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 Southwestern Native Aquatic Resources and Recovery Center
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 May 2018



Title: Quantifying the effective number of breeders (N_b) of Flannelmouth Sucker (*Catostomus latipinnis*) and expanding the spatial sampling of larvae below the waterfall to evaluate potential reproductive bottlenecks of Razorback Sucker (*Xyrauchen texanus*) in the San Juan River

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Introduction & Justification

Comparing the effective number of breeders (N_b) among closely-related, co-occurring species has the potential to inform conservation programs when the evolutionary potential of a certain species is at risk (Bernos et al. 2017; Yates et al. 2017). In particular, comparing the ratio between adult census size (N_a) and N_b can provide insight into how factors shape this relationship because N_a is governed by ecological processes (e.g., recruitment, competition, and mortality) while N_b is shaped by contemporary eco-evolutionary process (e.g. sexual selection, density dependence, variance in reproductive success). Certainty in the relative magnitude of N_b/N_a within natural populations is often unknown. Available data on 62 N_b/N_a estimates were found to vary considerably (0.01-0.95) across taxa with a median estimate of 0.225 (Palstra and Fraser 2012). Although, the magnitude of N_b/N_a can differ among populations within and among species, quantifying the magnitude of these differences among coexisting, closely-related taxa has the potential to inform the ecological significance of N_b/N_a estimates.

Augmentation of the San Juan River Razorback Sucker (*Xyrauchen texanus*) population using captive-reared individuals began in the mid-1990s and continues as a recovery action (USFWS 2005; USFWS 2015). Population monitoring has been annually supported through the San Juan River Basin Recovery Implementation Program (SJRRIP). Successful reproduction of hatchery-released individuals has been documented (Farrington et al. 2015), but recruitment remains extremely limited. Conversely, the two most common sucker species, Flannelmouth Sucker (*Catostomus latipinnis*) and Bluehead Sucker (*Catostomus discobolus*), consistently recruit to the juvenile stage (Zeigler and Ruhl 2016). Estimates of N_b for Razorback Sucker between 2009-2015 were consistently low. Overall, mean N_b estimates averaged 82 across years (range of 65-109) with similar results observed among years; conversely, mean N_a (4,571; range = 3,817-5,410) among years was relatively high (Schleicher 2016; SJRRIP 2017). Consequently, the relative contribution of adults appears consistently low with an observed annual N_b/N_a ratio less than 0.03 (Diver and Wilson 2018). These results suggest factors are limiting the reproductive potential of the Razorback Sucker population in the San Juan River. Nonetheless, questions remain regarding the ecological context of this ratio as well as potential factors affecting its magnitude.

Among-species comparisons of sympatric species can provide insight into factors affecting N_b/N_a because both species are likely subjected to similar climatic and environmental conditions that structure natural populations (Kanno et al. 2015; Bernos et al. 2017). Annual N_b and N_a estimates have been obtained for Razorback Sucker in Lake Mohave between 1997 and 2010, and results showed a significant increase in both N_b (e.g. $N_b = 743$ [1998]; $N_b = 49,984$ [2005]) and N_b/N_a (mean 0.708) over a fourteen year period (Dowling et al. 2013; Carson et al. 2016); however, this population is not subjected to the same environmental conditions or management activities as the San Juan River population. Although, direct comparison of a closely-related species can be problematic due to different life-history strategies and demographic characteristics, both populations of Razorback Sucker and Flannelmouth Sucker in the San Juan River are generally constrained to similar environmental conditions. Thus, annual estimates of N_b for this closely related species that successfully recruits could inform the ecological context of the presumed low N_b/N_a ratio in Razorback Sucker. For example, if N_b/N_a

is relatively high for Flannelmouth Sucker in the San Juan River, it would suggest other biological and/or environmental factors are limiting the reproductive potential of Razorback Suckers in the system.

Objective:

1. Quantify the effective number of breeders (N_b) for Flannelmouth Sucker for five years (2015-2019) and compared to Razorback Sucker estimates.
2. Continue N_b monitoring for both Razorback Sucker and Colorado Pikeminnow collected in the San Juan River in 2019.
3. Expand upon N_b estimates for Razorback Sucker and Colorado Pikeminnow larvae captured below the waterfall for 2018 and 2019.

Methods

Larval fish surveys are conducted annually along a 140 mile section of the San Juan River between Shiprock, NM, and Clay Hills, UT. Approximately 600 larval Flannelmouth Sucker samples representing the spatial and temporal distribution of larval sampling efforts will be examined for N_b estimates. These estimates will be provided for the five most recent years of larval sampling (2015-2019). Estimates of effective number of breeders for both Razorback Sucker and Colorado Pikeminnow collected during 2018 larval monitoring was funded in fiscal year 2018. As a continuation of the N_b dataset, samples collected in 2019 from the San Juan River will be included in this scope of work. In 2018, larval sampling downstream of the waterfall located in the San Juan arm of Lake Powell commenced. Although sampling has not begun, up to 120 samples for both endangered species will be included. Estimates for 2018 and 2019 will be included in this scope of work in order to address questions related to larval emigration and adult spawning below the waterfall. Genetic sampling will strive to comprise a minimum of three ($N = 40$) or four ($N = 30$) spawning periods (e.g., April, May, June, July) to ensure samples are not collected from a single spawning bout. In addition, samples collected during each spawning period will cover the relative abundance and distribution of larval collections to reduce the potential bias caused by the collection of siblings at any one site.

Genomic DNA will be extracted from tissues following standard protocols used at Southwestern ARRC. Nineteen microsatellite loci were used for Razorback Sucker; twelve were developed at Southwestern ARRC for evaluating the genetic diversity of the Razorback Sucker broodstock (Wilson 2012), and seven were developed by and Dowling et al. (2011). These are the same loci used to estimate N_b for Razorback Sucker. Samples will be processed on an ABI 3500xl Genetic Analyzer and scored using GeneMapper™ 4.0 software (Applied Biosystems). A second researcher will perform a 10% quality assurance/quality control of samples to ensure accuracy. Departures from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) will be tested using Genepop v4.2 (Raymond and Rousset 1995), and alpha (0.05) will be adjusted for multiple comparisons using the Benjamini and Yekutieli (2001) method false discovery rate (Narum 2006). Effective number of breeders will be calculated using Effective number of breeders (N_b) was estimated using the sibship-assignment (SA) method in COLONY version 2.0.4.0 (Jones and Wang 2010). Adult census size for Flannelmouth Sucker will be estimated from adult monitoring efforts using catch per unit effort (CPUE) data.

Schedule:

Completion of genetic analysis	January 1, 2020
Final Report	March 30, 2020

Intended Method of Information Dissemination:

Dissemination of the results will include a final report and presentation of project results at the San Juan Researcher's meeting.

Budget Narrative

The requested project funds will be used to purchase replacement items as needed. Example items in each materials/supplies category: Extractions – sample tubes, DNA extraction kits, sample storage plates, ethanol; PCR Reactions – polymerase, oligonucleotides, buffer, magnesium, PCR plates, plate sealing film, primers; Genetic

Analyzer Costs – size standard, buffer, polymer, reaction plates, formamide, capillary array; Other – filtered tips, gloves, tubes, solution basins, storage boxes.

Unlike other projects, purchases of supplies are not kept for one project alone. Supplies are kept on hand and used as projects are started. Many of the items have expiration dates, so as a project is started in our work flow we utilize inventory we have on hand. Project funds are used to replace what is used and needed at that point in time. Over the years we have estimated the cost of each item used per sample and calculated a per sample cost. For example, the array in the genetic analyzer (\$1,000/per array) will last for a few thousand samples so we use it for multiple projects until we need to replace it. It does not make sense to replace it after every project. Likewise, we purchase items such as plastics (tips, plates, tubes) and ethanol in bulk to reduce prices, so again these are shared across projects but we have estimated how much is used per sample. If purchased in small quantities for a single project prices would go up. In doing things this way, sharing across projects and purchasing in bulk, money is saved, efficiency is increased, and waste decreased.

Detailed Spending Plan

1. PERSONNEL		
A. Laboratory Work		
1 Bio/Geneticist (GS 9; 93 hours -3.0 pay periods) @\$34.05/hr		\$3,167
1 Bio/Geneticist (GS 9; 148 hours -3.0 pay periods) @\$34.05/hr		in-kind
B. Report Writing		
1 Bio/Geneticist (GS13; 45 hours -1.63 pay periods) @\$42.15/hr		\$1,897
1 Bio/Geneticist (GS13; 85 hours -1.63 pay periods) @\$42.15/ hr		in-kind
	Subtotal Personnel	\$5,063
2. MATERIALS/SUPPLIES		
A. Extractions		\$3,601
B. PCR Reactions		\$22,695
C. Genetic Analyzer Costs		\$7,807
D. Other (tubes, tips, etc.)		\$10,228
	Subtotal Supplies	\$44,331
	Total	\$49,394
Southwestern ARRC Utilities		
-Electrical, (approx. 4,259 KW/h @ 0.34569 per KW/h) =		\$1,000
		\$50,394
Administrative and Overhead Costs Regional Office @ 3%		\$1,512
Project Total FY2019		\$51,906
Project Total FY2020		\$0

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Response to comments on:**New SOW - Quantifying the effective number of breeders (N_b) of Flannelmouth Sucker (*Catostomus latipinnis*) while expanding the spatial sampling of larvae below the waterfall to evaluate potential reproductive bottlenecks of Razorback Sucker (*Xyrauchen texanus*) in the San Juan River****Below we rebut specific comments (in regular font) with our responses (in bold).**

PO: “We think this SOW could be altered substantially to improve its value to the Program. We suggest the number of years Flannelmouth Sucker are analyzed should be reduced to the most recent five (2012-2019). This reduction in effort should allow for the analysis of Razorback Sucker and Colorado Pikeminnow larvae captured below the waterfall and in the river proper in 2018 and 2019. We think this change would aid our understanding of larval emigration as well as any reproduction that may be occurring below the waterfall.”

We have modified our SOW by reducing the number of years we will examine Flannelmouth Sucker. Instead of the proposed 10 years (2009-2018), we will obtain N_b estimates for the most recent five years (2015-2019). These years should provide a robust comparison of spatial sampling due to the extension of larval sampling efforts in 2017 and 2018. Effective number of breeder estimates for the 2018 San Juan River larval collections were funded for FY18; therefore, we could only include estimates for both endangered species collected in 2019. At the request of the PO, and other reviewers, we will also obtain N_b estimates for up to 120 larval Razorback Sucker and Colorado Pikeminnow collected below the waterfall. This should allow us to examine larval emigration and reproduction below the waterfall. If these collections are short of the 120 individuals of each species, then we will include additional sampling of Razorback Sucker, Colorado Pikeminnow or Flannelmouth Sucker in the river proper to ensure our budget remains neutral. If this occurs, we will address this concern with the SJRRIP when larval sampling is completed.

Wesche: “The proposal is well written, clear, concise and understandable to someone such as I with little background in such matters. I was glad to see reference made to work done with razorback sucker from Lake Mojave. This raised the question if there might be a possibility to include razorbacks from the Upper Basin Program in this analysis?”

Thank you for the recommendation. We think this information could be very valuable to the Program; however, at the moment we would have to further investigate what samples are available and the additional cost of obtaining those estimates from the Upper Basin. If comparative Upper Basin estimates are of interest to the Program, we would be willing to submit this proposed question to the SJRRIP for FY20.

Lamarra: “This study is extremely important to “calibrate” the results of the previously razorback investigations. This study should have a high priority.”

Thank you for your support.

Davis: “More explanation regarding how the comparison of N_b/N_a ratios of Flannelmouth Sucker and Razorback Sucker will help the Program better manage for Razorback Sucker would be informative. If we assume that the ratio is high for Flannelmouth Sucker how will this help in the identification of other biological and/or environmental factors limiting reproductive potential of Razorback Sucker? I fully realize the risk in this assumption, given that I assumed N_b of Razorback Sucker would be higher than what it was, but outside of providing a comparative context I’m unclear on how this analysis would aid us in tackling the potential problem of low N_b with Razorback Sucker. Without population estimates how will you calculate N_b/N_a ratio for Flannelmouth Sucker?”

Our current approach for obtaining N_b/N_a ratios for Flannelmouth Sucker is to use CPUE data. In order to put N_b estimates in context with Razorback Sucker, we will also use CPUE estimates for Razorback Sucker in addition to the previously reported N_a estimates. Assuming efficiency of catches don’t differ between the species, we think this should provide estimates at the resolution necessary to evaluate potential differences in reproductive output between the species.

The present goal of the SOW is to provide comparative context for Razorback Sucker to help us better understand the significance of the low N_b estimates already obtained. The scope of this work lacks the ability to solve the potential problem of low N_b estimates. Instead, we seek to obtain a better understanding as to why we are observing annually low N_b . For example, if spatial analyses of Flannelmouth Sucker suggest a majority of the population is spawning relatively high in the system, then results could suggest their recruitment success is due, in part, to access to adequate spawning habitat. Conversely, it could suggest the low N_b estimates observed for Razorback Sucker could be due to apparent barriers (e.g., PNM weir) resulting in their inability to access suitable spawning habitat.

Hubert: “Representative sampling is a major concern. First, can the researchers be assured that the larval samples available to them represent the full range of times and locations where Flannelmouth Sucker larvae are present in the river? Even with large numbers of preserved larvae, it cannot be assured that the samples represent they full genetic composition of the larvae that were present in the river because of the short duration of sampling efforts. Second, in drawing the subsample of about 1,200 larvae for genetic analysis, can the full array of the genetic composition of the larger be assured? It is good that the researchers will be sure to include fish from the 3 - 4 indicated spawning periods in the sample. However, what is the relative contribution of larvae during each sampling period? Similarly, the full spatial distribution of larvae in the samples needs to be considered in the drawing of the subsample.

It is possible the low N_b estimates for Razorback Sucker are due to a larval sampling problem (i.e., a representative sample of all larvae produced was not made available for genetic analyses) and insights into that problem may be elucidated by such “what if” games with the Flannelmouth Sucker data. For example, if larvae were sampled from only one known spawning period, what would the estimate of N_b be in comparison to the N_b from the full subsample?

We do not disagree with the concern that representative sampling could bias our estimates if larval sampling problems exist; however, we cannot address that within the context of this work. Although that is a major assumption and caveat to our results, if the larval sampling that already occurs is not representative, then current larval monitoring methods are potentially inadequate for addressing questions related to annual reproduction of the San Juan River fishes. We, however, are working under the assumption that this long term monitoring program adequately reflects spawning. Nonetheless, we acknowledge the need for additional investigations into these potential sampling biases.

Our ability to summarize all the spatial and temporal variation of spawning endangered fishes in the San Juan River is currently limited by the extent of larval sampling. At present, our approach to adequately

reflect the relative contribution of larvae is to ensure our samples represent the relative abundance of the larval monitoring samples both spatially and temporally. For example, if one larval collection (i.e., site) comprises 10% of the total number of larval fish collected that year, we will ensure our sampling is reflective of that 10%. We chose this approach in order to reduce individual biases. If a large collection is representative of a larger spawning event, then we do not want to misrepresent those densities, and by assumption, genetic contribution.

The recommendation of testing “if larvae were sampled from only one known spawning period, what would the estimate of N_b be in comparison to the N_b from the full subsample?” is a good question. We will try and address this question with the Flannelmouth Sucker dataset in our FY19 SOW. This may have the potential to elucidate or resolve concerns pertaining to subsampling.

Zeigler “The technical aspects of this project are well described and will meet the objectives as outlined in the SOW. No alterations to the technical aspects are needed.

Minor Comments

- Line 17: Insert “to” between potential and inform”

Thank you for your support and notifying us of our grammatical error. It has been corrected.