Proposal: Recruitment limitations and trophic dynamics of Colorado Pikeminnow in the Colorado River Basin

Principal Investigator

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Background

Altering flow regimes through impoundment and water withdrawal can have a marked impact on trophic structure and ecosystem properties in fluvial systems. Specifically, changes in flow can alter primary production and macroinvertebrate assemblages having a “bottom-up” impact on higher trophic levels (Chester and Norris 2006). The introduction of invasive predators can increase predation pressure on lower trophic levels and create competitive interactions with native predators (Johnson and Agrawal 2003). In addition, invasion of lower trophic levels can alter native prey abundance through competition, change prey availability, or reduce food quality, leading to reduced fitness of native predators (Carlsson et al. 2009). Most often this occurs when an invasive prey is less nutritionally optimal or predation on invasive prey is limited.

The San Juan and Upper Colorado River Basins have undergone invasions of predators and prey species along with alteration of the natural flow regime (Holden and Wick 1982; Tyus et al. 1982). These changes have likely altered trophic linkages which could partly explain the declines of Colorado Pikeminnow (*Ptychocheilus lucius*; CPM). In the San Juan River, extensive augmentation of age-0 CPM has failed to establish natural recruitment and few stocked fish apparently reach adulthood (Durst 2015). Stocked CPM are typically recaptured at age-1 and age-2, but high mortality makes larger size classes less abundant. Other Upper Colorado River Basin drainages have a more equal distribution of size classes with a greater distribution of adult fish compared to the San Juan River (Figure 1; unpublished data).

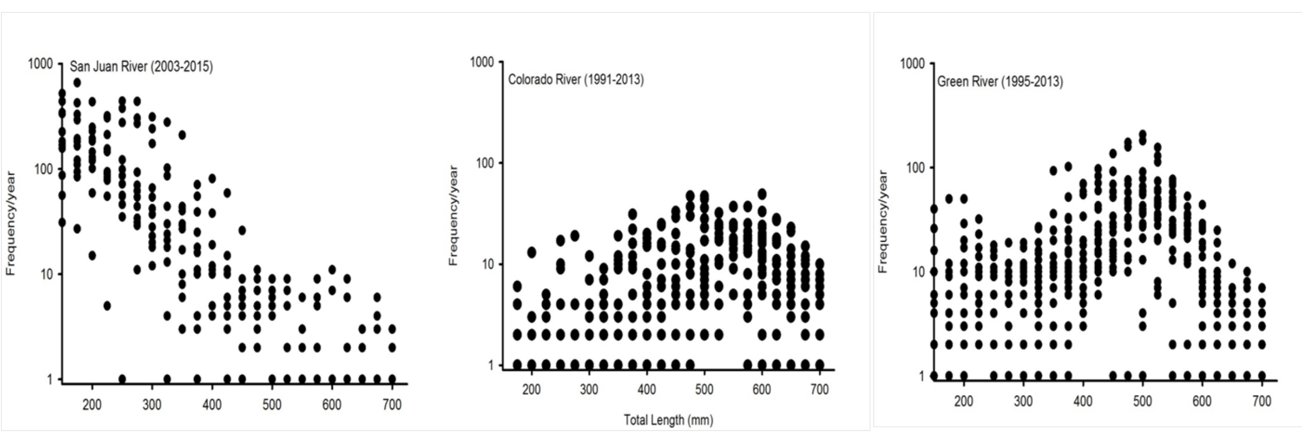

Figure 1. Length-frequency scatter plot showing the size distribution in the San Juan River (2003-2015; Left), Colorado River (1991-2013; center) and Green River (1995-2013). Unpublished data.

The sharp decline in abundance of stocked CPM coincides with a historically observed ontogenetic diet shift from primarily invertebrates to piscivory (Vanicek and Kramer 1969). Additionally, recent stable isotope analyses from the San Juan River failed to document the hypothesized shift in trophic position (Franssen 2014) although there was a gradual increase in trophic position with total length. These results indicate that CPM may be experiencing prey limitations that could be attributed to the extirpation of native prey species (Roundtail Chub, *Gila robusta*) or gape limitations for predation on non-native small bodied fishes (Franssen 2007; Franssen 2014).

Compared to the San Juan River, the Colorado and Green Rivers have natural (unstocked) populations of CPM including fish in larger size classes and potentially have a greater abundance of native prey. In order to attribute lower trophic position and lack of diet shifts to prey limitation, comparisons between the San Juan River, Colorado, and Green River populations would be useful. In addition, to better understand the trophic positon of CPM in the San Juan River, Colorado, and Green Rivers it is necessary to determine the composition of prey items in the diet. As traditional stomach content analysis is not feasible with rare and endangered fish, we will use alternative methods to qualitatively assess difference in diet between the Green, Colorado, and San Juan Rivers. Specifically, this proposal will seek to address two main questions: 1) Do CPM exhibit previously observed ontogenetic diet shift in the Green or mainstem Colorado Rivers that was not observed in the San Juan River? and 2) what fish and non-fish prey are contributing to the diet of CPM? We hypothesize that populations with greater recruitment (Green, Colorado) will exhibit expected diet shift with adults being exclusively piscivorous. In addition, we hypothesize that adult Colorado Pikeminnow in the SJR will have a higher reliance on benthic macroinvertebrates compared to Colorado and Green River populations.

Objectives

1) Compare the trophic position of Colorado Pikeminnow using carbon and nitrogen stable isotope signatures in the San Juan River, Green River, and Upper Colorado River.
2) Measure and compare the composition of fatty acids in CPM tissue lipids as an indicator of nutrient flow pathways, diet, and fish health among these three rivers.
3) Conduct stable isotope and fatty acid analysis on common primary producers, invertebrates, and fishes in order to compare trophic structure and diet.
4) Assess differences in prey availability (density and assemblage) between basins using respective small bodied fish monitoring data.

Methods

Study Site

Sampling will be conducted in the Upper Colorado River, Green River, and the San Juan River. A 128 km sample reach will be selected within each basin where Colorado Pikeminnow are the most abundant based on annual monitoring efforts. Sample reaches in the upper basin will be upstream of the Green/Colorado River confluence. San Juan samples will be collected from each reach corresponding to Franssen et al. (2014).

Tissue Sampling

Colorado Pikeminnow and other large-bodied fishes will be collected via raft-based electrofishing during monitoring, population estimates, and non-native removal efforts conducted by the San Juan River Recovery Implementation Program (SJRIP) and the Upper Colorado River Endangered Fish Recovery Program (UCRP). Small-bodied and juvenile fishes will be collected via seining. Invertebrates will be sampled in all locations using mesh screens in riffles and backwater habitats and preserved in salt. Algae will be collected from cobble, cleaned, and preserved in the field. Terrestrial vegetation will also be collected to account for carbon sources including cottonwood, willow, grasses, Russian olive (leaves and seeds).

Stable isotopes signatures for carbon and nitrogen ($\delta^{15}N$ and $\delta^{13}C$) will be assessed from fin clips preserved in table salt for large-bodied species and those of conservation concern (*C. latipinnis, C. discobolus, I. punctatus, M. dolomieu, G. robusta, and P. lucius*) (Arrington and Wineminner 2004). Small-bodied fishes (juvenile *C. latipinnis*, juvenile *C. discobolus*, juvenile *M. dolomieu*, *R. osculus*, *C. lutrensis*, and *P. promelas, I. punctatus)* will be preserved whole in table salt. Fatty acid signatures will be accessed via a muscle biopsy from the dorsal surface for large bodied fishes and those of conservation concern (see list above). Small bodied fishes will be preserved whole. All samples for fatty acid analysis will be preserved in a chloroform and antioxidant solution. Samples will be stored on wet ice in the field and placed in a -80 °C freezer for storage.

In order to account for seasonal variation in diet and trophic position all samples will be collected during late summer-fall sampling efforts. Stable isotope data from the Colorado and Green Rivers will be collected in fall and compared to samples collected by Franssen et al. (2014). Fatty acids samples will be collected from all study sites in fall. We will seek to collect at least 15 samples from each river within each category (n=33) of primary producers, prey, and fish species (by size class for large bodied fishes; Table 1).

Laboratory Analyses

Stable isotope analysis methodology will follow those outlined in Franssen et al. (2014). Samples will be washed, dried, and ground prior to analysis at the University of New Mexico's Center for Stable Isotopes to remain consistent with the previous study. Both isotopic values will be reported in the common notation:

$$\delta^{15}N = [(R_{sample}/Rs_{tandard})\text{-}1] \times 10^{3}$$

$$\delta^{13}C = [(R_{sample}/R_{standard})\text{-}1] \times 10^{3}$$

Where R is the ratio between heavy and light isotopes ($^{13}C/^{12}C$; $^{15}N/^{14}N$).

Trophic position will be determined by comparing Colorado pikeminnow relative to a secondary consumer (*R. osculus)* and based on fractionation rates established in experimental trials in Franssen et al. (2014). Trophic position will be calculated as:

$$TP = [(\delta^{15}N\ \textit{P. lucius} - \delta^{15}N\ \textit{R. osculus})/\ 2.48] + 3$$

Where the mean fractionation rate for *P. lucius* is 2.48

Fatty acid samples (whole fish and muscle biopsy) will be frozen and homogenized prior to analysis. Fatty acid analyses will follow methods outlined in Budge et al. (2006) and Feiner et al. (2016). Fatty acids will be extracted from samples, transformed into fatty acid methyl esters (FAME) and separated by gas chromatography with Flame Ionization Detection. Fatty acids of interest will be identified and quantified by comparison to known standards and the abundance of each fatty acid will be reported as mass percentage of total identified FAME.

Statistical Analysis

A multivariate analysis of variance (MANOVA) will be used to simultaneously test for differences in $\delta^{15}N$ and $\delta^{13}C$ for Colorado Pikeminnow among rivers and size classes. Univariate analysis of variance will be used to assess individual differences of the stable isotopes. NMDS will be used to visualize the differences in fatty acid profiles among species and among rivers; the robustness of apparent divisions between groups will be tested using ANOSIM. SIMPER analysis will be used to contrast the fatty acids that show the greatest differences between groups.

Table 1. Number of samples per year that will be collected for stable isotope and fatty acid analysis. Stable isotope samples do not include samples previously collected in the SJR.

Taxa	Size Classes	Stable Isotope	Fatty Acid
Primary producers			
Algae	-	30	45
Cottonwood	-	30	45
Grasses	-	30	45
RO seeds	-	30	45
RO leaves	-	30	45
Invertebrates			
Crayfish	-	30	45
Chironomids	-	30	45
Ephemeroptera	-	30	45
Megaloptera	-	30	45
Odonata	-	30	45
Native Fishes			
C. discobolus	2	60	90
C. latipinnis	2	60	90
G. robusta	2	60	90
P. lucius	5	150	225
R. osculus	1	100	150
Non-native Fishes			
C. lutrensis	1	30	45
E. lucius	2	60	90
I. punctatus	2	60	90
M. dolomieu	2	60	90
N. stramineus	1	30	45
P. promelas	1	30	45

Total	31	1000	1500

Small Bodied Fish Availability

In order to understand any observed variation in the trophic position or fatty acid signatures of pikeminnow among the San Juan River and Upper Colorado basins we will assess differences in small bodied prey fish density and assemblage. To do this, we will compile the available small bodied fish data from the monitoring efforts in the San Juan River and Upper Colorado Basin. Differences in prey density and assemblage will help to explain differences between sites. Analysis of Variance will be used to assess differences in small bodied prey densities among basins. Non-metric Multidimensional Scaling and Multi-response Permutation Procedure will be used to test for differences in small bodied fish assemblages between basins.

Schedule for Completion

Inter-basin comparison sampling will begin in September of 2017 and extend into October. Timing of sampling will be determined by monitoring and non-native removal scheduled by US Fish and Wildlife Service. Stable isotope samples will be delivered to University of New Mexico Stable Isotope Lab in November of 2017 and completed by January 2018. Fatty Acid Analyses will also begin in November of 2017 and be completed by January 2018. Fatty Acid analyses will begin in November 2017 and continue until August 2018. Results will be presented to the SJRIP in February of 2019. Final reports and publication drafts will be completed by June 2019.

Literature Cited

Albrey Arrington, D., and K. O. Winemiller. 2002. Preservation Effects on Stable Isotope Analysis of Fish Muscle. Transactions of the American Fisheries Society 131:337–342.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22(4):759-801.

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Chester, H., and R. Norris. 2006. Dams and flow in the Cotter River, Australia: Effects on instream trophic structure and benthic metabolism. Hydrobiologia 572(1):275–286.

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Vanicek, C. D., and R. H. Kramer. 1969. Life history of the Colorado Squawfish, Ptychocheilus lucius, and the Colorado Chub, Gila robusta, in the Green River in Dinosaur National Monument, 1964-1966. Transactions of the American Fisheries Society 98(2):193–208

Budget Justification

The full cost of the stable isotope analyses will be contracted through the University of New Mexico's Center and samples will be shipped when needed. Fatty Acid analyses will be conducted at the Purdue University Aquatic Ecology Laboratory. A field technician will be hired for four months to assist with preparation and with collecting tissue and environmental samples in the field. An additional technician will be hired to conduct fatty acid analyses. A portion of the lab manager's annual salary is budgeted to oversee the fatty acid analyses and conduct data processing. Both the manager and FA technician will be paid per sample.

Travel will cover mileage, lodging costs, and attendance at two biology committee meetings to provide updates and results. Mileage will include two round trips to Lafayette, IN to sites in the Colorado River Basin. Mileage also covers driving between sample locations. Lodging and per diem will cover trips to meetings as well as between-trip lodging in the field. The majority of lodging will be camping and only per diem will be needed.

The majority of sample equipment will be available from federal and tribal agencies. Sample equipment will also be available from Purdue University. Also requested is the purchase of two dry boxes and two coolers to facilitate the transport of samples. Other material costs include preservatives and sample vials.

In-kind contribution will include one year of a graduate student assistantship which includes salary, tuition, benefits, and administrative costs. In-kind funding will be provided by Purdue University through National Science Foundation's Graduate Research Fellowship Program.

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Recruitment limitations and trophic dynamics of Colorado Pikeminnow in the Colorado River Basin				
Task Description		**Cost/unit**	**Units/hours**	**Total**
Task	**Item**			
Salaries				
Undergraduate Field Technician	Bi-weekly	$1,000.00	$8.00	$8,000.00
Fringe benefits				
Undergraduate Field Technician	1.00%	$10.00	$13.00	$130.00
Travel				
Travel: 2 X 15 day trips on San Juan River for Task 2 field work (2 people each trip)	Per diem (person/day)	$20.00	$90.00	$1,800.00
Hotels, 4 nights on each field trip	Lodging-Bluff, UT (person/day)	$75.00	$8.00	$600.00
	Vehicle mileage (mile: 3000 miles round trip Lafayette, IN to Bluff, UT and to field sites)	$0.50	$6,000.00	$3,000.00
Travel: Durango, CO for San Juan Researchers meeting (2 people)	Per diem (person/day)	$46.00	$4.00	$184.00
	Lodging-Durango, CO	$100.00	$4.00	$400.00
	Airfare (Lafayette, IN to Durango, CO)	$600.00	$2.00	$1,200.00
Supplies				
Field Sampling Gear	Dry boxes	$500.00	$2.00	$1,000.00
	Cooler	$500.00	$2.00	$1,000.00
	Ice	$2.00	$100.00	$200.00
	Stable Isotope sample vials	$150.00	$3.00	$450.00
	Fatty acid sample vials	$90.00	$15.00	$1,350.00
	Preservatives	$250.00	$1.00	$250.00
	Whirlpacks (24 oz, box of	$105.00	$10.00	$1,050.00

	500)			
	Shipping (FedEx samples to processing lab)	$250.00	$1.00	$250.00
Sample processing				
Stable Isotope sample analysis	Processing fee per sample	$12.50	$1,000.00	$12,500.00
Fatty acit sample analysis	Processing fee per sample	$16.00	$1,500.00	$24,000.00
Office	Phone calls, xerox	$100.00	$1.00	$100.00
		Total		**$57,464.00**
		F&A		**$10,056.20**
		Incl KSU 17.5%		**$67,520.20**