Habitat Suitability Criteria for Zuni Bluehead Sucker *Catostomus discobolus yarrowi* and Navajo Nation Genetic Subunit Bluehead Sucker *Catostomus discobolus* and Comparing Efficiency of AFS Standard Snorkeling Techniques to eDNA Sampling Techniques

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ABSTRACT

I quantified habitat selection for the endangered Zuni Bluehead Sucker *Catostomus discobolus yarrowi* and the Navajo Nation Genetic Subunit (NNGS) Bluehead Sucker *Catostomus discobolus* - a recent taxon described from genetic information. Both taxa are found in northern Arizona and New Mexico border regions. I examined fish [≥50 millimeters (mm) total length (TL)] selection of microhabitat conditions (i.e., water velocity, substrate size, overhead cover, water depth, instream cover, and mesohabitat conditions [i.e., pool, run riffle], during summer base flow conditions for NNGS Bluehead Suckers, and during both summer base flow and high spring flow conditions for Zuni Bluehead Suckers in six streams). Electrofishing, seining, and snorkeling were used to evaluate fish occupancy. From this information, I developed stream specific habitat suitability criteria (HSC) and then generalized HSC for each taxon, and tested transferability of the generalized HSC to individual streams. Zuni Bluehead Suckers and NNGS Bluehead Suckers occupied similar habitats: low velocity pools; sand, silt, and pebble substrate; high percent of instream cover; and water temperatures ranging from 2-21°C. However, Zuni Bluehead Suckers selected for low (0-25%) overhead cover where as NNGS Bluehead Sucker selected for high (0-75%) overhead cover. This was likely due to the source of instream cover – aquatic macrophytes that required sunlight in the Zuni Bluehead Sucker streams, and large woody debris falling from overhead branches in the NNGS Bluehead Sucker streams. Suggestions for managers includes maintaining existing cover or artificially construct additional instream cover; promote overhead cover (e.g., maintaining large trees along streams) and pool mesohabitats.

In addition to this work I also tested the new method of environmental DNA (eDNA) to further help conservation efforts for these taxa. Environmental DNA has typically been used to detect invasive species in aquatic environments through water samples. I compared the efficacy

of eDNA methodology to American Fisheries Society standard snorkeling surveys to detect presence of a rare fish species. My study site included three streams on the Navajo Nation in northern Arizona and northern New Mexico containing Navajo Nation Genetic Subunit Bluehead Sucker *Catostomus discobolus* and the Zuni Bluehead Sucker *Catostomus discobolus yarrowi*. To determine sample sites, I first divided entire wetted area of streams into 100-m consecutive reaches. I systematically selected 10 of those reaches for snorkel and eDNA surveys. Water samples were taken in 10-m sections within each 100-m reach, and fish presence via snorkeling was noted in each 10-m section as well. Water samples were collected at the downstream starting point of each reach, and continued upstream in each section 5 to 8 m ahead of the snorkeler. A qPCR was run on each individual water sample in quadruplicate to test for sucker presence or absence. I was able to positively detect both species with eDNA sampling techniques in two out of three streams. Snorkeling resulted in positive detections of both species in all three streams. In streams where fish were detected with eDNA sampling, snorkeling detected fishes at 11-29 sites per stream, where as eDNA detected fish at 3-12 sites per streams. My results suggested that AFS standard snorkeling was more effective at detecting target fish species than eDNA. To improve eDNA sampling, the amount of water collected and tested should be increased. Additionally, filtering water on site may improve eDNA techniques for detecting fish. Future research should focus on standardizing eDNA sampling to provide a widely operational sampling tool similar to electrofishing, netting, and hydroacoustics.

CHAPTER 1. HABITAT SUITABILITY CRITERIA FOR ZUNI BLUEHEAD SUCKER *Catostomus discobolus yarrowi* AND NAVAJO NATION GENETIC SUBUNIT BLUEHEAD SUCKER *Catostomus discobolus*

INTRODUCTION

Fishes of western deserts are among the most endangered fishes in the United States (Williams 1985; Fagan et. al. 2005). Critical habitat needs are poorly understood for many species. Zuni Bluehead Sucker *Catostomus discobolus yarrowi*, a fish recently listed as endangered under the U.S. Endangered Species Act (1973) (Federal Register 2014), is one such species. Zuni Bluehead Suckers are important ecologically, but also culturally, for various tribal and Anglo communities adjacent to streams in which the fish occur. Their populations are located in streams on the Navajo Nation in Arizona and within the Zuni Pueblo in New Mexico, and on adjacent U.S. Forest Service and private lands in both states (Propst and Hobbes 1996).

Zuni Bluehead Sucker are found in the Little Colorado River Watershed, Arizona/New Mexico, specifically in sub-watersheds of the Zuni River and Kinlichee Creek (Federal Register 2014). Accounts from European settlers show the Zuni River and Kinlichee Creek Watersheds were historically covered with Ponderosa Pine *Pinus ponderosa* forests and other woodland species (McCallum 1981). Between the late 1800s and early 1900s, logging and overgrazing became more widespread in the Zuni River Watershed, resulting in eroded soil, formation of gullies, altered vegetation structure, and declining water levels. These anthropogenic changes to the environment severely degraded habitat for Zuni Bluehead Sucker (Propst and Hobbes 1996).

The speciation of Zuni Bluehead Suckers is thought to have resulted from natural hybridization between Bluehead Suckers *C. discobolus* and Rio Grande Suckers *C. plebeius*

(Smith and Koehn 1983). Zuni Bluehead Suckers are valued because of their rarity and unique genetics (Smith and Koehn 1971; Smith et al. 1983; Crabtree and Buth 1987).

Another Bluehead Sucker has recently been described from tributaries to the Little Colorado River, specifically the sub-watershed of Canyon de Chelly. These fish, which were once thought to be Zuni Bluehead Suckers, have been classified as a Genetic Subunit of the Bluehead Sucker. Unpublished genetic data collected by Schwemm and Dowling (2008), and reanalyzed in 2014 (Federal Register 2014), suggest that suckers found in the sub-watershed of Canyon de Chelly do not show evidence of Rio Grande Sucker genes, nor do they share the same unique genetic background of the Zuni Bluehead Sucker (Federal Register 2014). Therefore, these suckers are now considered a new taxon, hereafter referred to as the Navajo Nation Genetic Subunit Bluehead Sucker (NNGS Bluehead Sucker).

On January 25, 2013 the U.S. Fish and Wildlife Service (USFWS) proposed to designate approximately 472 km (293 mi) of streams as critical habitat for Zuni Bluehead Sucker in Arizona and New Mexico (Federal Register 2014). On July 25, 2014 the USFWS published a final listing for the Zuni Bluehead Sucker, and due to the new genetic information regarding the NNGS Bluehead Sucker the amount of critical habitat was revised to 226 km (141 mi). Currently, little is known about the habitat requirements of Zuni Bluehead Sucker and even less about the NNGS Bluehead Sucker. My research will allow managers to identify and conserve existing habitat, and if needed, modify existing streams to create suitable conditions. Some information is available for habitat requirements of the closely related Bluehead Sucker (Ptacek et. al. 2005; Stewart et al. 2005), and for Zuni Bluehead Sucker in the Rio Nutria (Propst et. al. 2001), but specific habitat requirements of the Zuni Bluehead Sucker over a wider range, and the NNGS Bluehead Sucker remain undefined.

Habitat suitability criteria (HSC) are used to relate environmental characteristics of individual streams to indices of habitat quality for fishes (Bovee 1986; Thomas and Bovee 1993). Identification of specific preferences allows us to develop HSC for numerous habitat parameters in areas fish occupy. Habitat suitability criteria have been developed for numerous fish species and applied in many instream flow studies (Barrett and Maughan 1995; Jowett 2002; Strakosh et al. 2003).

Species-specific HSC can differ among streams (Bozek and Rahel 1992), so investigation of the variability in HSC among streams is important. Variability can be due to competition and predation from co-occurring species, and differences in available food. Habitat partitioning (Werner et al. 1977) may result in some species displacing others into less-desirable habitat. Comparing habitat usage in depauperate streams to usage in streams with high fish species diversity provides managers information on variability of HSC resulting from interactions with co-occurring species. Examining the transferability of HSC for a species among streams is commonly conducted to evaluate the overall quality of HSC (Thomas and Bovee 1993; Freeman et al. 1997). Development of habitat criteria for Zuni Bluehead Sucker and NNGS Bluehead Sucker and evaluation of how such criteria can transfer among streams is critical for the USFWS to conserve the species.

Objectives for my study were to: (1) develop stream specific habitat suitability criteria (HSC) during base flows for Zuni Bluehead Sucker and NNGS Bluehead Sucker; (2) because of its endangered species status, develop additional stream specific HSC for Zuni Bluehead Sucker during high spring flows, thought to be when the majority of spawning occurs; (3) From stream specific criteria HSC develop a generalized HSC for each taxon and evaluate its transferability to

individual streams during both base and high flows; (4) rank relative importance of habitat variables for each taxon.

METHODS

Study Sites

My study area included six streams on the Navajo Nation in northeastern Arizona and northwestern New Mexico (Figure 1.1). Streams were selected through joint consensus among U.S. Fish and Wildlife Service (USFWS), Navajo Nation Fish and Wildlife (NNFW), U.S. Geological Survey (USGS), and University of Arizona (UA) biologists. Kinlichee Creek, Black Soils Wash, and Scattered Willows Wash on the Defiance Plateau contained Zuni Bluehead Sucker; Whiskey, Crystal, and Tsaile creeks in the Chuska Mountains contained NNGS Bluehead Sucker; Streams were selected based on the range of environmental characteristics available, and presence of either Zuni Bluehead or NNGS Bluehead Suckers. I selected sampling sites from the entire wetted length of each stream. These streams included three of the ten streams containing Zuni Bluehead Sucker and three of the six streams containing the NNGS Bluehead Sucker. Some streams had other species that also occurred in them aside from our target species. For example, Kinlichee Creek containing Zuni Bluehead Sucker also contained Fathead Minnow *Pimephales promelas*. All three streams containing NNGS Bluehead Sucker also had Speckled Dace *Rhinichthys osculus,* while Whiskey Creek had Brown Trout *Salmo Trutta,* Crystal Creek had Fathead Minnnow, and Tsaile Creek had Rainbow Trout, *Oncorhynchus mykiss.*

Fish and Habitat Sampling

Streams were sampled during daylight hours and during base flow conditions (0.0 - 0.8 m/s) in two consecutive years (May 27 - July 16, 2014) and (May 25 - May 28, 2015). Because Zuni Bluehead Suckers are listed as endangered and critical habitat areas were formally

designated under the Endangered Species Act, I also quantified their habitat use during spring high flow conditions (0.0 - 1.4 m/s) (March 21 through April 1, 2015), which corresponded with the majority of their spawning activity.

At each sampling time, in each stream, I examined fish [≥50 millimeter (mm) total length (TL)] selection of microhabitat (i.e., water velocity, substrate size, overhead cover, water depth, instream cover and mesohabitat (i.e., pool, run riffle). I defined "habitat" to refer to stream conditions both occupied and unoccupied (available); my definition corresponds to the habitat definition of most HSC (Bovee 1986) and will be used henceforth. To develop HSC's, I captured or observed fish in the streams, measured habitat variables where fish were captured, and then measured the availability of those variables in the stream to evaluate whether fish were selecting for those specific characteristics.

I used electrofishing, seining, and snorkeling to evaluate fish use of various habitats. The efficiency of fish capture methods can vary, so the optimum method based on stream conditions at time of sampling was selected. In Whiskey Creek during base flows and Kinlichee Creek during high flows, I selected 300 random electrofishing sites from the entire wetted length of stream. I used a backpack electrofisher (Smith-Root, Model 12, Vancouver, Washington) as a modified prepositioned aerial electrofishing device (PAED). To avoid fright bias, PAED's are usually placed in the water and left for a minimum of 11 min (Bain et al. 1985; Rinne 1985). However, suckers were not observed moving from their location when electrofishing equipment was placed in water. This was verified by snorkel survey and underwater video footage from a stationary mounted underwater camera (GoPro Hero 3, San Mateo, California). Therefore, samplers were able to move to the stream edge and apply current immediately with minimal fish movement. To conduct the procedure, one person, with a backpack electrofisher, moved directly

to the stream edge and quickly placed the cathode and anode into the stream, 1 m apart, and applied current immediately. Individuals held the anode in one hand and an extender claw gripped the cathode in the other. A person present downstream netted shocked fish that were occupying that specific site. Fish collected were identified to species, counted, and measured (TL, mm). I recorded data on individual fish and released them back into the stream.

Fish were enumerated by snorkeling in Crystal Creek, Tsaile Creek, Black Soils Wash, and Scattered Willows Wash during base flows and Black Soils Wash and Scattered Willows Wash during high flows. Snorkeling can be used to observe fish in streams without causing disturbance (Bovee 1986) and was possible at these sites because of high underwater visibility (>3 m). To sample, I divided the entire wetted length of each stream into 100 m consecutive reaches. I systematically selected 10 reaches for snorkel surveys, each separated by 350 m. The wetted length of Black Soils Wash during base flows was 400 m; therefore, the entire creek was censused. During the second sampling season, coinciding with high flows, an additional two miles were sampled from Black Soils Wash. However, the additional two miles of sampling did not encompass entire stream length. The same snorkeler sampled all streams, moving upstream slowly, and examining the entire stream width. Sites occupied by suckers were marked with a flagged, weighted washer. The snorkeler recorded number of suckers at each site, and presence of other fish species.

Kinlichee Creek was seined with a standard stream seine (6.5-mm bar mesh, 3.0 ×1.5 m) because the entire stream was comprised of isolated pools with low visibility (< 0.1 m). Pools (i.e., sampling sites) were connected by water trickles (depths <3 cm, width <25 cm) that could not hold fish. I systematically seined every second pool to encompass the entire wetted stream length. Two samplers at the most downstream point of the pool stretched a seine net to sample

its entire width. The net was dragged upstream across the entire pool, and once to the other side, the net was pulled up and out to capture fish. Suckers were counted and measured (TL, mm) before release.

After fish capture, I quantified habitat in each stream. A meter stick was used to measure water depth (cm); a Marsh-McBurney flow meter (Global Water Flow Probe, Gold River, California) to measure water velocity (m/s); a spherical densiometer to measure overhead cover (% shaded; Lemon 1956); visual observation to quantify proportion of instream cover (% cover of aquatic macrophytes, boulders, undercut banks, and debris under which fish could hide); an ISO calibrated liquid-in-glass thermometer to measure water temperature in Celsius (°C); and a measuring tape to measure stream width (cm). Pool was defined as moderate to deep water with low velocities that showed no turbulence at the surface; run was defined as moderately deep water with moderate velocities that was somewhat turbulent at the surface; riffle was defined as shallow water and fast velocities that showed clear turbulence at the surface (Armstrong and Parker 2000).

I used a different sampling design to quantify available and occupied habitat depending on the method of fish capture. Because Whiskey Creek during base flows and Kinlichee during high flows were sampled with modified prepositioned electrofishing, and Kinlichee Creek during base flows was sampled by seining, I measured environmental characteristics at all sampling sites (electrofishing grids or seined pools) whether or not suckers were captured. For electrofishing sites, characteristics were measured in 1×1 m sampling sites corresponding to the electrofishing site. Three measurements were taken along the diagonal of the plot and averaged. For seining sites, characteristics were measured in three locations across the diagonal of the sampled pool and averaged. Because Crystal, Tsaile, Black Soils, and Scattered Willows creeks

were measured with snorkel surveys, habitat availability was measured in 1×1 m sampling sites systematically placed every 10 m starting from the bottom of the reach.

Data Analysis

Development of Stream Specific and Generalized Habitat Suitability Criteria - To develop stream specific habitat suitability criteria, I plotted frequency distributions of fish occupancy of subdivisions of various habitat variables. I defined the central 50% of conditions occupied by fish as optimal habitat, the central 50-90% as useable habitat, the combination of optimal and useable (central 90%) as suitable, and outside the central 90% as unsuitable habitat per Bovee (1986). The optimal and suitable values were used to develop the optimal and suitable HSC for each stream. I used nonparametric analyses (one-sided chi square test) to test significance of fish selection for specific conditions (Bovee 1986; Thomas and Bovee 1993). This nonparametric analysis allowed us to compare conditions in which fish were found to conditions available throughout the stream to identify conditions for which they were selecting. I also plotted suitability indices showing the selection variability among streams within habitat variables. The figures were created by dividing the occupied ranges of each habitat variable, by the available ranges of each habitat variable and standardizing to a maximum of 1.0 (Bovee 1986) (Figure 1.2, 1.3, and 1.4).

Next, I created generalized HSC for both base flow and high flow conditions. I averaged the minimums of all ranges for each habitat variable from all streams to obtain a minimum generalized criterion. I averaged the maximums of all ranges for each habitat variable from all streams to obtain a maximum generalized criterion. I created generalized criteria for both optimal and suitable habitat ranges.

Transferability of Generalized Habitat Suitability Criteria - I followed the procedures of Thomas and Bovee (1993) to evaluate the transferability of my generalized HSC among streams.

First, I categorized sites from individual streams with my generalized criteria into optimal, useable, unsuitable, and suitable. I wanted to test how well my generalized HSC corresponded to habitat that was optimal or suitable in each individual stream. I did this by testing two hypotheses in 2×2 contingency tables (Conover 1980; Thomas and Bovee 1993):

(1) Ho: Optimal sites were occupied in the same proportion as useable sites, and
(2) Ho: Suitable sites were occupied in the same proportion as unsuitable sites.

Regression Analysis - Multiple logistic regression was used to relate fish presence to the relative importance of habitat variables, and was conducted for both taxa during base flows and Zuni Bluehead Sucker during high flows (Ahmadi-Nedushan et al. 2006). Fit for the model was reported with R^2 values (Ramsey and Schafer 2002). All habitat variables from streams were continuous. Significant habitat variables were indicated by *P*-values <0.05. The logistic regression equation used to predict the binary response of fish presence or fish absence was:

$$\text{logit}(\pi) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6$$

$\beta_0 =$ Intercept
$\beta_1 X_1 =$ Depth (cm)
$\beta_2 X_2 =$ Velocity (m/s)
$\beta_3 X_3 =$ Substrate (Wentworth Scale)
$\beta_4 X_4 =$ Temperature (°C)
$\beta_5 X_5 =$ Instream Cover (%)
$\beta_6 X_6 =$ Overhead Cover (%)

I used JMP's prediction profiler tool (independent re-sampled inputs) to rank habitat variable importance (Amyrgialaki 2014). All multiple logistic regression were run with JMP®, Version 12 (JMP 12®, SAS Institute Inc., Cary, NC).

RESULTS

Base Flow Conditions for Zuni Bluehead Sucker

Habitat Suitability Criteria – Zuni Bluehead Suckers occupied similar habitat in all three streams during summer base flow conditions (Figure 1.2). In all three streams Zuni Bluehead Suckers occupied low (0.0-0.1 m/s) velocity pools; silt and sand substrates; water of 13-22°C; sites with 30-60 % instream cover comprised of aquatic macrophytes as primary cover; and a low percent overhead cover (0-25%) (Table 1.1).

Generalized Habitat Suitability Criteria and Transferability – Black Soils Wash and Scattered Willows Wash were very similar in size, elevation, vegetation and substrates present. Kinlichee Creek was deeper, wider, and lacked instream cover. When comparing generalized HSC to each individual streams HSC during base flow conditions, two out of three streams HSC containing Zuni Bluehead Suckers were transferable. I rejected both null hypotheses for Scattered Willows Wash and Black Soils Wash. Conversely, I failed to reject the null hypothesis for Kinlichee Creek because there were no optimal sites when compared to my generalized HSC. Therefore, Kinlichee Creek's HSC was not transferable.

Logistic Regression Analysis – The results were a moderate fit (R^2=0.443). Significant habitat variables in the model according to *P*-values for Zuni Bluehead Sucker during summer base flow conditions were: water depth (cm), velocity (m/s), and instream cover (%) (Table 1.2). The variable importance report ranked the habitat variables as follows: Instream cover, velocity, and depth. (Figure 1.3)

Base Flow Conditions for NNGS Bluehead Sucker

Habitat Suitability Criteria – NNGS Bluehead Suckers occupied similar habitat variables in all streams during summer base flow conditions (Figure 1.4). In all three streams, NNGS

Bluehead Suckers were found in low velocity pools; silt, sand, and pebble substrates; water of 10-21°C; sites with 40-60% instream cover comprised of woody debris as primary cover; and a high percent overhead cover (75-100%; Table 1.3).

Generalized Habitat Suitability Criteria and Transferability – I found the generalized HSC was transferable to all three streams individual HSC's (Figure 1.4). I rejected both null hypotheses for Whiskey, Crystal, and Tsaile creeks after evaluating each individual stream against my generalized HSC.

Logistic Regression Analysis – The results were a moderate fit (R^2=0.429). Significant habitat variables in the model according to *P*-values for NNGS Bluehead Suckers during summer base flow conditions were: overhead cover (%), velocity (m/s), instream cover (%), and water depth (cm) (Table 1.4). The variable importance report ranked the habitat variables as follows: Depth, instream cover, overhead cover, and velocity (Figure 1.3)

High Flow Conditions for Zuni Bluehead Sucker

Habitat Suitability Criteria – Zuni Bluehead Suckers occupied similar habitat variables in all three streams during spring high flow conditions (Figure 1.5;). They occupied low velocity pools; silt, sand, and pebble substrates; water of 2-14°C; sites with 40-70 % instream cover comprised of aquatic macrophytes as primary cover; and a low percent overhead cover (0-25%; Table 1.5).

Generalized Habitat Suitability Criteria and Transferability – Black Soils Wash and Scattered Willows Wash were very similar in size, elevation, vegetation, and substrates present. Kinlichee Creek was deeper, wider, and lacked instream cover. When comparing generalized HSC to each individual stream HSC during high flow conditions, two out of three streams HSC containing Zuni Bluehead Suckers were transferable. I rejected both null hypotheses for

Scattered Willows Wash and Black Soils Wash. Conversely; I failed to reject the null hypothesis for Kinlichee Creek because there were no optimal sites when compared to my generalized HSC. Therefore, Kinlichee Creek's HSC was not transferable.

Logistic Regression Analysis – The results were a moderate fit (R^2=0.401). Significant habitat variables in the model according to *P*-values for Zuni Bluehead Sucker were: water depth (cm), instream cover (%), velocity (m/s) (Table1.6). The variable importance report ranked the habitat variables, in order of importance, as follows: Instream cover, velocity, and depth (Figure 1.3).

DISCUSSION

Zuni Bluehead Suckers selected pools with low velocities; high levels of instream cover; silt and sand substrates; and in areas with low percent overhead cover during base and high flows (Figure 1.2 and 1.5). My findings are similar to those of Propst et al. (2001), who found that Zuni Bluehead Suckers in New Mexico streams occupied pools and pool-runs with low velocities, and areas of periphytic algae or aquatic macrophytes.

Because Zuni Bluehead Suckers are the ancestral hybrid cross of Bluehead Sucker and Rio Grande Sucker, I compared my findings to habitat studies involving these two species. Holden (1973) found that Bluehead Suckers select for rocky substrates, and Holden and Stalnaker (1975) found that Bluehead Suckers prefer runs and riffles to pools. Bluehead Suckers are also found in a variety of stream sizes with varying velocities ranging from < 0.05 m/s to >300 m/s (Smith 1966). Calamusso (1996) found that Rio Grande Suckers in New Mexico streams preferred pools and glides (i.e., runs). They occupied low velocity areas (e.g., <0.2 m/s) and preferred cobble and boulder substrates. Habitat preferences of Zuni Bluehead Suckers are

more similar to Rio Grande Suckers than Bluehead Suckers, with the exception of preference for substrate.

The NNGS Bluehead Sucker used similar habitat characteristics during base flows as Zuni Bluehead Suckers. They were found in deep pools; with low velocities; preferred instream cover; and were found over silt, sand, and pebble substrates (Figure 1.4).

One difference between the taxa was the type of instream cover used, and seemingly different preferences for overhead cover. NNGS Bluehead Suckers preferred woody debris as instream cover (e.g., branches), likely because of abundant trees lining banks of the streams in which they were found. These trees were necessary to provide branches to the instream cover, thus that NNGS Bluehead Suckers were present under these tree canopies (high percentage of overhead cover) is not surprising. However, streams containing Zuni Bluehead Sucker were in sunny areas, with few trees lining the banks. Abundant aquatic macrophytes and algae were found in these streams and provided instream cover. These plants grow best in areas with slow current and low shade from overhead canopies. Therefore, although instream cover was important for both taxa, how instream cover was provided differed. The relatively deep pools in which both taxa were found may also help conceal them from terrestrial, or avian predators. For example, Shortnose Suckers *Chasmistes brevirostris* occupy sites deeper than 2 m because these depths provide protection from avian predators (Banish 2009). Especially when instream cover is sparse, depths greater than 2 m can provide concealment from predators (Findholt and Anderson 1995; Derby and Lovvorn 1997). I did not observe pools that approached 2 m in depth, however, even shallow pools in my streams may have provided some degree of cover.

Both taxa were found over a variety of substrates, but were most frequently found occupying silt and sand substrates. This is contradictory to many sucker species, which prefer

larger substrate sizes (Holden 1973; Calamusso 1996). Overgrazing may have led to soil erosion and sedimentation, particularly in pools where there was little to no flow. Study sites showed signs of grazing including barren ground, and high amounts of silt and sand substrate where target species were found. Perhaps selecting for pool mesohabitats overrides the selection for a particular substrate type.

Temperature was not a significant characteristic in predicting presence of either taxa. I documented Zuni Bluehead Suckers in temperatures ranging from 4 to 22° C and NNGS Bluehead Sucker in temperatures ranging from 10 to 20° C. Temperatures recorded depended on time of day and time of year sampled. I captured or observed fish throughout the entirety of the streams and throughout the day, finding them at both the minimum and maximum recorded temperatures.

Limitations of Study

Eliminating all bias from measuring fish habitat preference is not possible (Bovee 1986). Ideally, data should be obtained from unexploited streams at carrying capacity when developing HSC (Mathur et al. 1985; Bovee 1992). I observed both taxa in varying densities at measured sites, which can make measuring preferences for habitat variables challenging. High density of individuals at one site can decrease the use of that site by other fish. When optimal environments are occupied, those individuals still in search of available habitat may settle in suboptimal areas (Power 1984). And if density of a population is low, not all optimal sites are being utilized (Power 1984). Endangered species, like the Zuni Bluehead Sucker, typically occur at low densities that may skew the results of habitat preference for a species (Strickler 2014).

If streams are affected anthropogenically, then fish may use areas that are less optimal but are abundantly available due to human impacts. Streams in this study were affected by

sedimentation, possibly due to livestock grazing. Grazing livestock can adversely affect streams by increasing erosion rates. Sediment losses are significantly greater from grazed stream banks than ungrazed stream banks (Platts and Nelson 1985; Elmore and Beschta 1987). High rates of riparian grazing can modify stream channels or banks because vegetation is removed which can increase erosion (Trimble and Mendel 1995). Both of these alterations over time can drastically change habitat quality and availability.

My sampling occurred solely during daylight hours. Studies on HSC usually do not sample diel variation in habitat preference (Davey et al. 2011). This can lead to bias in development of HSC (Mathur et al. 1985; Heggenes 1996; Davey et al. 2011). To expand understanding of habitat preferences for the target species, conditions used could be measured during night, at other life stages, and during different times of the year.

Conclusions and Management Implications

I found both species used low-velocity stream pools with high amounts of instream cover. Therefore, adding these types of conditions to streams will increase the amount of habitat available to the adult life stage of both species. Suggestions for managing habitat for my target species include constructing livestock exclusion fences around sections of stream to reduce bank erosion, which has likely contributed to the widening and shallower depths of streams. Although I found both species primarily on silt and sand substrates, this may have been due to high availability of these small-particle substrates likely because of grazing impacts. Because instream cover was a predictor for target species presence, managers should maintain existing cover or artificially construct additional instream cover. Strategies to maintaining instream cover include promoting overhead cover (e.g., maintaining large trees along streams). To promote more pools,

managers should consider placing large objects like boulder, logs, culverts, and possibly gabion barriers to provide more habitat for the target species.

Future research to better define habitat needs should focus on surveys during both daytime and nighttime hours. Diel surveys will contribute a better understanding of how species environmental preferences may change throughout the day. Previous studies have shown that habitat preferences may differ for some species in the morning, compared to at night (Muhlfeld et al. 2001; Roussel and Bardonnet 2008; Bradford and Higgins 2011).

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TABLES

Table 1.1- Generalized habitat suitability criteria for Zuni Bluehead Sucker during base flows, including optimal (central 50%) and suitable ranges (central 95%) and *t*-values for chi square tests of habitat suitability criteria for habitat variables, combined from all streams. Substrate Scale (0=silt,1= sand, 2=gravel, 3=pebble, 4=cobble, 5=boulder).

	Optimal Range		**Suitable Range**			
Habitat Parameter	Min.	Max.	Min.	Max.	$t_{optimal}$	*P*
Depth (cm)	28	59	19	62	4.21	<0.01
Mean Velocity (m/s)	0	0	0	0.17	4.11	<0.01
Substrate (Wentworth)	0	1.2	0	3.2	2.53	<0.05
Instream Cover (%)	30	60	10	80	2.11	<0.01
Overhead Cover (%)	0	27	0	81	0.34	<0.05
Temperature (°C)	14	20	13	22	0.46	>0.10

Table 1.2- Parameter estimates and associated values for explanatory variables in logistic regression analysis for Zuni Bluehead Sucker presence during base flows in Black Soils Springs, Kinlichee Creek, and Scattered Willows Wash. Asterisk (*) indicates significant *P* value (<0.05). Odds ratio shows the odds of individual habitat variables at predicting fish presence.

Variable	**Parameter Estimate**	**SE**	***t*-ratio**	**Odds Ratio**	***P***
Intercept	0.217	0.065	3.340	1.240	0.0009
Depth (cm)	0.003	0.001	3.520	1.001	0.0045*
Velocity (m/s)	0.255	0.313	0.810	1.290	<0.0001*
Substrate (Wentworth)	-0.027	0.017	-1.560	1.027	0.1193
Temperature (°C)	0.008	0.003	2.610	1.001	0.9211
Instream Cover(%)	0.059	0.020	2.980	1.050	0.0001*
Overhead (%)	-0.001	0.007	-0.150	0.999	0.8813

Table 1.3- Generalized habitat suitability criteria for Navajo Nation Genetic Subunit Bluehead Sucker during base flows, including optimal (central 50%) and suitable ranges (central 95%) and *t*-values for chi square tests of habitat suitability criteria for habitat variables, combined from all streams. Wentworth Substrate Scale (0=silt,1= sand, 2=gravel, 3=pebble, 4=cobble, 5=boulder).

	Optimal Range		**Suitable Range**			
Habitat Parameter	Min.	Max.	Min.	Max.	$t_{optimal}$	*P*
Depth (cm)	21	45	14	75	4.76	<0.01
Mean Velocity (m/s)	0	0.13	0	0.21	4.53	<0.01
Substrate (Wentworth)	0	2.4	0	4.2	2.43	<0.05
Instream Cover (%)	40	60	10	70	3.74	<0.01
Overhead Cover (%)	24	83	0	97	0.67	<0.05
Temperature (°C)	12	19	10	21	0.95	>0.1

Table 1.4- Parameter estimates and associated values for explanatory variables in logistic regression analysis for Navajo Nation Genetic Subunit Bluehead Sucker presence in Crystal, Tsaile, and Whiskey Creeks. Asterisk (*) indicates significant *P* value (<0.05). Odds ratio shows the odds of individual habitat variables at predicting fish presence.

Variable	**Parameter Estimate**	**SE**	***t*-ratio**	**Odds Ratio**	***P*-value**
Intercept	-0.075	0.046	-1.630	0.927	0.1036
Depth(cm)	0.006	0.001	6.780	1.000	<.0001*
Velocity (m/s)	-0.111	0.051	-2.170	0.895	0.0300*
Substrate (Wentworth)	0.001	0.007	0.11	1.001	0.9108
Temperature (°C)	0.001	0.002	0.560	1.001	0.5780
Instream Cover(%)	0.068	0.012	5.870	1.070	<.0001*
Overhead (%)	0.001	0.000	5.050	1.001	<.0001*

Table 1.5- Generalized habitat suitability criteria for Zuni Bluehead Sucker during high flows, including optimal (central 50%) and suitable ranges (central 95%) and *t*-values for chi square tests of habitat suitability criteria for habitat variables, combined from all streams. Substrate Scale (0=silt,1= sand, 2=gravel, 3=pebble, 4=cobble, 5=boulder).

	Optimal Range		**Suitable Range**			
Habitat Parameter	Min.	Max.	Min.	Max.	$t_{optimal}$	*P*
Depth (cm)	43	60	34	68	3.23	<0.01
Mean Velocity (m/s)	0	0	0	0.24	5.83	<0.01
Substrate (Wentworth)	0	1.5	0	2.6	3.11	<0.05
Instream Cover (%)	40	70	20	80	4.12	<0.01
Overhead Cover (%)	0	21	0	76	1.56	<0.05
Temperature (°C)	6	12	4	14	0.72	>0.10

Table 1.6- Parameter estimates and associated values for explanatory variables in logistic regression analysis for Zuni Bluehead Sucker presence during high flows in Black Soils Springs, Kinlichee Creek, and Scattered Willows Wash. Asterisk (*) indicates significant *P* value (<0.05). Odds ratio shows the odds of individual habitat variables at predicting fish presence.

Variable	**Parameter Estimate**	**SE**	***t*-ratio**	**Odds Ratio**	***P***
Intercept	-0.009	0.044	-0.210	0.991	0.8346
Depth(cm)	0.001	0.001	2.750	1.001	0.0062*
Velocity (m/s)	-0.284	0.106	-2.690	0.752	0.0074*
Substrate (Wentworth)	-0.023	0.017	-1.310	0.977	0.1912
Temperature (°C)	0.005	0.004	1.390	1.005	0.1653
Instream Cover(%)	0.171	0.028	6.090	1.186	<0.0001*
Overhead (%)	0.000	0.001	0.000	1.000	0.9985

FIGURES

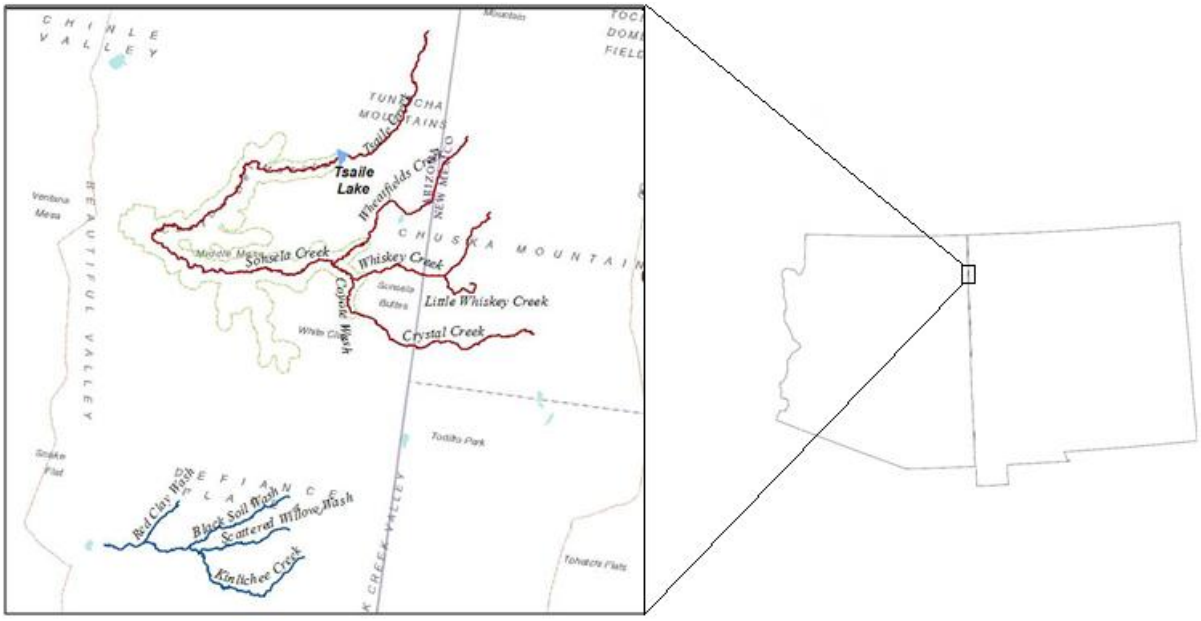


Figure 1.1— Streams that support Zuni Bluehead Sucker and Navajo Nation Genetic Subunit Sucker on the Navajo Nation. Zuni Bluehead Sucker inhabit Black Soils Wash, Scattered Willows Wash, and Kinlichee Creek. NNGS Bluehead Sucker inhabit Tsaile, Crystal, and Whiskey Creek.

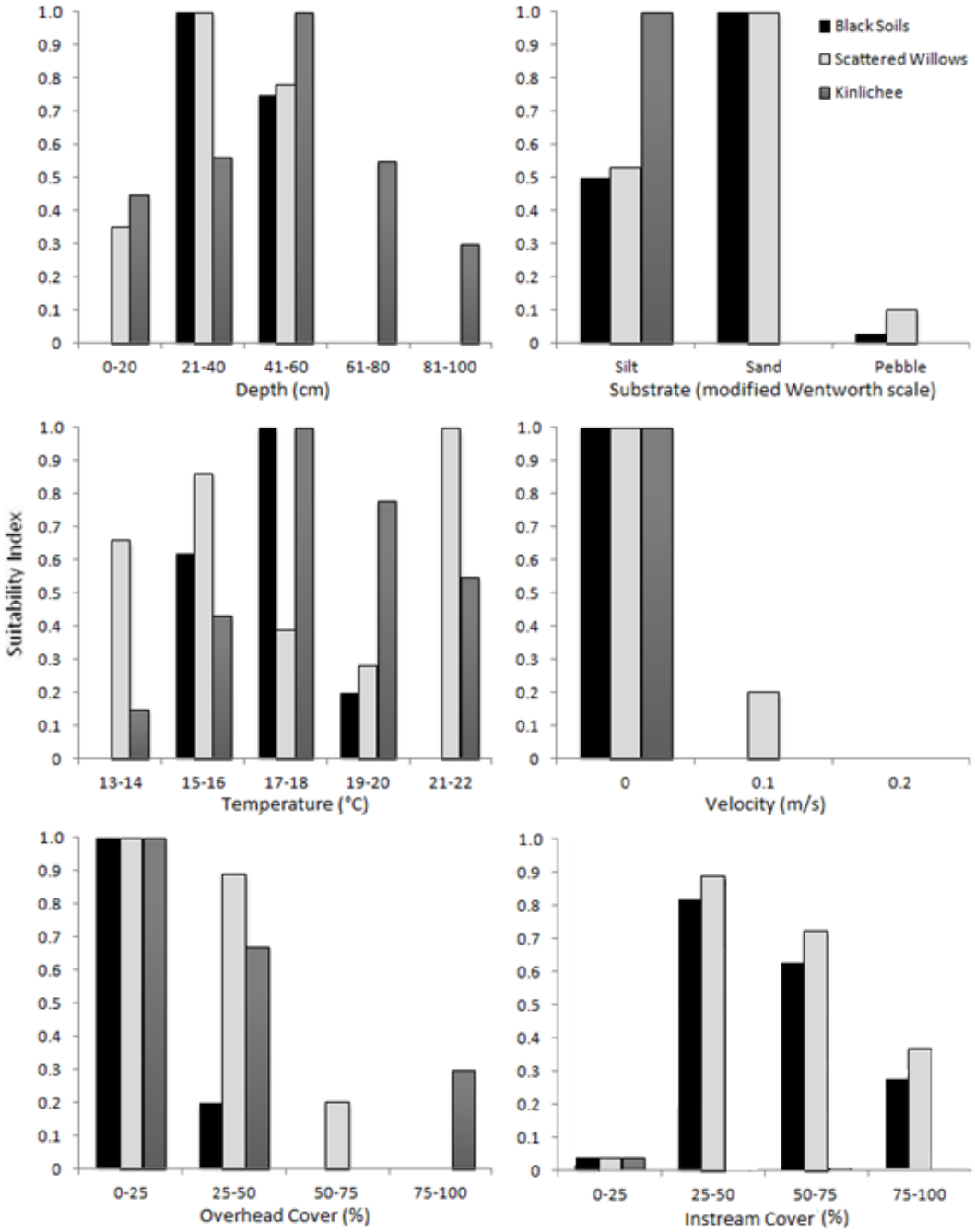
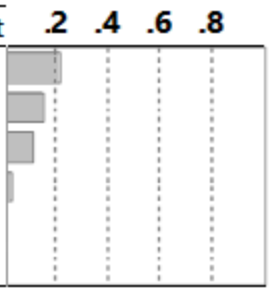


Figure 1.2—Stream-specific Zuni Bluehead Sucker habitat suitability indices for Black Soils Springs, Scattered Willows Wash and, Kinlichee Creek during base flows for depth (cm), substrate type, temperature (°C) at time of capture, water velocity (m/s), overhead cover (%) , and instream cover (%). Suitability Index approaching one indicates habitat most preferred.

NNGS Bluehead Sucker: Low Flows

Variable Importance Report

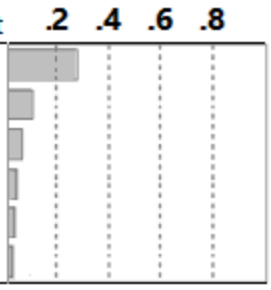
Variable	Main Effect
Depth (cm)	0.221
Instream Cover (%)	0.146
Overhead (%)	0.109
Velocity (m/s)	0.023
Temperature (°C)	0.002
Substrate	0.001



Zuni Bluehead Sucker: Low Flows

Variable Importance Report

Variable	Main Effect
Instream Cover (%)	0.263
Velocity (m/s)	0.120
Depth (cm)	0.078
Substrate	0.049
Overhead (%)	0.045
Temperature (°C)	0.032



Zuni Bluehead Sucker: High Flows

Variable Importance Report

Variable	Main Effect
Instream Cover (%)	0.321
Velocity (m/s)	0.074
Depth (cm)	0.067
Substrate	0.021
Temperature (°C)	0.016
Overhead (%)	0.001

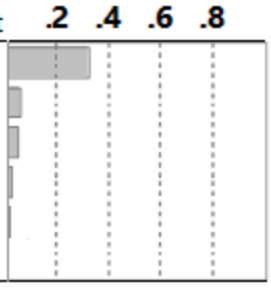


Figure 1.3- Independent variable importance reports for Navajo Nation Genetic Subunit (NNGS) Bluehead Sucker and Zuni Bluehead Suckers during low and high flows. Substrate units are on a modified Wentworth Scale (0=silt,1= sand, 2=gravel, 3=pebble, 4=cobble, 5=boulder). Main effect is the importance index that reflects the relative contribution of that factor alone to fish presence, not in combination with other factors.

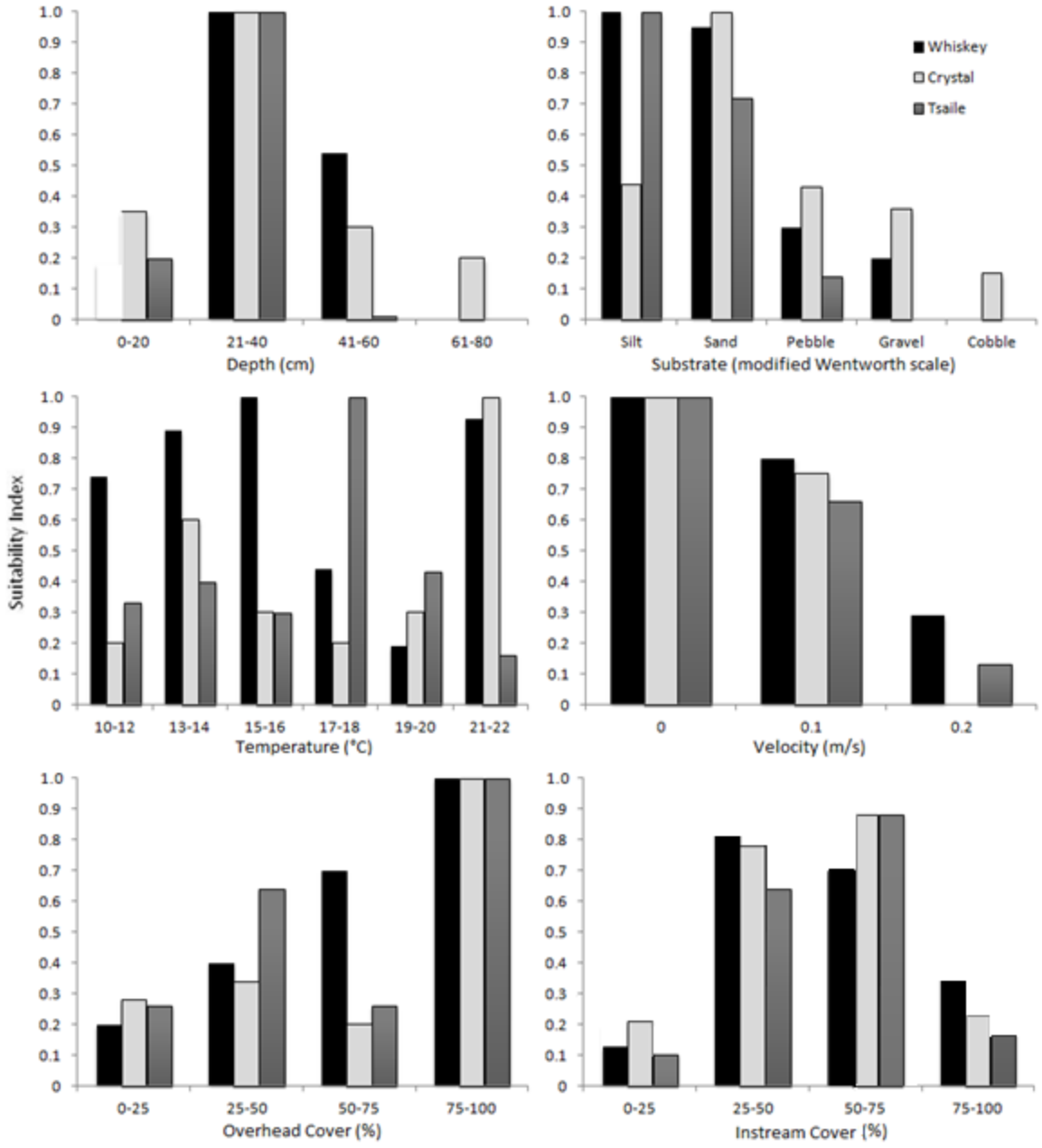


Figure 1.4—Stream-specific Navajo Nation Genetic Subunit Bluehead Sucker habitat suitability indices for Crystal Creek, Tsaile Creek and, Whiskey Creek during base flows for depth (cm), substrate type, temperature in Celsius at time of capture, water velocity (m/s), overhead cover (%) , and instream cover (%). Suitability Index approaching one indicates habitat most preferred.

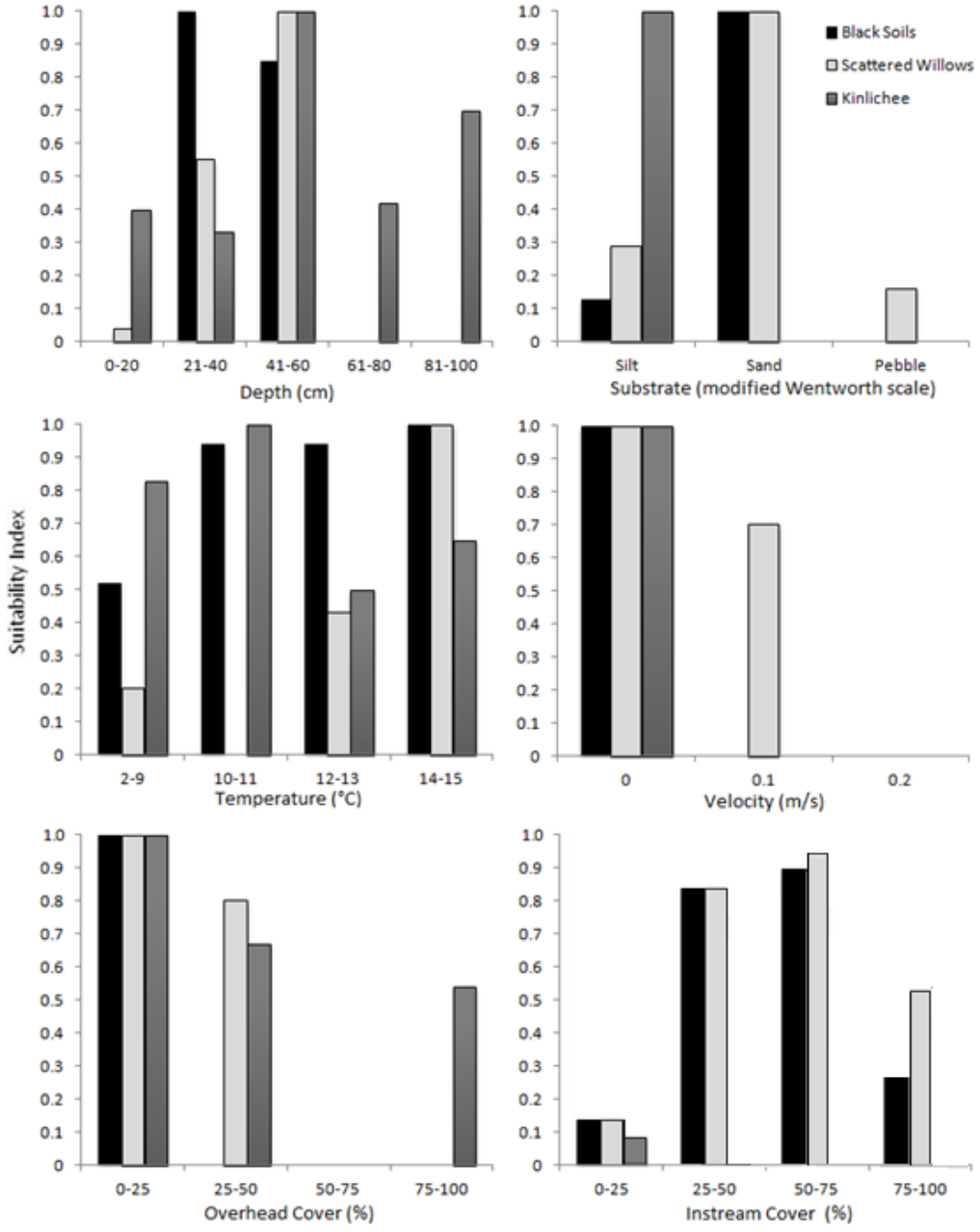


Figure 1.5— Stream-specific Zuni Bluehead Sucker habitat suitability indices for Black Soils Springs, Scattered Willows Wash, Kinlichee creek, and my generalized criteria during high flows for depth (cm), substrate type, temperature in Celsius at time of capture, water velocity (m/s), overhead cover (%) , and instream cover (%). Suitability Index approaching one indicates habitat most preferred.

CHAPTER 2. Comparing efficiency of AFS standard snorkeling techniques to eDNA sampling techniques in streams with Zuni Bluehead Sucker *Catostomus discobolus yarrowi* and Navajo Nation Genetic Subunit Bluehead Sucker *Catostomus discobolus*

INTRODUCTION

Advances in technology have recently enabled biologists to detect organisms in aquatic environments by testing water samples. This new method of species monitoring is referred to as environmental DNA (eDNA). Environmental DNA techniques are advantageous because they offer a non-invasive way to monitor or detect species in aquatic environments. Environmental DNA is nuclear or mitochondrial DNA from an organism that is released into the environment, including air, soil, and water; and comes from many sources including scales, mucous, feces, or gametes. Currently, most eDNA applications are used for detecting species in aquatic systems (e.g., Takahara et al. 2012; Thomsen et al. 2012); however, recent research suggests eDNA may show promise for monitoring biomass or relative abundance (Takahara et al. 2012).

Many uncertainties exist concerning the use of eDNA that need to be addressed before this tool can be broadly applicable. The persistence and rate of degradation of eDNA in various environments is not well known. Previous studies demonstrate that eDNA can persist in an aquatic environment between 7-21 d (Dejean et al. 2011). Temperature, water flow, and ultraviolet radiation (UV) are all known to affect persistence and degradation of eDNA, but exactly how these factors change the availability of eDNA remains unclear. High temperatures degrade eDNA faster because of increased microbial enzymatic activity (Zhu 2006). Ultraviolet radiation can also accelerate degradation of eDNA because radiation disrupts DNA base pair bonds (Pilloid 2013). Density of the target population can also affect the ability to detect eDNA in aquatic systems (Dejean 2011). Greater knowledge of how these factors and others affect

eDNA detectability, and their corresponding implications to sampling design, is vital to managers who wish to evaluate the use of eDNA methods to accurately assess population presence and abundance (Pilliod 2013).

Environmental DNA data must be collected, processed, and examined carefully in a sterile environment because the technique can produce false positive detections. Kwok (1989) describes the PCR process used to amplify DNA as extremely sensitive and that it is prone to resulting in false positive detections. Additionally, eDNA samples are very prone to contamination during the collection, processing, and analyzing of the water sample (Kwok 1989). Therefore, it is imperative that field samples are collected according to strict protocols to help minimize risk of contamination.

Environmental DNA surveys may save fisheries managers time, labor, and project funds. However, given the uncertainties associated with eDNA sampling, data provided by eDNA sampling should be compared to that provided by traditional fish sampling techniques. My study addressed the accuracy of eDNA methods to detect species presence in streams by comparing observational data, collected through snorkel surveys, to positive detections of eDNA. The two target species were Zuni Bluehead Sucker *Catostomus discobolus yarrowi*, and Navajo Nation Genetic Subunit Bluehead Sucker *Catostomus discobolus*. The Zuni Bluehead sucker is federally endangered. The Navajo Nation Genetic Subunit Bluehead Sucker (NNGS Bluehead Sucker) is a new taxon that is considered genetically distinct from Zuni Bluehead Suckers (Federal Register 2014). My objectives were to (1) evaluate if eDNA can be used to detect two rare sucker species, Zuni Bluehead Sucker and NNGS Bluehead Sucker, in small lotic systems; and (2) if successful, compare the efficacy of eDNA methods to AFS standard snorkeling methods (Bonar et al. 2009) in detecting species presence.

METHODS

Study Sites

My study area included three streams on the Navajo Nation in northeastern Arizona and northwestern New Mexico: Crystal Creek, Tsaile Creek, and Black Soils Wash (Figure 2.1). Crystal and Tsaile creeks are located in the Chuska Mountains and contain NNGS Bluehead Sucker. Black Soils Wash is located on the Defiance Plateau and contains Zuni Bluehead Sucker. Streams were selected through consultation with U.S. Fish and Wildlife Service (USFWS), Navajo Nation Fish and Wildlife, U.S. Geological Survey (USGS), and University of Arizona (UA) biologists who based selection of streams on known presence from prior surveys of either Zuni Bluehead Sucker or NNGS Bluehead Sucker. The three streams selected were small and mountaine with widths rarely exceeding 2 to 3 m. Streams were sampled between May 27, and July 16, 2014, during summer base flow conditions. The selected streams included one of ten streams known to contain Zuni Bluehead Suckers, and two of six streams known to contain NNGS Bluehead Sucker. For the Zuni Bluehead Sucker streams Kinlichee Creek also contained Fathead Minnow *Pimephales promelas*. For the NNGS Bluehead Sucker streams all three contained Speckled Dace *Rhinichthys osculus* along with Whiskey Creek having Brown Trout *Salmo Trutta*, Crystal Creek having Fathead Minnnow, and Tsaile Creek having Rainbow Trout, *Oncorhynchus mykiss.*

Environmental DNA Sampling

I divided the entire wetted length of each stream into 100-m consecutive reaches. I systematically selected 10 reaches from Tsaile and Crystal creeks. The wetted length of Black Soils Wash during base flows was 400 m; therefore, the entire stream was sampled. To sample eDNA, 50 ml water samples were collected every 10 m within the 100-m reach, starting at the

downstream end and continuing upstream. I subsampled each water sample taking 15 ml from each 50-ml sample. I took 111 water samples from Crystal Creek totaling 1.66 L; 111 water samples from Tsaile Creek totaling 1.66 L; and 41 water samples from Black Soils Wash totaling 0.61 L.

My water collection methods closely followed protocol of the USFWS (2013). Sanitation and strict quarantine are essential when collecting eDNA samples. Therefore, when collecting water samples, the same person, who was fully versed in collection procedures, took samples for the entirety of the stream to avoid bias or contamination. Prior to handling any water sampling materials, the individual put on powderless latex gloves to help avoid contamination of the sample. I collected water samples by submerging 50 mL conical plastic vials in the center of the stream on an extender claw. Fifty milliliters of water were collected in each vial and were promptly capped and labeled. During field sampling, water samples were kept on ice to preserve DNA collected. At the end of the field day, all water samples were processed at a sterile laboratory station. Processing included taking a 15 ml subsample from each vial and adding 1.5 mL of 3 M sodium acetate, and 33 mL of 200 proof ethanol to each vial to further preserve samples, which were then frozen. I also sent a control sample (containing deionized water) to help identify any contaminants. Within 1 to 3 d following collection, water samples were sent on ice via overnight courier to the USGS Upper Midwest Environmental Sciences Center in Lacrosse, Wisconsin (UMESC) for eDNA processing.

Snorkeling Methods

Snorkeling can be used to observe fish in streams without causing disturbance (Bovee 1986). Therefore, the snorkeler moved 5 to 8 m behind the eDNA sampler (roughly 5 to 10 min) allowing any frightened fish to return to their original location before the snorkeler sampled. The

same snorkeler sampled all streams and counted fish in each 10-m section above where the water sample was collected. The snorkeler moved upstream slowly, examining the entire stream width. The snorkeler recorded number of suckers ≥50 mm total length (TL) at each site.

Habitat Variables

Habitat conditions were measured every 10 m within the 100-m reach after the eDNA and snorkel surveys were conducted. Habitat conditions measured included water velocity, substrate size, over-head cover, water depth, instream cover, and mesohabitat type (i.e., pool, run riffle). A meter stick was used to measure water depth (cm); a Marsh-McBurney flow meter (Global Water Flow Probe, Gold River, California) was used to measure water velocity (m/s); a spherical densiometer to was used to measure overhead cover (% shaded; Lemon 1956); visual observation was used to quantify proportion of instream cover (% cover of aquatic macrophytes, boulders, undercut banks, and debris under which fish could hide); an ISO calibrated liquid-in-glass thermometer was used to measure water temperature (°C); and a measuring tape was used to estimate stream width (cm); and snorkeling observations were used to measure fish density between each 10-m reach. Pools were defined as moderate to deep water areas with low velocities, which showed no turbulence at the surface. Runs were defined as areas with moderately deep water with moderate velocities, which were somewhat turbulent at the surface. Riffles were defined as shallow water areas with fast velocities, which were turbulent at the surface (Armstrong and Parker 2000).

DNA Extraction from the Water Samples

The following procedures on DNA extraction were written by the UMESC (John Amberg, UMESC, Personal Communication): “Water samples (15 mL water + 33.5 mL pure ethanol + 1.5 mL sodium acetate) in 50 mL tubes were centrifuged at 5000 x *g* for 30 min upon

arrival at UMESC. Supernatants were discarded and the samples were inverted for 10 min. Sample pellets were lysed by pipetting in 250 µl of GSB buffer from the gMAX Mini Genomic DNA Kit (IBI Scientific, Peosta, IA). The lysates were then transferred to 1.5 mL microcentrifuge tubes and frozen at -80°C until further processing. DNA was later extracted according to the kit manufacturer's instructions. The final elution volume was 100 µL."

qPCR Amplification and eDNA Quantification

The following procedures on qPCR amplification and eDNA quantification were written by the UMESC (John Amberg, UMESC, Personal Communication): "Primers (forward- GTTGCCACTACTGCCTTGGT; reverse- CAGTTGAGTGGATCGGGTTC) and a probe (sequence-6FAM- AGTGACTAATTCTGCAAGAACTAGCTAAACAG -TAMRA) capable of detecting both Zuni Bluehead Sucker (GenBank: JX488852.1) and Bluehead Sucker (GenBank: KJ441308.1) were designed within the ND2 gene of the mitochondrial genome. This marker set was originally designed to be specific to Zuni Bluehead Sucker, but validation experiments revealed complete cross-reactivity with Bluehead Sucker. Further specificity testing was performed with DNA from the following off-target species, and no cross-reaction was observed under the qPCR cycling conditions: Speckled Dace *Rhinichthys osculus*, Rainbow Trout *Oncorhynchus mykiss*, Brown Trout *Salmo trutta*, Fathead Minnow *Pimephales promelas*, Bluegill *Lepomis macrochirus*, Largemouth Bass *Micropterus salmoides*, Rio Grande Sucker *Catostomus plebeius*, Sonora Sucker *C. insignis*, and Flannelmouth Sucker *C. latipinnis*. Off-target species samples were obtained from Museum of Southwestern Biology, Albuquerque, New Mexico. Qualitative PCR cycling conditions were: 95°C for 2 min followed by 55 cycles of 95°C for 15 s, 66°C for 15 s, 72°C for 20 s, and a final hold at 4°C. Reactions had a final volume of 20 µl and contained 1 µL of template DNA, 500 nM forward and reverse primers, 250

nM probe, and 10 µL of 2x SensiFAST Probe No-ROX Mix (Bioline USA Inc., Taunton, MA). Each water sample was analyzed in quadruplicate. Each plate had additional no template controls. I used a 7-point calibration curve with synthetic DNA standards of 31,250, 6,250, 1,250, 250, 50, 10, and 0 copies per reaction. The synthetic DNA standard was the 246 base pair (bp) segment of the Zuni Bluehead Sucker ND2 gene (GenBank: JX488852.1) bound by the primers described above. Efficiency for qPCR was 91% in standard curve validation experiments. Amplification was detected by probe fluorescence at 520 nm, and DNA counts were calculated by Mastercycler ep Realplex software (version 2.2, Eppendorf North American, Hauppauge, NY).

Data Analysis

A two sample paired *t*-test was run comparing number of positive snorkeling site detections to the number of eDNA positive site detections in all streams. Confidence intervals were established at 95%. The null hypothesis was there would be no difference between detection of Zuni Bluehead Sucker or NNGS Bluehead Sucker when comparing snorkeling and eDNA. If the null hypothesis was rejected, a one tailed *t*-test was preformed to check directionality of the difference.

Next, to analyze the influence of habitat variables on eDNA detections, I used a multiple logistic regression to assess the effect of habitat variables on sites with positive eDNA detections. Multiple logistic regression was used to relate positive eDNA detections to the relative importance of habitat variables, and was conducted for both taxon during base flows (Ahmadi-Nedushan et. al. 2006). Each stream was run independently, and all habitat variables were continuous with the exception of mesohabitat. All multiple logistic regressions were run with JMP®, Version 12 (JMP 12®, SAS Institute Inc., Cary, North Carolina)

RESULTS

eDNA Detection

I detected the taxa in Black Soils Wash and Crystal Creek with eDNA sampling methods, but did not detect any sucker species in Tsaile Creek (Table 2.1; Figure 2.2). In Black Soils Wash, 3 water samples out of 41 total water samples (0.615) L detected suckers. As sample collection progressed upstream in Black Soils Wash, suckers were not detected until I had sampled 0.045 L of water. In Crystal Creek, 12 water samples out of 111 total water samples (1.665 L) detected suckers. As sample collection progressed upstream in Crystal Creek, suckers were not detected until I had sampled 0.855 L of water. In Tsaile Creek, 0 water samples out of 111 total water samples (1.665 L) detected suckers.

Snorkeling Detections

I detected 288 Zuni Bluehead Suckers in Black Soils Wash, 415 NNGS Bluehead Suckers in Crystal Creek, and 47 NNGS Bluehead Suckers in Tsaile Creek with snorkel surveys (Table 2.1; Figure 2.2). The catch per unit effort (CPUE) for Black Soils Wash (20 fish/100 m^2) and Crystal Creek (18 fish/100 m^2) were similar (Table 2.1), though total stream length differed (400 m compared to 1000 m, respectively). The lowest number of fish were observed in Tsaile Creek, which had the correspondingly lowest CPUE (3 fish/100 m^2) of the three streams (Table 2.1).

eDNA and Snorkeling Comparison

Snorkeling observations were more effective in detecting suckers than eDNA sampling techniques. I rejected the null hypothesis for my two sample paired *t*-test because there was a significant difference between the number of detections during snorkel surveys and eDNA surveys. The results of the one tailed *t*-test showed that snorkeling detected fish at a higher number of sites than eDNA in all streams ($t = 4.86$, df = 2, $P = 0.01$) (Table 2.2).

Relation between eDNA detections and habitat variables

The results were a moderate fit (R^2=0.446). Significant habitat variables in the model according to *P*-values for the taxa were: fish density (m^2) and depth (cm) (Table 2.3).

DISCUSSION

AFS standard snorkeling techniques outperformed eDNA sampling for detecting Zuni Bluehead Sucker and NNGS Bluehead Sucker. Furthermore, data obtained from snorkeling was available immediately compared to eDNA methods where I had to wait for shipping and processing of samples. Snorkeling also allowed biologists to distinguish between living and nonliving animals, as well as different life stages (Rees 2014). Although the number of positive eDNA detections seemed roughly related to the density of suckers, there was not a statistically significant relationship. Furthermore, eDNA did not detect suckers in Tsaile Creek despite observing them while snorkeling. (Thomsen et al. (2011) similarly found that eDNA methods sometimes do not detect species that are known to be present.

Though my findings show that snorkeling outperformed eDNA sampling methods, there have been many other studies that used eDNA methods to successfully detect a species. Dejean et al. (2012) used visual surveys on ponds to detect Bullfrogs *Lithobates catesbeianus* and, like my study, only took 15 mL water samples. His eDNA results showed positive detection in all areas where bullfrogs were seen. Takahara et al. (2013) also used eDNA methods to successfully detect a bluegill *Lepomis macrochirus* in ponds in which it had been previously observed.

Fish Density Thresholds for Positive eDNA Detection

Results suggest that fish density relates to positive eDNA detections (Figure 2.3) (Table 2.3) When fish populations exist at low densities, the abundance of DNA sources is low and can hinder eDNA detectability. The rate of detection can be affected by the species being targeted,

the density of the population, and the type of water body being sampled (Rees et al. 2014). Pilloid et al. (2013) found a positive relationship between the densities of two amphibian species and positive eDNA detections. Thus, the rarity of the target species likely contributed to the low number of positive eDNA detections, suggesting that a certain number of fish may need to be present in system (relative to the volume of the stream) to accurately produce positive detections.

Volumes of Water Samples Relating to eDNA Detections

Because eDNA techniques are a new methodology, much remains to be learned about the optimal volume of water to take for samples. A standardized method to compute the volume of water necessary to detect eDNA does not currently exist. I sampled a total of 1.66 L per stream in Crystal and Tsaile Creeks and 0.61 L in Black Soils Wash. Many studies have taken individual 15 mL water samples as I did (Ficetola et al. 2008; Dejean et al. 2012; Foote et al. 2012; Collins et al. 2013). However, other studies have taken total volume samples ranging from 1 to 20 L (Goldberg et al. 2013; Pilloid et al. 2013; Rees et al. 2014; Jane et al. 2015). Many studies on ponds and lakes take three water samples at each sampling point to help increase chances for detection, as opposed to the one sample that I took at each point (Dejean et al. 2012; Takahara et al. 2012; Thomsen et al. 2015; Goldberg et al. 2013). The numbers of samples required for rivers, streams, lakes, lagoons, and seawater will be dependent on the size of the environment under study and are difficult to specify (Reese 2014). Aquatic systems vary, as do the conditions (e.g., remote field sites) and limitations (e.g., labor) for each study. Thus the volume of water samples needs to be appropriately adjusted. Previous research has shown that eDNA detection rates are lower in lotic than lentic systems because flows may flush eDNA out of a system (Rees et al. 2014). Therefore, greater volumes of water are usually taken in lentic systems to account for any loss of eDNA during its time in the flowing system (Goldberg et al. 2011; Jerde et al.

2011; Pilliod et al. 2013). However, in my study, 15 ml water samples were thought to be of adequate size due to smallness of stream, low water velocities during time of sampling, and low turbidity to interfere with eDNA (USGS UMESC Personal Communication). My small sample volumes were also necessary due to remoteness of field locations and limitations of adequate containers (e.g., ice chests) to store and preserve water samples on ice in the field. The smaller volumes of water samples may have decreased the number of detections in my study. Collecting larger volumes of water in the future and perhaps filtering in the field rather than just collecting water may increase detection rates.

Future Research

Future research should concentrate on quantifying the persistence of eDNA in an environment and analyze how eDNA is transported in lotic environments. Temperature, UV radiation, pH, microbial community, and flow rate all influence how quickly eDNA degrades and the probability of detection (Goldberg 2011; Taberlet 2012; Wilson 2014; Wilcox 2015). Understanding eDNA persistence in different environments is crucial to furthering this new technique (Barnes et al. 2014; Rees et al. 2014). Estimating the relationship between water collected and eDNA detection, and identifying an appropriate amount of water to be collected from different bodies of water is an important area for new research. Furthermore, knowledge of the needed sample size of water samples, and their spatial placement in different water bodies is also required.

Environmental DNA sampling methods require meticulous procedures and a sterile environment to prevent contamination (Yu et al. 2012). If procedures are not strictly followed, then samples may be exposed to contamination that could result in false positives (Darling and Mahon 2011; Bohmann et al. 2014; Rees et al. 2014). I used every known precaution to avoid

contamination, and through the use of control samples, I was able to conclude that I avoided contamination. Anyone working in remote settings should be cognizant of the environment in which they are collecting and processing samples. Future research on the procedures to reduce contamination and eliminate false positives in samples collected in exceptionally isolated areas is also important.

Conclusion

In conclusion, I found that eDNA methods could detect both Zuni Bluehead Sucker and NNGS Bluehead Suckers, yet these methods were not as effective as snorkeling in the detection surveys I employed. Lack of detections may have resulted from low densities of our target species, or inadequate amounts of water sampled for test, so research to identify an appropriate amount of water to be collected is warranted. I believe eDNA should not replace traditional forms of surveys for detection until the reliability of technique is further developed. The amount of fish and eDNA being shed into the water was likely sparse, resulting in few positive species detections of eDNA. Furthermore, I would not have detected any suckers in one of the streams, had I not used snorkel surveys as well. Environmental DNA sampling cannot be substituted for field observation by an experienced ecologist (Thomsen et al. 2011). Managers should continue to use traditional sampling techniques, especially if study sites are remote and methods of keeping samples viable are limited. Detection rates will vary, depending on target species, density of populations, and water body type (Rees et al 2014). Environmental DNA will need to be further refined, biases understood, and standardized (e.g., Bonar et al. 2009) to provide a widely operational sampling tool similar to electrofishing, netting, and hydroacoustics.

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TABLES

Table 2.1- Comparison of established snorkeling and eDNA fish detection methods. eDNA sample size is number of water samples taken per stream used in analysis. Black Soils contained Zuni Bluehead Suckers, Crystal and Tsaile creeks contain Navajo Nation Genetic Subunit Bluehead Sucker.

Stream	Total Stream length Sampled (m)	Number of fish observed snorkeling	Mean Snorkeling CPUE (fish/100 m)	CPUE Standard Deviation	eDNA Sample Size (*N*)	Positive eDNA Detection	Mean Positive eDNA Detection (100 m stream length)	Positive eDNA Detection Standard Deviation
Tsaile	1000	47	3	1.85	111	0	0.00	0.0
Black Soils	400	288	20	16.79	41	3	0.75	1.1
Crystal	1000	415	18	19.33	111	12	1.20	4.3

Table 2.2 A two sample paired *t*-test comparing number of positive snorkeling site detections to the number of eDNA positive site detections. Confidence intervals were established at 95%. Prob > |t| is from two tailed *t*-test. Prob < *t* is from the one tailed t-test showing that snorkeling detected fish at a higher number of sites than eDNA in all streams

	Snorkeling positive site detections	eDNA positive site detections	*t*	df	Prob > I*t*I	Prob < *t*
Crystal	29	12				
Black Soils	11	3	4.86	2	0.03	0.01
Tsaile	13	0				

Table. 2.3- Parameter estimates and associated values for variables in logistic regression analysis comparing habitat variables to positive eDNA detections for all three streams. The results were a moderate fit ($R^2 = 0.446$). Asterisk (*) indicates significant *P* value (<0.05)

Variable	Parameter Estimate	SE	*t*	*p*
Intercept	0.130	0.043	3.040	0.003*
Temperature (C°)	-0.004	0.002	-1.800	0.091
Depth (cm)	-0.002	0.001	-2.860	0.004*
Velocity (m/s)	-0.074	0.039	-1.870	0.092
Substrate (Wentworth)	0.014	0.007	1.980	0.249
Instream (%)	-0.019	0.021	-0.920	0.357
Overhead (%)	0.000	0.000	-0.690	0.490
Fish Density (10 m^2)	0.005	0.002	2.950	0.003*

FIGURES

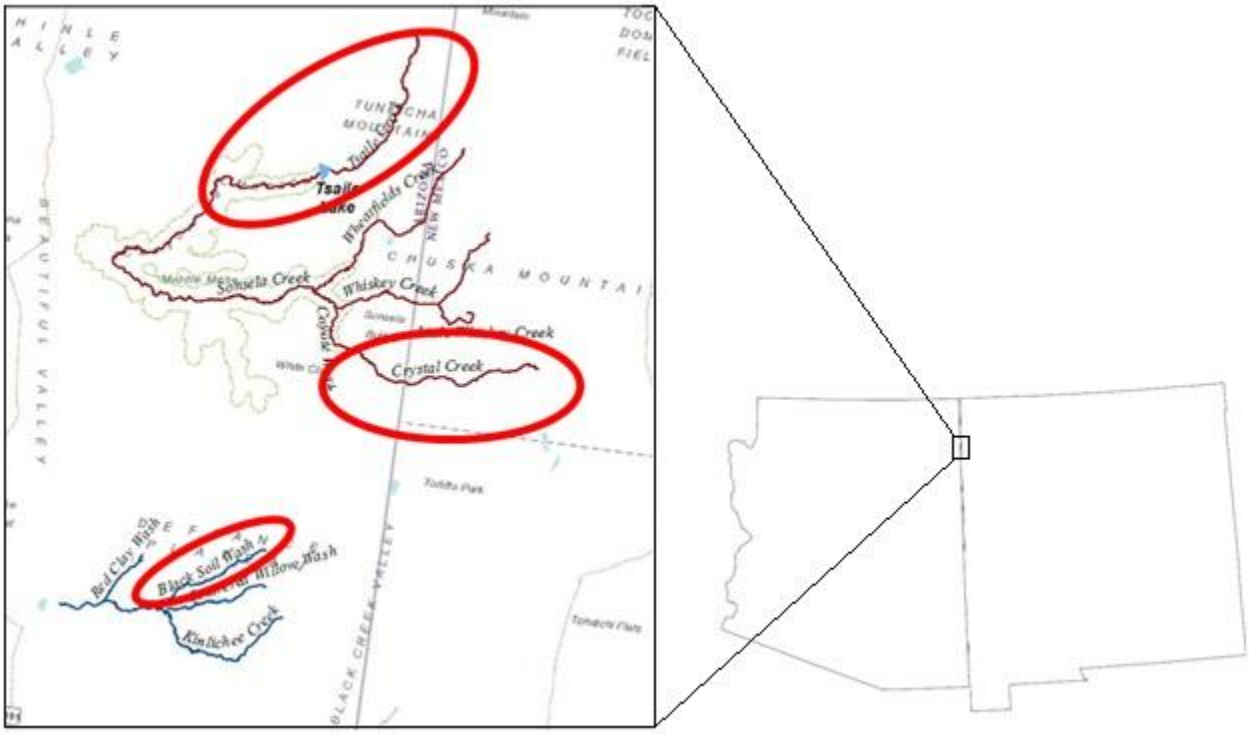

Figure 2.1- Black Soils Wash supports Zuni Bluehead Sucker; Crystal and Tsaile Creeks support Navajo Nation Genetic Subunit Bluehead Sucker on the Navajo Nation.

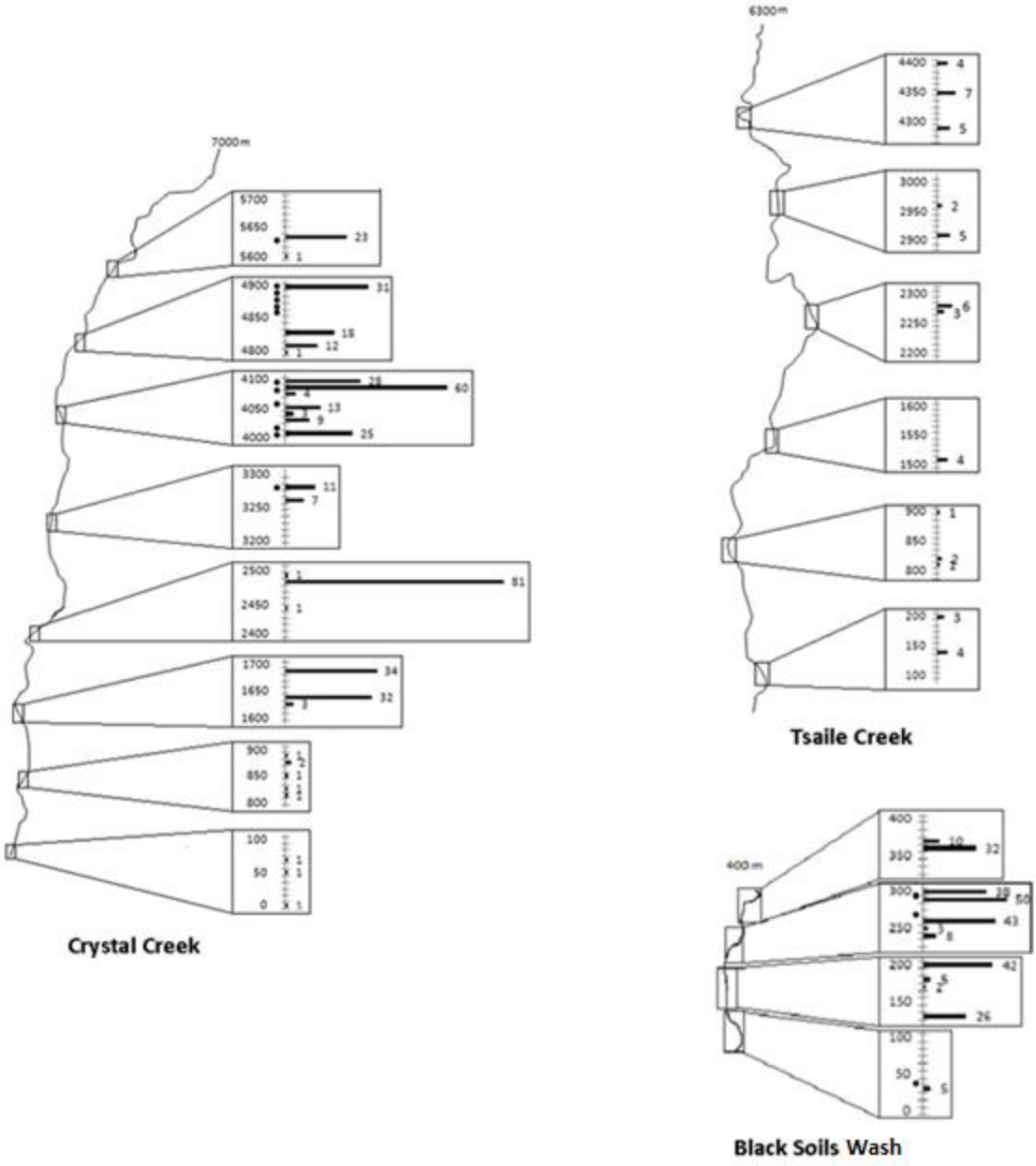

Figure 2.2- Snorkel survey observations compared to eDNA positive detections on Crystal, Tsaile, and Black Soils. Black circles to the left of the Y axis indicate positive eDNA detection in each 10-m segment, numbers to the right of the y axis are number of fish observed during snorkel surveys in each 10-m segment.

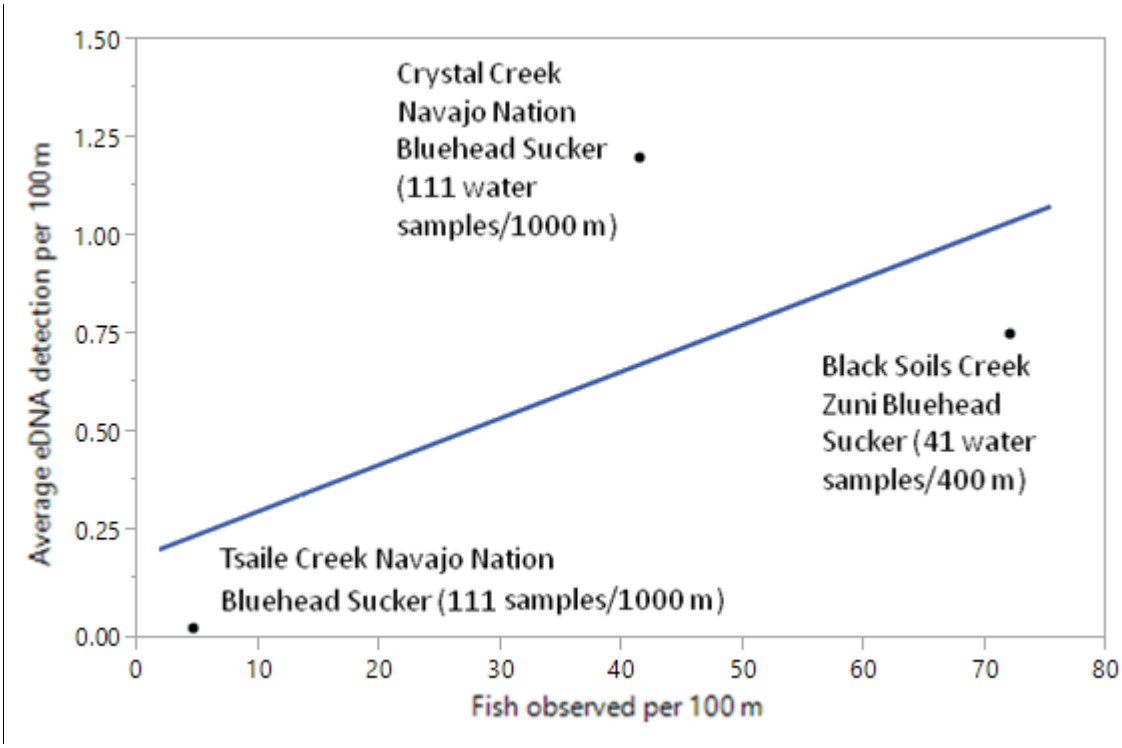

Figure 2.3- Fish observed per 100-m section during snorkel surveys compared to the average number of positive eDNA detections per 100-m section.