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NOTE

Dietary assessment of age-0 pallid sturgeon and shovelnose sturgeon: implications for surrogacy

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ABSTRACT: Validation is critical when considering potential surrogates for endangered species research. The pallid sturgeon *Scaphirhynchus albus* is federally endangered in the USA, whereas the congeneric shovelnose sturgeon *S. platorynchus* is common. Inadequate food availability during early life history is a hypothesized factor limiting recruitment of pallid sturgeon in the lower Missouri River, USA, and an established surrogate relationship with shovelnose sturgeon would dramatically expedite research efforts related to potential food limitation. During 2018, 4 exogenously feeding pallid sturgeon larvae were captured concurrently with multiple shovelnose sturgeon, providing a critical opportunity to evaluate diet surrogacy using individuals captured from the same locations. We found that both species primarily consumed ephemeropterans but chironomids were also frequent prey. Over 90% of the pallid sturgeon prey items belonged to taxa also consumed by shovelnose sturgeon. Additionally, we often observed high diet similarity between these congeners. As for total prey weight, pallid sturgeon consumption was similar or higher compared to similarly sized shovelnose sturgeon. Although the sample size was limited, this study supports the hypothesis that larval pallid sturgeon are capable of finding food in the lower Missouri River and exhibiting diet similarity with shovelnose sturgeon. We also provide the first quantification of prey weight for wild-produced larval pallid sturgeon, which consumed relatively large amounts of prey in the wild during the critical transition from endogenous to exogenous feeding.

KEY WORDS: Pallid sturgeon · Shovelnose sturgeon · Diet · Surrogacy · Age-0

1. INTRODUCTION

Endangered species recovery efforts are often guided by data from surrogates, which are 'species that are used to represent other species or aspects of the environment to attain a conservation objective' (Caro 2010). The surrogate species approach is an attractive option for managers given the limited information available for at-risk species (Murphy et al. 2011). However, this approach depends on extrapolation, which involves considerable risk unless the relationship between species has been validated (Caro 2010, Murphy & Weiland 2019). Murphy et al. (2011) cautioned that an increasing body of research indicates only marginal success when using a surrogate to guide recovery efforts because a valid surrogate relationship is often assumed. With validation, the likelihood of success increases as does the potential to benefit a variety of at-risk species.

The shovelnose sturgeon *Scaphirhynchus platorynchus* is a potential surrogate for the federally endangered pallid sturgeon *S. albus* (US Fish and

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Wildlife Service 1990), and researchers have recently developed a suite of hypotheses highlighting potential factors that may be limiting pallid sturgeon recruitment to age-1 (Jacobson et al. 2016). Inadequate food availability during early life history is one of the hypotheses garnering recent attention (Gosch et al. 2016, 2018, Civiello et al. 2018, Gemeinhardt et al. 2019) and is currently the focus of habitat restoration efforts on the lower Missouri River (LMOR; Gavins Point Dam, South Dakota, downstream to the Mississippi River confluence at St. Louis, Missouri). Additionally, some of the habitat restoration targets were based on age-0 *Scaphirhynchus* sturgeon capture data due to the scarcity of pallid sturgeon (Gemeinhardt et al. 2019). Sampling crews first documented wild age-0 pallid sturgeon in the LMOR with the capture of 4 drifting free embryos and 3 exogenously feeding larvae in 2014 (Gosch et al. 2018). These 3 individuals provided critical information by confirming that wild-produced pallid sturgeon progeny are capable of finding food after the free-embryo drift period. They also provided a valuable opportunity to assess potential diet surrogacy with the congeneric shovelnose sturgeon. Given the high availability of age-0 shovelnose sturgeon, an established surrogate relationship would dramatically expedite research efforts focused on understanding potential pallid sturgeon food limitations. Gosch et al. (2018) reported that all 3 exogenously feeding larvae contained food and consumed similar prey types as similarly sized shovelnose sturgeon.

Subsequent sampling in the LMOR from 2015–2017 failed to yield additional wild age-0 pallid sturgeon despite the capture of over 6000 congeners. Efforts continued in 2018, yielding over 4400 age-0 *Scaphirhynchus* sturgeon (referred to as age-0 sturgeon hereafter), and 4 of these individuals were genetically identified as pallid sturgeon (Table A1 in the Appendix). Given the extremely limited diet data available for wild age-0 pallid sturgeon throughout the species range (Gosch et al. 2016, 2018) and the relevance to current recovery efforts, these new captures are critical for documenting wild age-0 pallid sturgeon prey use and further evaluating potential diet surrogacy with shovelnose sturgeon during the critical transition from endogenous to exogenous feeding. A fundamental tenet of surrogate validation is establishing that both species respond similarly when subjected to the same environmental conditions (Murphy & Weiland 2014). Unlike 2014, each age-0 pallid sturgeon was concurrently captured with multiple shovelnose sturgeon individuals during 2018. Thus, our primary objective was to compare diet composition (at the lowest possible taxonomic level) between wild age-0 pallid sturgeon and shovelnose sturgeon captured from the same sampling locations. We also provide the first documented comparison of prey weight consumed during the early life history of these species.

2. MATERIALS AND METHODS

We used a benthic otter trawl (4.9 m wide with 4 mm mesh) to capture age-0 sturgeon from a previously established sampling reach located between river km 494 and 526 of the LMOR (Reach 1 hereafter; Gosch et al. 2017, Civiello et al. 2018). All age-0 sturgeon were preserved in 100% ethanol and placed on ice after capture. In the laboratory, each individual was blotted dry and weighed (mg). We measured fork length or total length (excluding the caudal filament) depending on the presence or absence of a distinct fork (Braaten et al. 2007), and fin tissue from each individual was sent for genetic identification (Dr. Edward Heist, Southern Illinois University). Four individuals captured from separate trawl samples (conducted at different locations on different dates) in early to mid-June were identified as pallid sturgeon (see Table A1 for genetic results and Table A2 for more detail on each trawl). We removed the lower esophagus and stomach, which were then blotted dry and weighed (mg). Prey items were removed and each stomach was reweighed to estimate the prey weight, including unidentifiable material (Hyslop 1980). We stratified by size to randomly select 5 age-0 sturgeon from each trawl sample that captured a pallid sturgeon (total of 20 individuals) for comparative diet analysis; 18 were genetically confirmed as shovelnose sturgeon (Table A1), and the other 2 were excluded from diet analyses. All prey items were identified to the lowest possible taxonomic (often genus and species) level to provided increased confidence when evaluating potential diet surrogacy (Gosch et al. 2018). Identification depended on the level of digestion and availability of taxonomic keys (see Harrison et al. 2014 for more detailed information).

We used PRIMER software (version 7, PRIMER-E) to compare diet composition between pallid sturgeon and shovelnose sturgeon. We used the 'standardise' pre-treatment option in PRIMER to convert count data for each prey taxon to a relative percentage of the total prey count removed from each stomach. This step accounts for variability in the total amount of prey consumed among individuals (Clarke & Gorley

2006) due to the inherent limitations of a snapshot dietary assessment at the moment of capture. These percentages were square root transformed to balance contributions from abundant and rarer species (Clarke & Gorley 2006). We then used the 'resemblance' option to construct a Bray-Curtis similarity matrix, which was used to conduct a 1-way ANOSIM comparing diet composition between species. The ANOSIM analysis is analogous to a univariate ANOVA without the parametric assumptions. This analysis yields a p-value and an R statistic, which typically ranges from 0 (high similarity) to 1 (low similarity). Clarke & Warwick (2001) advised that R-values are at least equally as important as the p-values when assessing similarity. Furthermore, Clarke & Gorley (2006) suggested that the R-values are more useful for interpretation of ANOSIM because R is 'an absolute measure of differences between 2 (or more) groups' whereas p is 'always hijacked by the sample size.' Additionally, we used the Bray-Curtis similarity matrix data to conduct non-metric multidimensional scaling (NMDS) to complement the ANOSIM by comparing diet composition among pallid sturgeon and shovelnose sturgeon in 2-dimensional space. Clarke & Warwick (2001) suggested that stress levels <0.2 provide a useful 2-dimensional representation of the data.

We compared the amount of prey consumed by pallid sturgeon and shovelnose sturgeon by constructing scatter plots of prey weight and body weight; these weights were measured from preserved samples which may limit comparison with future studies using unpreserved specimens. Using the shovelnose sturgeon data, we conducted linear regression with associated 95% prediction intervals to provide context to the pallid sturgeon prey weight values. We also calculated the consumption percentage (prey weight divided by body weight multiplied by 100; Gemeinhardt et al. 2019) for each pallid and shovelnose sturgeon and used a *t*-test to compare the amount of prey consumed between species. Additionally, we constructed box plots to visually compare pallid sturgeon consumption percentage values with shovelnose sturgeon captured from the same trawl sample as well as additional age-0 sturgeon captured from Reach 1 during 2014–2018 to provide additional context regarding the amount of prey consumed by age-0 pallid sturgeon. Data from the individuals captured during 2014–2015 were included in previous studies (Civiello et al. 2018, Gosch et al. 2018, Gemeinhardt et al. 2019); data from the individuals captured during 2016–2018 were previously unpublished, but sampling and laboratory methods were consistent among all years. We obtained all required state and federal sampling permits prior to study initiation. We also followed the handling procedures of the US Fish and Wildlife Service Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon and the American Fisheries Society's Guidelines for the Use of Fishes in Research.

3. RESULTS

Every pallid sturgeon (n = 4, length range 20–25 mm) and shovelnose sturgeon (n = 18, length range 20–26 mm) consumed prey. Both species primarily consumed ephemeropterans, but chironomids were also frequent prey (Table S1 in the Supplement at www.int-res.com/articles/suppl/n040p321_supp.pdf). Of the 48 prey items removed from pallid sturgeon stomachs, 45 (94%) belonged to taxa also consumed by shovelnose sturgeon (Table S1). Of the 351 prey items removed from shovelnose sturgeon stomachs, 336 (96%) belonged to taxa also consumed by pallid sturgeon (Table S1). The 1-way ANOSIM yielded a low R value and non-significant p-value for species (R = 0.002, p = 0.47), suggesting high diet similarity between species. Additionally, the NMDS plot (2-dimensional stress = 0.12) showed that pallid sturgeon often had high diet similarity with at least 1 shovelnose sturgeon (Fig. 1). The pallid sturgeon

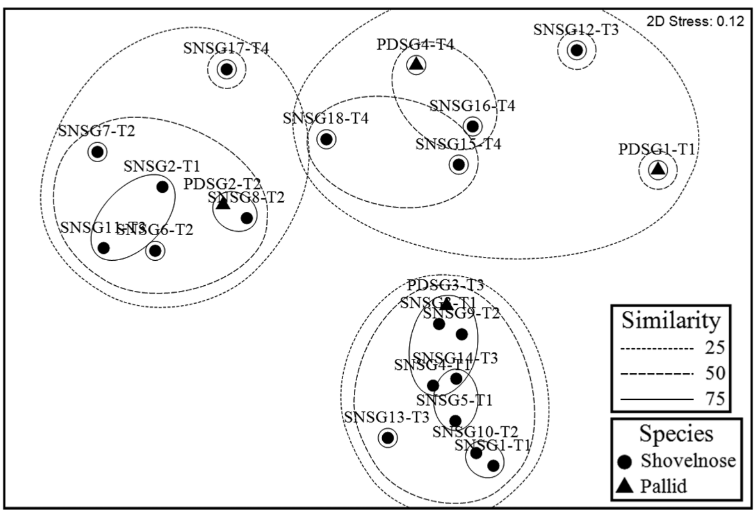

Fig. 1. Prey composition for age-0 pallid sturgeon and shovelnose sturgeon captured from the same trawl samples during 2018. Contours depict percent similarity among individuals and the last number of each individual's label identifies the trawl of capture (e.g. T1 refers to Trawl 1)

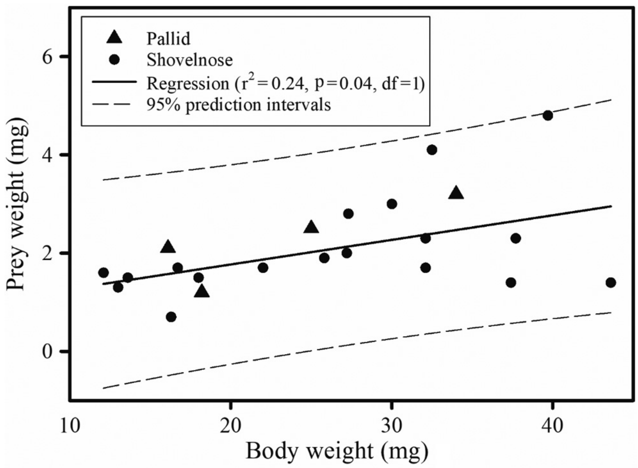

Fig. 2. Prey weight and sturgeon body weight for age-0 pallid sturgeon and shovelnose sturgeon captured from the same trawl samples during 2018

from Trawl 3 had 75% diet similarity with 4 shovelnose sturgeon captured in Trawls 1 to 3 (Fig. 1). The same was true for a pallid sturgeon and shovelnose sturgeon from Trawl 2 (Fig. 1). A pallid sturgeon and shovelnose sturgeon from Trawl 4 yielded 50% diet similarity. The pallid sturgeon from Trawl 1 and 2 shovelnose sturgeon from Trawls 3 and 4 had the lowest similarity values as none of these individuals yielded ≥50% diet similarity with any other individual regardless of species (Fig. 1).

As for total prey weight consumed, the shovelnose sturgeon linear regression was significant, and all 4 pallid sturgeon prey weights were within the 95% prediction intervals of similarly sized shovelnose sturgeon captured from the same trawl sample (Fig. 2). When comparing consumption percentage values, pallid sturgeon (9.8 ± 2.6%; mean ± SD) and shovelnose sturgeon (8.3 ± 3.0%) were not significantly different (t = 0.9, p = 0.40), and box plots had a high degree of overlap during 2018 (Fig. 3a). Additionally, the consumption percentage values for pallid sturgeon during 2018 (Fig. 3a) were relatively high compared to other similarly sized age-0 sturgeon captured from Reach 1 during 2014–2018 (Fig. 3b).

4. DISCUSSION

Gosch et al. (2018) provided the first evidence that wild age-0 pallid sturgeon and shovelnose sturgeon consume similar prey during early life history. Our findings were consistent with past results providing additional evidence of diet similarity between these congeners in the LMOR. Although consumption of similar prey items by these species was observed during both 2014 and 2018, we also documented differences in the primary prey consumed between years. During 2014, both species almost exclusively preyed on chironomids (Gosch et al. 2018), whereas ephemeropterans were the primary prey consumed during 2018. Although prey availability was not measured, these findings may indicate a change in prey availability between years as previous research has reported that chironomids were preferred by age-0 pallid sturgeon in laboratory trials (Rapp 2014) as well as age-0 sturgeon in the middle Mississippi River (Sechler et al. 2012). As such, the change in primary prey consumed between 2014 and 2018 provides additional evidence of diet surrogacy by demonstrating that both species exhibited a similar diet shift in 2018.

Despite instances of high diet similarity with shovelnose sturgeon, none of the 4 pallid sturgeon larvae exhibited high intraspecific diet similarity. This may be explained by differences in capture location and capture date as all 4 pallid sturgeon were captured in separate trawl samples on different dates (Table S1); however, individuals captured in the same trawl sample did not consistently group together in the NMDS plot (Fig. 1). Interestingly, some shovelnose sturgeon also yielded low intraspecific diet similarity. As such, the relatively low intraspecific diet similarity observed among pallid sturgeon is

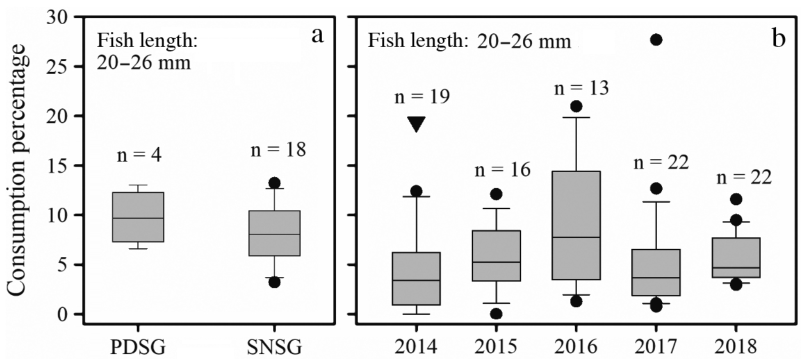

Fig. 3. Consumption percentage (prey weight divided by body weight multiplied by 100; Gemeinhardt et al. 2019) for (a) age-0 pallid sturgeon (PDSG) and shovelnose sturgeon (SNSG) captured from the same trawl samples during 2018 and for (b) age-0 sturgeon captured from Reach 1 during 2014–2018. One PDSG (20 mm) was captured from Reach 1 during 2014 (▼). Horizontal line in each box indicates the median, box dimensions represent the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and black dots are outliers

likely a function of the limited sample size and the inherent limitations of a snapshot assessment of an individual's diet at the moment of capture.

Assessing the amount of prey consumed by each species is also important when evaluating potential surrogacy. Even if individuals from both species are consuming similar types of prey, a large disparity in the weight of prey consumed could indicate a weak surrogate relationship as one species may be better able to utilize prey resources. During 2018, however, age-0 pallid sturgeon appeared equally capable of finding and consuming prey compared to shovelnose sturgeon captured from the same trawl sample when looking at the prey weight and consumption percentage values. The same was true when comparing the 2018 pallid sturgeon to additional age-0 sturgeon captured from Reach 1 over multiple years (2014–2018). Interestingly, the only pallid sturgeon (length of 20 mm) captured from Reach 1 during 2014 also yielded one of the highest consumption percentage values during the entire 5 yr period (Fig. 3). During 2014, a 24 mm pallid sturgeon was captured roughly 500 river km downstream, near the Mississippi River confluence (Gosch et al. 2018), with comparable prey weight (2 mg) and consumption percentage (5.5%) values (US Army Corps of Engineers [USACE] unpubl. data) relative to the other specimens examined during this study. This observation provides further evidence that pallid sturgeon in the 20 mm size class are not struggling to find food relative to other age-0 sturgeon. Twelve days later in 2014, a 48 mm pallid sturgeon was captured from the same area of the LMOR near the Mississippi River confluence (Gosch et al. 2018). This individual contained 2.5 mg of prey (USACE unpubl. data), which is on the lower end of prey weights consumed by similarly sized age-0 sturgeon (41–60 mm) in the LMOR (Civiello et al. 2018). This individual also had a relatively low body weight for its size, resulting in a moderate consumption percentage value (2.0%; USACE unpubl. data) compared to other age-0 sturgeon in the LMOR (Gemeinhardt et al. 2019). Currently, no other age-0 pallid sturgeon of similar or larger size have been captured for comparison. Given the natural variability in feeding, the relatively low prey weight observed for the 48 mm individual may be a function of the inherent limitations of a snapshot dietary assessment at the moment of capture, especially considering that some age-0 sturgeon have been observed with similarly low prey weights (Civiello et al. 2018). Alternatively, it is possible that the 48 mm individual was struggling to find food; however, growth was an estimated 2 mm d^{-1} based on the day of capture and difference in length relative to the 24 mm sibling. This is rapid growth for age-0 pallid sturgeon (Braaten et al. 2012), suggesting that food acquisition was likely not an issue, but additional captures of 40 to 50 mm pallid sturgeon would provide valuable context regarding the amount of prey consumed by this larger individual.

Overall, this study provides additional evidence of diet similarity between pallid sturgeon and shovelnose sturgeon as well as the first quantification of prey weight for wild-produced larval pallid sturgeon. During the critical transition period (endogenous to exogenous feeding, Hjort 1914), these individuals consumed large amounts of prey in the wild relative to an abundant congener that is self-sustaining in the highly modified LMOR. One factor not evaluated during this study was potential interspecific differences in the ability to metabolize food, which could jeopardize surrogacy. A recent laboratory study evaluating larger pallid sturgeon (156–242 mm, average = 161 mm) and shovelnose sturgeon (120–237 mm, average = 166 mm) suggested that subtle interspecific physiological differences (e.g. alimentary canal length) contribute to differences in simulated growth rate potential (GRP) between these species (Porreca et al. 2017a). Specifically, pallid sturgeon required a 15% larger increase in prey energy density, relative to shovelnose sturgeon, to maintain their GRP when transitioning from an all gravel to all sand environment (Porreca et al. 2017a). In contrast, they also reported that pallid sturgeon GRP was higher than shovelnose sturgeon in gravel-dominated substrate (~80% or more gravel). Although sand is usually the dominant habitat in large modified rivers containing pallid sturgeon (Porreca et al. 2017b), dunes often form that may provide energetic refugia. Another pallid sturgeon (140–170 mm) laboratory experiment documented the energetic benefits of dune habitat as oxygen consumption was 16 to 20% higher in swim tunnel trials without a dune (Porreca et al. 2017b). Given that sand dunes are widely available and often a preferred habitat for pallid sturgeon (Porreca et al. 2017b), additional information regarding the influence of dunes on GRP would provide valuable context regarding physiological differences and surrogacy. Regardless of the potential influence of dunes, the metabolic rate of pallid sturgeon does not necessarily preclude survival in the wild. Using LMOR capture data from 2003 to 2015, Steffensen et al. (2019) found evidence of limited natural recruitment estimating that 8% of the pallid sturgeon captured were likely the result of natural reproduction, which indicates that some wild pallid sturgeon were capable of recruiting to the population despite the potential metabolic differences with shovelnose sturgeon.

Although sample size limitations inherent to endangered species research persist, current diet data indicate that a valid surrogate relationship likely exists between these species during early life history, but the Missouri River Recovery Program has an adaptive management framework (Fischenich et al. 2017) in place to account for new findings and adjust recovery actions if future work yields different results. To our knowledge, the 7 pallid sturgeon captured in the LMOR during 2014 and 2018 are the only genetically confirmed and wild-produced exogenously feeding individuals ever captured during the first year of life for this species. Murphy & Weiland (2019) commented that 'the best available scientific information on the scarcest of the imperiled species receiving federal protection often is not much scientific information at all.' As such, this diet data is invaluable especially considering the uncertainty regarding future age-0 captures of this endangered species. If future captures occur, diet evaluation will be important regardless of the place of capture (Gosch et al. 2018). Additionally, the collection of larger age-0 specimens would provide valuable insight regarding surrogacy throughout this life stage; however, this is challenging considering the rarity of age-0 pallid sturgeon coupled with the general decline in age-0 sturgeon captures as length increases (Gosch et al. 2017). We also encourage complementary diet analysis of sympatric shovelnose sturgeon specimens captured concurrently from pallid sturgeon locations if possible to provide the most accurate dietary comparisons between these species.

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Appendix.

Table A1. Genetic identification results from the microsatellite analysis conducted by Dr. Edward Heist, Southern Illinois University. Probability values for each individual are reported for each possible category: pallid sturgeon (PDSG), shovelnose sturgeon (SNSG), or hybrid. Individuals with a strong probability assignment (≥0.95) to the PDSG or SNSG categories were included in diet analyses

Length (mm)	Capture date (mo/d/yr)	PDSG	Hybrid	SNSG
22	6/4/2018	0.998	0.002	0.000
25	6/5/2018	0.998	0.002	0.000
20	6/13/2018	0.999	0.001	0.000
22	6/15/2018	1.000	0.000	0.000
20	6/4/2018	0.000	0.000	1.000
24	6/4/2018	0.000	0.004	0.996
25	6/4/2018	0.000	0.000	1.000
20	6/4/2018	0.000	0.006	0.994
22	6/4/2018	0.000	0.010	0.990
26	6/5/2018	0.000	0.003	0.997
24	6/5/2018	0.000	0.002	0.998
24	6/5/2018	0.000	0.001	0.999
24	6/5/2018	0.000	0.012	0.988
24	6/5/2018	0.000	0.001	0.999
20	6/13/2018	0.000	0.001	0.999
20	6/13/2018	0.000	0.001	0.999
20	6/13/2018	0.000	0.000	1.000
20	6/13/2018	0.000	0.002	0.998
20	6/13/2018	0.000	0.196	0.804
22	6/15/2018	0.000	0.005	0.995
20	6/15/2018	0.000	0.000	1.000
22	6/15/2018	0.000	0.000	1.000
24	6/15/2018	0.000	0.000	1.000
23	6/15/2018	0.000	0.226	0.774

Table A2. Details for each trawl sample capturing an age-0 pallid sturgeon in 2018. Discharge was taken from the nearest US Geological Survey gage station (06894650) at Napoleon, Missouri. Depth was recorded at the beginning, middle and end of each trawl run and averaged; velocity was recorded near the bottom with a Marsh-McBirney flow meter at the midpoint of each trawl. The sample size represents all age-0 *Scaphirhynchus* sturgeon, including pallid sturgeon, captured in each trawl

Date (mo/d/yr)	River km	Discharge ($m^3 s^{-1}$)	Distance (m)	Depth (m)	Velocity ($m s^{-1}$)	Sample size
6/4/2018	504	2158	134	4.2	0.60	12
6/5/2018	504	2217	88	3.9	0.29	29
6/13/2018	500	2161	158	2.7	0.40	110
6/15/2018	500	2350	123	3.3	0.55	33

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