear structure between Badger and Jackass and Spanish Bottom and Red Lake Canyon, suggesting that migration and associated gene flow among these populations is limited.

This analysis supports our hypothesis that the river acts as a partial barrier to movement and gene flow in most cases.

The statistically significant genetic structure between populations on river right and populations on river left implies that common side-blotched lizard gene flow does not span long distances on the same side of the river. This suggests that common side-blotched lizard gene flow is limited along the river and rugged canyon terrain can fragment lizard habitat. The topography of these areas may decrease migration and gene flow between populations of common side-blotched lizards on the same side of the river, but on a much larger scale than the fragmentation created by the Colorado River. Genetic structure at cross-river sites separated by ~70 m of Colorado River is approximately the same as same-side sites separated by ~445 km (~275 river miles) of harsh canyon terrain. This suggests that common side-blotched lizards are existing in small patches of favorable habitat, unable to cross the Colorado River in most cases or traverse certain terrain to access new populations. High levels of heterozygosity suggests that these lizards are genetically distinct but geographically isolated. Further research may shed light on the relative role isolation by distance and unsuitable habitat conditions have on these genetic patterns.

There was significant genetic differentiation across the river in two study sites, but we were surprised at the connectivity between the Tanner-Basalt study area (Figure 12). We offer three possible explanations about this low genetic divergence at Tanner and Basalt. First this may be due to the lack of rapids or strong currents between these two sampling areas (Figure 4C). In contrast, strong currents and rapid flows from Badger Creek rapid separate the sampling sites in the Badger-Jackass area (Figure

4B), which also show a higher degree of genetic differentiation (Figure 11). Badger Creek Rapid is a class five/six rapid on the Grand Canyon scale with a 3.6 meter drop and is considered the first significant rapid in Marble Canyon (Martin and Whitis 2019). This suggests that the presence of cold, fast rapids in the Grand Canyon may play a role in limiting gene flow. Second, we speculate that lizards could be swept downstream from Tanner and wash up on the shore of Basalt, surviving due to the lack of white water and the short river distance between the two sites. We encountered two lizards in the river during field work, one floating about one meter from the shore at Jackass, alive but unable to swim, and the other dead about 1.5 meters from shore at Badger. The occurrence of a live lizard in the water suggests that they might survive being swept down river to wash up on the opposite shore. A third explanation is that the Black and Silver Bridges at Phantom Ranch, 29-30 km (18-19 miles) downriver from Tanner-Basalt, could provide an avenue for gene flow. Lizards have been observed opportunistically on these bridges, supporting our theory that they cross the river at Phantom Ranch. There are two cross-river bridges in Marble Canyon (Navajo Bridge) that are 5 km (3 miles) upriver from Badger and Jackass, however these bridges are at the canyon rim, 142 m (467 feet) above the river. Black and Silver Bridges are the only river-level crossings on the Colorado River through the Grand Canyon. As few as one migrant every two generations is sufficient to maintain healthy levels of heterozygosity and five or more immigrants per generation would halt genetic drift within a population (Lacy 1987). A single lizard successfully reaching Basalt from Tanner every two years could maintain connectivity between the two populations.

Because Cataract Canyon is the least dam-altered stretch of the Colorado River mainstem in terms of water temperature, natural flow regimes, and sediment discharge, we had expected more cross-river gene flow there than at the other two study areas. Our results suggest that the Colorado River is as much of a barrier to movement and gene flow in the Cataract Canyon study area as it is in the cold-water sites below the dam. Perhaps this is caused by the impacts of Flaming Gorge Dam and other dam-created impoundments upstream from Cataract Canyon or the regular high flow rates through this canyon (Figure 5A/B). Contrasting these results with mitochondrial DNA analysis would illuminate whether differentiation existed before dam completion.

Movement of common side-blotched lizards is inhibited by the Colorado River in Canyonlands and Grand Canyon National Parks. The dammed Colorado River is a barrier to gene flow and migration at a small geographic scale. Although cross-river movement was highest in the Tanner-Basalt study area, several factors may facilitate river crossings for animals in this area and small sample sizes may have influenced this result. If future research can identify these factors, this understanding might help lessen the impacts of dams on wildlife movement. Measuring and monitoring movement and gene flow is an important part of maintaining viable populations that have sufficient genetic variation for adapting to changing climate, habitat loss, and environmental shifts.

Future Research

The presence of rapids at study sites like Badger and Jackass may limit gene flow, while river-level crossings such as the bridges at Phantom Ranch may facilitate cross-river migration and gene flow. Further genetic studies on wildlife in certain areas of the canyon, such as above and below Phantom Ranch and along either side of the river with a rapid separating them, will be beneficial to further our understanding of rivers as barriers to gene flow, and animals' abilities to adjust to human-altered landscapes.

Data on flow rates, discharge, and temperature at specific cross-river sites will be beneficial to determine how moving water inhibits lizard movement. In addition, our results suggest that canyon topography is also a barrier to movement, but on a much larger scale. More sampling locations are necessary to determine if sheer cliffs and rugged terrain disrupt gene flow on a single side of the river for this species.

Future studies should include mitochondrial DNA in conjunction with microsatellite loci to determine if there are different haplotypes above and below the dam and the direction of gene flow between two populations (i.e. Tanner and Basalt) (Travis and Keim 1995). These data could provide important insight on how a maternally-inherited marker is distributed above and below the dam, and corresponding populations on either side of the river, leading to more informed management strategies within Grand Canyon and Canyonlands National Parks.

Project Conclusions

The Colorado River is one of the most manipulated and regulated bodies of water in the United States, with over a dozen large dams and thousands of small impoundments between its headwaters in the southern Rocky Mountains of Colorado to its historical endpoint in the Gulf of California (Stevens 2012). It provides the water for agricultural, industrial, and domestic uses of over 35 million people in the western United States and Mexico and is now a necessity for life in the Southwest (Tillman et al. 2020). Impoundments, dams, and irrigation along the Colorado River have changed historic sediment transport, flood dynamics, water temperature and chemistry, hydraulic flow, and the distribution and abundance of native biota (Stevens 2012; Sankey et al. 2014; Schmidt et al. 1998).

The apparent inability for the common side-blotched lizard to migrate across the river in most cases and the genetic structure we observed suggests that lizards may be limited to isolated patches of favorable habitat, that they may suffer loss of diversity within and between populations if habitat degradation continues, and that river-level crossing points might mitigate such impacts. Although the common side-blotched lizard is a common and widespread species, dams could have more profound impacts on rare endemic species. Further studies are needed to determine the full extent of dam impacts to wildlife in the southwest to manage wildlife as effectively as possible.

TABLES

Table 1. Research site and sample collection data including site name, UTM, site location, river side (right or left), and the number of individual common side-blotched lizards collected at each research site.

Site	UTM	Location	River Side	# Samples
Spanish Bottom	12 S 593457 E 4223754	Canyonlands NP, Utah	Right	20
Red Lake Canyon	12 S 594067 E 4223839	Canyonlands NP, Utah	Left	49
Badger Canyon	12 S 441337 E 4069939	Grand Canyon NP, Arizona	Right	64
Jackass Canyon	12 S 441563 E 4069818	Grand Canyon NP, Arizona	Left	58
Tanner Canyon	12 S 424924 E 3995651	Grand Canyon NP, Arizona	Left	34
Basalt Creek	12 S 424351 E 3994857	Grand Canyon NP, Arizona	Right	16

Table 2. Microsatellite loci, repeat motif, and forward (F) and reverse (R) primers for *Uta stansburiana*.

Locus	Repeat Motif	Primer Sequence
10,000 M's	(CA) ₁₂	F: AACCATACAGCATAGCAGTGAT R: AAGCATCATGTTTACCAGATGC
BRtt	(CA) ₁₀	F: CATTTCATGCACTCTTGATGCAC R: GGGCACAGAAGATGTTATAGAC
IGs	(CA) ₁₄	F: GAGCTAGGCAATATGTACTTAATG R: TGTACCATCCTGCAACGTTGTT
MCC	(CA) ₆ A(CA) ₆	F: TCAGCTGTAACACCCAGAAAC R: TTAAGTGCCAGAAAAGGACCG
NGff	(CA) ₁₀	F: GGGAATCAGGCAGCACACAAT R: TTGTCAGCAAACCTCCAGCGG
PLkn	(CA) ₁₇	F: GTACCTTGTGACTGCAGTGCT R: TTGAGACACAGGAGGCAGAAG
SMcL	(CA) ₁₅	F: TAATCTTTCATACTGAGAT R: CCTAGCACATCTCTAGTAAG
Sphill	(CA) ₉	F: GATCATATACTGGTTTAAGACA R: TCACACATCGACTCCAAAGTCAG
Sveg	(CA) ₃ CG(CA) ₆	F: TGGTTGCAATGAGCATATCAG R: TCATTCACCATGCTAGCAA

Table 3. Population genetic parameters for *Uta stansburiana* in Grand Canyon and Canyonland National Parks, USA, including mean number of individuals genotyped at each locus, the average number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness, rarified allelic richness, and the number of private alleles observed in each population.

Site	Mean Individuals per locus	Alleles per Locus $\pm$ SE	$H_o \pm$ SE	$H_e \pm$ SE	Allelic Richness $\pm$ SE	Rarified Allelic Richness	Private Alleles
Badger	63.5	5.125 $\pm$ 1.008	0.475 $\pm$ 0.105	0.502 $\pm$ 0.114	5.375 $\pm$ 1.224	4.09	3
Jackass	57.8	4.625 $\pm$ 0.925	0.493 $\pm$ 0.109	0.518 $\pm$ 0.109	5.500 $\pm$ 1.118	4.05	4
Basalt	16	3.625 $\pm$ 1.034	0.406 $\pm$ 0.131	0.381 $\pm$ 0.122	3.625 $\pm$ 1.034	3.63	2
Tanner	34	4.750 $\pm$ 1.221	0.382 $\pm$ 0.122	0.395 $\pm$ 0.125	4.750 $\pm$ 1.221	4.19	3
Red Lake Canyon	48.9	5.000 $\pm$ 1.195	0.454 $\pm$ 0.124	0.433 $\pm$ 0.120	5.000 $\pm$ 1.195	4.06	2
Spanish Bottom	20	4.125 $\pm$ 0.990	0.494 $\pm$ 0.143	0.467 $\pm$ 0.133	4.125 $\pm$ 0.990	3.94	1

Table 4. Pairwise population fixation indexes between river right and river left populations in Canyonlands and Grand Canyon National Parks. F_{ST} values highlighted to show population differentiation between cross-river study sites.

Pairwise Population F_{ST} Values						
	Badger	Basalt	Spanish Bottom	Jackass	Tanner	Red Lake
Badger	0.000					
Basalt	0.234	0.000				
Spanish Bottom	0.143	0.146	0.000			
Jackass	0.124	0.237	0.182	0.000		
Tanner	0.221	0.053	0.158	0.249	0.000	
Red Lake	0.205	0.250	0.126	0.245	0.255	0.000

Table 5. Analysis of Molecular Variance (AMOVA) results showing the level of genetic differentiation among different populations on river right and river left in Canyonlands and Grand Canyon National Parks. 23% of variation was due to differences among populations and 77% of variation was due to differences within individuals.

Summary AMOVA Table					
Source of Differentiation	df	SS	MS	Est. Var.	%
Variation among Populations	4	161.553	40.388	0.534	23%
Variation among Individuals	235	432.463	1.840	0.007	0%
Variation within Individuals	241	440.000	1.826	1.826	77%
Total Variation	481	1058.407		2.367	100%

Table 6 (A). Pairwise population fixation indexes between sites on river right: Badger, Basalt, and Spanish Bottom. F_{ST} values highlighted to show population differentiation between river right sites.

Pairwise Population F_{ST} Values – River Right			
	Badger	Basalt	Spanish Bottom
Badger	0.000		
Basalt	0.234	0.000	
Spanish Bottom	0.143	0.146	0.000

Table 6 (B). Pairwise population fixation indexes between sites on river left: Jackass, Tanner, and Red Lake Canyon. F_{ST} values highlighted to show population differentiation between river left sites.

Pairwise Population F_{ST} Values – River Left			
	Jackass	Tanner	Red Lake
Jackass	0.000		
Tanner	0.249	0.000	
Red Lake	0.245	0.255	0.000

FIGURES

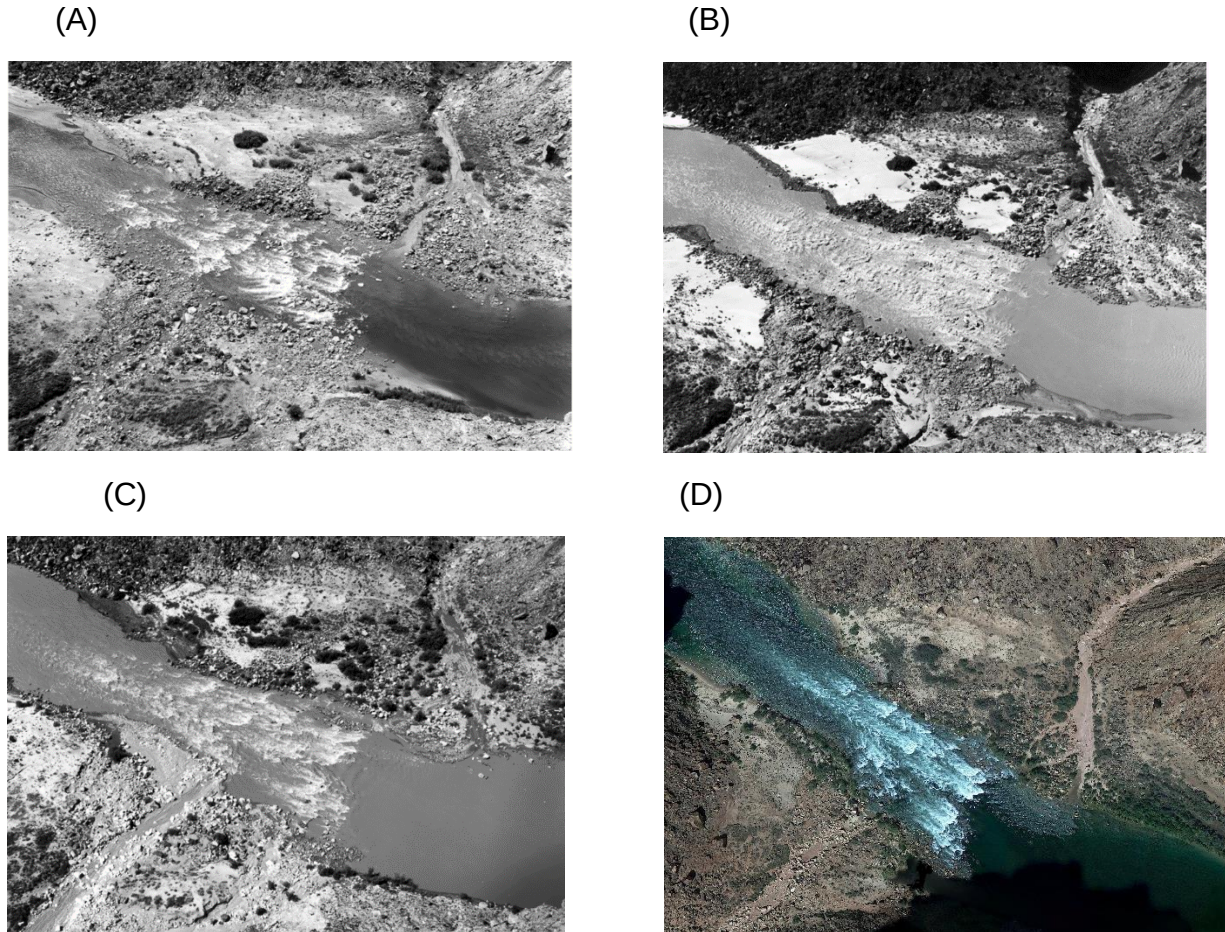


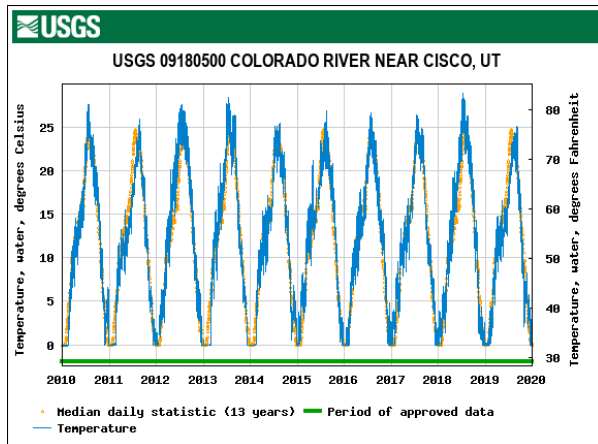
Figure 1. (A) Badger Creek Rapid in August 1964, soon after the March 1963 completion of Glen Canyon Dam. Low water in the Colorado River reveals many rocks that create Badger Creek Rapid. The shores of the river are bare and have little vegetation. Photograph by Tad Nichols (Webb et al. 2002).

(B) Badger Creek Rapid in October 1968 with increased shoreline vegetation, specifically tamarisk. Photograph by Tad Nichols (Webb et al. 2002).

(C) Badger Creek Rapid in September 1994. Debris flow from Jackass Creek constrict Badger Creek Rapid. Higher water levels have completely covered most of the rocks in this rapid, closely resembling present-day conditions. Decreased sediment influx and consistent flow rates have resulted in smaller sandbars and increased riparian vegetation along the riverbanks. Photograph by Robert Webb (Webb et al. 2002).

(D) Badger Creek Rapid in July 2019. No rocks are visible in the first major rapid in Marble Canyon. Both sides of the river are covered in vegetation and depleted sand bars have resulted in limited beach habitat. Photo from Google Earth.

(A)



(B)

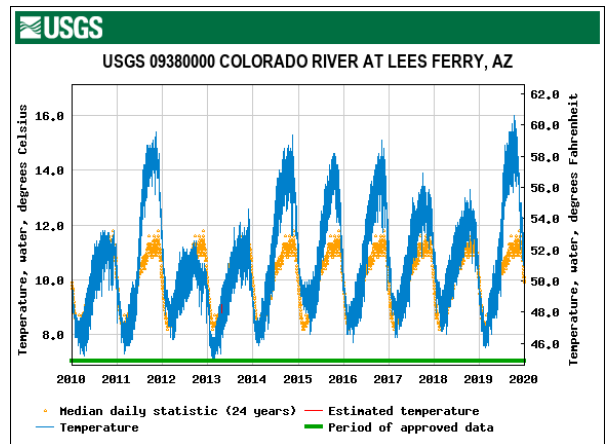


Figure 2. (A) USGS median daily water temperature of the Colorado River above Glen Canyon Dam and its impoundment from January 1, 2010 to January 1, 2020. The orange line is the median daily temperature for that day of the year from 2010-2020. Source: USGS Water Resources (2021).

(B) USGS median daily water temperature of the Colorado River at Lee's Ferry, Arizona, 14 river miles (22.5 km) below Glen Canyon Dam from January 1, 2010 to January 1, 2020. The orange line is the median daily temperature for that day of the year from 2010-2020. Source: USGS Water Resources (2021).

(A)



(B)



Figure 3. (A) Warm water research sites (green) above Glen Canyon Dam in Cataract Canyon, Canyonlands National Park, Utah. Spanish Bottom and Red Lake Canyon, 66 km (41 miles) upriver from Hite Marina (pink) at the northeast end of Lake Powell and 305 km (190 miles) upriver from Glen Canyon Dam (blue).

(B) Cold water research sites below Glen Canyon Dam (blue) in Grand Canyon National Park, Arizona. Badger and Jackass (yellow), 37 km (23 miles) downriver from Glen Canyon Dam and Tanner and Basalt (teal), 135/136 km (84/85 miles) downriver from Glen Canyon Dam.

(A)



(B)



(C)

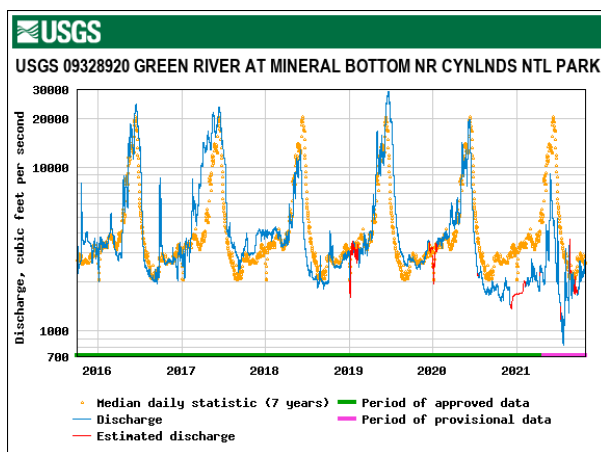


Figure 4. (A) Spanish Bottom and Red Lake Canyon, Cataract Canyon, Canyonlands National Park, Utah, 5 km (3 miles) downriver from the confluence of the Green and Colorado Rivers, 71 km (44 river miles) from Hite Marina at the northeast end of Lake Powell, and 305 km (190 miles) upriver from Glen Canyon Dam. Map is orientated by direction of river flow. Due north can be found at in the bottom right of the map.

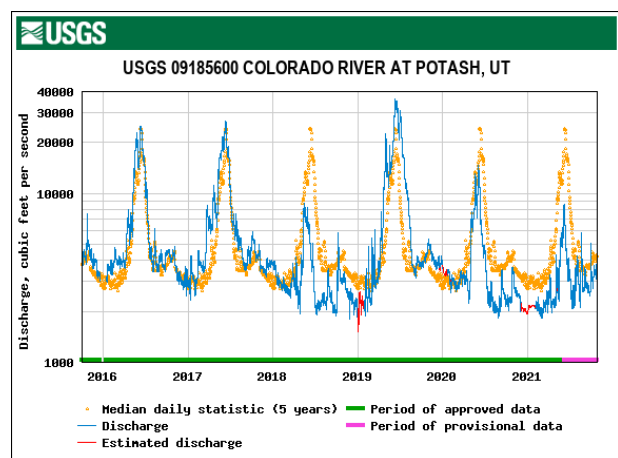
(B) Badger Canyon and Jackass Canyon, separated by Badger Creek Rapid, Grand Canyon National Park, Arizona, 37 km (23 miles) downriver from Glen Canyon Dam. Map is orientated by direction of river flow. Due north can be found at in the bottom right of the map.

(C) Tanner Beach and Basalt Creek, Grand Canyon National Park, Arizona, 29 km (18 miles) downriver from the confluence of the Little Colorado and Colorado Rivers, 98 km (61 miles) downriver from Badger and Jackass, and 135 km (84 miles) downriver from Glen Canyon Dam. Map is orientated by direction of river flow. Due north can be found at in the bottom right of the map.

(A)



(B)



(C)

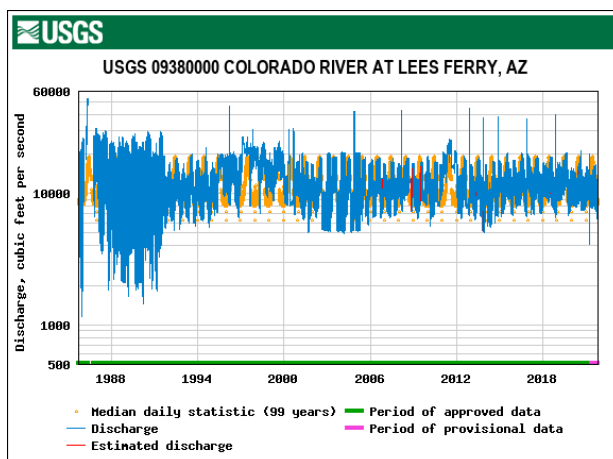


Figure 5. (A) USGS median annual discharge rate at Mineral Bottom on the Green River in Canyonlands National Park, Utah from 2015 to today. Annual discharge rate ranges from 2,000 ft³/sec to 35,000 ft³/sec. Mineral Bottom is 84 km (52 miles) upriver from Spanish Bottom and Red Lake Canyon.

(B) USGS median annual discharge rate at Potash on the Colorado River in Moab, Utah from 2015 to today. Annual discharge rate ranges from 2,000 ft³/sec to 35,000 ft³/sec. Potash is 82 km (51 miles) upriver from Spanish Bottom and Red Lake Canyon.

(C) USGS median annual discharge rate at Lee's Ferry, Arizona at the entrance of Grand Canyon National Park from 1988 to today. Apart from a few low flow years between 1988 and 1992, the annual discharge rate at Lee's Ferry below the dam is between 7,000 ft³/sec to 20,000 ft³/sec.

(A)



(B)

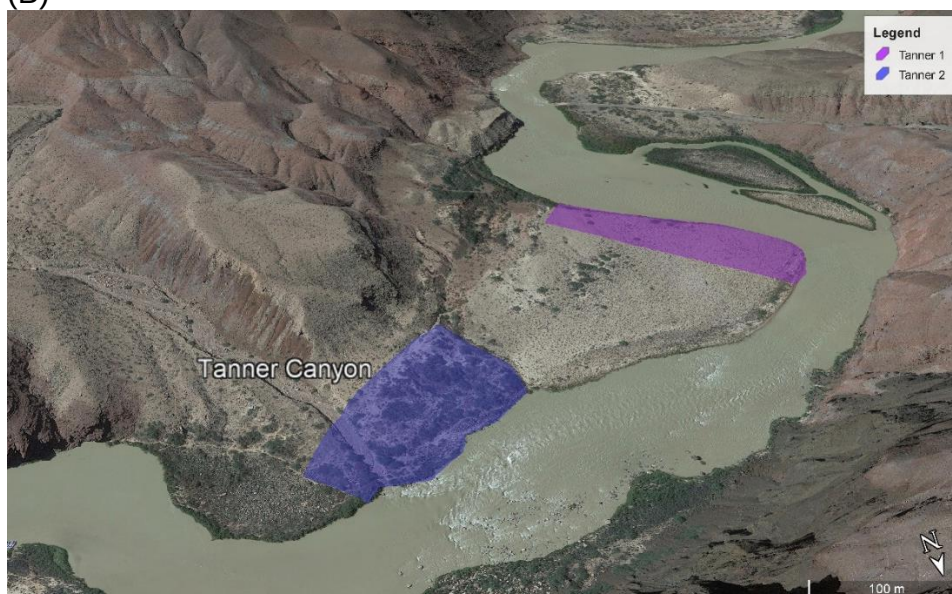


Figure 6. (A) Sampling areas at Badger Canyon and Jackass Canyon (37 km downriver from Glen Canyon Dam), Marble Canyon, Grand Canyon National Park. Badger and Jackass Canyons are separated by Badger Creek Rapid, the first significant rapid in Marble Canyon. The two sampling areas on one side of the river are about a river's width apart.

(B) Sampling areas at Tanner Canyon (135 km from Glen Canyon Dam), Grand Canyon National Park. Tanner 1 (pink) and Tanner 2 (purple) are ~500m apart.

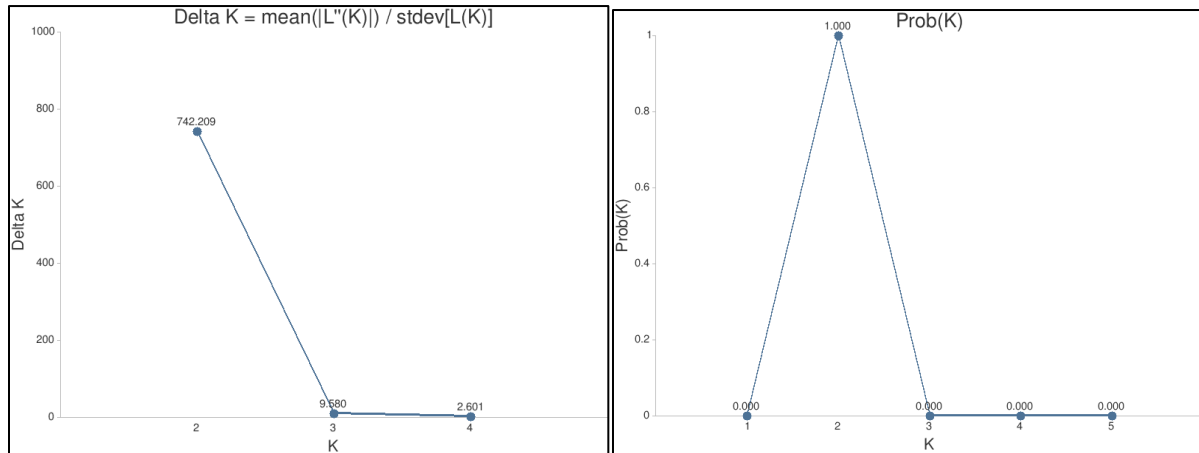


Figure 7. Male common side-blotched lizard (*Uta stansburiana*) sunning himself on a sandstone slab, displaying a prominent blue-black splotch and blue speckling. Photo taken April 2021 at South Canyon, Grand Canyon National Park by T. Corsetti.



Figure 8. Common side-blotched lizard (*Uta stansburiana*) marked with a non-toxic, xylene-free paint pen in colors consistent with natural coloring to prevent recapture, double sampling, and unnecessary stress to the animal. Photo taken June 2020 at Jackass Canyon, Grand Canyon National Park by T. Corsetti.

(A)



(B)

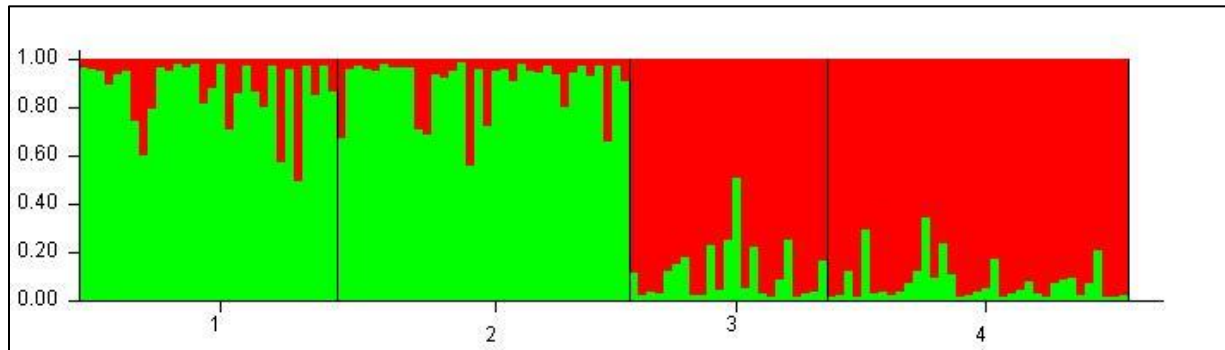
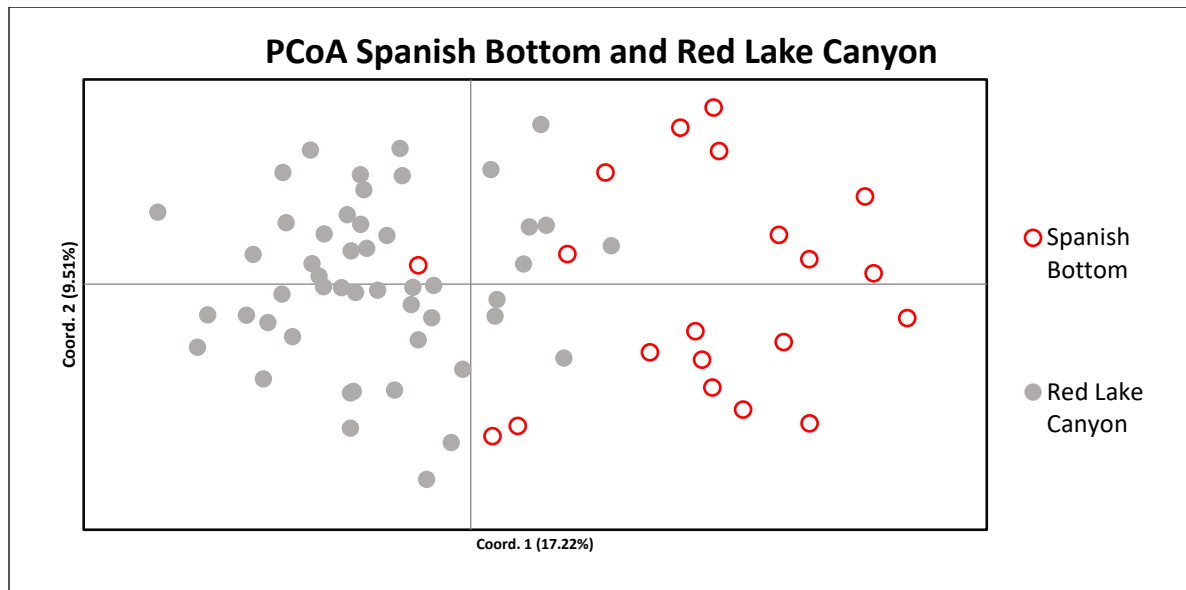


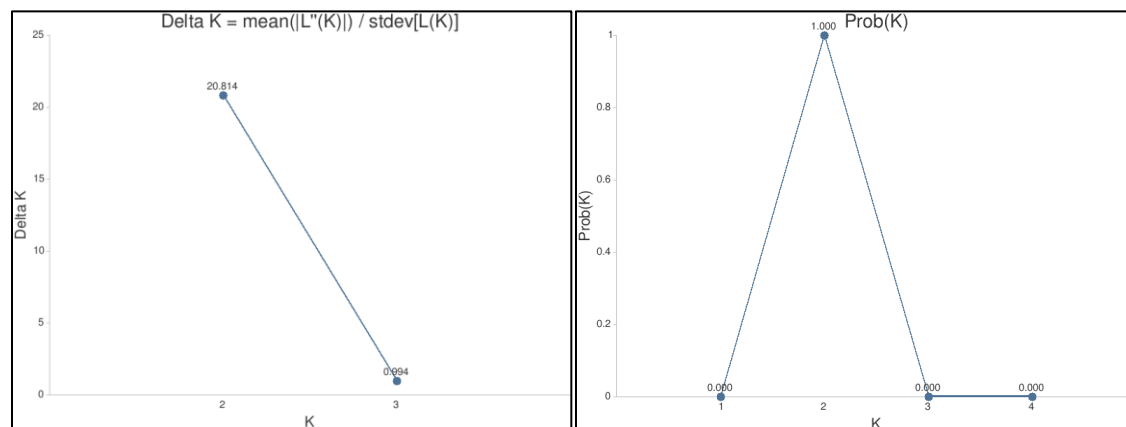
Figure 9. (A) CLUMPAK Best K analysis for Delta K (Evanno et al. 2005) and probability by K (Pritchard et al. 2000) of Badger1 and Badger 2 and Jackass1 and Jackass 2 for 122 individuals between two populations. Estimations of populations of *Uta stansburiana* in Grand Canyon NP derived Δk for k from 1 to 4.

(B) STRUCTURE results for Badger (1 and 2) and Jackass (3 and 4) showing distinct genetic structure between Badger and Jackass and lack of genetic structure between sampling sites on one side of the river (K= 2). Genotype for each individual is represented by a vertical bar, with red and green indicating the relative proportion of assignment to each genetic cluster.

(A)



(B)



(C)

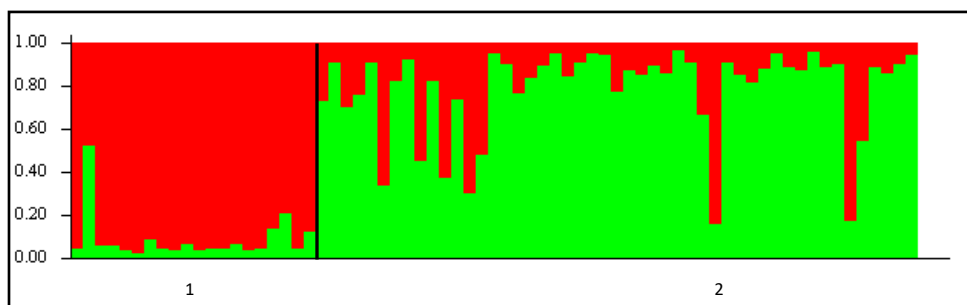
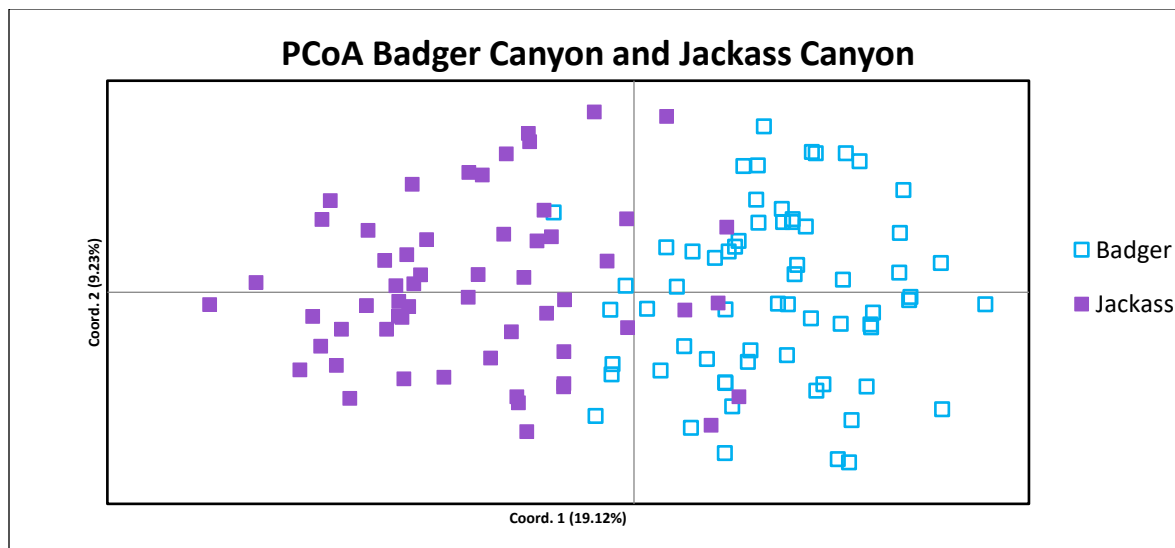


Figure 10. (A) Principal coordinates analysis showing genetic structure between Spanish Bottom and Lower Red Lake Canyon, on opposite sides of the Colorado River in Canyonlands, Utah.

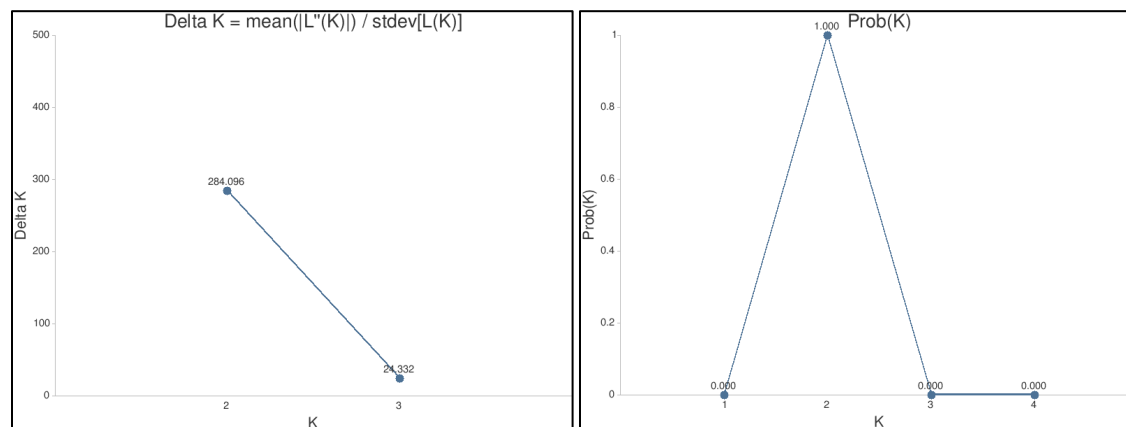
(B) Clumpak Best K analysis for probability by K (Pritchard et al. 2000; Evanno et al. 2005) grouped core set of 69 individuals into 2 populations by river side. Estimations of populations of *Uta stansburiana* at Spanish Bottom and Red Lake Canyon, Canyonlands NP derived Δk for k from 1 to 4. There are two assumed populations or genetic groups in this study, which is consistent with our data ecologically.

(C) STRUCTURE results based on two populations (K=2), Spanish Bottom (1) and Red Lake Canyon (2). Genotype for each individual is represented by a vertical bar, with red and green indicating the relative proportion of assignment to each genetic cluster.

(A)



(B)



(C)

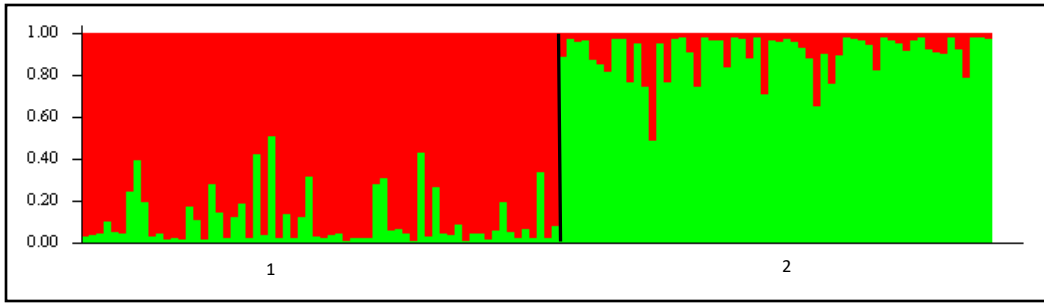
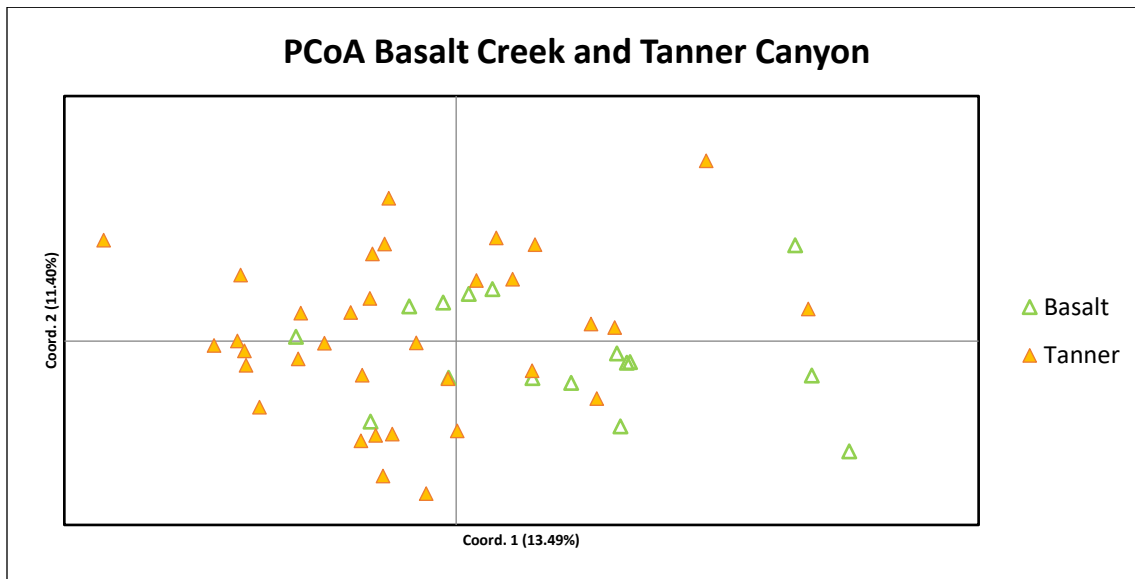


Figure 11. (A) Principal coordinates analysis showing genetic structure between Badger and Jackass Canyons on opposite sides of the Colorado River in Grand Canyon, Arizona.

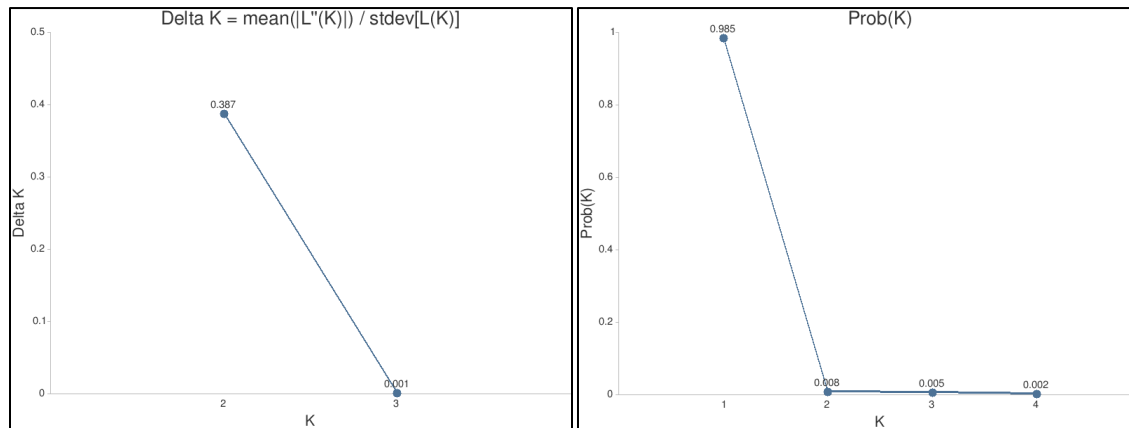
(B) Clumpak Best K analysis for probability by K (Pritchard et al. 2000; Evanno et al. 2005) grouped core set of 122 individuals into 2 populations by river side. Estimations of populations of *Uta stansburiana* at Badger Canyon and Jackass Canyon, Grand Canyon NP derived Δk for k from 1 to 4. There are two assumed populations or genetic groups in this study, which is consistent with our data ecologically.

(C) STRUCTURE results based on two populations (K=2), Badger (1) and Jackass (2). Genotype for each individual is represented by a vertical bar, with red and green indicating the relative proportion of assignment to each genetic cluster.

(A)



(B)



(C)

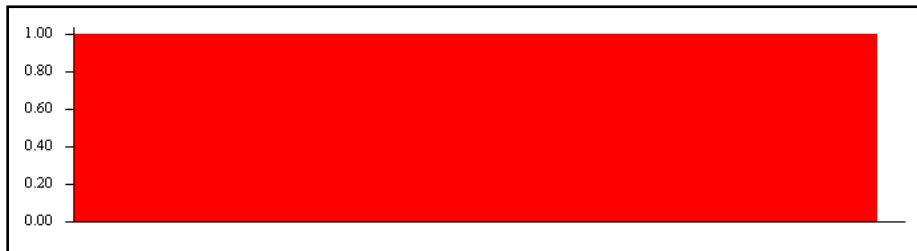


Figure 12. (A) Principal coordinates analysis of populations showing lack of population differentiation between Tanner Canyon and Basalt Creek, on opposite sides of the Colorado River Grand Canyon, Arizona.

(B) Clumpak Best K analysis for probability by K (Pritchard et al. 2000; Evanno et al. 2005) grouped core set of 50 individuals into a single breeding population. Estimations of populations of *Uta stansburiana* at Tanner Canyon and Basalt Creek, Grand Canyon NP derived Δk for k from 1 to 4. There one assumed population or genetic group between two sites, indicating that lizards can access and successfully breed with individuals on the opposite side of the river. Evanno et al. (2005) determined Delta K=2, there are two assumed populations or genetic groups in this study. Uneven sample sizes between Tanner and Basalt may be driving this result. Pritchard et al. (2000) determined K=1, there is one single population between these two sites. This is consistent with the rest of our data (see *Results – Complete Site Analysis*).

(C) STRUCTURE results based on one population (K=1) at Tanner Canyon and Basalt Creek. Red indicates a single genetic cluster.

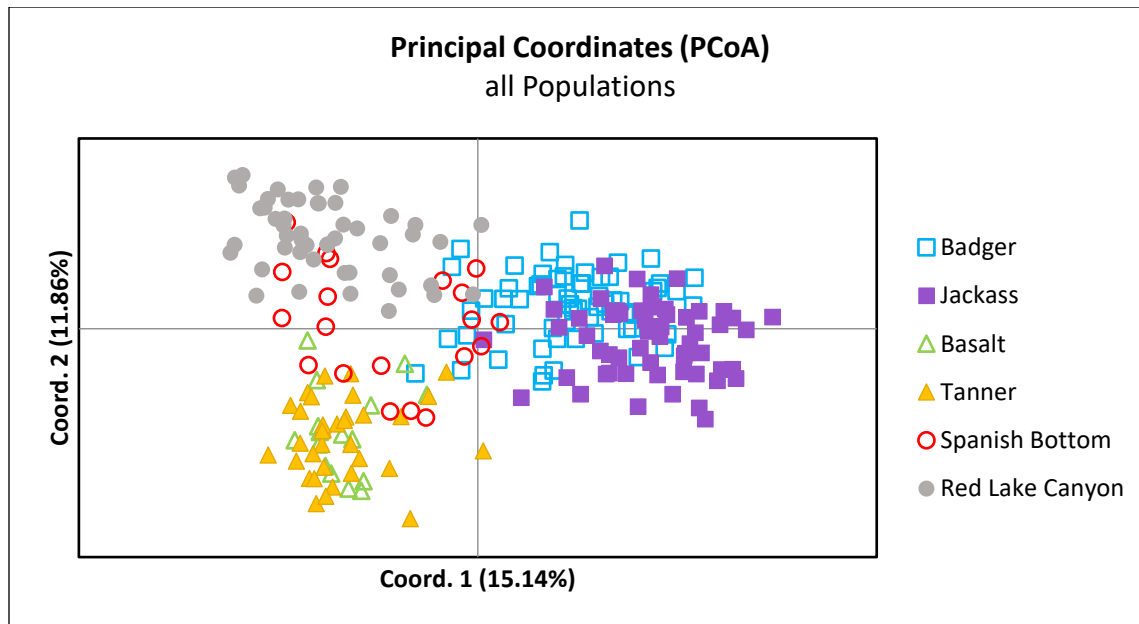


Figure 13. Principle coordinates analysis of all populations showing isolation by distance using a pairwise genetic distance matrix. Analysis shows clustering of populations consistent with their geographic location. Samples are color-coded by population. River right sites are no fill symbols, river left sites are filled symbols. Cross-river sites Badger and Jackass are identified by squares, Tanner and Basalt are diamonds, and Spanish Bottom and Red Lake Canyon are circles. Sites in PCoA span a length of ~275 river miles from warm water sites (Spanish Bottom and Red Lake Canyon) in Canyonlands to the second cold water site (Tanner and Basalt).

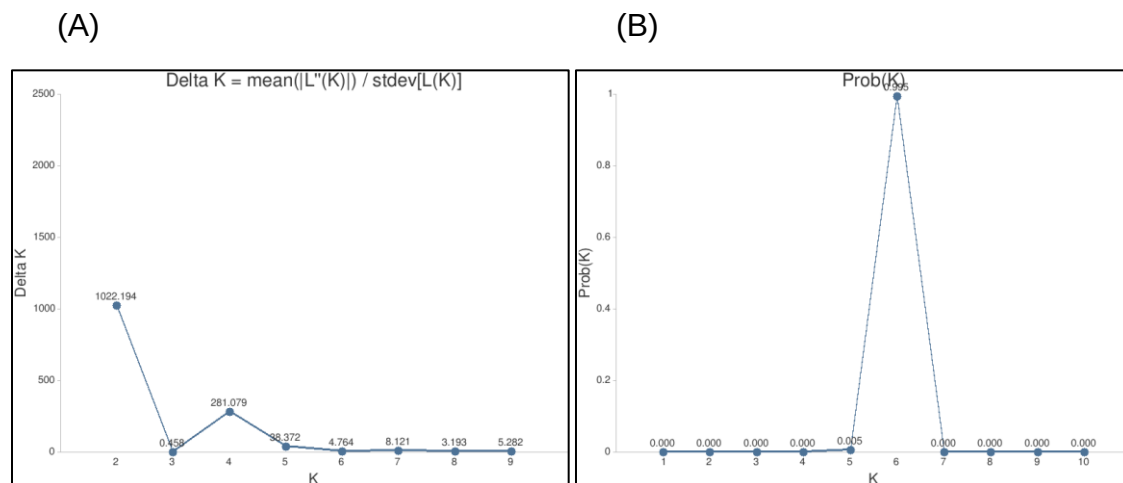


Figure 14. (A) Clumpak Best K analysis for Delta K (Evanno et al. 2005) grouped core set of 241 individuals into two populations. Estimations of populations of *Uta stansburiana* in Grand Canyon and Canyonlands NP derived Δk for k from 1 to 8. Evanno et al. (2005) determined Delta K=2, there are two assumed populations or

genetic groups in this study. Uneven sample sizes across multiple sites and geographic distance between populations may be driving this result.

(B) Clumpak Best K analysis for probability by K (Pritchard et al. 2000) grouped core set of 241 individuals into six populations. Estimations of populations of *Uta stansburiana* in Grand Canyon and Canyonlands NP derived Δk for k from 1 to 8. Prichard et al. (2000) determined Prob(K)=6, there are six assumed populations or genetic groups in this study, which is consistent with our data ecologically.

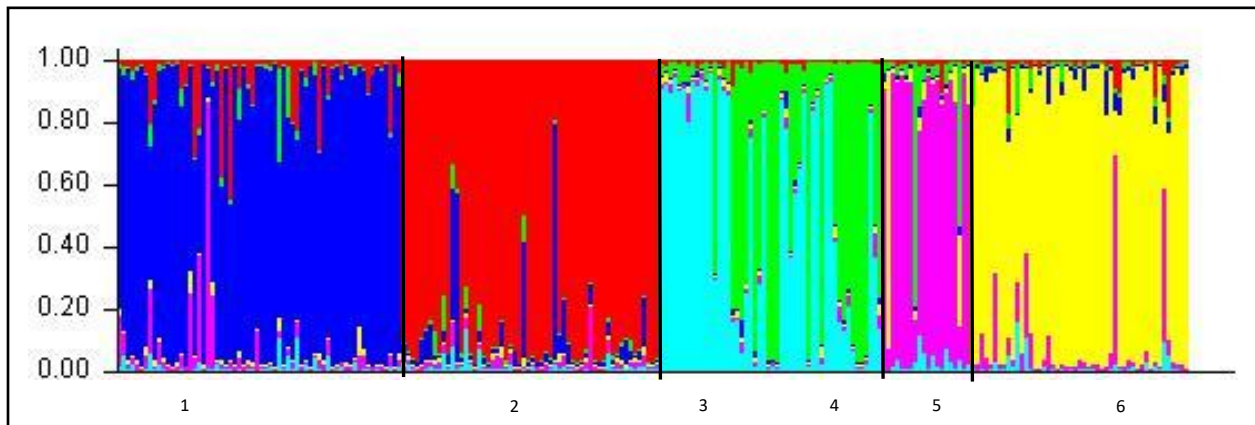


Figure 15. STRUCTURE result based on six populations (K=6) in Canyonlands and Grand Canyon National Parks. Each individual sample is represented by a vertical bar with colors indicating the proportions of each genetic cluster. Bar plot shows genetic structure between Badger (1, blue) and Jackass (2, red) and Spanish Bottom (5, pink) and Red Lake Canyon (6, yellow). It shows overlap of populations at Tanner (3, teal) and Basalt (4, green) suggesting that the two populations are a single breeding population.

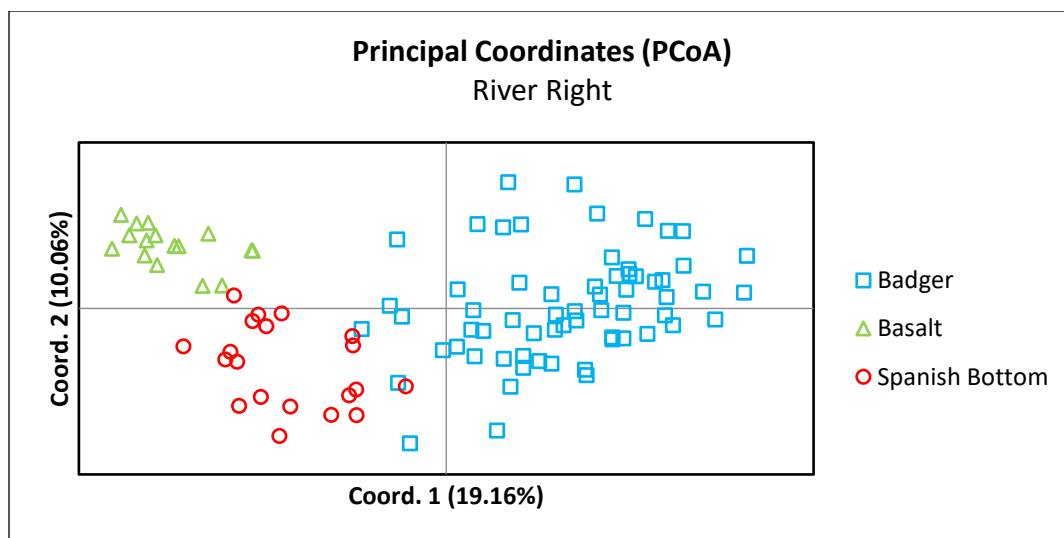


Figure 16. PCoA for research sites on river right only, showing isolation by distance over 442 km (275 miles). Samples are color-coded by population. Significant clustering can be observed in all river right sites through Grand Canyon and Canyonlands, suggesting genetic differentiation on same-side sites.

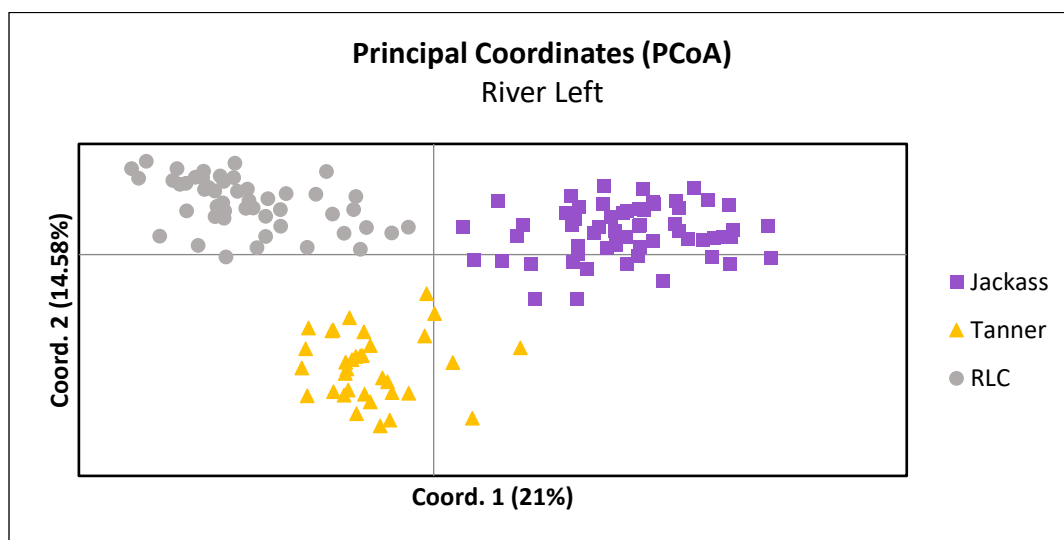


Figure 17. PCoA for research sites on river left showing isolation by distance over 442 km (275 miles). Samples are color-coded by population. Significant clustering can be observed in all river left sites through Grand Canyon and Canyonlands, suggesting genetic differentiation on same-side sites.

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