Habitat Characteristics and Resource Availability in Secondary Channel and Mainstem Backwaters of the San Juan River: Implications for Environmental Flows Management and Imperiled Fish Conservation

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Principal Investigator
James E. Whitney, PhD, Department of Biology, Pittsburg State University, Pittsburg, KS 66762

Background

Backwaters are the preferred habitat of early life stage (i.e., larvae and juvenile) Razorback Suckers (*Xyrauchen texanus*) (Minckley et al. 1991; Mueller 2006). For example, in the San Juan River larvae were captured at higher densities in backwaters when compared to other mainstem habitats such as low velocity runs (Farrington et al. 2016). Furthermore, the only juvenile Razorback Sucker captured from the San Juan River in the last 18 years during small-bodied fish monitoring was from a large backwater in fall 2015 (Zeigler and Ruhl 2016). Backwaters are important habitat for early life stages of other imperiled fishes as well. Of the juvenile Colorado Pikeminnow (*Ptychocheilus lucius*) captured in 2016, approximately 48% were captured in large backwaters compared to 17% captured in the main channel and 35% in secondary channels (Zeigler and Ruhl 2016). The importance of backwater habitat to early life stages of imperiled fishes has made the restoration of this habitat an important management goal throughout the Colorado River Basin (CRB) (USFWS 2002a; USFWS 2002b). As in other parts of the CRB, backwater formation in the San Juan River has been reduced by flow regulation and concomitant geomorphic changes in the river channel (Holden 1999).

Razorback Sucker can spawn successfully in the San Juan River, as larvae are present in the river and its backwaters during April through June (Farrington et al. 2016). However, these young-of-year (YOY) fishes (i.e., both larvae and juvenile age-0 fishes) are largely absent from the river by August, suggesting some environmental factor or factors are limiting recruitment to the juvenile stage of the population (i.e., a recruitment bottleneck) (Farrington et al. 2016). Several hypotheses may explain this impediment to recovery, including a lack of backwater nursery habitat for development, inadequate physicochemical conditions in backwaters, starvation, and/or predation in backwaters. Identifying the environmental factor(s) responsible for the recruitment bottleneck of Razorback Suckers has important management implications, because once the source of a roadblock is identified, actions can be taken to alleviate that problem.

Although backwaters are important habitat for the early life stages of imperiled fish, there is a high degree of variability among backwaters in terms of habitat characteristics. For instance, Bliesner and Lamarra (2000) demonstrated that geomorphological characteristics and resource availability varied in backwaters along the longitudinal gradient (i.e., upstream to downstream) of the San Juan River. Backwaters positioned more upstream tended to have the highest resource availability, although their upstream location resulted in higher gradient and water velocity in the adjacent mainstem, which presumably prevented larvae from settling in these backwaters (Bliesner and Lamarra 2000). Although Bliesner and Lamarra (2000) examined longitudinal and temporal changes in backwaters generally, they did not investigate differences among different types of backwaters. Long-term habitat mapping in the San Juan River has identified three types of backwaters, including: 1) backwaters associated with secondary channels, 2) island backwaters, and 3) point bar backwaters (Fig. 1; Lamarra et al. In prep). Secondary channels are narrower and receive less flow than the main channel, and when they stop flowing, form secondary channel backwaters that are connected to the main channel at their downstream end, but are disconnected at their upstream end (Fig. 1A; Landers et al. 2002; Yager et al. 2013). Island backwaters are zero-velocity habitats that form in off-channel habitats between an island and the river bank, or at the downstream end of an island (Fig. 1B). Point bars are formed by alluvial deposits on the inside bend of a river (Legleiter et al. 2011). When zero-velocity habitats form on the downstream end of a point bar, a backwater is formed (Fig. 1C). Island and point bar backwaters exhibit high connectivity with the main river channel, whereas secondary channel backwaters are less connected to the main channel (Lamarra et al. In prep). Furthermore, backwaters associated with secondary channels increase in size with increasing base flows, but total backwater area does not change since there is an associated reduction in island and point bar backwaters (from hereon, main channel backwaters) as base flow increases (Lamarra et al. In prep). Thus, changes in base flow result in variation in the size, stability, and type of backwater, meaning the amount and type of backwater habitat in the San Juan River is dependent on flow regulation (Lamarra et al. In prep).

It is currently unclear whether early life stages of imperiled fish exhibit a preference for secondary channel over mainstem backwaters. For instance, age-0 fish may prefer secondary channel backwaters because they function as refugia from flow fluctuations because of their lower connectivity to the mainstem. As such, secondary channel backwaters are less prone to scouring events during the monsoon season in July through September (Adams and Comrie 1997), a time period when nearly all age-0 Razorback Sucker disappear from the river. High-velocity monsoonal flows in main channel backwaters could displace imperiled YOY fish to the main channel where they would experience higher mortality (Robinson et al. 1998; Valdez et al. 2001; Gido and Propst 2012). Further, mainstem backwaters that experience a greater frequency of silt-laden monsoonal flows may have higher turbidity, total suspended solids, and siltation compared to secondary channel backwaters (Bliesner and Lamarra 2000; Heins et al. 2004). High turbidity may hinder YOY feeding because of poorer water clarity (De Robertis et al. 2003; Manning et al. 2014), plus high suspended sediment loads can clog and damage gills, thus interfering with respiration (Sutherland and Meyer 2007; Clark Barkalow and Bonar 2015). In fact, larval Razorback Sucker exhibit a preference for clear water over turbid water (Johnson and Hines 1999). However, nonnative predators feed more effectively on age-0 Razorback Sucker in clear water compared to turbid water, thus, higher turbidity may also benefit YOY fish by reducing predation (Johnson and Hines 1999).

More-frequent and higher magnitude monsoonal flows in mainstem backwaters compared to secondary channel backwaters may cause lower resource availability, which could influence the recruitment success of imperiled fishes (Papoulias and Minckley 1990; Bestgen 1996). For instance, monsoonal floods could scour the substrate and water column of benthic and pelagic invertebrates, respectively, more often in main channel compared to secondary channel backwaters (Speas 2000). Benthic (e.g., chironomids) and pelagic (e.g., cladocerans and copepods) macroinvertebrates are the most frequently occurring prey items in the diets of YOY Razorback Sucker (C. Pennock unpublished data), leaving them potentially more prone to starvation in main channel backwaters. Furthermore, differences among backwaters in water clarity and substrate size may limit basal productivity in main channel versus secondary channel backwaters. More stable secondary channel backwaters would exhibit lower turbidity that allows for greater light penetration that could stimulate photosynthesis of phytoplankton and periphyton, and may also have greater substrate size (e.g., pebble and cobble rather than silt and sand) that provides a stable attachment surface for periphyton (Burkholder 1996; Hillebrand 2002). In turn, greater algal productivity in secondary channel backwaters may exert bottom-up control on the availability of benthic and pelagic macroinvertebrates, as algal productivity is a primary factor influencing invertebrate secondary production (Wotton 1988; Whitney et al. 2014).

Secondary channel backwaters are located nearer the riparian zone and channel margin of the San Juan River, and as such they may receive greater shading from canopy cover and canyon walls. More shading may result in more benign physicochemical conditions in secondary channel backwaters compared to main channel backwaters that receive more direct sunlight. For instance, direct solar radiation in main channel backwaters could cause water temperatures to exceed the upper thermal limit of imperiled age-0 fishes, resulting in hyperthermia, heat stress, and heat-induced mortality (Sweeney 1993; Smale and Rabeni 1995; Poole and Berman 2001; Kappenman et al. 2010; Deslauriers et al. 2016). Further, higher water temperatures may result in hypoxia and anoxia that could suffocate fish (Carlson and Siefert 1974; Fontenot et al. 2001), since the solubility of oxygen decreases as water temperatures increase. Both hyperthermia and hypoxia could results in greater YOY mortality in main channel compared to secondary channel backwaters.

Water temperature may interact with basal resource availability to influence YOY recruitment success. Warmer water temperatures result in greater fish metabolic rates (Fry 1947), meaning fish need more food at warmer temperatures relative to cooler temperatures (Houde 1989). Fish can also attain higher growth rates at warmer temperatures (Houde 1989), but only if enough food is available to sustain their growth (Bestgen 2008). As such, fish in warm water with high rates of resource productivity may exhibit extremely high growth rates, but fishes in warm water with lower productivity may be more prone to starvation (Houde 1989). Investigating resource availability and its relationship with backwater type may inform conservation. For instance, if it is found that low resource availability from frequent monsoonal flooding coupled with high temperatures from direct sunlight occur in main channel backwaters, this unsuitable age-0 habitat could be reduced by increasing base flows (Lamarra et al. In prep). Higher base flows creates more secondary channel backwaters (Lamarra et al. In prep), which may have greater resource availability because of infrequent flooding and cooler temperatures from riparian and canopy shading.

Predation pressure is a final explanation for the variation in recruitment and abundance of YOY Razorback Suckers among backwaters. For instance, several small-bodied nonnative fishes occur in backwater habitat and can prey heavily on native age-0 fishes, including Red Shiner (*Cyprinella lutrensis*), Fathead Minnow (*Pimephales promelas*), and Western Mosquitofish (*Gambusia affinis*) (Tyus and Haines 1991; Ruppert et al. 1993; Brandenburg and Gido 1999; Tyus and Saunders 2000). In fact, Bestgen et al. (2006) concluded that predation by Red Shiners interacting with environmental factors limited the recruitment success of Colorado Pikeminnow in the Green River. These small-bodied nonnative predators are abundant in secondary channel backwaters in the San Juan River, although their densities decreased in between 1999 and 2012 (Franssen et al. 2015).

A lack of large, stable, zero-velocity nursery habitat without large-bodied nonnative predators is one explanation for the recruitment bottleneck of Razorback Suckers in the San Juan River. However, even if this ideal nursery habitat were present in the San Juan River, it is currently unclear whether Razorback Sucker larvae could successfully recruit from the larval to juvenile stage. To test this hypothesis, the Phase III Habitat Restoration Project is proposed to be implemented in 2019 to create more quality nursery habitat via the construction of a ~1 hectare artificial wetland near river mile (RM) 107 (Gori et al. 2018). The Phase III project also aims to measure physicochemical conditions and larval predation pressure (i.e., via small-bodied fish monitoring) in the constructed wetland, but does not plan to measure resource availability. If benthic and/or pelagic algae and macroinvertebrates are scarce in the artificial wetland relative to demand, this lack of food for imperiled larval fishes could hinder the success of this project. Monitoring is needed to assess resource availability in the Phase III wetland.

We have provided several explanations for why secondary channel backwaters may be superior habitat for imperiled YOY fishes relative to main channel backwaters, although high abundances of nonnatives in secondary channel backwaters could reduce their quality. However, it is currently unknown whether stability, habitat quality, resource availability, and/or predation pressure actually differ between backwater types. As such, differences between secondary channel and mainstem backwaters are in need of investigation before environmental flows management can be implemented, as it would be ineffective to increase the area of secondary channel backwaters via elevated base flows if this is not better habitat than main channel backwaters (Lamarra et al. In prep). If secondary channel backwaters do indeed exhibit greater habitat quality and resource availability for imperiled fishes, then environmental flows management (Tharme 2003; Propst and Gido 2004) could be used to increase the coverage of this habitat because of the positive relationship between base flows and amount of secondary channel backwaters (Lamarra et al. In prep).

Objectives and Hypotheses

The objective of the proposed work is to compare stability, physicochemical characteristics, resource availability, and small-bodied predator density between secondary channel and main channel backwaters (i.e., island and point bar) in the San Juan River. To accomplish this objective we will test five hypotheses and their associated predictions (Fig. 2).

Hypothesis and Prediction #1: Stability differs between secondary channel and mainstem backwaters because secondary channel backwaters exhibit lower connectivity with the San Juan River mainstem; secondary channel backwaters will exhibit greater stability compared to main channel backwaters because they experience less frequent, lower magnitude, and shorter duration monsoonal flows.

Hypothesis and Prediction #2: Shading differs between secondary channel and mainstem backwaters because secondary channel backwaters are located nearer the margin of the San Juan River mainstem; secondary channel backwaters will exhibit greater shading compared to main channel backwaters because of greater canopy cover and canyon wall influence.

Hypothesis and Prediction #3: Physicochemical characteristics differ between secondary channel and mainstem backwaters because of differences in stability and shading; secondary channel backwaters will have lower turbidity, larger substrate, cooler water temperatures, and higher dissolved oxygen concentrations because of greater stability and shading.

Hypothesis and Prediction #4: Resource availability differs between secondary channel and mainstem backwaters because of differences in stability and shading; secondary channel backwaters will have greater algal and macroinvertebrate biomass because of greater stability. Also, we will test for differences in resource availability among the Phase III artificial wetland, secondary channel, and mainstem backwaters as part of this hypothesis.

Hypothesis and Prediction #5: The density of small-bodied nonnative predators differs between secondary channel and mainstem backwaters; secondary channel backwaters will have more nonnative predators (Franssen et al. 2015).

Study Area, Site Selection, and Sampling Regime

This study will be conducted in backwaters of the San Juan River located between RM 149 (Shiprock, NM) and RM 93 (Montezuma Creek, UT) (Fig. 3). This stretch of river was chosen because it contains a high density of secondary channel backwaters (Lamarra et al. In Prep). Within this 56-mile stretch we will attempt to sample 10 secondary channel and mainstem backwaters per sample trip (20 total backwaters per sample trip). Potential backwater sites will be scouted using recent habitat maps and surveys in April-May. Furthermore, we will add an initial float trip to scout for potential study sites at the beginning of the field season. During early July before sampling for this project starts, we will float our sample reach to visit every backwater habitat. Every backwater visited will be categorized as secondary channel or mainstem (island and point bar) and will then have its surface area and depth (both mean and maximum) measured. Following this initial scouting trip, the 10 largest secondary channel and mainstem backwaters (20 total) will be selected as sample sites during a study year. The largest backwaters will be selected since they will likely be more permanent and have a lower probability of drying up over the course of the field season. We will also sample resource availability (i.e., Hypothesis #4) in the Phase III artificial wetland at RM 107, in addition to all of the other study backwaters. Ten backwaters per category per sample trip (i.e., 20 total) was chosen because Lamarra et al. (In Prep) indicated that this is a realistic estimate of the total number of available backwaters in our study reach during late spring, summer, and early fall. However, given the dynamic and ephemeral nature of backwaters and their dependence on flow in the mainstem (Bliesner and Lamarra 2000; Lamarra et al. In Prep), the total number of backwaters sampled per trip may differ from 10 per category. Also, the variable nature of backwaters may result in some backwaters being sampled only once while others get sampled repeatedly. This will be an issue for the testing of Hypothesis #1 since it relies on deployed data loggers (see below), but will not be an issue in testing Hypotheses #2 - #5. So long as we have replication of each backwater type regardless of the identity of any individual backwater, our study design will allow us to test Hypotheses #2 - #5. Sampling will commence when discharge of the San Juan River falls below ~1,500 cubic feet per second (cfs), as most secondary channel backwaters will likely be flowing when discharge is greater than this. As such, sampling will typically start around July 15th, but may be earlier or later depending on flow conditions. Sample trips will then be conducted every 14 days until the end of September, resulting in ~6 sample trips per year in 2019 and 2020. If flows become elevated above 1,500 cfs during our sample period because of monsoonal rainfall or otherwise, a sample trip will be delayed until flows drop back below 1,500 cfs threshold. During each sampling trip we will raft from backwater to backwater collecting data as described below. Each sample trip will take ~5 days to complete.

The length and area of sample sites will depend upon backwater size. A general recommendation in stream sampling is to survey a reach length equal to 40 times the average width to provide a representative sample, with a maximum length of 300 m for a sample reach (Klemm and Lazorchak 1994; Lazorchak et al. 1998). We will follow these guidelines when sampling San Juan River backwaters. In a database containing information on 332 secondary channel and 2,057 main channel backwaters (2,389 total) in the San Juan River, the mean length and width of secondary channel backwaters was 240 m (median = 169 m; range = 13 – 1,442 m) and 3.2 m (2.72 m; 0.22 – 10.10 m), respectively, while that of main channel backwaters was 58 m (29 m; 1 – 1,161 m) and 1.3 m (0.92 m; 0.10 – 8.03 m), respectively (N. Franssen; unpublished data). Therefore, since most of the backwaters in the San Juan River are of a smaller size, our size criteria will result in a large proportion of total backwater area being sampled for most backwaters, allowing us to accurately characterize backwater conditions.

Materials and Methods

Hypotheses #1: Backwater Type and Stability

In each backwater a HOBO MX2001 data logger (Onset Company, Bourne MA) will be deployed in the deepest part of the backwater excluding the mouth, with the deepest point determined via an initial depth survey. The logger will be set to record water level every 30 minutes. To reduce the influence of the mainstem, the logger will be deployed some distance from the backwater mouth, with that distance dictated by backwater length. The logger will be housed in a PVC casing and will be attached to a t-post to lessen the chance that it will be displaced during high flows. When possible, data loggers will be placed in inconspicuous locations to decrease the probability of being noticed and potentially vandalized, although depth will be the primary determinant of logger placement. Additionally, we will measure the frequency and duration of flow from the mainstem into backwaters using electrical resistance sensors (Jaeger and Olden 2012). Based on the change in relative conductivity, these sensors (i.e., customized Onset TidbiT Temperature Data loggers) can determine whether the logger is dry (low relative conductivity) versus submersed by water (high relative conductivity) where the sensor is deployed in the substrate (Blasch et al. 2002; Goulsbra et al. 2009). We will deploy a sensor such that it will be able to detect when water is flowing from the mainstem into the backwater. For secondary channels this location will be near the upstream head of the backwater, and in mainstem backwaters the sensor will be located in the backwater mouth. Sensors will be placed in the lowest elevation point in-between the main channel and the backwater (determined visually) to ensure a flow connection is recorded when present. Electrical resistance sensors will be housed in a PVC casing and will be attached to a t-post to prevent them from being displaced during high flows. When possible, resistance sensors will be placed in inconspicuous locations to decrease the probability of being noticed and potentially vandalized. Data from water level loggers and resistance sensors will be downloaded every sample trip. From the continuous measurements collected by electrical resistance sensors we can calculate the frequency and duration of flow into backwaters, and with the water level loggers we can calculate the magnitude and variability (using coefficient of variation) of flow events from changes in water level. Finally, we will also deploy an electrical resistance sensor by the water level logger in the deepest part of a backwater located some distance from the backwater mouth (depending on backwater length) to assess whether or not a backwater dries completely in-between sampling events.

Hypotheses #2 and #3: Shading and Physicochemical Variables

Several habitat variables will be quantified along equally-spaced transects in a backwater. The distance between transects will equal 10% of backwater length (i.e., in a 300 m backwater, transects will occur every 30 m), and as such will result in 10 transects per backwater (Klemm and Lazorchak 1994; Lazorchak et al. 1998). To limit the influence of the mainstem on habitat measurements, transects will not be positioned in the mouth of the backwater where it connects to the mainstem (if applicable). Shading resulting from canopy cover and canyon walls will be quantified at each transect midpoint in a backwater using a spherical concave densiometer, providing an estimate of percent canopy coverage. The wetted surface area (m^2) of a backwater will be calculated by multiplying backwater length (m) by the mean wetted width (m) of a backwater measured at each transect. Also, along each transect five measurements of depth (measured with a Hach topset wading rod) and substrate (e.g., clay, silt, sand, gravel, pebble, cobble, boulder, and bedrock) will be taken. The five locations per transect will be at river right (#1), then 25% (#2), 50% (i.e., transect midpoint; #3), and 75% (#4) of the distance from river right to river left, and then finally at river left (#5) (Klemm and Lazorchak 1994; Lazorchak et al. 1998). The substrate at a transect location will be determined with visual and tactile examination. If there is a mixture of substrates at a location, the location will be assigned the category of whichever substrate is dominant. These measurements will generate an estimate of mean depth, maximum depth, and percent coverage of fine (clay + silt + sand) and coarse substrates (gravel + pebble + cobble + boulder + bedrock) for each backwater. Mean backwater depth (m) will also be multiplied by backwater surface area (m^2) to calculate backwater volume (m^3). Water temperature in a backwater will be measured every two hours using the HOBO MX2001 data logger, which records temperature in addition to water level. Backwater turbidity in nephalometric turbidity units (NTUs) will be measured using an Aquaflor handheld fluorometer (Turner Designs, San Jose, CA), and dissolved oxygen (mg/l and % saturation) will be measured using an Extech heavy duty dissolved oxygen meter (FLIR Commercial Systems, Nashua, NH).

Hypothesis #4: Resource Availability

Algal biomass will be measured by quantifying pelagic and benthic chlorophyll *a* concentrations. Phytoplankton concentrations will be measured by taking a 500 ml water sample from each backwater. The water sample will be collected at approximately 8 cm depth in the water column at multiple backwater locations until a 500 ml Nalgene collection jar is filled, thus providing a representative sample. The water sample will then be filtered through a Whatman glass microfibre filter (grade GF/C), with chlorophyll *a* then extracted from the filter by submersing it in 95% ethanol for 12 hours. Chlorophyll *a* concentration (µg/L) will then be measured using an Aquaflor handheld fluorometer followed by correction for sample volume (Wetzel and Likens 2000; Rice et al. 2017). In fine substrates (i.e., silt and sand) benthic chlorophyll *a* samples will be collected using a core sampler (3.2 cm inside diameter), and in coarse substrates (i.e., gravel, pebble, and cobble) whole rocks will be collected. Benthic samples will be collected from six habitat transects per backwater (approximately every other transect); three replicates will be collected along each transect near river right, river left, and the transect midpoint, with either whole rocks or core samples pooled into the same transect Whirl-Pak. The composition of the pooled transect sample (e.g., transect #1 = 2 cores + 1 whole rock; transect #3 = 3 whole rocks) will be recorded in the field notebook. Samples will be kept in the dark in a cooler with dry ice, and then transported back to the lab where they can be frozen. 95% ethanol will be used to extract chlorophyll *a* from benthic samples for 12 hours, with chlorophyll *a* concentration (µg/cm^2) determined with a fluorometer followed by correction for sample surface area (core size or rock size) (Sartory and Grobbelaar 1984; Steinman et al. 2017). This sampling will be performed in backwaters and in the Phase III artificial pond.

The availability of larval prey will be estimated by collecting benthic and pelagic backwater macroinvertebrates. Benthic macroinvertebrates residing in fine-textured benthic substrate (i.e., clay, silt and sand) will be sampled using an Eckman grab, whereas benthic macroinvertebrates residing coarser substrates (i.e., gravel, pebble, cobble, and boulder) will be sampled by scrubbing whole rocks in a bucket (Hauer and Resh 2017). If large woody debris (LWD) is present in a backwater transect, it will be sampled by enclosing a subsection of LWD and then removing macroinvertebrates from it (Whitney et al. 2014; Whitney et al. 2015). Benthic macroinvertebrate samples will be taken from six habitat transects per backwater (approximately every other transect); three replicates will be collected along each transect near river right, river left, and the transect midpoint, with whole rocks, core samples, and LWD samples pooled into the same transect bucket, which will then be sieved and stored in 10% formalin in a Whirl-Pak. The composition of the pooled transect sample (e.g., transect #1 = 2 cores + 1 whole rock; transect #3 = 2 whole rocks + 1 LWD) will be recorded in the field notebook. Pelagic macroinvertebrates (e.g., Copepods and Cladocerans) will be sampled using a Wisconsin plankton sampler equipped with a mechanical flow meter that will measure the volume of water sampled (De Bernardi 1984), with three pelagic macroinvertebrate samples collected per backwater per sample date. Macroinvertebrates will then be identified, enumerated, and measured for total length in the laboratory under a dissecting microscope. Published length-mass relationships (Burgherr and Meyer 1997; Benke et al. 1999; Sabo et al. 2002) will then be used to estimate biomass in grams of dry mass (DM) for each macroinvertebrate taxon, then biomass of macroinvertebrate taxa will be summed separately for benthic and pelagic samples to provide estimates of benthic and pelagic resource availability. This sampling will be performed in backwaters and in the Phase III artificial pond.

Hypothesis #5: Small-Bodied Nonnative Predators

The abundance of potential predators in each backwater will be assessed using a combination of backpack electrofishing with 1-2 dipnetters (Smith-Root LR-20B backpack electrofisher) and seining (4.6m wide X 1.8 m tall; 3.2 mm mesh). To prevent escape of fishes from our sample reach, we will use blocknets to separate our sample reach from the mainstem if necessary, and in longer backwaters, from the section of the backwater not sampled. All potential larval predators will be identified to species, measured for total length, and then returned to the backwater from whence they came. Any native fishes captured by this sampling will also be identified, measured, and released. The density of backwater larval predators for each species and in total will be calculated by dividing the number of individuals caught by backwater area sampled (m^2), giving an estimate in $\#/m^2$.

Our ability to capture fishes may vary among backwaters because of differences in habitat characteristics related to depth, turbidity, and substrate size (Tyre et al. 2003; Gu and Swihart 2004; Falke et al. 2010). As such, differences in sampling efficiency among backwaters could result in different fish density estimates among backwaters independent of the effect of backwater environmental characteristics on fish presence and abundance. To account for differences in sampling efficiency among backwaters, we will perform depletion sampling during one sample trip per month (i.e., every other trip). Depletion sampling will involve sampling each backwater site with multiple repeated passes; after each pass all captured fishes will be retained and won't be released until all passes have been completed. This design will allow for the calculation of absolute population size ($\widehat{N}$), catchability (*q*; Hayes et al. 2007) and detection probability (*p*; MacKenzie et al. 2002), which can then be modeled according to habitat characteristics (e.g., depth, turbidity, substrate size). If capture efficiency is found to vary among backwaters, we will use our catchability and detection probabilities to correct our fish density estimates, thus making estimates comparable among backwaters.

Data Analysis

Our interrelated hypotheses operate through several intermediary pathways of cause and effect relationships, and contain multiple predictor and response variables (Fig. 2). As such, covariance structure analysis, otherwise known as structural equations modeling, is conducive to statistically testing our hypotheses (Bollen 1989; Shipley 2000; Infante and Allan 2010). Covariate structure analysis (CSA) is a multivariate technique that allows for the modeling of relationships among sets of direct and indirect predictors and response variables. This technique is similar to multiple regression analysis or canonical ordination, but differs in the fact that it accounts for interrelatedness of predictor variables and indirect effects. In order to model relationships, a priori hypotheses describing the structure among variables and direct and indirect effects must first be completed. These a priori hypotheses (i.e., Fig. 2) are then combined with collected sample data to model relationships via path analysis, which then provides output describing model fit and the strength of relationships. Model fit will be examined using chi-squared analysis, root mean squared error approximation, and the normed fit index, whereas strength and direction of relationships will be evaluated using multiple correlation and slope coefficients (Burcher et al. 2007; Perkin et al. 2014). Covariate structure analysis will be done with the flexible partial least squares approach (PLS; Perkin et al. 2014) using the functions in the plspm package (Sanchez et al. 2017) in program R version 3.3.3 (R Core Team 2017). Results will be displayed using a path diagram.

We will use one-way multivariate analysis of variance (MANOVA) to test for differences in resource availability among secondary channel backwaters, main channel backwaters, and the Phase III wetland. The three categories of wetland or backwater will serve as the predictor variable, and response variables will include phytoplankton biomass (chlorophyll *a* in μg/L), periphyton biomass (chlorophyll *a* $\mu g/cm^2$), benthic macroinvertebrate biomass ($g\ DM/m^2$), and zooplankton biomass (g DM/L). The MANOVA assumptions of multivariate normality and equality of variance-covariance matrices will be tested using a Shapiro-Wilk test of multivariate normality and Box's M test, respectively. If assumptions are violated, Box-Cox transformations will be applied to the data. If the MANOVA detects significant differences, we will use separate one-way ANOVAs coupled with Tukey's honest significance difference (HSD) to determine which resource availability variables actually differ among predictor variable categories.

Deliverables

An annual report will be provided each year of the study using the same timeline as reports required for the San Juan River Basin Recovery Implementation Program (SJRIP) and Reclamation. Likewise, an annual oral report will be given at the SJRIP Annual Biology Committee (BC) meeting in February. At the completion of the project a final report will be delivered to both the SJRIP and Reclamation. Finally, when the project is completed all data files will be provided to the Program Office in digital format.

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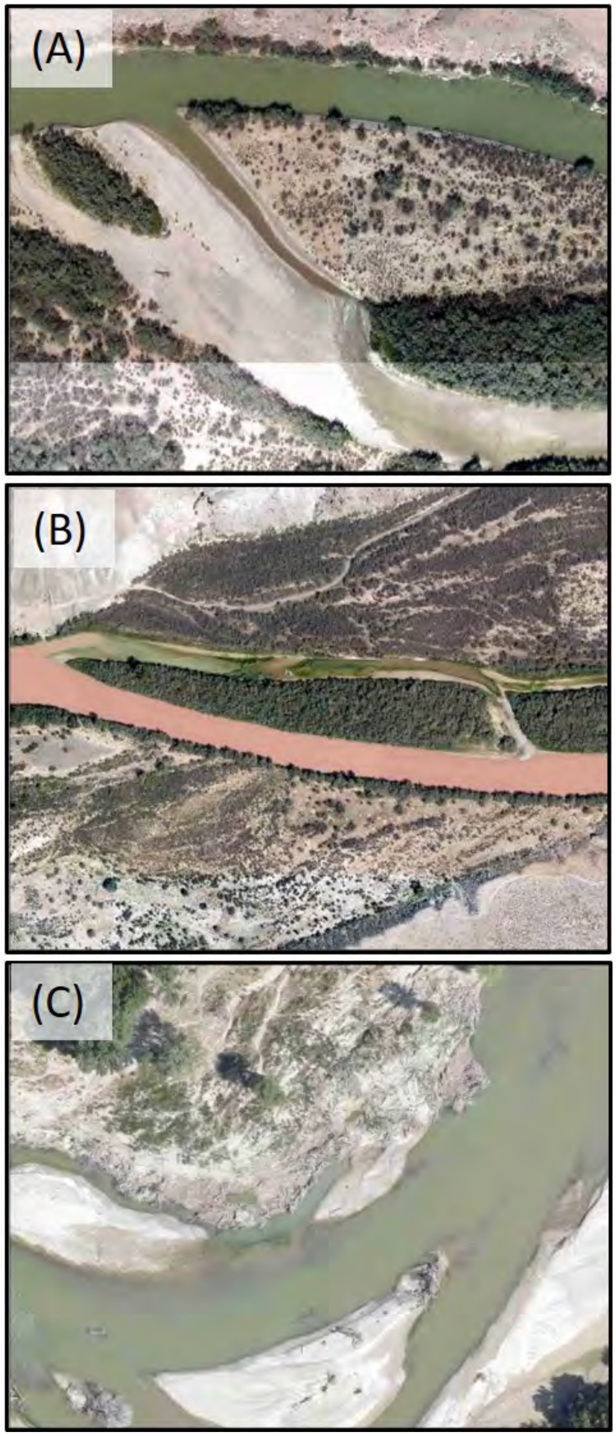

Figure 1. Examples of secondary channel (A), island (B), and point bar (C) backwaters in the San Juan River. Island and point bar backwaters are two types of main channel backwater. (Photos courtesy of Dan and Vince Lamarra).

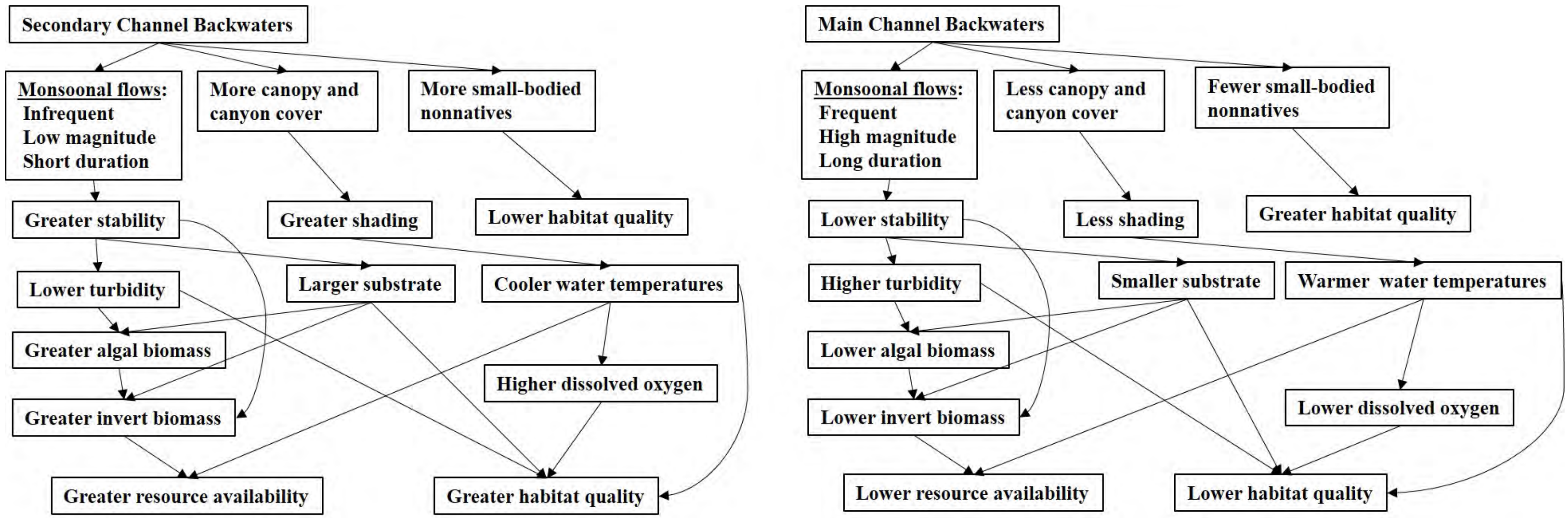

Figure 2. Conceptual model illustrating pathways and hypothesized differences in habitat quality, resource availability, and nonnative (NN) small-bodied predators between secondary channel and main channel backwaters in the San Juan River.

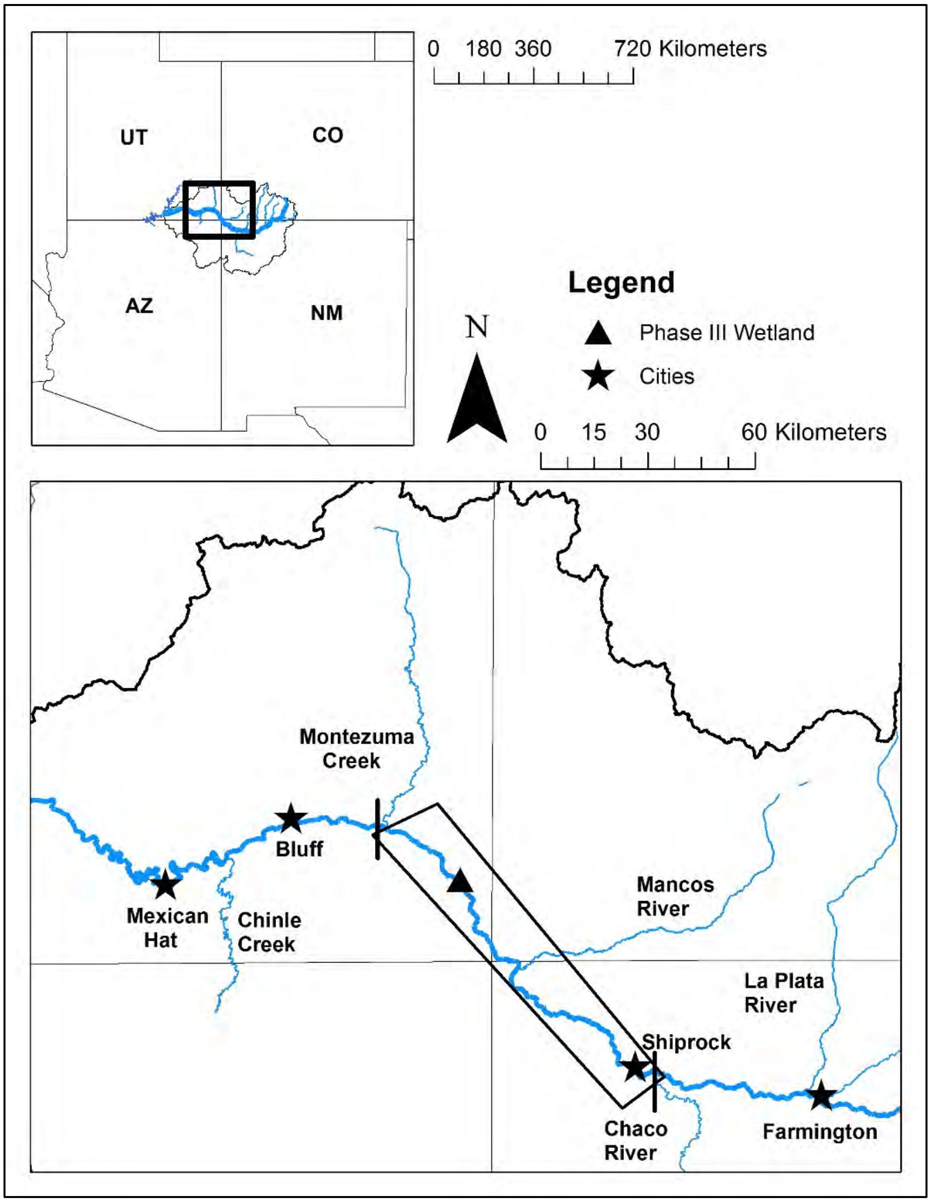

Figure 3. Map of the San Juan River Basin (broad extent) and the section of the San Juan River where backwaters will be studied for this project (fine extent), with Shiprock denoting the upstream end of the study reach and the confluence with Montezuma Creek marking the downstream end of the study reach.

Table 1. Detailed budget for fiscal year 2021 (year 3) of the project. GS = graduate student.

Category	Type	Expense	Explanation
Personnel	GS Stipend	$10,000.00	6-month stipend working 0.5 time (20 hrs/week; $19.23/hr)
	GS Fringe	$433.60	GS stipend *0.04336
Travel	SJRIP BC Meeting	$2,000.00	2 people; $1200 airfare; $400 rental car; $400 hotel
Supplies	None	$0.00	
Equipment	None	$0.00	
Other	Tuition and fees	$4,161.00	$4,161.00/semester for the GRA
Total Personnel		**$10,433.60**	
Total Overhead		**$1,825.88**	Overhead rate = 17.5% on personnel costs
Total Travel		**$2,000.00**	
Total Supplies		**$0.00**	
Total Equipment		**$0.00**	
Total Other		**$4,161.00**	
Grand Total		**$18,420.48**	

Budget Justification

Personnel – Funds are requested to support a graduate research assistant in year 3 such that they can complete data analysis and write up the results of the project.

Travel –Funds are requested in year 3 for the graduate student and PI to travel to the SJRIP Biology Committee (BC) Meeting.

Indirect Costs – The overhead rate at Pittsburg State University is 47% on personnel costs, but PSU is going to charge 17.5% on personnel costs in year 3 of the project.

Response to BC Comments

Bill Miller, Southern Ute Indian Tribe, BC member

How can the technical aspects of this SOW be improved?

A deliverable for this SOW does not list the delivery of all data files in digital format to the Program Office. Preservation of the original data files for all projects is needed in all Scopes of Work. This is the third year for funding this project and no work has yet been completed due to contracting issues. It was originally proposed as a three year project so the deliverable needs to include the final report. The annual report to the BC should be at the winter meeting not the May meeting. The scope should be revised to update the deliverables and presentation of annual results.

Response: *We have added a sentence at the end of the Deliverables section stating that "when the project is completed all data files will be provided to the Program Office in digital format." Also, we changed the Deliverables section to indicate that an annual report will be given to the Biology Committee during the winter meeting rather than in May.*

What is this SOW's contribution to recovery?

It has the potential to provide data on productivity of low velocity habitats.

Wayne Hubert, Peer Reviewer

How can the technical aspects of this SOW be improved?

The background section of the SOW is impressive with a substantial use of literature. The need to understand differences between secondary channel and main channel backwaters is well developed.

The application of hypotheses (H) and predictions is an impressive component of the proposal. They are essentially null hypotheses with specific alternative hypotheses upon rejection of the null. The five hypotheses and associated predictions are well stated. Predictions associated with H 1-4 all predict secondary channel backwaters to be more stable, have more shade, have better physicochemical conditions, and have higher biological productivity, than main channel backwaters and to be more conducive to better survival of larvae and juveniles fishes. However, the prediction associated with H 5 is that secondary channel backwaters will have higher densities of nonnative predators than main channel backwaters. If the predictions are true, it is unclear how insights obtained for the project may lead to management strategies for enhancing backwaters or managing discharge from Navajo Dam that benefit Razorback Sucker or Colorado Pikeminnow.

Response: *If the predictions for H1-H4 all turn out to be true while the prediction for H5 proves false, releases from Navajo Dam could be manipulated to increase the availability of secondary channel backwaters to the potential benefit of Razorback Sucker and Colorado Pikeminnow. However, some or all of the predictions for H1-H4 could be incorrect, while that of H5 could be true, limiting the efficacy of using environmental flows management with regards to secondary channel backwaters as a conservation strategy. However, we will not know whether any of these predictions are true until we actually collect the data, which is the purpose of conducting the study in the first place. Furthermore, if all predictions prove true, the magnitude of difference between secondary channel and mainstem backwaters will dictate how useful environmental flows management is to native fish conservation. If nonnative fish densities are only slightly higher in secondary channel compared to mainstem backwaters, then the benefits to native fishes of residing in secondary channel backwaters (e.g., greater food availability; more benign physicochemical conditions) may far outweigh the costs associated with nonnative fish predation. But if nonnative densities are much higher in secondary channel versus mainstem backwaters, then the predation costs to native fishes of living in these habitats may be too high, limiting their conservation value. But, the study needs to be completed before we will know the answer to these questions.*

The description of the study design raises many questions.

1. It is unclear how the sampling sites for secondary channel and main channel backwaters will be selected. The description indicates that 10 of each category may be near the total number available in the study reach, but the number may more or less. Will all of the sites of each category encountered during the first sampling trip be identified and sampled? Or, will it be the first 10 encountered of each category? If there are substantially more than 10 sites in a category within the study reach, how will a random sample be selected? As discharge varies over the sampling season, will all of the sites identified on the first trip be assessed and sampled if water is present? There is indication in the SOW that additional sites will be added during the sampling season. What does this do to the sampling design and ability to conduct statistical analyses?

Response: *The dynamic and ephemeral nature of backwater habitats definitely complicates study site selection for this project. As such, we have decided that we will add an initial float trip to scout for potential study sites at the beginning of the field season. During early July before sampling for this project starts, we will float our sample reach to visit every backwater habitat. Every backwater visited will be categorized as secondary channel or mainstem (island and point bar) and will then have its surface area and depth (both mean and maximum) measured. Following this scouting trip, the 10 largest secondary channel and mainstem backwaters (20 total) based on surface area and depth will be selected as* *sample sites during a study year. The largest backwaters will be selected since they will likely be more permanent and have a lower probability of drying up over the course of the field season. We have indicated this change in study site selection in the statement of work.*

2. The definition of a suitable sampling site is not clear. The SOW indicates that "a general recommendation for stream sampling is to survey a reach length equal to 40 times the average width to provide a representative sample." Backwaters are not streams with riffles, pools, runs, etc. Habitat heterogeneity within secondary channel or main channel backwaters is unlikely to be as diverse as streams, but it is also likely to change in a systematic manner from the head to the mouth of a backwater. It is stated that a surface area of > 30 m2 will be a criteria for sample site selection. A much more objective and systematic set of criteria for identification and selection of both secondary channel and main channel backwaters is needed to assure the reader that the sites sampled are representative of the universe of available sites within the study reach.

Response: *See the previous response for our updated sample site selection protocol. Our study site selection procedure is not random since we are selecting the largest, most permanent backwaters. However, this study site selection strategy is necessary to ensure that we are able to sample repeatedly the study sites we select during a given year.*

3. The descriptions of sampling designs, sampling methods, computation of variables, and statistical analyses are unclear, incomplete, and confusing. Some of the most prominent concerns include:

(a) For H 1, the location of HOBO loggers will be at the "deepest" point in the backwater but some distance from the mouth. This seems to be a contradiction given that the deepest point is likely to be at the mouth of most of these backwaters. How will the deepest point be identified? Further, the loggers are to be located at "inconspicuous" sites. How, is this compatible with being in the deepest spot? How will monsoons, vandalism, and sample-site drying affect the likelihood of systematic data being obtained through the sampling seasons? What are the metrics describing "stability" that will be computed from the logger data and use in statistical analyses?

Response: *The logger will be deployed in the deepest part of the backwater excluding the mouth. The deepest part of the backwater will be determined using an initial depth survey. When possible, data loggers will be placed in inconspicuous locations to decrease the probability of being noticed and potentially vandalized, although depth will be the primary determinant of logger placement. Stability will be calculated using the coefficient of variation calculated from changes in water level. Some loggers may or may not be lost due to* *unpredictable forces like flooding and vandalism, but the deployment of multiple loggers and replication will help us overcome these challenges.*

(b) For H 1, it is intriguing that electrical resistance will be measured using modified loggers and enable identification of flow through backwaters. The ability to effectively utilize this method depends on identifying the "lowest elevation poing in-between the main channel and the backwater." How will this specific location be identified in the field when the first trip is made in July when relatively high flows are likely to occur? What are the metrics to be computed from these data?

Response: *The lowest elevation point will be determined visually. The frequency and duration of flow connections between the main channel and a backwater will be calculated with these data.*

(c) For H 1, modified loggers will be located in the deepest point to identify if or when a backwater dries up. Will these be the same locations as in (a) above?

Response: *Yes.*

(d) For H 2 and 3, the transect sampling design is unclear. The number and spacing of transects appears to vary among sampling sites and within sampling sites among trips. Given the criteria that transects will be 10 m apart in backwaters < 150 m in length, some backwaters may only have a single transect sampled. How can a representative sample of a backwater by be assured? How many points (and where) on a transect will be sampled? What will be measured at each point? How will time of day affect the sampling of physicochemical features? What are the metrics that will be computed and used in the statistical analyses?

Response: *We have modified our transect sampling design such that the distance between transects will always be 10% of backwater length, resulting in 10 total transects for every backwater. Number of transect points, point location, and variables measured are described later on in the paragraph being commented upon. Time of day will have minimal influence on transect measurements.*

(f) For H 2 and 3, five transects will be sampled to determine extent of shade. How will these transects be located? Why are transects used for physicochemical sampling not used for sampling shade? How will shade be measured? How will time of day affect shade measurements?

Response: *Percent canopy coverage as measured using a densiometer will be quantified at 10 transects, and these will be the exact same transects as the depth and substrate transects. Time of day does not affect percent canopy cover.*

(g) For H 4, chlorophyll a will be measured in a single 500-ml sample obtained from various depths. How will the depth composition affect the estimate? Sampling at a consistent depth would provide a more precise estimate to enable comparisons among sites and over time. How much spatial variation occurs in a backwater and how will that affect the estimates? Benthic chlorophyll a will be sampled using a core sampler and whole rocks. Samples from cores and rocks will be pooled for analysis? What is the metric that will be computed and how precise will it be? Similarly, macroinvertebrates will be sample using an Eckman dredge, by scrubbing rocks, isolating sections of large woody debris. Again, sample for various sources will be pooled. What kind of metric will be attaind and how precise will it be?

Response: *We have changed our phytoplankton sampling protocol such that water collection will occur at approximately 8 cm depth in all backwaters. Since water will be collected from multiple backwater locations, that will help account for spatial variation within backwaters. Pooling of chlorophyll a samples will occur at the transect level, such that there will be six replicates per site per sample period. This replication will allow us to calculate precision (e.g., calculating standard deviation) of chlorophyll a biomass in units of µg/cm^2. Similarly, we'll have six macroinvertebrate replicates per site per sample period, which will allow us to calculate precision of macroinvertebrate biomass in g of dry mass per m^2.*

(h) For H 5, small bodied fish will be sampled by electrofishing and seining. The two techniques differ substantially in their selectivity. What kind of sampling design will be used using each gear? It is stated that sample with the gears will be pooled and density (number/m2) will be used as the metric for density. How precise will the estimate be given the differences in selectivity and difficulty in computing the area sampled with each gear? It is stated the depletion sampling will be conducted to calibrate the electrofishing and seining samples. Insufficient information is provided to enable an assessment of how this will be achieved.

Response: W*e acknowledge that seining and backpack electrofishing target different components of a fish community. It is specifically because of this reason why pooling together electrofishing and seining data is more accurate that treating each sampling technique independently. We are not interested in comparing the sampling efficiencies of these two different gears, but rather we are trying to accurately determine the total population size of nonnatives in backwaters.*

4. Overall, the metrics to be computed from the sampling data have not been described. The accuracy and precision of various metrics are questionable given the sampling designs that are described. Given the error that is likely in the measurements of many of the variables, statistical testing of the hypotheses is likely to be affected.

What is this SOW's contribution to recovery?

It is unclear how insights obtained for this project may lead to management strategies for enhancing backwaters or managing discharge from Navajo Dam that benefit Razorback Sucker or Colorado Pikeminnow.