

FEEDING ECOLOGY OF PALLID STURGEON IN THE MISSOURI RIVER ABOVE FORT
PECK RESERVOIR, MONTANA

by

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of

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ABSTRACT

Age and size at sexual maturity of juvenile Pallid Sturgeon (*Scaphirhynchus albus*) in the Missouri River above Fort Peck Reservoir is driven by growth; therefore, understanding the mechanisms for the variation in growth is crucial for conserving Pallid Sturgeon. For this research, we addressed the following questions: 1) "Does the level of piscivory differ among year-classes," 2) "Has the level of piscivory changed from 2003 and 2004 to 2023 and 2024," and 3) "Is trophic position related to age, length, and weight?" Pallid Sturgeon were sampled for stable isotopes (i.e., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), gut contents, and fecal material (i.e., DNA metabarcoding of prey species) in the Missouri River above Fort Peck Reservoir. Data were analyzed by year-class, size (length and weight), and age to better understand the factors influencing the use of food resources in the Missouri River. For all year-classes pooled, piscivory only accounted for 11.84 mean percent by weight and benthic macroinvertebrates accounted for 88.16 mean percent by weight. Simuliidae was the most common diet item and had the greatest mean percent by weight of all fish sampled. DNA metabarcoding of fecal material detected eleven additional fish species that were not observed in the gastric lavage samples, including *Carpiodes carpio*, *Ictiobus bubalus*, *Polyodon spathula*, *Sander canadensis*, and *Cycleptus elongatus*, which have not been recorded in Pallid Sturgeon diets previous to the present study. The 1997 year-class (oldest year-class; 28 years old) had the highest mean trophic position of 4.17, which is indicative of an apex predator. Conversely, the 2021 year-class (youngest; 4 years old) had the lowest mean trophic position of 3.18, which is consistent with an insectivore. Pallid Sturgeon were observed introducing fish into their diet at 439 mm, 308 g, and 3.5 years old and transitioning exclusively to fish at 674 mm, 1,450 g, and 18.4 years old. Trophic position was strongly associated with Pallid Sturgeon length, suggesting that the switch to piscivory increases growth rate. This dietary transition ultimately shapes when individuals reach sexual maturity, which has implications for Pallid Sturgeon conservation.

FEEDING ECOLOGY OF PALLID STURGEON IN THE MISSOURI RIVER ABOVE FORT PECK RESERVOIR, MONTANA

Introduction

Pallid Sturgeon *Scaphirhynchus albus* are an endemic species native to the Mississippi and Missouri rivers and their tributaries (Jordan et al. 2016). Multiple large-scale dams constructed in the upper Missouri River during the early 1900s altered natural flow regimes, sediment transport, and blocked fish passage (USFWS 1993). Historic sport and commercial harvest combined with large-scale anthropogenic habitat modifications within drainages occupied by Pallid Sturgeon led to large-scale population declines (USFWS 1993). Thus, Pallid Sturgeon were listed as an endangered species in 1990 (Federal Register 1990).

The population of Pallid Sturgeon in the upper Missouri River (reach of Missouri River above Fort Peck Dam to Morony Dam) has not successfully recruited for decades — probably since Fort Peck Dam was completed in the late 1930s. The lack of recruitment in the upper Missouri River was a consequence of Pallid Sturgeon larvae drifting into anoxic conditions at the headwaters of Fort Peck Reservoir (Guy et al. 2015). The lack of recruitment by Pallid Sturgeon prompted the development of the Pallid Sturgeon Conservation Propagation Program (USFWS 2014). The first cohort of Pallid Sturgeon from this program was stocked in the upper Missouri River in 1998. Since the first cohort was stocked, 14 additional cohorts have been stocked in the upper Missouri River.

In accordance with the Pallid Sturgeon Conservation Propagation Program, 142,917 fingerling and yearling Pallid Surgeon have been stocked in the upper Missouri River to date

(MTFWP, unpublished data 2023). To increase survival after stocking, Pallid Sturgeon are stocked as fingerlings or yearlings. Stocking densities of Pallid Sturgeon cohorts in the upper Missouri River generally do not exceed 10,000 individuals. However, the 2007, 2008, and 2009 year-class stocking densities exceeded 30,000 individuals per cohort (Figure A.1). Year-classes of stocked Pallid Sturgeon are composed of several family-lot combinations and broodstock are selected to reduce relatedness. For example, the 2005 year-class was from eight family lots. The estimated survival rate of stocked Pallid Sturgeon in the upper Missouri River is about 80% (Rotella 2017).

The propagation program offers a unique opportunity to analyze individual growth rates over time, because most fish are marked and measured for length and weight prior to stocking and at each recapture. The 1997 year-class was the first cohort stocked in the Missouri River and has the fastest growth rate in length and weight after age 6 (Figures A.2 and A.3). Growth among year-classes stocked after 1997 is highly variable (Figures A.2 and A.3). In addition to variation in growth rates among year-classes, considerable variation within year-classes has been observed (Figure A.4 and A.5). The observed variation in growth within and among year-classes has population-level implications especially in terms of age at first sexual maturity because age is linked to the length of Pallid Sturgeon (Holmquist et al. 2018). The observed variation in growth undoubtedly influences recovery goals for the Pallid Sturgeon population in the upper Missouri River (See appendix A).

The observed variation in growth rates among and within year-classes of Pallid Sturgeon prompted the question "What are the mechanisms for the variation in growth?" The mechanism for the differences in growth among year-classes of Pallid Surgeon is unknown, but one of the leading hypotheses is related to diet and the transition from feeding on benthic aquatic

macroinvertebrates to piscivory. That is, the Pallid Sturgeon that exhibit the fastest growth rates may have switched to piscivory at a younger age than those with slower growth rates.

Ontogenetic transition to piscivorous feeding increased growth rates of freshwater piscivores and other sturgeon species (Buckel et al. 1998, Mittelbach and Persson 1998, Muir et al. 1988). The mechanisms that initiate the transition to piscivory are not well understood and often vary for individuals of a given species and among populations. Previous literature suggested that juvenile Pallid Sturgeon in the upper Missouri River switch to piscivory at about 530 mm (Gerrity 2005), below Fort Peck Reservoir and the lower Yellowstone River at 300 mm (Dutton 2018), and in the middle Missouri River at 600 mm (Grohs et al. 2009).

The objective of this study was to better understand the feeding ecology of juvenile Pallid Sturgeon to provide insights into the observed variation in growth. I hypothesized a relationship exists between the level of piscivory and body size and age. I also hypothesized that the level of piscivory has changed from 2003 and 2004 to 2023 and 2024. More specifically, I addressed the following questions: 1) "Does the level of piscivory differ among year-classes," 2) "Has the level of piscivory changed from 2003 and 2004 to 2023 and 2024," and 3) "Is trophic position related to age, length, and weight?"

Methods and Materials

Study Area

The Missouri River is the longest river in the United States flowing about 3,768 kilometers (Galat and Lipkin 2000). The study area for this project consisted of the Missouri River in Montana from the confluence with the Marias River (river km 3,303) to the upper end of

Fort Peck Reservoir (river km 3,010) (Figure 1). Upstream impoundments regulate discharge, but seasonal influxes from unregulated tributaries (i.e., Judith River, river km 3,194) allow for modest surges in the hydrograph. In response to habitat alterations in the form of large-scale dams, the United States Fish and Wildlife Service (USFWS) established four areas throughout the Missouri River drainage as high priority management units for Pallid Sturgeon (USFWS 2014). My research was conducted in the Great Plains Management Unit, the most northwestern portion of the Pallid Sturgeon native range (USFWS 2014).

Numerous benthic aquatic macroinvertebrate and freshwater fish species reside in the upper Missouri River. Thirteen orders of macroinvertebrates have been described in this reach; Diptera, Ephemeroptera, and Trichoptera are the most abundant (Berg 1981). Fifty-three freshwater fish species reside in the mainstem river representing 14 families comprising Acipenseridae, Catostomidae, Centrarchidae, Cottidae, Esocidae, Gadidae, Gasterosteidae, Hiodontidae, Ictaluridae, Leuciscidae, Percidae, Polyodontidae, Salmonidae, and Sciaenidae (Brown 1971).

Fish Collection

Pallid Sturgeon were sampled in April of 2023 and 2024 by Montana Fish, Wildlife and Parks (MTFWP) using set lines (Hubert et al. 2012) and from May through September of 2023 and 2024 by Montana State University (MSU) using multifilament trammel nets (Hubert et al. 2012). Set lines were deployed at 90 randomly selected locations from the bow of the boat perpendicular to the river. Set lines were set in the afternoon and pulled the following day. Anchors on each end allowed for sets to remain perpendicular to the flow of the river throughout the night. Set lines were 30.5 m long and each was set with 20 octopus circle hooks, sizes 3/0,

2/0, and 2. Hooks were baited with common earthworms (*Lumbricus terrestris*) and fresh cut bait, primarily Flathead Chub (*Platygobio gracilis*), White Sucker (*Catostomus commersonii*), and *Hybognathus* spp. Trammel nets were 45.7 m by 1.8 m, with lead lines weighing 13.6 kg and 9.5 mm diameter foam core float lines. Two different trammel net sizes were used. One had an inner panel mesh size of 25.4 mm and the other had an inner panel mesh size of 101.6 mm; both had 254 mm outer panel meshes. Trammel nets were drifted perpendicular to the flow of the river. Drift duration was water temperature dependent and never exceeded ten minutes. Trammel nets were deployed from the bow of the boat and included a buoy connected to each side of the trammel net as a visual reference. One of the buoys had a lead that remained tethered to the deck. Sampling locations were a combination of “hotspots” (i.e., locations where Pallid Sturgeon were commonly sampled by MTFWP) and MSU-selected locations. Montana State University selected locations that appeared visually optimal, such as inside bends or areas where radio-transmitted Pallid Sturgeon (radio tagged by MTFWP for a concurrent study) were present. The boat was equipped with an antenna and Lotek Telemetry Receivers (SRX 400 and SRX 800) to locate areas occupied by radio-transmitted Pallid Sturgeon. Fork lengths (mm) and weights (g) of all sampled Pallid Sturgeon were measured. Each individual was assessed for visual marks (removed scutes and elastomer marks in the ventral rostrum), dorsal and peritoneal passive integrated transponder (PIT) tags (23 mm FDX and 32 mm HDX), and radio transmitters.

Gastric Lavage

Gastric lavage (Haley 1998) was performed following the collection of length and weight to extract stomach contents. Fish ≤ 650 mm were lavaged using a two-gallon pressurized garden sprayer. The vinyl tubing of the sprayer had a 9.5 mm inside diameter and was connected to a

trigger nozzle. Flexible vinyl tubing was connected to the output end of the trigger nozzle and was selected based on the size of the esophageal opening (outer diameter in numerous sizes varied from 6 mm to 13 mm). The size of the vinyl tubing was selected to allow for insertion and for stomach contents to exit within the esophagus (Haley 1998). Fish > 650 mm were lavaged using a 12-volt, 18.3 liters per minute transfer pump to produce adequate water pressure for retrieval of stomach contents. Water used for lavage was site-specific river water strained through a 500- μ m sieve in a five-gallon bucket connected to the transfer pump by polymer tubing. The outtake water flowed through the polymer tubing to the pump, into polymer tubing to a pressure regulator that allowed pressure regulation depending on the size of the fish, into tubing to a trigger nozzle, and finally into fish-size dependent vinyl tubing. A small volume of water was discharged through the tubing prior to inserting it into the esophageal opening to prevent introducing air bubbles into the digestive tract.

Fish were held ventral side up and the vinyl tubing was inserted slowly into the esophagus until it reached the first bend in the stomach, where resistance occurred (Brosse et al. 2002). The vinyl tubing was retracted slightly (about 5 mm) to prevent perforating the pyloric ceca. Fish were held laterally above the 500- μ m sieve, with the rostrum angled down at about 45° (Wanner 2006). Forward pressure was applied externally to the abdomen while flushing water into the stomach to help induce the removal of stomach contents (Crossman et al. 2016). Flushing occurred for 20-30 seconds or until the discharged water contained no contents. The fish was returned to the tank for a short period (about one minute), and the procedure was repeated once more on the same fish. All dietary contents were stored in 70% ethanol until examination in the laboratory.

Fecal Collection and Primers

Fecal material was collected to identify prey fish species DNA. The colonic flushing apparatus consisted of a 5 mL or 10 mL syringe containing 5% saline fluid, connected to a medical-grade catheter (Cure Medical Intermittent Catheter, Size: F10, Product ID: CURF10-30 or Cure Medical Intermittent Catheter, Size: F16, Product ID: CURF16-30). The lower abdomen and vent of each fish was cleared of any debris and dried before sample collection. I wore nitrile rubber gloves and unpackaged catheters immediately prior to the procedure to minimize contamination with human DNA. The F10 catheters contained 2-3 mL of 5% saline fluid and were used on fish ≤ 650 mm whereas the F16 catheters contained 5-6 mL of 5% saline fluid and were used on fish > 650 mm to prevent perforation of the lower digestive tract (Hoover 2007). The catheter was inserted about 5 cm into the anus, the saline fluid was injected, and the syringe plunger was retracted slightly to collect the saline fluid and visible amounts of fecal matter. The fluid and fecal matter mix were injected into a screw-top 10 mL vial and placed on ice until subsequently placed in a freezer. River water was collected in a 0.9 kg Nalgene bottle and analyzed for any fish primers as a baseline for contamination of river water when collecting fecal material.

Basal Trophic Level Taxa Tissue Samples and Capture

Basal taxonomic groups and potential Pallid Sturgeon prey species (algae, freshwater mussels, benthic macroinvertebrates, and fish) were collected in August of 2024 for stable isotope analysis to calculate Pallid Sturgeon trophic levels. Algae (*Cladophora* spp.) were collected from drifted trammel nets, Fatmucket (*Lampsilis siliquoidea*) were collected by hand from a shallow bank, Ephemeroptera species were collected from MTFWP beam trawls,

Sicklefin Chub (*Macrhybopsis meeki*) and Flathead Chub were collected using a bag seine (10.7 m by 1.8 m with 5-mm mesh diameter), and Stonecat (*Noturus flavus*) were bycatch from a trammel net targeted for Pallid Sturgeon. A 1 cm² tissue biopsy was collected from each individual, placed in a labeled screw-top 5 mL microcentrifuge vial, immediately put on ice, frozen when access to a freezer became available, and analyzed in the laboratory.

Historical Comparison

We compared the stomach contents from gastric lavage methods of 56 age 6- and 7-year-old Pallid Sturgeon from our study to the only other Pallid Sturgeon diet data (Gerrity et al. 2006) that exist in the upper Missouri River to address our second question “Has the level of piscivory changed from 2003 and 2004 to 2023 and 2024?” The previous stomach contents research was conducted in 2003 and 2004 using gastric lavage methods, on only 28 individuals from the 1997 year-class (i.e., 6- and 7-years old).

Tissue Biopsy

A 5 mm pelvic fin biopsy was removed from both pelvic fins (equating to 1 cm²) using forceps and dissecting scissors. A 1 cm² tissue biopsy was collected from each individual, placed in a labeled screw-top 5 mL microcentrifuge vial, immediately put on ice, frozen when access to a freezer became available, and analyzed in the laboratory. Vial identification was recorded on the data sheet and cross-referenced with the individual Pallid Sturgeon. Any biological tissue remaining on forceps and dissecting scissors was removed using 70% ethanol to prevent cross contamination of samples.

Laboratory Analyses

Diets

Count (N), length (mm), and dry mass (g) were recorded for all items in the diets. Using a dissecting microscope (Leica M165C), macroinvertebrates were identified to the lowest taxon. Macroinvertebrates were measured to the nearest millimeter, excluding the cerci and antennae (Appendix Table A.1). If a diet exceeded 30 individuals of the same taxonomic group, the first 30 were measured and the remaining were counted, and average length was applied. Partial macroinvertebrates were measured and identified if possible. Fish in diets were identified to species. All fish were measured to the nearest millimeter for standard length (Appendix Table A.1.). Identification of digested fish was attempted based on vertebrae counts and identifiable parts such as pharyngeal teeth or cleithrum. Scales, bones, and other parts were measured, counted, and recorded as unknown fish parts.

Frequency of occurrence (O_i) and mean percent by weight (MW_i) were calculated to compare diets among year classes of Pallid Sturgeon sampled (Garvey and Chipps 2012) as

$$O_i = \frac{J_i}{P} * 100,$$

$$W_i = \left(\frac{w_i}{\sum_{i=1}^Q w_i} \right), \text{ and}$$

$$MW_i = \frac{1}{P} \sum_{j=1}^P \left(\frac{w_{ij}}{\sum_{i=1}^Q w_{ij}} \right) * 100,$$

where J_i was the number of fish containing prey i , P was the number of fish with food in their stomachs, Q was the number of food types, W_i was the weight of the given prey type i , and j was the individual fish. To allow for comparisons, W_i was calculated for the individual fish and averaged for each prey type to obtain MW_i (Garvey and Chipps 2012).

Fecal Samples

Prey fish were identified from fecal samples using DNA metabarcoding, amplification, and next-generation sequencing at the Pisces Molecular Wildlife and Conservation Molecular Genetics Lab in Boulder, CO. Raw fecal samples were lysed and DNA was extracted using the standard reagents and protocol from the Qiagen DNeasy Blood & Tissue Kit. DNA extracts were then prepared as NGS libraries in the following steps: 1) target region of fish mitochondrial 12S amplified by PCR in triplicate (to mitigate stochastic sampling error) using MiFish-U (universal) primers with Illumina Nextera adapter overhangs, then triplicate PCR products pooled per sample, 2) the first round PCR products were cleaned with ExoI/rSAP plus the restriction enzyme BamHIhf to cut any amplified Pallid Sturgeon 12S molecules. The restriction cutting was done to decrease the expected predominance of host, Pallid Sturgeon, reads found during sequencing. Fortuitously, an alignment of 12S sequences from GenBank for Pallid Sturgeon and all potential prey species indicated that only the Pallid & Shovelnose Sturgeon 12S amplicon sequences contained a BamHI recognition site, 54 base pairs from the 3' end of the 12S MiFish amplicon (Figure A.6). Next, a second round of PCR was performed to complete the sequencing library construct, appending the final Illumina sequencing adapters and integrating a sample-specific, Illumina Nextera XT 12-nucleotide index sequence, and 3) final libraries were cleaned by magnetic bead purification, quantified by Qubit, and normalized to an appropriate concentration for sequencing. Final libraries were then pooled and sequenced at Pisces Molecular on an Illumina MiSeq (San Diego, CA) using a v2 300-cycle reagent kit on a Nano flow cell (Illumina catalog # MS-103-1001).

Pisces Molecular conducted sequence data quality control and analysis, largely within a QIIME2 workflow for provenance tracking. Prior to downstream analysis, MiFish-U primer

sequences were removed from the reads using Cutadapt. Reads were then denoised into Actual Sequence Variants (ASVs) using DADA2. Taxonomy was assigned to each ASV by performing a BLAST+ search with a 98% identity threshold in two ways: 1) against a custom, location-based database curated from the original MitoFish database to include only sequences from 40 potential Pallid Sturgeon diet taxa (fish species known to be present in the Missouri River, plus sequences for Pallid Sturgeon and Shovelnose Sturgeon (*Scaphirhynchus platyrhynchus*), and 2) against the full MitoFish database, which identified a few taxa at high confidence that were not on the “potential diet species” list but which appear to possibly be sympatric with Pallid Sturgeon (e.g., Paddlefish, *Polyodon spathula*). Finally, the results of the above were combined and a few remaining ASVs that were not assigned taxonomy from the MitoFish database were then manually BLASTed against the full NCBI GenBank database and only hits with >98% identity were considered (in the results spreadsheet, these are in red and considered presumptive).

Frequency of occurrence (O_i , see equation above) of prey fish was calculated to compare diets among year classes of Pallid Sturgeon that were sampled using DNA metabarcoding.

Stable Isotopes: Pallid Sturgeon

Stable isotope analyses were performed to analyze individual Pallid Sturgeon trophic positions. Tissue samples collected for stable isotopic analysis were freeze-dried for 36 hours using a Labconco Freezone 1 (Labconco Cooperation, Kansas City, MO, USA), ground into powder using a mortar and pestle, and returned to the corresponding vial. The ground samples were analyzed by the Wyoming Stable Isotope Facility using a Thermo Finnigan Delta Plus XP, Costech 4010 and Carlo Erba 1110 Elemental Analyzer, Costech Zero Blank Autosampler, and

Finnigan Conflo III interface. Standard ellipses were created for 13 year-classes of Pallid Sturgeon using SIBER (Stable Isotope Bayesian Ellipses) metrics in R to analyze trophic position overlap (Jackson et al. 2011). Trophic position analysis uses baseline signatures from lower-level prey (i.e., primary consumer). Trophic position (TP) was calculated for each individual (Post 2002) as

$$TP = \left(\frac{\delta^{15}N_{pallid\ sturgeon} - 8.859836}{3.4} \right) + 2,$$

where $\delta^{15}N_{pallid\ sturgeon}$ was the individual $\delta^{15}N$ value, 8.859836 was the $\delta^{15}N_{mussel}$ value, 3.4 was the ΔN value used for standard bulk tissue samples in freshwater systems, and 2 was the trophic level of mussels, a primary consumer. Using the Regression Models with Break-Points/Change-Points Estimation package in R, segmented regression models were used to analyze multiple relationships among trophic position and length, weight, and age (Muggeo 2026). Segmented regression allowed for multiple slopes at breaking points for the independent variable.

Stable Isotopes: Basal Trophic Level Taxa

Stable isotope analyses were performed on all basal trophic level taxa sampled following the procedure described above (see Stable Isotope: Pallid Sturgeon).

Results

Gastric Lavage: Frequency of Occurrence and Mean Percent by Weight

Piscivory accounted for 11.84 mean percent by weight and benthic aquatic macroinvertebrates accounted for 88.16 mean percent by weight for all year classes pooled in stomach content data collected from 296 Pallid Sturgeon (Table 1). Fish were uncommon relative to macroinvertebrates in the diets of Pallid Sturgeon (Table 2; Figure 2). One year class consumed only fish (1997), four year-classes consumed exclusively macroinvertebrates (2005, 2007, 2010, and 2013), and nine year-classes consumed both macroinvertebrates and fish (2006, 2008, 2009, 2012, 2014, 2015, 2016, 2017, and 2021) (Table 2). Five orders of macroinvertebrates (Coleoptera, Diptera, Ephemeroptera, Hymenoptera, and Trichoptera) were identified in the diets (Table 2; Figure 2, Table A.1). Simuliidae was the most common diet item and had the greatest overall mean percent by weight (45.23) (Table 2).

Four families of fish (Acipenseridae, Ictaluridae, Leuciscidae, and Salmonidae) were identified in the diets (Table 2; Figure 2, Table A.1). Ictaluridae was the highest mean percent by weight (7.43) when all year classes were pooled. Seven species of fish were identified in the diets of Pallid Sturgeon using the gastric lavage method (Figure 3). The 1997 year-class was the only year-class that was exclusively piscivorous; Stonecat (92.88) and Channel Catfish (*Ictalurus punctatus*) (7.12) were the only two species observed in the stomach contents (Table 2). Sicklefin Chub was observed in the diets of four year-classes and made up 0.97 percent by weight (Table 2). No Sturgeon Chub (*Macrhybopsis gelida*) were observed. Unexpectedly, one Cisco (*Coregonus artedii*) was observed in the diet of a Pallid Sturgeon from the 2009 year-class (Table 2; Figure 3). The smallest Pallid Sturgeon that had a fish in its diet was 378 mm long (2021 year-class).

DNA Metabarcoding: Frequency of Occurrence

More fish species were identified in the diets of Pallid Sturgeon using DNA metabarcoding ($n = 71$; Table 1) than using the gastric lavage method (Figure 3). DNA from seven families (Catostomidae, Cottidae, Ictaluridae, Leuciscidae, Percidae, Polyodontidae, and Salmonidae) and 14 species were observed in fecal material (Figure 3). The top three species observed were Channel Catfish (21.17), Sturgeon Chub (16.36), and Emerald Shiner (*Notropis atherinoides*; 11.64) when all year classes were pooled (Table 3). Emerald Shiner, Sturgeon Chub, Flathead Chub, Channel Catfish, Sicklefin Chub, and Paddlefish (*Polyodon spathula*), were observed in five or more year-classes, perhaps indicating an important prey item for Pallid Sturgeon. The 1997 year-class had the most diversity of families or species observed in fecal matter, with 11 unique DNA hits (Table 3). River Carpsucker (*Carpiodes carpio*; 10.00), *Hybognathus* spp. (10.00), and Sauger (*Sander canadensis*; 10.00) were only found in fecal material of the 1997 year-class (Table 3). The 2015 year-class had the second highest amount of diversity of species with nine unique DNA hits; Lake Chub (*Couesius plumbeus*; 12.50) and Shorthead Redhorse (*Moxostoma macrolepidotum*; 12.50) were only observed in the diets of this year-class. River Carpsucker, Smallmouth Buffalo (*Ictiobus bubalus*), Blue Sucker (*Cycleptus elongatus*), Paddlefish and Sauger DNA had never been recorded in the diets of Pallid Sturgeon prior to our study.

Historical Comparison

Mean fork length and weight of 6- and 7-year-old Pallid Sturgeon were similar between the Gerrity et al. (2006) study and the present study, although more variation in fork length and

weight of fish was reported in the Gerrity et al. (2006) study (Figure 4). Pallid Sturgeon sampled by Gerrity et al. (2006) had a mean fork length of 538 mm and a weight of 518 g, and in the present study, Pallid Sturgeon averaged 517 mm and 427 g. Overall, fish were uncommon in the diets of 6- and 7-year-old Pallid Sturgeon in the present study ($O_i = 12.9$) relative to Gerrity et al. (2006) ($O_i = 54.0$; Figure 5). Conversely, 6- and 7-year-old Pallid Sturgeon examined in our study consumed more macroinvertebrates, especially in the family Ephemeroptera ($O_i = 61.5$), than observed by Gerrity et al. (2006) ($O_i = 32.0$; Figure 5). Frequency of occurrence of Chironomidae was the most similar between Gerrity et al. (2006) and the present study (Figure 5). Sturgeon Chub and Sicklefin Chub were the primary fish species observed in diets of Pallid Sturgeon by Gerrity et al. (2006) ($O_i = 79$). No Sicklefin Chub or Sturgeon Chub were observed in the stomach contents of 6- and 7-year-old Pallid Sturgeon examined using gastric lavage in the present study (Table 2); however, Sturgeon Chub were observed in the fecal material (Figure 3). In addition to Sicklefin Chub and Sturgeon Chub; Flathead Chub, Channel Catfish, Shorthead Redhorse, and Sand Shiner (*Notropis stramineus*) were observed by Gerrity et al. (2006). Sicklefin Chub and Channel Catfish were the only species observed in the stomach contents of 6- and 7-year-old Pallid Sturgeon from the present study. Sand Shiner was not observed in either diet methods from the present study.

Stable Isotope Ellipses and Trophic Positioning

The six sampled basal taxa varied in $\delta^{15}\text{N}$ from 8.75 (algae) to 15.70 (Stonecat) and in $\delta^{13}\text{C}$ from -29.71 (Fatmucket) to -24.27 (Flathead Chub) (Figure 6). The 239 Pallid Sturgeon analyzed for stable isotope signature had a centroid value of $\delta^{15}\text{N}$ ellipses varying from 16.25 for the 1997 year-class to 12.86 for the 2021 year-class (Figure 6). Ellipses for $\delta^{15}\text{N}$ overlapped for

all year-classes, except 1997 (Figure 6). The centroid value of $\delta^{13}\text{C}$ ellipses varied from -25.21 (2015) to -23.70 (1997) and ellipses for $\delta^{13}\text{C}$ overlapped for all year-classes (Figure 6). Despite overlap of ellipse centroid values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among year-classes, some individuals obtained $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures outside the ellipses suggesting variation in feeding habits within a year-class.

Trophic position varied from 2.86 (2021) to 4.52 (1997) (Table 4). The 1997 year-class had the highest mean trophic position (4.17) with the highest range (0.98) (Table 4). The 2021 year-class had the lowest mean trophic position of 3.18, and the 2014 year-class had the lowest range (0.27) (Table 4). Trophic position was related to age, and two break points were identified at age 3.5 (break point 95% CI = 2.9 – 4.2) and age 17.3 (break point 95% CI = 16 – 18.5) (overall model: intercept = 2.64, $r^2 = 0.73$, residual standard error = 0.14, df = 229) (Figure 7). Trophic position was positively correlated from age 2 to 3.5 ($p = 0.00013$, slope = 0.21, CI = 0.11 – 0.32) and from age 3.5 to 17 but with a flatter slope ($p = 0.003$, slope = 0.008, CI = 0.00 – 0.14). Fish older than 17 had a positive relationship with trophic position ($p < 0.0001$, slope = 0.07, CI = 0.06 – 0.08). Age-2 Pallid Sturgeon had a mean trophic position of 3.18 and Pallid Sturgeon $\geq$ age 26 had a mean trophic position of 4.17. Pallid Sturgeon were 2.6 years old when they exceeded trophic position 3.2 and 18.4 years old when they exceeded trophic position 3.6 (trophic position < 3.2 indicates feeding primarily on benthic macroinvertebrates, 3.2 - 3.6 indicates feeding on both benthic macroinvertebrates and fish, and trophic position > 3.6 indicates feeding primarily on fish) (Table 5). Trophic position was positively correlated with length and only one break point was identified at 439 mm (break point 95% CI = 369 – 509) (overall model: intercept = 2.03, $r^2 = 0.80$, residual standard error = 0.12, df = 230) (Figure 7). Trophic position increased from 311 mm to 439 mm ($p = 0.0014$, slope =

0.002, CI = 0.001 – 0.004). Once Pallid Sturgeon exceeded 439 mm, trophic position continued to increase with length but with a slightly flatter slope ($p < 0.0001$, slope = 0.001, CI = 0.001 – 0.001). Pallid Sturgeon were 393 mm when they exceed trophic position 3.2 and 674 mm when they reach trophic position 3.6 (Table 5). Trophic position was positively correlated with weight with two break points identified at 308 g (break point 95% CI = 256 – 360) and 5,126 g (break point 95% CI = 3,479 – 6.772) (overall model: intercept = 2.77, $r^2 = 0.81$, residual standard error = 0.12, df = 228). Trophic position increased with weight from 106 g to 308 g ($p < 0.0001$, slope = 0.002, CI = 0.00 – 0.00) (Figure 7). Weight was correlated with trophic position from 308 g to 5,126 g ($p < 0.0001$, slope = 0.0001, CI = 0.002 – 0.002). After 5,5126 g, weight was not correlated with trophic position ($p = 0.507$, slope = 0.00, CI = 0.00 – 0.00). Pallid Sturgeon were 203 g when they exceed trophic position 3.2 and 1,450 g when they exceed trophic position 3.6 (Table 5).

Discussion

I predicted the level of piscivory would vary by year-class given that piscivory would be related to age and size. That is, older year-classes would exhibit more piscivorous feeding habits than younger year-classes. We observed the 1997 year-class consuming exclusively fish, whereas the younger year-classes (2005-2017) exhibited some piscivory but largely consumed benthic macroinvertebrates and the youngest year-class (2021) almost exclusively consumed macroinvertebrates. I observed within year-class variation in the level of piscivory that was strongly related to Pallid Sturgeon length and weight. In general, the prediction was supported with the oldest, largest, and mostly mature year-class (1997) having the highest trophic level that was associated with a top-level apex predator.

Benthic macroinvertebrates were the primary diet content of smaller, younger Pallid Sturgeon. The macroinvertebrate order Diptera made up the highest mean percent by weight, and Ephemeroptera occurred most frequently. More specifically, Simuliidae was the most common family in the diets and made up the highest overall mean percent by weight. The primary diet item for Pallid Sturgeon varied across the range of the species. For example, in the middle Missouri River between Fort Peck Dam and Lake Sakakawea, Chironomidae and Emerald Shiner made up the highest mean percent by weight (Dutton 2018). Ictaluridae and Cyprinidae species were common diet items in the lower Missouri River (Winders et al. 2014), and in the Mississippi River, Hydropsychidae, Cyprinidae, Sciaenidae, and Clupeidae were common in diets (Hoover et al. 2006). Shovelnose Sturgeon, which occupy similar river habitats as Pallid Sturgeon, consume exclusively benthic aquatic macroinvertebrates (primarily Chironomidae), and little diet overlap exists with Pallid Sturgeon (Gerrity et al. 2006, Dutton 2018, Rapp et al. 2011, DeVries 2020).

Prey fish species identified in Pallid Sturgeon diets by the gastric lavage and DNA metabarcoding methods differed substantially. Given that DNA metabarcoding of fecal material is a novel method, literature comparing DNA metabarcoding to other diet analysis methods is limited; however, a wider diet breadth of prey species was observed in fish diets assessed with DNA metabarcoding Berry et al. (2015), Maes et al. (2022), and Roy et al. (2024). The difference in results between methods I observed may be related to digestion and efficiency of the gastric lavage. The efficiency of gastric lavage methods on retrieving diet items from Lake Sturgeon (*Acipenser fulvescens*) was optimal four hours post consumption ($75.8\% \pm 14.1\%$ stomach content retrieval); it was lower at two, six, and 12 hours and unretrievable after 12 hours because contents had been digested past the pyloric ceca (Naznin and Genz 2022). Furthermore,

the efficiency of gastric lavage on White Sturgeon (*Acipenser transmontanus*) was only 60% (Crossman et al. 2016). I applied gastric lavage to 409 individuals in the field. Of those, 107 (26%) were empty, perhaps due to gastric lavage limitations or absence of contents to retrieve. DNA of prey fish was present in 71 of the 91 (73%) of the Pallid Sturgeon sampled.

The application of DNA metabarcoding for species identification in fecal material can provide more insight to diet but also has limitations. DNA metabarcoding is a relatively new method in fisheries research. The DNA metabarcoding results from the ten individuals sampled from the 1997 year-class indicated 11 unique prey fish species were consumed, whereas the gastric lavage indicated two. Similarly, for the 2015 year-class, DNA metabarcoding indicated seven prey fish species and one species from the gastric lavage. I expected the application of DNA metabarcoding to provide additional insight to piscivorous habits, but I did not expect to see large differences between the methods. Several of the species from the metabarcoding DNA have never been observed in Pallid Sturgeon diets outside of the present study, such as River Carpsucker, Smallmouth Buffalo, Paddlefish, Blue Sucker, and Sauger. As stated above, the traditional gastric lavage methods used for diet studies provide some insight to diet, but have many limitations, especially if these methods are performed after prey has been digested. DNA metabarcoding also has limitations, such as providing only presence versus biomass. In addition, the taxonomic resolution of DNA metabarcoding can be limited as a result of incomplete available reference data, which was problematic for marine fishes (Berry et al. 2015). The application of DNA metabarcoding provided additional insight into the feeding ecology of Pallid Sturgeon that otherwise would not have been detected using exclusively gastric lavage methods. Furthermore, results from DNA metabarcoding suggest Pallid Sturgeon exhibit a more generalist feeding habitat on fishes than previously described by Gerrity et al. (2006). I recommend DNA

metabarcoding be paired with gastric lavage in future Pallid Sturgeon diet studies to better understand the diversity of diet items consumed.

Research on the feeding ecology of Pallid Sturgeon in Missouri River above Fort Peck Reservoir is limited. The only research in addition to the present study was conducted in the early 2000s on the 1997 year-class by Gerrity et al. (2006). Diet composition results using gastric lavage methods for Pallid Sturgeon differed considerably between the present study and Gerrity et al. (2006). More species were observed in the diets by Gerrity et al. (2006) than observed in same aged fish during the present research. Age 6- and 7-year-old Pallid Sturgeon were more piscivorous in the early 2000s than currently, despite having similar mean length and weight. This may be why more variation in fork length and weight of fish is observed in the Gerrity et al. (2006) study. This suggests a change in the abundance of prey fish species, a change in the feeding habitats as a result of subsequent stockings and an increase in Pallid Sturgeon density, or both. The growth trajectory of the 1997 year-class is much higher than subsequent year-classes, especially after age 6, and this may be a result of the high level of piscivory at age 6 and 7. A higher level of piscivory at a younger age (age 6 and 7) may have set a higher growth trajectory after age 6 for the 1997 year-class compared to years-classes that consume mostly macroinvertebrates at this age. The shift from feeding on zooplankton or benthic macroinvertebrates to feeding on fish at a younger age has been observed increasing growth rates of juvenile freshwater and marine fish (Buckel et al. 1998; Colchen et al. 2020; Jonsson et al. 1999; Post 2003).

The level of piscivory or trophic level was correlated best with length. Pallid Sturgeon from the 2006 year-class that were < 610 mm consumed fish and macroinvertebrates, but Pallid Sturgeon longer than 610 were mostly piscivorous. The length when a Pallid Sturgeon was

consistently considered a top-level predator ($TP > 3.6$) was about 830 mm, which individuals from three years-classes were considered exclusively as top-level predators (1997, 2005, and 2006). Other top-level predator freshwater species such as Northern Pike (*Esox lucius*) and Largemouth Bass (*Micropterus nigricans*) have been observed in $TP > 3.7$ (Vander Zanden et al. 1996), comparable to predatory Pallid Sturgeon. Comparable trophic positions were observed in Lake Sturgeon in the Mississippi and St. Croix rivers ranging from 2.7 – 4.2 (Ziegeweid et al. 2021). The transition to piscivory occurred around 439 mm where fish are incorporated into the diet, but macroinvertebrates are still an important component of the diet. After 439 mm, a gradual increase in length with an increase in piscivory, for every increase in length of 100 mm trophic position changed by 0.112. Similar trophic level – length relationships were found for Pallid Sturgeon throughout their sympatric range, suggesting that Pallid Sturgeon < 500 mm feed at lower trophic levels and transition to higher trophic levels with increases in length (DeVries 2020).

The transition to piscivory in fishes is widely associated with increases in growth (Buckel et al. 1998; Colchen et al. 2020; Jonsson et al. 1999; Mittelbach and Persson 1998, Muir et al. 1988). As fish grow larger, more energy-rich prey is needed to satisfy energy requirements (Wooten 1992), and this was observed for Pallid Sturgeon in the upper Missouri River. The size of Pallid Sturgeon is closely linked to the switch to piscivory and an early adoption of a piscivorous feeding habitat increases the growth trajectory. It is unknown why year-classes stocked after the 1997 year-class delay switching to piscivory. It is also unknown why some individuals within a year-class switch exclusively to piscivory and other individuals consume fish and macroinvertebrates. Differences among and within year-classes of feeding behavior have been observed in other species (Pelham et al. 2001; Tesfaye et al. 2025,). The delay in

switching to piscivory within and among year-classes has conservation implications for Pallid Sturgeon related to the timing of sexual maturity. It is well documented in the upper Missouri River that Pallid Sturgeon reach sexual maturity around 1,000 mm (18 years old; Holmquist et al. 2018) and the data that informed that estimate came from the 1997 year-class. Thus, subsequent year-classes that delay the transition to piscivory may have reduced growth rates, which would delay the age and time to sexual maturity. For example, given the current growth trajectory of the 2008 year-class, they would be about 35 years old when they reach 1,000 mm.

Although Pallid Sturgeon reproduce in the upper Missouri River (Holmquist et al. 2018, Cox et al. 2023), recruitment has not been documented. Water-level management actions are continually being evaluated in upper Missouri River to achieve recruitment, making the presence of sexually mature Pallid Sturgeon from multiple year-classes critical for species recovery. Therefore, piscivory plays a crucial role in Pallid Sturgeon life history and conservation. Understanding prey availability and its relationship to Pallid Sturgeon density and growth should be a priority for advancing conservation of this iconic large-river species.

Tables

Table 1: Sample size, minimum, median, and maximum fork lengths (FL) and weights (Wt) by diet sampling method and year-class of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024.

Year-class	Unit	Diet - Gastric Lavage				Diet - DNA Metabarcoding				Stable Isotope - Trophic Position			
		N	Minimum	Median	Maximum	N	Minimum	Median	Maximum	N	Minimum	Median	Maximum
1997	FL (mm)	2	810	NA	1,156	10	1,121	1,180.5	1,280	19	810	1,197	1,280
	Wt (g)		2,050	NA	7,400		7,200	8,925	12,200		2,050	8,850	12,200
2005	FL (mm)	8	385	556.5	925	4	557	580	991	10	522	610	991
	Wt (g)		189	593.5	3,800		582	700	4,375		486	798	4,375
2006	FL (mm)	24	529	590.5	956	9	555	642	789	24	520	620	956
	Wt (g)		494	720	4,375		557	860	1,840		494	745.5	4,375
2007	FL (mm)	3	500	538	546	1	527	NA	527	5	500	527	630
	Wt (g)		466	489	592		525	NA	525		455	489	815
2008	FL (mm)	25	542	579.5	611	7	564	581	825	24	542	582	825
	Wt (g)		544	667	768		641	678	2,205		353	678	2,205
2009	FL (mm)	23	507	579	657	9	530	580	657	26	530	583.5	657
	Wt (g)		420	649	1,000		558	688	823		420	666.5	980
2010	FL (mm)	4	525	603.5	647	1	605	NA	605	3	525	602	605
	Wt (g)		504	726.5	886		751	NA	751		504	702	751
2012	FL (mm)	4	493	525	595	3	493	501	595	7	493	549	595
	Wt (g)		445	530.5	692		445	485	680		425	545	692
2013	FL (mm)	10	495	528.5	590	2	495	NA	530	11	495	526	590
	Wt (g)		400	458	625		414	NA	441		400	441	625

Table 1 continued.

2014	FL (mm)	11	490	533	692	1	692	NA	692	10	497	534	692
	Wt (g)		374	496	690		625	NA	625		374	480	625
2015	FL (mm)	43	450	524	575	8	500	521	570	25	450	517	575
	Wt (g)		335	466	615		415	443.5	575		300	445	615
2016	FL (mm)	34	476	539.5	608	4	511	563	584	22	476	528	587
	Wt (g)		308	479	698		485	554.5	650		308	481.5	650
2017	FL (mm)	20	475	517.5	570	5	475	520	570	28	469	515	570
	Wt (g)		362	437	529		387	473	59		335	422.5	529
2021	FL (mm)	85	343	445	508	7	370	423	500	25	311	388	497
	Wt (g)		110	270	411		191	253	390		106	175	385

Table 2: Mean percent by weight and frequency of occurrence (in parenthesis) of diet items for all year-classes of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024. Diet taxa are generally grouped by order, fish diet items are grouped by family (i.e., Ictaluridae). Sample size (in parenthesis) is displayed under each year-class, sample size is for Pallid Sturgeon with diet contents. Mean percent by weight values ≥ 10.00 are indicated in bold.

Diet Item	Year-class													
	1997	2005	2006	2007	2008	2009	2010	2012	2013	2014	2015	2016	2017	2021
	(2)	(7)	(12)	(2)	(16)	(11)	(3)	(2)	(8)	(7)	(25)	(14)	(11)	(65)
Acipenseridae														
Egg	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.71 (6.25)	<0.00 (9.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Coleoptera														
Elmidae	0.00 (0.00)	0.00 (0.00)	0.18 (8.33)	0.00 (0.00)	0.72 (6.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.24 (7.14)	0.00 (0.00)	0.05 (1.54)
Diptera														
Ceratopogonidae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.22 (3.08)
Chironomidae	0.00 (0.00)	6.12 (28.57)	0.08 (8.33)	71.49 (50.00)	0.51 (18.75)	1.30 (36.36)	30.03 (33.33)	100.00 (50.00)	0.40 (12.50)	12.22 (28.57)	1.24 (24.00)	13.43 (42.86)	1.55 (27.27)	31.30 (58.46)
Ephydriidae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.12 (4.61)
Simuliidae	0.00 (0.00)	32.25 (71.43)	11.07 (50.00)	0.90 (50.00)	13.27 (68.75)	17.05 (63.64)	69.97 (66.67)	0.00 (0.00)	20.00 (75.00)	24.22 (57.14)	8.02 (80.00)	49.29 (57.14)	35.21 (63.64)	3.85 (73.77)
Unknown	0.00 (0.00)	0.36 (14.29)	0.25 (8.33)	0.00 (0.00)	<0.00 (6.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.05 (12.50)	0.00 (0.00)	1.50 (4.00)	0.02 (7.14)	0.00 (0.00)	0.00 (0.00)
Ephemeroptera														
Baetidae	0.00 (0.00)	0.00 (0.00)	0.28 (16.66)	0.00 (0.00)	0.00 (0.00)	0.28 (9.09)	0.00 (0.00)	0.00 (0.00)	3.05 (37.50)	2.73 (28.57)	0.74 (28.00)	8.19 (21.43)	6.46 (27.27)	0.55 (32.31)
<i>Ephemerella</i> spp.	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.31 (4.00)	1.56 (7.14)	0.00 (0.00)	0.07 (3.08)
Heptageniidae	0.00 (0.00)	0.00 (0.00)	0.35 (8.33)	0.00 (0.00)	0.05 (6.25)	6.45 (27.28)	0.00 (0.00)	0.00 (0.00)	3.17 (25.00)	28.76 (14.29)	0.00 (0.00)	4.96 (14.29)	3.57 (9.09)	0.34 (4.62)
<i>Traverella</i> spp.	0.00 (0.00)	55.66 (42.86)	63.62 (50.00)	0.00 (0.00)	40.19 (43.75)	16.86 (27.28)	0.00 (0.00)	0.00 (0.00)	14.48 (12.50)	7.79 (14.29)	16.65 (32.00)	12.90 (35.71)	19.70 (40.00)	11.98 (30.77)
<i>Ephoron</i> spp.	0.00 (0.00)	0.00 (0.00)	14.38 (9.09)	26.03 (50.00)	8.22 (6.25)	29.31 (18.18)	0.00 (0.00)	0.00 (0.00)	21.76 (12.50)	24.27 (14.29)	36.13 (12.00)	0.00 (0.00)	18.94 (9.09)	41.89 (15.38)
Unknown	0.00 (0.00)	5.61 (14.29)	1.97 (25.00)	0.00 (0.00)	2.34 (18.75)	0.87 (9.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.96 (28.00)	9.49 (21.43)	4.40 (18.18)	0.37 (7.69)

Table 2 continued.

Hymenoptera														
Formicidae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.18 (7.14)	0.00 (0.00)	0.00 (0.00)
Ictaluridae														
<i>Ictalurus punctatus</i>	7.12 (50.00)	0.00 (0.00)	0.05 (8.33)	0.00 (0.00)	<0.00 (6.25)	<0.00 (9.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.02 (4.62)
<i>Noturus flavus</i>	92.88 (50.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Unknown	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.09 (12.50)	0.16 (9.09)	0.00 (0.00)	<0.00 (50.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.01 (6.56)
Leuciscidae														
<i>Macrhybopsis meeki</i>	0.00 (0.00)	0.00 (0.00)	0.11 (8.33)	0.00 (0.00)	0.00 (0.00)	1.73 (9.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.18 (8.00)	0.00 (0.00)	0.00 (0.00)	0.01 (1.54)
Salmonidae														
<i>Coregonus artedii</i>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.20 (9.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Trichoptera														
Brachycentridae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.58 (50.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.44 (4.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Hydropsychidae	0.00 (0.00)	0.00 (0.00)	7.65 (25.00)	0.00 (0.00)	31.05 (25.00)	21.75 (36.36)	0.00 (0.00)	0.00 (0.00)	37.14 (25.50)	0.00 (0.00)	32.60 (16.00)	0.00 (0.00)	10.15 (9.09)	8.99 (21.54)
Unknown	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.24 (4.00)	0.00 (0.00)	0.00 (0.00)	0.33 (3.08)
Unknown Fish Part														
Scale	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<0.00 (14.29)	<0.00 (8.00)	0.00 (0.00)	<0.00 (9.09)	<0.00 (1.54)
Trunk	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.199 (6.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<0.00 (8.00)	<0.00 (7.14)	0.04 (9.09)	<0.00 (6.15)
Vertebrae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<0.00 (1.54)

Table 3: Frequency of occurrence (%) of species observed in fecal material from DNA metabarcoding by year-class of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024. Sample size of fecal samples with contents by year-class (in parenthesis) displayed under each year-class. Frequency of occurrence values $\geq 30.00\%$ are indicated in bold.

	Year-class													
	1997	2005	2006	2007	2008	2009	2010	2012	2013	2014	2015	2016	2017	2021
Family or Species	(10)	(4)	(9)	(1)	(7)	(9)	(1)	(3)	(2)	(1)	(8)	(4)	(5)	(7)
River Carpsucker	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sculpin Family	0.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lake Chub	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00
Blue Sucker	10.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Catfish Family	10.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00
Channel Catfish	0.00	0.00	33.33	0.00	28.57	44.44	0.00	33.33	100.00	100.00	12.50	25.00	60.00	28.57
Smallmouth Buffalo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	0.00
Minnow Family	10.00	25.00	11.11	0.00	14.29	11.11	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00
Brassy/Western Silver/Plains Minnow	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sturgeon Chub	0.00	0.00	22.22	0.00	14.29	33.33	0.00	66.67	0.00	100.00	25.00	50.00	20.00	28.57
Sicklefin Chub	0.00	0.00	22.22	0.00	14.29	11.11	100.00	0.00	0.00	0.00	12.50	0.00	0.00	28.57
Shorthead Redhorse	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.50	0.00	0.00	14.29
Emerald Shiner	10.00	50.00	33.33	100.00	0.00	11.11	0.00	0.00	0.00	0.00	37.50	0.00	0.00	14.29
Stonecat	40.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00
Flathead Chub	10.00	0.00	33.33	100.00	0.00	0.00	0.00	66.67	0.00	0.00	0.00	25.00	0.00	0.00
Paddlefish	20.00	50.00	0.00	0.00	28.57	22.22	0.00	0.00	0.00	0.00	0.00	0.00	40.00	14.29
Longnose Dace	0.00	25.00	22.22	0.00	0.00	11.11	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00
Trout Family	30.00	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.00	0.00	12.50	25.00	0.00	0.00
Sauger	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4: Mean, minimum, maximum, and range of trophic position values by year-class for Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024.

Year-class	Trophic position			
	Mean	Minimum	Maximum	Range
1997	4.17	3.54	4.52	0.98
2005	3.65	3.35	4.05	0.70
2006	3.52	3.20	4.00	0.90
2007	3.48	3.17	3.68	0.51
2008	3.52	3.36	3.80	0.44
2009	3.51	3.30	3.73	0.43
2010	3.43	3.26	3.55	0.29
2013	3.45	3.31	3.73	0.42
2014	3.46	3.33	3.60	0.27
2015	3.47	3.29	3.72	0.43
2016	3.40	3.10	3.62	0.52
2017	3.43	3.22	3.71	0.49
2021	3.18	2.86	3.44	0.58

Table 5: Age (years), length (mm), and weight (g) of segmented regression break values and where metrics reached trophic position values 3.2 and 3.6 for Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024.

Metric	Segmented Regression Break 1	Segmented Regression Break 2	Trophic Position 3.2	Trophic Position 3.6
Age	3.5	17.3	2.6	18.4
Length	439	NA	393	674
Weight	308	5,126	203	1,450

Figures

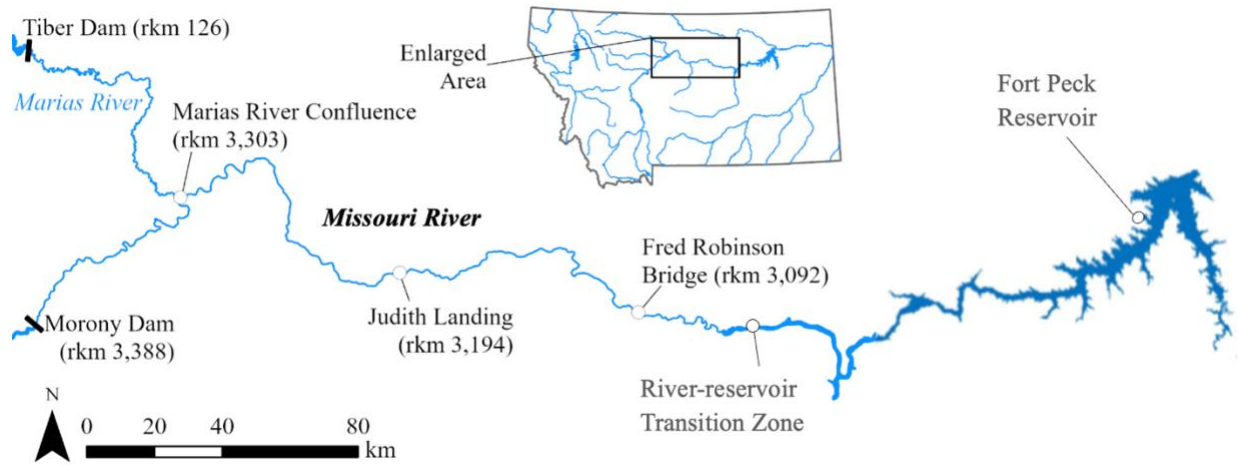


Figure 1: Map of the Missouri River above Fort Peck Reservoir, Montana, USA. The sampling area was from Judith Landing to the transition zone of Fort Peck Reservoir. Map revised from Cox et al. (2023).

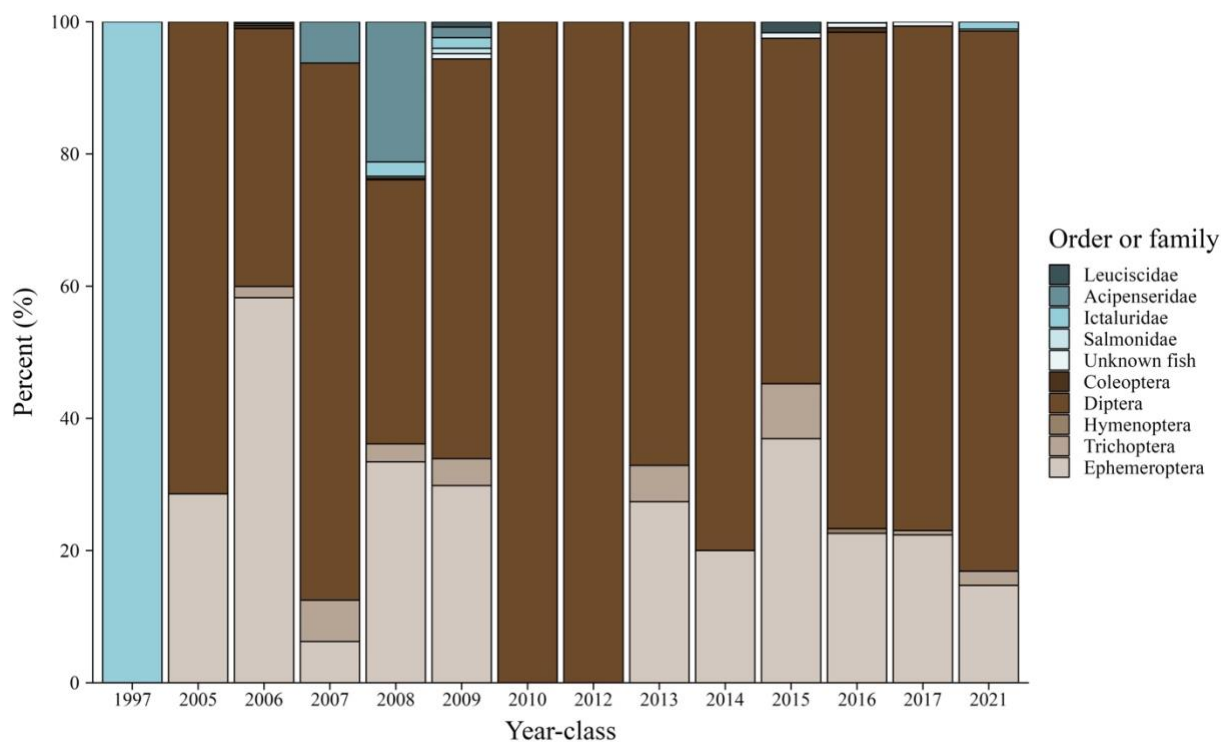


Figure 2: Percent (%) of diet items by order or family (blue gradients represent fish, brown gradients represent macroinvertebrates) of each year-class of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024.

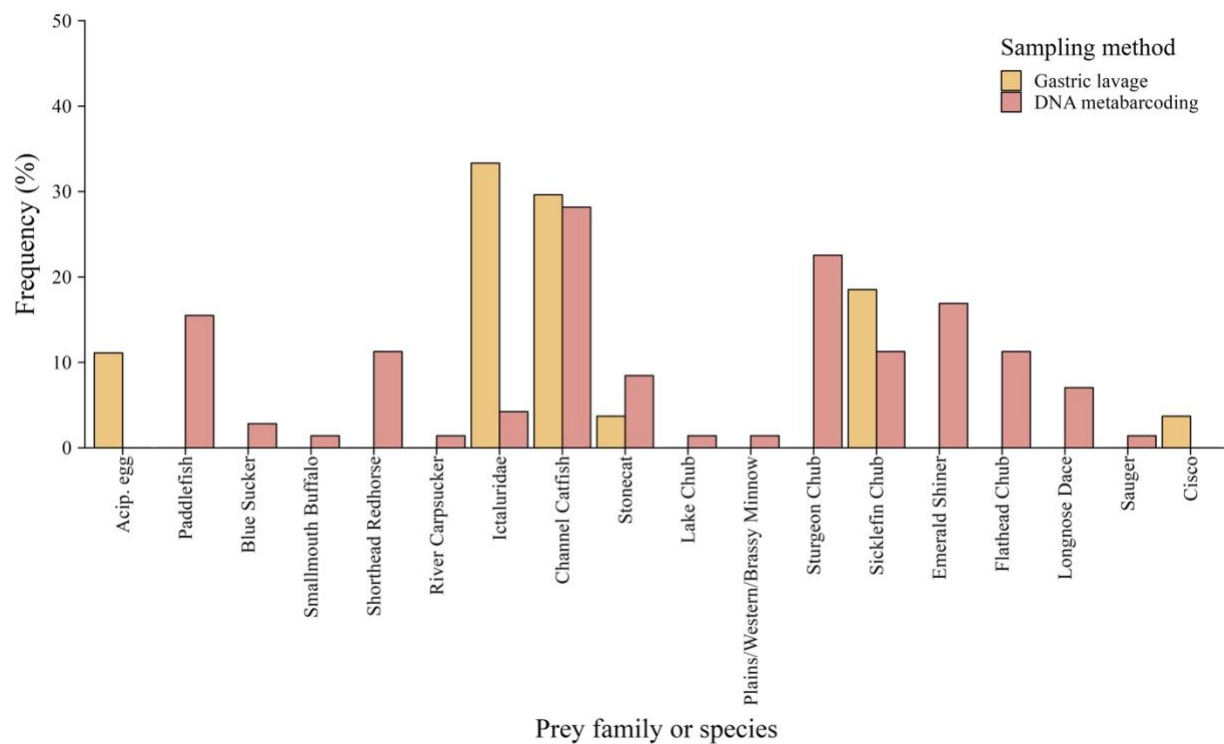


Figure 2: Frequency of occurrence of 18 diet items observed using gastric lavage and fecal DNA metabarcoding methods for Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024 (gastric lavage N = 185, fecal DNA metabarcoding N=71).

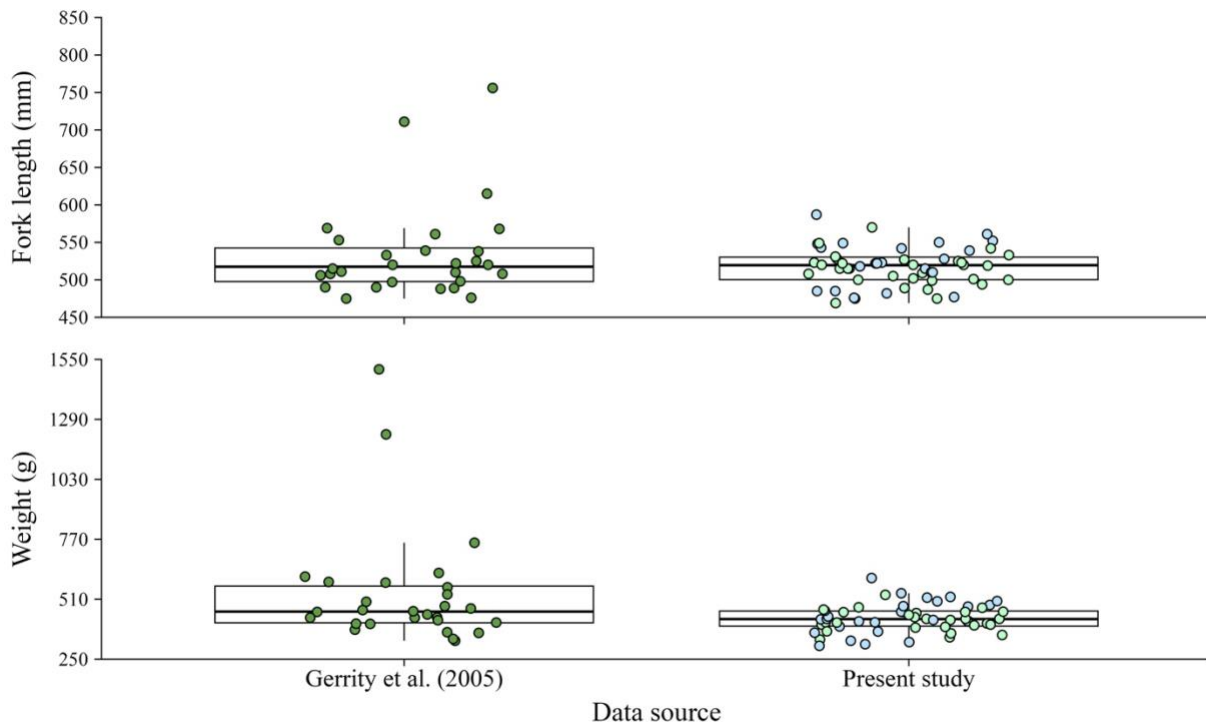


Figure 3: Fork length (mm; top panel) and weight (g; bottom panel) of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2003 and 2004 (Gerrity et al. 2006; N = 28) and 2023 and 2024 (present study; N = 56). Frequency of occurrence of fish in the diet of Pallid Sturgeon was 54.0% in Gerrity et al. (2006) and 12.9% in the present study. Green points represent the 1997 year-class, light blue (2016), and light green (2017).

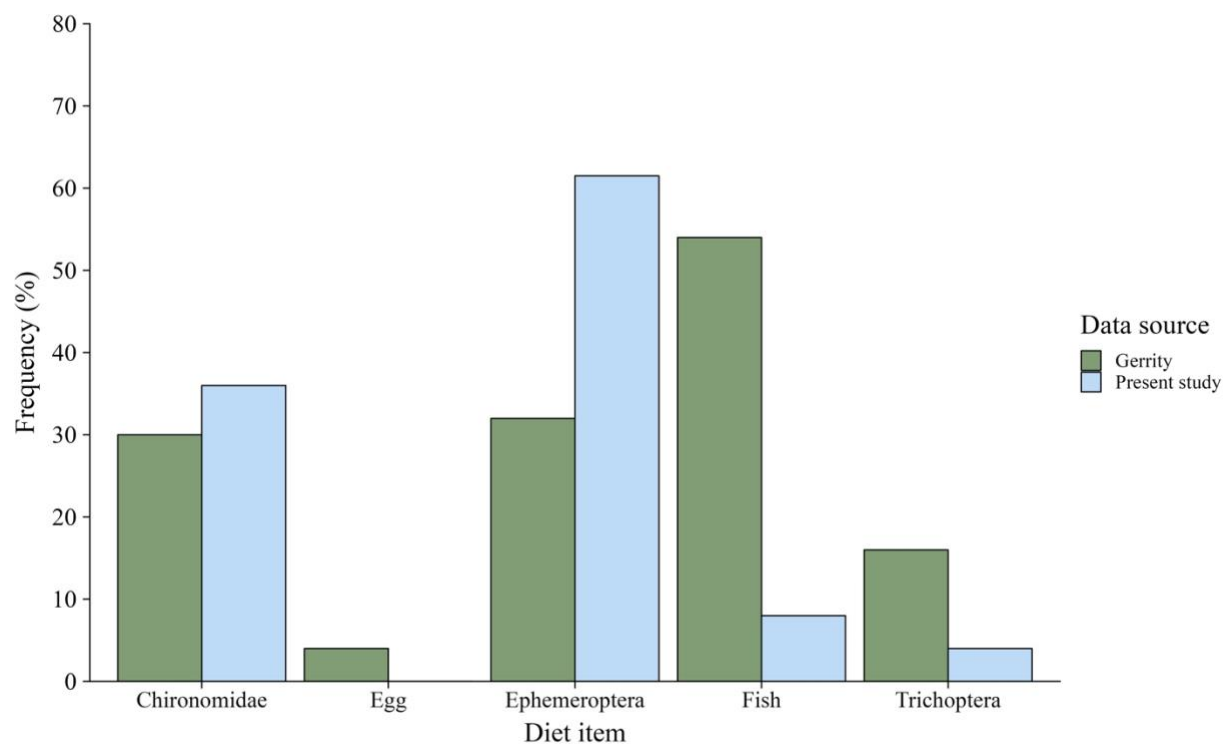


Figure 4: Frequency of occurrence for Chironomidae, Fish eggs (Egg), Ephemeroptera, Fish, and Trichoptera in the diets of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2003 and 2004 (Gerrity et al. 2006; N = 28) and 2023 and 2024 (study; N = 56). Frequency of occurrence of fish in the diet of Pallid Sturgeon was 54.0% in Gerrity et al. (2006) and 12.9% in the present study.

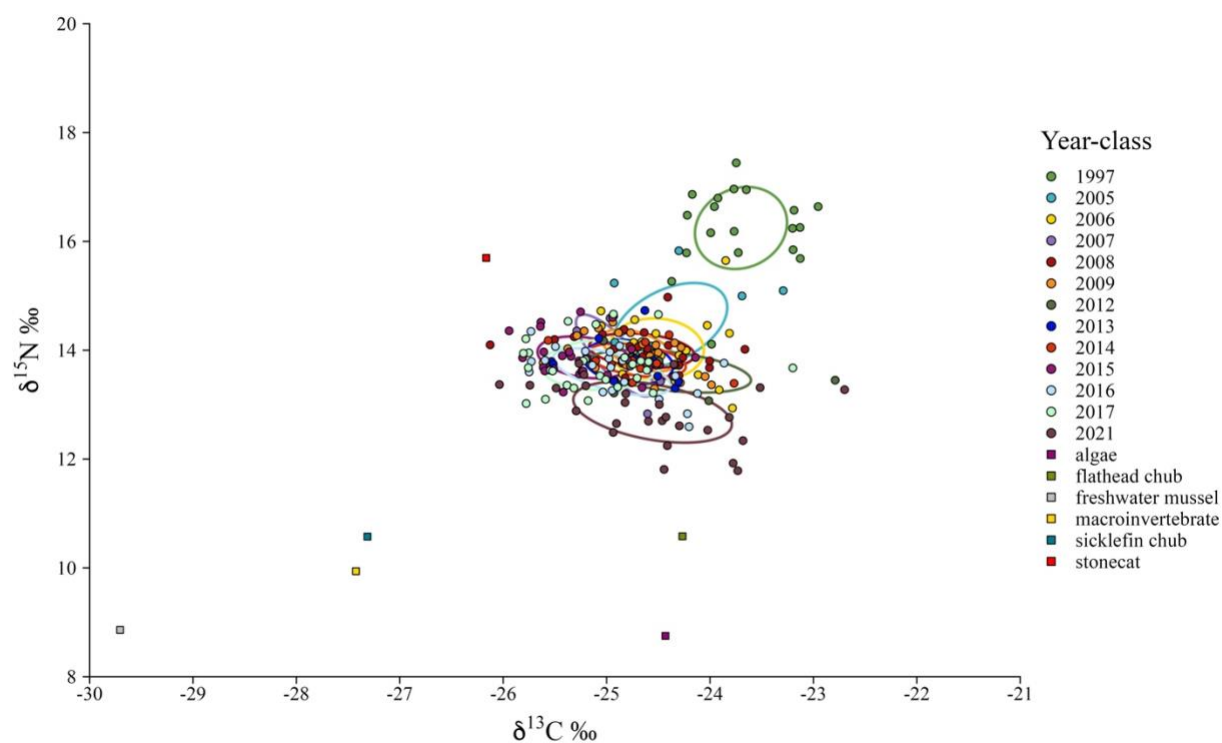


Figure 5: Stable-isotope plot for year-classes of Pallid Sturgeon, basal food web taxa (i.e., algae, benthic macroinvertebrates, and freshwater mussel), and potential Pallid Sturgeon diet items (i.e., Flathead Chub, Sicklefin Chub, and Stonecat). Samples were collected from the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024. Ellipses represent 40% of Bayesian standard areas for stable isotope signatures.

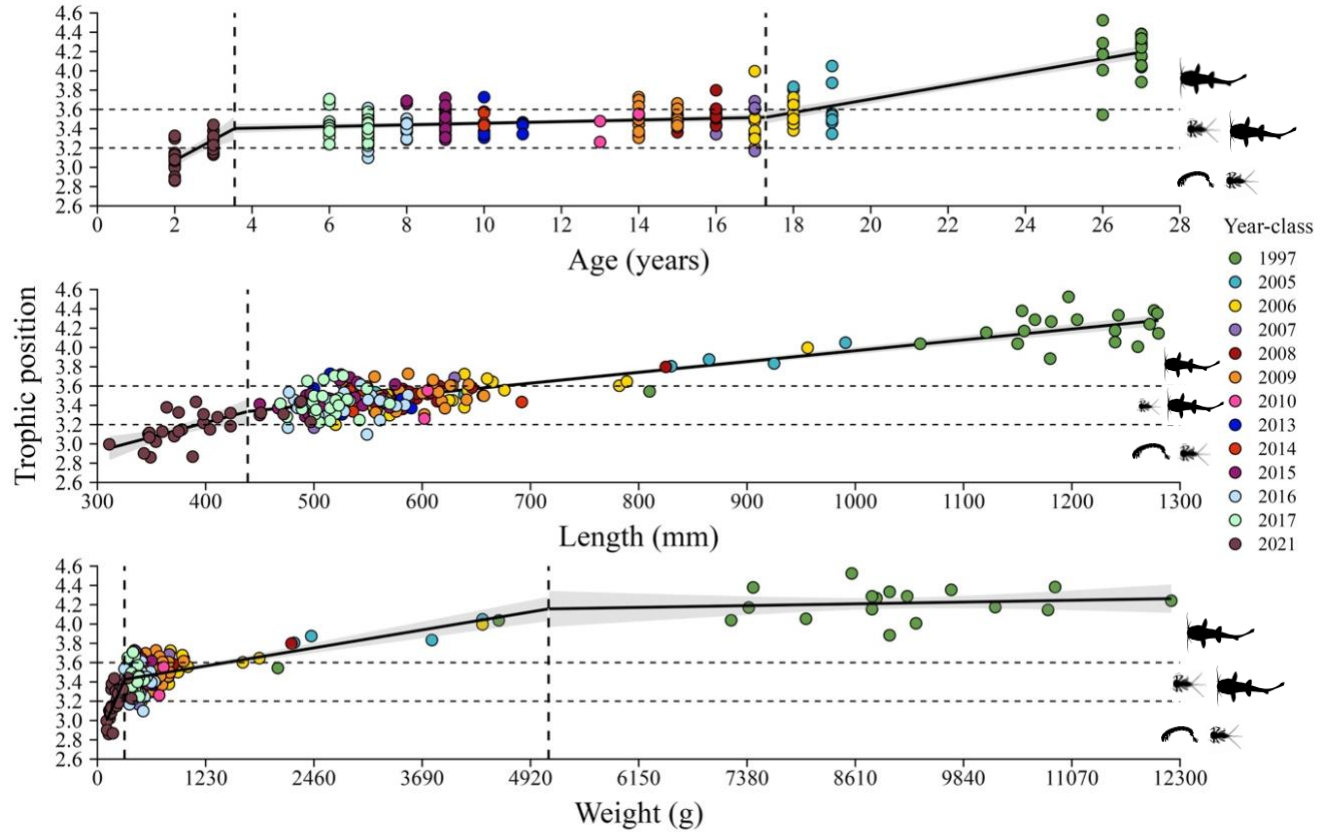


Figure 6: Relationships of trophic position and age, length, and weight of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana, USA in 2023 and 2024. Horizontal dashed lines delineate trophic position categories (i.e., trophic position < 3.2 indicate primarily feeding on benthic macroinvertebrates, $TP \geq 3.2$ and ≤ 3.6 indicate feeding on a mix of benthic macroinvertebrates and fish, and $TP > 3.6$ indicate feeding primarily on fish). Segmented regression analysis (see methods) was used to evaluate the relationship between trophic position and age (years), length (mm), and weight (g). Segmented regression line is solid black line with ± 95 CI bands in gray. Segmented breaks are shown as the vertical dashed line.

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APPENDIX

Methods

Growth Analyses

Predicted Fork Lengths and Weights

Montana Fish, Wildlife and Parks maintains a database of recapture events for every Pallid Sturgeon, including length, weight, age, and unique PIT tag identification.

Therefore, individual growth and predicted length and weight-at-age were calculated for all Pallid Sturgeon in the database. 5,515 individual Pallid Sturgeon were recaptured, representing 12 year classes (1997, 2005 – 2010, 2013 – 2017). Length -at-age was predicted using the von Bertalanffy growth model (von Bertalanffy 1938):

$$L_t = L_{\infty} [1 - e^{-k(t-t_0)}],$$

where L_t is the length at time t , L_{∞} is the asymptotic length, k is the growth rate, and t_0 is the assumed length at age 0. Weight-at-age was predicted using a log-log linear regression model:

$$\log(W_i) = a + b \log(A_i),$$

where W_i is the observed weight, A_i is the age of the fish i . Predicted weights were subsequently back-transformed to provide weights on the initial scale.

Individual Growth

Individual growth was calculated for 213 recaptured Pallid Sturgeon representing 12 year-classes (1997, 2005 – 2010, 2012, 2014 – 2017). Individual growth was calculated using the formula revised from Dunnigan and Terrazas (2021):

$$AGP_{l,w} = \frac{\left(\frac{L_2 - L_1}{L_1}\right)}{t} * 100,$$

where AGP is annual growth percentage, l is length, w is weight, L_2 is the most recent capture size, L_1 is the first capture size, and t is the fraction of the year (number of days at large, may exceed 1). If $t < 1$ or if the AGP < 0.05 , fish were removed from the analysis. AGP quantifies the annual percent growth in body mass (length or weight) standardized by the first size at capture.

Results

Growth Analyses

Predicted Fork Lengths and Weights

The 1997 year-class was the first cohort stocked in the Missouri River and has the fastest growth rate in length and weight after age 6 (Figures A.2 and A.3). The 2005 and 2006 year-classes begin to have increased growth rates compared to other year-classes beginning at age 9, especially the 2005 year-class with regard to weight (Figures A.2 and A.3). In addition to variation in growth among year-classes, considerable variation within year-classes also exists (Figures A.4 and A.5). For example, age-8 Pallid Sturgeon in the 2005 year-class varied from 441 mm to 614 mm (Figure A.4). Another example, the 2009 year-class varied in percent length acquired annually from 0.47% to 6.98% (Figure A.5).

Individual Growth

Previous research in this reach indicated that first spawning and maturity of hatchery-origin Pallid Sturgeon females was about 1,115 mm (18 years old) and for males was about 1,076 mm (14.5 years old) (Holmquist et al. 2018). Today, individuals from the

1997, 2005, 2006, and 2007 year-classes have reached maturity and express reproductive behaviors (MTFWP unpublished data). The observed variation in growth rates of Pallid Sturgeon could cause variability in the timing of sexual maturation for both males and females. Consequently, the estimated length for sexual maturity in this reach provided by Holmquist et al. (2018) may not be reached for many individuals until nearly 30 years of age. We estimated this age by finding the intersect between year-class length and the dashed line of estimated maturity provided by previous research (Figure A.2).

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Tables

Table A.1: Minimum, median, and maximum lengths of all macroinvertebrates (to the nearest hundredth and fish (to the nearest tenth) observed in the diets of Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, Montana in 2023 and 2024.

Macroinvertebrate Taxa	Length (mm)		
	Minimum	Median	Maximum
Coleoptera			
Elmidae	2.08	3.75	5.78
Diptera			
Ceratopogonidae	5.39	7.82	7.89
Chironomidae	1.68	5.38	14.86
Ephydriidae	6.96	8.51	8.63
Simuliidae	1.50	3.98	7.14
Ephemeroptera			
Baetidae	1.03	3.48	6.38
<i>Ephemerella</i> spp.	3.90	4.16	4.42
Heptageniidae	2.07	4.03	7.77
<i>Traverella</i> spp.	2.07	5.85	10.14
<i>Ephoron</i> spp.	7.36	12.31	15.29
Hymenoptera			
Formicidae	3.41	3.41	3.41
Ictaluridae			
<i>Ictalurus punctatus</i>	14.0	30.0	14.0
<i>Noturus flavus</i>	124.0	NA	124.0
Leuciscidae			
<i>Macrhybopsis meeki</i>	25.0	47.0	76.0
Salmonidae			
<i>Coregonus artedii</i>	123.0	NA	123.0
Trichoptera			
Brachycentridae	3.38	4.21	5.04
Hydropsychidae	4.14	8.02	16.12

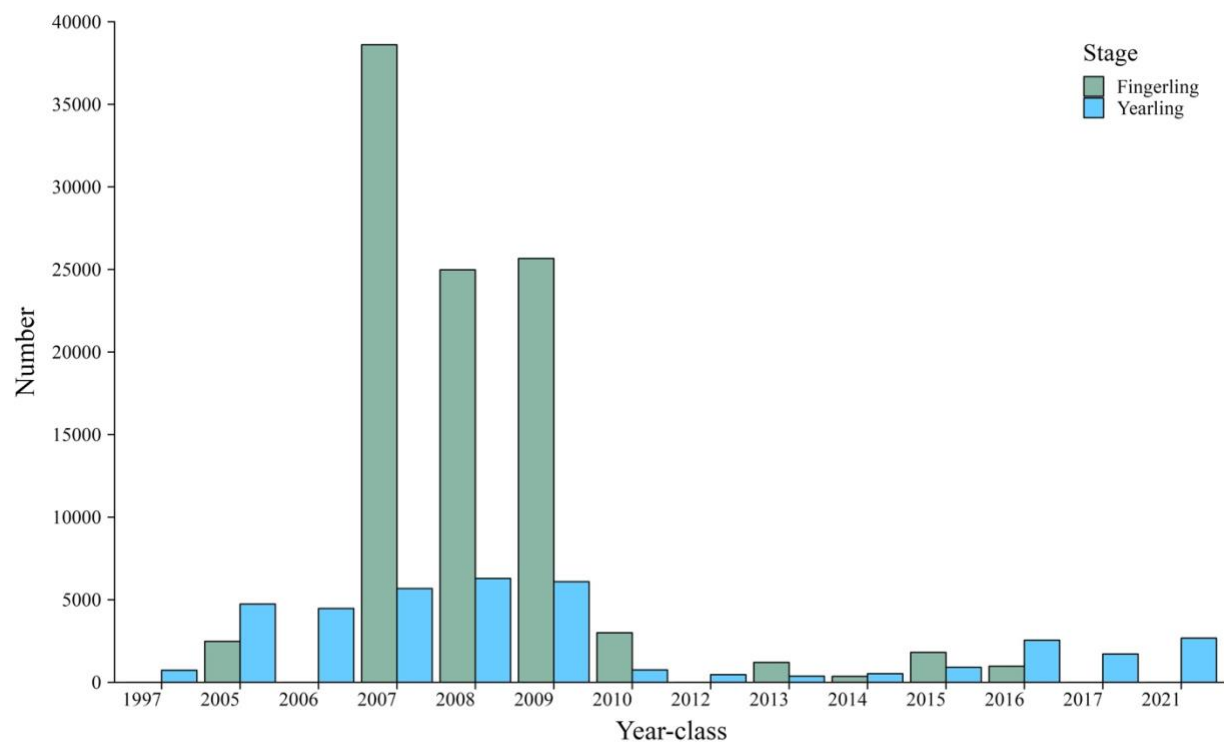
Figures

Figure A.1: Stocking densities by year of fingerling and yearling Pallid Sturgeon in the Missouri River above Fort Peck Reservoir, Montana.

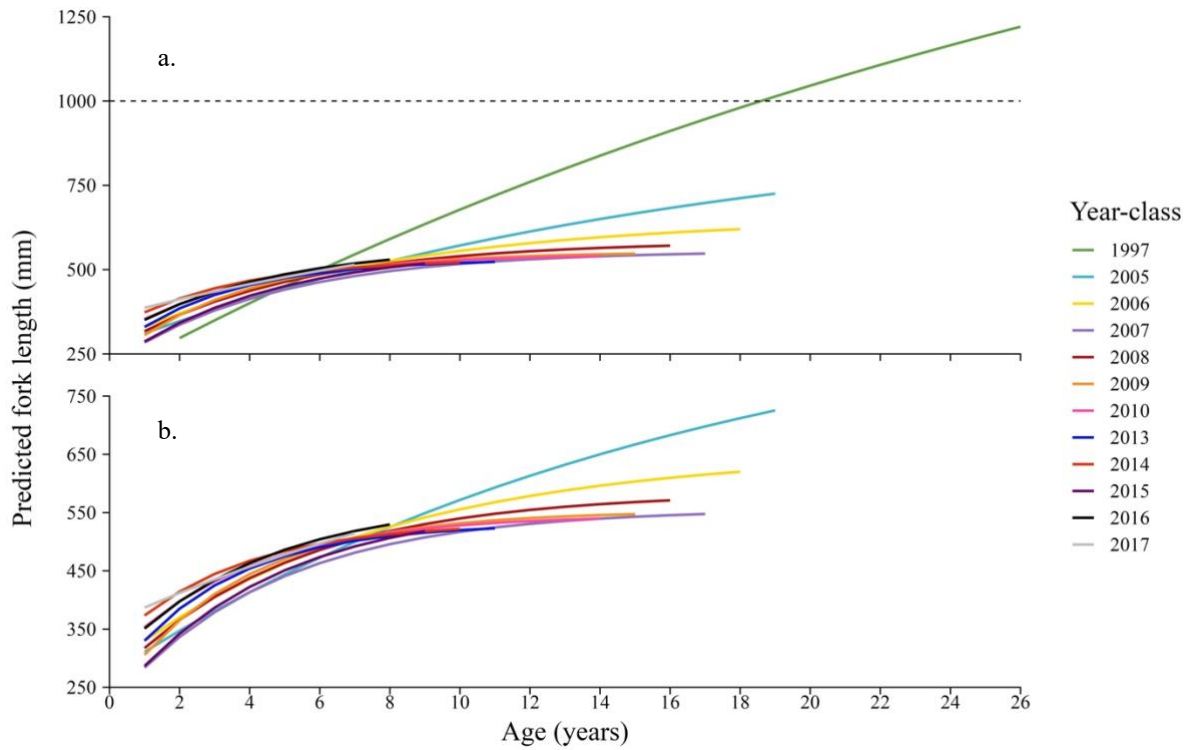


Figure A. 2: Mean predicted length-at-age for all year-classes of Pallid Sturgeon in the Missouri River above Fort Peck Reservoir, Montana sampled in 2023 and 2024. Panel a: mean predicted length-at-age for all year-classes of Pallid Sturgeon estimated using the von Bertalanffy growth model. The dashed line at 1,000 mm FL indicates the estimated length at maturity (Holmquist 2018). Panel b: mean predicted length-at-age for all year-classes excluding the 1997 year-class of Pallid Sturgeon estimated using the von Bertalanffy growth function model.

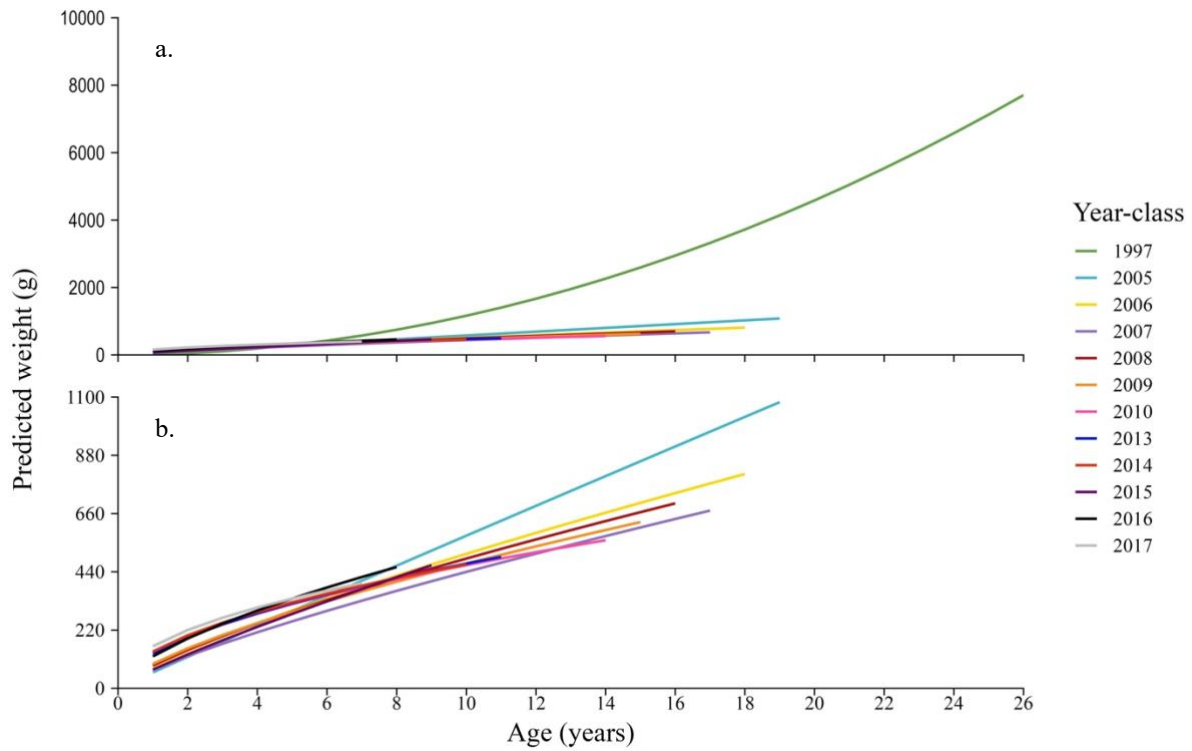


Figure A. 3: Mean predicted weight-at-age for all year-classes of Pallid Sturgeon in the Missouri River above Fort Peck Reservoir, Montana sampled in 2023 and 2024. Panel a: mean predicted weight-at-age for all year-classes of Pallid Sturgeon estimated using a linear weight-at-age model. Panel b: mean predicted weight-at-age estimates for all year-classes excluding the 1997 year-class of Pallid Sturgeon using a linear weight-at-age model.

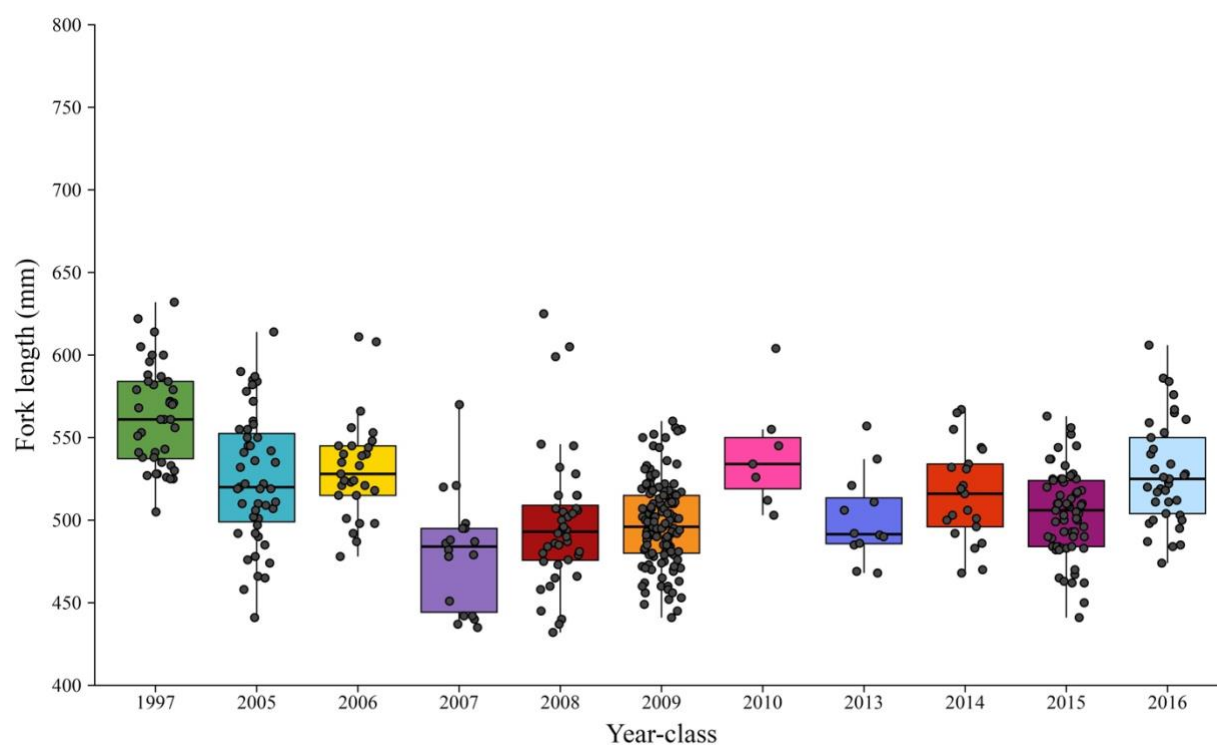


Figure A. 4: Fork lengths (mm) among and within year-classes of age 8 (years) Pallid Sturgeon in the Missouri River above Fort Peck Reservoir, Montana sampled in 2023 and 2024. Data points within a year class represent individual pallid sturgeon.

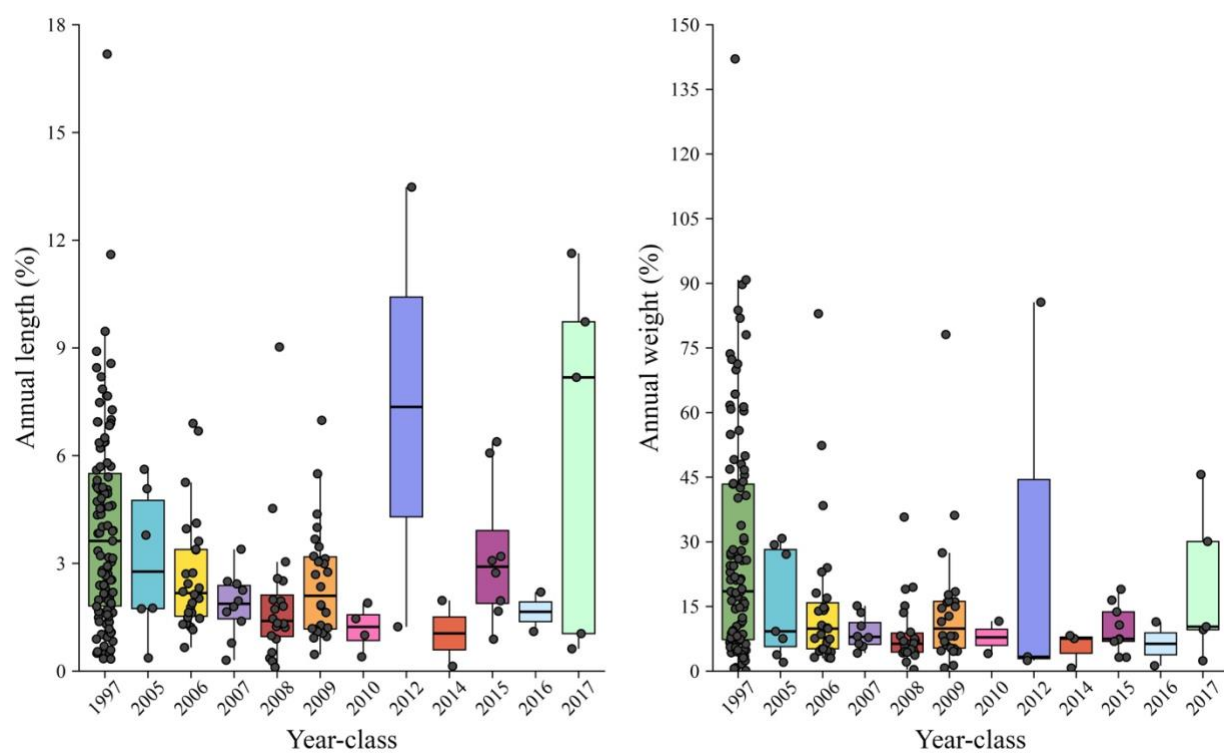


Figure A. 5: Growth rate, as a percent of annual length (mm; left panel) and weight (g, right panel), by year class for Pallid Sturgeon sampled in the Missouri River above Fort Peck Reservoir, MT sampled in 2023 and 2024.

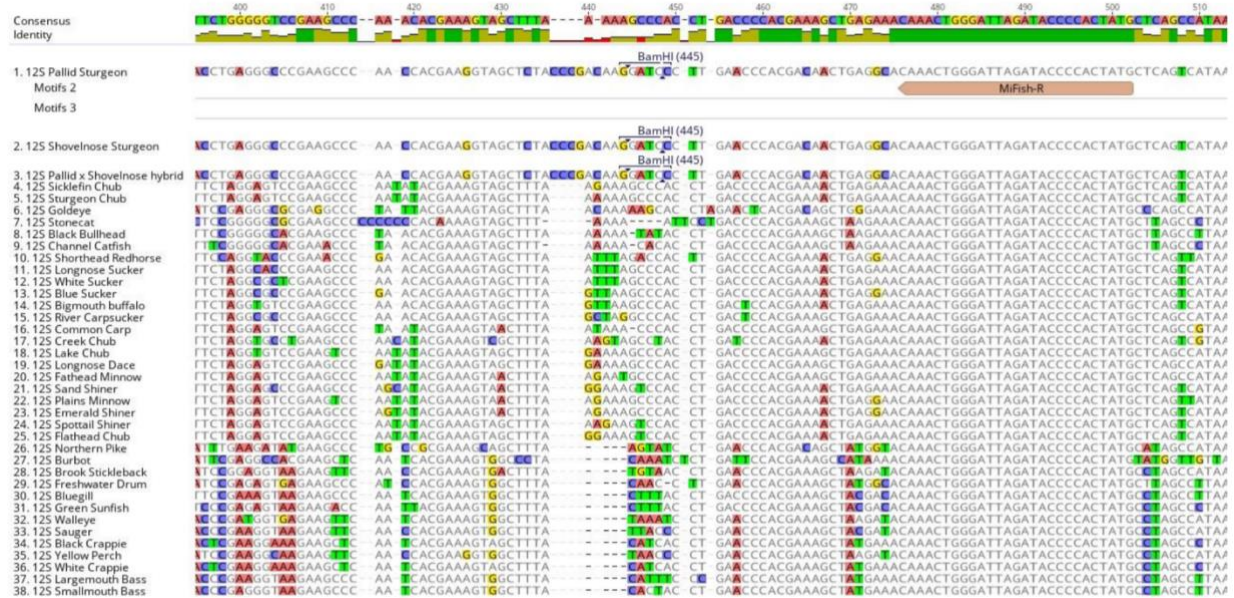


Figure A.6: Pallid Sturgeon and prey species DNA metabarcoding 12S alignment.