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Article

Spawning Locations of Pallid Sturgeon in the Missouri River Corroborate the Mechanism for Recruitment Failure

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Abstract: Conservation propagation of pallid sturgeon (*Scaphirhynchus albus*) upstream of Fort Peck Reservoir, MT, USA, has successfully recruited a new generation of spawning-capable pallid sturgeon where there would otherwise be fewer than 30 remaining wild reproductively mature pallid sturgeon. Successful recovery of pallid sturgeon will now rely on the behavior of pallid sturgeon (e.g., successful spawning in locations that provide adequate drift distance for larvae to recruit). We used location data of pallid sturgeon during four putative spawning seasons to answer the following questions: Where do pallid sturgeon spawn? Are spawning locations related to discharge? Are substrate characteristics at the spawning locations similar to other river reaches? Do spawning-capable females, spawning-capable males, and female pallid sturgeon undergoing mass ovarian follicular atresia use the river similarly? Additionally, we considered if spawning locations are far enough from the river–reservoir transition zone to provide adequate drift distance for larvae to recruit. Spawning-capable pallid sturgeon did explore upstream locations, and four spawning-capable pallid sturgeon were located in the Marias River during the spawning season in 2018 when discharge was at an unprecedented high. Pallid sturgeon exited the Marias River and moved downstream prior to spawning, and when spawning occurred, it was not far enough upstream to prevent larvae from entering the transition zone of Fort Peck Reservoir. Thus, management of discharge and water temperature to mimic 2018 conditions may increase use of the Marias River by pallid sturgeon during the spawning season, which would increase drift distance available to larvae and increase the probability of successful recruitment.

Keywords: pallid sturgeon; recruitment failure; spawning substrate; spawning location; spawning movement

Key Contribution: Spawning locations of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir do not provide adequate drift distance for free embryos. This finding corroborates the mechanism for recruitment failure and anoxic conditions at the river–reservoir transition zone.

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1. Introduction

Conservation propagation of endangered species has been used to prevent the extirpation or extinction of many taxon, such as plants [1], insects [2], birds [3], mammals [4], amphibians [5], and fish [6]. However, individuals from a conservation propagation program may not perform well (i.e., survive and reproduce such that the population can self-sustain) in the wild such that recovery of the population is never achieved. For example, the Trumpeter Swan (*Cygnus buccinator* [Richardson]) conservation propagation resulted in increased abundance; however, young swans from the captive breeding program also had to be trained to effectively migrate to wintering areas or they would have perished during the winter [7]. Therefore, it is imperative to study the behavior of individuals from conservation propagation programs after they are placed in the natural environment to assess performance as it relates to recovery objectives, where performance is defined as reproductive output and survival that result in population persistence.

Conservation propagation of fishes has increased abundance and prevented the extirpation of several species (e.g., cutthroat trout or *Oncorhynchus clarkii* (Richardson) [8], lake sturgeon or *Acipenser fulvescens* (Rafinesque) [9], white sturgeon or *Acipenser transmontanus* (Richardson) [10], and razorback sucker or *Xyrauchen texanus* (Abbott) [11]). However, not all conservation propagation of fishes has been successful, and an analysis of case studies on endangered fish reintroductions (i.e., conservation propagation and translocations from existing populations) found 42% were unsuccessful [12]. Unforeseen behavioral deficits associated with hatchery-origin fish, such as a lack of predator avoidance [13,14], limited migratory behavior [15,16] and reduced foraging ability [17], may result in poor performance of an augmented population (e.g., in stocked populations). Therefore, the success of conservation propagation programs for species recovery requires an understanding of the behavior of the augmented population to ensure that behavior results in adequate performance as it relates to achieving recovery objectives.

Conservation propagation is being used to conserve pallid sturgeon (*Scaphirhynchus albus* [Forbes & Richardson]) because recruitment failure has reduced the abundance of wild pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir [18], such that there are probably fewer than 30 individual wild pallid sturgeon [19]. Pallid sturgeon were first stocked upstream of Fort Peck Reservoir in 1998 with pallid sturgeon hatched in 1997 [18]. As the abundance of wild pallid sturgeon continues to decline, recovery of pallid sturgeon upstream of Fort Peck Reservoir has begun with hatchery-origin pallid sturgeon becoming reproductively mature, successfully spawning, and contributing recruits to the population.

Recruitment failure of pallid sturgeon in the upper basin of the Missouri River is related to the spawning location juxtaposed with the location of the river–reservoir transition zone [20,21]. Thus, the behavior of hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir regarding spawning movements and location will determine if successful recruitment is plausible. In the past, a lack of successfully ovulating hatchery-origin female pallid sturgeon has prevented spawning locations and spawning-related movements of successfully spawning females from being characterized [22].

If hatchery-origin pallid sturgeon spawn in upstream locations of the upper Missouri River or Marias River, the drift distance available to free embryos (i.e., pallid sturgeon in the developmental stage between hatching and exogenous feeding) will be optimized. Otherwise, spawning in downstream locations will result in inadequate drift distance, which could necessitate management actions for successful recruitment to occur. Increasing discharge has been suggested as a potential method to prompt upstream migration prior to spawning. However, it is unknown if discharge is correlated with the spawning location of pallid sturgeon. Discharge in the Marias River was shown to be associated with spawning of shovelnose sturgeon (*Scaphirhynchus platorynchus* [Rafinesque])—spawning occurred when discharge was greater than 28 m^3/s [23]. Furthermore, spawning location could be limited by substrate composition, which has been associated with the spawning location of other sturgeon species [24]. If discharge, substrate composition, or other abiotic factors affect spawning location, management actions that improve drift distance available to free embryos could be implemented.

Recovery of pallid sturgeon upstream of Fort Peck Reservoir was initiated by successfully augmenting the population using conservation propagation. However, successful recovery of endangered species using conservation propagation requires augmented populations to perform such that recovery criteria are eventually met. For pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir, reaching reproductive maturity and spawning in locations that provide adequate drift distance for free embryos are the necessary next steps if the population is going to be self-sustaining as outlined in the Revised Recovery Plan for Pallid Sturgeon [18].

We designed this study to answer the following questions about the hatchery-origin pallid sturgeon: (1) Do spawning-capable females, spawning-capable males, and female pallid sturgeon undergoing mass ovarian follicular atresia use the river similarly? (2) Where do pallid sturgeon spawn? (3) What are substrate characteristics at the spawning locations? Additionally, we considered if the spawning locations are far enough from the river–reservoir transition zone such that there would be adequate drift distance for free embryos to survive and recruit, and we considered if discharge is related to spawning locations. Answering these questions will inform management decisions such as how to manage discharge and reservoir water-surface levels to promote successful recruitment of progeny from hatchery-origin pallid sturgeon and thereby increase the likelihood of recovery.

2. Materials and Method

2.1. Study Area

The study area is located in the Great Plains Management Unit described by the United States Fish and Wildlife Service (USFWS, 2014) [18] and consists of the Missouri River from the upstream end of Fort Peck Reservoir to Morony Dam (river kilometer [rkm] 3010 to rkm 3388) and the Marias River from the confluence with the Missouri River to Tiber Dam (rkm 0 to rkm 126, Figure 1). The study area described here represents the furthest upstream distribution of pallid sturgeon in the Missouri River basin [18].

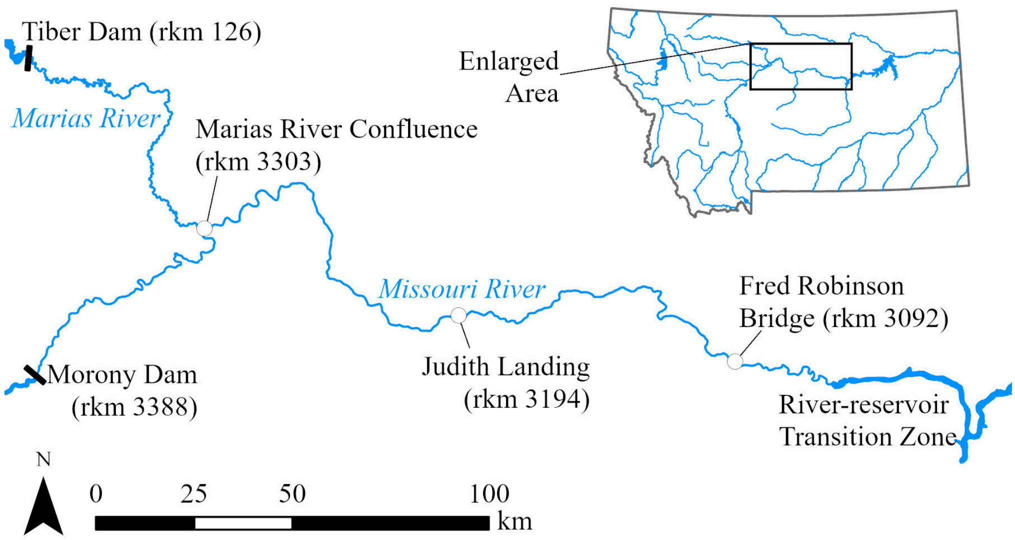

Figure 1. Map of the Missouri River from the river–reservoir transition zone at Fort Peck Reservoir, MT, USA, to Morony Dam, MT, USA (river kilometer [rkm] 3010–rkm 3388), and the Marias River from the confluence with the Missouri River to Tiber Dam, MT, USA (rkm 0–rkm 126). Dams are denoted by ▬, and points of reference are denoted by ○.

The population of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir is bound by anthropogenic upstream and downstream barriers. Upstream movement beyond river kilometer 3388 is prevented by Morony Dam. Prior to construction of Morony Dam, upstream movement was naturally prevented by the Great Falls of the Missouri River fewer than 7 km upstream of Morony Dam [18]. Downstream movement of pallid sturgeon is limited by the transition to lacustrine conditions at the headwaters of Fort Peck Reservoir near river kilometer 3010. The location of the Fort Peck Reservoir river–reservoir transition zone varies depending on reservoir elevation.

Discharge in the Missouri River upstream of Fort Peck Reservoir is influenced by unregulated tributaries (i.e., the Smith, Teton, and Judith rivers; and the Belt, Arrow, Dog, and Cow creeks [25]), impounded tributaries, and mainstem impoundments. Upstream of Fort Peck Reservoir, the mainstem Missouri River contains nine dams. Eight of the nine dams have negligible influence on the hydrograph because outflows are maintained roughly equal to inflows [26,27]. Canyon Ferry Dam (rkm 3626) is the exception and is used to store water and regulate discharge in the Missouri River [28]. Tiber Dam on the Marias River and Gibson Dam on the Sun River are also used to store water and regulate discharge, which can reduce discharge in the Missouri River during the spring and summer [28]. Peak discharge typically occurs between late May and late June in the Missouri River.

The first five mainstem impoundments upstream of Fort Peck Reservoir have little effect on the downstream water temperature other than reducing the daily variation in water temperature [29]. The effects of the Canyon Ferry, Hauser, and Holter reservoirs have not been thoroughly evaluated. Water temperature in the Marias River can be decreased by hypolimnetic water releases from Tiber Dam [30]. However, use of the surface spillway, an auxiliary water outlet completed at Tiber Dam in 1969, or a combination of the two could counteract cold-water temperatures from the hypolimnetic release.

Substrate within the Missouri River upstream of Fort Peck Reservoir transitions from larger substrate in the upstream reaches to smaller substrate in the downstream reaches. Substrate is primarily cobble from river kilometer 3340 to 3130 [31]. At river kilometer 3,130, composition shifts to mostly gravel for several kilometers before transitioning into fine and sandy substrate [31]. Turbidity and substrate are likely influenced by the upstream impoundments, as sediment trapping at impoundments is a ubiquitous occurrence, and mitigation of sediment trapping is typically not focused on returning downstream sediment loading to preimpoundment conditions [32].

2.2. Sampling

Pallid sturgeon were sampled between early May and late July of 2018 and 2019. Pallid sturgeon targeted for sampling had been previously radio telemetered by Montana Fish, Wildlife & Parks as part of a concurrent study [22]. Pallid sturgeon were captured during the prespawning season (i.e., prior to the peak of the hydrograph) to determine sex and stage of maturity, and spawning-capable females were recaptured at the end of the spawning season (i.e., when water temperatures neared 24°C) to determine ovulatory outcome. Discharge and water temperature were used to define the spawning season because spawning of pallid sturgeon typically occurs in late spring to early summer on the descending limb of the hydrograph [33,34], and water temperatures during spawning are estimated to be 12–24 °C based on embryo survival [35].

Prespawning-season sampling was prioritized using reproductive assessment data from 2011 through 2017. Pallid sturgeon known to be female that experienced reproductive activity in the past were considered high priority and were targeted for recapture first. After sampling high-priority pallid sturgeon, other known females were targeted. Male pallid sturgeon and pallid sturgeon of unknown sex were assigned lower priority and were sampled opportunistically.

Pallid sturgeon locations were estimated using radio telemetry, and trammel nets 45.7 m long and 1.8 m deep with a 10.16 cm inner bar mesh and a 25.4 cm or 20.32 cm outer bar mesh were used to capture relocated pallid sturgeon. Smaller mesh trammel nets 45.7 m long and 1.8 m deep with a 5.08 cm inner bar mesh and a 25.4 cm outer bar mesh were occasionally used if the larger mesh trammel nets were ineffective at capturing an individual. Biological samples were collected from all captured pallid sturgeon. Handling and sampling procedures conformed to protocols developed for pallid sturgeon [36]. Blood was sampled from the caudal vasculature of each pallid sturgeon using a 3 mL syringe. Blood samples were immediately transferred to a 7 ml lithium heparinized vacutainer, stored in a cool environment, and transported to the field station the same day. Once at the field station, blood samples were centrifuged at 1228$\times$ g (relative centrifugal force) for 5 min to separate blood plasma from red and white blood cells. Blood plasma was transferred to 1.5 mL vials and stored at −20 to −80 °C until analyzed at the USFWS Bozeman Fish Technology Center.

Gonadal tissue was sampled from pallid sturgeon of unknown sex, and ovarian follicles were sampled from females that were known or expected to be spawning capable. A small abdominal incision (1–2 cm) was made anterior to the urogenital pore between the midline and the ventral scutes. An otoscope was used to identify the gonad, and gonadal tissue samples were taken through the otoscope specula using a Miltex biopsy cup [37]. The otoscope speculum was angled to collect gonadal tissue from three different locations to account for gonadal heterogeneity. All tools used for the collection of gonadal tissue were disinfected with 70% isopropyl alcohol and were rinsed with sterile saline prior to use. Incisions were closed with 1–3 evenly spaced single interrupted sutures using size 0 absorbable suture material attached to a CP-1 suture needle (Ethicon PDS*II). Ovarian follicles and gonadal tissue were preserved in 10% phosphate-buffered formalin.

2.3. Sex and Stage Determination

Blood, ovarian follicles, and gonadal tissue were analyzed at the USFWS Bozeman Fish Technology Center. Sex steroids (testosterone [T] and estradiol-17β [E2]) were extracted from blood plasma using methods described in Fitzpatrick et al. (1987) [38]. An extraction solvent (2 mL of diethyl ether, extracted twice) was added to tubes with 100 µL of the plasma and vortexed. The aqueous phase was removed by snap-freezing with liquid nitrogen, and ether was allowed to evaporate overnight in a chemical hood. The extract was reconstituted in 1 mL of phosphate-buffered saline with gelatin (PBSG). Following this, 10 or 50 µL of reconstituted steroid extract was analyzed using radioimmunoassay as described in Fitzpatrick et al. (1986) [39] and modified by Feist et al. (1990) [40]. A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L PBSG) was used for all assays. Testosterone and E2 concentrations were validated by verifying that serial dilutions were parallel to standard curves. Recovery efficiency was determined by adding tritiated steroids to tubes containing plasma (n = 4), which were extracted as described above. Recovery efficiencies were 91–95% for T and 83–93% for E2. All steroid assay results were corrected for recovery. Nondetectable plasma sex steroid concentrations (i.e., not quantifiable) were assigned half of the minimum quantifiable concentration for statistical purposes (0.10 ng/mL for T and 0.05 ng/mL for E2) [41]. The intra- and interassay coefficients of variation for all assays were less than 5% and 10%, respectively.

Plasma sex-steroid concentrations were used to assign sex and stage of maturity to pallid sturgeon prior to spawning. Concentrations of T greater than 38 ng/mL and E2 less than 0.3 ng/mL were used to assign a reproductively active male pallid sturgeon, with reproductively active male pallid sturgeon considered to be spawning capable. A spawning-capable male pallid sturgeon based on steroid concentrations would have testicular cysts with germ cells that were meiotic (spermatocytes, spermatids, and/or spermatozoa). Concentrations of T greater than 10 ng/mL and E2 greater than 0.3 ng/mL were used to assign a reproductively active female pallid sturgeon. Reproductively active females were vitellogenic or spawning capable (Figure 2, Level 4) and were differentiated via collection of ovarian follicles that were examined under a microscope, processed histologically, or both. Vitellogenic females would not be able to spawn during the year they were sampled and were not included in this study. Reproductively active females classified as spawning-capable females were capable of spawning during the year they were sampled; however, not all spawning-capable females successfully spawned. Therefore, an additional subclassification was added (Figure 2, Level 5), and during analyses females that underwent mass ovarian follicular atresia (hereafter referred to as atretic females) were grouped separately from females that successfully ovulated (hereafter referred to as spawning females). Histological analysis of gonadal tissue collected postspawning season was used to differentiate spawning females from atretic females. During histological analysis, postovulatory ovarian follicles indicated successful spawning (spawning females), and mass ovarian follicular atresia (atretic females) was indicated by >50% of the ovarian follicles undergoing atresia [42] and indicated spawning failure. One hatchery-origin male pallid sturgeon classified as reproductively active using steroid concentrations but without corresponding gonadal tissue was removed from analysis due to a total lack of movement during spawning season (see Holmquist et al. 2019 [22]). Pallid sturgeon that had completed puberty—as determined by prior sampling—were considered reproductively mature (Figure 2, Level 1) irrespective of the reproductive classification at any given time.

Figure 2. Hierarchical classification of reproductively mature pallid sturgeon.

2.4. Tracking

Pallid sturgeon were tracked for the duration of the spawning season using radio telemetry equipment. Tracking began in the fifth week of May in 2018 and the fourth week of May in 2019 before peak discharge. Tracking ended in the first week of July in 2018 and the second week of July in 2019 after all spawning-capable female pallid sturgeon had been determined to have experienced ovulatory success or failure. Tracking equipment included boat-mounted, handheld, and land-based receiver systems with three- or four-element Yagi antennas. Each system used Lotek SRX 400 telemetry receivers (Lotek Wireless, Inc.; Newmarket ON, Canada) to receive and decode radio transmitter signals. Boat-mounted and handheld systems were used manually and provided real-time information about transmitters in range. Land-based systems were autonomous and recorded data for each transmitter that passed through the field of detection. Transmitters had a unique code allowing each pallid sturgeon to be individually identified.

Signal directionality and signal strength were used to maximize accuracy when locating a pallid sturgeon. Direction of a pallid sturgeon was determined by rotating the handheld antenna until finding the bearing that resulted in maximum signal strength. The boat was then moved in the direction of the pallid sturgeon until passing over the fish, which was indicated by a sudden decrease in signal strength. When the boat was positioned over a pallid sturgeon, a GPS location was recorded.

Pallid sturgeon were systematically tracked based on priority given the sex and stage of maturity. The relocation of spawning-capable female pallid sturgeon was attempted every two days. Relocation of reproductively mature male pallid sturgeon was attempted once per week. Land-based telemetry stations were used to aid in searching for undetected pallid sturgeon by documenting if a fish left the last known location.

In addition to this study, location data collected by Holmquist et al. (2019) [22] were combined with these data to produce larger sample sizes and better answer the questions posed. Holmquist et al. (2019) [22] used similar methods to this study and located pallid sturgeon weekly. Individual pallid sturgeon located for fewer than half the weeks tracked in a given year were excluded from analyses because minimal data on individual fish may not accurately represent movement or location of the individual.

2.5. Data Analysis

Locations of pallid sturgeon collected during this study and by Holmquist et al. (2019) [22] were used to estimate movement rates of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir. Movement rates were quantified for each relocation of individual pallid sturgeon as net movement per day (km/day). Then, the median net-movement rate per day was calculated for each individual within specified reproductive classifications (i.e., atretic female, spawning female, or spawning-capable male). A negative median net movement rate indicated a downstream movement, and a positive median net movement rate indicated an upstream movement. Total movement by individual pallid sturgeon was calculated as the sum of distances between locations (i.e., river kilometers) throughout a putative spawning season. Total movement for pallid sturgeon tracked during two putative spawning seasons was determined by calculating total movement for each season and averaging the values. The median net movement rate and total movement were calculated using the minimum distance moved between relocations because pallid sturgeon might have made undetected movements between relocations. The median net movement rates and total movements of pallid sturgeon were summarized using reproductive classifications (i.e., atretic female, spawning female, and spawning-capable male). Summarized data were visualized in box plots for comparison, and the interquartile range (IQR) was used to describe variation within classifications. Interquartile ranges were calculated as the difference between the 25th and 75th quantiles (i.e., the difference between the lower and upper quartiles).

Location data collected in this study and by Holmquist et al. (2019) [22] were used to summarize and compare locations among reproductive classifications. The median location was calculated as the median river kilometer among all recorded locations of an individual pallid sturgeon during specified reproductive classifications (i.e., atretic female, spawning female, and spawning-capable male). The maximum upstream location was calculated as the most upstream location among all recorded locations of an individual pallid sturgeon during specified reproductive classifications (i.e., atretic female, spawning female, and spawning-capable male). Median locations and maximum upstream locations of pallid sturgeon were summarized for atretic females, spawning females, and spawning-capable males. Summarized data were visualized in box plots for comparison, and the IQR was used to describe variation within classifications.

Putative spawning reaches of pallid sturgeon that successfully ovulated were estimated using kernel densities. Using ArcMap 10.5.1 (ESRI, Redlands, CA, USA), a kernel-density estimate map was created from locations of individual spawning females after the initial peak of the hydrograph. The cell values were scaled to represent the continuous relative density between one and zero, where a value of one was the maximum relative density of locations of the individual and zero represented no locations. Reaches scoring relative density values approaching one and the area between reaches scoring relative density values approaching one were included in the estimated putative spawning reach. Locations of mature male pallid sturgeon were overlaid on the kernel density map to verify if mature males had occupied the delineated spawning reaches.

Substrate imagery (Figure 3) was collected in 2019 in three suspected spawning locations of pallid sturgeon—one 2018 suspected spawning location and two 2019 suspected spawning locations. Locations were considered suspected spawning locations when spawning-capable female pallid sturgeon were observed within 0.25 km of mature male pallid sturgeon locations. All suspected spawning locations that were mapped were within putative spawning reaches delineated by kernel-density analyses. Substrate was mapped for the full width of the river and ~0.5 km above and below suspected spawning locations. Substrate mapping was completed by adapting methods described in Kaeser Litts (2010) [43]. A sonar and GPS unit (Humminbird HELIX 7 CHIRP MEGA SI GPS G3) was used to record side-scan sonar images of the riverbed and record GPS coordinates. Image recordings were georeferenced using SonarTRX (SonarTRX, Honolulu, HI, USA) and imported to ArcMap 10.5.1. Substrate types were manually delineated as polygons representing sand, gravel, and cobble (Figure 3). Delineated substrate types were verified at opportunistic locations by collecting substrate samples. The surface area of the polygons at each site was calculated, and the relative proportion of each substrate type was estimated. The central tendency and variation of the proportional substrate types were characterized using the median and IQR.

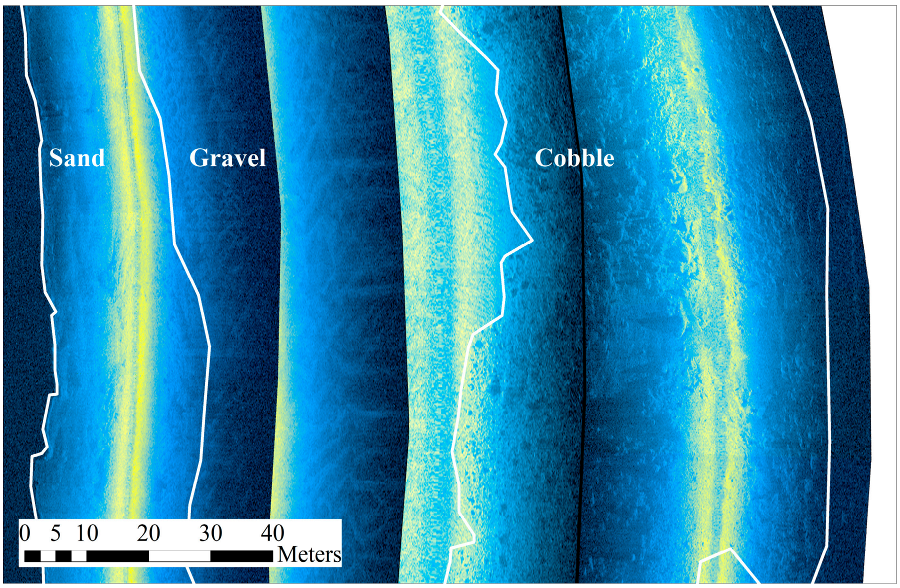

Figure 3. Example of substrate imagery and delineation of substrate types (sand, gravel, and cobble). Substrate imagery was collected in the Missouri River near river kilometer 3117. Substrate imagery was collected and polygons were delineated using methods adapted from Kaeser & Litts (2010) [43].

Mean daily discharge data from the U.S. Geological Survey stream gages were used to characterize discharge in the Missouri and Marias rivers from April through July [44]. The mean daily discharge in the Missouri River near Landusky, MT, USA (stream gage 06115200), from 1956 through 2019 was summarized as the median monthly discharge by calculating the median of the daily mean discharge values for each month of each year. The mean daily discharge in the Marias River near Loma, MT, USA (stream gage 06102050), from 1960 through 2019, excluding 1973 through 2001 when discharge data were not recorded, was summarized as historical median monthly discharge by calculating the median of daily discharge values for each month of each year. Historical monthly discharge values were summarized for each river by calculating the 10th, 25th, median, 75th, and 90th quantiles for each month, and the summary of historical discharge data were compared with the discharge when tracking occurred.

The mean daily temperature data from the U.S. Geological Survey stream gages were used to characterize the temperature in the Missouri and Maris rivers from April through July during years that tracking of pallid sturgeon occurred. The mean daily temperature data for the Missouri River near Landusky, MT, USA (stream gage 06115200), and the Marias River near Loma, MT, USA (stream gage 06102050), were summarized as the median monthly temperature from April through July of 2015, 2016, 2018, and 2019 by calculating the median of daily temperature values for each month of each year.

3. Results

In general, the median discharge of the Missouri River declined from the mid-1960s to 2019, and discharge during the spring was particularly low during the early 2000s (Figure 4). The median monthly discharge in the Missouri River in 2015 and 2016 was below the historical median from April through July and was below the historical 25th quantile in May 2015, June 2016, and July 2016 (Table 1). Conversely, the median monthly discharge in the Missouri River in 2018 exceeded the historical 90th quantile in April and May, exceeded the historical 75th quantile in June, and exceeded the historical median in July (Table 1). In 2019, the median monthly discharge in the Missouri River exceeded the historical 75th quantile in April and May and was between the 25th and 75th quantiles in June and July (Table 1). During April, May, and June, the median monthly water temperature in the Missouri River was slightly warmer in 2015 and 2016 than in 2018 and 2019, but in July, the median monthly water temperature was coldest in 2015 and 2019 and warmest in 2016 and 2018 (Table 2).

Table 1. The 10th, 25th, median, 75th, and 90th quantiles of monthly median discharge (m^3/s) in the Missouri River from 1956 through 2019 from April through July, and the median discharge from April through July for 2015, 2016, 2018, and 2019. Data from the U.S. Geological Survey stream gage station near Landusky, MT, USA (stream gage 06115200).

Month	1956–2019					2015	2016	2018	2019
	10th	25th	Median	75th	90th	Median			
April	152.3	196.4	246.2	293.1	408.3	210.7	205.3	511.1	406.3
May	187.3	253.2	351.1	441.0	610.2	209.5	280.9	869.3	487.0
June	194.3	299.5	407.1	653.1	868.8	301.6	238.7	831.1	375.2
July	139.8	173.5	248.2	332.0	512.0	184.3	171.3	322.8	303.0

Table 2. Median water temperature (°C) in the Missouri River from April through July of 2015, 2016, 2018, and 2019 excluding April of 2015 and 2018 when the water temperature data were not collected and June of 2018 when the stream gage was inoperable due to flooding. Data from the U.S. Geological Survey stream gage station near Landusky, MT, USA (stream gage 06115200).

Month	Median Water Temperature (°C)			
	2015	2016	2018	2019
April	–	11.4	–	10.4
May	14.0	14.8	13.8	12.0
June	19.9	20.7	–	19.0
July	21.4	23.8	23.5	22.7

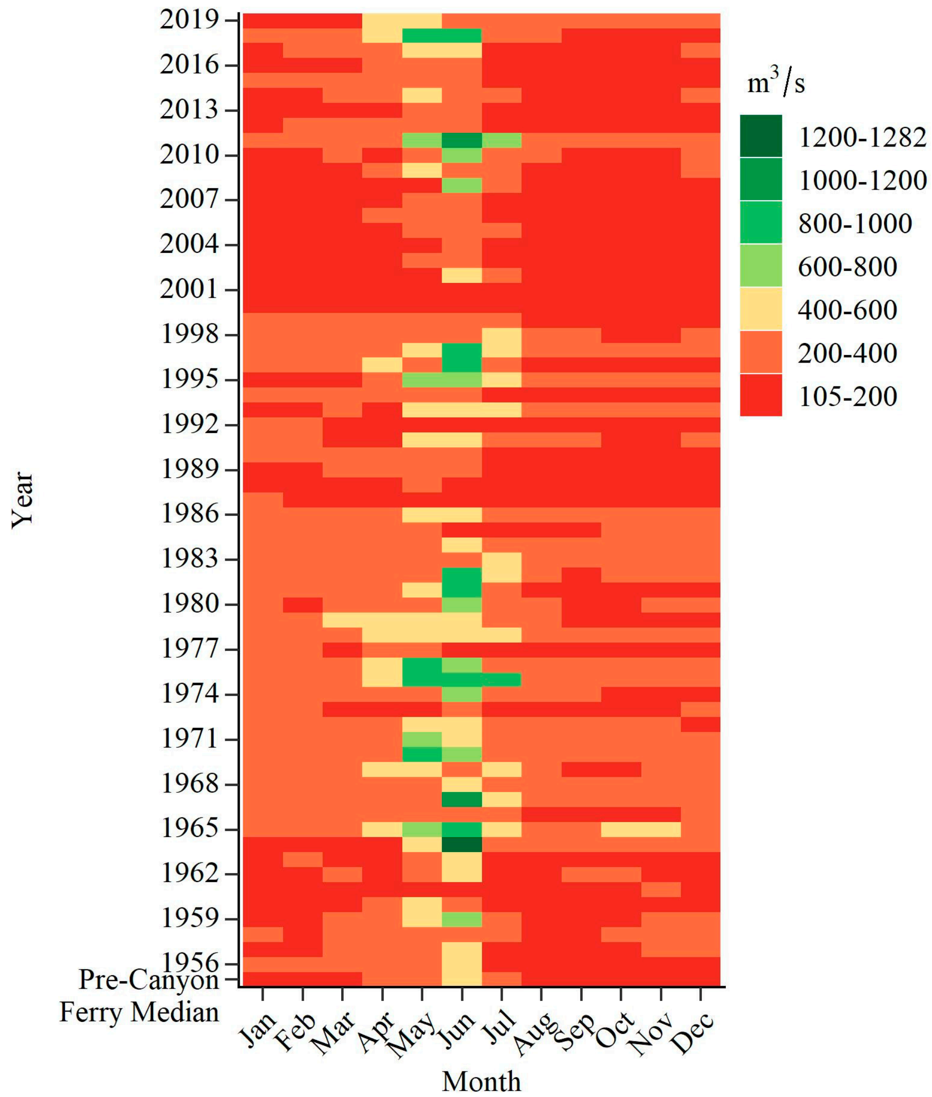

Figure 4. Median discharge (m^3/s) in the Missouri River by month from 1956 through 2019. Additionally, the median of monthly median discharge from pre-Canyon Ferry Dam (1934–1953) is included. Data from the U.S. Geological Survey stream gage station near Landusky, MT, USA (stream gage 06115200).

In general, the spring discharge in the Marias River has been relatively low throughout the 2000s as compared to the discharge during the 1960s and 1970s (Figure 5). The median monthly discharge in the Marias River was between the historical 25th and 75th quantiles from April through July of 2015 and was at or below the 25th quantile from April through June of 2016 (Table 3). Conversely, the median monthly discharge in the Marias River reached an unprecedented high in 2018 exceeding the historical 90th quantile during April and May and exceeding the median in June and July (Table 3). The median monthly discharge in the Marias River in 2019 remained between the historical 25th and 75th quantiles from April through June of 2019 but rose to above the 90th quantile in July of 2019 (Table 3). The median monthly temperature in the Marias River in 2015 was within the range of the temperature in other years of the study. However, from April through July of 2016, the median monthly temperature was higher than in 2015, 2018, and 2019 (Table 4). In 2018, the median monthly temperature in the Marias River was lower from April through June than it was during the other years of the study but reached temperatures similar to those of other years during July (Table 4). In 2019, the median monthly temperature was within the range of other years from April through June but was colder than in the other years of the study during July (Table 4).

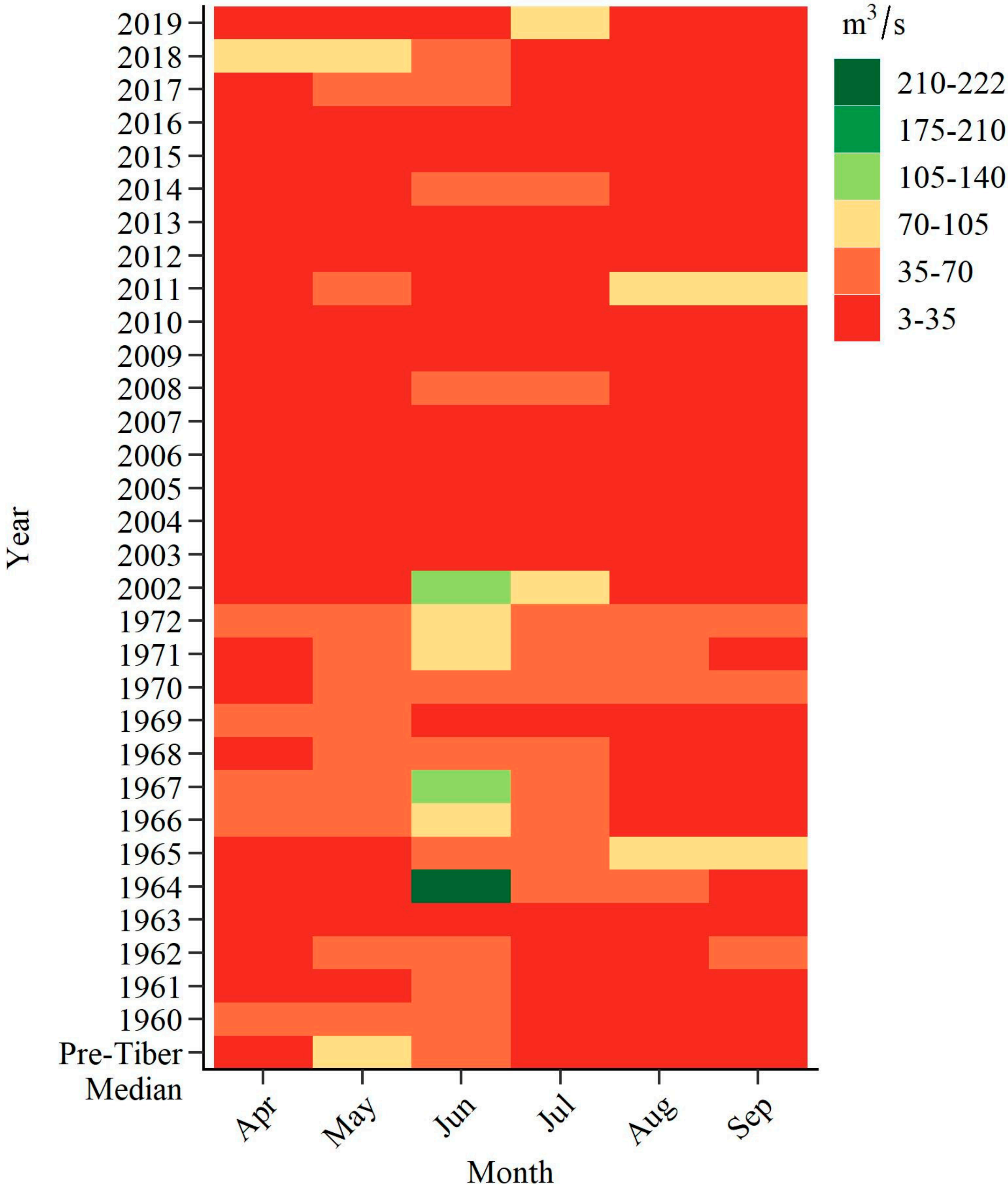

Figure 5. Median discharge (m^3/s) in the Marias River by month from 1960 through 2019, excluding 1973 through 2001 when discharge data were not recorded. Data from the U.S. Geological Survey stream gage station near Loma, MT, USA (stream gage 06102050). Additionally, the median of monthly median discharge from pre-Tiber Dam (1922–1956; data recorded at U.S. the Geological Survey stream gage 06102000) is included.

Table 3. The 10th, 25th, median, 75th, and 90th quantiles of the monthly median discharge (m^3/s) in the Marias River from 1960 through 2019, excluding 1973 through 2001 when discharge data were not recorded, summarized by month, and the median discharge from April through July for 2015, 2016, 2018, and 2019. Data from the U.S. Geological Survey stream gage station near Loma, MT, USA (stream gage 06102050).

Month	1960–1972 and 2001–2019					2015	2016	2018	2019
	10th	25th	Median	75th	90th	Median			
April	11.5	14.0	15.2	27.1	37.1	15.7	14.0	81.8	18.9
May	13.3	14.9	30.9	40.2	56.6	15.1	13.9	90.3	20.4
June	13.7	18.0	34.5	54.4	79.2	18.3	13.6	50.7	20.4
July	10.0	15.2	19.4	39.7	57.6	18.2	14.9	21.5	75.3

Table 4. Median water temperature (°C) in the Marias River for April through July of 2015, 2016, 2018, and 2019. Data from the U.S. Geological Survey stream gage station near Loma, MT, USA (stream gage 06102050).

Month	Median Water Temperature (°C)			
	2015	2016	2018	2019
April	10.7	11.0	6.0	9.6
May	14.7	15.6	11.9	12.3
June	20.3	20.4	16.5	18.9
July	20.8	22.8	20.0	19.9

Twenty spawning or atretic hatchery-origin pallid sturgeon were tracked during this study. Of the 20 pallid sturgeon tracked, two females were tracked during years that culminated in two different reproductive classifications (i.e., atretic and spawning) resulting in 22 individual classifications with tracking data—12 were classified as spawning-capable males, five as spawning females, and five as atretic females.

The medians of median net movement rates were similar among the reproductive classifications and consisted of upstream movements less than 1 km/day (Figure 6). The median net movement rates had little variation within classifications, and all movements were between −3.3 and 4.1 km/day (Figure 6). The largest variation in median net movement rate was for spawning-capable males, with an IQR of 1.7 km/day. In contrast to the median net movement rates, the medians of total movement rates varied considerably among the reproductive classifications. Spawning female pallid sturgeon had the highest median total movement of 269.9 km, which was 176.3 km more than that of spawning-capable males (Figure 7). Atretic females had a median total movement of 142.3 km—less than that of spawning females but 48.8 km more than that of spawning-capable males (Figure 7). The total movement of atretic females had large variation with the 25th and 75th quantiles varying from 101.3 km to 221.6 km (IQR = 120.3; Figure 7).

The median of the median locations of spawning females was within one km of the median of the median locations of spawning-capable males (Figure 8). The median locations of atretic females were highly variable (IQR = 203.0) compared to those of the spawning females (IQR 13.4) and spawning-capable males (IQR = 19.7). Furthermore, the median locations of atretic females overlapped the other classifications (Figure 8). The median locations tended to be in the lower reaches of the study area, with 19 of the 22 median locations downstream of Judith Landing (rkm 3194); however, three individual pallid sturgeon had median locations upstream of Judith Landing including one atretic female in the Missouri River, one atretic female in the Marias River, and one spawning-capable male in the Marias River (Figure 8)—all locations in the Marias River occurred in 2018 when discharge was high (Table 3; Figure 5). Interestingly, no pallid sturgeon had median locations between rkm 3126 and rkm 3293.

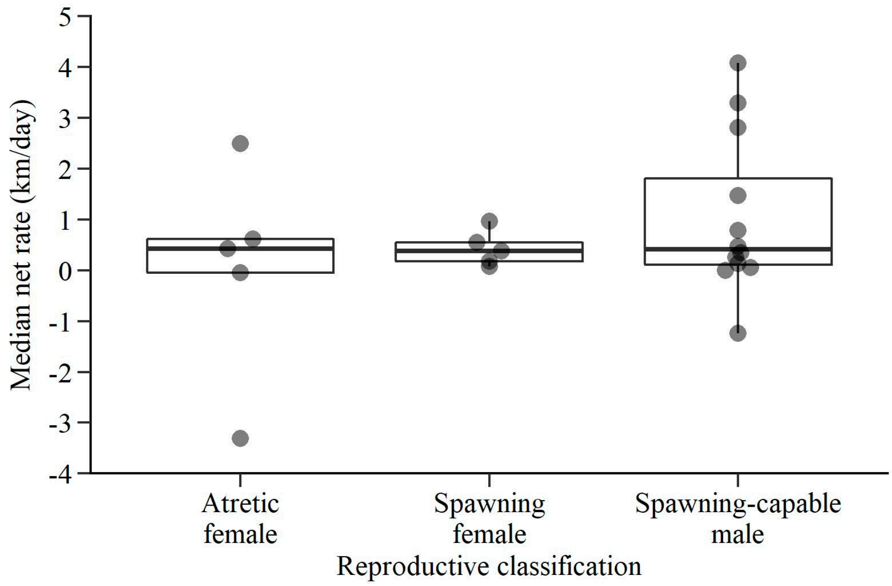

Figure 6. Median net movement rate (km/day, circles represent individuals) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as atretic female, spawning female, and spawning-capable male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 × interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 × IQR from the 25th quantile.

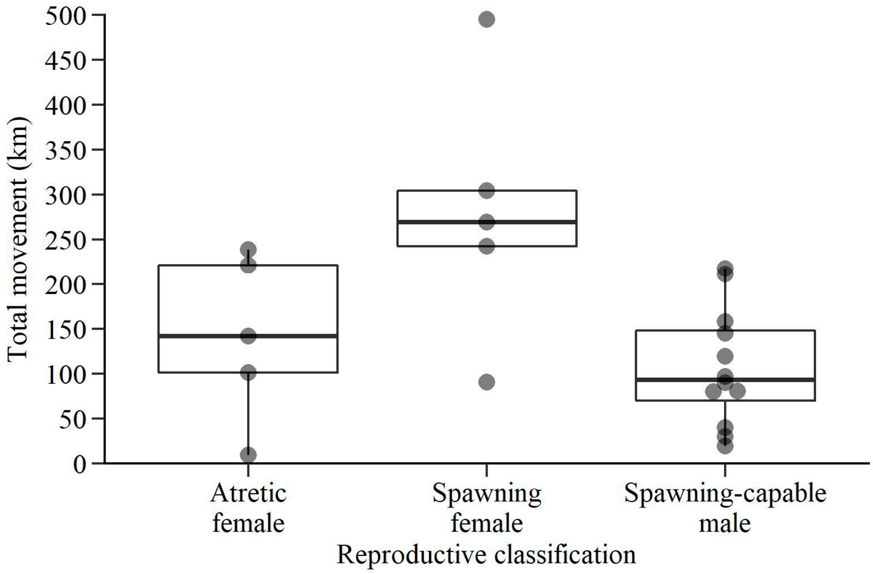

Figure 7. Total movement (km; circles represent individuals) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as atretic female, spawning female, and spawning-capable male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 × interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 × IQR from the 25th quantile.

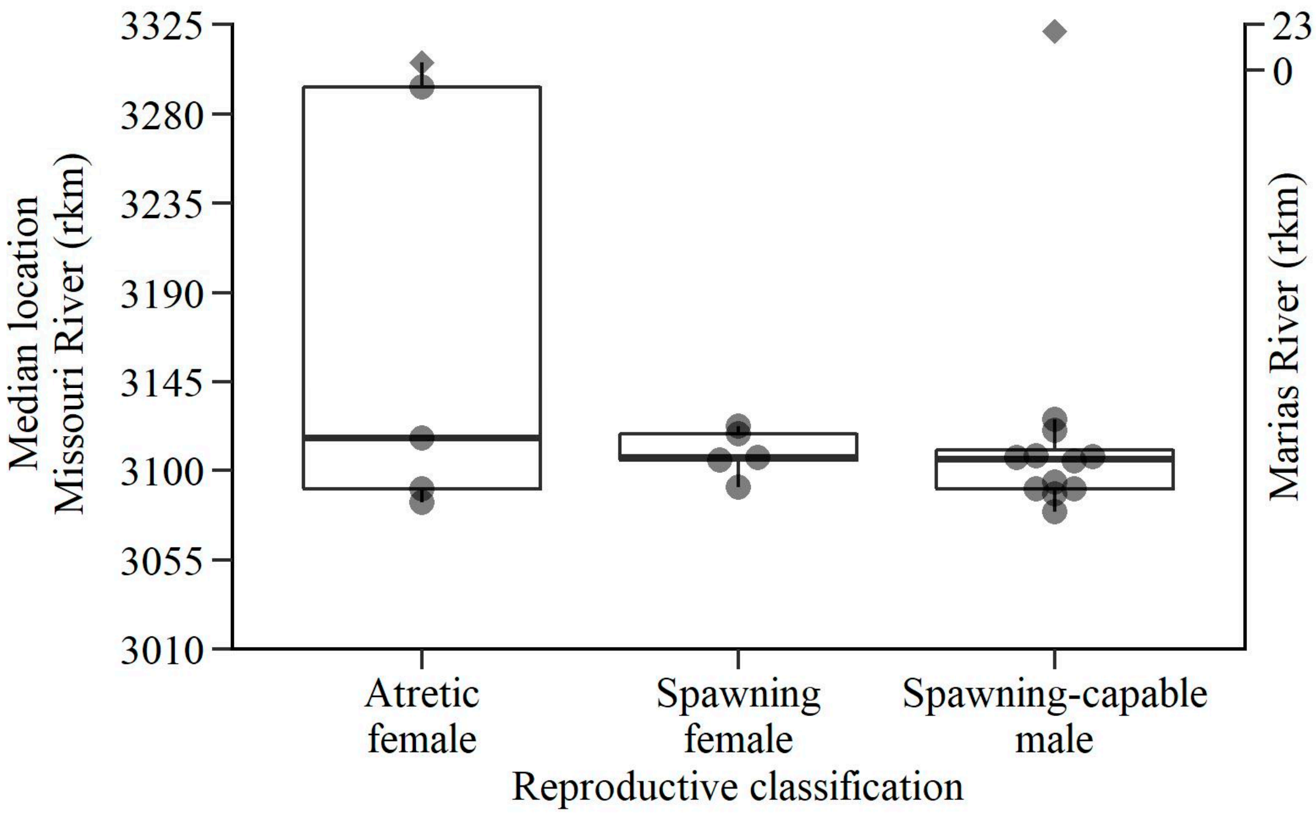

Figure 8. Median location (rkm; circles represent locations in the Missouri River, and diamonds represent locations in the Marias River) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 upstream of Fort Peck Reservoir. Individuals were classified as atretic female, spawning female, and spawning-capable male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 × interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 × IQR from the 25th quantile.

Atretic females had the highest median maximum upstream location (median = 3292.6 rkm), spawning-capable males had the lowest (median = 3162.3 rkm), and spawning females were between the other classifications (median = 3207.0 rkm, Figure 9). In 2018, maximum locations were recorded for four pallid sturgeon in the Marias River—one spawning female, two atretic females, and one spawning-capable male (Figure 9).

The putative spawning reaches for five spawning females were between rkm 3072 and rkm 3141 (Figures 10 and 11). The lower bound of spawning reaches were further downstream in 2019 than in 2018. In 2019, two free embryos were captured downstream of the putative spawning reaches and were linked to a spawning female (ID 8_92) by genetic analysis [45]. The substrate was similar among the mapped reaches and was predominantly composed of gravel and sand with a small amount of cobble (Table 5). The furthest upstream mapped reach (rkm 3117.5–3119.0) contained the largest proportion of cobble (Table 5).

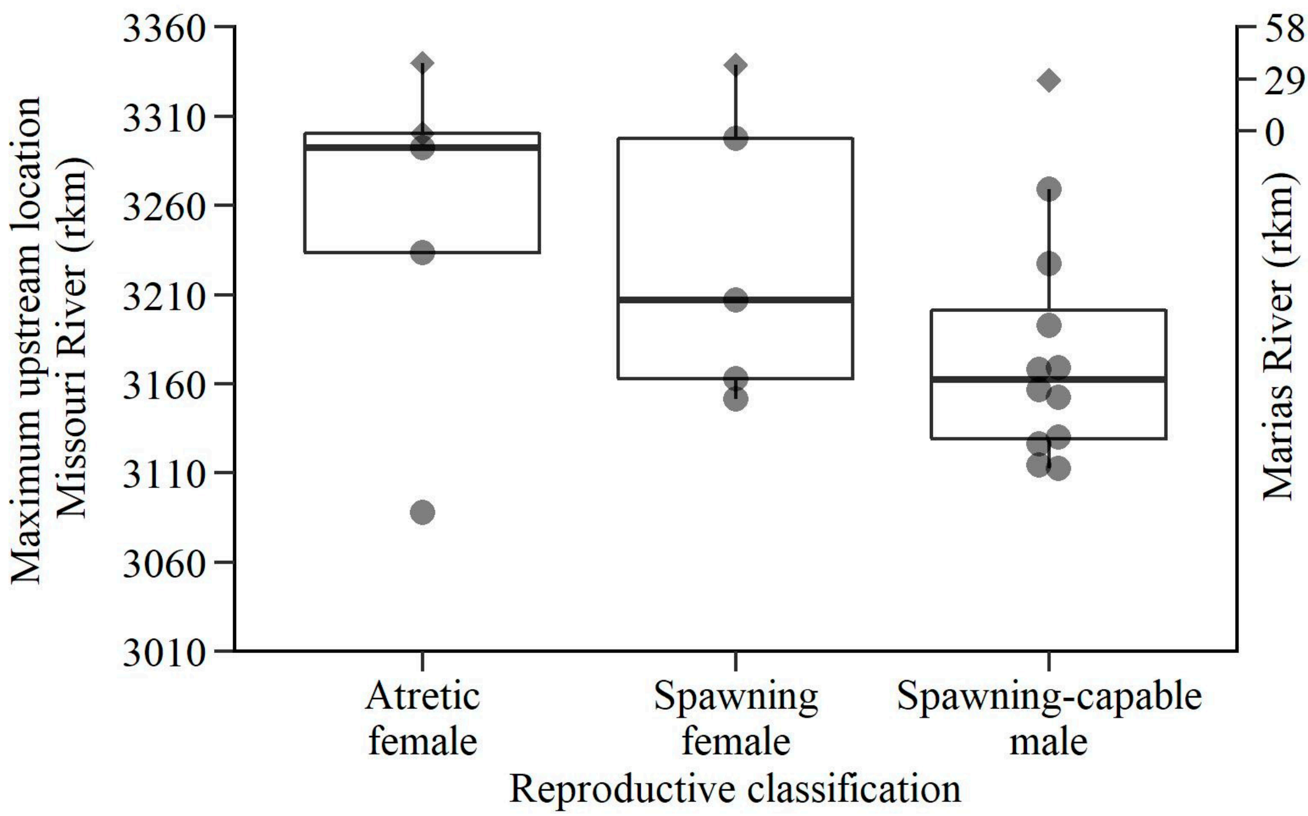

Figure 9. Maximum upstream location (rkm; circles represent locations in the Missouri River and diamonds represent locations in the Marias River) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as atretic female, spawning female, and spawning-capable male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 × interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 × IQR from the 25th quantile.

Table 5. Proportion of substrate types for three mapped reaches in the Missouri River upstream of Fort Peck Reservoir and the median of proportion of substrate type at locations with the interquartile range (IQR) in parentheses. The river kilometer (rkm) of the upper and lower boundary of the mapped reaches is denoted. The mapped reaches were within the putative spawning reaches (see Figures 10 and 11) and were selected for mapping when spawning-capable female pallid sturgeon were observed interacting with mature male pallid sturgeon. The mapped reaches included a ~0.5 km distance above and below where interactions were observed.

Mapped Reach (rkm)	Proportion Substrate Type		
	Sand	Gravel	Cobble
3081.5–3084.0	0.43	0.56	0.01
3088.0–3090.0	0.41	0.53	0.06
3117.5–3119.0 [a]	0.38	0.49	0.13
Median (IQR)	0.41 (0.02)	0.53 (0.04)	0.06 (0.06)

[a] Substrate imagery collected one year after spawning occurred.

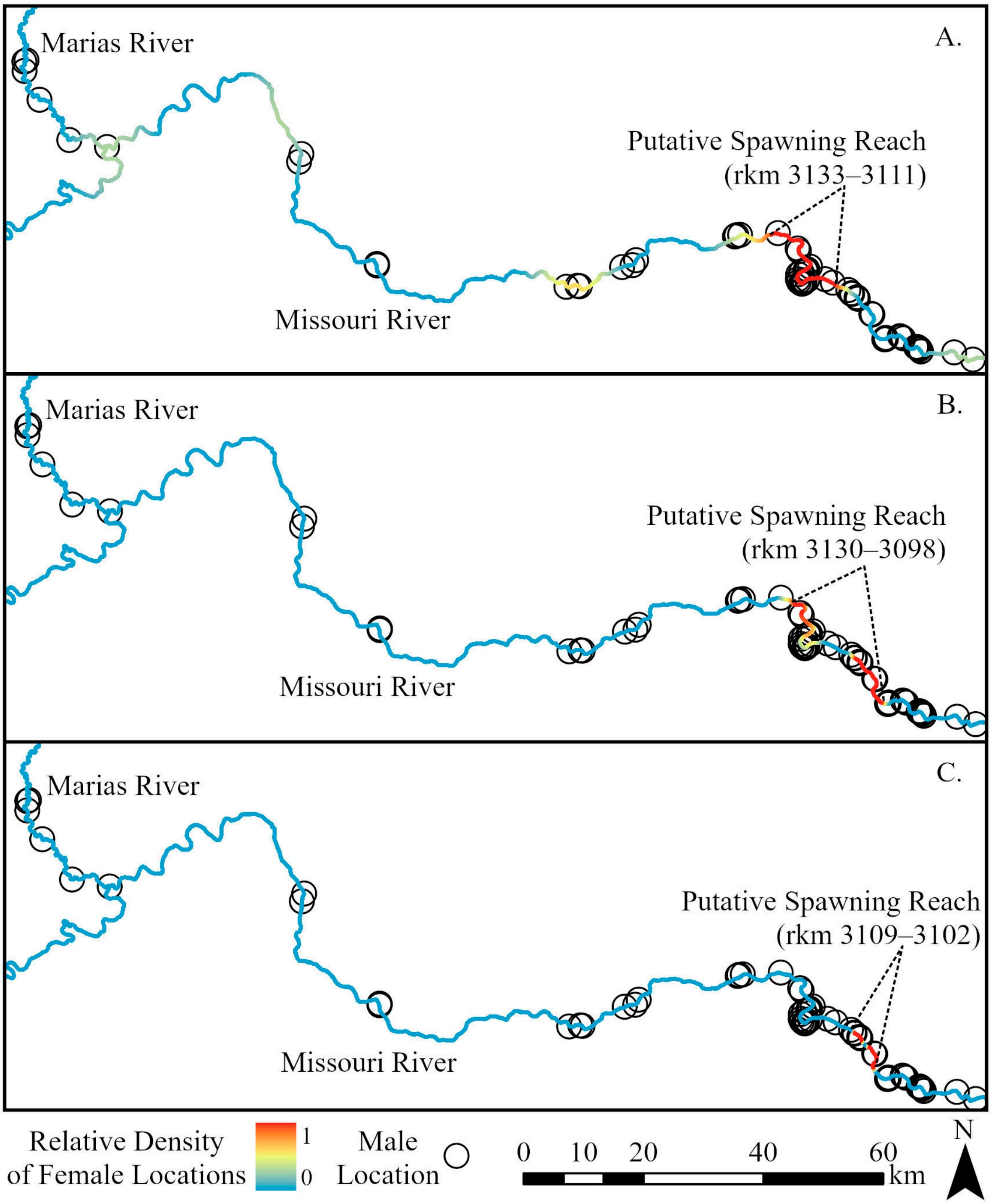

Figure 10. Kernel-density maps show the relative densities of locations for individual females that successfully ovulated (spawning females) during the spawning season 2018 (**A**. ID 9_161, **B**. ID 9_163, and **C**. ID 9_171). Relative densities closer to one indicate areas where females were most frequently located. The lower and upper bounds of the putative spawning reach are indicated by the dashed lines, and the river kilometer (rkm) is in parentheses. Mature male locations during the spawning season are indicated by open circles.

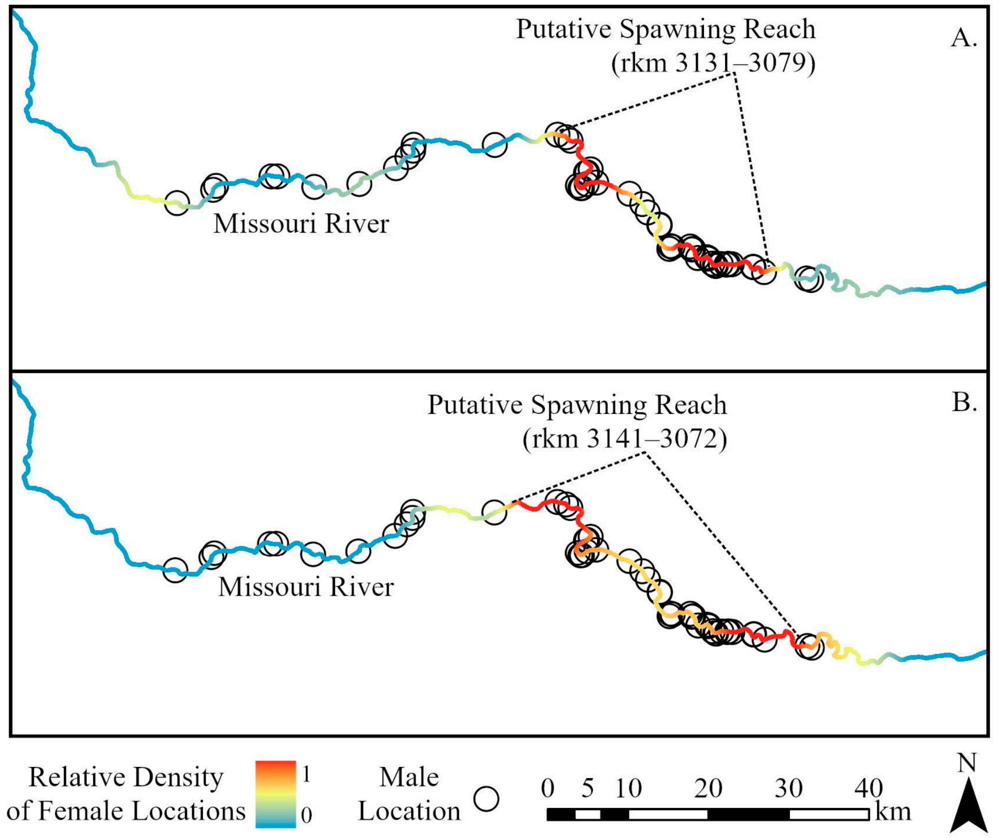

Figure 11. Kernel-density maps show relative densities of locations for individual females that successfully ovulated (spawning females) during the spawning season 2019 (**A**. ID 8_86 and **B**. ID 8_92). Relative densities closer to one indicate areas where females were most frequently located. The lower and upper bounds of the putative spawning reach are indicated by the dashed lines, and the river kilometer (rkm) is in parentheses. Mature male locations during the spawning season are indicated by open circles.

4. Discussion

We characterized movement and identified spawning locations of successfully ovulating pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir for the first time. In general, spawning females made large movements during the putative spawning season but had median net movement rates that were less than 1 km/day and upstream. Spawning-capable males and atretic females had similar median movement rates as those of spawning females. However, the total movements by spawning-capable males and atretic females were fewer than those of spawning females. Interestingly, atretic females had a large variation in total movement rate where some individuals moved similar distances as spawning females did, while others moved much shorter distances. River locations used were similar between spawning females and spawning-capable males, which is the result of both classifications being located near putative spawning locations. However, maximum river location differed, where spawning females had further upstream locations than did spawning-capable males. The disparity in maximum upstream location between spawning females and spawning-capable males indicates that spawning-capable male pallid sturgeon do not move as far upstream as do spawning females during the putative spawning season.

We documented putative spawning reaches of spawning female pallid sturgeon upstream of Fort Peck Reservoir for the first time. Distances from putative spawning reaches to the transition zone at the upstream end of Fort Peck Reservoir were less than the estimated drift distance required for ontogenetic development of pallid sturgeon free embryos. Downstream of Fort Peck Reservoir, the minimum required drift distance has been estimated to be 245 km and is positively correlated with water velocity [20]. Therefore, the required drift distance upstream of Fort Peck Reservoir may be further than 245 km. Pallid sturgeon putative spawning reaches described here were between 62 km and 131 km from the transition zone indicating that even the most upstream spawning reaches provided far less than the required drift distance. Substrate at suspected spawning locations within spawning reaches was a gravel–sand mosaic with little to no cobble or larger substrate. Substrate in the Missouri River transitions from smaller substrate at downstream reaches to larger substrate moving upstream [31]. Therefore, the substrate types we observed at spawning reaches are not commonly found at further upstream locations in the Missouri River. The Marias River may contain substrate compositions similar to what we documented at suspected spawning locations, but the substrate in the Marias River has not been characterized. Additionally, the sediment input at tributary mouths may provide more heterogenous substrate favorable to spawning in upstream areas that are generally dominated by cobble.

Pallid sturgeon were located in the Marias River and upstream of Judith Landing in the Missouri River. Upstream locations and locations in the Marias River occurred during the 2018 putative spawning season, which coincided with uncharacteristically high discharge. However, pallid sturgeon did not spawn while at upstream locations and instead spawned in lower portions of the Missouri River. A scarcity of spawning-capable male pallid sturgeon in the upstream portions of the Missouri River or in the Marias River may preclude upstream spawning opportunities even if female pallid sturgeon could spawn at those locations. Determining why spawning-capable male pallid sturgeon are apparently less likely to be located in upstream reaches during the putative spawning season could help elucidate ways to encourage spawning further upstream. Spawning in the Marias River or upstream of Judith Landing in the Missouri River would increase available drift distance, and given the apparent association between discharge and upstream location, future research and management efforts could consider discharge as a way to increase use of upstream reaches and tributaries. In closely related shovelnose sturgeon, discharge is linked to spawning in the Marias River [23]. When we observed spawning, female, atretic female, and spawning-capable male pallid sturgeon located in the Marias River in 2018, and the Marias River had a historically high discharge, which peaked at 157.2 m^3/s on June 1, 2018. After peaking, the discharge was rapidly reduced to aid in flood control in the Missouri River and reached 20.3 m^3/s by June 28, 2018. During the rapid decrease in discharge, all spawning-capable pallid sturgeon exited the Marias River.

Pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir spawned in locations where the substrate is a mosaic of sand and gravel, which is similar to other populations of pallid sturgeon [42,43]. Upstream of where we observed spawning, potential spawning substrate transitions to larger substrate types (e.g., cobble and gravel) [31]. If sand is an important substrate for spawning pallid sturgeon, the lack of sand further upstream could limit the likelihood of spawning in locations with adequate drift distance, regardless of discharge. However, the Marias River may contain preferable spawning substrate. If appropriate spawning substrate is present in the Marias River, discharge and temperature manipulation at Tiber Dam may be a viable management action to promote upstream spawning.

Pallid sturgeon, like other sturgeon species (e.g., white sturgeon [46]), have adhesive eggs that probably evolved to adhere to hard substrates, and hard substrate, such as the large proportion of gravel observed in spawning reaches during this study, may be the preferred spawning habitat. However, why pallid sturgeon spawn in areas with high proportions of sand remains unknown. In the lower Missouri River, spawning reaches were dominated by sand, but selection coefficients were highest for hard substrates (i.e., gravel, cobble, boulder, or bedrock) and lowest for sand [47]. Spawning of pallid sturgeon in the Yellowstone River has occurred in reaches that are mostly sand [34], and white sturgeon have been documented spawning over sand; however, it was hypothesized that white sturgeon spawning over sand may be due to an absence of a preferred habitat [48]. Perhaps a gravel–sand mosaic of substrate the preferred habitat for reasons other than spawning. For example, in the lower Platte River, diet items of adult shovelnose sturgeon and age-0 shovelnose sturgeon and pallid sturgeon are associated with sand substrate [49,50]. Furthermore, age-0 pallid sturgeon have been shown to use alluvial sand dunes as velocity refugia [51]. However, downstream drift of free embryos makes the link to spawning site selection and age-0 pallid sturgeon intangible.

The variation of movement by atretic females is likely associated with the timing of ovarian follicular atresia. Ovarian follicular atresia is associated with a drop in sex steroid concentrations [52], and sex steroids (e.g., testosterone or estradiol) are closely associated with endocrine signaling that drives spawning-related behavior. For example, upstream migration can be induced in immature landlocked sockeye salmon (*Oncorhynchus nerka* [Walbaum in Artedi]) by implanting individuals with testosterone [53]. In pallid sturgeon, a decrease in sex steroids at the onset of follicular atresia would result in decreased drive to undergo spawning-related movements. Therefore, pallid sturgeon that initiate ovarian follicular atresia early in the spawning season would behave as though they were not reproductively active while pallid sturgeon that initiate atresia late in the spawning season would behave more similarly to spawning females. The movement of pallid sturgeon that are not reproductively active consists of slower movement and shorter distances compared to that of reproductively active pallid sturgeon (i.e., spawning females, atretic females, and spawning-capable males) [22]. The large variation in total movement by atretic females and the difference in total movement between atretic females and spawning females indicates that grouping atretic females with spawning females should be avoided when observing the behavior of pallid sturgeon.

5. Conclusions

Here, we report the first observations of hatchery-origin pallid sturgeon spawning in the Missouri River upstream of Fort Peck Reservoir. In addition, we found that spawning-capable pallid sturgeon will use the Marias River, but we only observed this during an unprecedented discharge event. Recovery of pallid sturgeon as defined in the Recovery Plan will only be possible if pallid sturgeon spawn further upstream than the spawning locations identified here. Management actions such as modified water releases from upstream dams to encourage use of upstream locations, lowering Fort Peck Reservoir to provide more drift distance, or both may be necessary to promote successful recruitment as outlined in the Recovery Plan. Furthermore, we found that pallid sturgeon spawned in locations with a gravel–sand mosaic of substrate, which is negatively associated with distance upstream in the upper Missouri River. The Marias River may contain a more suitable spawning habitat provided that discharge and temperature regimes are managed to construct suitable spawning conditions. Management actions such as modifying discharge at Tiber Dam, altering reservoir levels at Fort Peck Reservoir, or constructing spawning habitat in desired spawning locations may be necessary to ensure spawning occurs in locations with an adequate drift distance for free embryos.

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