Feeding Ecology of Early Life Stage Razorback Sucker Relative to Other Sucker Species in the San Juan River, Utah

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Abstract

Low survival of early life stage (ELS) Razorback Sucker Xyrauchen texanus in the Colorado River basin, USA, is thought to cause a recruitment bottleneck. Conversely, two other native sucker species, the Bluehead Sucker Catostomus discobolus and Flannelmouth Sucker C. latipinnis, successfully recruit to adulthood. To explore a potential factor leading to this survival discrepancy among species, we used museum-catalogued specimens collected from the San Juan River, Utah, to investigate trophic resource use of co-occurring ELS suckers. We evaluated diet diversity and composition, expecting high overlap in diets among sucker species. All species were significantly different in median SL and varied in the ranges of developmental stages present in collections, with Razorback Sucker generally being the smallest and least developed. Size differences among sampled individuals coincided with a 1.7× higher mean diet richness for Bluehead Sucker and Flannelmouth Sucker compared to Razorback Sucker. The Bluehead Sucker was the only species for which diet richness increased significantly with fish size. Discriminant function analysis was able to distinguish Razorback Sucker from the other two species based on a low frequency of occurrence of all diet items. Nine of twelve diet item categories differed significantly in frequency of occurrence among species. Bluehead Sucker and Razorback Sucker had higher mean carbon and nitrogen stable isotope ratios (δ13C and δ15N) relative to Flannelmouth Sucker, and variation in δ13C was high among individual Flannelmouth Sucker across sites. The δ13C and δ15N of Bluehead Sucker decreased significantly with fish size, but no significant relationship was found for Flannelmouth Sucker or Razorback Sucker. The confounding issue of fish size and species identity in this data set prevented us from completely isolating either of these two effects on differences in diet richness, δ13C, and δ15N. Nevertheless, differences in diet item occurrence across sizes and species reflect differences in resource acquisition among ELS suckers that might be related to recruitment bottlenecks.

Recruitment of freshwater fishes can be greatly influenced by trophic interactions of juveniles (Houde 1994). These early life stage (ELS) fish experience drastic ontogenetic shifts with size and trait development (e.g., gut, fins, and mobility) over relatively short time periods, creating a “critical period” when newly hatched larval fish must acquire resources after yolk absorption to support rapid growth and escape predation (Hjort 1914; Miller et al. 1988; Holzman et al. 2015). Failure to do so can diminish fish survival and potentially cause a recruitment bottleneck.
(Werner and Gilliam 1984). Interspecific differences in ELS fish responses to abiotic and biotic factors can also influence recruitment success (Miller et al. 1988). For instance, some species might be more susceptible to starvation due to lower energy reserves in their yolks (Hunter 1981), while other species might be more susceptible to predation due to smaller size or delayed mobility (Bailey and Houde 1989). Despite the importance of ELS fish survival for population stability (Starrett 1951; Schlosser 1985; Freeman et al. 2001), studies of the trophic ecology of fishes during this life stage are limited (Ross 1986; Childs et al. 1998; Pease et al. 2006; Starks et al. 2016). Increasing our understanding about trophic ecology of co-occurring fishes could help to predict why some species might be unable to successfully transition through this critical period of early life in altered ecosystems.

Colorado River basin fishes in the American Southwest are highly imperiled due to intense water development that has caused rangewide population declines. Consequently, many species were listed under the Endangered Species Act after the construction of large hydropower dams (Minckley and Deacon 1991; Minckley et al. 2003). Native fishes must now contend with highly altered habitats and heavily managed flow regimes that include changes in geomorphology following nonnative plant establishment and artificially restricted movements and habitat loss from river fragmentation (Tyus and Karp 1990; Franssen et al. 2014; Cathcart et al. 2018). Additionally, a suite of nonnative species has been introduced throughout the basin, and they are hypothesized to prey on and compete with native fishes, increasing the imperilment of native species in altered habitats (Minckley et al. 2003). These altered conditions have led some species to experience recruitment bottlenecks despite intensive recovery efforts (Schooley and Marsh 2007).

The Razorback Sucker *Xyrauchen texanus* is an endangered “big-river” species that is maintained in the wild through intense stocking efforts throughout the Colorado River basin. Evidence of successful recruitment (i.e., survival to adulthood) of wild-spawned Razorback Sucker in remnant river fragments is almost nonexistent (except for Lake Mead; Albrecht et al. 2010). Conversely, two native sucker species that co-occur with Razorback Sucker, the Bluehead Sucker *Catostomus discobolus* and Flannelmouth Sucker *Catostomus latipinnis*, have relatively stable, self-sustaining populations despite also being extirpated from most of their historical range (Bezzederes and Bestgen 2002; Budy et al. 2015). Currently, there is no confirmed mechanism explaining the recruitment bottleneck experienced by Razorback Sucker, but investigations into why other native suckers are recruiting could further our understanding on species’ resistance to habitat degradation. Several competing hypotheses have been proposed to explain the lack of recruitment to maturity by Razorback Sucker, including reduced habitat availability (Tyus and Saunders 2000; Clarkson et al. 2005; Bestgen 2008), altered flow and temperature regimes (Bestgen 2008), introduction of nonnative species (Minckley et al. 2003; Marsh and Pacey 2005), habitat degradation (Horn 1996; Franssen et al. 2014), and food limitation (Papoulias and Minckley 1990, 1992; Horn 1996). The presence of multiple and potentially compounding mechanisms affecting the recruitment of imperiled fishes makes it difficult for researchers and managers to mitigate any single factor.

In the San Juan River of New Mexico, Colorado, and Utah, wild-spawned ELS Razorback Sucker have been collected during annual monitoring efforts during every year since 1998 (Barkstedt et al. 2018), yet survival of these fish past their first winter is extremely rare. Early life stages of Bluehead Sucker and Flannelmouth Sucker are also collected during these surveys, but age-1 and older (age-1+) fish of these species are commonly collected (Farrington et al. 2018). The limited detection of juvenile and subadult Razorback Sucker suggests that high mortality is occurring in early life (Guttermuth et al. 1994; Modde 1996; Bestgen et al. 2017). Other studies have been conducted on trophic resource use by ELS Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker, but these studies either have been conducted in lentic habitats (i.e., for Razorback Sucker in reservoirs; Marsh and Langhorst 1988) or did not include some species due to a lack of occurrence (Muth and Snyder 1995; Childs et al. 1998; Seegert et al. 2014). To our knowledge, this is the first study to assess trophic resource use by ELS Razorback Sucker from riverine habitats. As adults and juveniles, Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker generally overlap in diet, but they also have differences in morphology that allow for specialization on particular food items. For instance, Bluehead Sucker have been described as feeding mainly on attached algae by using specialized mouthparts adapted for scraping (Taba et al. 1965; Muth and Snyder 1995; Snyder and Muth 2004), but they also consume macroinvertebrates, detritus, and terrestrial vegetation (Seegert et al. 2014). Flannelmouth Sucker are considered the most general feeders of the three species, and their diets include macroinvertebrates, algae, organic matter, zooplankton, terrestrial vegetation, and bottom ooze (Taba et al. 1965; Muth and Snyder 1995; Seegert et al. 2014). Razorback Sucker feed on a variety of items, including algae, bottom ooze, zooplankton, macroinvertebrates, and detritus (Marsh 1987; Horn 1996), and are thought to be more planktivorous than the other two species, aided by a larger number of gill rakers and a slightly less terminal mouth (Snyder and Muth 2004). Given these differences in morphology as juveniles and adults, it is possible that varying morphologies exist in ELS suckers, allowing each species to specialize on particular food items.
Co-occurring sucker species (family Catostomidae) are common throughout North America, and their continued persistence is challenged by a multitude of threats (Cooke et al. 2005). Increasing knowledge on the natural history and ecology of members of this diverse and imperiled family may assist conservation efforts (Cooke et al. 2012; Covich et al. 2015; Matthews 2015). Our objective was to compare trophic resource use among co-occurring ELS Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker by using museum-voucheried specimens collected from the San Juan River, Utah. Based on gut content and stable isotope analyses, we tested for differences in diet richness and composition among the three sucker species. Previous studies have found high diet overlap between Bluehead Sucker and Flannelmouth Sucker (Childs et al. 1998; Seegert et al. 2014); therefore, we predicted minimal differences in trophic resource use among species. We expected to find a higher proportion of empty stomachs among Razorback Sucker because overwinter survival (i.e., survival to age 1) has been extremely limited in the wild for this species. However, Razorback Sucker also hatch at smaller sizes compared to Bluehead Sucker and Flannelmouth Sucker (Snyder and Muth 2004), which might also limit foraging success due to gape limitation. Size-dependent effects on ELS fish can be very important for survival, likely differ among species, and are not well understood for many species (Werner and Gilliam 1984; Graeb et al. 2004; Bestgen et al. 2006). Furthermore, ELS fish are typically limited in the size of prey items available to them (Graeb et al. 2004).

METHODS

Study area and larval fish collections.—The San Juan River begins in the southern Rocky Mountains of Colorado and flows south and west before its eventual confluence with the Colorado River in southern Utah. Along its course, the San Juan River is fragmented by a major dam (Navajo Dam, forming Navajo Reservoir) and several smaller low-head weirs designed to divert water for agricultural and industrial use (Figure 1). The historical confluence of the San Juan and Colorado rivers is now inundated by Lake Powell, the second-largest reservoir in the United States (volume at full capacity >32 km$^3$; https://www.usbr.gov/uc/rm/crsp/gc/). The San Juan River is a snowmelt-driven system; however, contemporary flows in the river are heavily managed by dam operations at Navajo Reservoir (Propst and Gido 2004; Franssen et al. 2007; Gido et al. 2013). Undammed tributaries still provide natural flow regime cues (e.g., Animas River) and spawning habitat (e.g., McElmo Creek) for some of the native fishes (e.g., Cathcart et al. 2015). Recovery efforts for endangered fishes in the San Juan River include nonnative predator removal (Franssen et al. 2014; Pennock et al. 2018), flow management (Propst and Gido 2004; Gido and Propst 2012), and stocking of hatchery-reared fish (Furr 2016).

As part of an effort to document spawning and recruitment by native fishes occurring in the San Juan River, larval fish monitoring has been conducted every year from 1991 to 2018 (Farrington et al. 2018). These collections consist of seining all available low-velocity habitats along the course of the river to document the occurrence and relative abundance of ELS fish in the San Juan River. Collections are generally made during April–August along 220 km of river (see Barkstedt et al. 2018 for more details). The first collection of Razorback Sucker larvae during these monitoring efforts occurred in 1998. All collections are catalogued in the research collection at the Division of Fishes, Museum of Southwestern Biology (MSB), University of New Mexico, and are made available for use. Throughout this article, we use the term “ELS” to describe fish at various stages of larval development and recently transformed juveniles (Snyder and Muth 2004).

Gut content analysis.—To assess differences in diets among the three sucker species, we first searched the FishNet2 online database housed at the MSB to identify collections in which all three species were captured from the same locality (e.g., backwater) on the same date. We further limited collections to those that contained at least 10 individuals of each species (Table 1). In hopes of reducing variation in observed diets due to spatial or temporal differences in available trophic resources, we chose collections that occurred close together in both space and time. The specimens we chose were collected in May ($n = 1$ collection) and June ($n = 4$ collections) 2007 from five sites. From these five collections, we chose 10 individuals of each species that represented the SL range of captured fish (i.e., if more than 10 fish were collected). Individuals were classified by stage of development according to Snyder and Muth (2004) and were measured to the nearest millimeter SL prior to gut content analysis ($n = 50$ fish per species; Figure 2). The entire digestive tract was removed from each fish, and the contents were placed on a microscope slide. Gut contents were viewed under a compound microscope at 40–200× magnification. Because of subjective limitations with quantifying diets (Baker et al. 2014), we chose to describe diet composition by using frequency of occurrence. This involved recording the presence of various diet items for each individual fish. Diet items were classified into 12 categories, and items occurring in less than 5% of all individuals were grouped into the “rare” category (Table 2). The “detritus” category included all unidentifiable organic material.

Stable isotope analysis.—Stable isotopes are commonly used to assess energy flow in aquatic systems relative to trophic resource use (Finlay 2001; Gido et al. 2006; Pease et al. 2006). Unlike gut content analysis, which provides a
FIGURE 1. The San Juan River basin is located in the Four Corners region of the American Southwest (top-left panel). The river is fragmented by a hydropower dam (Navajo Dam) and associated water storage reservoir (Navajo Reservoir [Res.]) and several smaller weirs located between the Animas and Mancos River confluences (top-right panel; identified with arrows). Sites of larval sucker collections (S1–S5) occurred in a downstream, canyon-bound portion of the river (bottom panel).

TABLE 1. Abundance (relative abundance [%] in parentheses) of early life stage Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker collected in five backwaters of the San Juan River, Utah. River kilometers (rkm) were calculated using the Piute Farms Waterfall (Catheart et al. 2018) as rkm 0 to match river maps used by biologists with the San Juan River Basin Recovery Implementation Program. The total abundance of all fish and the percent sucker abundance are also presented. Specimens were catalogued in the Division of Fishes, Museum of Southwestern Biology (MSB) research collection (University of New Mexico).

<table>
<thead>
<tr>
<th>MSB catalogue numbers</th>
<th>Site</th>
<th>rkm</th>
<th>Bluehead Sucker abundance</th>
<th>Flannelmouth Sucker abundance</th>
<th>Razorback Sucker abundance</th>
<th>Total abundance</th>
<th>Percent sucker</th>
</tr>
</thead>
<tbody>
<tr>
<td>63698, 63699, 63700</td>
<td>1</td>
<td>109.0</td>
<td>331 (73)</td>
<td>22 (5)</td>
<td>17 (4)</td>
<td>454</td>
<td>82</td>
</tr>
<tr>
<td>63712, 63713, 63714</td>
<td>2</td>
<td>100.3</td>
<td>602 (89)</td>
<td>29 (4)</td>
<td>13 (2)</td>
<td>680</td>
<td>95</td>
</tr>
<tr>
<td>63496, 63497, 63498</td>
<td>3</td>
<td>39.4</td>
<td>45 (49)</td>
<td>11 (12)</td>
<td>13 (14)</td>
<td>92</td>
<td>75</td>
</tr>
<tr>
<td>63530, 63531, 63532</td>
<td>4</td>
<td>18.5</td>
<td>58 (52)</td>
<td>10 (9)</td>
<td>19 (17)</td>
<td>112</td>
<td>78</td>
</tr>
<tr>
<td>63460, 63461, 63462</td>
<td>5</td>
<td>16.1</td>
<td>36 (18)</td>
<td>109 (56)</td>
<td>17 (9)</td>
<td>196</td>
<td>83</td>
</tr>
</tbody>
</table>
snapshot in time of an individual’s diet, stable isotopes typically reflect resource use over longer time periods. Relative amounts of $^{15}$N in fish tissue can provide an estimate of trophic level (Vander Zanden et al. 1997). Although $^{13}$C varies little across trophic levels, it allows for differentiation among organic matter sources (e.g., benthic algae versus detritus). After dietary tracts were removed, whole bodies of fish (minus the heads) were retained for isotope analysis. Fish were dried (60°C for ~8 h), either ground whole after freezing with liquid nitrogen or broken up into small pieces, packed into tin capsules, and weighed. Samples were analyzed for carbon and nitrogen stable isotope ratios at the Stable Isotope Mass Spectrometry Laboratory, Kansas State University, using an Elementar Vario Pyro Cube elemental analyzer coupled to an Elementar Vision mass spectrometer with continuous flow capabilities (Elementar Americas, Mt. Laurel, New Jersey). Data are reported on a per mille basis ($\%e$) in delta ($\delta$) notation. Delta values were calculated using the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1,000,$$

where $R$ is equal to $^{13}C/^{12}C$ and $^{15}N/^{14}N$. We used laboratory standards calibrated against international standards: Pee Dee Belemnite as the standard for carbon and atmospheric molecular nitrogen as the standard for nitrogen. Measurement error on routine analysis of laboratory standards was less than 0.1 $\%e$ for both $\delta^{13}C$ and $\delta^{15}N$. Because formalin fixation and ethanol preservation predictably affect carbon and nitrogen isotopic signatures in fish tissue, we corrected the isotopic ratios of our samples by adding 1.1 $\%e$ to observed $\delta^{13}C$ and by subtracting 0.5$\%e$ from observed $\delta^{15}N$ (Edwards et al. 2002).

**Statistical analysis.—** All analyses were conducted in R version 3.5.1 (R Core Team 2018). Fish length data did not meet parametric assumptions, so differences in SL among species were tested with a Kruskal–Wallis one-way ANOVA on ranks and pairwise comparisons were assessed with Tukey’s honestly significant difference (HSD) test. Using individual fish as replicates, we tested for differences in mean diet richness (number of diet item categories in gut contents) among species by using generalized linear mixed-effects models with a Poisson distribution and a log-link function, including site as a random effect. Although we were mainly interested in assessing differences among species, fish size can be a strong determinant of diet and ELS fish in our study exhibited a wide range of SLs and minimal overlap among species. Therefore, we compared models including only the fixed effect of species against models with fixed effects of species and SL and their interaction effects using likelihood ratio tests. We used the “glmer” function in the lme4 package (Bates et al. 2015) to build and run generalized mixed-effects models. The “lmer” function in the nlme package (Pinheiro et al. 2017) was used to compare nested models and an intercept-only model with likelihood ratio tests (Zeileis and Hothorn 2002). We assessed model fit using the “rsquared” function in the piecewiseSEM package (Lefcheck 2016), which calculates the proportion of variance explained by only fixed factors (marginal $R^2$) and by both fixed and random factors combined (conditional $R^2$). Least-squares means and 95% confidence intervals (CIs) for species were calculated using the “effect” function from the Effects package (Fox and Weisberg 2018, 2019). We also assessed the relationship between diet richness and SL for each species independently by using general linear models with a Poisson distribution and log-link function.
To assess differences in diet composition among species, we used discriminant function analysis (DFA) in the flipMultivariates package (https://github.com/Displayr/flipMultivariates/). To assess model accuracy, we first split our data set into a testing portion and a training portion by randomly selecting 70% (n = 105) of observations with replacement to train the model, and we then used the remaining “out-of-sample” data (n = 45) to test the model. Significance of individual predictor variables (i.e., diet item categories) was assessed with multivariate ANOVA, and P-values were corrected for multiple tests by using a false discovery rate correction (Benjamini and Hochberg 1995). Significant P-values are shown in bold italics.

### TABLE 2. Mean frequency of occurrence (FO) of diet item categories for Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker (n = 50 fish per species) collected from five backwaters in the San Juan River, Utah. Diet item categories were used as predictor variables in a discriminant function analysis (DFA) to classify sucker species (LD1, LD2 = coefficients of linear discriminant functions 1 and 2). Overall, the DFA model classified sucker species correctly 65% of the time. The P-values for multiple comparisons were corrected using a false discovery rate correction (Benjamini and Hochberg 1995). Significant P-values are shown in bold italics.

<table>
<thead>
<tr>
<th>Diet item category</th>
<th>Bluehead Sucker FO</th>
<th>Flannelmouth Sucker FO</th>
<th>Razorback Sucker FO</th>
<th>P-value</th>
<th>Total FO</th>
<th>LD1</th>
<th>LD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>0.80</td>
<td>0.78</td>
<td>0.58</td>
<td>0.019</td>
<td>0.72</td>
<td>−0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Detritus</td>
<td>0.90</td>
<td>0.94</td>
<td>0.68</td>
<td>&lt;0.001</td>
<td>0.84</td>
<td>−0.78</td>
<td>0.08</td>
</tr>
<tr>
<td>Diatom</td>
<td>0.80</td>
<td>0.58</td>
<td>0.46</td>
<td>0.002</td>
<td>0.61</td>
<td>−0.81</td>
<td>1.17</td>
</tr>
<tr>
<td>Diptera (a)</td>
<td>0.20</td>
<td>0.32</td>
<td>0.00</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>−1.46</td>
<td>−0.16</td>
</tr>
<tr>
<td>Diptera (i)</td>
<td>0.62</td>
<td>0.84</td>
<td>0.42</td>
<td>&lt;0.001</td>
<td>0.63</td>
<td>−0.63</td>
<td>−0.99</td>
</tr>
<tr>
<td>EPT</td>
<td>0.02</td>
<td>0.12</td>
<td>0.12</td>
<td>0.114</td>
<td>0.09</td>
<td>−0.25</td>
<td>−0.49</td>
</tr>
<tr>
<td>Nematode</td>
<td>0.14</td>
<td>0.24</td>
<td>0.06</td>
<td>0.061</td>
<td>0.15</td>
<td>−0.39</td>
<td>−1.61</td>
</tr>
<tr>
<td>Pollen</td>
<td>0.60</td>
<td>0.44</td>
<td>0.20</td>
<td>&lt;0.001</td>
<td>0.41</td>
<td>−0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>Protist</td>
<td>0.42</td>
<td>0.28</td>
<td>0.20</td>
<td>0.065</td>
<td>0.30</td>
<td>−0.24</td>
<td>0.73</td>
</tr>
<tr>
<td>Sand</td>
<td>0.22</td>
<td>0.24</td>
<td>0.00</td>
<td>&lt;0.001</td>
<td>0.15</td>
<td>−0.81</td>
<td>−0.51</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>0.72</td>
<td>0.84</td>
<td>0.62</td>
<td>0.076</td>
<td>0.73</td>
<td>−0.87</td>
<td>−0.78</td>
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<tr>
<td>Rare</td>
<td>0.24</td>
<td>0.20</td>
<td>0.06</td>
<td>0.037</td>
<td>0.17</td>
<td>−0.39</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*Diptera (a) represents adult stages.*

*Diptera (i) represents immature stages (pupae and larvae).*

*Ephemeroptera, Plecoptera, and Trichoptera.*

*Items with less than 5% frequency of occurrence across all individuals: blue-green algae (0.01), euglena (0.03), macroinvertebrate eggs (0.01), aquatic mite (0.04), plant material (0.03), terrestrial insect (0.04).*

RESULTS

### Collection Composition, Fish Lengths, and Diet Richness

Relative abundance of Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker varied across the five collection sites, ranging from 75% to 95%, and suckers made up 87% of the total individuals collected (Table 1). All fish examined had identifiable material in their guts. Lengths of ELS fish were significantly different among species (ANOVA on ranks: *H* = 115.8, df = 2, *P* < 0.001). Lengths of Bluehead Sucker (median = 12.0 mm SL; interquartile range [IQR] = 11.3–13.0 mm SL), Flannelmouth Sucker (median = 19.5 mm SL; IQR = 15.0–22.0 mm SL), and Razorback Sucker (median = 22.0 mm SL; IQR = 19.5–25.0 mm SL) were used as predictor variables in a discriminant function analysis (DFA) to classify sucker species (LD1, LD2 = coefficients of linear discriminant functions 1 and 2). Overall, the DFA model classified sucker species correctly 65% of the time. The P-values for multiple comparisons were corrected using a false discovery rate correction (Benjamini and Hochberg 1995). Significant P-values are shown in bold italics.
mm SL), and Razorback Sucker (median = 9.0 mm SL; IQR = 9.0–10.0 mm SL) were all significantly different (Tukey’s HSD test: \( P < 0.05 \); Figure 2). Species differed significantly in mean diet richness (Figure 3). The model with only the fixed effect of species was significantly different from the intercept-only model (likelihood ratio = 39.65, df = 2, \( P < 0.001 \), and models including the additive effect of SL or the additive and interactive effects of SL were not significantly different from the species-only model (\( P > 0.05 \); Table 3). Mean diet richness for Bluehead Sucker (least-squares mean = 5.7; 95% CI = 5.2–6.2) and Flannelmouth Sucker (least-squares mean = 5.8; 95% CI = 5.3–6.3) was 1.7\times higher than that of Razorback Sucker (least-squares mean = 3.4; 95% CI = 2.9–3.9). The marginal \( R^2 \) of the species-only model was 0.22 and the conditional \( R^2 \) was 0.22, suggesting that the proportion of variance explained by the fixed effect of species accounted for nearly all of the variance (>99%) explained by the model. Adding the effect of SL only increased the marginal and conditional \( R^2 \) to 0.23–0.24, suggesting that the effect of SL explained very little additional variation. Diet richness for Bluehead Sucker (df = 48, \( P = 0.029 \)) showed a significant positive relationship with SL, but there was no significant relationship for Flannelmouth Sucker (df = 48, \( P = 0.158 \)) or Razorback Sucker (df = 48, \( P = 0.460 \); Figure 3).

**Diet Composition**

The trained DFA model had an overall classification accuracy of 57% on out-of-sample data. The first linear discriminant function (LD1) explained 76% of the variation among species and somewhat separated Razorback Sucker from Bluehead Sucker and Flannelmouth Sucker. No diet item categories loaded positively on the LD1 axis, and the categories were opposite of the centroid for Razorback Sucker (Figure 4). The second linear discriminant function (LD2) explained the remaining 24% of the variation and was driven by separation between Bluehead Sucker and Flannelmouth Sucker. On the LD2 axis, diatoms, pollen, and rare items loaded heavily on the positive end (associated more with Bluehead Sucker), and Diptera immature life stages, Diptera adults, and sand loaded heavily on the negative end (aligned more with Flannelmouth Sucker). Overall, 9 of the 12 diet item categories differed significantly (\( P < 0.05 \); Table 2) in mean frequency of occurrence among species. This was mostly driven by the relatively low frequency of occurrence of all diet items in the gut contents of Razorback Sucker. With the exception of one category (i.e., Ephemeroptera, Plecoptera, and Trichoptera [EPT]), Razorback Sucker had the lowest frequencies of occurrence for all diet item categories.

**Stable Isotopes**

Isotopic signatures also indicated diet differences among species, despite overlap in isotopic space (Figure 5). Flannelmouth Sucker had the highest isotopic niche breadth, which was evident from high variation in isotopic space (Figure 5, left panel). Flannelmouth Sucker also had the largest standard ellipse area, corrected for sample size. Ellipses for Bluehead Sucker and Razorback Sucker were 33% and 51% that of Flannelmouth Sucker, respectively. Bluehead Sucker and Razorback Sucker overlapped most
TABLE 3. Model comparison results using likelihood ratio tests to sequentially compare nested mixed models for diet richness, $\delta^{13}C$, and $\delta^{15}N$ of Blue- head Sucker, Flannelmouth Sucker, and Razorback Sucker. Marginal $R^2$ values represent the proportion of variation explained by only fixed factors; conditional $R^2$ values represent the proportion of variation explained by fixed and random effects. "(1|Site)" represents the random effect of site following model syntax in R.

<table>
<thead>
<tr>
<th>Model</th>
<th>Marginal $R^2$</th>
<th>Conditional $R^2$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>Likelihood ratio</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet richness $\sim 1 + (1</td>
<td>Site)$</td>
<td>0.22</td>
<td>0.22</td>
<td>2</td>
<td>39.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diet richness $\sim$ Species + (1</td>
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<td>0.23</td>
<td>1</td>
<td>3.32</td>
<td>0.068</td>
</tr>
<tr>
<td>Diet richness $\sim$ Species + SL + (1</td>
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<td>0.24</td>
<td>2</td>
<td>3.77</td>
<td>0.152</td>
</tr>
<tr>
<td>Diet richness $\sim$ Species + SL + (Species $\times$ SL) + (1</td>
<td>Site)</td>
<td>0.44</td>
<td>0.73</td>
<td>2</td>
<td>2.72</td>
<td>0.256</td>
</tr>
</tbody>
</table>

$\delta^{13}C$ $\sim 1 + (1|Site)$

<table>
<thead>
<tr>
<th>Model</th>
<th>Marginal $R^2$</th>
<th>Conditional $R^2$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>Likelihood ratio</th>
<th>$P$-value</th>
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<tbody>
<tr>
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<td>0.71</td>
<td>2</td>
<td>57.88</td>
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</tr>
<tr>
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<td>2</td>
<td>2.72</td>
<td>0.256</td>
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</tbody>
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$\delta^{15}N$ $\sim 1 + (1|Site)$

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<tr>
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<th>Conditional $R^2$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>Likelihood ratio</th>
<th>$P$-value</th>
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<td>0.24</td>
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FIGURE 4. Discriminant function analysis biplot of early life stage diet compositions among Bluehead Sucker (CATDIS), Flannelmouth Sucker (CATLAT), and Razorback Sucker (XYRTEX). The first linear discriminant function (LD1) explained 76.22% of the variation among groups and largely separated Razorback Sucker from both Bluehead Sucker and Flannelmouth Sucker. The second linear discriminant function (LD2) separated Bluehead Sucker from Flannelmouth Sucker and explained the remaining 23.78% of the variation. See Table 2 for significance of individual diet item categories (diptera.A = adult Diptera; diptera.I = immature life stages of Diptera). Ellipses are 95% confidence ellipses calculated from a multivariate normal distribution. [Color figure can be viewed at afsjournals.org.]

in isotopic space, while differences in Flannelmouth Sucker $\delta^{13}C$ across sites varied substantially. Species differed significantly in mean $\delta^{13}C$ (likelihood ratio = 57.88, df = 2, $P < 0.001$), and again models with the effects of SL did not differ significantly ($P > 0.05$) from the species-only model (Table 3). Bluehead Sucker (least-squares mean = $-21.7\%e$; 95% CI = $-21.2\%e$ to $-22.1\%e$) and Razorback Sucker (least-squares mean = $-20.9\%e$; 95% CI = $-20.4\%e$ to $-21.3\%e$) both had higher $\delta^{13}C$ than Flannelmouth Sucker (least-squares mean = $-22.2\%e$; 95% CI = $-21.8\%e$ to $-22.7\%e$). The fixed effect of species accounted for 63% of the variation explained by the model (marginal $R^2 = 0.45$), and the random effect of site accounted for approximately 37% of the variation (conditional $R^2 = 0.71$). The $\delta^{13}C$ decreased significantly with SL for Bluehead Sucker (df = 48, $P = 0.014$), but no significant relationship was found for Flannelmouth Sucker (df = 45, $P = 0.928$) or Razorback Sucker (df = 46, $P = 0.723$).

Species differed significantly in mean $\delta^{15}N$ (likelihood ratio = 30.06, df = 2, $P < 0.001$); as with diet richness and $\delta^{13}C$, models including the effects of SL were not significantly different ($P > 0.05$) from the species-only model (Table 3). Bluehead Sucker (least-squares mean = 12.4$\%e$; 95% CI = 11.9–12.9$\%e$) and Razorback Sucker (least-squares mean = 12.2$\%e$; 95% CI = 11.7–12.7$\%e$) had higher $\delta^{15}N$ than Flannelmouth Sucker (least-squares mean = 10.9$\%e$; 95% CI = 10.4–11.4$\%e$). The conditional $R^2$ for the species-only $\delta^{15}N$ model was 0.24, and the fixed effect of species accounted for 75% of the variation explained by the model (marginal $R^2 = 0.18$). Similar to $\delta^{13}C$, the $\delta^{15}N$ of Bluehead Sucker decreased significantly with SL (slope = $-0.375$, df = 48, $P < 0.001$) but did not differ for Flannelmouth Sucker (slope = 0.002, df = 45, $P = 0.964$) or Razorback Sucker (slope = $-0.315$, df = 46, $P = 0.077$).

DISCUSSION

Rather than finding high overlap in the diets of ELS suckers, we found differences in diet richness and
composition among the three species. Specifically, intraspecific variation in diet item composition across individual Razorback Sucker drove the majority of variation among species. Although diet differences among species were confounded by differences in SL (see below), the complete lack of certain diet item categories from Razorback Sucker gut contents—categories that were present in the diets of the other two sucker species—is particularly interesting. For example, adult dipterans (e.g., chironomids) were not present in Razorback Sucker diets but were present in the diets of 20% of Bluehead Sucker and 32% of Flannelmouth Sucker, suggesting that these individuals foraged at the water surface. Conversely, sand was also absent from Razorback Sucker gut contents but was present in the diet for 22% of Bluehead Sucker and 24% of Flannelmouth Sucker, indicating benthic feeding. These results suggest that differences in the feeding ecology of ELS suckers might include differential habitat use and resource partitioning (Markle and Clauson 2006), whereby Bluehead Sucker and Flannelmouth Sucker feed at both the water surface and in the benthos, while Razorback Sucker are more limited in their foraging behavior. Markle and Clauson (2006) found ontogenetic diet shifts in ELS Shortnose Sucker *Chasmistes brevirostris* and Lost River Sucker *Deltistes luxatus*, indicating a transition from surface feeding to more benthic feeding as fish grew larger. Thus, diet items that are suggestive of both surface and benthic feeding could be related to a wider range of individuals from different developmental stages and sizes rather than to the partitioning of resources by these species.

Potential differences in resource use could be explained by the differences in size and developmental stage exhibited by the fish in our study rather than by innate differences in feeding ecology across species. It has been hypothesized that ELS fish increase their trophic niche breadth as they grow, which leads to higher chances of survival (Fuiman 2002). Werner and Gilliam (1984) referred to this as the ontogenetic niche, which describes changes in resource use patterns across developmental stages. One potential change in the ontogenetic niche occurs when the range of prey types (i.e., diet richness) increases with body size, but the niche of larger individuals includes that of smaller individuals (Wilson 1975; Werner and Gilliam 1984; Gill 2003). This idea might apply to the results from this study, where Razorback Sucker were the shortest and least developed fish and had the lowest diet richness. Conversely, Bluehead Sucker and Flannelmouth Sucker had the highest diet richness and also had a wider range of SL and developmental stages relative to Razorback Sucker. However, Flannelmouth Sucker were larger on average than Bluehead Sucker, but we did not observe differences in diet richness between these two species. Thus, the ontogenetic niche of Bluehead Sucker and Flannelmouth Sucker was larger but included the niche of Razorback Sucker. Based on ontogenetic niche shifts, differences in species life history (e.g., egg size, size at hatching, and developmental rate) could play

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**FIGURE 5.** Bivariate plot of $\delta^{13}$C and $\delta^{15}$N (left panel) of co-occurring early life stage Bluehead Sucker (CATDIS), Flannelmouth Sucker (CATLAT), and Razorback Sucker (XYRTEX) collected from five sites in the San Juan River, Utah. Sites are represented by different symbols, and standard ellipse areas were corrected for sample size (SEA) and calculated using the package Stable Isotope Analysis in R (Parnell and Jackson 2013). Flannelmouth Sucker had the largest isotopic niche breadth (SEA = 9.15) compared to Bluehead Sucker (SEA = 3.06) and Razorback Sucker (SEA = 4.65). The $\delta^{13}$C (top-right panel) differed among species. Bluehead Sucker and Razorback Sucker had higher $\delta^{15}$N (bottom-right panel) than Flannelmouth Sucker. Values of $\delta^{13}$C and $\delta^{15}$N represent least-squares means ($\pm$95% confidence interval). [Color figure can be viewed at afsjournals.org.]
an important role in determining resource use during early life, which could ultimately affect recruitment.

In addition to differences in diet composition based on gut content analysis, we also found significant differences (despite substantial overlap) among species based on stable isotope analysis. Flannelmouth Sucker displayed the most variation in trophic resource use, and this appeared to be driven by variation in δ¹³C across sites. Flannelmouth Sucker at sites 2 and 4 (S2 and S4 in Figure 1) had lower δ¹³C compared to individuals of all three species across the other sites. However, individuals with the lowest δ¹³C among Bluehead Sucker and Razorback Sucker also occurred at site 4. Gido et al. (2006) found that δ¹³C of primary consumers (e.g., EPT) decreased from upstream to downstream in the San Juan River, possibly due to differences in current velocity among habitats (Findlay et al. 1999). Because all five sites in the current study occurred in a canyon-bound, high-gradient reach of the river, we might expect minimal variation in δ¹³C, but differences in local site characteristics (e.g., connection to the main channel) could have contributed to the observed variation in δ¹³C. Another possibility is that the contribution of riparian plant resources to the food web could differ spatially along the river and across sites. Based on δ¹⁵N of fish in this study, it appears that ELS fish are not feeding directly on primary producers and that variation in δ¹³C could be driven by variation in diets of lower-trophic-level groups, such as aquatic insects or zooplankton. Pease et al. (2006) hypothesized that δ¹³C variation in larval fish could be due to diet switching by invertebrate grazers between benthic algae and other carbon sources (e.g., emergent macrophytes). Benthic algae are thought to be the major carbon source for fish in arid-land rivers (Bunn et al. 2003; King 2004; Pease et al. 2006), but Gido et al. (2006) concluded that detritus from terrestrial sources was the major carbon source for juvenile and adult fish in the San Juan River. The δ¹³C of ELS fish measured in this study suggests that carbon sources can vary substantially even within river reaches having similar habitat characteristics, and such variation could be driven by variation in the diets of lower-trophic-level consumers.

Differences in δ¹⁵N among species were consistent across sites but seem to have been related to ontogenetic stage rather than diet. Specifically, larger Flannelmouth Sucker had significantly lower δ¹⁵N relative to Bluehead Sucker and Razorback Sucker. Although we lack data on baseline trophic resources, higher δ¹⁵N in Bluehead Sucker and Razorback Sucker indicates that these species are feeding at a higher trophic level, which was not supported by gut content data. Alternatively, smaller and less developed ELS fish might be more susceptible to starvation (i.e., in the “critical period”) such that the higher mean δ¹⁵N in Bluehead Sucker and Razorback Sucker could be due to the catabolism of body tissues (Gannes et al. 1997; Gaye-Siesssegger et al. 2007). Gaye-Siesssegger et al. (2007) found that starved Nile Tilapia Oreochromis niloticus and those fed a maintenance-only ration had higher δ¹⁵N values than fish receiving a ration above maintenance. Another, more likely, alternative is that ELS fishes have high δ¹⁵N and experience rapid depletion of the heavy nitrogen isotope as they transition to juveniles (Vander Zanden et al. 1998). Vander Zanden et al. (1998) found a strong pattern in ELS Smallmouth Bass Micropterus dolomieu, with δ¹⁵N being high in embryos but declining rapidly until larvae made the transition to the juvenile stage. Parental contributions of δ¹⁵N were predicted to be the cause of this pattern. A similar phenomenon was observed in penguins, as chicks exhibited higher δ¹⁵N relative to juveniles and adults (Forero et al. 2002). Although we only found a significant relationship between δ¹⁵N and fish size for Bluehead Sucker, the lower mean δ¹⁵N of Flannelmouth Sucker might have been caused by individuals being farther along in development relative to the other two species. Flannelmouth Sucker spawn earlier, have larger eggs, and are typically larger per developmental stage compared to other catostomids in the Colorado River basin (Weiss et al. 1998; Snyder and Muth 2004). Assessing the relative trophic level of ELS fish might be problematic because δ¹⁵N can be biased by lag effects from maternal signatures and can change rapidly across developmental stages (Vander Zanden et al. 1998).

During annual monitoring efforts in the San Juan River, few if any juvenile Razorback Sucker have been collected, whereas all stages of Bluehead Sucker and Flannelmouth Sucker, including age-1+ fish, are common. This lack of more developed Razorback Sucker in ELS fish collections has been consistent over the entirety (1998–present) of larval fish monitoring in the San Juan River (Barkstedt et al. 2018). In 2007, when fish in this study were assessed, only 200 Razorback Sucker were collected compared to 16,535 Flannelmouth Sucker and 7,996 Bluehead Sucker. Whether this low relative abundance of Razorback Sucker is due to differences among species in their propensity to drift, their use of different spawning habitats (i.e., main channel versus tributaries; Tyus 1987; Cathcart et al. 2015), a lack of spawning adults (Diver and Wilson 2018), or other factors is unknown. Few data exist on resource availability and potential limitation in rivers of the Colorado River basin, but the timing and amount of trophic resources are critically important to the success of ELS fish (Cushing 1990; Humphries et al. 2013). Future studies could consider linking the dynamics of resource availability and ELS fish success in rivers of the Colorado River basin.

Currently in the San Juan River (and elsewhere in the Colorado River basin), nursery habitats for ELS fishes (e.g., backwaters) are rare as a result of flow regime modification and establishment of nonnative riparian
vegetation. The completion of Navajo Dam brought on decreased spring discharge and increased summer flows in addition to lowered water temperatures from hypolimnetic water releases (Franssen et al. 2007). In the San Juan River, invasive saltcedar _Tamarix_ spp. and Russian olive _Elaeagnus angustifolia_ have displaced native tree species, such as Fremont's cottonwood _Populus fremontii_ and Goodding's black willow _Salix gooddingii_. A change in bank vegetation coupled with reduced annual flow peaks has contributed to armoring of riverbanks and the loss of backwater habitats (Nagler et al. 2011; Franssen et al. 2014). This loss of rearing habitat has reduced available space and has likely concentrated ELS fish into remaining habitats, where competition or predation could be intense. Alterations to main-stem rivers elsewhere have been linked directly to recruitment bottlenecks experienced by imperiled species (Humphries et al. 2002; Guy et al. 2015), but our understanding of how river regulation has impacted ELS fish is lacking for many species.

In this study, diet differences among seemingly similar ELS fishes suggest differences in feeding ecology. How fish respond to the critical period of early life—whether they succumb to it or survive it—can influence population and community dynamics (Houde 1994; Freeman et al. 2001). Early life stage fish are largely presumed to rely on similar resources, but differences among species during these delicate life stages could explain differential responses at the population level to habitat alteration and repatriation efforts (e.g., Mueller and Wydoski 2004). Negative interactions with nonnative fishes are the most commonly hypothesized factor contributing to the imperilment of native fishes in the Colorado River basin (Minckley et al. 2003). If predation by nonnative fishes is the primary cause for the complete lack of recruitment by Razorback Sucker, then why have Bluehead Sucker and Flannelmouth Sucker not suffered similar fates? Habitat alteration and loss have also been hypothesized as contributing to the decline of “big-river” species in the Colorado River basin, but thus far a mechanistic understanding of specific factors limiting the recruitment of species like the Razorback Sucker remains elusive. Along with altering habitats for fishes, river regulation has impacted prey communities of ELS fish (e.g., Kennedy et al. 2016), potentially leading to mismatches in ELS fish occurrence and prey availability. Other studies have assessed trophic resource use by ELS Razorback Sucker in artificial habitats (e.g., reservoirs; Marsh and Langhorst 1988; Papoulias and Minckley 1992), but this is the first study to quantify trophic resource use in riverine habitats and compare it with that exhibited by other native species. Comparative studies that include similar species showcasing differential responses to habitat alteration can help to place results in a context that would otherwise be lacking from single-species investigations.

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**REFERENCES**


