DETERMINING THE NATAL ORIGIN OF SAN JUAN RIVER RAZORBACK SUCKER THROUGH ISOTOPIC SIGNATURES AND ELEMENTAL ANALYSIS OF FIN RAYS
FISCAL YEAR 2016 PROJECT PROPOSAL

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Introduction:

Razorback Sucker, *Xyrauchen texanus,* was listed as endangered under the Endangered Species Act in 1991. Due to low numbers and natural recruitment to spawning age (Minckley 1983, Bestgen et al. 2002), population supplementation is provided by hatcheries. Hatcheries that produce Razorback Suckers and supplement wild populations in the San Juan River are Southwestern Native Aquatic Resources and Recovery Center (SNARRC), Uvalde National Fish Hatchery (NFH), and Ouray NFH-Grand Valley Unit (GVU). As of 2014, Razorback Sucker from Uvalde National Fish Hatchery were no longer raised for stocking into the San Juan River. To more effectively manage this endangered species, it is necessary to differentiate between wild spawned versus hatchery stock Razorback Sucker in the San Juan River. Wild fish are individuals naturally spawned in the San Juan River and hatchery fish are specimens propagated in a hatchery.

While it is easy to determine natal origin of fish with passive integrated transponder (PIT) tags, it is not always possible to ascertain if fish captured without PIT tags are actually wild fish as tags may have been lost or specimens not tagged. If fish captured without tags are considered wild, wild fish numbers may be inflated and may not accurately represent natal origin composition in the San Juan River. The inability to differentiate between wild and hatchery fish (i.e., determine natal origin) can hinder progress in recovery of the species (Barnett-Johnson et al. 2007). The percent of non-PIT tagged Razorback Sucker captured in the San Juan River has fluctuated between 38% in 2006 and 6.7% in 2014. Of the 1,256 sub-adult and adult Razorback Sucker collected in the San Juan River in 2014, 90 were not PIT tagged (Table 1).

Otolith microchemical analysis can be used to determine natal origins of fish, but this technique requires euthanizing specimens. Alternatively, fin ray microchemistry offers a non-lethal method to determine natal origins of fish. Fin rays are calcified structures that can be collected without sacrificing fish. Like otoliths, fin rays accrete isotopic and elemental materials that are linked with environmental conditions and can be used to ascertain natal origin and other details of their life history. The results of our 2014 study examining isotopic signatures and elemental analysis showed that, in the San Juan River, fin ray microchemistry is better correlated than otolith microchemistry with that of the environment.

In 2014, in the San Juan River, we completed creation of a Razorback Sucker fin ray and otolith microchemistry library, and used a combination of isotopic ($^{87}Sr/^{86}Sr$) and elemental (Sr/Ca, Ba/Ca) microchemistry to successfully categorize Razorback Sucker to their hatchery of origin (SNARRC, Uvalde NFH, Ouray NFH-GVU) or the San Juan River. This validity of the technique was determined by generating fin ray based “blind classifications” of PIT tagged Razorback Sucker with known natal origins. In addition to assigning Razorback Sucker to hatchery of origins, we were also able to confirmation the collection of a wild spawned Razorback Sucker.

Table 1. Number of sub-adult and adult razorback sucker collected per year and the number of specimens lacking PIT tags.

Adult Razorback Sucker Collected					
Sample Year	**# without PIT tags**	**# with PIT tags (Stocked only)**	**Total Collected**	**Percent without PIT tags**	**# Larval Razorback Sucker collected**
2004	34	381	415	8.2	41
2005	34	307	341	10.0	19
2005	213	338	551	38.7	202
2007	357	708	1,065	33.5	200
2008	184	382	566	32.5	126
2009	184	440	624	29.5	272
2010	164	873	1,037	15.8	1,251
2011	254	1,379	1,633	15.6	1,065
2012	318	1797	2115	14.4	1778
2013	134	1616	1750	7.1	979
2014	90	1256	1346	6.7	612

San Juan River Project Objectives:

1. Use previously developed microchemistry dataset to differentiate between wild spawned and hatchery reared Razorback Sucker.
2. Use previously developed microchemistry dataset to determine natal origin of hatchery reared Razorback Sucker.
3. Report results, accuracy of statistical model, and all pertinent findings.

San Juan River Arm of Lake Powell Project Objectives:

1. Develop a robust dataset of isotopic and elemental measurements from hatchery specimens of Razorback Sucker and water samples from Lake Powell (and it's tributaries).
2. Use isotopic and elemental concentration data generated from LA-ICP-MS to determine feasibility of discriminating natal origin of Lake Powell collected Razorback Sucker through statistical modeling.
3. Test discriminating power of the isotopic and elemental dataset developed under Objectives 1 and 2 for accuracy by using known natal origin fin rays (PIT tagged) from Razorback Sucker collected in the Lake Powell that are not included in the hatchery dataset.
4. Report results, accuracy of statistical model, and all pertinent findings.

Study Area:

The study area is the San Juan River and San Juan River Arm of Lake Powell.

Methods:

Field — Fin rays will be removed from study specimens using antiseptic techniques. Field crews will be provided a water-proof fin ray sampling kit containing sampling instructions, special fin ray clippers, water-proof pens and pencils, isopropyl wipes, and pre-labeled sample envelopes. The fin-ray clippers will be used to remove a 10-15 mm portion of the second fin ray from the right pectoral fin. After the fin ray is removed from an individual fish, it will be placed in a pre-labeled # 1 coin envelope (2.25 inches x 3.5 inches). The species, date of collection, PIT tag number, length (standard and total), weight, and location (river mile) of the captured individual will be recorded on each envelope. The fin ray clippers will be cleaned with an isopropyl wipe each time fin rays have been removed from a specimen.

Laboratory processing of fin rays – After removal and return to the laboratory, fin rays will be sonicated in Milli-Q water for and subsequently embedded in epoxy resin and cut transversely using an Isomet low-speed saw with diamond wafering blades to expose annuli. Fin rays will be mounted on microscope slides using Krazy glue, sonicated in Milli-Q water for five minutes, and air-dried for 24 hours. Sample slides will be placed in clean plastic petri dishes and taken to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility, Woods Hole, Massachusetts, for microchemical analysis.

Laboratory – (Woods Hole Oceanographic Institution) – For the San Juan Arm of Lake Powell, water samples will be analyzed using inductively coupled plasma mass spectrometry for strontium isotopes (isotopic analysis) and elemental concentrations of barium, calcium, magnesium, manganese, and strontium to determine if microchemical signatures of sources (hatcheries or wild) differ enough from each other to be detectable in our fin ray samples. This process was completed for microchemistry work in the San Juan River in 2014; however, due to the many possible sources of fish in the San Juan Arm of Lake Powell (Table 2), these analyses will be needed for any Lake Powell work.

Fin rays will be analyzed at WHOI via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for strontium isotopes (isotopic analysis) and elemental concentrations of barium, calcium, and strontium. Fin rays obtained from hatcheries will serve as reference and create a reference data set of measurements for known origin fish. Fin rays from non-tagged Razorback Suckers will be compared to the data set of known origin fish to determine natal origin.

Precision and accuracy of sample analyses are determined by periodic analysis of reagent blanks and Canadian (FEBS-1; National Research Council [Canada] Institute for National Measurement Standards; Sturgeon et al. 2005) and Japanese certified otolith reference materials (NIES-022; Japan National Institute for Environmental Studies fish otolith; Yoshinaga et al. 2000). These materials are not only analyzed at the beginning and end of the daily session but are also introduced to the mass spectrometer (analyzed) after every fifth fin ray has been sampled. Analysis of the blanks and standards is the same as performed on the fin rays (i.e., same five elements and same 10 isotopes per element). As these samples are a liquid, they are not ablated but instead transported into the analytic chamber via argon gas and analyzed at approximately one-second intervals for about one-minute. These data are used to determine and correct (if necessary) the "drift" in the mass spectrometer during the daily session so that adjustments can be made to the elemental values of the individual fin rays.

Analysis:

Data Analysis — Because of the complicated nature of the data generated, we were strongly advised to have an expert in analytical chemistry review our data before attempting analyses and interpretation. ASIR will hire an expert to perform this review of the data prior to and after analyses to ensure that our interpretations are sound. Data analysis will include importing all data into a useable format for analysis using statistical software. Elemental concentration readings for each fin ray will be examined for analytical suitability. Adequate fin rays are those with readings above the limit of detection (LOD) for each element. The LOD for each element will be determined after blanks are run for each element. Following data manipulation, to establish natal origin signature from concentrations, a predictive model will be created and tested for classification accuracy. For the San Juan River, fin rays will be categorized to site of propagation and rearing by comparing fin ray microchemistry against the microchemistry dataset produced in 2014 (Table 2). For San Juan River Arm of Lake Powell, a data set (or library) of known natal origin fish fin rays will be created to determine if fish fin rays from unknown origin fish can be correctly classified to their site of propagation and rearing (specific hatchery or wild). Lists of sources that will need to be analyzed to complete the microchemistry dataset for the San Juan Arm of Lake Powell are included in Table 2.

Table 2. Status of microchemical dataset (isotope and elemental data) for the San Juan River[1] portion of the study and for the expansion of the proposed study to include Lake Powell[2].

Natal Origin/Source		
Hatcheries	**Isotopic Analysis**	**Elemental Analysis**

SNARRC [1]	Completed	Completed
Uvalde NFH [1]	Completed	**Incomplete**
Ouray NFH-GVU [1]	Completed	Completed
Ouray NFH [2]	**Needed**	**Needed**
Lentic and Lotic Systems	**Isotopic Analysis**	**Elemental Analysis**
San Juan River [1]	Completed	Completed
NAPI Ponds [1]	Completed	Completed
Colorado River [2]	**Needed**	**Needed**
Green River [2]	**Needed**	**Needed**
Yampa River [2]	**Needed**	**Needed**
Escalante River [2]	**Needed**	**Needed**
Lake Powell – San Juan River Arm [2]	**Needed**	**Needed**
Lake Powell – Colorado River Arm [2]	**Needed**	**Needed**

Products:

A draft report will be presented to the San Juan River Basin Biology Committee for review by 31 March 2017. Upon receipt of written comments, that report will be finalized and disseminated to members of the San Juan River Basin Biology Committee by 30 June 2017. Electronic copies of the data will be transferred to the San Juan River database manager. Fish fin rays collected from this study will be curated in the Division of Fishes, Museum of Southwestern Biology (MSB), Department of Biology, at the University of New Mexico under a MSB contract with the SJRBRIP.

Meetings:

Researchers are required to attend a minimum of two meetings annually and report on annual monitoring projects. The two meetings (February and May) require researchers present PowerPoint presentations outlining the results and that years findings. Each meeting lasts about three days (which includes travel time). No additional costs will be required for the presentation of this material as it will be incorporated into the San Juan River larval fish monitoring presentation.

Literature Cited:

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Project Title: Determining the natal origin of SAN JUAN RIVER Razorback Sucker through microchemical signatures of fin rays

Proposed budget based on fin ray samples from 200 fish and isotopic/elemental analysis performed on one fin ray per fish

Personnel			
FIELD WORK			
MATERIAL GATHERED UNDER CURRENT SOW'S			
Adult Monitoring	no charge	$	0
Non-native Removal	no charge	$	0
PNM Fish Ladder	no charge	$	0
FIN RAY PREPARATION (200 fish, 200 fin rays)			
Fisheries Technician	5 staff days	$	1,366
TASKS: *Class 100 clean room processing of fin rays: selection, examination, sonification, preparation, mounting, and accounting of sample materials*			
WHOI ANALYTICAL RUNS (200 fish, 200 fin rays)			
Fisheries Technician	10 staff days	$	2,733
10 staff days per trip x 1 trip (1 staff; includes travel &12 hr days)			
TASKS: *Perform analytical runs of fin rays*			
Fisheries Biologist I	10 staff days	$	4,442
10 staff days per trip x 1 trip (1 staff; includes travel &12 hr days)			
TASKS: *Perform analytical runs of fin rays*			
OFFICE WORK (ANALYSIS OF DATA & REPORT PRODUCTION)			
Fisheries Biologist I	20 staff days	$	8,883
Office effort – 20 staff days			
TASKS: *Post-ablation data processing (photography, review, lengths), data analysis, draft report preparation, review redraft and submission, development of presentation of study for annual meetings*			
	Personnel: Total	**$**	**16,058**
Materials and Supplies			
Fin Ray Preparation (Class 100 cleaning facility)			

Slides and mounting media		$ 100
Washing/cleaning (sonicator, hydrogen peroxide, HCl, etc.)		$ 200
Non-metallic (ceramic) cleaning and mounting tools		$ 100
Isomet 5" saw blade (11-4254)		$ 154
Buehler EpoThin^2 Resin and Hardener (48 oz)		$ 200
		$
	Fin Ray Preparation: Subtotal	$ 754

Analysis at WHOI (published rates) for two five-day trips		
Element 2 argon plasma mass spectrometer (daily user fee) x 2	$ 1,435/day	$ 2,870
Neptune isotope plasma mass spectrometer (daily user fee) x 2	$ 1,700/day	$ 3,400
193 nm LASER (daily user fee) x 4	$ 125/day	$ 500
"Night" argon daily user fee (for long analytical sessions) x 4	$ 125/day	$ 500
	Mass Spectroscopy: Subtotal	$ 7,270
	Materials and Supplies: Total	$ **8,024**

Travel and Per Diem		
Elemental Analysis at WHOI		
Off-season rates (15 November - 15 April)		
Travel - (airlines; Albuquerque, NM to Providence, RI)	$ 700/r.t.	$ 1,400
Round-trip (r.t.) tickets x 2 staff x 1 trip		
Travel - (car rental and fuel)	$ 90/day	$ 450
Five days per trip x 1 trip		
Per Diem - (food and expenses)	$ 50/day	$ 500
Five days per trip x 2 staff x 1 trip		
Hotel - (Falmouth/Cape Cod)	$ 100/night	$ 1,000
Five days per trip x 2 staff x 1 trip		
	Travel and Per Diem (WHOI): Total	$ **3,350**

Personnel Total	$	**16,058**
Materials and Supplies Total	$	**8,024**
Travel and Per Diem Total	$	**3,350**

Project Subtotal	$	**27,432**
IDC (20%)	$	**5,486**
2016 Estimated Costs: GRAND TOTAL	$	**32,918**

Project Title: Determining the natal origin of LAKE POWELL Razorback Sucker through microchemical signatures of fin rays

Proposed budget based on fin ray samples from 500 fish and isotopic/elemental analysis performed on one fin ray per fish

Personnel			
FIELD WORK			
MATERIAL GATHERED UNDER CURRENT SOW'S			
Lake Powell Project		no charge	$ 0
FIN RAY PREPARATION (500 fish, 500 fin rays)			
Fisheries Technician		10 staff days	$ 2,733
TASKS: *Class 100 clean room processing of fin rays: selection, examination, sonification, preparation, mounting, and accounting of sample materials*			
WHOI ANALYTICAL RUNS (500 fish, 500 fin rays)			
Fisheries Technician		20 staff days	$ 5,466
10 staff days per trip x 2 trips (1 staff; includes travel &12 hr days)			
TASKS: *Perform analytical runs of fin rays*			
Fisheries Biologist I		20 staff days	$ 8,883
10 staff days per trip x 2 trips (1 staff; includes travel &12 hr days)			
TASKS: *Perform analytical runs of fin rays*			
OFFICE WORK (ANALYSIS OF DATA & REPORT PRODUCTION)			
Fisheries Biologist I		30 staff days	$ 13,325
Office effort – 30 staff days			
TASKS: *Post-ablation data processing (photography, review, lengths), data analysis, draft report preparation, review redraft and submission, development of presentation of study for annual meetings*			
	Personnel: Total		**$ 27,674**
Materials and Supplies			

Fin Ray Preparation (Class 100 cleaning facility)			
Slides and mounting media		$	100
Washing/cleaning (sonicator, hydrogen peroxide, HCl, etc.)		$	200
Non-metallic (ceramic) cleaning and mounting tools		$	In SJR budget
Isomet 5" saw blade (11-4254)		$	In SJR budget
Buehler EpoThin^{2} Resin and Hardener (48 oz)		$	200
		$	
	Fin Ray Preparation: Subtotal	$	500

Analysis at WHOI (published rates) for two five-day trips			
Element 2 argon plasma mass spectrometer (daily user fee) x 5	$ 1,435/day	$	7,175
Neptune isotope plasma mass spectrometer (daily user fee) x 5	$ 1,700/day	$	8,500
193 nm LASER (daily user fee) x 10	$ 125/day	$	1,250
"Night" argon daily user fee (for long analytical sessions) x 10	$ 125/day	$	1,250
Isotopic Analysis of Lake Powell water samples (x 8)	$ 250/each	$	2,000
	Mass Spectroscopy: Subtotal	$	20,175
	Materials and Supplies: Total	$	**20,675**

Travel and Per Diem			
Elemental Analysis at WHOI			
Off-season rates (15 November - 15 April)			
Travel - (airlines; Albuquerque, NM to Providence, RI)	$ 700/r.t.	$	2,800
Round-trip (r.t.) tickets x 2 staff x 2 trips			
Travel - (car rental and fuel)	$ 90/day	$	900
Five days per trip x 2 trips			
Per Diem - (food and expenses)	$ 50/day	$	1,000
Five days per trip x 2 staff x 2 trips			
Hotel - (Falmouth/Cape Cod)	$ 100/night	$	2,000
Five days per trip x 2 staff x 2 trips			
	Travel and Per Diem (WHOI): Total	$	**6,700**

Personnel Total	$	**27,674**
Materials and Supplies Total	$	**20,675**
Travel and Per Diem Total	$	**6,700**

Project Subtotal	$	**55,049**
IDC (20%)	$	**11,010**
2016 Estimated Costs: GRAND TOTAL	$	**66,059**