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Rediscovery of the Pallid Shiner, *Hybopsis amnis*, in the Black River System of Arkansas and Missouri Including Notes on Ecology and Life History

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Abstract

The Pallid Shiner, *Hybopsis amnis*, is a rare and understudied minnow with little information about its ecology. This species is listed as a Species of Greatest Conservation Need (SGCN) throughout much of its range and is generally considered to be declining. It had not been detected in the Black River system of Missouri and Arkansas in over 75 years, or the state of Missouri in over 60 years. We sampled over 100 sites in the Black River system between 2017 and 2020 to assess temporal trends in fish assemblage structure and to update the status of SGCN species in this drainage. We collected 226 *H. amnis* at seven different sites in the Black River system. We measured total lengths to estimate age classes and year of spawning. Corresponding habitat and year class data indicate this species may spawn in late winter to early spring and rely on floodplain habitat for spawning and recruitment. The apparent decline of *H. amnis* in other systems may be a result of reduced floodplain connectivity.

Cover Page Footnote

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INTRODUCTION

The Pallid Shiner, *Hybopsis amnis* (Hubbs and Greene 1951), is a slender, slightly compressed minnow with a blunt snout and small subterminal mouth. It is widely distributed in the Mississippi River basin, ranging from Minnesota and Wisconsin south to Louisiana. Its range extends eastward up the Cumberland River in Kentucky and westward into the Arkansas and Red river drainages in Oklahoma (Page and Burr 2011; Robison and Buchanan 2020). It is generally considered a rare and declining species except for isolated local populations, and constitutes less than one percent of the minnow population in the upper Mississippi River basin (Hubbs 1951; Becker 1983). *Hybopsis amnis* is listed as a Species of Greatest Conservation Need (SGCN) in nine of the 13 states it occurs in, and thought to be extirpated from Missouri where it had not been collected since 1957. Originally this record was reported in 1956 (Pflieger 1997; Cieslewicz 2004); however, Dr. Pflieger's personal data sheets indicate this collection occurred in 1957. Although reasons for its decline across its range are unknown, it is speculated they do not tolerate excessive siltation (Hubbs 1951; Clemmer 1980; Pflieger 1997; Ross 2001; Page and Burr 2011).

In Arkansas, *H. amnis* inhabits lowland streams in the Coastal Plains physiographic province, and is known throughout the state in the lower White, St. Francis, lower Arkansas, Poteau, Little, Red, Saline, and Ouachita drainages (Robison and Buchanan 2020). McCallister et al. (2010) sampled streams throughout Arkansas to update its status. They collected individuals in 25 of 75 counties in Arkansas including all major rivers in the West Gulf Coastal Plain and Mississippi River Alluvial Plain physiographic provinces, except the St. Francis River. However, they were detected from the St. Francis River drainage in 2015 by Tumlison et al. (2016). McCallister et al. (2010) identified nine new county records for *H. amnis* in Arkansas, indicating populations are stable but localized. Though McCallister et al. (2010) did not detect any specimens in the Black River system, it was previously reported from a single location at the confluence of the Spring and Black rivers in Lawrence County, Arkansas prior to the 1940s (Hubbs 1951; Buchanan 1973).

Throughout the Black River system, *H. amnis* has only been collected at four localities, including the single site previously mentioned in Arkansas (Cieslewicz 2004; Robison and Buchanan 2020). The other three collections were made during 1941 from Missouri: Cane Creek in Butler County (1 specimen), Black River in Butler County (2 specimens), and Little Black River (a tributary of the Current River) in Ripley County (6 specimens) (Pflieger 1997; Cieslewicz 2004). None of these specimens were reportedly vouchered. As a result of its extended absence from the system, the International Union for Conservation of Nature (IUCN) lists *H. amnis* as extirpated from the Black River system (NatureServe 2013). Robison and Buchanan (2020) hypothesized its absence from the Black River system is due to surrounding land use practices and associated siltation in the watershed.

The Black River is a relatively under-studied watershed with most survey data collected from the 1960s to 1980s. At the University of Central Arkansas (UCA), we recently conducted intensive sampling (2017 to 2020) to assess temporal trends in fish assemblage structure and assess the status of SGCN species in the Black River system. Additional opportunistic sampling was conducted in the Missouri portion of the system during 2019 by Robert Hrabik and colleagues. The objectives of this paper are to update the distribution of *H. amnis* and provide ecology and life history data given the paucity of information about this species.

Methods

Between 1960 – 1990, multiple different collectors extensively sampled tributaries within the Black River system and none reported collections of *H. amnis.* We resampled a total of 101 of these sites between 2017 and 2020 to analyze temporal trends in fish assemblage structure in the Black River system: 13 sites in the Fourche River drainage, 16 sites in the Strawberry River drainage, 31 sites in the Spring River drainage, 21 sites in the Eleven Point River drainage, and 20 sites in the Current River drainage. We sampled sites with seines having mesh size 0.48 cm (2.44 by 1.83 m, 3.66 by 1.83 m, and 9.14 by 2.44 m), and all fish vouchered were preserved in 10% formalin before being transferred to 70% ethanol and stored in the University of Central Arkansas Fish Collection. We vouchered all captured *H. amnis* individuals except for specimens collected at Cane Creek ditch and measured total length (TL) and standard length (SL) for each individual. Total length was used for age analysis for direct comparison with Becker (1983) and Kwak (1991).

Habitat data were also measured at each site with corresponding fish collections. Air temperature was measured using a thermometer. Water temperature and dissolved oxygen (DO) were measured at the site level using a Yellow Springs Instrument Model 85. Depth measurements were taken in three transects in each macrohabitat (riffle, run, and pool) with a meter stick (mm). Each transect consisted of five equidistant measurements from bank to bank, or from bank to as far as was sampleable in deeper reaches. Velocity measurements were taken in one transect in each macrohabitat using a Hach FH950 flow meter connected to a top set graduated wading rod. Velocity transects were measured similar to depth transects. Substrate was visually assessed for each macrohabitat in percentages of clay (C), sand/silt (SS), gravel (G), pebble (P), cobble (CO), boulder (B), and bedrock (BR). Robert Hrabik and colleagues sampled three sites within the Cane Creek system during 2019 using seines as part of an Ichthyology class field trip and collected corresponding environmental data.

Results and Discussion

Our study documents the rediscovery of *H. amnis* in the Black River system and in the state of Missouri (Figure 1). During our surveys we recorded the first collection of *H. amnis* in the Fourche and Current river drainages in Arkansas, and in the Spring River above its confluence with the Black. This is also the first record of *H. amnis* in Randolph County, Arkansas (Figure 2). These are the first records of *H. amnis* in the Black River system in over 75 years, and the first documentation of it in Missouri since it was last collected in 1957 from the Meramec River. We collected a total of 226 individuals from seven sites during sampling from 2018 to 2020 in the months of June, August, September, October, and December. We collected

Figure 1. *Hybopsis amnis* collected from the Current River in the summer of 2020.

19 from the Current River system, 27 from the Fourche, 80 from the Spring, and we estimate that at least 100 were collected from Cane Creek Ditch in Butler County, Missouri. We were only able to measure total length of 33 *H. amnis* collected at Cane Creek Ditch.

We collected *H. amnis* in habitats with depths ranging from 0.29 – 1.86 m and velocities ranging from 0 – 75 cm/s. Our depth findings are similar to those reported by Kwak (1991), who reported individuals from depths of 0.40 – 1.50 m; however, we detected *H. amnis* in habitats with greater velocities than Kwak (1991) who reported individuals at velocities of 0 – 5.2 cm/s. Stream temperatures for *H. amnis* range from qualitative reports of "cold" and "warm" stream temperatures (Becker 1983), and Kwak (1991) reported them from temperatures of 20.4 – 30.0°C. These temperature reports are from summer months, so our findings of individuals in streams with temperatures ranging from 2.8 – 35.5°C are not comparable. We also detected *H. amnis* at dissolved oxygen levels of 5.75 – 12.1 mg/L, similar to the range 6.9 – 12.9 mg/L reported by Kwak (1991). We collected individuals at air temperatures ranging from 5.6 – 36.1°C. Again, these temperatures were taken spanning summer and winter months so they are not comparable to Kwak (1991) who found a relationship between air temperature and catch of *H. amnis* during summer months. We collected specimens in streams that ranged from 7 – 89 m in width. The dominant substrate within each habitat ranged from 100% clay to 65% gravel (Table 1).

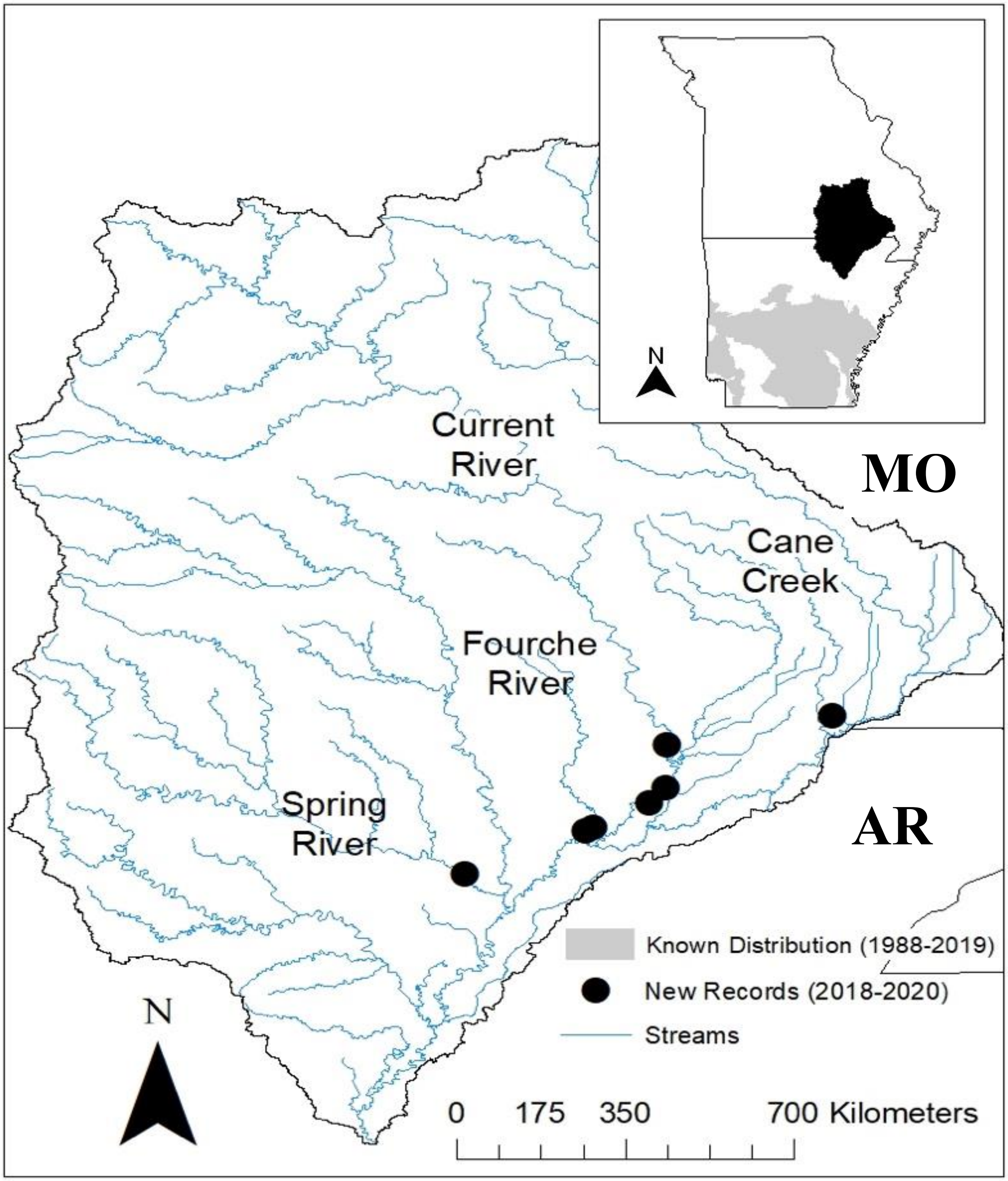

Figure 2. Known distribution of *H. amnis* and new records collected within the Black River system. Gray shading represents distribution within Arkansas (AR) and Missouri (MO) between 1988 – 2019 based on Robison and Buchanan (2020) and Pflieger (1997). Black shading represents the Black River system. Black points represent new collections between 2018 – 2020.

Table 1. Habitat data for sites with *H. amnis* collections between 2018 – 2020. Substrate was visually estimated as percent clay (C), sand/silt (SS), gravel (G), pebble (P), cobble (CO), boulder (B), and bedrock (BR). FR = Fourche River drainage, CR = Current River drainage. (*) Indicates *H. amnis* numbers that were estimated.

Stream	Latitude	Longitude	# Pallid Shiners collected	Habitat	% Substrate	Air temp. (° C)	Water temp. (° C)	DO (mg/L)	Avg. width (m)	Avg. sampled depth (m)	Avg. velocity (m/s)
Spring River	36.1932	-91.1550	71 9	Pool Run	65G 20S 15P 65G 15S 15P	11.1 11.1	15.4 15.4	8.21 8.21	30 25	1.86 0.44	0.37 0.75
Main Ditch (FR)	36.2873	-90.9157	21	Pool	100C	33.3	35.5	8.45	12	0.29	0.01
Fourche River	36.2807	-90.9296	1 5	Pool Run	90SS 10C 100SS	32.2 32.2	28.4 28.4	5.94 5.94	17 12	0.85 0.60	0.12 0.20
Current River	36.3686	-90.7796	14	Pool	95SS 5G	25.6	23.7	5.75	80	0.70	0.32
Unnamed Tributary (CR)	36.4561	-90.7776	4	Pool	100SS	5.6	2.8	12.1	7	0.32	0
Current River	36.3375	-90.8105	1	Pool	90SS 5G 5BR	36.1	26.4	6.18	89	0.75	0.18
Cane Creek Ditch	36.5167	-90.4673	100*	Pool	NA	28.9	NA	NA	18.2	0.60	NA

Ninety-four percent of *H. amnis* we collected were primarily captured in slow moving, sandy and silty pools, and 6% were collected in runs. They generally did not constitute a large percentage of the minnow community (<1 – 12%) (Table 2). This supports previous observations that *H. amnis* prefer slower velocity habitats with sandy and silty substrate, and is rarely present in high abundance (Hubbs 1951; Becker 1983; Kwak 1991; Pflieger 1997; Ross 2001; Robison and Buchanan 2020). Juveniles collected during 2018 at Main Ditch were the only young of the year (YOY) collected in our study, and the habitat was characterized by a 100% clay substrate with an average velocity of 0.01 m/s, average depth of 0.29 m, and a water temperature of 35.5°C (Table 1). This is the shallowest and warmest habitat where we collected specimens, suggesting *H. amnis* utilize shallower, warmer waters for nursery habitat. Juvenile presence in more turbid habitats could be a result of preference for shallow, warm waters which are often more turbid, as nursery habitat.

Complete fish community data were available from the six sites sampled in Arkansas. Across these sites, *H. amnis* was associated with 9 species. *Hybognathus nuchalis, Notropis atherinoides, Notropis texanus, Labidesthes sicculus, Fundulus olivaceus,* and *Lepomis macrochirus* were present at all six sites where *H. amnis* was collected, and *Cyprinella venusta*, *Pimephales vigilax*, and *Lepomis megalotis* were present at five of six sites. All of the species associated with *H. amnis* in the Black River system utilize lentic or slow flowing lotic environments, and have been associated with *H. amnis* in Arkansas, Wisconsin, and/or Illinois with the exception of *C. venusta* (Becker 1983; Kwak 1991; Naus and Adams 2018). Many of the species associated with adult and juvenile *H. amnis* utilize features of floodplains for nursery habitat, namely tributaries and oxbow lakes.

Like most minnows, *H. amnis* is hypothesized to reach maturity at age one and have a relatively short lifespan with a maximum age of 2 to 3 years (Becker 1983; Kwak 1991). Studies of northern populations found juveniles (Age-0) ranged from 0 – 34 mm in total length, Age-1 individuals generally ranged from 35 – 49 mm total length, and Age-2 individuals were greater than 49 mm in total length (Becker 1983; Kwak 1991). Our size frequency histogram indicates a likely presence of three age classes in our study (Figure 3). Juveniles ranged in size up to 38 mm, Age-1 were 41 – 56 mm, and Age-2 were greater than 56 mm in total length. Size discrepancies in age class between the northern (Wisconsin and Illinois) and Black River populations could be because sampling in the Black River system occurred later in the year than northern populations, and/or they experienced more optimal growing conditions at lower latitudes.

Total length data on 158 specimens (one specimen could not be measured for total length because its caudal fin was destroyed) suggested three age classes were present in the Black River system: juveniles (Age-0), Age-1, and Age-2 (Figure 3). We collected a total of 17 juveniles, 129 Age-1, and 12 Age-2 specimens (Table 2). By subtracting the age of specimens from their year of sampling, we estimated the year individuals were spawned. We estimated four different spawning years that produced the *H. amnis* collected during this study: 2016 (1), 2017 (20), 2018 (136), and 2019 (1) (Figure 4). Unequal sampling occurred across years; therefore, these data may not accurately reflect quality of annual recruitment across years. However, it is intriguing the only year that Age-0 individuals were collected was during 2018 and that most older fish collected, spanning two years and multiple locations, were also estimated to have been spawned during 2018.

Table 2. Date of collections, habitat, number of *H. amnis* collected, age estimations, sizes, and percent of minnow populations by site for individuals collected in the Black River system between 2018 – 2020. FR = Fourche River drainage, CR = Current River drainage. (*) Indicates *H. amnis* numbers that were estimated. One specimen could not be measured from the Spring River because its caudal fin was destroyed.

Stream	Latitude	Longitude	Month Sampled mm/dd/yy	# Pallid Shiners collected	Habitat	Age 0	Age 1	Age 2	Size range (TL mm)	% Minnow pop.
Spring River	36.1932	-91.1550	10/13/19	71 9	Pool Run	0 0	68 8	2 1	44.3 – 59.5 50.4 – 56.1	6 ---
Main Ditch (FR)	36.2873	-90.9157	06/14/18	21	Pool	14	6	1	20.9 – 57.5	1
Fourche River	36.2807	-90.9296	08/13/18	1 5	Pool Run	1 2	0 3	0 0	35.0 37.7 – 49.5	<1 ---
Current River	36.3686	-90.7796	09/21/19	14	Pool	0	13	1	45.7 – 57.2	4
Unnamed Tributary (CR)	36.4561	-90.7776	12/11/19	4	Pool	0	0	4	58.7 – 63.7	<1
Current River	36.3375	-90.8105	08/10/20	1	Pool	0	1	0	44.5	<1
Cane Creek Ditch	36.5167	-90.4673	09/28/19	33 (100*)	Pool	0	30	3	42.0 – 58.0	12*

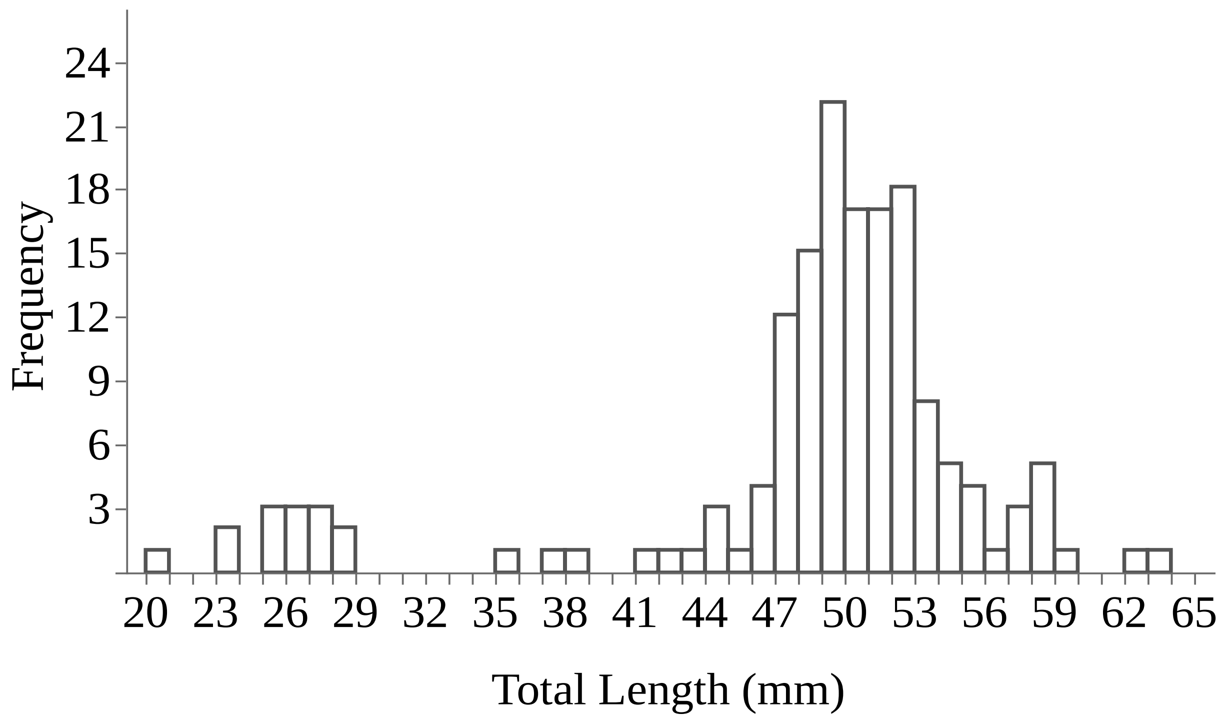

Figure 3. Frequency histogram for the total length (mm) of *H. amnis* collected in the Black River system between 2018 – 2020. Clustering around 27 mm and 50 mm indicate two-year classes. The precipitous drop in individuals from 52 mm to 56 mm and the clustering around 58 mm indicates the presence of a third-year class.

In the Arkansas portion of the Mississippi River, evidence from ripe individuals suggests spawning occurs in late winter to early spring (Clemmer 1980; Robison and Buchanan 2020). Naus (2015) sampled larval/juvenile fish communities in the lower Fourche LaFave River system of central Arkansas, and collected larval *H. amnis* in light traps on 7 April 2012 (5 mm TL; 22.1° C) and from 15-20 May 2012 (5-10 mm TL; 25.9-30.6° C). The earliest juveniles were collected was 17 May (16-18 mm TL), but larger juveniles were collected about the same time in May (25-30 mm TL) (Naus 2015). We dissected four large individuals collected from the Current River drainage in December 2019 and one large individual collected from the Fourche River drainage in June 2018. The four individuals collected from the Current River were females with eggs in ovaries at an early developmental stage. Corresponding lengths and gonadosomatic index (gonad mass/eviscerated body mass X 100) values were as follows: 62.2 mm TL (5.1%), 58.8 mm TL (4.8%), 63.7 mm TL (4.0%), and 58.7 mm TL (2.5%). The female collected during June (57.5 mm TL) had spent ovaries. Our early life stage data from the Fourche LaFave River support a late winter/early spring spawn period that could extend into April, and the gonads examined in this study also support a late winter/early spring spawn period that ends prior to June.

Kwak (1991) hypothesized access to adjacent floodplain, and therefore flooding, was important for its spawning and recruitment. He found that abundance of juveniles was higher when minimum discharge in the Kankakee River was elevated during May-June, and total catch of *H. amnis* was positively associated with maximum discharge in March (Kwak 1991). Reproductive success of river floodplain fishes is predicted to be highest when connecting floods correspond with the spawning season (coupling of discharge and temperature) (Junk et al. 1989). Therefore, reproductive success of *H. amnis* is predicted to be high when floods occur during the late winter/spring spawning period and when extended flooding/elevated discharge occurs in late spring/early summer facilitating recruitment of young (sensu Kwak 1991). The vast majority of individuals collected from the Black River during our study were estimated to be part of the 2018 reproductive cohort. We plotted daily average discharge data from the main stem Black River in the vicinity of the *H. amnis* collections (Black Rock, Arkansas) for 2016 to 2019 (Figure 5). Black River discharge during the reproductive period in 2018 matches the predicted regime from Kwak (1991) whereby a flood occurred in early March (probably facilitated spawning) and discharge exceeded flood levels from April to mid-May (probably facilitated early recruitment) (Figure 5). Elements of beneficial discharge for *H. amnis* reproduction was observed in other years, and we found evidence of recruitment in all years. Based on discharge patterns, we predict 2019 was another good year for reproduction but our sampling was sparse in that year. Information on specific spawning location, mode, and egg characteristics (e.g., adhesive vs. semi-buoyant) are needed to better evaluate the dependency of *H. amnis* on availability of floodplain habitat for spawning.

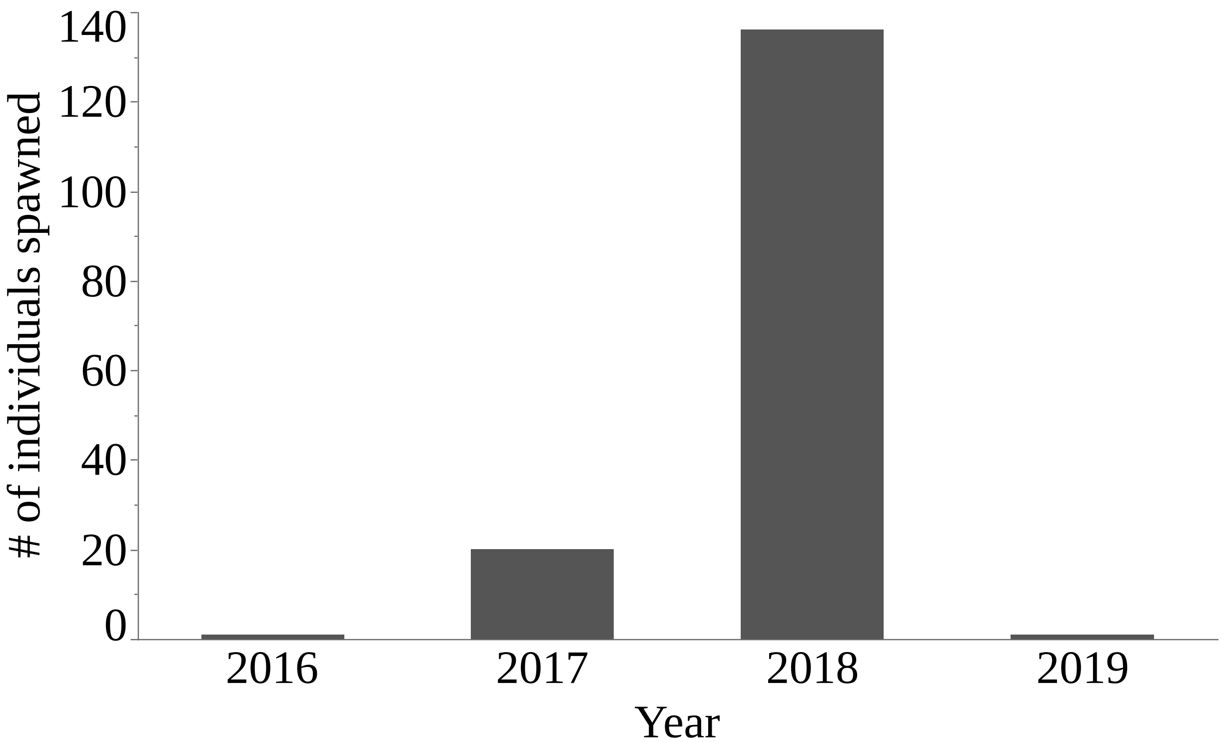

Figure 4. The number of *H. amnis* and their estimated year of spawning in the Black River system between 2016 – 2019.

Over the last century the Black River system has experienced conversion of forest to pasture or row crop agriculture, channelization of some tributaries, and construction of levees. Although land use surrounding the Black River system has changed very little since the 1970s, large amounts of pasture still exist as a chronic perturbation. Robison and Buchanan (2020) hypothesized the absence of *H. amnis* from this system was a result of excessive siltation associated with land use practices. However, presence of individuals in the lower Black River system, namely in Main Ditch does not support this hypothesis. Main Ditch is a channelized tributary to the Fourche River constructed to drain agricultural land. Substrates were composed of soft clay and turbidity was high. It is plausible both land use modification in floodplains (e.g., leading to increased turbidity and siltation) and disruption of river-floodplain connectivity (e.g., levees) have played a major role in decline of *H. amnis* throughout its range. In altered river floodplain systems, tributaries play an important role in allowing fish access to at least fringing, adjacent habitat (Naus and Adams 2018). For example, juvenile *H. amnis* were found utilizing lower reaches of floodplain tributaries to the Fourche LaFave River and adults are often found in the main stem at tributary confluences (Naus and Adams 2018). Connectivity of the Black River with smaller systems such as Fourche River and Cane Creek probably enhances fish access to floodplain habitat. Observations of relatively high abundances of *H. amnis* by R. Hrabik in the Hatchie River near Jackson, Tennessee further support the hypothesis floodplain connectivity is important for their populations. This segment of the Hatchie River is characterized by a mosaic of off-channel and floodplain habitats that could be accessible to fish during flooding. Therefore, floodplain connectivity should be conserved and restored to benefit *H. amnis* and species with similar life histories.

It remains unclear whether presence of *H. amnis* in the Black River system is a recent phenomenon indicating range expansion, or if they were historically present in low numbers and not detected or misidentified. Bigeye Chub (*Hybopsis amblops*) occurs in the Black River system and has been confused with *H. amnis* in Illinois and Arkansas collections (Warren and Burr 1988; Robison and Buchanan 2020). The tributary sites where *H. amnis* was recently detected were historically sampled between the 1960s and 1980s, with none of those efforts yielding specimens, suggesting it was absent from these tributary systems for a period of time. However, the mainstem Black River in Arkansas has even fewer historical samples than its tributaries, with only 64 collections on 27 different days between 1965 and 2014. Given the sporadic nature with which *H. amnis* is collected (Kwak 1991), this low sampling effort may not have been enough to detect it. We hypothesize resident *H. amnis* populations have persisted in the main stem Black River but were not detected due to under sampling, and that recent detection in tributaries to the Black River is probably a result of increased movement and recruitment associated with flood events. Though presence of *H. amnis* populations in the Black River system is encouraging, it is unlikely that it is making a permanent comeback in Missouri. Recent detection in Cane Creek may be transient (e.g., a response to recent flood regimes) given the ditch network in the Missouri Bootheel is still well-maintained preventing connectivity to floodplains and the continued presence of other anthropogenic disturbances associated with modified land use.

This paper is meant to present the rediscovery of *H. amnis* in Missouri and the Black River system and to provide valuable habitat and year class data to support future studies. We present evidence that access to floodplain habitat may be crucial to the spawning and recruitment of this species. This study was not designed specifically to assess life history characteristics of *H. amnis*. Sites were sampled at different times of the year in different locations making it difficult to completely assess year classes and recruitment. More research is needed to determine what factors have contributed to the recent recruitment of *H. amnis* into these systems, and to understand why it is so rare and seems to be declining (Becker 1983; Page and Burr 2011). Increased sampling effort in the Black River system and St. Francis River may yield additional specimens (e.g., in reaches of the Current and St. Francis rivers in Missouri).

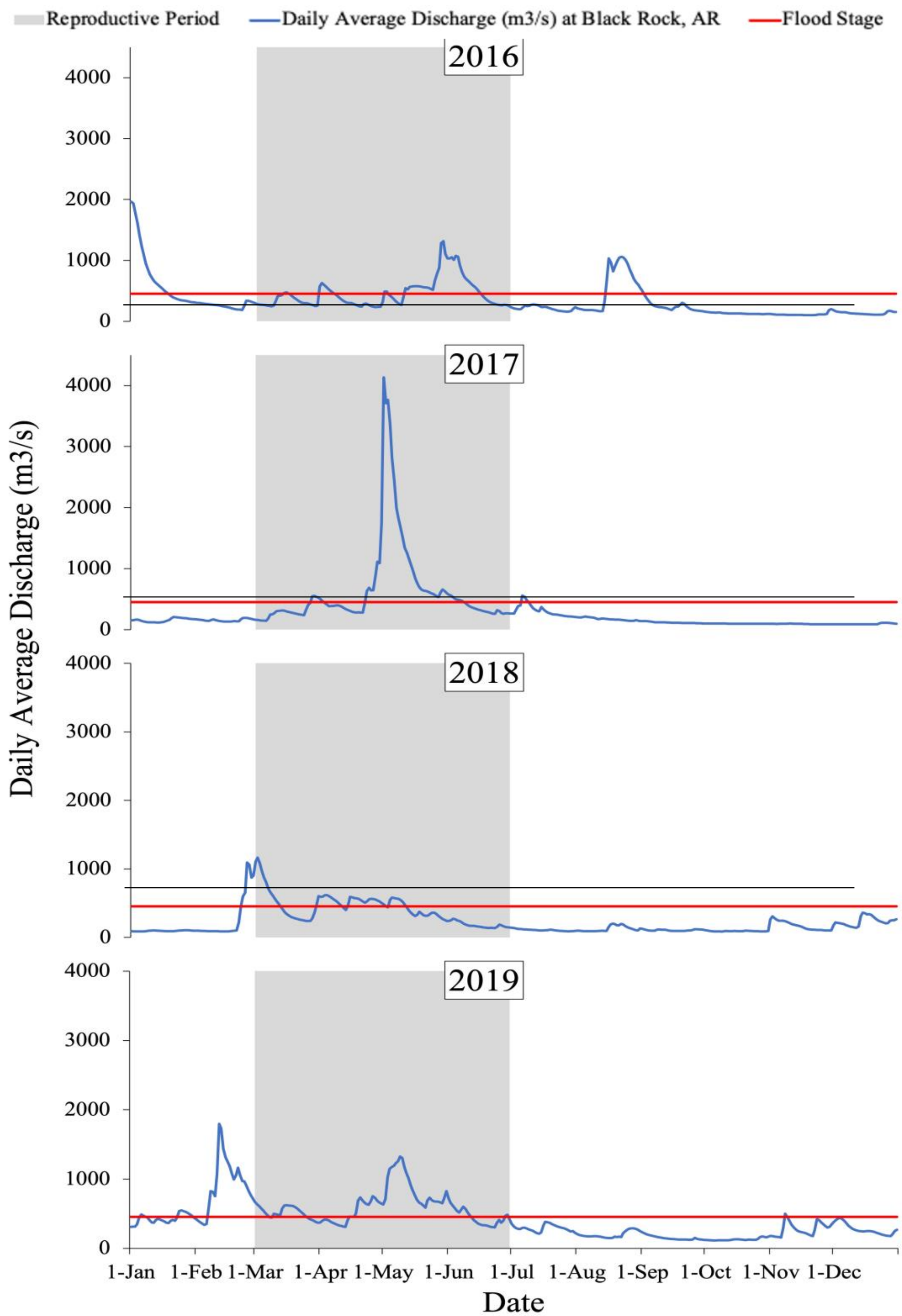

Figure 5. Hydrograph of daily average discharge (m^3/s) at Black Rock, Arkansas between 2016 – 2019 (USGS 2020). The red line represents flood stage levels for this gage (453 m^3/s) and the shaded area indicates reproductive/recruitment time period for *H. amnis.*

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