Age, Growth, and Reproductive Biology of Buffalofishes (*Ictiobus* spp.) in the lower Red River catchment

by

Daniel M. Bryant

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 14, 2024

Keywords: fisheries, river ecology, histology

Approved by

Dr. Shannon Brewer, Chair, Unit Leader, Research Professor, U.S. Geological Survey, Alabama Cooperative Fish and Wildlife Unit

Dr. Ian Anthony Earnest Butts, Associate Professor of Fisheries,
Aquaculture, and Aquatic Sciences

Dr. Matthew Catalano, Associate Professor of Fisheries,
Aquaculture, and Aquatic Sciences

Abstract

Many North American freshwater fishes are of conservation concern. Riverine non-game fishes are understudied with many knowledge gaps in life history such as factors influencing spawning, hatching, and growth of individuals. Buffalofishes, such as Bigmouth Buffalo *Ictiobus cyprinellus*, Black Buffalo *I. niger*, and Smallmouth Buffalo *I. bubalus* are emblematic of these conservation concerns and knowledge gaps. My research objectives were to relate environmental covariates to adult buffalo growth and assess population demographics of three sympatric buffalofishes and determine the hatch and spawning chronology of female buffalofishes in the lower Red River catchment. I collected 598 Bigmouth Buffalo, 343 Black Buffalo, and 1,153 Smallmouth Buffalo from March 2022 to May 2024 using gillnets and boat electrofishing. I collected gonadosomatic index from 565 females that were used for histological analyses. Additionally, I collected 67 age-0 buffalofishes using mini-fyke nets, beach seine, and a Siamese trawl. On average, Black Buffalo and Bigmouth Buffalo were larger than Smallmouth Buffalo. The oldest individual was a 61-year-old Bigmouth Buffalo. My results indicate low mortality and variable recruitment among species with Bigmouth Buffalo having the least stable recruitment Successful hatches were positively associated with precipitation, while environmental factors such as air temperature and discharge variability influenced post-ovulatory follicle presence. Juvenile hatch success varied across years and locations, with Smallmouth Buffalo showing the longest spawning period and Bigmouth Buffalo and Black Buffalo having more restricted timelines. These results suggest an opportunistic spawning strategy with limited successful hatches during the warmwater season, emphasizing the need for winter sampling to capture coldwater season dynamics.

Acknowledgements

I am extremely grateful to everyone who contributed to the success of this project and offered their time and expertise. First, I would like to thank my advisor, Dr. Shannon Brewer, for the opportunity and for shaping me into the fisheries scientist I am today. I am thankful for her guidance, high standards, and for teaching me to think critically about the ecology of my study fishes. I would also like to thank my committee members Dr. Matt Catalano for helping improve my coding and Dr. Ian Butts for letting me use the histology lab.

I would like to thank the Arkansas Game and Fish Commission, Oklahoma Department of Wildlife Conservation, and Texas Parks and Wildlife for providing the funding and making this research possible. I am extremely indebted to John Datillo for his massive contribution running crews and sampling year-round. John brought great energy and problem-solving skills (i.e., Johnny Logistics) to fieldwork and was always ready to catfish in the downtime. I would like to acknowledge the hard work and good spirits of the field research assistants Daniel Paulson, Brock Pearson, Jesse Woytowick and lab technicians Eli Wilson, John Peters, and Olivia Wilkes. I would like to thank Phil Carson for his friendship and teaching a Colorado kid how to float and fish these Alabama creeks. I am thankful for Jay Datillo's assistance identifying juvenile catostomids, getting me out of Swingle and into the woods, and for all wrenching help.

I am thoroughly grateful for my predecessors and lab mates Ben Birdsall, Jordan Ramey, Paul Ramsey, and Jamie Rogers for all the advice and shannonagins. Your friendships mean more than you know. I would not have made it without the friendship, support, and advice of my lab mates Sam Delaney, Zane Fuqua, Lisa Fermin, Maria Vilchez, Jeff Stevens, Blake Rummage, and Brian De La Torre. Aiden Maddux, it has been an honor and a privilege to go through grad school and work on the Red River together. I am extremely thankful for having a

good buddy to share the grad school experience with, and more importantly a great friend to chase some Redeye with.

Finally, I would like to send my deepest gratitude to my family. I would not be in natural resources without my parents, Bruce and Stephanie Bryant, getting me outside skiing, hiking, fishing, and teaching me to see the beauty in nature wherever I go. I will be forever thankful for their support. I owe my wife, Tiffany Eskew, so much for her emotional support over the years, limitless hours of fish talk, and putting up with long distance and myself. Finally, to the corgis, Montana and Kimber, and my daily rider Ducati, thank you for always being there every day

Table of Contents

List of Tables

List of Figures

Chapter I. General Introduction

Native fishes are declining at a rapid rate; unfortunately, a disproportionate focus has been placed on sportfishes, leaving large gaps in our basic understanding of other species including Catostomids. Native fish populations are declining due to many threats including harvest, introduction of invasive species, habitat fragmentation, flow alteration, and habitat alteration (Cooke et al. 2005; Jelks et al. 2008; Vörösmarty et al. 2010). Invasive species such as Bighead Carp *Hypopthalmichthys nobilis* and Silver Carp *H. nobilis* are an additional challenge to native fishes. Sampson et al. (2009) found diet overlap between the Asian carp and two native planktivores Bigmouth Buffalo and Gizzard Shad *Dorosoma cepedianum* in the Illinois and Mississippi rivers revealing a perceived decrease in abundance in the native species. Catostomids, in particular, are among the most imperiled fishes in North America with 40% at risk (Cooke et al. 2005; Quist and Spiegel 2012; Harris et al. 2014; Bagley et al. 2018). Unfortunately, our knowledge of non-game fishes basic biology and ecology is lacking. Rypel et al. (2021) conducted a Google Scholar literature review and found an eleven-fold difference in the number of publications between traditional sport fishes and non-game fishes. The authors found that 27 sportfishes had an average of 1,698 publications per species compared to 28 non-game fishes with an average of 149 publications per species. For example, Bigmouth Buffalo *Ictiobus cyprinellus* lapilli otoliths were verified only recently as ageing structures (Lackmann et al. 2019). Likewise, Smallmouth Buffalo *I. bubalus* lapilli otoliths were not verified until 2023 (Long et al., 2023). Alternatively, more popular sportfishes such as Largemouth Bass

Micropterus salmoides had their ageing structured verified in 1982 (sagittal otoliths, Taubert and Tranquilli 1982).

Buffalofishes endemic to the United States comprise Bigmouth Buffalo, Smallmouth Buffalo, and Black Buffalo *I. niger* (Miller and Robison 2004; Snow et al. 2020; Lackmann et al. 2021). These species are primarily found in the Mississippi River basin, extending from the Great Lakes region south to the gulf coast. Smallmouth Buffalo and Black Buffalo extend east and southwest into Texas gulf coast drainages such as the Colorado and Brazos Rivers. Bigmouth Buffalo extend further north in Canada and lower Hudson drainages.

Buffalofishes are targeted in both commercial and recreational fisheries, thereby increasing demand on these species but also new threats to their persistence. Commercial fisheries exist throughout the buffalofishes range with some having operated for decades (Klein et al. 2018). Buffalofishes are commonly harvested commercially across North America (e.g., North Dakota, Arkansas, and Louisiana) (Love et al. 2019; Scarnecchia and Schooley 2020; Lackmann et al. 2021). Buffalofishes comprised 26% of harvest in Upper Mississippi River (UMR); the average total value of the fishery is reported at $5 million (Klein et al. 2018). Klein et al. (2018) reported the UMR commercial fishery is stable (i.e., average annual harvest was stable around mean (standard deviation) 4,543 (854) values metric tons) and can support an increase of 3,000 tons of harvest annually. Additionally, the fluctuations in the UMR fishery are likely attributed to market demand rather than overfishing. Recreational bowfishing by tournament and non-tournament recreational anglers is an emerging threat to buffalofishes due to high catch rates and indiscriminate size of harvest (Quinn 2010; Scarnecchia and Schooley 2020; Scarnecchia et al. 2021). Quinn (2010) found relatively high harvest rates (mean ± SD; 3.8 ± 1.1 fish/h) across six Arkansas bowfishing tournaments. An additional challenge for native fishes during bowfishing tournaments is the identification of the fish in the water prior to harvest (e.g., 2018 U.S. Open Bowfishing Tournament, Scarnecchia and Schooley 2020). Despite the growing interest in these fisheries (e.g., Apache Lake, Arizona, Lackmann et al. 2023; seasonal sucker fishery in SE AL, Scarnecchia et al. 2021), only two (i.e., LA, AR) of 34 states where buffalofishes reside have any harvest regulations (Lackmann et al. 2021). The lack of basic information or historical management attention makes buffalo fishes difficult to manage despite the growing threats.

Unfortunately, many freshwater inland fisheries are managed without stock assessments simply because they lack the necessary data. Stock assessments, such as catch-at-age models, are commonly used in marine fisheries to help inform management decisions for the fishery (Lordan et al. 2011; Lorenzen et al. 2016). Marine stock assessments commonly rely on fishery-dependent data such as onboard observers and port surveys where landings can be sub-sampled while inland fisheries (Lordan et al. 2011). For example, length-based indicators (LBI) are common in marine fisheries as they require a representative sample of population length structure rather than age data (Welcomme 1999; Fitzgerald et al. 2018). Inland fisheries, although commonly data-deficient, are not excluded from quantitative assessment. Fisheries assessments in freshwater use similar approaches to marine fisheries and rely heavily on time series data (Lorenzen et al. 2016). Ecological investigations into understudied small scale inland fisheries can produce ecologically relevant research without fishery-dependent sampling as freshwater ecosystems are often underrepresented when compared to marine systems (Smith et al. 2021). Simple indicators of fishery health (e.g., size structure indicis or trend analysis) can be accomplished with minimal data including catch, effort, or length composition of the target species (Lorenzen et al. 2016). Correspondingly, the goal of my thesis research was to provide data to help reduce Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo knowledge gaps that inhibit the development of quantitative fisheries models. My specific study objectives were: 1) to document longevity, age, and growth and use these data to relate environmental conditions to growth, and 2) use juvenile hatch data and histology of adult fish ovaries to identify spawning periodicity and successful hatching during the warmwater portion of the spawning period. Buffalofishes spawning periodicity is understudied (Johnson 1963; Osborn 1966; Guy and Allen 2018; Lackmann et al. 2024); however, the species are presumed to spawn during the spring high discharge events (Johnson 1963; Lackmann et al. 2019, 2022b).

Methods

Study Area

The Red River catchment drains approximately 233,000 km² making it the second largest in the Southern Great Plains (Benke and Cushing 2011; Mollenhauer et al. 2021) The river originates in the Texas panhandle, an area characterized by the Western Short Grasslands ecoregion, with annual precipitation ranging from 26 to 29 cm (Benke and Cushing 2011). The predominant land use in the region is rangeland and crops (Benke and Cushing 2011). As the river flows southeast, it forms the Oklahoma-Texas border, passing through the Central and Southern Mixed Grasslands ecoregions. Here, the catchment is influenced by salt springs and weeps, leading to wide variations in salinity, with additional inputs from oil and gas development (Ward 1963; Whittemore 1995). The catchment is divided into upper and lower portions by Lake Texoma, a reservoir impounded by Denison Dam in 1944 for flood control and hydropower (Riggs and Bonn 1959). The upper catchment, receiving less precipitation, is prone to droughts interrupted by high discharge events (Mollenhauer et al. 2021). The lower catchment in the east receives more rainfall, ranging from 127 cm to 152-cm annually (Benke and Cushing 2011; McCorkle et al. 2016). The lower catchment includes major tributaries including Bois D'Arc Creek, Blue River, Kiamichi River, and the Muddy Boggy River, and transitions through the Central Forest Grassland Transition Zone into the Piney Woods Forests ecoregion (Benke and Cushing 2011). The Red River continues downriver to its confluence with the Atchafalaya River, a distributary of the Mississippi River, in Louisianna.

The flow and sediment conditions of the Red River and major tributaries are influenced by several water-control structures. Below Dennison Dam, the mainstem Red River is relatively clear, downcut to bedrock, and the water is cooler from hypolimnetic water releases. The river returns to its turbid state from the red clay sediment of the river channel (Christman et al. 2018) and is free flowing until Shreveport, Louisiana, where there is a series of five navigation locks and dams (Torres and Harrelson 2012). The releases from Dennison Dam are typically flood flows or flows associated with power generation (i.e., no minimum flow releases). The mainstem river is braided in many sections with large woody debris frequently found in the channel. The Red River is a low gradient river characterized by its large flood plain with connected backwaters and oxbow lakes (Benke and Cushing 2011). The majority of the major tributaries are impounded except for the Muddy Boggy River. The Kiamichi River is impounded by Hugo Reservoir and tends only to release flood flows during certain times of the year. There is a low head dam on the Blue River that may be passable by fishes during high flow periods. The Little River is impounded by Millwood Dam for flood control purposes. Lastly, Bois D'Arc Creek was recently impounded in 2021. Like the mainstem Red River, the tributaries tend to have rocky outcrops below the dam but transition quickly to narrower and deeper channel with fine substrates in their lower extents. In Arkansas, the Red River has been heavily modified by dredging and contains several wing dike structures to maintain deep water.

Fish sampling

I sampled adult and juvenile buffalofishes during different times depending on the sampling objective. Regardless, all sampling occurred during daylight hours at a variety of locations that were within reasonable distance of access points given much of the catchment is privately owned (Figure 1). I collected adult buffalofishes year-round beginning April 1, 2023, and concluding on May 1, 2024, to collect 1-year of adult ovary samples. I sampled 1-2 times per month to ensure I was able to capture the spawning periodicity of the species. My goal was to sample 20 females of each species per month including mainstem and tributary sites. These sampling events also allowed me to collect otoliths for age and growth (see below). Alternatively, juvenile buffalofishes were sampled during the spring and summer of 2023. My collection dates ranged from May 15—August 30, 2023 following the high spring floods and during which time I would expect fish to be using nursery habitat prior to winter. According to Johnson (1963), buffalofishes are expected to spawn during the spring flood pulse making this an ideal time for collection of YOY fishes. Additionally, Smallmouth Buffalo spawning was first observed during April, 1978 in Rough River Lake in west-central Kentucky (Hoyt et al. 1979).

Adult buffalofishes were sampled using experimental gill nets and electrofishing from reaches approximately 1.5 km in length. I used experiment sinking gillnets that varied by length depending on sampling location. My nets were 54.8-m long for the Red River and 30.5-m long for the tributaries. All nets were 3.65-m tall with 8.9, 10.16, and 10.8-cm bar-length mesh panels. I deployed the gillnets in 6-hour sets that were distributed throughout the site to avoid entanglement in large woody debris (LWD). Gillnetting was not conducted during high-water events due to the risk of drifting debris damaging or sinking gear. I used an 80-amp high conductivity Midwest Lakes Electrofishing Systems (Midwest Lakes; Polo, Missouri) unit using pulsed DC current to sample the site. Because conductivity is higher in the Red River than the tributaries, I used high range (>300 volts, 60Hz, pulsed DC) for the tributaries and low range (<300 volts, 60Hz, pulsed DC) for the Red River. I began electrofishing at the upriver end of each site (INFO) shocking downstream in a cloverleaf patter with power applied in 10-second bursts with 5-seconds off power between bursts. Electrofishing continued downstream while attempting to push fishes into the gillnets. I quantified electrofishing effort by time (sec).

All buffalofishes collected were euthanized with a lethal dose of tricaine mesylate (MS-222) prior to otolith extraction, morphometric measurements, and ovary extraction. Buffalofishes are variable in length-at-age (Auburn University Animal Use and Care 2023-5345). Bigmouth Buffalo, as an example, are thought to grow fast to asymptotic size and then live for decades at or near that size (Lackmann et al. 2019). Variability in length-at-age across ages creates biased length-age keys (Westrheim and Ricker 1978), thereby introducing imprecision in von Bertalanffy parameter estimates (Coggins et al. 2013). Total length (mm, ± 1 mm), weight (g, ± 10 g), sex, and ovary weight (g ± 2 g) were recorded for all adult buffalofishes. I removed both lobes of the ovary, patted dry the tissue, and weighed the gonad (g, ± 2 g) for gonadosomatic index (GSI) (see Chapter 2). I removed a 2-cm wide subsection from each ovarian lobe using dissecting scissors and fixed them in 10% neutral buffered (i.e., buffered with sodium phosphates to a pH of 7) formalin (NBF) in a 250-mL bottle for laboratory processing at Auburn University. If the gonad appeared to be immature (i.e., clear, small, and threadlike with indistinct blood vessels, Brown-Peterson et al. 2011), I kept the whole gonad and stored it in a 250-mL bottle of 10% NBF.

Sampling for juvenile buffalofishes was completed using mini-fyke nets, beach seining, a Siamese trawl, and subsurface larval tow. Sampling for juveniles was conducted over a 300-m reach. I began juvenile sampling by setting three mini-fyke nets (0.6 m X 4.5 m lead, 0.6 X 1.2 m trap, 3-mm mesh) in < 2 m of water adjacent to shoreline to target juvenile fishes (Eggleton et al. 2010; Ramsey et al. 2024). Then, I used a beach seine using a modified encirclement and sample all wadeable habitat (Bayley and Herendeen 2000; Ramsey et al. 2024). I limited seine hauls to 25 m to maintain sampling efficiency (Lombardi et al. 2014). I quantified seine distance, river width, and the number of hauls. After seining, I used a Siamese trawl (trawl, 3-m mouth, 4.88-m long, 38-mm and 4mm meshes; Innovative Net Services, Milton, LA) to collect young-of-year (YOY) and juvenile buffalofishes in the Red River and tributaries. Prior to trawl deployment, I used a Humminbird Helix 12 side-scan sonar (Humminbird, Racine WI) to identify debris on the river bottom to avoid damage to the net, otter boards, and ropes. If the river bottom appeared free from large debris, I conducted three tows with the trawl targeting non-wadeable (i.e., > 1-m) benthic habitats in backwaters and main channel to sample areas otherwise avoided by mini-fykes and seining. Trawling was conducted by reversing the boat downriver at a speed faster than river velocity (approx. 5-kph). Towing the trawl in reverse is safer for the crew and gear in case of an abrupt snag, the bow provides more freeboard than the stern (if towing from the stern), thereby reducing the risk of swamping the boat. Additionally, using forward gear to hold the boat stationary during retrieval is more stable than using reverse. The trawl tow ropes (2.54-cm diameter, 30.48-m length) were tied to hard-mounted (e.g., welded or riveted) points on the boat. I followed the 1:7 ratio for deployment; 7-m of rope between the otter boards and boat for every 1-m of depth (G. Faulker, personal communication, Innovative Net Services, Milton, LA). I quantified trawl effort by time beginning when the towlines became taut until net retrieval (approximately 100 m trawled per 1-minute). A sub-surface larval tow (0.5-m mouth diameter, 1.65-m length, 500-μm mesh) was used to sample for larval buffalofishes. I conducted one 10-min stationary (i.e., anchor the boat and deploy the net) tow in deeper water not accessible by mini-fykes or seining in flowing water. I quantified the volume of water filtered with a flow meter attached to the mouth of the net General Oceanics Mechanical Flowmeter Model 2030R). Collected larval fishes were preserved in 70% ethanol for laboratory processing and identification using the Hoyt (1979) larval key. All juvenile buffalofishes were preserved in 50-mL tubes or 250-mL bottles in 70% ethanol.

Chapter 2. Population demographics of three sympatric buffalofishes in the lower Red River catchment

Introduction

Accurate age and growth data are necessary to understand life-history characteristics and therefore is useful to managers (Ricker 1975; de Roos et al. 2006). Hard structures such as otoliths are used to estimate age structure of populations (Fournier 1983). These age data serve multiple purposes in fisheries research and management. For example, age data can show sexual dimorphic differences in growth and size, as well as to calculate age or size at maturity (Trippel and Harvey 1991; Parker 1992; Trippel 1995; Lackmann et al. 2019). Furthermore, otolith ages and annuli increment widths are necessary components of common fisheries management growth models (von Bertalanffy 1957; Stevenson and Campana 1992; Weisberg et al. 2010). Recreational fisheries commonly use length limits restricting fish available to harvest where prior knowledge of a population's growth rate is needed (Coggins Jr et al. 2007; van Poorten et al. 2013; Gwinn et al. 2015). For example, Bull Trout *Salvelinus confluentus* exhibit sexually dimorphic growth (Nitychoruk et al. 2013) and are susceptible to overfishing (Carl et al. 1989). Size at maturity and growth are valuable to managers to avoid sex ratio skewing and overharvest (Nitychoruk et al. 2013). The collapse of the Canadian Grand Banks Cod fishery can be attributed to overfishing and the slow recovery of age 2 and age 3 recruitment and changes in natural mortality (M) over time has influenced the lack of recovery (Myers et al. 1997; Martell et al. 2008). Reeves (2003) found in simulation analysis that age reading errors in the Baltic Cod stock created discrepancies in fishing mortality and catch forecasts. Fishes have different lifespans. For example, some species of African killifish (family Nothobranchiidae) have adopted an annual lifecycle such that they hatch, spawn, and die in one season (Furness 2016). For long lived fishes—commonly defined as a maximum age greater than 30 years—to be successful, they need to maximize recruitment to replenish the population (Leaman and Beamish 1984; Froese 2004). Population demographics including mortality, recruitment, growth, and age are informative data to managers (Maceina 1997; Pine et al. 2003; Winemiller 2011; Honsey et al. 2017).

Estimates of mortality (i.e., natural and fishing) are important in stock assessments and recovery plans for endangered species (Simpfendorfer 2000; Allen and Hightower 2010). Mortality is commonly reported in stock assessments (Maunder et al. 2023) and life-history investigations (Braaten and Guy 2002). However, accurate estimates of mortality are often difficult and require high-quality data. Radford et al. (2021) estimated Blue Sucker population mortality with catch curves using lapilli otoliths and fin rays and found a significant difference in estimates: 22.9% with fin rays versus 4.5% with otoliths. Effective preservation of species relies on accurate and precise population demographic data. Mortality can be size or age dependent in fishes and assuming constant mortality will bias results (Lorenzen 2022), Furthermore, knowledge and understanding of population dynamic rates can facilitate restoration efforts (Fausch et al. 2002).

Recruitment is a complex process that is paramount to population persistence, yet it can vary across species and habitats. (Rothschild 2000; Maunder and Thorson 2019). Recruitment is unpredictable factor influencing population growth and stability (Rothschild 2000; Allen and Hightower 2010; Maunder and Thorson 2019). For instance, Lackmann et al. (2022) observed that Bigmouth Buffalo in a Canadian lake had one successful year class recruit to the population since 1955. Similarly, Radford et al. (2021) found Blue Suckers with a trimodal age distribution (using otoliths) and suggested episodic recruitment in the population. Likewise, Paddlefish *Polyodon spathula* is also an episodic spawner in the Yellowstone-Sakakawea stock (Scarneccia et al. 2014); thus, episodic recruitment may be common for fishes characterized as late-maturing, long-lived, high-fecundity, oviparous spawners such as buffalofishes.

Current management for buffalofishes in the United States is minimal to non-existent. Most state agencies treat buffalo as “rough fish” often with few or no harvest regulations. For example, Buffalofishes occur in 34 states; only Arkansas and Louisiana have a minimum length limit for recreational and commercial harvest at 404.6mm (i.e., 16 in) where all buffalofishes are managed together. Louisiana manages buffalofishes collectively with a daily creel limit of 25 fishes (Louisiana Department of Wildlife and Fisheries 2024). In Oklahoma, Black Buffalo is a species of concern and has a daily harvest limit of one fish with mandatory reporting (Oklahoma Department of Wildlife Conservation 2024). Bigmouth Buffalo are state listed in Pennsylvania with take and possession prohibited (Pennsylvania Fish and Boat Commission 2024). Bigmouth Buffalo in Canadian waters became a species of special concern in 2009 and no take or possession is permitted (Johnson and McAleer 1987; COSEWIC 2009; Lackmann et al. 2022, Manitoba Fisheries 2024). Some state agencies have public support for non-game fisheries management. For example, the Oklahoma Department of Wildlife Conservation conducted a creel survey on bowfishing values towards non-game fishes and their management and found that 86% (n = 223) of bowfishers and 94% (n = 957) non-bow anglers trusted the state agency to manage native non-game fishes (York et al. 2022). Collectively, the status of buffalofishes suggests we need to better understand the demographics of these populations so we can improve our ability to manage these populations where necessary.

Correspondingly, the first objective of my thesis was to determine longevity, age, and growth of buffalofishes in the Red River catchment. My three target species are Smallmouth Buffalo, Bigmouth Buffalo, and Black Buffalo. These species co-occur within the catchment but vary in abundances leading to hypothesized differences among the species demographics and responses to human effects.

Methods

Using an ageing structure that has been verified against the species age is important to estimating population parameters. Bigmouth Buffalo is the only buffalo species with verified ageing structures. Lackmann et al. (2019) verified all three otoliths for ageing using bomb-carbon dating. Lapilli otoliths have also been validated in several closely related species such as White Sucker *Catostomus commersoni* and Blue Sucker *Cycleptus elongates* (Thompson and Beckman 1995; Quist et al. 2007; Radford et al. 2021). Although several hard structures have been used to age catostomids including scales, the opercle, and the cleithrum, these structures typically lack precision (Quist et al. 2007). Radford et al. (2021) found lapilli otoliths yielded a greater age range and more precision when compared to scales and fin rays. Additionally, they found a significant difference in population mortality estimates when using otoliths (4.5%) versus fin rays (22.9%). Therefore, I used the lapilli otolith to determine age and growth for buffalofishes in my study.

Otolith processing

I removed the lapilli otoliths following Lackmann et al. (2019). Lapilli otoliths in buffalofishes were accessed with a cut at the posterior of the skull in line with the preopercle.

Otoliths were extracted using forceps and placed in labeled coin envelops for drying and storage before laboratory analyses.

In the laboratory, otoliths were sectioned and prepared for ageing. I marked the nucleus of the otolith using a ballpoint pen, and I placed the marked otolith into epoxy and allowed 24 hours to cure (105-A resin and 205 hardener, West Systems, Bay City, Michigan). After curing, I sectioned the otolith using a low speed isomet saw (Buehler IsoMet Low Speed Precision Cutter, Lake Bluff, Illinois) including the nucleus within 0.3-0.5-mm cross section. Next, I polished the otolith section using 3-µm diamond lapping paper (Diamond Lapping Film, 8" diameter, plain backing, Electron Microscopy Sciences, Hatfield, PA). After polishing, I used thermoplastic cement to mount the section to a labeled glass microscope slide. The slide was placed under a dissection microscope with a digital camera and light source. I imaged the otoliths using camera equipped microscope (Luminera Infinity 2, Tyledyne Luminera, Ontario) for later incremental growth analysis.

Ageing

Two readers separately enumerate the annuli of each sectioned otolith using a dissection microscope with transmitted light (Figure 2). I aged the lapilli otoliths along the from the along the longest axis within the otolith such that the axis was perpendicular to the annuli. Annulus counts were tracked using a 1x4 multiple-tally counter (The Denominator Company, Waterbury, CT) and were recorded. Fish collected after June 1st will have the edge included as year presuming buffalofishes spawn once in the spring (Johnson 1963; Lackmann et al. 2021). The readers will have no knowledge of length, weight, or sex to avoid reader bias. Different assigned ages between readers will be rectified with a concert read; if no agreement can be made the age will be excluded. Once aged, I imaged each otolith on a compound microscope under 40x magnification using a digital camera (Luminera Infinity 2, Tyledyne Luminera, Ontario). These images will be used for back-calculation of lengths and annular increments.

Analysis

I analyzed recruitment and mortality of the buffalofishes using a catch-curve and recruitment variability index (RVI). The RVI or similar approaches are often the only methods available in infrequently sampled populations and was developed for populations with missing year-classes (Isermann et al. 2002). I analyzed each buffalo species separately to calculate vital rates among species. I used a Chapman-Robson peak-plus catch-curve corrected for overdispersion to estimate annual and instantaneous mortality for each buffalo species.

$$\mathrm{CR(Z)} = \log\left(\frac{1+T-Tc-\frac{1}{N}}{T-Tc}\right) - \frac{(N-1)(N-2)}{N[N(T-Tc)+1][N+N(T-Tc)-1]}$$

Where T is the mean age of fish equal to and greater than Tc; Tc is the age of complete recruitment; and N is sample size greater than or equal to Tc. Peak-plus catch-curves only use age classes occurring after the most abundant age class. If the difference between the penultimate maximum age and maximum age is greater than three years, I removed it from the catch curve to reduce bias (Smith et al. 2012).

$$\mathrm{RVI} = \left[\frac{S_N}{N_m + N_P} - \frac{N_m}{N_P}\right],$$

Where S_N is the sum of cumulative relative frequencies across year-classes in the sample; N_m is the number of missing year classes, and N_p is the number of present year-classes (Guy and Willis 1995).

I used the Dahl-Lea method for back-calculated length at age rather than the Fraser-Lee due to a lack of biological intercept (Heidarsson et al. 2006; Quist and Isermann 2017).

$$\text{The Dah-Lea: } L_i = \left(\frac{S_i}{S_c}\right) * L\text{c}$$

Where L_i is length at age I, S_i is otolith radius at age i, S_c is the otolith radius to the edge, and Lc is length-at-capture.

I fit a von Bertalanffy growth model (VBGM) to three buffalo species using observed length-at-age data. I separated individuals of each species into one of three categories: male, female, and not sexed (including immatures). Bigmouth Buffalo exhibits sexually dimorphic growth with females reaching larger sizes similar to other fishes (Lackmann et al. 2019). Von Bertalanffy parameters are used widely and allow for population comparisons including Bigmouth Buffalo populations in Minnesota and North Dakota (Lackmann et al. 2021, 2022b) and Smallmouth Buffalo in the upper Mississippi River (UMR) and likely the first reported VBGM parameters for a sympatric buffalo assemblage (Klein et al. 2018). The von Bertalanffy equation:

$$\text{TL} = L_\infty[1 - e^{-k(t-t0)}]$$

where L_∞ is the theoretical maximum length for the population, k is the coefficient of growth, t is the age, and t_0 is the age when fish length is zero (Katsanevakis and Maravelias 2008; Maceina and Sammons 2016).

I used a Weisberg mixed-effects model to relate environmental conditions to buffalo growth (Weisberg et al. 2010). Mixed-effect modeling is useful as it allows accounting of the length, age, and differences in growth rates among individuals (Weisberg et al. 2010; van der Sleen et al. 2018). Additionally, landscape-scale changes (e.g., water temperature and hydrologic changes) can be related to interannual growth. For example, in Australian temperate lakes, fish growth declined as water level declined (Morrongiello et al. 2011). Following Weisberg et al. (2010):

$$Y_{tnj} = X_j + V_{t+j-1} + F_{tn} + e_{tnj},$$

Where Y_{tnj} is the annular increment j for fish n in cohort t; X_j is the annular increment for the fish in year j; $V_t+j-1$is the environmental effect for year X=t+j-1, which is the year that a fish in year class t was age j; Ftn is the effect of fish n in the year class t; $etnj$ is the model error (Weisberg et al. 2010). I modeled age and environmental factors as fixed effects and individual fish and cohort as random effects (Watkins et al. 2017).

All analyses were conducted using Program R statistical software v. 4.2.2 (R Core Team 2015). I used the r packaged "lme4" (Bates et al. 2014), "dataRetrival" (Hirsch and Cicco 2015), "AICcmodavg" (Mazerolle 2017), and "MuMIn" (Barton 2015).

Environmental Covariates

I obtained environmental data useful to developing meaningful metrics to relate to fish growth. Due to the prolonged lifespan of buffalofishes and data availability (i.e., oldest available USGS gage data begins in 1986), I used discharge and temperature data from 1986 to 2024. I collected mean daily discharge (m^3/s) and air temperature (°C) from the USGS Arthur City gage (07335500) and National Oceanic and Atmospheric Administration (NOAA) Texarkana station (USC00418942) for environmental covariate calculation. I used air temperature as a proxy for water temperature as this catchment experiences large fluctuations in water levels making use of water temperature loggers difficult. However, the lower basin has limited groundwater input and thus, approaches air temperature during the warm months (Caissie et al. 2001; Morrill et al. 2005).

I developed flow and temperature metrics that I hypothesized related to buffalofishes growth (Table 1). I developed several flow metrics using discharge: number of days above the

75th percentile of discharge (m^3/s) (i.e., high flows, hereafter high flows), number of days below the 25th percentile of discharge (i.e., low flows, hereafter low flows), number of days between 50th and 70th percentiles (i.e., sustained flows, hereafter mod flows), and the week of year that peak flow occurs (i.e., 1-52, hereafter peak week). Furthermore, I included cv of discharge from Jan 1–May 1 (i.e., winter and spring, hereafter cv winter-spring) and the 75th percentile of discharge while gonads are resting i.e., (July 1—Sept 30, hereafter summer75). I also included a temperature metric that reflected the number of days the air temperature was above 25°C, week (i.e., 1-52) of peak discharge occurrence (hereafter, peak week).

I considered several interactions between my covariates may affect buffalofish growth. I hypothesized that cv of winter-spring discharge had a quadratic relationship to buffalofishes growth as buffalofishes can persist in both impoundments and rivers (Martin et al. 1964; Minckley et al. 1970; Love et al. 2019; Prull et al. 2023; Lackmann et al. 2023) suggesting they may tolerate a broad range of flow variability but perhaps not extremes. I included an interaction between the number hot days and the number of low flow days as I hypothesized buffalofishes growth may be influenced by both temperature and flows as early fish growth has been associated with river temperatures and flows (Tonkin et al. 2011). During periods of high temperatures (hot days), fish might experience faster metabolism or increased food availability and potentially increased growth. However, if these hot days coincide with large numbers of low flow days, the low flows could limit access to productive habitats reducing growth. Conversely, in the absence of low flows (i.e., greater than the 25th percentile) with warm temperatures fish may grow more due to more habitat accessibility, temperature refugia, and increased forage accessibility (Ouellet et al. 2024).

I natural-log transformed the number of days below 25th discharge percentile, cv of Jan 1 – May 1 discharge, and the 75th percentile of discharge while gonads are resting because these data were skewed. I did not transform the number of days above 75th discharge percentile, number of days between 50th and 70th percentiles of discharge, number of days above 25C, and peak week. Next, I checked my covariates for multicollinearity ($| r | > 0.60$). I removed the number of days above 75th percentile of discharge because it was multicollinear with the number of days below the 25th percentile of discharge (-0.73) and with the 75th percentile of discharge when gonads are resting (0.68, Table 2). Finally, I standardized the remaining covariates to a mean of zero and a standard deviation of 1.

I created species-specific mixed-effect models to relate environmental factors to growth following Weisberg et al. (2010). I began by fitting a complex (global) model for each species that included all retained covariates. The complex model included the fixed effects of age (i.e., age of fish during growth season) and the environmental factors of the 75th percentile of discharge, the number of low flow days, the number of medium flow days, peak discharge week of year, coefficient of variation (cv) of discharge from Jan 1-May 1 (winter-spring discharges), the number of days above 25°C were included. I included an interaction term between the number of days above 25°C and the number of low flow days because I hypothesized that low flow days combined with number of days of 25°C could limit habitat availability and food access, thus effecting growth (Tonkin et al. 2011). Additionally, I incorporated a quadratic term for cv of discharge from Jan 1-May 1, to test if extreme discharge variability adversely impacts growth in buffalofish, which tolerate a broad range of flows (Table 3). I included the random effects of individual and cohort to explain individual growth differences and differences in growth among cohorts (Watkins et al. 2017).

Once each complex model was fit, I evaluated the estimates and confidence intervals for each covariate and removed covariates with small effect sizes and retained covariates with reasonable effect sizes. After removing all covariates with a small effect size, I then fit the simplified final model. This model selection method, similar to one-step backwards selection, ensured parsimony without losing predictors in with my small number of retained covariates. For each simplified final model, I calculated the marginal r^2 (R^2_m) and conditional r^2 (R^2_c), number of parameters (k), and Akaike's Information Criterion correlated for small sample sizes (AICc).

Results

I collected buffalofishes over a range of ages and sizes that were sampled from the Red River catchment in Oklahoma and Arkansas. I aged 598 Bigmouth Buffalo, 343 Black Buffalo, and 1,153 Smallmouth Buffalo from March 2022 to May 2024 (Figure 3,4). Among the three species, Smallmouth Buffalo were the smallest, on average (mean TL: 523 mm, range: 182–960-mm). Bigmouth Buffalo had an average length of 609 mm (range: 220–990-mm), whereas Black Buffalo were the largest, with a mean length of 657 mm (range: 338–884-mm). The mean age of both Smallmouth Buffalo and Bigmouth Buffalo was 15 years, whereas Black Buffalo were older, averaging 17 years. The oldest individuals sampled were a 61-year-old Bigmouth Buffalo, a 52-year-old Smallmouth Buffalo, and a 45-year-old Black Buffalo.

Catch curve analysis indicates low mortality among buffalofishes whereas the RVI indicates variable recruitment. I fit three peak-plus catch curves using ages 11-49 for Bigmouth, 10-45 for Black, and 11-52 for Smallmouth Buffalo because ages 10, 9, and 10 were the most abundant in my sample, respectively. Mortality was low for all buffalo species, with total instantaneous mortality (Z) estimates of 0.11 for Bigmouth Buffalo, 0.08 for Black Buffalo, and

0.11 for Smallmouth Buffalo (Table 4). The total mortality (e.g., fishing and natural mortality) rates were similar among species (Bigmouth, 0.10; Smallmouth, 0.09, Black Buffalo, 0.07). Recruitment variability indices varied among the three species with Smallmouth and Black buffalos having relatively stable recruitment (Smallmouth, 0.49; Black, 0.46), whereas Bigmouth Buffalo appear to have low recruitment stability (-0.12).

Growth differed by species of buffalofish and sex. Von Bertalanffy growth coefficients (k) were highest for Black Buffalo (0.23 females, 0.18 males), followed by Smallmouth (0.12 females, 0.15 males), and Bigmouth (0.10 females, 0.12 males). Smallmouth had the smallest in L_{inf} estimates, while Bigmouth and Black Buffalo were similar in theoretical maximum size (Table 5, Figure 5). In all three species, females had larger theoretical maximum lengths than males.

Environmental Measures

Discharge and air temperature patterns varied significantly over the 37 years in the catchment. The number of high flow days had a mean of 90.4 days, ranging from 0 to 222 days (Table 6). Hot days averaged 93.2 days per year, ranging from 64 to 127 days. Medium flow days had a mean of 73.1 days, ranging from 10 to 147 days. The peak week of maximum discharge varied throughout the year, with a mean of week 22.1 (appx. June 1st), ranging from week 1 to week 52. The CV of winter-spring discharge was highly variable, with a mean of 83.1, ranging from 29.041 to 160.11. The 75th percentile of summer flow when gonads are resting had a mean of 240.1 m^3/s, ranging from 21.245 to 1363.5 m^3/s.

Factor related to fish growth

The factors related to bufflofish growth varied by species (Table 7). For the final simplified Black Buffalo model, I retained the covariates age, hot days, low flows, cv winter-spring, and summer 75th percentile. Winter-spring discharge variability was positively associated with growth, whereas age had the largest effect size and was negatively associated with growth. Hot days, low flows, and summer 75th percentile were not related to fish growth. The fixed effects explained 68% of the variability in the model (marginal $R^2_m = 0.68$), whereas the random effects accounted for an additional 5% (73% total) (conditional $R^2_c = 0.73$). For Smallmouth Buffalo, I retained the following covariates of age, hot days, low flows, and cv winter-spring for the final simplified model. Age had a negative association with growth, whereas hot days and low flows were positively associated with growth. Variability of Discharge (i.e., cv winter-spring) from Jan 1 – May 1 was negatively associated with growth. The fixed effects explained 69% of the variability ($R^2_m = 0.69$) and the inclusion of random effects explained an additional 5% of the variability ($R^2_c = 0.74$). For Bigmouth Buffalo, I retained age, quadratic cv winter-spring, Moderate flows, and summer 75th percentile for the final model. Age was negatively associated with growth, whereas quadratic cv winter-spring and summer 75th percentile were positively associated with growth. Moderate flows were not related to growth. The fixed effects explained 69% of the variability ($R^2m = 0.69$) and the inclusion of random effects explained an additional 4% of the variability ($R^2c = 0.735$).

Discussion

Flows are an important component of riverine fish growth as they can affect species differently. The flow regime primarily drives ecological processes in lotic systems (Poff et al. 1997; Poff and Zimmerman 2010). Flow variability and magnitude are frequently associated with positive growth and recruitment by fishes (Tonkin et al. 2011, 2021; Jacquemin et al. 2015). Large non-wadable rivers are among the least studied ecosystems and catostomids knowledge is lacking (Cooke et al. 2005; Flotemersch et al. 2006; Rypel et al. 2021). For example, Northern Hogsucker *Hypentelium nigricans*, River Carpsucker *Carpiodes carpio*, and Golden Redhorse *Moxostoma erythrurum* growth was positively associated with the number of days annually above the 75th percentile of discharge in Iowa's rivers (Quist and Spiegel 2012).

Minimal information has been published on basic life-history characteristics and population demographics of catostomids, thus limiting comparisons among populations. Frequently, studies on *Ictiobus* spp. focus exclusively on Bigmouth Buffalo (Johnson 1963; Burr and Heidinger 1983; Irons et al. 2007; COSEWIC 2009; Lackmann et al. 2019; Prull et al. 2023) or Smallmouth Buffalo (Martin et al. 1964; Adams and Parsons 1998; Klein et al. 2018; Love et al. 2019; Rypel et al. 2021; Long et al. 2023) whereas any research on Black Buffalo is scarce (Johnson and Minckley 1972; Guy and Allen 2018; Lackmann et al. 2023). Recent age structure investigations in non-native buffaflofish populations in Apache Lake, Arizona found Black Buffalo displayed extreme longevity with individuals over 100 years old (n = 2) (Lackmann et al. 2023). Recent investigations into Black Buffalo ecology have focused on use in aquaculture systems. For example, wild caught individuals were successfully induced to spawn and juvenile diet and growth was quantified (Guy and Allen 2018). Furthermore, research on sympatric buffalo populations is notably rare (Minckley et al. 1970; Bart et al. 2010; Lackmann et al. 2023).

Buffalo recruitment in the lower Red River catchment varied among species, but Bigmouth Buffalo appears to have much higher variability in recruitment. Smallmouth Buffalo recruitment in Texas rivers was variable among years (e.g., 2013 low recruitment and 2015 high recruitment) and positively associated with higher flow events during the spring (Winemiller et al. 2024). Bigmouth Buffalo in northern populations (e.g., North Dakota and Saskatchewan, CA) recruit episodically; only one successful recruitment event since 1948 in a Canadian lake and very few observed age classes from 1993-2000 in a North Dakota reservoir (Lackmann et al. 2022b). Likewise, I found variable recruitment in the Red River population (Figure 6). Factors related to this variability are magnitude, variability, and timing of discharge. This has been observed in other freshwater fishes (Tonkin et al. 2021; Mignien and Stoll 2024) and attributed to a myriad of factors including spring discharge magnitude (Tonkin et al. 2021), persistent low discharges (Goto et al. 2015), increasing discharges (Roberts et al. 2008; Tonkin et al. 2011) and maximum flow duration (Tonkin et al. 2011; Mignien and Stoll 2024).

I found lower Red River buffalofishes have low mortality rates similar to other long-lived fishes. For example, middle Mississippi River Smallmouth Buffalo have total instantaneous mortality estimates ranging from 0.04 to 0.30 and annual mortality estimates from 4.0 to 26.17% (Love et al. 2019; Maxson et al. 2024). My estimates indicate southern populations may have estimates on the lower end of this range. Paddlefish in the upper Missouri River had annual mortality rates of 12% for females and 15% for males. A southern population of Quillback Carpsucker *Carpiodes cyprinus* also have low annual mortality (4.9%, Lackmann et al. 2022a).

The age structure of buffalofish populations in the lower Red River reveals important insight into their growth and recruitment. In the middle Mississippi River (i.e., pools 4, 8, 13, 26), the theoretical maximum length of Smallmouth Buffalo varied among pools from 488 to 730-mm TL, however the fished were not sexed (Love et al. 2019; Maxson et al. 2024). Smallmouth Buffalo in the lower Red River reached maximum lengths of 639-mm TL for females and 551-mm TL for males similar to estimates Mississippi River populations (Maxson et al. 2024) Similarly, Bigmouth Buffalo in northern populations exhibited theoretical maximum lengths of 834 to 882-mm TL for females and 732 to 746-mm TL for males (Lackmann et al. 2019, 2021, 2023). In comparison, I found female Bigmouth Buffalo in the lower Red River reached 732-mm TL, whereas males measured 641-mm TL.

Where data are available, growth of buffalofishes in the Red River appear comparable to other populations. The von Bertalanffy growth coefficient (k) for Smallmouth Buffalo in the Red River was 0.12 for males and 0.15 for females, compared to values ranging from 0.03 to 0.17 in the middle Mississippi River (Maxson et al. 2024). For Bigmouth Buffalo in the Red River, k values were 0.10 for males and 0.12 for females, whereas northern populations showed ranges from 0.10 to 0.14 for females and 0.10 to 0.17 for males. Black Buffalo in the lower Red River exhibited the highest *k* estimates of the three species, with values of 0.23 for females and 0.18 for males. However, Black Buffalo had the smallest sample size and included one immature female (age 1) was collected, which likely influenced the model fit.

Like other large river populations, I found Buffalofishes of the lower Red River appear shorter lived compared to more northerly populations. Countergradient growth, where the somatic growth rates of a species increase with latitude even when adjusted for temperature-related growth potential, may describe these observations. Despite colder temperatures slowing growth, species in higher latitudes grow faster during critical life stages compared to their southern latitude counterparts (Rypel 2012, 2014). Smallmouth Buffalo longevity has recently been documented in Oklahoma. A 61 year old Smallmouth Buffalo (1,015-mm TL) was collected from Broken Bow Reservoir, OK and shown to have hatched prior to impoundment (Snow et al. 2020). Smallmouth Buffalo populations in the Brazos, Colorado, and Trinity Rivers of Texas had a maximum age of 65 (Winemiller et al. 2024 and refereces within). Bigmouth

Buffalo in northern populations have been documented with extreme longevity with multiple individuals aged over 110 years old (Lackmann et al. 2019, 2022b, 2023).

The growth and recruitment of buffalofishes is influenced by flow regimes. Studies have shown that discharge variability and magnitude are crucial to riverine fish ecology, positively affecting fish growth and recruitment (Tonkin et al. 2011, 2021; Jacquemin et al. 2015; Dattilo et al. 2021). However, large non-wader rivers remain understudied and catostomid knowledge limited (Cooke et al. 2005; Flotemersch et al. 2006; Rypel et al. 2021). Despite the absence of data on Black Buffalo, their longevity has been documented (i.e., > 106 years old) and juvenile diet investigated (Guy et al. 2019; Lackmann et al. 2023). The longevity and recruitment patterns of buffalofishes vary significantly across regions, with northern populations of Bigmouth Buffalo exhibiting extreme longevity and episodic recruitment (Lackmann et al. 2019, 2022b, 2023), while the Red River population showed episodic recruitment but shorter lifespans. These differences highlight the importance of the natural flow regime and its components on flow-dependent life history strategists.

My growth model for Smallmouth Buffalo revealed negative associations with age and positive relationships with environmental factors such as hot days and low flows. The negative association with age suggests that as Smallmouth age growth declines. Conversely, the positive effects of hot days and low flows indicate that warmer temperatures and sustained flow conditions enhance growth, possibly by increasing metabolic rates and improving foraging opportunities. The negative association with cv of winterspring suggests that increased variability during this period may decrease growth. Bigmouth Buffalo age was a significant predictor of growth, with a negative association. Interestingly, the quadratic term for cv of winterspring suggests a non-linear relationship where moderate variability may not be detrimental, but larger fluctuations could influence growth. The effects of Moderate flows and summer 75th percentile was not statistically significant, suggesting that low flows and temperature during summer may have minimal impacts on growth when considered as separate fixed effects. Black Buffalo the negative association with age mirrors the patterns observed in the other species. The cv of winterspring showed a positive association indicating that increased variability may positively influence growth in this species. This may reflect adaptive strategies that allow Black Buffalo to persist under dynamic conditions. Both hot days and low flows did not show significant effects on Black Buffalo growth, suggesting that their growth may be less sensitive to these factors compared to Smallmouth Buffalo. Bigmouth Buffalo were found to have no physiological declines with age as age had no effect on telomere length yet more stressed individuals had shorter telomeres (Sauer et al. 2021).

My results indicate that species specific differences occur in how environmental factors influence growth of buffalofishes. Smallmouth Buffalo share a positive association with growth between number of low flow days and the number of hot days. Increasing hot days promotes growth potentially due to increased forage and fish metabolism. For example, in Lake Texoma impounded by Denison Dam, 71 species of zooplankton were found during 1996-1997 sampling with densities the highest in April and May (Franks et al. 2001). The negative association of cv winterspring on growth is complex as smallmouth may be spawning-capable during those months (Burr and Heidinger 1983; Lackmann et al. 2024). Winter-spring variability may benefit spawning females with available spawning habitat, such as inundated floodplain or increased forage (Burr and Heidinger 1983; Lackmann et al. 2024). Bigmouth Buffalo positive association with quadratic cv of winterspring suggests a non-linear effect of variable winter-spring discharges. For example, Murray Cod *Maccullochella peelii* were found to have stage-specific responses to annual discharge such that no single discharge value is as beneficial as the variability in the natural flow regime (Stoffels et al. 2020). Summertime flows (i.e., summer75, 75th percentile of flow when gonads are resting July 1 - Sept 30) positive association with growth supports my hypothesis that energy recruitment while individuals are regenerating gonads may be more valuable than in different parts of the year. Furthermore, an increase in somatic growth rates post spawning have been observed in cod populations in contrast to the "reproductive drain hypothesis" (Pauly et al. 2023). Black buffalo had shared weak positive associations between growth and hot days, low flows, summer75, and cv of winterspring. Variability of winterspring discharge has a positive association with growth. Spring time discharge and variability have been linked to freshwater cod growth and supported the flood pulse concept (Junk et al. 1989; Junk and Wantzen 2004; Tonkin et al. 2017) as flooding and variability in flows are necessary for productive and sustainable systems. Fish growth can influenced by flow events and timing of optimum water temperatures (Junk et al. 1989; Tockner et al. 2000).

As non-game native fishes continue to gather research interest my results build upon catostomids ecology and are useful for conservation efforts. Together with recent studies (Lackmann et al. 2019, 2023, 2024; Love et al. 2019; Maxson et al. 2024) my results emphasize the necessity of flows and flow variability for buffalo growth. This may assist managers where Bigmouth Buffalo populations are declining, if water management and environmental flows are possible it may benefit the bigmouth. It appears recent management concerns on the effects of bowfishing are warranted (Quinn 2010; Scarnecchia and Schooley 2020; York et al. 2022). Red River buffalofishes are long-lived, slow growing, exhibit sexually dimorphic maximum lengths, and have variable recruitment. However, further research on angler preference, fishing habits, and harvest across the catchment would be beneficial to future decisions about harvest concerns.

Table 1. Environmental covariates used to relate environmental conditions to growth using a mixed effects model Resolution, hypothesis, and references are included for each covariate. Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo were collected from the lower Red River catchment in Oklahoma, Texas, and Arkansas.

Covariate (abbreviation)	Resolution	Hypothesis
Number of days Red River flow greater than 75th percentile (high flow)	1 Day	Stable access (i.e., more days) to inundated floodplain during will promote growth. [4,5,8]
Number of days above 75th percentile temperature (hot days)	1 Day	More days indicates a longer growing season and better growth.[3,4,5]
Number of days below 25th percentile discharge (low flow)	1 Day	Less days will promote growth as there is more water and less environmental stressors. [5,6,8]
Number of days discharge between 50 and 70 percentiles (moderate flow)	1 Day	More days indicates sustained flows allowing fish to move and feed. High-flow non-flood events promote growth. [4,6,8]
Peak flow week of year (peak week)	Week	Calander week of year (i.e., 1-52) that peak discharge occurs. Seasonality in peak occurrence may effect growth for

		remainder of year. May peaks may be more beneficial than November. [1,5,6]
Coefficient of variation of Jan 1 - April 30 mean scaled discharge	CV	Too much variability during the spawning season may be detrimental to growth. [1,5,6]
75th percentile of flow when gonads are resting July 1 - Sept 30 (75Q)	m^3/s	Energy recruitment is more important for growth after the cessation of spawning and prior to the winter gonad development. [1,5,6]
Age	Year	Growth will decline with age. [2,4]

[1] Poff et al. 2010, [2] Morrongiello and Thresher 2015, [3] Catalano et al. 2015 [4] Tonkin et al. 2017 [5] Tonkin et al. 2019 [6] Tonkin et al. 2021

Table 2. Pearson's Correlation matrix (rho values) for environmental covariates used for Weisberg growth model. Parameters are: high_flows = number of days Red River flow was greater than 75th percentile, mod_flows = number of days discharge was between 50 and 70 percentiles, NL_low_flows = log (NL) transformed number of days below 25th percentile discharge, hot_days = number of days air temperature was above 75th percentile, log_CVws = log transformed coefficient of variation (CV) of the winter/spring spawning season (Jan 1 - April 30); peak_week = peak flow occurrence week of year, and NL_sum75 = log transformed 75th percentile of discharge when gonads are resting July 1 - Sept 30.

	High_flows	Med_flows	NL_low_flows	Hot_days	NL_CVws	Peak_week	NL_sum75
High_flows	1	0.395	-0.729	-0.159	-0.427	0.047	0.678
Med_flows		1	-0.532	-0.200	-0.326	0.129	0.574
NL_low_flows			1	0.135	0.518	-0.109	-0.461
Hot_days				1	0.209	-0.138	-0.325
NL_CVws					1	0.167	-0.170
Peak_week						1	0.131
NL_sum75							1

Table 3. Models considered during species-specific model construction. I built complex models first including all covariates, a quadratic term for cv of winterspring discharge, and an interaction between the number of hot days and the number of low flow days. Final models were made after evaluating complex model estimates and removing variables with small effect sizes in one step. All models were fit with random effects for individual and cohort. K is the number of parameters, R^2_C is the conditional R^2 and R^2_M is the marginal R^2 value.

Species	Model	formula	k	R^2_C	R^2_M
Smallmouth Buffalo	Complex	~ age + Hot_days + NL_low_flows + NL_CVws + NL_CVws ^{2}+ Med_flow + NL_sum75+ Peak_week + Hot_days : NL_low_flows	12	0.742	0.69
	Final	age + Hot_days + NL_low_flows + NL_CVws	7	0.737	0.687
Bigmouth Buffalo	Complex	~ age + Hot_days + NL_low_flows + NL_CVws + NL_CVws ^{2}+ Med_flow + NL_sum75+ Peak_week + Hot_days : NL_low_flows	12	0.737	0.696
	Final	age ++ NL_CVws ^{2} + Mod_flows + NL_sum75	7	0.733	0.694
Black Buffalo	Complex	age + Hot_days + NL_low_flows + NL_CVws + NL_CVws ^{2}+ Med_flow + NL_sum75 + Peak_week + Hot_days : NL_low_flows	12	0.736	0.679
	Final	age + Hot_days + NL_low_flows + NL_CVws + NL_sum75	8	0.728	0.672

Table 4. three peak-plus catch curves using ages 11-49 for Bigmouth, 10-45 for Black, and 11-52 for Smallmouth Buffalo Parameter estimates from peak-plus catch curves for lower Red River Buffalofishes. The first age group for each species that was one year older than the age of peak abundance was 11, 10, and 11 for Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo, respectively. Estimates are provided for total instantaneous mortality (z), annual survival (S) , and total annual mortality (A).

Species	z	S	A
Bigmouth Buffalo	0.108	0.898	0.102
Black Buffalo	0.075	0.928	0.072
Smallmouth Buffalo	0.105	0.900	0.099

Table 5. Model estimates from von Bertalanffy growth model on buffalofishes from the lower Red River catchment. Estimates for females, males, and all (includes un-sexed individuals) fish. Linf is the theoretical asymptotic size, k is the von Bertalanffy growth coefficient, t0 is the time at length zero, and n is sample size.

Species	Sex	linf	k	t0	n
Smallmouth	Female	638.59	0.12	-3.04	372
	Male	551.66	0.15	-3.18	336
	All	618.69	0.11	-5.24	1096
Bigmouth	Female	731.75	0.1	-8.28	200
	Male	640.9	0.12	-5.71	173
	All	678.73	0.12	-6.79	575
Black	Female	781.76	0.06	-20.38	161
	Male	641.71	0.18	-6.4	152
	All	679.47	0.26	-1.17	343

Table 6. Summary statistics (mean, minimum [Min], maximum [Max], and standard deviation [SD]) of the environmental conditions for the lower Red River catchment near Texarkana, Arkansas from 1986 through 2023. High flows are number of days above 75th percentile of flow; Moderate flows, number of days between 50-70 percentiles; low flows, number of days below 25th percentile; hot days, number of days above 75th percentile; peak week, the week of year (1-52) that max discharge occurs; cv_Winterspring, coefficient of variation of spawning season discharge Jan 1 - April 30; and summer75, 75th percentile of flow when gonads are resting July 1 - Sept 30.

Metric	Mean	Min	Max	SD
High_flows	90.4	0	222	59.913
Hot_days	93.2	64	127	12.112
Med_lows	73.1	10	147	33.402
Peak_week	22.1	1	52	14.424
CV WinterSpring	83.1	29.041	160.11	28.837
Summer 75	240.1	21.245	1363.5	266.63

Table 1. Model estimates from final species-specific mixed-effect models to relate environmental factors to growth for lower Red River Buffalofishes. Model estimates with p-values (p) and 95% confidence intervals reported. For parameter list see Table 5.

Species	Coefficient	Estimate	P	2.5%	97.5%
Smallmouth Buffalo	age	-0.847	$p < 0.001$	-0.858	-0.837
	Hot_days	0.026	$p < 0.001$	0.017	0.036
	NL_low_flows	0.039	$p < 0.001$	0.028	0.051
	NL_CVws	-0.018	0.001	-0.030	-0.008
Bigmouth Buffalo	age	-0.857	$p < 0.001$	-0.873	-0.842
	quad_CVws	0.012	0.098	-0.002	0.026
	Med_flows	-0.007	0.409	-0.023	0.010
	NL_sum75	0.016	0.059	-0.001	0.032
Black Buffalo	age	-0.861	$p < 0.001$	-0.881	-0.841

Hot_days	0.014	0.176	-0.006	0.033
NL_low_flows	0.014	0.250	-0.010	0.039
NL_CVws	0.028	0.015	0.006	0.050
NL_sum75	0.012	0.276	-0.010	0.034

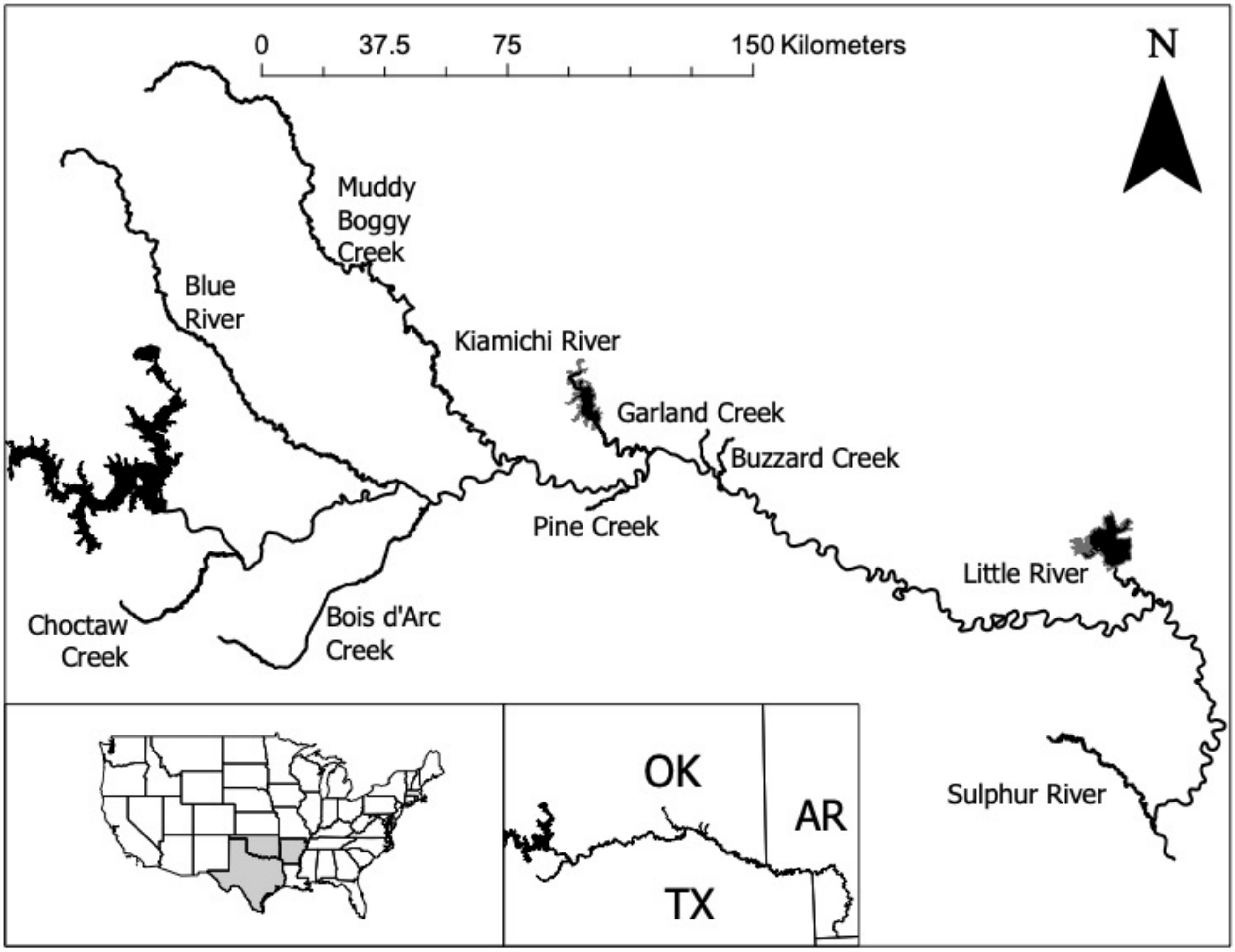

Figure 1. The lower Red River catchment from Lake Texoma to the to the Arkansas – Louisiana state line. Major tributaries and their impoundments, Bois D'arc Creek impoundment began filling in 2021.

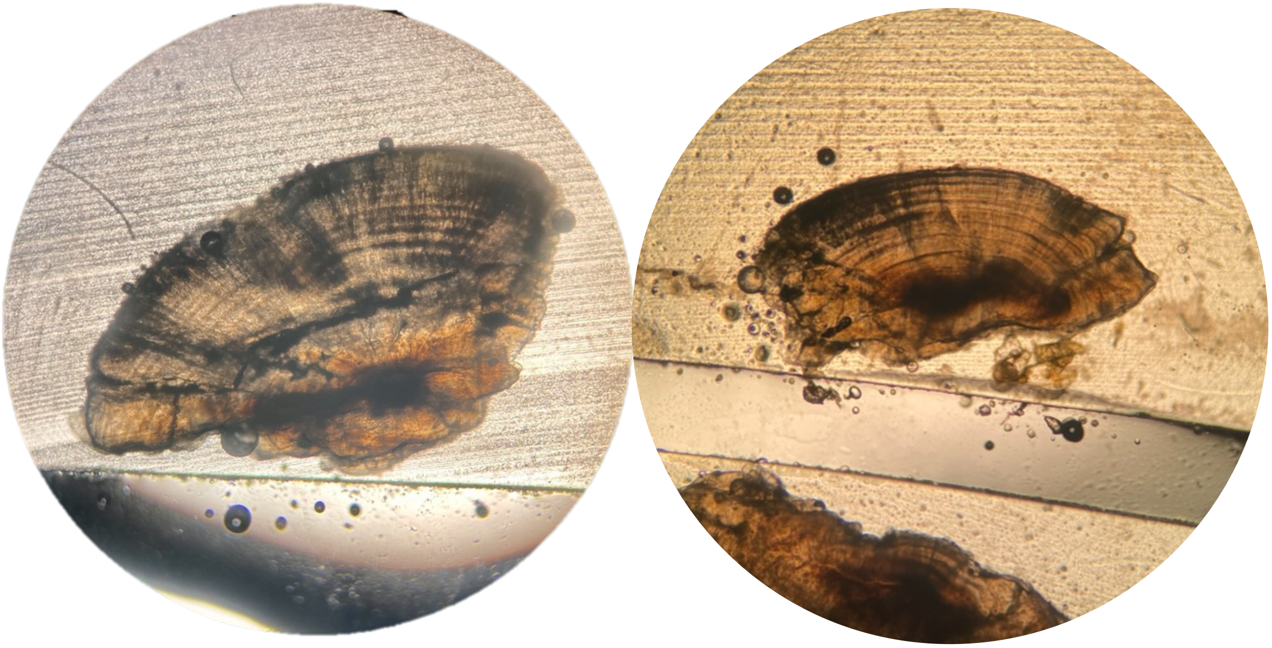

Figure 2. An example of two thin-sectioned (.5-mm) Bigmouth Buffalo lapilli otolith collected from the lower Red River in 2023.

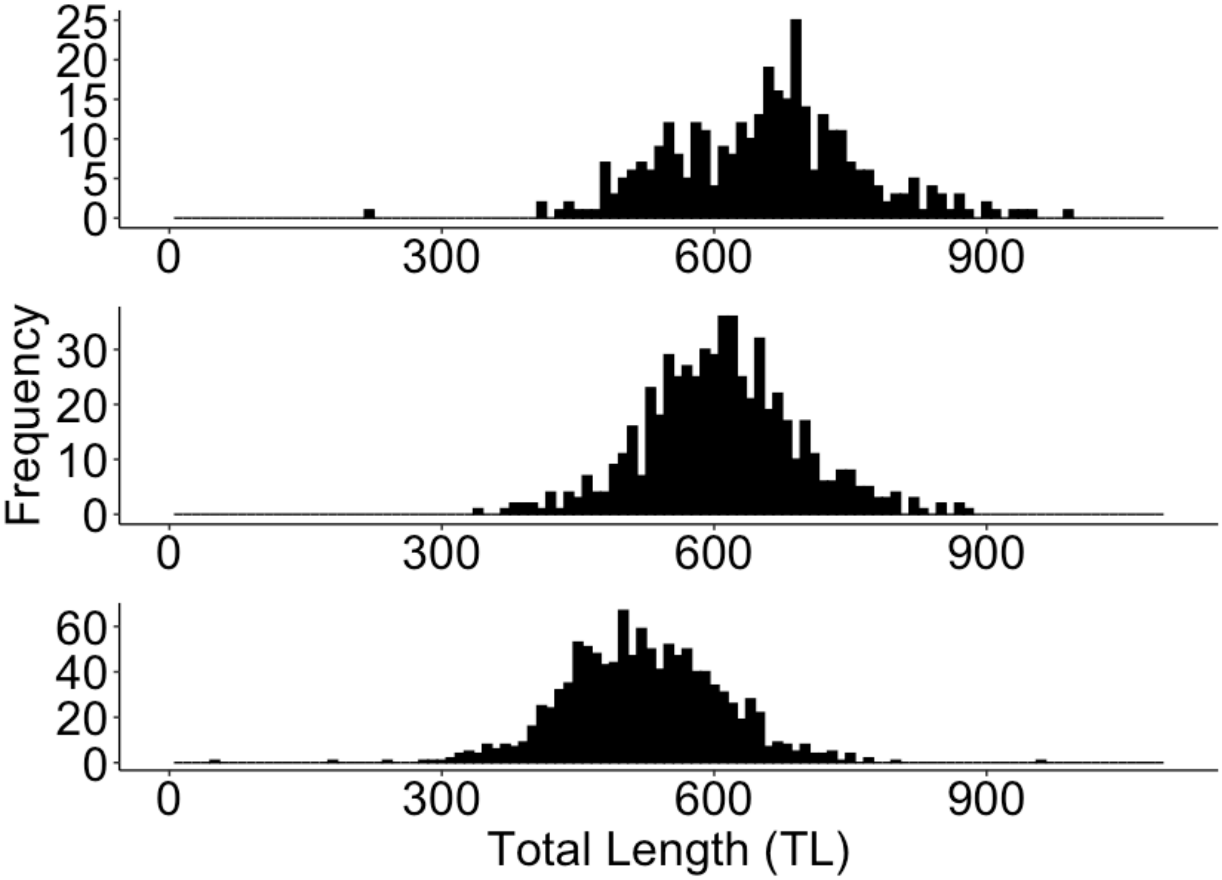

Figure 3. Length (mm) frequency of buffalofishes collected from mainstem lower Red River and several tributaries from March 2022 to May 2024. Black Buffalo are the top panel, Bigmouth Buffalo in the middle, and Smallmouth Buffalo on the bottom. 10-mm bins.

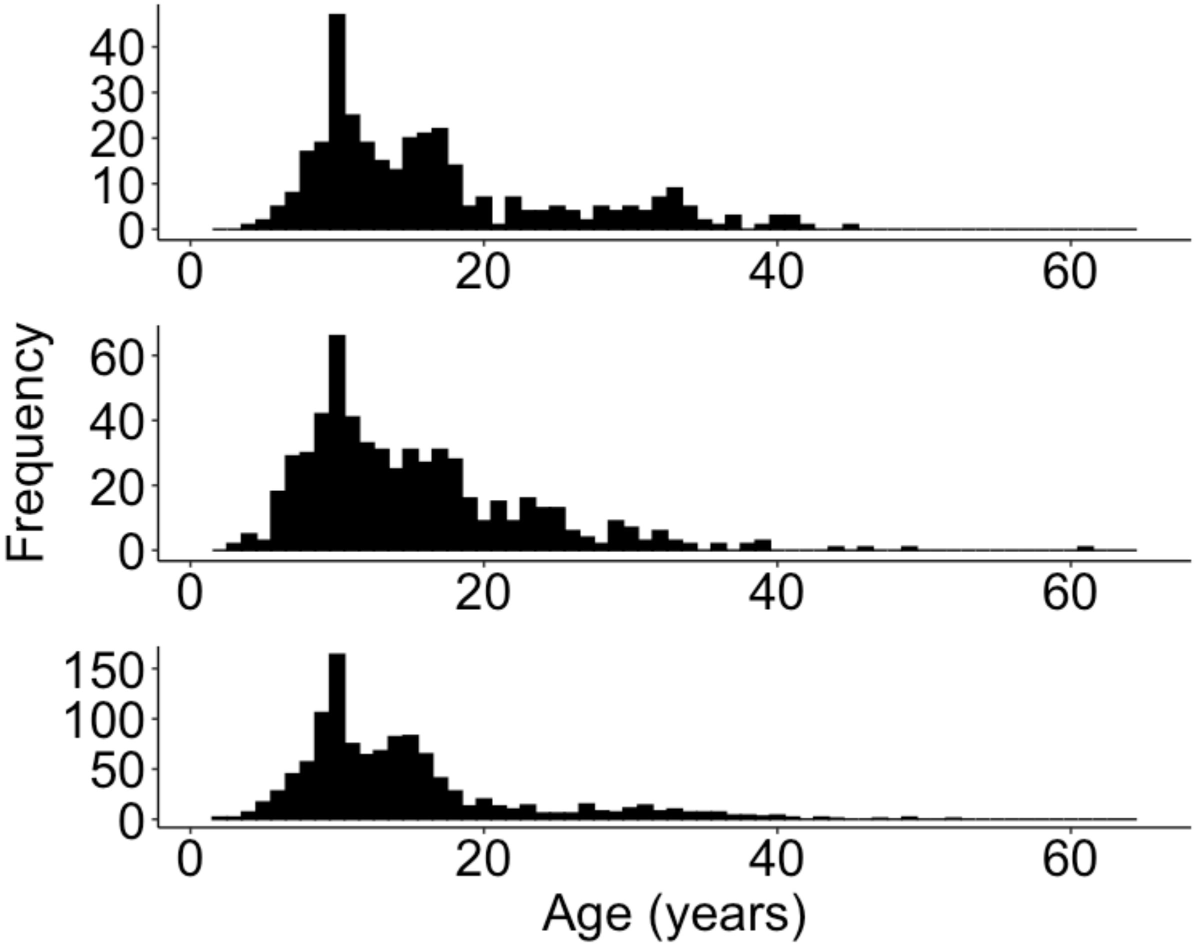

Figure 4. Age frequency plot for Black Buffalo (top), Bigmouth Buffalo (middle), and Smallmouth Buffalo (bottom). Oldest individual was a 61-year-old Bigmouth Buffalo collected in 2022 in Choctaw Creek. Fishes were collected year-round in mainstem Red River and tributaries from March 2022 through May 2024. Ages from two independent readers ageing lapilli otoliths via transmitted light.

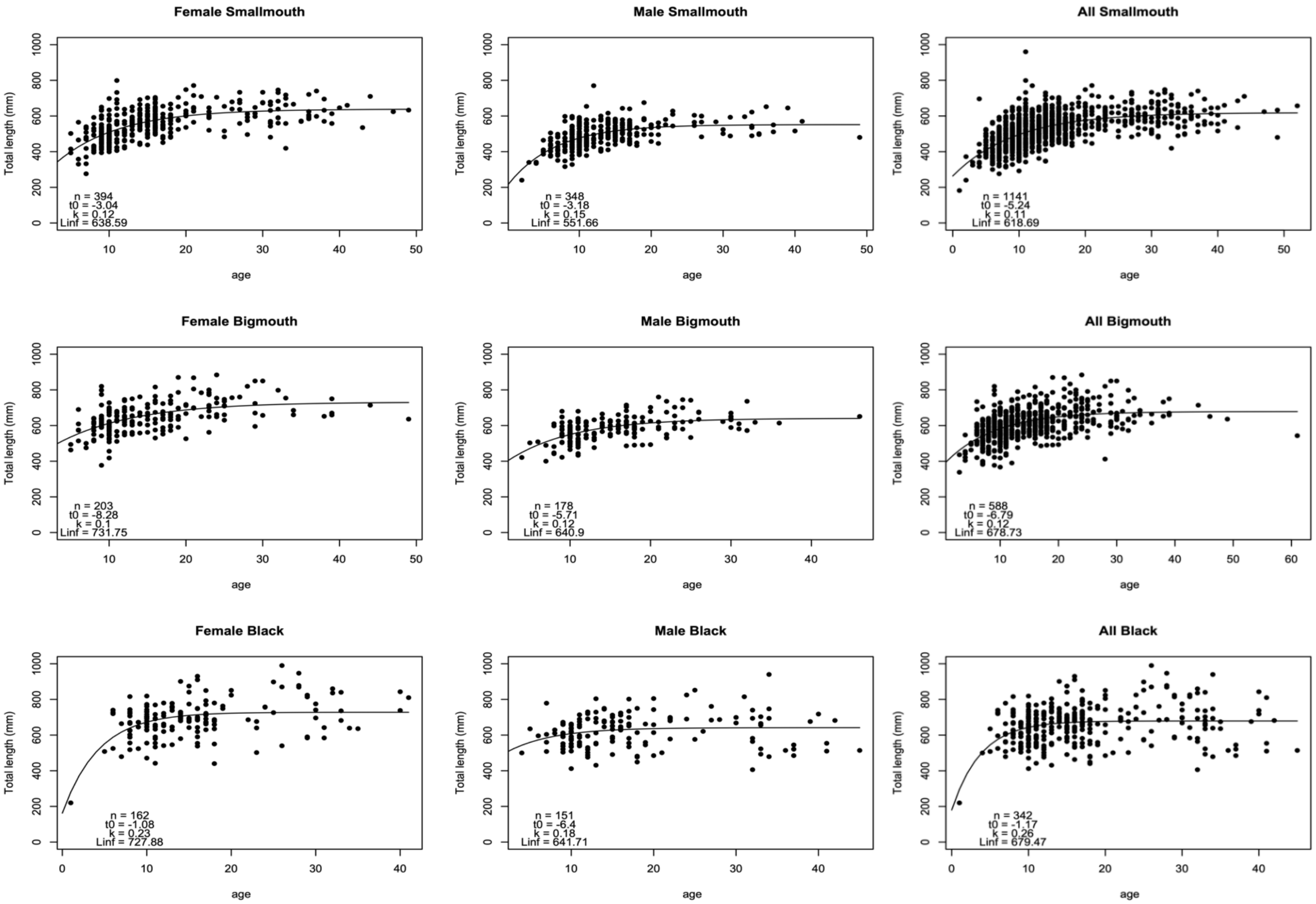

Figure 5. Plots by sex and species of von Bertalanffy growth models. Sample size and estimates of t0, k, and Linf are displayed in the lower left of each plot.

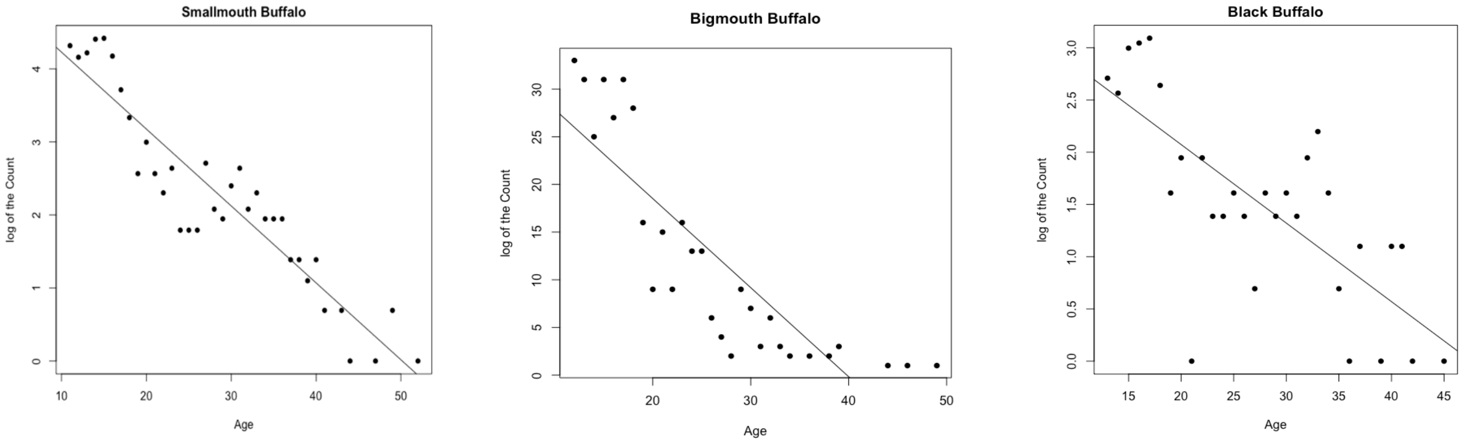

Figure 6. Peak-plus catch curves for buffalofishes assessing mortality and recruitment in the lower Red River catchment. I used ages 11-52 for Smallmouth Buffalo, 11-49 for Bigmouth Buffalo, and 11-52 for Black Buffalo.

Chapter 3. Determine the spawning and hatch chronology of female Buffalofishes in the Red River

Introduction

Fishes have developed a wide range of reproductive strategies to thrive in diverse aquatic environments. Notable examples include salmonid spp that complete anadromous migrations, that help reduce competition and predation on their young (Keeley 2001). Likewise, the diversity of strategies is impressive in freshwater environments where, for example, Bluegill *Lepomis macrochirus* use complex social structures and sneaker males are part of a complex reproductive strategy (Gross 1982). As another example, Goldfish *Carassius auratus* is an indeterminate batch spawner that continually recruit oocytes and can spawn multiple times, whereas Shovelnose Sturgeon *Scaphirhynchus platorynchus* has been found to skip spawning events altogether while some participated in a second spawning event during the fall (Tripp et al. 2009; Brown-Peterson et al. 2011). Many species produce large quantities of eggs to ensure that at least some offspring survive in environments where predation is high (Andersen et al. 2008) or the environment is particularly challenging (Winemiller 2005). Likewise, pelagic broadcast spawning minnows have evolved to exploit a hard environment where a large proportion of the offspring drift hundreds of kilometers during development (Worthington et al. 2018),and some species take advantage of different spawning habitats depending on drought and flood cycles (e.g., Prairie Chub, Wedgeworth et al. 2023)). In contrast, many *Micropterus* spp provide parental care via nest guarding to the eggs and larvae until they disperse from the nest (Steinhart et al. 2005;

Hanson et al. 2009). By adapting reproductive strategies to suit specific habitats, fishes enhance their chances of passing on their genes in sometimes physiochemically harsh and competitive ecosystems.

Understanding the components of species-specific reproductive biology such as age-at-maturity, fecundity, and spawning strategy (e.g. determinate spawners, group synchrony) are important for management and conservation actions (Brown-Peterson et al. 2011; Fish 2021). For example, age-at-maturity varies among species and directly affects population growth rates where early-maturing species may recover quickly from population declines (Ross et al. 1985; Dodds et al. 2004), whereas late-maturing species may need extended protection (e.g., sturgeons, Colombo et al. 2007). An emblematic example is the declining stocks of Atlantic Cod *Gadus morhua* where spawning closures were suggested as an important aspect to avoid disruption of spawning activity and the extirpation of semi-discrete spawning components (Zemeckis et al. 2014). Fecundity levels also inform how resilient a species might be under environmental pressures where species having low fecundity may require more careful management. Reductions in fertilization rates, for example, may occur with highly skewed sex ratios and need be carefully evaluated if interested in population recovery (Heppell et al. 2006). Additionally, spawning strategies, such as determinate spawning (i.e., a fixed number of eggs develop in a season) and group synchrony (i.e., spawning occurs in synchrony to maximize fertilization success), can influence the timing and spatial planning of conservation actions (Lowerre-Barbieri et al. 2011; Ganias 2013). Understanding these reproductive characteristics helps managers develop plans that align with the species' biology via specific fishing regulations, habitat protection, and population recovery efforts (Brown-Peterson et al., 2011; Fish 2021).

Understanding a species' reproductive biology is not only important for understanding a fish's life history but also of economic importance for exploited fishes (e.g., commercial harvest or aquaculture production). Inaccurate macroscopic analysis of Cod gonads from the Kattegat region led to an overestimation of spawning stock biomass (SSB) by up to 35% (Vitale et al. 2006) whereas misclassification of Atlantic Herring *Clupea harengus* gonads in the North Sea underestimated SSB by 27% (McPherson et al. 2011). To accurately adjust SSB calculation in stock assessments, managers need a time series of skip spawning data from the population. More accurate methods for estimating the proportion of mature individuals at a given age or length are needed to improve stock assessments and ensure sustainable fisheries management (Flores et al. 2019).

Several long-lived riverine fishes, including buffalofishes, are thought to spawn during higher-water periods and be episodic spawners. Buffalofishes are presumed to spawn once annually during the spring flood pulse and broadcast spawn adhesive eggs in inundated vegetation (Johnson 1963; Lackmann et al. 2022b). Lackmann et al. (2019, 2022) suggests that Bigmouth Buffalo skip spawn and recruit episodically based on age structure data and the number of missing year classes present. Skip spawning can be inferred through age composition data; however, identification of skipped spawning needs ovarian histology to be quantified (Rideout and Tomkiewicz 2011). The migration and duration of spawning by tagged Common Snook *Centropomus undecimalis* was found to be consistent with previous life-history studies, however, the authors reported that 40% of the tagged fish remained within the study area and forwent spawning (Trotter et al. 2012). Skip spawning is perceived more common among species and populations as more histological analyses are published (Rideout et al. 2005; Rideout and Tomkiewicz 2011). Current research suggests longevity in fishes is correlated with skip spawning (Rideout et al. 2005). Secor (2008) found that moderate to high longevity of iteroparous fishes with lifespans greater than 15 years are associated with skipping spawning. Three types of skipped spawning have been described: retaining, resorbing, and resting (Rideout et al. 2005; Rideout and Tomkiewicz 2011). Retaining spawning omission is when eggs are fully formed but not released (Rideout and Tomkiewicz 2011). Resorption omission is when oocytes begin to form for the upcoming spawn but are interrupted and vitellogenic oocytes are resorbed by follicular atresia (Rideout and Tomkiewicz 2011). Resting omission is when the oocytes remain in pre-vitellogenic form (Rideout and Tomkiewicz 2011).

There appear to be several physicochemical and physiological factors that influence skip spawning by individuals or proportions of populations. For example, skip-spawning White Suckers were positively correlated with hypoxia and negatively correlated with chironomid forage (Trippel and Harvey 1989). Sitar et al. (2014) evaluated skip spawning in Lake Superior Lake Trout *Salvelinus namaycush* and reported 58% of siscowet Lake Trout in southern Lake Superior populations do not reproduce annually. Skipped spawning in large populations, such as the Northeast Artic haddock *Melanogrammus aeglefinu* stock was associated with energy reserves in the fish such that skipping increased when energy levels were low (Skjæraasen et al. 2020). Additionally, skipped spawning became more frequent with increasing population age (Skjæraasen et al. 2020). Potamodromous fishes have also been documented to skip spawn in North America. Humpback Chub *Gila cypha*, for example, skip spawn which makes the fish unavailable to annual sampling potentially biasing survival estimates associated with conservation efforts (Pearson et al. 2015).

There are several ways to estimate spawning periodicity in fishes, each having different advantages and disadvantages. Two common approaches are the gonadosomatic index (GSI) and histological analyses. GSI has been used successfully to estimate stages of maturity in fishes such as the Atlantic Herring (McPherson et al. 2011; Skjæraasen et al. 2012). Flores et al. (2015) reported agreement of 87% on Chilean Hake *Merluccius gayi* between GSI and histological results; GSI ogives were closer to histology derived ogives when compared to macroscopic examination. GSI is helpful when practical limitations prevent other examinations (i.e., access to equipment, higher cost) and certainly more valuable when validated with histological analysis (Devlaming et al. 1982). Histological analysis of ovaries provides more valuable data than macroscopic observations alone. For example, immature ovaries and non-reproductive ovaries can be difficult to distinguish without histology (Rideout et al. 2005; McPherson et al. 2011). Temporal aspects of sample collection are essential for assessing maturity stages with histology is important as a female retaining eggs and skipping spawning can be difficult to delineate from a pre-spawn female (Rideout et al. 2005). Especially important, histology allows for the identification of post-ovulatory follicles (POFs) which indicate spawning occurred (Rideout et al. 2005; Brown-Peterson et al. 2011). Ovarian histology allows for oocyte size (area, µm) frequency counts that can be used to describe reproductive strategies of the species such as spawning pattern by tracking modal patterns (Fish et al. 2020). Consequently, both GSI and histology can provide useful information about a species' reproductive timing, once GSI is validated.

The goal of my second thesis chapter was to assess the factors related to reproductive success of three species of buffalofishes. My study objectives were 1) to describe the reproductive development of female Buffalofishes (i.e., Smallmouth Buffalo *Ictiobus bubalus*, Bigmouth Buffalo *Ictiobus cyprinellus*, Black Buffalo *Ictiobus niger*) in the Red River using both ovarian histology and gonadosomatic index, and 2) to relate environmental conditions to successful hatching and spawning.

Methods

Fish collection

I sampled juvenile and adult buffalofishes from several reaches of the Red River catchment. Juvenile fishes were sampled from 19 reaches using a variety of sample gears. I sampled using mini-fyke nets, beach seining, Siamese trawl, and a subsurface larval tow. Juvenile Smallmouth Buffalo were also collected in 2021-2022 (n = 31) as part of another study on the Red River catchment (Ramsey 2023), and I was able to use those fish to increase my sample size of juvenile fishes. Juvenile fish sampling was completed in May-September to 1) be consistent with previous sampling, and 2) was supported by the existing literature of when we would expect spawning to have occurred (i.e., during spring, (Johnson 1963; Osborn 1966; Lackmann et al. 2022b). I collected adult female buffalofishes using the methods described in Chapter 2 (Fish Sampling). Briefly, I set three gillnets with 6-hour fishing times and boat electrofished each 1.0-1.5-km site. Fish were sampled every month of the year so I could examine the developmental stages related to spawning preparation. For larger fishes, I removed otoliths in the field (see below), whereas smaller fishes were preserved in 70% ethanol until later otolith extraction in the laboratory (see below).

Otolith processing

I extracted the lapilli otoliths from young-of-year (YOY) to estimate hatch dates. I used the lapilli otoliths as they form at hatch in other catostomids (Bundy and Bestgen 2001; Song et al. 2008). Lost River Sucker *Deltistes luxatus* and Shortnose Sucker *Chasmistes brevirostris* have lapilli otoliths formed at hatch and have age-validated daily rings, therefore, I did not subtract additional days from daily ring enumerations to calculate hatch date (Hoff et al. 1997). The Chinese Sucker *Myxocyprinus asiaticus* lapilli otoliths have also been verified for daily rings (Song et al. 2008).

I mounted and polished the lapilli otoliths to quantify daily bands and estimate daily growth. I mounted the whole otoliths to a glass slide using thermoplastic quartz cement (Lakeside No. 70C, Monee, IL) (Secor et al. 1992; Long and Grabowski 2017). I melted the cement on a microscope slide using a hot plate set to 65°C to reduce bubbles. I placed the whole otolith onto the cement and allowed it to cool and harden. I polished the mounted otoliths by hand using a combination of 3 and 1-µm diamond lapping films (Diamond Lapping Film 8", plain backing, Electron Microscopy Sciences, Thermo Fisher Scientific), from coarse to finer, to increase clarity. I applied light pressure and polished in small circular motions using my index finger and frequently checked progress under the microscope to ensure I did not over polish. I spent approximately ten minutes to process each otolith depending on the amount of cement used. An otolith was considered adequately polished when a complete transect of daily bands were visible from the nucleus to the otolith margin. I estimated mean daily growth rates (mm/day) from hatch date to collection date by dividing TL by daily age (Sammons et al. 2021) TL (0.01 mm) was measured prior to otolith extraction and age was the final mean daily ring count (days).

Morphometric data and gonadosomatic index (GSI)

I collected morphometrics data, measured GSI in the adult buffalofishes, and stored the ovaries for later histological analysis. I collected total length (1 mm), wet body weight (± 10 g), blotted gonad weight (± 2 g), and removed lapilli otoliths. I collected ovary weights to track GSI of female buffalofishes over a one-year period. I classified individual females as mature (i.e., macroscopically visible oocytes and prominent blood vessels) or immature based on macroscopic analyses (i.e., small, densely pack oocytes, absent muscle bundles, only one oocyte stage present, and a thin ovarian wall), for length-at-maturity estimation and recorded a macroscopic observation of the ovary size and color (Brown-Peterson et al. 2011). Ovary size was classified as large, intermediate, and immature. The color was indicated as yellow (often with visible oocyte), orange, pink, and brown. I calculated GSI where GSI = $\frac{Gonad\ Wieght}{Total\ Body\ Weight} \times 100$ (Fontoura et al. 2009). Due to the large size of gonads from buffalofishes, I subsampled ovarian tissue from each fish and stored it in 10% neutral buffered formalin (NBF) in a 250mL vial. I removed and stored the entire ovary from immature fish visually identified as small, firm, clear tubes located dorsally in the body cavity (Brown-Peterson et al. 2011) whereas spawning-capable females would carry visible oocytes. I collected two approximately 2-cm wide samples from the central third of each ovarian lobe per female buffalo. I assumed that oocyte development was relatively homogenous throughout the ovary and was representative of spawning condition.

Histology

The ovary tissue was processed to dehydrate and embed my samples in paraffin wax. Histology processing of ovarian samples was completed at the Auburn University Aquatic Reproductive Physiology Histology Lab. The ovary subsections were retained in NBF for a minimum of 5 days to ensure adequate fixation, then samples were transferred from NBF through a series of tap water rinses to remove excess NBF prior to storing them in 70% ethanol (EtOH) to begin the dehydration process. I used an Epredia STP120 Tissue Processor (Epredia, Kalamazoo, MI) to automate the tissue dehydration process (Table 1). Tissue dehydration is necessary to remove water and facilitate wax infiltration in biologic samples (Fournie et al. 2000; Mumford 2004). I loaded 50 to 60 cassettes (histology cassette (length x width x depth (mm)), 41 x 28 x 7, VWR, Radnor, PA) with ovary subsections and placed them into the tissue processor. The processor underwent a 12-hour dehydration cycle allowing for later embedding (Table 1). The ovaries were cycled in 1-hour intervals through 80%-100% ethanol. The samples were then rinsed in xylene to clear the ethanol to facilitate paraffin infiltration. The final three steps consisted of three soaks in paraffin wax.

Once dehydrated, I embedded the tissue in paraffin wax using an embedding station (Epredia Histostar, Kalamazoo, MI) and cryotable. First, a base layer or paraffin was dispensed and cooled to hold the tissue at the mold bottom. Using forceps, the tissue was placed onto the paraffin leaving space between the tissue edge and the mold, and the tissue cassette with lid removed was placed on top of the mold. I used stainless steel molds (VWR, Radnor, PA) with dimensions that varied based on ovary section size: 15mm x 15mm, 25mm x 25mm, and 25mm x 35mm with the smallest being the most preferable for cutting. Then, I dispensed paraffin until the mold and cassette were sufficiently filled. The molds were then cooled on a cryotable to solidify the wax prior to microtome sectioning.

Once embedded in paraffin, the ovaries were sectioned. Each ovary sample was removed from the mold once cooled and placed into ice water bath or face down on the cryotable to cool and harden the wax for sectioning. I sectioned each ovary at 5-µm using a Leica RM2125 RTS (Leica Biosystems, Deer Park, IL) rotary microtome using low-profile blades (Epredia Edge-rite, Epredia, Kalamazoo, MI) set at 5°. To begin, I trimmed the wax down to the tissue, I then allowed the cassette to float in a bowl of ice water or rest wax-up on the cryotable, I then began sectioning the tissue until a ribbon formed (approximately 3-5 sections) and used a paintbrush to transfer the ribbon onto a 45°C heated water bath (Leica HI1210, Deer Park, IL) to smooth the paraffin section. I labeled the slides using a #2 HB pencil and gently lifted the slide from under the floating tissue section at a 45-degree angle lifting the wax section out of the water onto the slide. This process was repeated until each ovarian sample had three slides. The tissue-mounted slides were placed vertically in a rack and allowed to dry overnight prior to staining and cover slipping.

I stained the slides and used an automated process for placing the coverslips (Table 2). Staining and cover slipping were completed using SAKURA Tissue-TekTissue Prisma Stainer and Tissue-Tek Film cover slipper (SAKURA, Torrance, CA). I followed the National Wild Fish Health Survey (NHFHS) Laboratory Procedures Manual Standard Hematoxylin and Eosin Schedule for staining (Table 2). The staining and cover slipping processes were automated (Tissue-Tek Prisma Stainer, Sakura Finetek USA, Torrance, CA). The hematoxylin and eosin staining process began with the slides being immersed in three separate xylene rinses to remove paraffin wax. This was followed by a rehydration process. Following rehydration, the slides were stained with hematoxylin (hematoxylin, VWR, Radnor, PA) and rinsed. Next, the slides were placed into Scott's Water (Electron Microscopy Sciences, Hatfield, PA) to facilitate the bluing of the nucleus (Scott and Dorling, 1965). After this, the slides were rinsed again, they were counterstained with eosin. The slides were dehydrated and cleared prior to applying cover slips via automation. The slides were then passed to the cover-slipper (Tissue-Tek Film, Sakura Finetek USA, Torrance, CA) using the LINK function from the stainer (Tissue-Tek Prisma, Sakura Finetek USA, Torrance, CA). The coverslips were 60-mm SAKURA glass. Finished slides were allowed to dry completing prior to viewing.

I assigned developmental stages to each ovary section using the most advanced oocyte (MAO, i.e., largest) present in the tissue. I examined ovarian follicles on a microscope using 4x-20x magnification and transmitted light. I categorized the developmental stages of the oocytes present using the standardized terminology described by Brown-Peterson et al. (2011). I quantified ovaries using a seven-stage scale: 1, primary growth, smallest in size, angular, and darkly stained; 2, cortical alveoli, larger and more circular than primary growth with the appearance of a white ring near the periphery of the cell; 3, early vitellogenic, two or three rings of yolk vesicles; 4, mid vitellogenic, globules fill inwards towards the nucleus from the periphery; 5, completed vitellogenesis, globules fill the ooplasm and coalesce into larger globules; 6, final maturation, germinal vesicle migration; and 7, post-ovulatory follicles, collapsed follicular wall remaining after a oocyte is released (Guzmán et al. 2017; Fish et al. 2020). Additionally, I recorded the presence or absence of atresia (i.e., atretic oocytes) within the sample and total number of oocyte developmental stages present. I considered ovaries containing resorbing follicles and degenerating oocytes where oocyte development was stopped before vitellogenesis as evidence of skip-spawning (Goetz et al. 2011; Sitar et al. 2014).

Environmental Covariates

I measured environmental variables that I hypothesized would be important to stream-fish reproduction to examine relationships associated with the three species of buffalofishes (Table 3). Discharge is often related to spawning initiation and recruitment success (Durham and Wilde 2006). I obtained mean daily discharge (1.00 m^3/sec) measurements from the USGS stream gage nearest to each of my sites (Table 3). I scaled discharge by dividing each measurement by the respective drainage area to allow more direct comparison of discharge values across sites with variable orders of magnitude. I collected average daily air temperature (1.0 ℃) and total daily rainfall (1.00 mm) from the weather station nearest to each of my sample reaches (Table 3). I used air temperature data as a proxy for water temperature as because temperature is a primary mechanism that governs spawning initiation and development among fishes (Graham and Orth 1986; Kjesbu 1994). I used air temperature as a proxy for water temperature as this catchment experiences large fluctuations in water levels making use of water temperature loggers difficult and the catchment has minimal groundwater inputs. I also collected precipitation data (Table 3) because weather patterns including drought may influence fish recruitment (Zeug and Winemiller 2008; Wedgeworth et al. 2023).

Analyses

Hatch model

I placed my hatch dates into bins and calculated environmental metrics. For my hatch analyses, I grouped observed buffalofish hatches, as a genus, into 7-day periods beginning in March and ending in September of 2023 because this range encompassed my observed hatches from both spring and summer sampling. The mean air temperature and mean scaled discharges were calculated seven days prior to the group hatch week. I calculated six other metrics: the coefficient of variation (cv, $\frac{standard\ deviation}{mean} \times 100$) of mean air temperature and cv of mean scaled discharge were both calculated seven days prior to the group hatch week, the number of days post spring flood (i.e., highest discharge peak from Jan 1 to April) 14-day rate of change (i.e., average difference between maximum and minimum discharge over 14 days prior to hatch week), calendar day (i.e., number of days from Jan 1 to start of hatch week), and the sum of precipitation 1-week prior to the hatch week (Table 4).

I checked for multicollinearity, examined my covariates to ensure I met the linear regression assumptions, made necessary transformation, and standardized my covariates prior to model building. I natural log transformed mean scaled discharge, cv of mean scaled discharge, and cv of mean air temperature to account for skewness. I used the Pearson correlation coefficients ($| r | < 0.60$) to retain orthogonal covariates for modeling. I removed cv of mean scaled discharge because it was multicollinear with the cv of mean air temperature ($r = 0.9$), I removed the number of days post spring flood because it was multicollinear with mean air temperature ($r = 0.75$), and lastly, I removed calendar day (Table 5). Prior to running the model, I standardized my data to a mean of zero and a standard deviation of one to aid in model convergence and interpretation (Gelman and Hill 2006).

I used a zero-inflated binomial (ZIB) model to analyze the probability of hatch success related to my environmental covariates. Results can be biased if excess zeros are not accounted for in the data (Blasco-Moreno et al. 2019). Zero-inflated models such as ZIB account for excess zeros and overdispersion (Lambert 1992; Martin et al. 2005). I aggregated the 19 sample locations from 2021-2023 into 6 sites for my analyses where sites shared environmental covariates (Figure 1). My model consisted of two components: first, the conditional model used to relate environmental conditions to hatch success and second, the logistic regression zero-inflated model the probability structural zeros. To avoid overfitting the zero-inflated component I held it constant with one variable, one week mean daily air temperature and did not include it in the conditional model. I used the “glmmTMB” R package (Brooks et al. 2017).

Conditional model:

$$logit(p_i) = \ln\left(\frac{p_i}{1 - p_i}\right) = \beta_0 + \alpha_1 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \gamma_t + e_{it} \sim N(0, \sigma)$$

Zero-inflated model:

$$logit(z_i) = \lambda_0 + \lambda_1 Z$$

Where:

p = is the probability of hatch i and site t,

β_0 = prime intercept,

α_1 = fixed effect for year where 2021 was the reference

β_i = intercept for environmental predictor variable X

X_i = environmental predictor variable

$z_i = 1$ indicates a structural zero,

λ_0 = intercept for the zero-inflation component,

Z = predictor for zero-inflated component,

λ_1 = coefficient for the predictor Z

I ranked my candidate model sets to determine which to include in my final models. I used Akaike's information criterion adjusted for small sample size (AIC_c) to select my top models based on the lowest AICc value (Burnham and Anderson 2003). I used combinations of covariates in my candidate model sets that were based on my hypotheses about factors that may relate to hatch by each species (Table 6). To account for annual variation, I included a categorical fixed effect for year (2021-2023) for the Smallmouth Buffalo model as that was the only species where I had multiple years of data from previous research efforts. I used Akaike weights (w_i) to determine the relative model support (Burnham and Anderson 2002). To avoid including uninformative parameters, I determined my top ZIB as the most parsimonious models within 2 AIC_c of the top model with the highest weight (Table 7).

Histological descriptions and GSI

I provided histological descriptions for the observed oocyte developmental stages of three species of buffalofishes. I report reproductive phases (Table 8), oocyte stage (Table 9), and briefly describe the oocyte. Briefly, there were reproductive phases that were developed based on criteria of macroscopic and histological features including the most advanced oocyte present, presence of muscle bundles, atresia presence, and (Brown-Peterson et al. 2011; Lowerre-Barbieri et al. 2011; Ganias and Lowerre-Barbieri 2018). I used 7 stages for oocyte development based on Guzmán et al (2017) and Fish et al. (2020). I modified their criteria by including a primary growth stage (Guzmán et al. 2017) and by simplifying the stages of primary growth and oocyte maturation to one stage each, respectively (Fish et al. 2020). My descriptions were made by visual examination of each stage underneath the microscope using transmitted (Nikon eclipse E400, 40x).

I calculated mean GSI per oocyte developmental stage (i.e., oocyte stages 1-7, Table 10) to investigate the use of GSI as a predictor of maturity when compared to histological assessment. I tested GSI values for normal distributions using the Shapiro-Wilks test, homogenous variances with Levene's test, and checked multicollinearity among independent variables using Pearson's correlation test and only retained variables with $r < 0.6$ (Roever et al. 2014).

To investigate the relationship between GSI and female maturity, I conducted an analysis of variance between GSI and histological stage. I calculated the mean GSI, standard error, and sample size by month by species. Then, I checked my GSI data for normality using the Shapiro-Wilk test. I used the Levene's test to check for unequal variances. I log transformed the GSI values because these data were not normally distributed (i.e., right skewed). I then used analysis of variance (ANOVA) to determine significant ($\alpha \leq 0.05$) differences between GSI and the most advanced oocyte stage. I used Tukey's Honest Significant Differences post-hoc test to determine where significant differences occurred.

Probability of POF presence

I calculated environmental covariates that I hypothesized would relate to spawning by the three species of buffalofishes (Table 11). I calculated mean daily air temperature (°C) of the four weeks prior to spawn (i.e., presence of a POF) , mean daily scaled discharge (m/s) four weeks prior to POF presence, cv of mean daily air temp 4 weeks prior to POF presence, cv of mean daily scaled discharge 4 weeks prior to POF presence, day of year where POF presence was observed (i.e., Jan 1 = 1), the number of days to the annual peak discharge event (i.e., highest discharge peak during that year), and the 75th percentile of scaled daily discharge one week prior to POF presence.

I considered one interaction term in all species models. I hypothesized there would be an interaction between mean temperature and mean discharge (4 weeks prior to POF presence). I hypothesize that buffalofishes in the lower Red River that spawned during the winter at lower flows and colder temperatures would have a higher probability of spawning compared to colder temperatures and higher discharges. Because the predation risk would be lower during winter, that may be an ideal time to spawn but combined with high discharge conditions may be too harsh and not provide offspring with increased food availability during that time. I also hypothesized that buffalofishes would have a higher likelihood of spawning during warmer air temperatures when discharge was higher, and offspring would have floodplain access.

I built a binomial generalized linear model (GLM) to relate environmental conditions to spawning using observed post-ovulatory follicles (POFs) of females buffalofishes. I aggregated POFs by week (7-days) to relate to my environmental metrics (calculated as 4 weeks prior to each POF week) prior to each hatch week. Post-ovulatory follicles were grouped by week beginning April 1st, 2023, and concluding May 31st, 2024, by species, as this was one year of continuous ovarian sampling (Table 12). I built three species-specific POF models (i.e., probability of POF) using the statistical computing software R (Version 4.2.2, R Core Team 2022). I used glm from the “stats” R package and “dataRetrival” package (Hirsch and Cicco 2015).The POF model can be expressed as:

$$\text{logit}(p_i) = \beta_0 + \beta_1 * X_1 + \cdots + \ e_i \sim N(0, \sigma)$$

Where:

p = the probability of a POF for observation i

β_0 = grand intercept

β_i = intercept for environmental factor $X_{1\text{-n}}$

e = error term that is normally distributed with a mean of 0 and standard deviation of σ.

I checked for multicollinearity, examined my covariates to ensure I met the linear regression assumptions, made necessary transformation, and standardized my covariates prior to model building (Table 13). I natural log transformed mean scaled discharge, cv of mean scaled discharge, and cv of mean air temperature to account for skewness. I used the Pearson correlation coefficients (| r | < 0.60) to retain orthogonal covariates for modeling. I fit my POF model using a simple backward selection approach. I first fit a global model with one interaction and all main effects. If the interaction was not significant, it was removed from the model and then refit the model with the main effects. Lastly, I removed any main effects that were not significant and then refit my final model. I repeated this process for each species (Table 14). My Black Buffalo final model included the fixed effects of day of year, mean daily air temperature, and mean daily scaled discharge. Bigmouth Buffalo final model retained day of year and mean daily air temperature. Lastly, the Smallmouth Buffalo final model retained day of year, mean daily air temperature, and mean daily scaled discharge.

Results

Fish collection

I completed a total of 85 surveys between in 2023 across 19 locations during my juvenile sample season (i.e., May – September; late spring and summer). Most sampling occurred in the mainstem Red River (67/85 surveys) but I also sampled buffalofishes in the Muddy Boggy, Kiamichi River, and Pine Creek. During the sampling season, I revisited each site approximately three times to capture multiple cohorts.

Detection of age-0 buffalofishes was variable among species and sites. I completed 19 of 86 surveys in three tributaries (i.e., Kiamichi River, Muddy Boggy, and Pine Creek) and the remaining 67 surveys in the mainstem Red River throughout the catchment. Age-0 Smallmouth Buffalo had the most detections (total n = 36) whereas Bigmouth Buffalo and Black Buffalo were much rarer in my samples (Bigmouth, Buffalo n = 14 and Black Buffalo, n = 16 in 2023). Bigmouth Buffalo juveniles were only detected by my sampling gears over 5 days in 2023 (May 19th - May 23rd). Black Buffalo juveniles were detected only in the mainstem Red River with successful hatches occurring in April and May across the mainstem sites with only one detection in June at the Spanish Bluffs site. Smallmouth Buffalo juveniles were detected in both mainstem and tributary sites (i.e., Muddy Boggy). Successful hatches of Smallmouth Buffalo occurred from April through June across the catchment.

Otolith processing

Otoliths that met agreement my criteria for ageing were retained for my hatch analyses. In total, I processed and aged 97 age-0 buffalofishes from 2021 through 2023 (example otolith image, Figure 2). I retained 16/16 age-0 Black Buffalo, 14/15 Bigmouth Buffalo, and 36/36 Smallmouth Buffalo otoliths for analyses. Ages of age-0 Black Buffalo that I processed ranged from 16 to 25 days, Bigmouth Buffalo ranged from 34 to 66 days, and Smallmouth Buffalo ranged from 15 to 63 days.

Buffalofishes exhibited distinct successful hatching periods that varied by species and year. In 2023, Bigmouth Buffalo had the earliest and briefest hatching period, with hatches detected from early March to April 5 (Figure 3). Smallmouth Buffalo hatching patterns differed among years where the earliest occurred in 2022, with 2023 hatches starting on March 29 and peaking the week of May 17 before concluding on June 21 (Figure 4). In 2021, Smallmouth Buffalo collected from the Muddy Boggy Creek exhibited a truncated successful hatching period from May 5 to May 25. Black Buffalo had a more extended hatching span compared to the other species in 2023 (April 12 and ending June 21, Figure 5). Overall, Bigmouth Buffalo exhibited the earliest and most condensed hatching window in 2023, whereas Smallmouth and Black Buffalo experienced more prolonged hatching periods.

Age-0 buffalofishes size and daily growth varied among species. Lengths of age-0 fishes for Smallmouth Buffalo and Black Buffalo ranged from 15.70 – 85.45 mm TL and 17.0 – 29.93 mm TL and Bigmouth lengths ranged from 26.21 – 40.85 mm TL. Daily growth rates ranged from 0.739 to 1.435 mm/day for Black Buffalo, 0.381 to 1.302 mm/day for Bigmouth Buffalo. Smallmouth Buffalo daily growth rates were similar among years, 2021 ranged from 0.922 – 1.799 mm/day, 2022 ranged from 0.991 – 2.030 mm/day, and 2023 ranged from 0.623 – 1.698 mm/day.

Morphometric data and Gonadosomatic Index

Adult buffalofishes sampled were variable in TL, weight, and GSI over time, with distinct sizes of fish having POFs. Female Smallmouth Buffalo had a mean age of 15 (range: 1 – 43 years), mean length 544-mm TL (range: 330-799-mm TL), and mean ovary weight of 175 (range: 2-1974g, standard deviation ± 348). Female Bigmouth Buffalo mean age of 16 (range: 5 – 49), mean length 649-mm TL (range: 377 – 884-mm) and mean ovary weight of 423g (range: 2-1646g, standard deviation ± 423). Female Black Buffalo had mean age of 16 (range: 1 – 41), the largest mean size of 688-mm TL (range: 220-990-mm), and largest mean ovary weight of 490g (range: 2-2190g, standard deviation ± 490) (Figure 6). Total length and weight were positively related as expected (Figure 7). I collected 582 ovaries from lower Red River Buffalofishes from April 1st, 2023, to May 1st, 2024, to evaluate spawning (Table 12). I retained 142 Bigmouth Buffalo, 121 Black Buffalo, and 302 Smallmouth Buffalo representing 565 ovarian samples after omitting samples that were not properly fixed. Monthly mean GSI values among species followed similar trends over time (Figure 8). Mean GSI decreased from April to July 2023 then began increasing in September with Bigmouth and Black Buffalo having a more pronounced peak compared to Smallmouth Buffalo. Only a relatively small proportion of fishes sampled had POFs (Smallmouth Buffalo, 38 of 300 sampled; Bigmouth Buffalo 14 of 128 sampled; Black Buffalo 17 of 123 sampled). Interestingly, only Bigmouth Buffalo greater than 500-mm TL, Black Buffalo greater than 600-mm TL, and Smallmouth Buffalo greater than 400-mm TL were observed with POFs.

Histology

I processed ovaries in proportion to the number of fish collected by species where Smallmouth Buffalo was the most common and Black Buffalo was the rarest in my samples. I processed 565 fishes with 53% representing Smallmouth Buffalo, 25% Bigmouth Buffalo, and 22% Black Buffalo. The distribution of oocyte developmental stages varied among the three buffalofishes. Black Buffalo had the highest number of individuals in stage five (36), followed by stage one (26). Bigmouth Buffalo showed a similar pattern, with the highest number in stage five (34) and stage one (30). Smallmouth Buffalo had the highest number in stage two (79), followed by stage five (54). I observed the presence of atretic oocytes (i.e., alpha atresia, Corriero et al. 2021) in 43 of 123 Black Buffalo, 63 of 128 Bigmouth Buffalo, and 80 of 300 Smallmouth Buffalo. I assigned oocyte developmental stages to all ovarian samples over time based on histological and macroscopic observations. Reproductive phases were variable through time among species. Smallmouth Buffalo had regressing ovaries (e.g., POFs may be present, post-spawn condition) observed in all but three months of sample collection November and December 2023 and May 2024 (Figure 9). Smallmouth Buffalo followed a similar patter with no spawning capable oocytes observed during June and July (Figure 10). Bigmouth Buffalo had spawning capable (e.g., presence of stage five) for nine months with no stage five oocytes observed in July – September (Figure 11). Interestingly, I observed actively spawning individuals (i.e., stage six, mature oocytes) of Black Buffalo and Bigmouth Buffalo first, in November followed by Smallmouth Buffalo in December (Figures 11-12).

Environmental Covariates

Discharge and air temperature patterns varied among years and sites (Table 15). In 2021, discharge in the Red River was characteristic of a wet spring, with flows exceeding 2,831-cms in May and June. This was followed by a prolonged period of decreasing flows, punctuated by smaller peaks, before reaching the annual minimum of 45-cms in December. In 2022, discharge patterns reflected longer-term patterns where there was a spring flood pulse and extended periods of relatively stable flows, interrupted by occasional smaller peaks. The discharge patterns in 2023 were marked by high spring flows and extreme fluctuations during the summer. There were several discharge spikes exceeding 900-cms, followed by flows below 200-cms. Compared to 2021, average discharge conditions in Muddy Boggy Creek were lower in 2023. Air temperatures across the study sites were cooler and more variable in 2023 than in 2022. At Arthur City, the mean air temperature in 2023 was 22.3°C, while at Texarkana, it was 24.8°C. Throughout the study, mainstream Red River sites were generally warmer, while Muddy Boggy sites were typically cooler.

Analyses

Hatch model

My top-ranked ZIB model for hatch probability included the cumulative one-week prior precipitation (hereafter, precip) and year (2021 as reference) in the conditional model whereas mean one-week daily air temperature (hereafter, temperature) was retained in the zero-inflated model. Precip had a weak positive association with hatch probability (Figure 12). The fixed effect of year indicated no significant difference in hatch probability in 2022 compared to 2021 whereas 2023 had a positive association with hatch probability. Temperature was significant predictor of excess zeros ($p < 0.001$) in the zero-inflated component. For my top ranked model I used the "DHARMa" package (Hartig 2017) to examine diagnostic residual plots (i.e., scatterplot of residual and fitted values and QQ plot, Figure 14).

Histological description and GSI

The presence of POFs indicated Bigmouth Buffalo has a relatively long spawning season that somewhat corresponds to changes in GSI values. Direct evidence of Bigmouth Buffalo spawning, indicated by the presence of POFs, was first observed in April 2023 and into June 2023 marking the conclusion of the 2023 spawn. I observed POFs marking the onset of the spawning season beginning in October and observed monthly until May 2024. Fish with spawning capable oocytes (i.e., stages five and six) were first observed in October 2023 and were the dominate oocyte stage found December 2023 through May 2024. corresponding with higher but somewhat variable mean monthly GSI values when compared to other months. Bigmouth Buffalo had relatively few oocyte stages present at any time indicating determinate fecundity and batch spawning over a protracted season beginning in early winter extending into the following spring and summer.

My GSI data were non normal with unequal variances. Shapiro-Wilk test on the GSI data indicated non-normal data ($p < 0.001$) and Levene's test supported unequal variances across most advanced oocyte stage present (ADV), stages ($p < 0.001$). Finally, a Pearson correlation analysis between GSI and ADV, yielding a moderate positive correlation ($| r | = 0.40$, $p < 0.0001$).

ANOVA was conducted on log-transformed GSI values across histological stages by species. Mean GSI of Bigmouth Buffalo increased across advancing ovarian stages, peaking in spawning capable stages five and six before decreasing in stage seven as spent gonads. Significant differences in mean GSI were observed between histological stages ($F_{6, 141} = 48.88$, $p < 0.001$). Post-hoc Tukey’s HSD test confirmed significant differences between GSI values and several stages ($P < 0.001$) (Figure 2). Stage four (i.e., secondary vitellogenesis) was significantly different from all other stages. There were no significant differences among Bigmouth Buffalo oocyte stages one, two, three, and seven. In contrast, stages five and six were significantly different from all other stages. Stage seven was significantly different from five and six and exhibited reduced GSI values (all $p < 0.001$). Significant differences amongst mean GSI values were identified in Black Buffalo ($F_{6, 111} = 26.66$, $p < 0.001$). GSI values associated with stages one, three, and seven were not different, whereas GSI of stages two and four were significantly different from all stages. GSI associated with stages five and six were significantly different from all other stages. Moreover, stage seven had significantly lower GSI compared to both stages five and six ($p < 0.001$). GSI of Smallmouth Buffalo had significant differences among histological stages. ($F_{6, 287} = 135.2$, $p < 0.001$). Specifically, Smallmouth Buffalo GSI at stage four (i.e., secondary vitellogenesis) was significantly different than at all other stages. In contrast, there were no significant differences in GSI among oocyte stages one, two, three, and seven. However, GSI at stages five and six was significantly different from that at all other stages. These differences included higher GSI at stage five compared to stage one, at stage six compared to stage one, at stage four compared to stage two, at stage five compared to stage two, and at stage six compared to stage two. Additionally, GSI at stage seven was significantly lower than at both stage five and stage six (all $p < 0.001$). Specifically, GSI at stage four (i.e., secondary vitellogenesis) was significantly higher than at all other stages. In contrast, there were no significant differences in GSI among oocyte stages one, two, three, and seven. However, GSI at stages five and six was significantly different from that at all other stages. These differences included higher GSI at stage five compared to stage one, at stage six compared to stage one, at stage four compared to stage two, at stage five compared to stage two, and at stage six compared to stage two. Additionally, GSI at stage seven was significantly lower than at both stage five and stage six (all $p < 0.001$).

Bigmouth Buffalo spawned over a relatively long season. The presence of POFs in Bigmouth Buffalo was observed monthly from May through August 2023. I sampled 14 of 128 Bigmouth Buffalo containing POFs. Spawning capable oocytes were observed were observed in all sampling months except July, August, and September of 2023. Interesting, actively spawning (i.e., stage six, mature oocytes) oocytes were absent from Bigmouth Buffalo collected in 2024 (n = 34). Mean GSI values began rising in October 2023 and peaking in April 2024 (n = 5).

Black Buffalo had a protracted spawning season. The presence of POFs indicated Black Buffalo has a relatively long spawning season that somewhat corresponded to changes in GSI values. I sampled 17 of 123 Black Buffalo that contained POFs. Black Buffalo evidence of spawning from POF presence was first observed in April and continued into June 2023 (Figure 7). The onset of the next spawn was identified by POF presence first observed in October and continuously through April 2024 indicating they spawn from late autumn into spring. Black Buffalo with spawning capable oocytes were observed April through June 2023 and October through Aril 2024 coinciding with the rise in mean GSI values. However, average GSI values across the population were not particularly reflective of the spawning timeline (Figure 6). Black Buffalo exhibited determinate fecundity and batch spawning over a protracted spawning season beginning in October and continuing to the following spring.

Smallmouth Buffalo spawned for many months of the year. Smallmouth Buffalo was observed with POFs in 2023 and 2024 over an extended time (Figure 9). In total, I only sampled Smallmouth Buffalo in five months (June and July 2023; October and November 2023 and May 2024) that were not actively spawning including May which had a very low sample size (n = 2) due to flooding. I only observed three months were POFs were absent: November and December 2023 and March 2024. Spawning capable fish were observed from August 2023 to May 2024 where monthly GSI values start to rise around October (Figure 6). Smallmouth Buffalo showed determinate fecundity, batch-spawning behavior, and a protracted spawning season beginning in Autumn and continuing through winter and spring.

Atretic oocytes were present among species with Bigmouth Buffalo having the highest relative proportion of atresia (0.44) followed by Black Buffalo (0.36), and Smallmouth Buffalo (0.27). In total, I examined eight immature fishes (via histology): six Smallmouth Buffalo (mean length 412 mm, range 330-505 mm), one Bigmouth Buffalo (418 mm TL), and one Black Buffalo (220-mm). I observed individuals of all three species with extreme atresia of large, developed oocytes such that only a few viable oocytes were left within the tissue section indicating potential skip-spawning. I observed individuals of all species exhibiting mass atresia (i.e., no observable viable oocytes in sections) 18 of 106 Black Buffalo, 21 of 106 Black Buffalo, and 14 of 128 Bigmouth Buffalo. Thus, I observed some individuals engaged in resorption skip spawning in three sympatric buffalofishes (Rideout and Tomkiewicz 2011).

Probability of POF presence

Metrics that I calculated using discharge and air temperature data from sites on the lower Red River catchment varied across the study period. The mean 2023 calendar day was 183.16 (range: 9 to 349). Mean water temperature over a four-week period was 20.79°C (range: 3.24°C to 30.96°C) with a coefficient of variation (CV) of 27.25% (range: 5.42% to 200.52%). Discharge, scaled by drainage area had a mean discharge of 0.0029 m/s (range: 0.00032 to 0.03498 m/s) and a CV of 60.41% (range: 0.48% to 175.56%). The one-week 75th percentile of scaled discharge was 0.0032 m/s (range: 0.00012 to 0.04676 m/s). The mean number of days from peak flow was 137.70 days (range: 25 to 278 days).

My final model for Bigmouth Buffalo probability of spawning contained the effects of day of year and mean daily air temperature (Table 16). Bigmouth spawning probability was negatively related to day of year whereas mean daily air temperature was positively related (Figures 15-16). Black Buffalo final model included day of year, mean daily air temperature, and cv of mean daily scaled discharge (Figures 17-19). Day of year had a negative relationship with whereas mean daily air temperature and cv of mean daily scaled shared a positive relationship with POF probability.

My final Smallmouth Buffalo POF model retained three covariates. I retained day of year, mean daily air temperature, and cv of mean daily scaled discharge in my final Smallmouth Buffalo model describing spawning (Table 16). Mean daily air temperature and cv of mean daily scaled discharge were both positively related to the probability of spawning (Figures 20-21). Day of year had a negative relationship with POF probability (Figure 22).

Discussion

There is an interesting parallel between spawning periodicity and the general lack of many strong relationships I found with POFs or successful hatching by buffalofishes. Although Smallmouth Buffalo is thought to prefer finer substrates, they appear to be most successful when they have floodplain access at higher flows (Becker 1983). Like the other species, their eggs are adhesive and attach to substrate, plants, or debris post spawn. Spawning high numbers of eggs over a relatively long period of time is considered a bet hedging strategy and species who develop those strategies sometimes have multiple life histories (e.g., pelagic broadcast spawning minnows have both migrant and resident populations, Rio Grande Silvery Minnow *Hybognathus amarus* Chase et al. 2005) It is unclear how the predominance of unstable substrates (i.e, sand) in the Red River relate to opportunistic spawning periodicity where survival is low but spawning output is high. It is interesting to consider the differences in buffalofishes, if any, that spawn during winter versus early to late spring. Female size has been related to earlier spawning activity (Wright 2013; Gunnarsson et al. 2016) and greater spawning duration (Evans-Powell et al. 2024) in marine fishes. Furthermore, size can influence spawning movements such as larger individuals migrating earlier than smaller individuals (García-Vega et al. 2018).

Analyzing spatial and temporal variation in hatching dynamics is necessary to understand how environmental variability affects recruitment. I sampled age-0 and juvenile buffalofishes from May through August 2023 and did not collect any fishes with hatches prior to March 1st or after June 21st (Bigmouth Buffalo were the earliest and Smallmouth Buffalo were the latest). Although the relationships between environmental factors and hatch probability were relatively weak, some patterns emerged that align with previous studies.

Flood events occurring at the onset of the monsoons have been observed cuing spawning in tropical stream fishes (Kramer 1978). For example, precipitation events have a positive relationship on hatches in the annual *Austrolebias* spp. (García et al. 2018). Likewise, precipitation events may be representative of other fine-scale processes in local spatial conditions. For example, rainfall induced water-level rise in coastal lagoon near Sydney, Australia increased nutrient inputs and resulted in a plankton bloom (Rissik et al. 2009). Additionally, extended drought conditions result in recruitment failure by Great Plain cyprinids (Perkin et al. 2019).The conditional model revealed interannual variability between 2023 and 2021 This interannual variability may reflect differences in sampling effort as age-0 buffalofishes were my target in 2023 whereas age-0 buffalofishes were collected as exploratory samples in 2021 and 2022. The zero-inflated component indicates the importance of temperature in predicting structural zeros, with higher temperatures associated with observing excess zeros. Temperature likely plays a dual role with sufficient temperatures needed for hatching to occur and however at extreme temperatures hatching may cease. For example, hybrid catfish *Ictalurus punctatus x I. furcatus* hatch success decreased from 40% at 26.6°C to 32% at 32.2°C (Myers et al. 2020). Furthermore, spawning cessation was negatively correlated with warmer water temperatures in Hudson Bay Estuary Stiped Bass *Morone saxatilis* (Nack et al. 2019).

Although my hatch results only reflect the environmental conditions of early spring and summer spawning, it is not surprising the zero-inflated component indicated temperature positively associated with excess zeros. Hatching success in big-river fishes is influenced by a combination of environmental and biological factors, often closely linked to river dynamics and habitat quality. Water temperature is often considered an important factor as it directly affects embryo development rates and survival (Aegerter and Jalabert 2004; Bobe and Labbé 2010). Discharge patterns are also considered important for many fishes that spawn in floodplain areas or depend on specific riverbed substrates for egg-laying (e.g., Smallmouth Buffalo, Becker 1983). For other pelagic fishes that do not rely on substrates, seasonal floods may provide access to important nursery habitats with ample food and shelter (Zeug and Winemiller 2008; Valdez et al. 2019). Water quality including turbidity and pollutant levels can influence hatching success, as suspended sediments can suffocate eggs (Auld and Schubel 1978) and pollutants can disrupt gonad development (Woodling et al. 2006) and egg development (Jezierska et al. 2009). Predation and competition within spawning habitats may also affect successful hatching (Greeley 1932; Krueger et al. 2014; Lackmann et al. 2024) and may be one factor to consider as important for possible winter spawning by buffalofishes.

My results further indicate that bet-hedging, common among periodic strategists, plays an important role in buffalofishes reproductive success. For instance, episodic recruitment (i.e., numerous missing age classes) has been documented in Canada populations of Bigmouth Buffalo (Winemiller 2005; Olden and Kennard 2010; Lackmann et al. 2022b). Female buffalofishes likely exhibit a bet-hedging by spreading their reproductive efforts across the protracted spawning. This strategy minimizes the risk of complete reproductive failure by distributing risk over multiple spawning attempts, as high mortality in early life stages can strongly affect recruitment and therefore year-class strength (Winemiller 2005; Olden and Kennard 2010). Moreover, the long lifespans and delayed maturity of buffalofishes enable them to allocate reproductive effort across multiple years, thus sustaining population stability despite environmental variability (Winemiller 2005; Olden and Kennard 2010).

My histology results indicate buffalofishes are spawning capable and actively spawning during winter and spring, indicative of a protracted season. This observed protracted spawning season aligns with periodic life-history traits, including longevity and variable recruitment (Winemiller 2005). Periodic life-history strategists (e.g., Paddlefish, Alligator Gar) typically have high fecundities, small eggs, and no parental care (Winemiller 2005; Winemiller et al. 2024). Studies in lowland North Rhine-Westphalia, Germany, found spawning success of periodic strategists was positively affected by high magnitude and high frequency of flows (Mignien and Stoll 2024).

The age structure of lower Red River buffalofishes indicates a predominance of younger age classes and extended longevity of fewer individuals. Bigmouth Buffalo in northern populations (i.e., Minnesota and Canada) have been repeatedly sampled over 100 years old and mature at five years old (Lackmann et al. 2019, 2022b, 2024) and have displayed centenarian longevity in introduced populations (Lackmann et al. 2023). Smallmouth Buffalo age or length at maturity used scales as the ageing structure, with maximum reported ages ranging from 11-18, when reported (Martin et al. 1964; Walburg and Nelson 1966; Padilla 1972; Nelson 1974).

I found buffalofishes spawned over a protracted period from around October through spring but successfully hatching during the duration of these spawns is unclear. Unfortunately, I did not sample buffalofishes until May and the earliest indicated hatch dates were in March. I am unable to say if buffalofishes successfully hatched over the earlier spring and winter seasons as I assumed the general spawning timeline provided in the literature (Johnson 1963; Lackmann et al. 2022b, 2024) which was not correct. Consequently, my analysis of hatch success is limited to the relatively warmer water period.

My zero model for buffalofishes, despite the small sample size, provides insight into the environmental factors influencing hatch. The positive relationship between precipitation and hatch probability may indicate a larger trend of hatching on the ascending limb of a flood pulse (Röpke et al. 2024). Interestingly, my hatch results complement the results from my Black Buffalo and Smallmouth Buffalo POF models where spawning probability had a positive relationship with the cv of mean daily scaled discharge. Moreover, floodplain habitats may be available to fry if spawning and hatching occurs on the rising limb (Röpke et al. 2024) and early spawned juveniles may better use the freshly inundated habitats (Ross et al. 1985).

Some of my results indicate that concerns expressed about persistence of non-game species over time may also be warranted for some species of buffalofishes. My data indicate there is a discrepancy between the reproductive effort the species are putting forth versus the representation of successful juveniles hatched. This is true of all species, but moreso for Black Buffalo and Bigmouth Buffalo where juveniles were only detected during 6 sample weeks, where Smallmouth Buffalo were detection in nine weeks. One reason this may be the case is simple related to gear detection probability. The probability of detection juvenile buffalo species may be low. Buffalofishes, known for their importance in both commercial and recreational fisheries, often inhabit large, turbid rivers and lakes where visibility and accessibility pose challenges for effective sampling. We have limited information on the ecology of juvenile buffalofishes; however, movements among habitats may make detection particularly difficult as they develop (Cooke et al. 2016) and as seasons change (Verhelst et al. 2023). Juvenile Bigmouth Buffalo and Smallmouth Buffalo are estimated to have lower than average detection probability in the Red River catchment while Bigmouth Buffalo juveniles had relatively low occupancy estimates compared to Smallmouth Buffalos (Ramsey et al. 2023). During the warm-water period, juvenile Bigmouth Buffalo and Smallmouth Buffalo shared similar habitat affinities except had opposite relationships with slope (positive for Bigmouth Buffalo) (Ramsey et al. 2023). It is unknown how habitat during other seasons may affect our sampling ability. Human-induced factors may also affect my observations.

Disproportionate effects of commercial fishing could also affect the successful hatch of juvenile buffalos. We do not have population estimates of these species, but it appears based on counts that Smallmouth Buffalo is most common followed by the other two species. No evaluation has been done that I am aware to evaluate how take relates to species and thus, how many fish are contributing to the reproductive output by these species. It may also be possible that the spawning season has become protracted to counteract anthropogenic effects (Wright and Trippel 2009; Wedekind and Küng 2010). Many species of fishes demonstrate some plasticity in reproductive timing. One example is Smallmouth Bass, where repeated spawning events occurs in response to higher flow events (Graham and Orth 1986). Likewise, Blue Sucker appears to adjust their spawning movements into tributaries based on flow and temperature conditions (Dyer and Brewer 2020). Although there are scant observations that some large river fishes can spawn during autumn such as Atlantic Sturgeon A*cipenser oxyrinchus oxyrinchus* (Balazik et al. 2012), there are very few freshwater fishes documented to spawn from autumn through spring. I acknowledge, however, that wintertime sampling is not common in fisheries research or management leading to holes in our understanding of many fishes.

Having an extensive spawning period may allow different portions of the population to take advantage of different factors that affect growth and survival. I observed spawning capable Black Buffalo both in October 2023 and in March 2024 from my histology analysis. This plasticity in timing reflects their ability to adjust spawning activities to prevailing environmental conditions, where colder conditions in early spawning periods could elevate mortality risks for larva (Michie et al. 2020) but also serve as a buffer against adverse conditions later in the season including predation (Litvak and Leggett 1992) , thus aligning with the bet-hedging strategy observed in other periodic strategists. Riverine periodic strategists may spawn early in the spring to allow offspring to exploit the rise in productivity following flood plain inundation (Winemiller and Rose 1992; Scharbert and Borcherding 2013). This pattern is consistent with the flood pulse concept, that periodic flooding events create optimal conditions for spawning and juvenile growth by increasing nutrient availability and habitat complexity (Junk et al. 1989; Poff et al. 1997; Junk and Wantzen 2004). Alternatively, winter spawning may reduce competition with other juvenile fishes for resources and also allow for reduced predation risk (Miranda and Hubbard 1994). Spawning over periods with different risk tradeoffs may be a strategy used to maximize reproductive success. Bet hedging strategies can provide risky (e.g., pelagic broadcast spawning minnows, (Worthington et al. 2018), especially for shorter lived fishes but the risk assessment differs for species that are long lived. Extreme longevity and late maturity allow individuals to allocate reproductive effort across many years while the adult persists (Winemiller 2005; Olden and Kennard 2010; Ferguson et al. 2014).

Future investigations of buffalofishes reproductive biology would benefit from examination of the male reproductive biology and other populations exhibiting extreme longevity. Knowledge of male gonad development will further increase the spatial and temporal resolution of spawning phenology of buffalo populations and its complementary role to female development. Using histological methods on testes and comparing spermatogenesis development over time, the identification of group-synchrony may be elucidated. Additional buffalofishes populations, such as those found in Minnesota, would also be helpful for evaluating female ovarian development through time where extreme longevity and episodic recruitment has been observed (Lackmann et al. 2022b).

The importance of discharge for spawning cues and age-0 growth is well documented for many fishes but are affected by human landscape changes. Discharge and temperature are important spawning cues for Blue Sucker populations in the Colorado River, Texas, where there may be potential mismatches in discharge and temperature regimes that limit spawning (Acre et al. 2023). Similarly, in the upper Missouri River, Blue Suckers rely on natural trends in discharge and water temperature to cue their spawning movements, with discharge being the most influential factor (Tornabene et al. 2020). Regulated rivers often have altered temperature regimes. In the upper Yangtze River in China, the construction of two dams and subsequent alteration of the temperature regime downstream was linked to two outcomes in *Coreius heterodon*. One, the spawning threshold temperature arrived late coinciding with a 66% reduction in spawning biomass and two, the mismatch in timing between critical spawning temperature threshold (i.e., lowest temperature to initiate spawning) and accumulated temperature threshold (i.e., minimum temperature accumulation for gonad maturation) inhibits spawning (Li et al. 2021). Catostomids typically spawn early in the year (e.g., Apalachicola Redhorse *Moxostoma poecilurum* and Spotted Sucker *Minytrema melanops*) (Grabowski et al. 2012). It is unclear if the fish I observed spawning during the winter is a natural phenomenon or related to the human-induced changes across the catchment. Flow and temperature patterns have changed substantially due to several dams, some of which generate power and do not have regulated releases. Moreover, it appears the fish assemblage has become more homogenous over time (Mollenhauer et al. 2022).

Using GSI as an indicator of spawning capable fishes may be possible for buffalofishes, though, GSI does not necessarily indicate successful spawning. For example, mean GSI of mature oocytes (stage six) in Bigmouth Buffalo had a mean of ~10, corresponding to fish collected in March, April, and May of 2024. The patterns of oocyte development in buffalofishes with one cohort of oocytes developing through time may allow managers to determine the spawning capable period of the population if collecting GSI over time. GSI has been found useful for documenting spawning capable periods in several other warmwater fishes such as Largemouth Bass *Micropterus salmoides* (Brown et al. 2019) and Suckermouth Minnow *Phenacobius mirabilis* (Brewer et al. 2008). Additionally, GSI could be used to identify mature individuals and the peak spring portion of the spawning season. For example, Smallmouth Buffalo and Bigmouth Buffalo both had oocyte stages five and six had significantly different mean GSIs from the other developmental stages whereas only stage six in Black Buffalo was significantly different from the rest. However, I found GSI trends over time were less helpful in identifying reproductive trends (but maybe select peaks) and individuals with atretic oocytes could still have relatively high GSI values. Thus, use of GSI for individual fish could be compared to the values reported here to provide some insight to individuals nearing spawning but GSI would lack the resolution to identify problems that can underlie failed spawning attempts. This is typically in agreement with others who have found GSI to be helpful with identifying stage of batch spawning fishes (Brewer et al. 2008; Flores et al. 2015, 2019).

Interestingly, my results on both spawning and hatching in buffalofishes is not particularly useful to developing current management strategies but does indicate cause for concern related to successful hatching, especially for Black Buffalo. If these species had only spawned during a small window, there may be options for seasonal closures that align with those key periods as seen for other species. For example, seasonal fishery closures have been effective at protecting large spawning aggregations (Grüss et al. 2014). Similarly, multi-species fishery closures may only benefit a few species if the closure does not align with active spawning among species (Samy-Kamal et al. 2015). Of course, Smallmouth Buffalo appears to be common in the basin and there would be no reason for a seasonal closure. However, it is unclear why the other two buffalofishes do not appear to be doing as well. It may be that take is higher on those two species and possibly associated with unintentional, disproportionate take by commercial fishers. Tracking adults of the three species over time would provide important insight to how they use the river both spatially and temporally. The disappearance of juvenile Bigmouth Buffalo after May 2023 combined with the poor recruitment observed is concerning (see Chapter 2). Bigmouth Buffalo are perceived to be declining throughout their range. For example, in Rice Lake, Minnesota age-0 Bigmouth Buffalo were detected in June and July of 2022 and 2023 and sparing after with the decline attributed to predation (Lackmann et al. 2024). With the relatively large spatial extent of my study area (i.e., ~600-km of mainstem river), attributing the lack of age-0 Bigmouth Buffalo detections to simply predation is unlikely. Lackmann et al (2022) found Bigmouth Buffalo from the Qu'Appelle system lived up to 125 years old, but there were only 13 observed year classes indicating very sparse episodic recruitment. Significant declines in catch have also been observed for Smallmouth Buffalo in the upper Mississippi River system (Maxon et al. 2024), but long-term data sets do not exist to allow this comparison for Red River buffalofishes. The danger with these species is it is relatively easy to capture adults but monitoring successful hatches is difficult leaving temporal gaps in our perceptions of whether the species is struggling in many basins.

To my knowledge, my study represents the first attempt to describe hatch phenology, female spawning chronology and explore the relationships between developmental stage, reproductive phase, and GSI in sympatric buffalofishes. Previous to my study, spawning was presumed to be occur concurrently with the spring flood pulse (Johnson 1963; Lackmann et al. 2019, 2022b). My results clearly show they spawn earlier and longer than previously documented but raise concerns about the stability of Bigmouth Buffalo and Black Buffalo given the low catch and limited successful hatch dates. Additional efforts to examine successful hatch during the cold-water season would be beneficial to either alleviating or elevating this concern.

Table 1. Tissue processing schedule used to dehydrate, clear, and infiltrate paraffin wax in buffalo using an Epredia STP120 Tissue Processor. Times used are for 50-60 samples. Tissues were stored in 70% ethanol after fixation.

Step	Reagent	Time
1	80% ethanol	1hr
2	85% ethanol	1hr
3	85% ethanol	1hr
4	95% ethanol	1hr
5	95% ethanol	1hr
6	100% ethanol	1hr
7	100% ethanol	1hr
8	Xylene	1hr
9	Xylene	1hr
10	Paraffin	1hr
11	Paraffin	1hr
12	Paraffin	1hr

Table 2. Stain schedule used to stain and counter stain adult female buffalofishes ovarian histology samples with hematoxylin and eosin, modified from Mumford (2004). Ovarian samples were collected from female Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo continuously from April 2023 through May 2024 in the lower Red River catchment.

Step	Reagent	Time (minutes)
1	Xylene	3:00
2	Xylene	3:00
3	Xylene	3:00
4	100% ethanol	2:00
5	100% ethanol	2:00
6	70% ethanol	2:00
7	Distilled water	2:00
8	Hematoxylin	4:00
9	Tap water rinse	10:00
10	Scott's water	2:00
11	70% ethanol	2:00
12	Eosin	2:00
13	70% ethanol	2:00
14	95% ethanol	2:00
15	95% ethanol	2:00
16	100% ethanol	2:00
17	100% ethanol	3:00

18	Xylene	3:00
19	Xylene	3:00

Table 3. Environmental data sources used to collect discharge, air temperature, and precipitation data for each of the grouped hatch locations (site, where 19 sample locations were collated into five sites based on shared environmental data). Discharge data were collected from USGS streamgages at or adjacent to each site. Air temperature and precipitation data were collected from either (Source) the Oklahoma mesonet or National Oceanic and Atmospheric Administration (NOAA). Location includes the nearest city to the site and station indications the name.

Site	USGS Streamgage	Location	Station	Source
Arthur City	07335500	Valliant, OK	VALL	Oklahoma mesonet
Muddy Boggy	07335300	Antlers, OK	ANT2	Oklahoma mesonet
Spanish Bluffs	07337000	Idabel, OK	IDAB	Oklahoma mesonet
Spring Bank	07344370	Texarkana Webb Airfield, AR	USW00013977	NOAA
Texarkana	07337000	Atlanta, TX	USC00410408	NOAA

Table 4. Covariates, data sources, hypothesis, and references used to develop my hatch probability models (i.e., zero inflated model) for three sympatric Buffalofishes in the lower Red River catchment.

Covariates	Data source	Hypotheses
Mean air temperature (°C) 1-week	NOAA OK Mesonet	Temperature is associated with spawning; probability of a successful hatch increase with temperature [1,2]
Scaled discharge (m/s) 1-week	USGS streamgage	Buffalo spawning is believed to be timed with spring floods [4,5,9]
Number of days post-peak spring flood	Days since maximum discharge in that year	Successful hatches occur during or following flood events during stability [6,7,8]
Rate of change scaled discharge 14-days prior to hatch week	USGS streamgage	Fluctuating water levels may influence hatch success e.g., extended floodplain connection [9,10]
Calendar day (days since Jan. 1)	Days since Jan 1 in that calendar year	Capture photoperiod and timing of hatch. Timing of a hatch within a spawning season may affect hatches [4,8]

[1] Fraser et al. 2019 [2] Clark Barkalow et al. 2020 [4] Peterson and Jennings 2007 [5] Bestgen 2016 [6] de Vlaming 1972 [7] Villamizar et al. 2009 [8] Quist and Spiegel 2012 [9] Lackmann et al. 2024 [10] Lackmann et al. 2019.

Table 5. Pearson's correlation matrix for weekly environmental covariates used for analyzing hatch probabilities of buffalofishes from the lower Red River catchment. Collected discharge (Q) measures were scaled to each site's respective drainage area. Included covariates represent mean air temperature (temp), coefficient of variation of mean air temperature (cv_temp), discharge (Q), coefficient of variation of mean Q, rate of change (roc) for preceding 14-days, the number of days post peak annual discharge (pp), week of year, and cumulative precipitation. Correlations were examined after necessary transformations were made.

	temp	cv_temp	Q	cv_Q	roc	pp	wk	rain
temp	1	-0.19	-0.36	-0.26	-0.05	0.75	0.80	-0.31
cv_temp		1	0.30	0.90	0.15	-0.18	-0.23	0.15
Q			1	0.30	0.38	-0.39	-0.38	0.25
cv_Q				1	0.07	-0.28	-0.34	0.13
roc					1	-0.04	-0.04	0.20
pp						1	0.93	-0.34
wk							1	-0.38
rain								1

Table 6. Rankings of all candidate zero-inflated binomial models to relate environmental factors to hatch probability of buffalofishes where Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo were modeled together. Precipitation is the cumulative sum of precipitation one week prior to hatch. CV of Discharge is the coefficient of variation of the one-week mean daily scaled discharge prior to hatch. Discharge is a categorical variable where 1 indicates high values of mean scaled discharge and 0 is absence. Year is a categorical variable with 2021 as the reference year. Temperature is the mean daily air temperature one week prior to hatch. The zero-inflated model was held constant with temperature. Degrees of freedom (df), number of parameters (k), log likelihood (logLik), Akaike's Information Criterion corrected for small sample sizes (AICc), delta AICc (difference in AICc), and model weight (weight) are reported for each model.

Model	Conditional	df	k	logLik	AICc	Delta	Weight
m6	Year + Precipitation	6	4	75.29	163.00	0.00	0.15
m1	Year	5	3	76.47	163.20	0.24	0.13
m5	Year + CV of Discharge	6	4	75.43	163.30	0.27	0.13
m11	Year + CV of Discharge + Precipitation	7	5	74.57	163.70	0.70	0.10
m7	Year + Discharge	6	4	75.85	164.10	1.13	0.08
m2	CV of Discharge	4	2	78.08	164.40	1.37	0.08
m3	Precipitation	4	2	78.19	164.60	1.59	0.07

m12	Year + CV of Discharge + Discharge	7	5	75.17	164.90	1.91	0.06
m8	CV of Discharge + Precipitation	5	3	77.37	165.00	2.04	0.05
m15	Year + CV of Discharge + Precipitation + Discharge	8	6	74.49	165.70	2.71	0.04
m4	Discharge	4	2	78.76	165.70	2.71	0.04
m9	CV of Discharge + Discharge	5	3	77.95	166.20	3.21	0.03
m10	Precipitation + Discharge	5	3	78.08	166.50	3.46	0.03
m14	CV of Discharge + Precipitation + Discharge	6	4	77.35	167.10	4.13	0.02

Table 7. Model estimates for top-ranked zero-inflated binomial model to relate environmental factors to hatch probability of grouped age-0 buffalofishes in the lower Red River catchment. The conditional model retained are fixed effect of year (2021 as reference) and the one-week cumulative precipitation prior to hatch. The zero-inflated component retained one-week mean daily air temperature. Fishes sampled 2021-2023. Estimate, standard error (SE), and 90% confidence intervals are reported.

Model Component	Variable	Estimate	SE	90% CI	Pr(>|z|)
Conditional	(Intercept)	-1.85	0.65	(-2.91, -0.78)	< 0.001
	Year2022	0.02	0.82	(-1.33, 1.36)	0.98
	Year2023	1.11	0.69	(-0.02, 2.25)	0.11
	Precipitation	0.42	0.28	(-0.05, 0.88)	0.14
Zero Inflated	(Intercept)	-1.4	0.9	(-2.88, 0.08)	0.12
	Temperature	3.64	1.26	(1.56, 5.72)	< 0.001

Table 8. Reproductive phase assigned to female buffalofishes used in histological analyses collected from the lower Red River April 2023 through May 2024. Terminology from Brown-Peterson et al. (2011).

Reproductive phase	Macroscopic	Histology
Immature	Clear and threadlike. No visible blood vessels	Only stage one present. Very densely packed. Oocytes small and irregular shapes
Developing	Some distinct blood vessels. Become more orange with development.	Stage twos may be present, often stage 3 or four. No POFs or stages five or six present.
Spawning capable	Mature. Large yellow ovary. Blood vessels distinct. Oocytes visible and distinct.	Stage five is the most advanced and prevalent. Atresia may and early stages of oocyte maturation may be present.
Actively spawning	Mature. Large yellow ovary. Blood vessels distinct. Oocytes visible and distinct.	Stage six oocytes (i.e., mature). Post-ovulatory follicles and atresia may be present. Stage five oocytes likely present.
Regressing	Ovary smaller, some oocytes distinct.	Atresia and post ovulatory follicles may be present, some stage one or two oocytes present. Tissues often open, muscle tissues present.

Regenerating	Ovary small, reduced blood vessels. Often brown in color.	Only stage one or two present. Old atresia or post ovulatory follicles may be present.

Table 9. Stages and brief descriptor and criteria used when quantifying most advanced oocyte present in ovarian samples collected from the lower Red River catchment in 2023 and 2024. Seven stages classification modified from Guzmán et al. (2017) and Fish et al. (2020).

Oocyte Stage	Description
1) Primary growth	Oocytes are small, angular, and darkly stained.
2) Cortical alveolar	Cortical alveoli appear in a white ring along the circumference. Oocyte is larger and rounder.
3) Primary vitellogenesis	Yolk globules appear, forming 2-3 rings within the cytoplasm.
4) Secondary vitellogenesis	Yolk globules fill the cytoplasm inwards toward the nucleus.
5) Tertiary vitellogenesis	Yolk globules completely fill the cytoplasm, vesicles appear at the periphery. Large size.
6) Final maturation	The nucleus begins to migrate, and large yolk globules are present.
7) Post-ovulatory follicles	Collapsed follicular cells following the expulsion of an oocyte.

Table 10. Mean gonadosomatic index (GSI) by oocyte developmental stage for three buffalofishes Black Buffalo (BBF), Bigmouth Buffalo (BMB), and Smallmouth Buffalo (SBF) in the lower Red River. For each species, mean GSI, standard error (SE) and sample size (n) are reported. Stages 1 through 7 represent the stages of the most advanced oocyte present when evaluated using histology. Oocyte stages modified from Guzmán et al. (2017)

Species	Stage	Mean GSI	SE	n
BBF	1	2.58	0.39	23
	2	1.64	0.52	15
	3	1.61	0.18	7
	4	4.63	0.62	11
	5	10.46	0.71	36
	6	11.44	2.11	10
	7	2.78	0.82	16
BMB	1	1.7	0.39	30
	2	1.76	0.41	23
	3	1.6	0.17	17
	4	4.69	0.46	15
	5	9	0.77	37
	6	9.61	1	12
	7	1.49	0.17	14
SBF	1	2.41	1.07	33
	2	1.61	0.28	74
	3	1.73	0.23	20

	4	5.93	0.69	36
	5	10.25	0.71	54
	6	12.97	0.92	41
	7	1.4	0.09	37

Table 11. Variables, data sources, hypotheses, and references for variables used to relate environmental factors to probability of post-ovulatory follicles in three sympatric buffalofishes from the lower Red River catchment. Females were collected year-round beginning in April 2023 and concluding in May 2024.

Variable	Data source	Hypotheses
Mean daily air temperature (°C) 4-week	Oklahoma Mesonet NOAA	Buffalofishes are more likely to spawn as the river warms.[1]
Mean daily scaled discharge (m/s) 4-week	USGS Streamgage	Larger flows will provide better spawning habitat in backwaters.[1,2]
Discharge*air temperature	-	Successful spawning at different times of year results in different environmental conditions for ova and resulting larva. Spawning early or late may be advantageous egg hatching and juvenile development.[3]
Coefficient of variation of Mean daily scaled discharge (m/s) 4-week	-	Variability in discharge could benefit spawning fishes (e.g., habitat availability/cue) [2,3]

Day	Day of year	Capture photoperiod/timing cue(s).[4]
Number of days from annual peak discharge	# of days	Presumed to spawn during the spring flood pulse.[5]
75th percentile of mean scaled discharge 1-week pre-spawn	USGS Streamgage	Finer-scale (1 week) flows influence spawning more once the fish is spawning capable
Coefficient of variation of Mean daily air temperature (°C) 4-week	-	Greater variation may hinder successful spawns.[1,2]

---

[1] King et al., 2015; [2] Scharbert and Borcherding, 2013; [3] Haworth and Bestgen, 2017; [4] de Vlaming, 1972; [5] Johnson 1963; [6] Craven et al., 2010

Table 12. Sample sizes by species by month of collected female Bigmouth Buffalo (Bigmouth), Black Buffalo (Black), and Smallmouth Buffalo (Smallmouth). I sampled 582 female buffalofishes for GSI and histological processing. Fishes were sampled from April 2023 through May 2024 in the lower Red River catchment.

Species	Collection month	Number of females collected
Bigmouth	April 2023	1
Bigmouth	May 2023	16
Bigmouth	June 2023	16
Bigmouth	July 2023	4
Bigmouth	August 2023	16
Bigmouth	September 2023	24
Bigmouth	October 2023	20
Bigmouth	November 2023	16
Bigmouth	December 2023	4
Bigmouth	January 2024	10
Bigmouth	February 2024	10
Bigmouth	March 2024	6
Bigmouth	April 2024	5
Bigmouth	May 2024	3
Black	April 2023	3
	May 2023	14

Species	Collection month	Number of females collected
Black	June 2023	22
Black	July 2023	10
Black	August 2023	6
Black	September 2023	12
Black	October 2023	19
Black	November 2023	4
Black	December 2023	2
Black	January 2024	4
Black	February 2024	12
Black	March 2024	16
Black	April 2024	1
Smallmouth	May 2023	37
Smallmouth	June 2023	20
Smallmouth	July 2023	23
Smallmouth	August 2023	57
Smallmouth	September 2023	28
Smallmouth	October 2023	44
Smallmouth	November 2023	15
Smallmouth	December 2023	9
Smallmouth	January 2024	22
Smallmouth	February 2024	25

Species	Collection month	Number of females collected
Smallmouth	March 2024	24
Smallmouth	May 2024	2

Table 13. Pearson's correlation matrix for environmental covariates used for spawning buffalofishes analysis in the lower Red River catchment. Collected discharge (Q) were scaled to each site’s respective drainage area. Included covariates represent day of year (Julian Day), mean air temperature 4-weeks prior to spawning (Mean Temp), coefficient of variation of temperature (cv temp), mean Q 4-weeks (mean Q), coefficient of variation of Q (cv Q), the 75the percentile of discharge 1-week (Q75), and the number of days to the annual peak discharge (Peak days). Correlations were examined after necessary transformations were made.

Variable	Julian Day	Mean Temp	cv temp	mean Q	cv Q	Q75	Peak days
Julian Day	1	0.59	-0.52	-0.35	-0.09	-0.08	0.85
Mean Temp		1	-0.96	-0.30	-0.31	-0.16	0.22
cv temp			1	0.23	0.18	0.17	-0.13
mean Q				1	0.42	0.42	-0.37
cv Q					1	-0.01	-0.02
Q75						1	-0.19
Peak days							1

Table 14. Initial model (complex) and simplified (final) model used to relate environmental conditions to the presence of observed post-ovulatory follicles from histologically processed adult female Bigmouth Buffalo, Black Buffalo, and Smallmouth Buffalo from the lower Red River. Covariates included represent the day of year (day), coefficient of variation of mean scaled discharge (cvQ), the 75th percentile of 1-week mean scaled discharge (p75), mean daily air temperature (temp), and mean daily scaled discharge (Q). The number of parameters (k), log-likelihood (logLik), Akaike's information criterion correlated for small sample sizes (AICc), the difference in AICc to the global (ΔAICc), and degrees of freedom (df) are reported for each species' model.

Species	Model	Formula	k	logLik	AICc	ΔAICc	df
Bigmouth Buffalo	Complex	day + cvQ + p75 + temp * flow	7	36.67	88.20	0	7
	Final	day + temperature	5	38.43	83.00	5.15	3
Black Buffalo	Complex	day + cvQ + p75 + temp * flow	7	38.86	92.7	4.66	7
	Final	day + temperature + cvQ	5	39.85	88.00	0.00	4
Smallmouth Buffalo	Complex	day + cvQ + p75 + temp * flow	7	96.21	206.80	5.32	7
	Final	day + temp + cvQ	4	96.67	201.50	0.00	4

Table 15. Environmental covariates used for zero-inflated binomial hatch model. Mean and range (in parentheses) of daily discharge (Discharge, 1.00 m3/s), daily air temperature (Temperature, 1.0 °C), and daily rainfall (Precipitation, 1-mm) by site for each sample year (2021-2023). Discharge data were collected from the nearest USGS stream gage to each site. Temperature and precipitation data were collected from the nearest NOAA environmental data station or Oklahoma, mesonet station (Table 13). Data represents environmental conditions from the lower Red River catchment in Oklahoma, Texas, and Arkansas.

Year	Site	Discharge	Temperature	Precipitation
2023	Arthur City	162.56 (31.04 - 731.39)	22.33 (9.44 - 29.55)	24.41 (0.1 - 76.96)
2021	Muddy Boggy	63.34 (1.11 - 390.49)	21.12 (9.43 - 27.53)	21.32 (0.01 - 104.54)
2023	Muddy Boggy	45.16 (0.59 - 213.54)	21.82 (8.78 - 29.32)	22.08 (0.1 - 125.22)
2023	Spanish Bluffs	42.74 (42.74 - 42.74)	24.17 (9.15 - 31.39)	50.62 (0.1 - 206.8)
2022	Spring Bank	420.33 (70.97 - 1409.47)	23.48 (6.95 - 31.26)	33.13 (0.1 - 128.3)
2023	Spring Bank	691.24 (106.65 - 2413.65)	23.55 (8.44 - 31.04)	29.48 (0.1 - 159.8)
2022	Texarkana	346.38 (50.86 - 1188.6)	24.82 (9.19 - 32.73)	42.42 (0.1 - 164)
2023	Texarkana	487.91 (93.59 - 1797.76)	24.81 (12.16 - 31.94)	29.48 (0.1 - 107.9)

Table 16. Estimates (logit) of each covariate included in the final model related to the probability of successful spawning for Bigmouth Buffalo (Bigmouth), Black Buffalo (Black), and Smallmouth Buffalo (Smallmouth) in the lower Red River catchment. The standard error (SE), p-value (p), and 95% confidence intervals are provided for each estimate. Included covariates represent day of year (day), mean daily air temperature (Temperature), and the cv of mean scaled daily discharge (cv of discharge).

Species	Variable	Estimate	Standard Error	p-value	2.5% CI	97.5% CI
Bigmouth Buffalo	Day	-2.58	1.09	0.02	-5.01	-0.78
	Temperature	2.55	0.94	0.01	0.97	4.62
Black Buffalo	Day	-1.03	0.66	0.12	-2.69	0.05
	Temperature	1.86	0.59	0.002	0.84	3.23
	cv of discharge	0.66	0.25	0.01	0.19	1.18
Smallmouth Buffalo	Day	-0.84	0.36	0.02	-1.65	-0.20
	Temperature	1.57	0.34	< 0.0001	0.95	2.28
	cv of discharge	1.00	0.26	< 0.0001	0.51	1.54

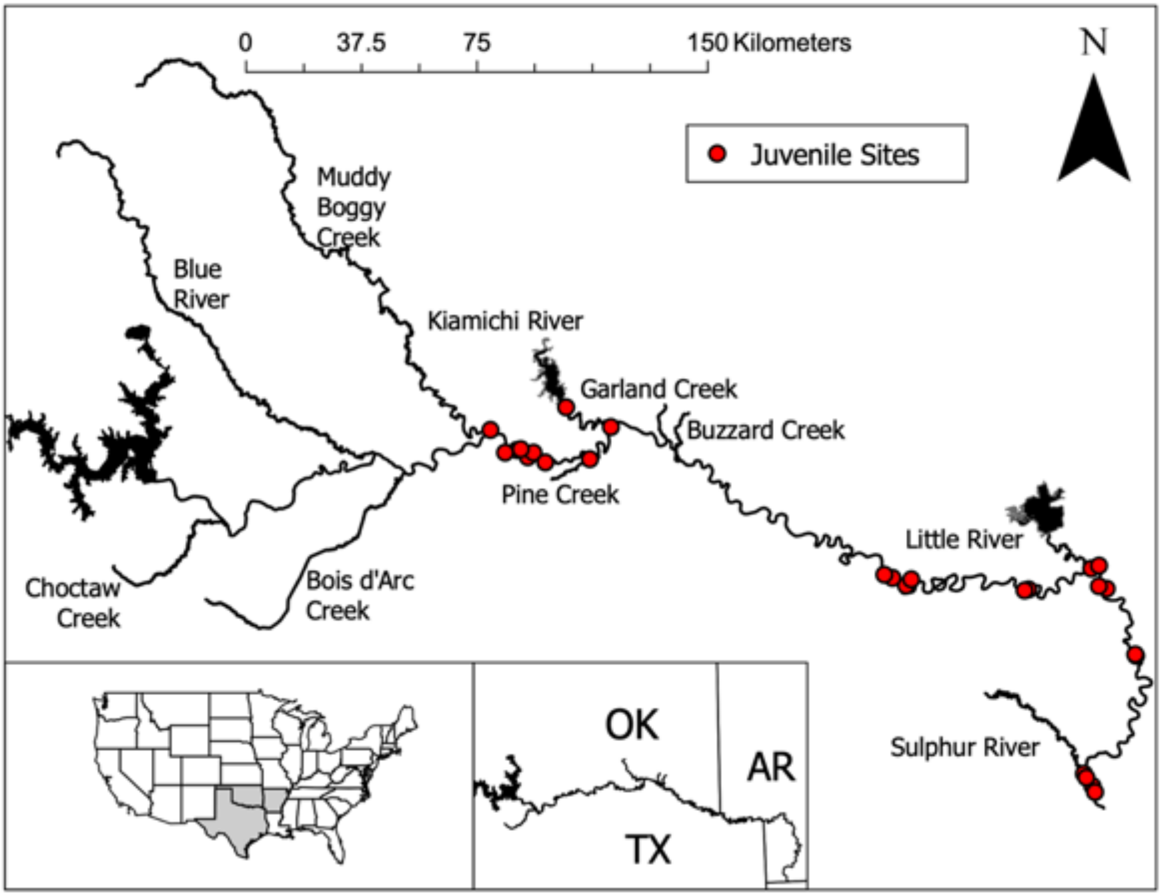

Figure 1. Sample sites in the lower Red River catchment were juvenile buffalofishes were sampled to determine factors related to successful hatch in 2021, 2022, and 2023. Each solid circle represents a sample location. Data were collected in 2021 and 2022 as part of another project that used similar sample gears.

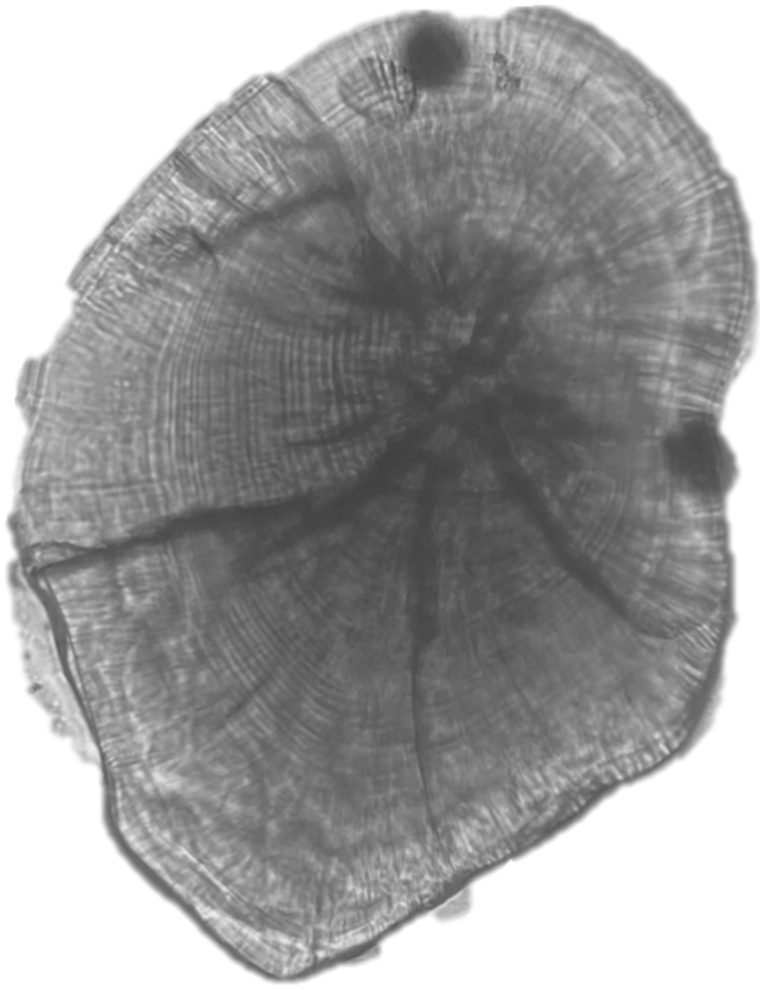

Figure 2. A lapilli otolith from an age-0 Bigmouth Buffalo *Ictiobus cyprinellus* estimated to be 40-days old. Collected May 19th, 2023 in the Red River downstream of the US 271 bridge using a mini-fyke net. Viewed under transmitted light at 40x magnification compound microscope (Nikon Eclipse E400 compound microscope)

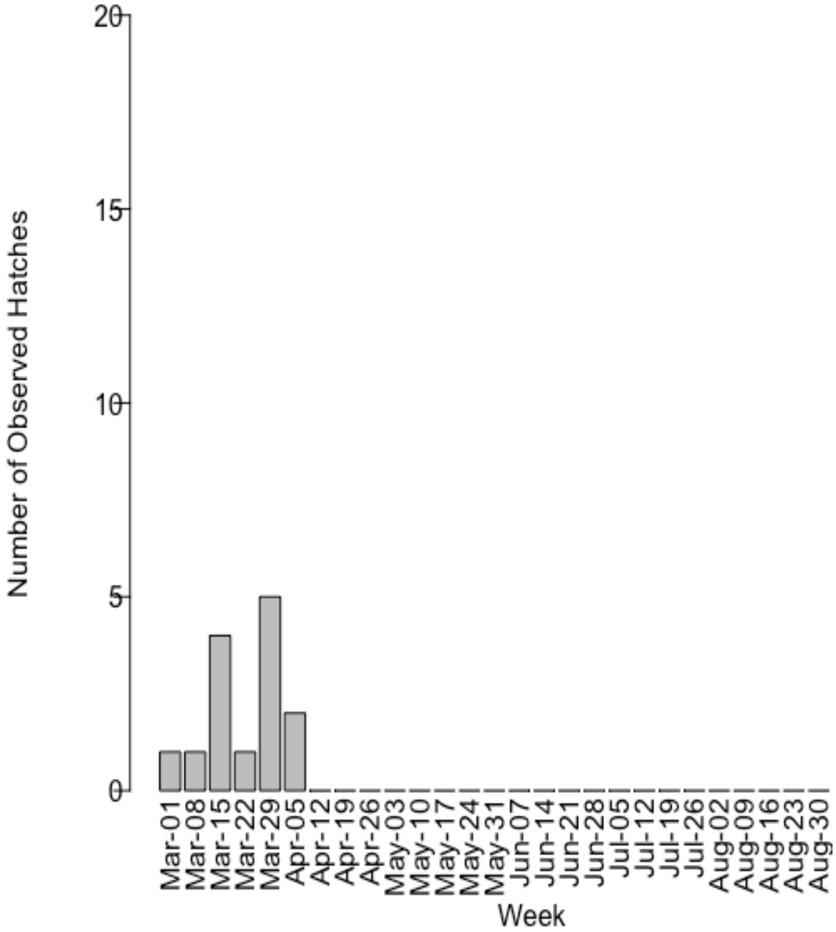

Figure 3. Bigmouth Buffalo hatch dates from age-0 collected in the lower Red River catchment. Sampling occurred from May through August 2023. Hatch dates have been aggregated to weeks.

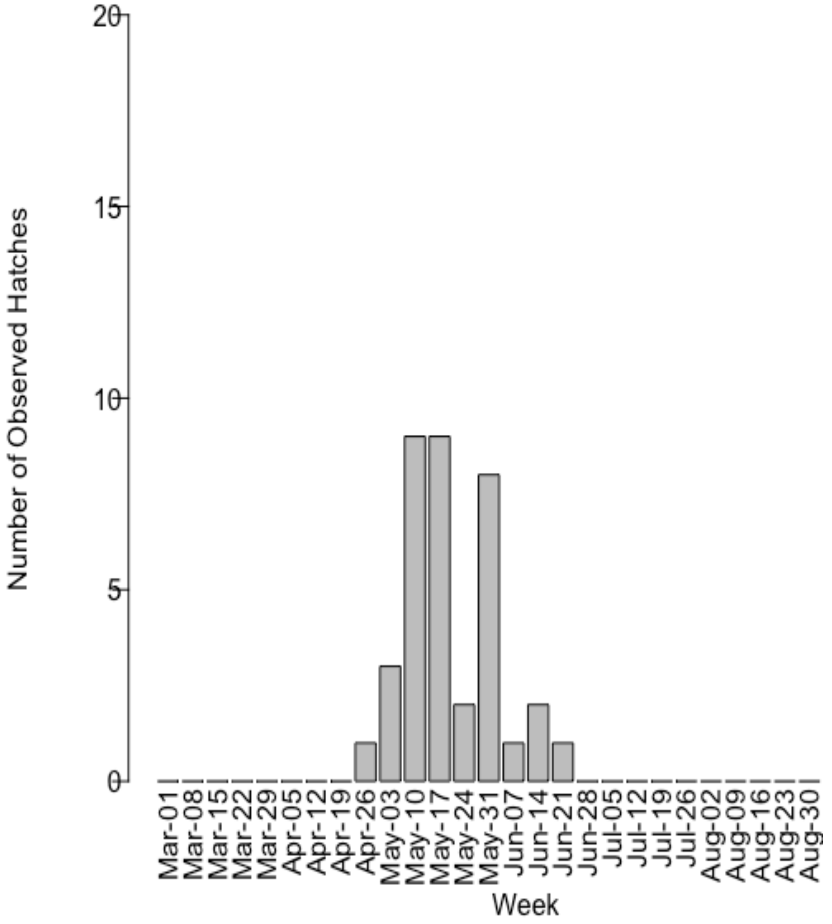

Figure 4. Smallmouth Buffalo hatch dates from age-0 collected in the lower Red River catchment. Sampling occurred from May through August 2023. Hatch dates have been aggregated to weeks.

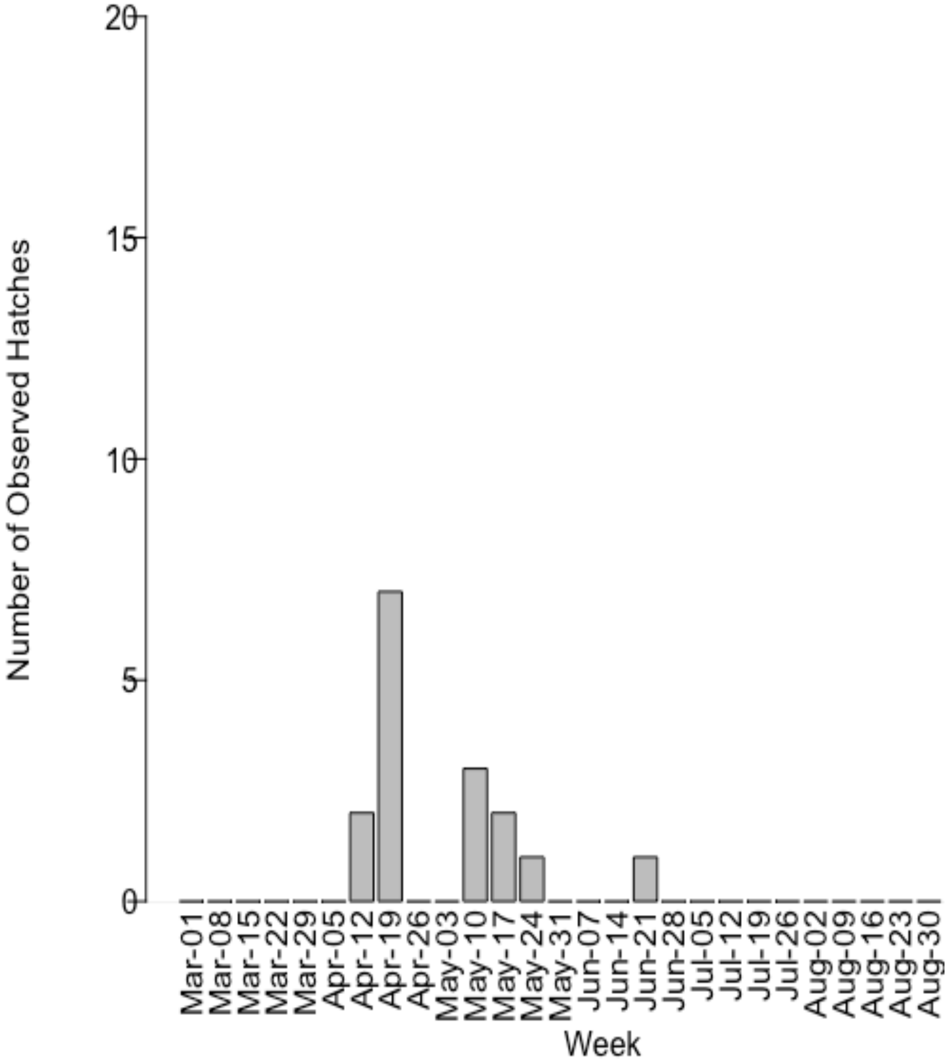

Figure 5. Black Buffalo hatch dates from age-0 collected in the lower Red River catchment. Sampling occurred from May through August 2023. Hatch dates were aggregated to weeks.

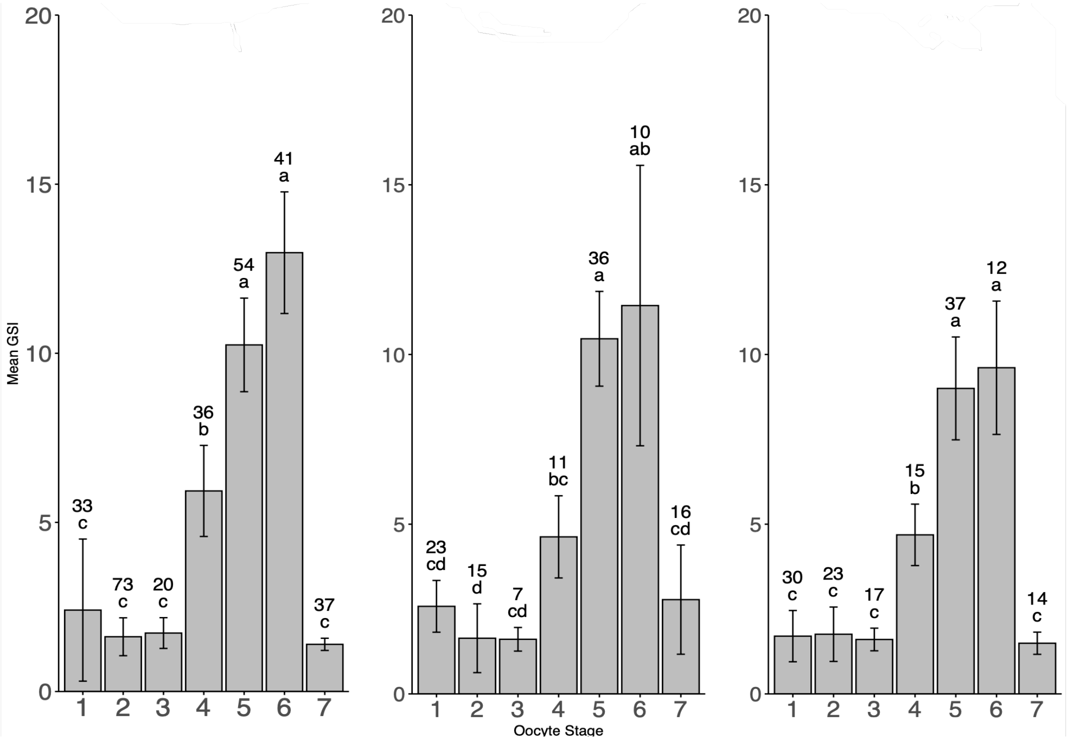

Figure 6. Mean gonadosomatic index values (± 95% confidence intervals) associated with each histological stage present in Smallmouth Buffalo (left), Black Buffalo (center), and Bigmouth Buffalo (right) in the lower Red River catchment. Numbers above each confidence interval represent sample size associated with each oocyte stage. Lower case letters above each bar indicate statistical significance ($p \leq 0.05$) among stages by species where stages with the same letter are not statistically different. Fishes were collected year-round from Aril 2023 through May 2024

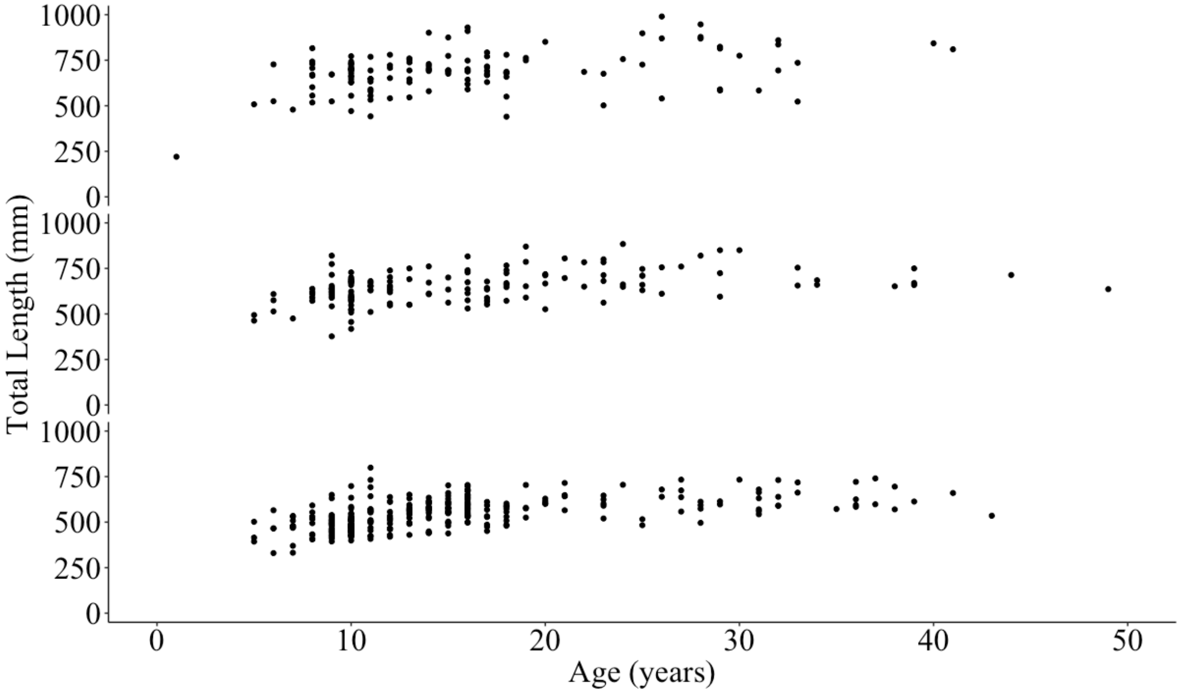

Figure 7. Length (mm) age plot of female Black Buffalo (top), Bigmouth Buffalo (center), and Smallmouth Buffalo (bottom) used in gonadosomatic index and histological analyses. Fishes were collected continuously April 2023 through May 2024 in the lower Red River catchment.

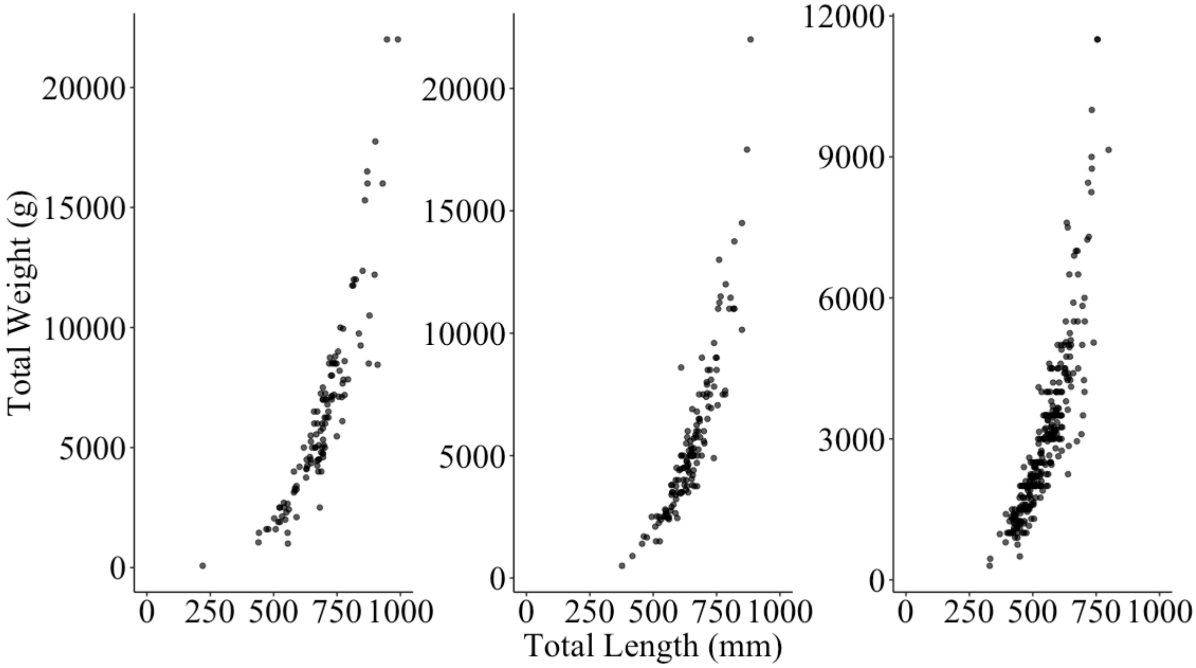

Figure 8. Length (mm) weight (g) plot of female Black Buffalo (left), Bigmouth Buffalo (center), and Smallmouth Buffalo (right) used in gonadosomatic index and histological analyses. Fishes were collected continuously April 2023 through May 2024 in the lower Red River catchment.

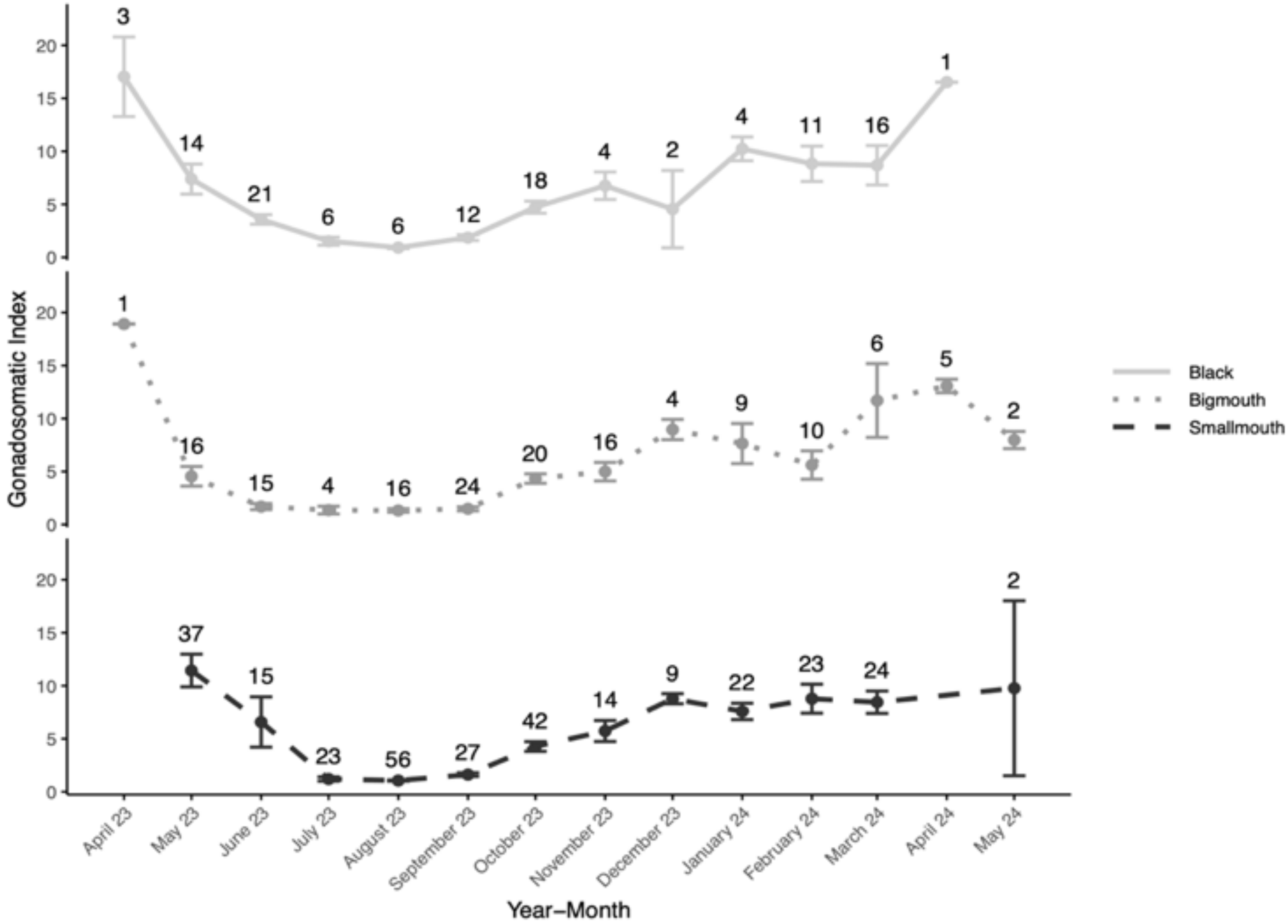

Figure 9. Mean monthly gonadosomatic index values for Black Buffalo (top, solid light gray), Bigmouth Buffalo (middle, dotted dark gray), and Smallmouth Buffalo (bottom, dashed black). The bars represent the standard error, and the numbers above represent that month's respective sample size. Data were collected continuously beginning in April 2023 and concluding in May 2024.

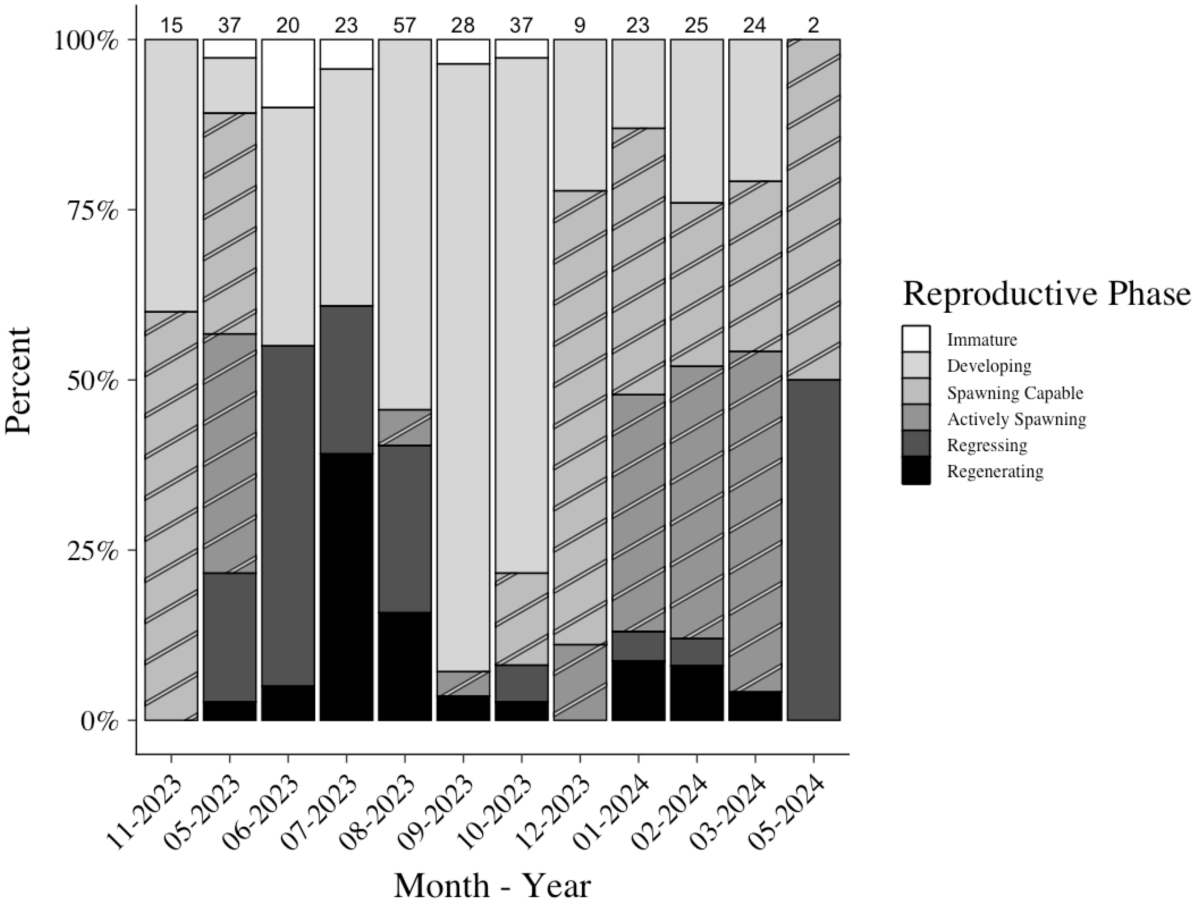

Figure 10. Relative proportions of Smallmouth Buffalo reproductive phase by month as observer from ovarian histology samples. Striped lines are to highlight spawning capable and actively spawning reproductive phases. Numbers above each column indicate the monthly sample size. Fish collected from the lower Red River catchment.

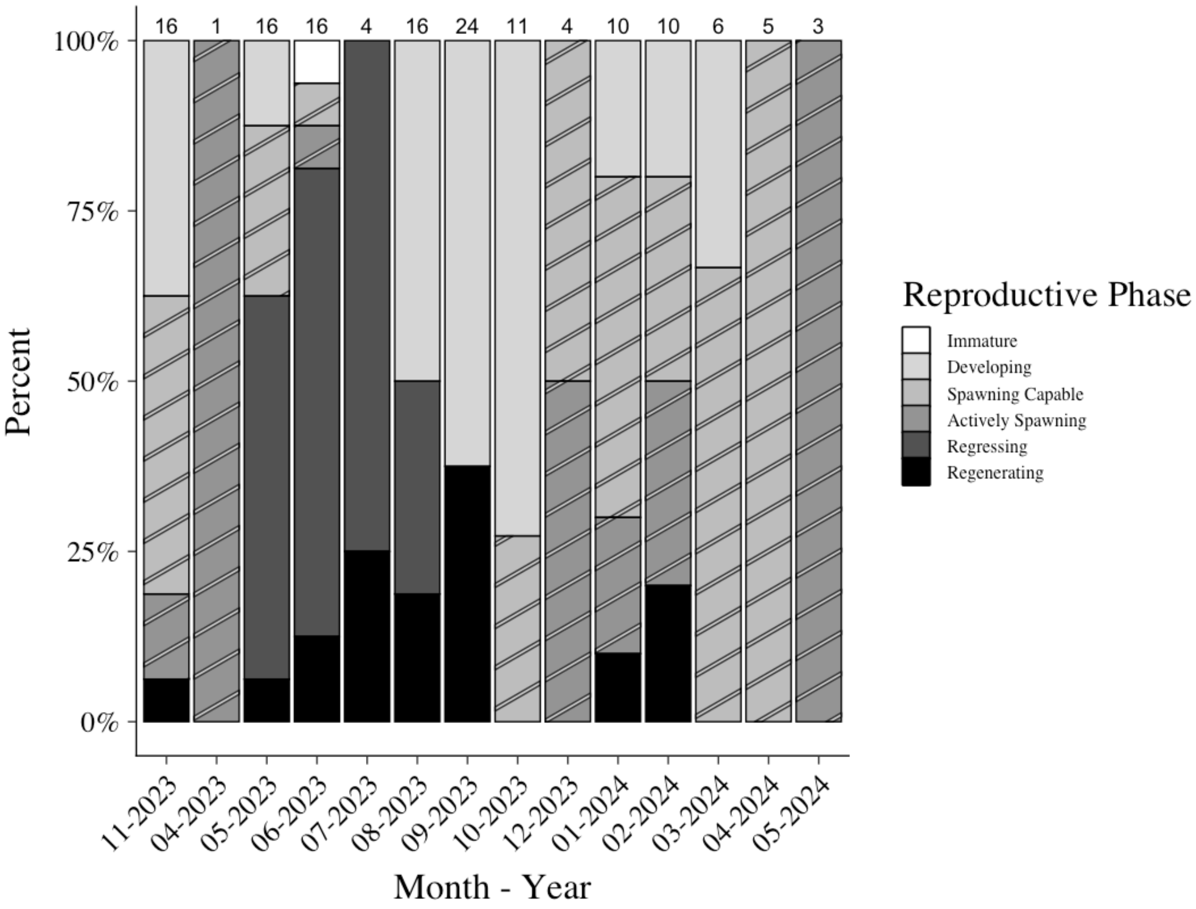

Figure 11. Percentages of Bigmouth Buffalo reproductive phase by month as observer from ovarian histology samples. Striped lines are to highlight spawning capable and actively spawning reproductive phases. Numbers above each column indicate the monthly sample size. Fish collected from the lower Red River catchment.

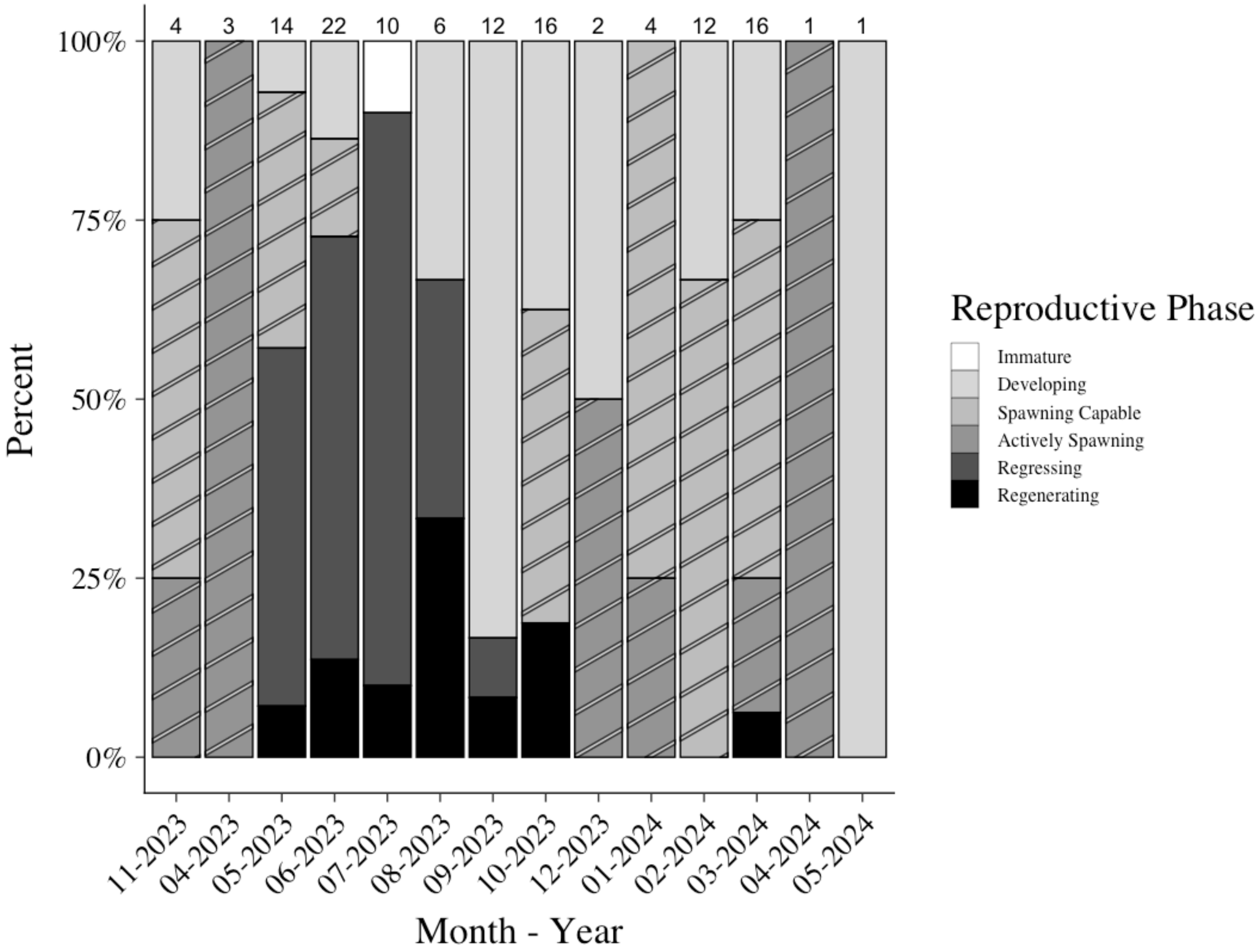

Figure 12. Percentages of Black Buffalo reproductive phase by month as observer from ovarian histology samples. Striped lines are to highlight spawning capable and actively spawning reproductive phases. Numbers above each column indicate the monthly sample size. Fish collected from the lower Red River catchment.

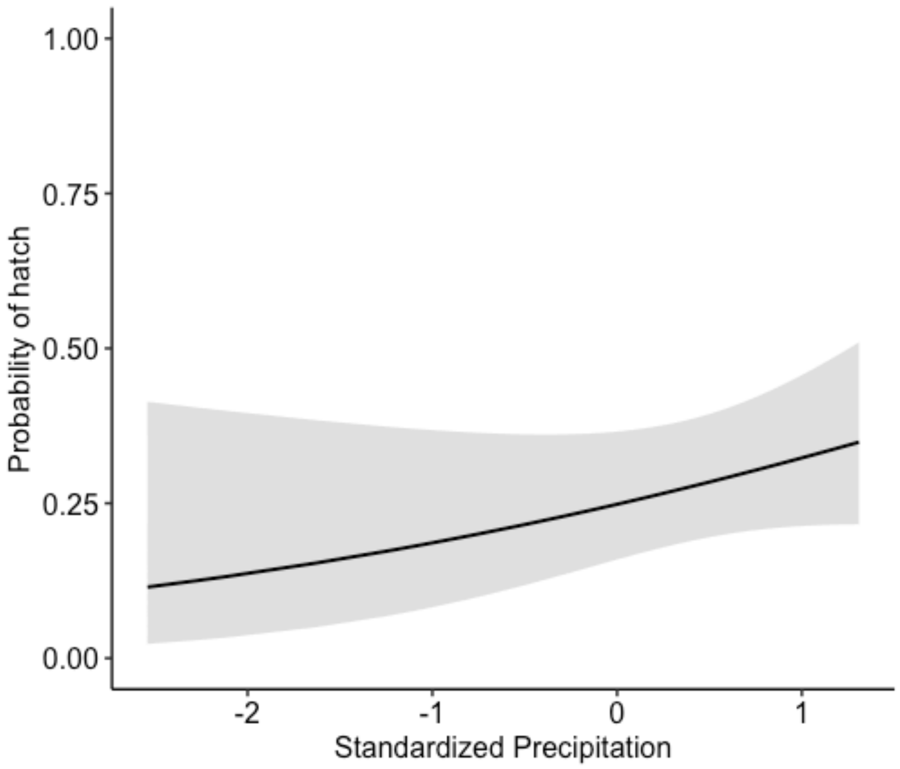

Figure 13. Relationship between hatch probability of standardized sum of precipitation 1-week prior to hatch for grouped buffalofishes from conditional model component of zero-inflated binomial model relating environmental factors to hatch. The gray shaded area represents 90% confidence intervals.

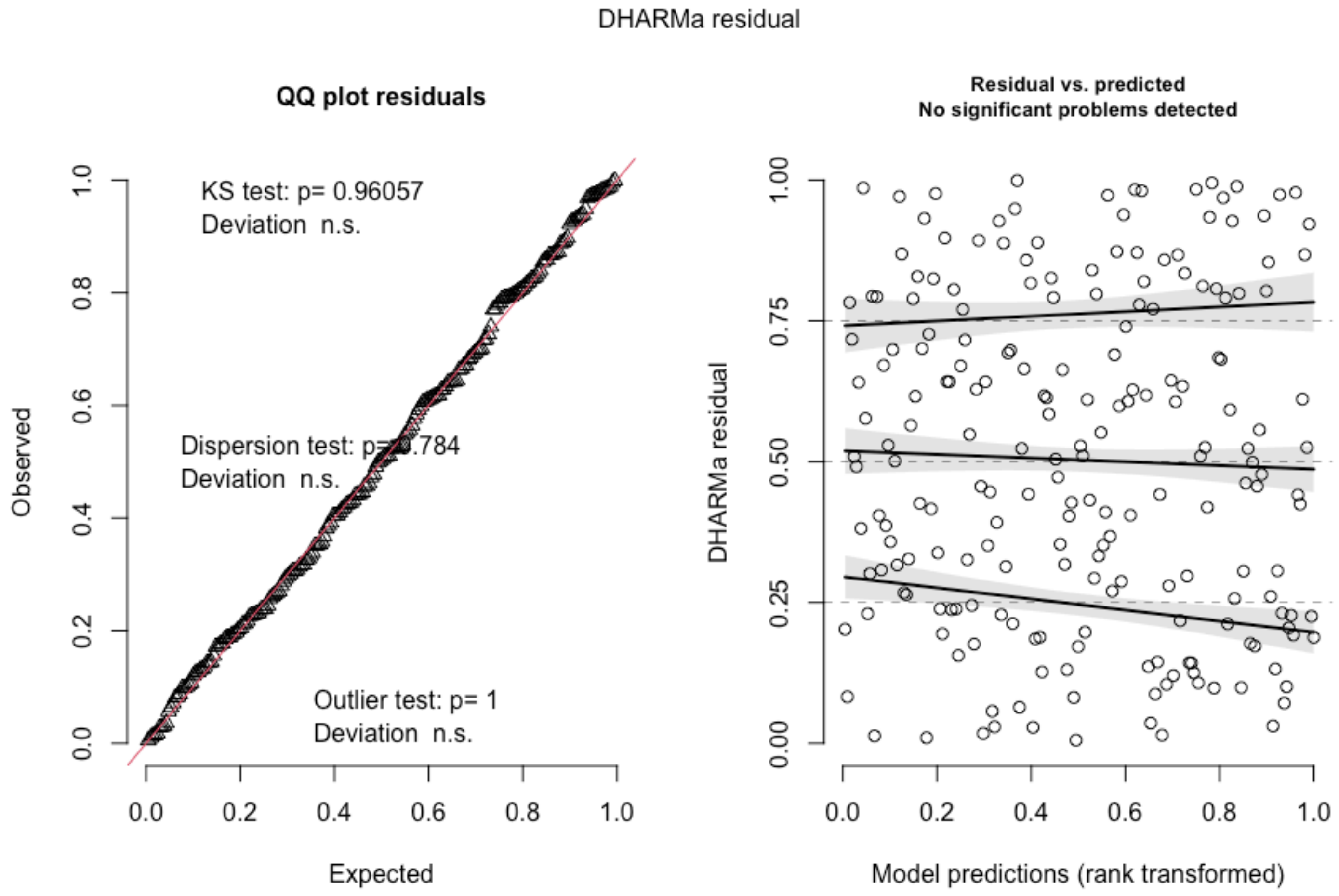

Figure 14. DHARMa residual diagnostic plots of the top ranked zero-inflated binomial model. Plots indicated adequate fit.

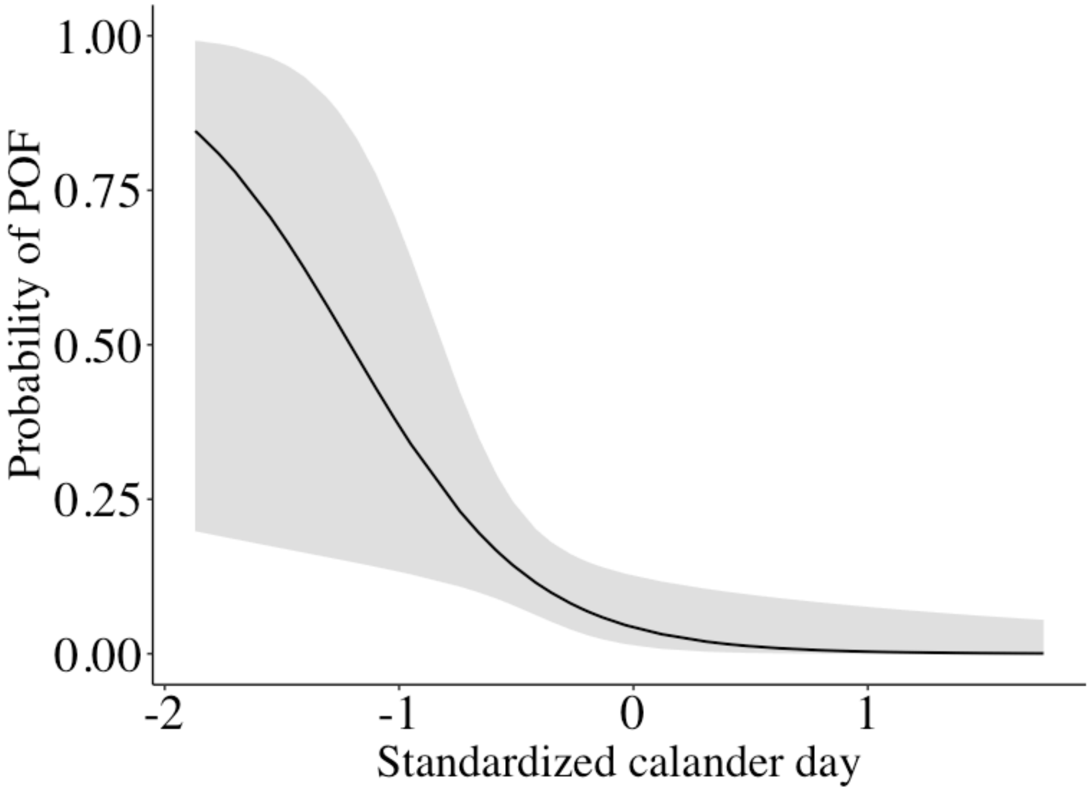

Figure 15. The negative relationship between probability of spawning (P(POF)) and calendar day for Bigmouth Buffalo in the lower Red River catchment. The gray represents the 95% confidence intervals.

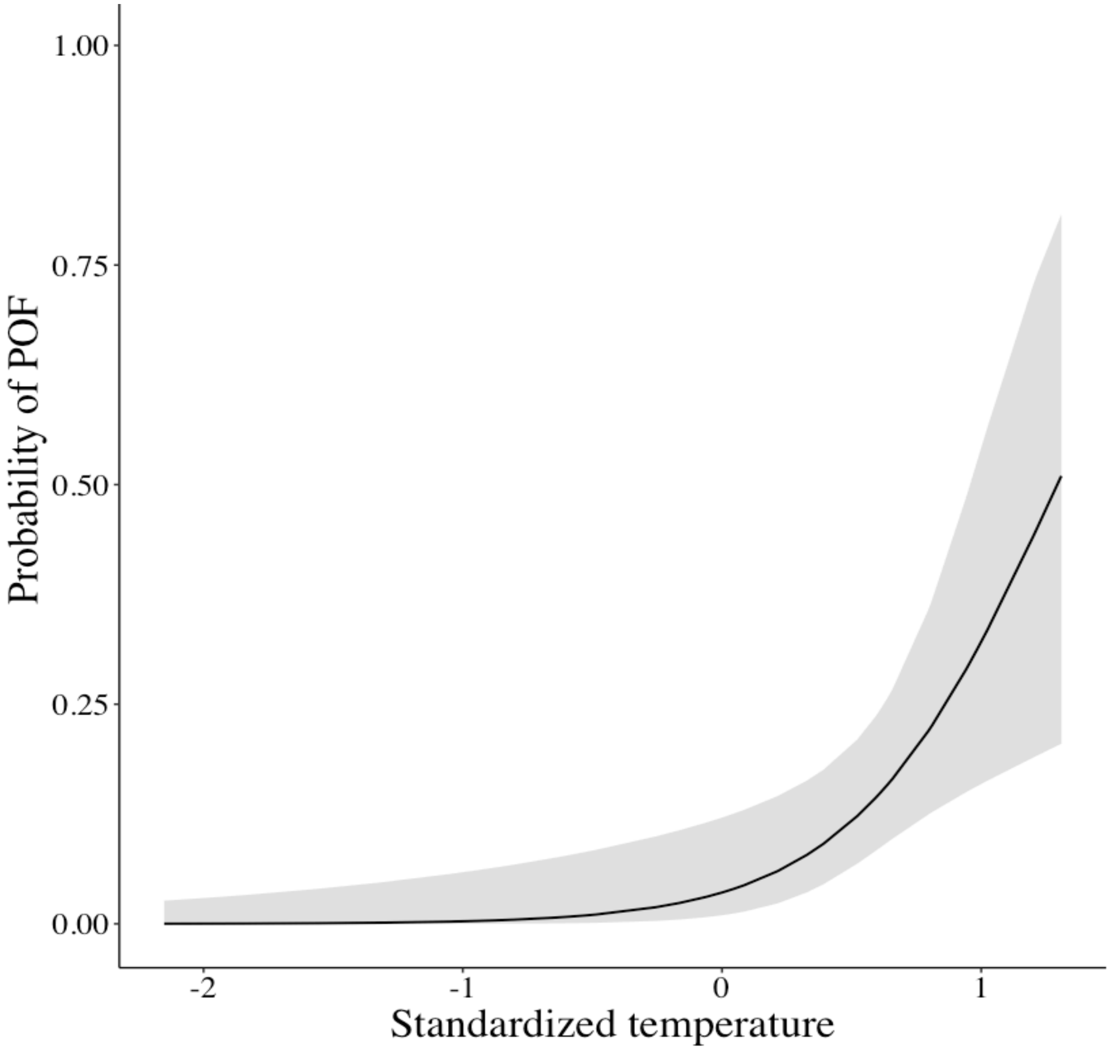

Figure 16. The positive relationship between probability of spawning (P(POF)) and mean daily air temperature for Bigmouth Buffalo in the lower Red River catchment. The gray represents the 95% confidence intervals

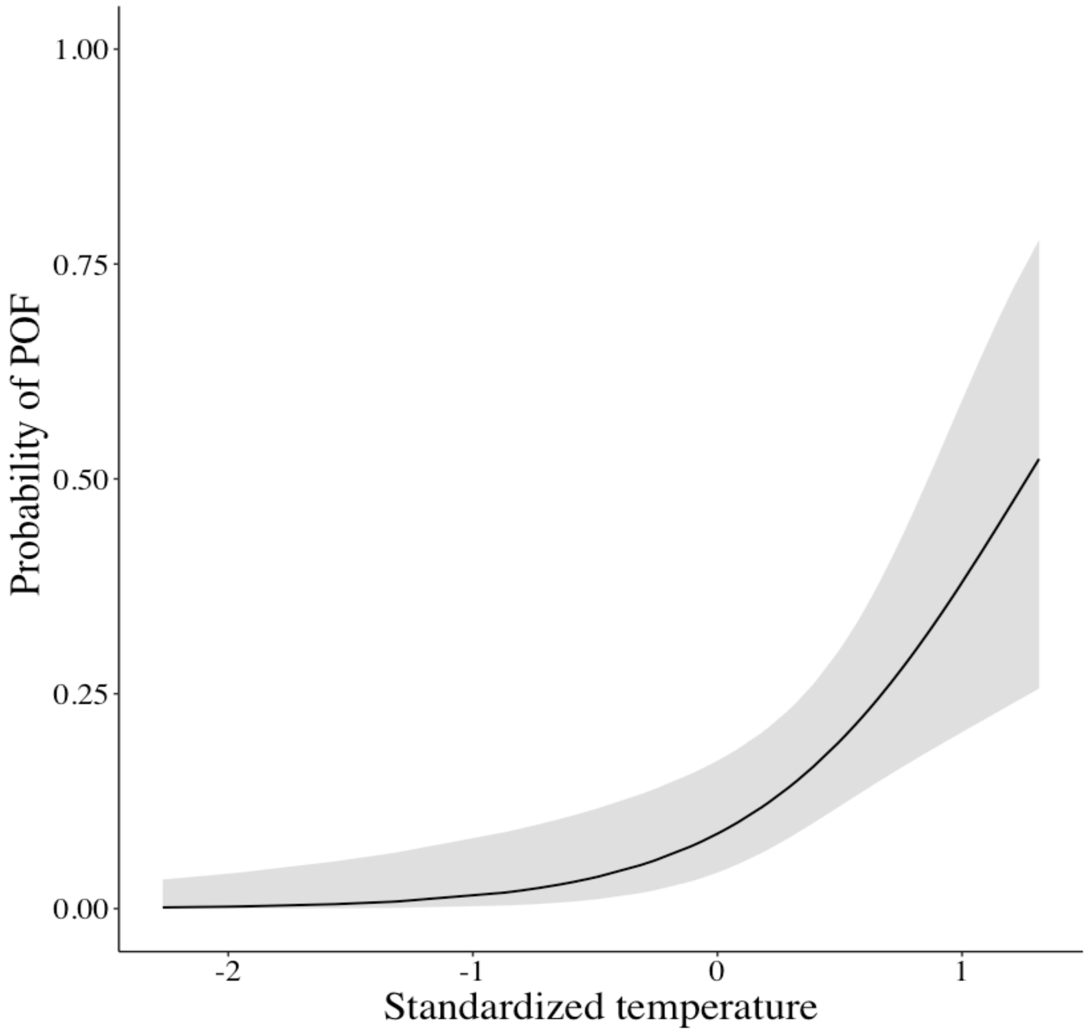

Figure 17. The positive relationship between spawning probability (probability of POF) and standardized mean daily air temperature in Black Buffalo from a binomial logistic regression. The shaded area represents the 95% confidence intervals.

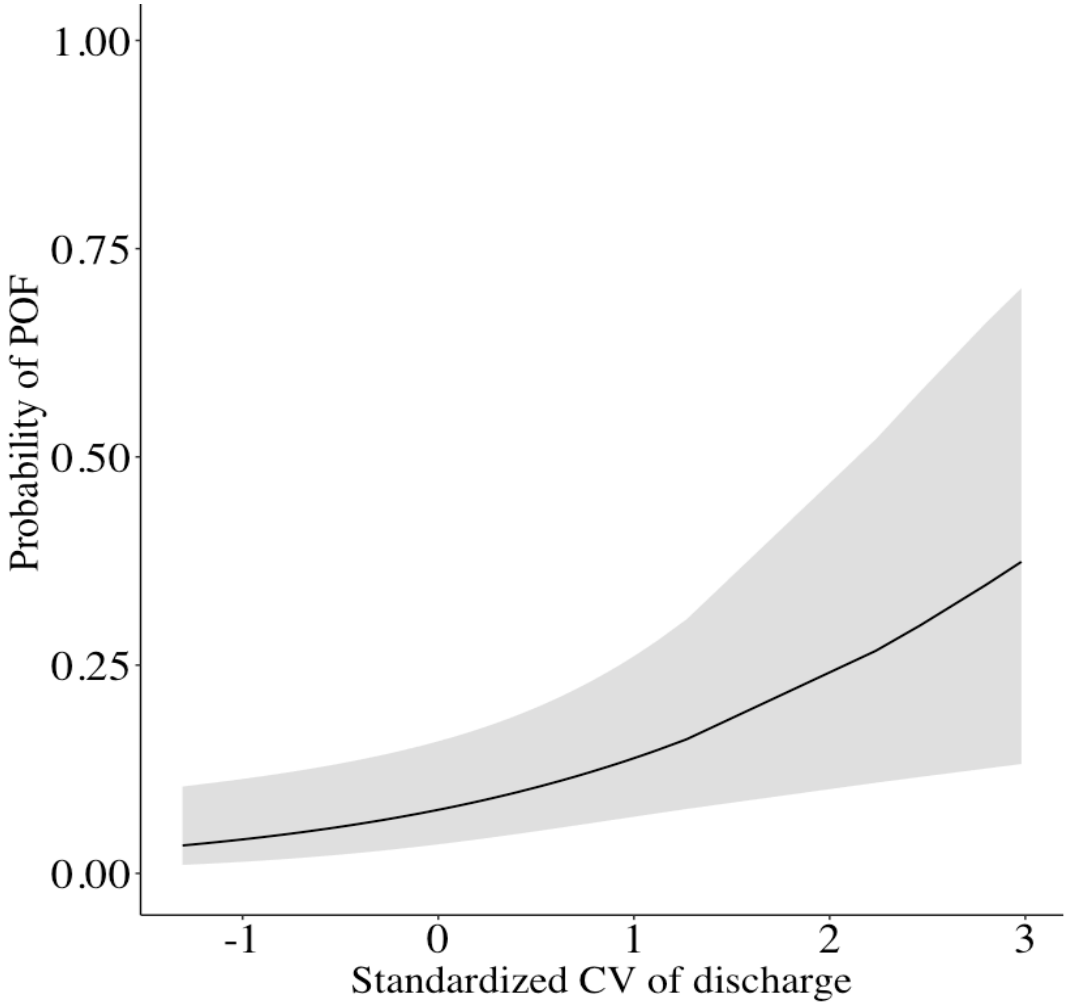

Figure 18. The positive relationship between spawning probability (probability of POF) and standardized coefficient of mean daily scaled discharge in Black Buffalo from a binomial logistic regression. The shaded area represents the 95% confidence interval

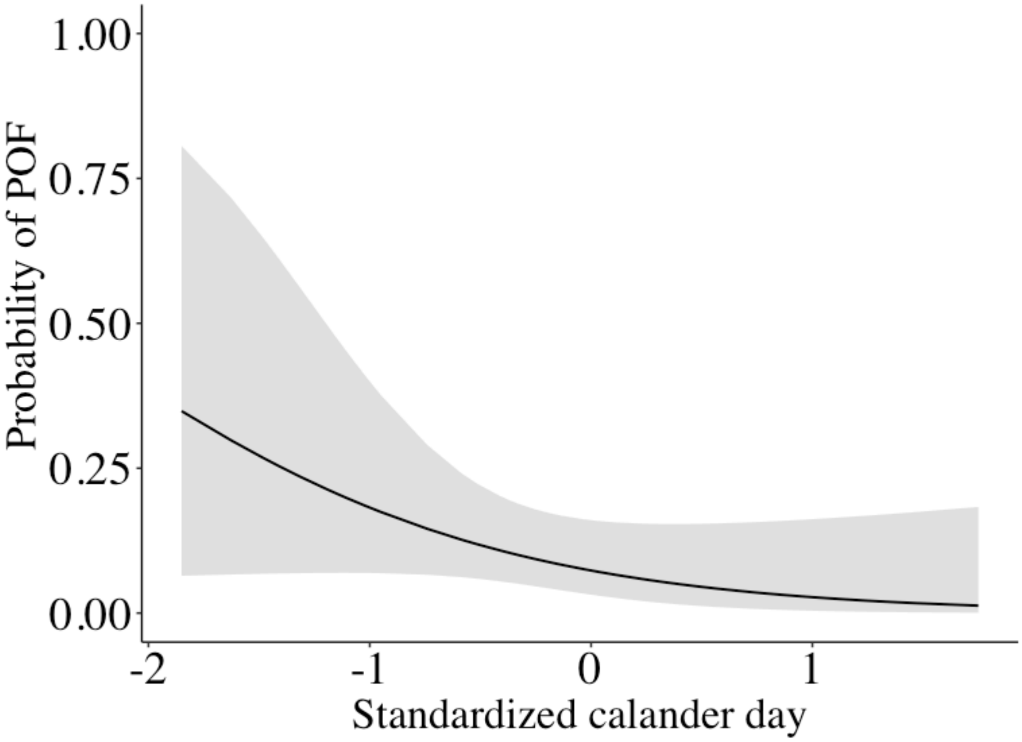

Figure 19. The negative relationship between spawning probability (probability of POF) and calendar day in Black Buffalo from a binomial logistic regression. The shaded area represents the 95% confidence intervals.

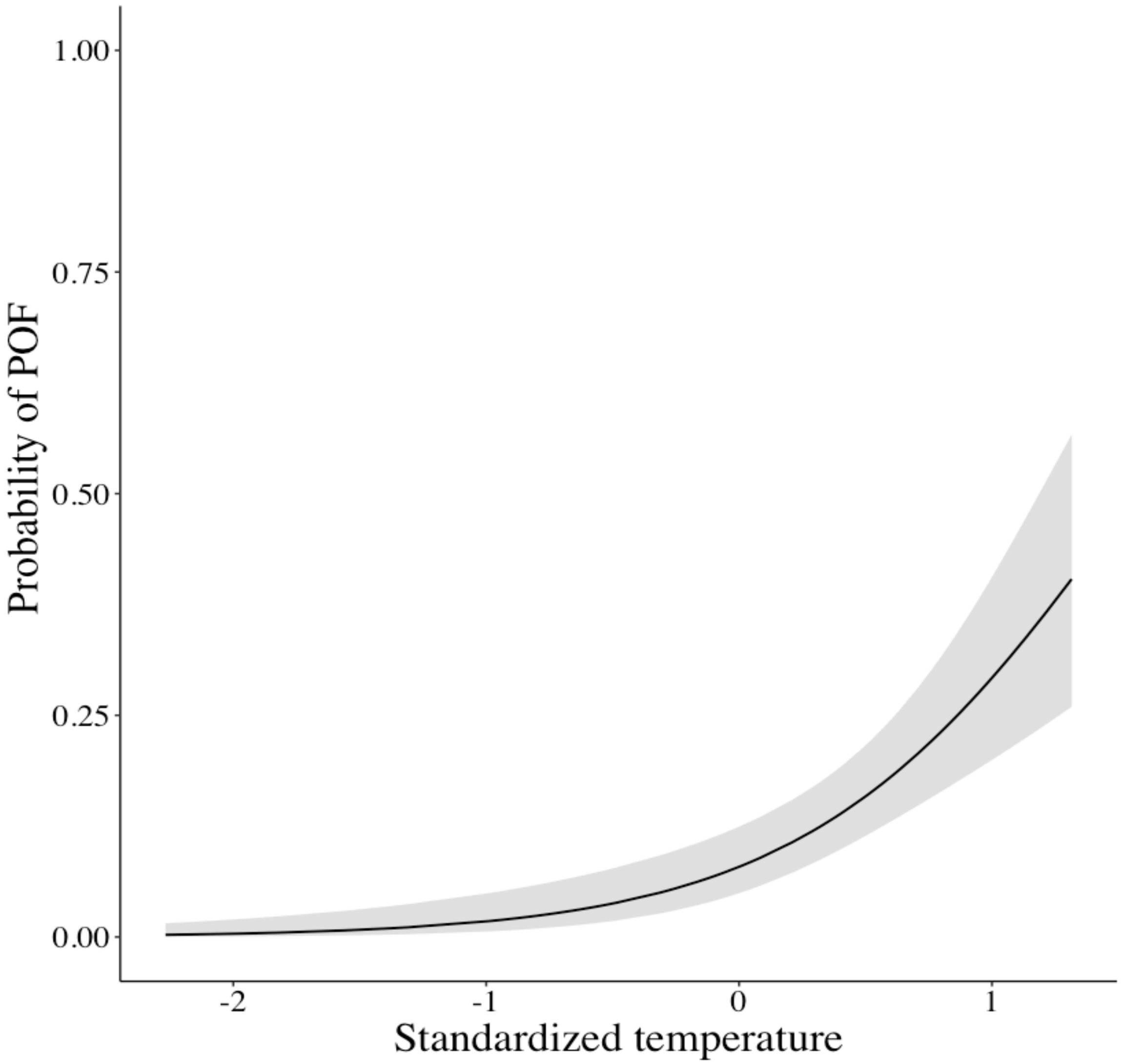

Figure 20. The positive relationship for between probability of spawning (P(POF)) and mean daily air temperature for Smallmouth Buffalo in the lower Red River catchment. The gray represents the 95% confidence intervals.

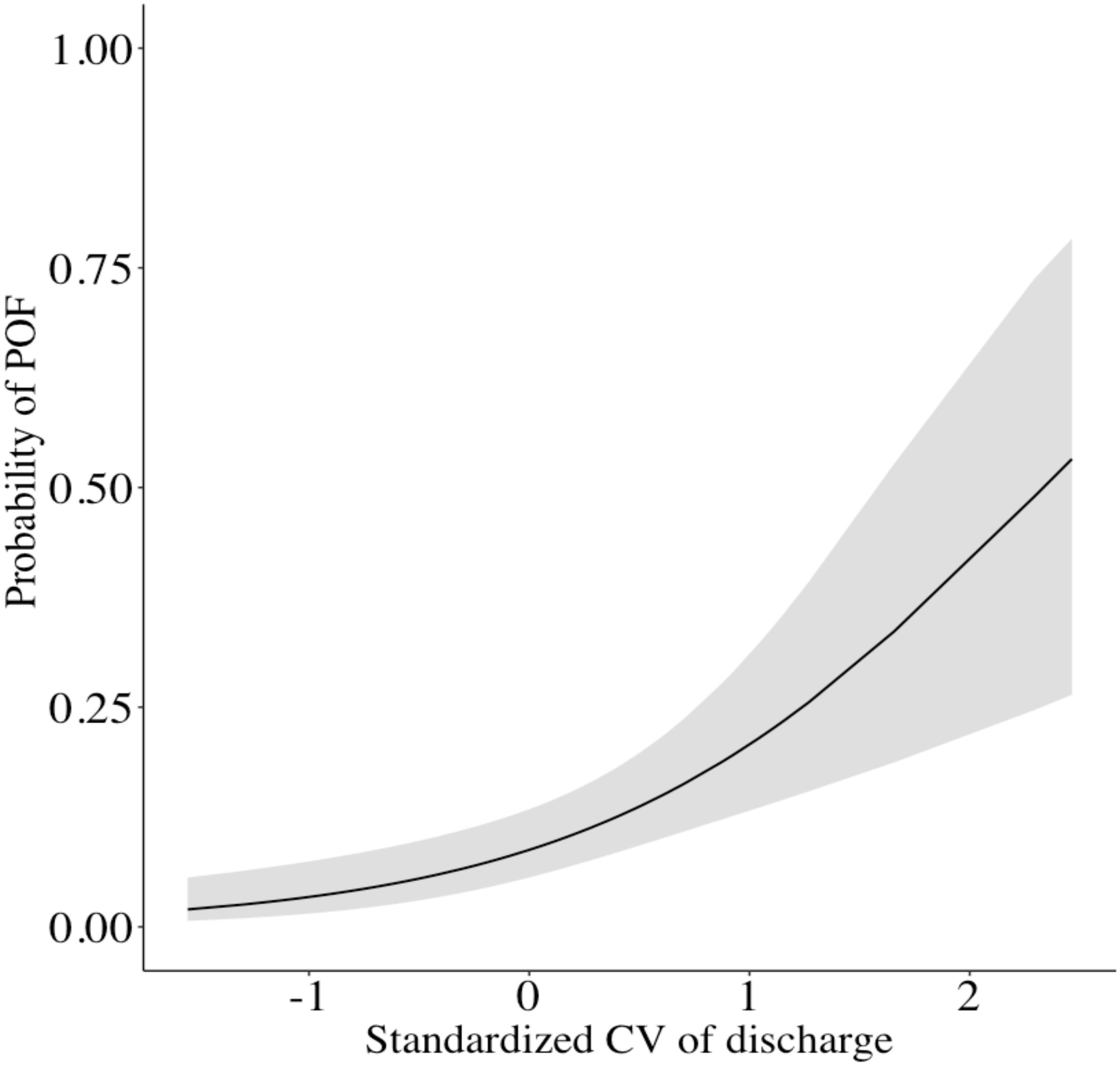

Figure 21. The positive relationship for between probability of spawning (P(POF)) and the coefficient of variation of variation of mean scaled daily discharge for Smallmouth Buffalo in the lower Red River catchment. The gray represents the 95% confidence interval.

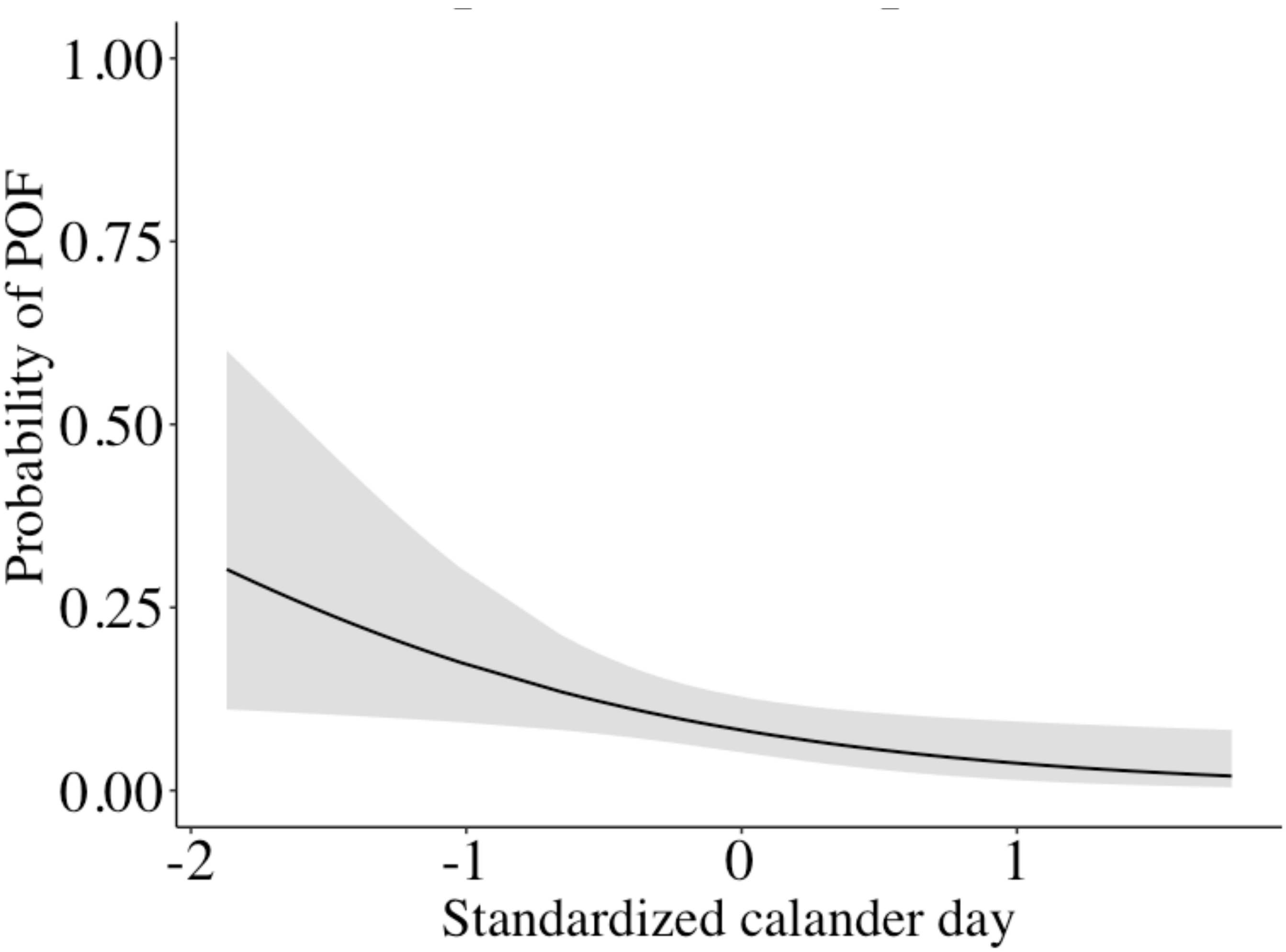

Figure 22. The negative relationship for between probability of spawning (P(POF)) and calendar day for Smallmouth Buffalo in the lower Red River catchment. The gray represents the 95% confidence intervals.

References:

Acre, M. R., T. B. Grabowski, D. J. Leavitt, N. G. Smith, A. A. Pease, P. T. Bean, and D. Geeslin. 2023. Mismatch between temperature and discharge disrupts spawning cues in a fluvial specialist, blue sucker Cycleptus elongatus. Ecology of Freshwater Fish 32(2):305–321.

Adams, S. R., and G. R. Parsons. 1998. Laboratory-Based Measurements of Swimming Performance and Related Metabolic Rates of Field-Sampled Smallmouth Buffalo (Ictiobus bubalus): A Study of Seasonal Changes. Physiological Zoology 71(4):350–358.

Aegerter, S., and B. Jalabert. 2004. Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 231(1):59–71.

Allen, M. S., and J. E. Hightower. 2010. Fish population dynamics: mortality, growth, and recruitment. Inland fisheries management in North America, 3rd edition. American Fisheries Society, Bethesda, Maryland:43–79.

Andersen, K. H., J. E. Beyer, M. Pedersen, N. G. Andersen, and H. Gislason. 2008. Life-history constraints on the success of the many small eggs reproductive strategy. Theoretical Population Biology 73(4):490–497.

Auld, A. H., and J. R. Schubel. 1978. Effects of suspended sediment on fish eggs and larvae: A laboratory assessment. Estuarine and Coastal Marine Science 6(2):153–164.

Balazik, M. T., G. C. Garman, J. P. Van Eenennaam, J. Mohler, and L. C. Woods III. 2012. Empirical Evidence of Fall Spawning by Atlantic Sturgeon in the James River, Virginia. Transactions of the American Fisheries Society 141(6):1465–1471.

Bart, H. L., M. D. Clements, R. E. Blanton, K. R. Piller, and D. L. Hurley. 2010. Discordant molecular and morphological evolution in buffalofishes (Actinopterygii: Catostomidae). Molecular Phylogenetics and Evolution 56(2):808–820.

Barton, K. 2015. Package ‘mumin.’ Version 1(18):439.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014, June 23. Fitting Linear Mixed-Effects Models using lme4. arXiv.

Bayley, P. B., and R. A. Herendeen. 2000. The Efficiency of a Seine Net. Transactions of the American Fisheries Society 129(4):901–923.

Benke, A. C., and C. E. Cushing. 2011. Rivers of North America. Elsevier.

von Bertalanffy, L. 1957. Quantitative Laws in Metabolism and Growth. The Quarterly Review of Biology 32(3):217–231.

Blasco-Moreno, A., M. Pérez-Casany, P. Puig, M. Morante, and E. Castells. 2019. What does a zero mean? Understanding false, random and structural zeros in ecology. Methods in Ecology and Evolution 10(7):949–959.

Bobe, J., and C. Labbé. 2010. Egg and sperm quality in fish. General and Comparative Endocrinology 165(3):535–548.

Braaten, P. J., and C. S. Guy. 2002. Life History Attributes of Fishes along the Latitudinal Gradient of the Missouri River. Transactions of the American Fisheries Society 131(5):931–945.

Brewer, S. K., C. F. Rabeni, and D. M. Papoulias. 2008. Comparing histology and gonadosomatic index for determining spawning condition of small-bodied riverine fishes. Ecology of Freshwater Fish 17(1):54–58.

Brooks, M. E., K. Kristensen, K. J. Van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Machler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R journal 9(2):378–400.

Brown, M. L., T. Kasiga, D. E. Spengler, and J. A. Clapper. 2019. Reproductive cycle of northern largemouth bass Micropterus salmoides salmoides. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 331(10):540–551.

Brown-Peterson, N. J., D. M. Wyanski, F. Saborido-Rey, B. J. Macewicz, and S. K. Lowerre-Barbieri. 2011. A Standardized Terminology for Describing Reproductive Development in Fishes. Marine and Coastal Fisheries 3(1):52–70.

Bundy, J. M., and K. R. Bestgen. 2001. Confirmation of Daily Increment Deposition in Otoliths of Young Razorback Suckers. The Southwestern Naturalist 46(1):34–40.

Burnham, K. P., and D. R. Anderson. 2003. Model selection and multimodel inference : a practical information-theoretic approach. Journal of Wildlife Management 67:655.

Burr, B. M., and R. C. Heidinger. 1983. Reproductive Behavior of the Bigmouth Buffalo Ictiobus cyprinellus in Crab Orchard Lake, Illinois. American Midland Naturalist 110(1):220.

Caissie, D., N. El-Jabi, and M. G. Satish. 2001. Modelling of maximum daily water temperatures in a small stream using air temperatures. Journal of Hydrology 251(1):14–28.

Carl, L. M., M. Kraft, and L. Rhude. 1989. Growth and taxonomy of bull charr, Salvelinus confluentus, in Pinto Lake, Alberta. Environmental Biology of Fishes 26(4):239–246.

Christman, J., K. Fields, S. Hebert, B. Kallenbach, M. Martinez, S. Poncik, A. Sumner, and E. Thomas. 2018. An Evaluation of the Causes, Consequences, and Potential Solutions to Increased Red River Flooding in the Caddo Parish Regions of Northwest Louisiana.

Clark Barkalow, S. L., M. A. Brandenburg, and S. P. Platania. 2020. Otoliths Reveal Spawning Ecology and Early Life History of Sympatric Catostomids. North American Journal of Fisheries Management 40(2):415–426.

Coggins Jr, L. G., M. J. Catalano, M. S. Allen, W. E. Pine III, and C. J. Walters. 2007. Effects of cryptic mortality and the hidden costs of using length limits in fishery management. Fish and Fisheries 8(3):196–210.

Coggins, L. G., D. C. Gwinn, and M. S. Allen. 2013. Evaluation of Age–Length Key Sample Sizes Required to Estimate Fish Total Mortality and Growth. Transactions of the American Fisheries Society 142(3):832–840.

Colombo, R. E., J. E. Garvey, N. D. Jackson, R. Brooks, D. P. Herzog, R. A. Hrabik, and T. W. Spier. 2007. Harvest of Mississippi River sturgeon drives abundance and reproductive success: a harbinger of collapse? Journal of Applied Ichthyology 23(4):444–451.

Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121(3):317–331.

Cooke, S. J., E. G. Martins, D. P. Struthers, L. F. G. Gutowsky, M. Power, S. E. Doka, J. M. Dettmers, D. A. Crook, M. C. Lucas, C. M. Holbrook, and C. C. Krueger. 2016. A moving target—incorporating knowledge of the spatial ecology of fish into the assessment and management of freshwater fish populations. Environmental Monitoring and Assessment 188(4):239.

Corriero, A., R. Zupa, C. C. Mylonas, and L. Passantino. 2021. Atresia of ovarian follicles in fishes, and implications and uses in aquaculture and fisheries. Journal of Fish Diseases 44(9):1271–1291.

COSEWIC. 2009. Assessment and update status report on the Bigmouth Buffalo Ictiobus cyprinellus Lakes—Great Lakes—Upper St. Lawrence populations Saskatchewan—Nelson River populations—in Canada. Committee on the Status of Endangered Wildlife in Canada Ottawa, ON, Canada.

Dattilo, J., S. K. Brewer, and D. E. Shoup. 2021. Flow Dynamics Influence Fish Recruitment in Hydrologically Connected River–Reservoir Landscapes. North American Journal of Fisheries Management 41(6):1752–1763.

Devlaming, V., G. Grossman, and F. Chapman. 1982. On the use of the gonosomatic index. Comparative Biochemistry and Physiology Part A: Physiology 73(1):31–39.

Dodds, W. K., K. Gido, M. R. Whiles, K. M. Fritz, and W. J. Matthews. 2004. Life on the Edge: The Ecology of Great Plains Prairie Streams. BioScience 54(3):205–216.

Dyer, J. J., and S. K. Brewer. 2020. Seasonal movements and tributary-specific fidelity of blue sucker in a Southern Plains riverscape. Journal of Fish Biology 97(1):279–292.

Eggleton, M. A., J. R. Jackson, and B. J. Lubinski. 2010. Comparison of Gears for Sampling Littoral-Zone Fishes in Floodplain Lakes of the Lower White River, Arkansas. North American Journal of Fisheries Management 30(4):928–939.

Evans-Powell, R. T., S. A. Hesp, A. Denham, and L. E. Beckley. 2024. Implications of big, old, fat, fecund, female fish (BOFFFFs) for the reproductive potential of a demersal teleost stock. Fisheries Research 272:106934.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes: A Continuous View of the River is Needed to Understand How Processes Interacting among Scales Set the Context for Stream Fishes and Their Habitat. BioScience 52(6):483–498.

Ferguson, G. J., T. M. Ward, A. Ivey, and T. Barnes. 2014. Life history of *Argyrosomus japonicus*, a large sciaenid at the southern part of its global distribution: Implications for fisheries management. Fisheries Research 151:148–157.

Fish, T. M. 2021. An Assessment of Female Pacific Halibut (Hippoglossus stenolepis) Reproductive Biology with Implications for Understanding and Characterizing Maturity. M.S.E.S., Alaska Pacific University, United States -- Alaska.

Fish, T., N. Wolf, B. P. Harris, and J. V. Planas. 2020. A comprehensive description of oocyte developmental stages in Pacific halibut, Hippoglossus stenolepis. Journal of Fish Biology 97(6):1880–1885.

Flores, A., R. Wiff, and E. Díaz. 2015. Using the gonadosomatic index to estimate the maturity ogive: application to Chilean hake (Merluccius gayi gayi). ICES Journal of Marine Science 72(2):508–514.

Flores, A., R. Wiff, K. Ganias, and C. T. Marshall. 2019. Accuracy of gonadosomatic index in maturity classification and estimation of maturity ogive. Fisheries Research 210:50–62.

Flotemersch, J. E., J. B. Stribling, and M. J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. US Environmental Protection Agency, Office of Research and Development ….

Fontoura, N. F., A. S. Braun, and P. C. C. Milani. 2009. Estimating size at first maturity (L50) from Gonadossomatic Index (GSI) data. Neotropical Ichthyology 7(2):217–222.

Fournie, J. W., R. M. Krol, and W. E. Hawkins. 2000. Chapter 34 - Fixation of Fish Tissues. Pages 569–578 *in* G. K. Ostrander, editor. The Laboratory Fish. Academic Press, London.

Fournier, D. 1983. Use of length and age data for estimating the age structure of a collection of fish. Can. Spec. Publ. Fish. Aquat. Sci 66:206–208.

Franks, J. L., G. A. Clyde, and K. L. Dickson. 2001. Zooplankton community structure and seasonal dynamics in Lake Texoma (Oklahoma-Texas). The Texas Journal of Science 53(3):203–221.

Fraser, G. S., K. R. Bestgen, D. L. Winkelman, and K. G. Thompson. 2019. Temperature—Not Flow—Predicts Native Fish Reproduction with Implications for Climate Change. Transactions of the American Fisheries Society 148(3):509–527.

Froese, R. 2004. Keep it simple: three indicators to deal with overfishing. Fish and Fisheries 5(1):86–91.

Furness, A. I. 2016. The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. Biological Reviews 91(3):796–812.

Ganias, K. 2013. Determining the indeterminate: Evolving concepts and methods on the assessment of the fecundity pattern of fishes. Fisheries Research 138:23–30.

Ganias, K., and S. Lowerre-Barbieri. 2018. Oocyte recruitment and fecundity type in fishes: Refining terms to reflect underlying processes and drivers. Fish and Fisheries 19(3):562–572.

García, D., M. Loureiro, E. Machín, and M. Reichard. 2018. Phenology of three coexisting annual fish species: seasonal patterns in hatching dates. Hydrobiologia 809(1):323–337.

García-Vega, A., F. J. Sanz-Ronda, L. Fernandes Celestino, S. Makrakis, and P. M. Leunda. 2018. Potamodromous brown trout movements in the North of the Iberian Peninsula: Modelling past, present and future based on continuous fishway monitoring. Science of The Total Environment 640–641:1521–1536.

Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge university press.

Goetz, F., S. Sitar, D. Rosauer, P. Swanson, C. R. Bronte, J. Dickey, and C. Simchick. 2011. The Reproductive Biology of Siscowet and Lean Lake Trout in Southern Lake Superior. Transactions of the American Fisheries Society 140(6):1472–1491.

Goto, D., M. J. Hamel, J. J. Hammen, M. L. Rugg, M. A. Pegg, and V. E. Forbes. 2015. Spatiotemporal variation in flow-dependent recruitment of long-lived riverine fish: Model development and evaluation. Ecological Modelling 296:79–92.

Grabowski, T. B., S. P. Young, J. J. Isely, and P. C. Ely. 2012. Age, Growth, and Reproductive Biology of Three Catostomids From the Apalachicola River, Florida. Journal of Fish and Wildlife Management 3(2):223–237.

Graham, R. J., and D. J. Orth. 1986. Effects of Temperature and Streamflow on Time and Duration of Spawning by Smallmouth Bass. Transactions of the American Fisheries Society 115(5):693–702.

Greeley, J. R. 1932. The Spawning Habits of Brook, Brown and Rainbow Trout, and the Problem of Egg Predators. Transactions of the American Fisheries Society.

Gross, M. R. 1982. Sneakers, Satellites and Parentals: Polymorphic Mating Strategies in North American Sunfishes. Zeitschrift für Tierpsychologie 60(1):1–26.

Grüss, A., J. Robinson, S. S. Heppell, S. A. Heppell, and B. X. Semmens. 2014. Conservation and fisheries effects of spawning aggregation marine protected areas: What we know, where we should go, and what we need to get there. ICES Journal of Marine Science 71(7):1515–1534.

Gunnarsson, Á., H. Björnsson, B. Elvarsson, and C. Pampoulie. 2016. Spatio-temporal variation in the reproduction timing of Atlantic Wolffish (*Anarhichas lupus* L) in Icelandic waters and its relationship with size. Fisheries Research 183:404–409.

Guy, C. S., and D. W. Willis. 1995. Population Characteristics of Black Crappies in South Dakota Waters: A Case for Ecosystem-Specific Management. North American Journal of Fisheries Management 15(4):754–765.

Guy, E. L., and P. J. Allen. 2018. Tank Acclimation and Induced Spawning of the Catostomid, Black Buffalo. North American Journal of Aquaculture 80(3):294–300.

Guy, E. L., C. C. Mischke, M. E. Colvin, and P. J. Allen. 2019. Zooplankton Selectivity by Black Buffalo in Fertilized Ponds. North American Journal of Aquaculture 81(3):215–221.

Guzmán, J. M., J. A. Luckenbach, M. A. Middleton, K. C. Massee, C. Jensen, F. W. Goetz, A. J. Jasonowicz, and P. Swanson. 2017. Reproductive life history of sablefish (Anoplopoma fimbria) from the U.S. Washington coast. PLOS ONE 12(9):e0184413.

Gwinn, D. C., M. S. Allen, F. D. Johnston, P. Brown, C. R. Todd, and R. Arlinghaus. 2015. Rethinking length-based fisheries regulations: the value of protecting old and large fish with harvest slots. Fish and Fisheries 16(2):259–281.

Hanson, K. C., A. Abizaid, and S. J. Cooke. 2009. Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*). Hormones and Behavior 56(5):503–509.

Hartig, F. 2017. Package ‘dharma.’ R package.

Heidarsson, T., T. Antonsson, and S. S. Snorrason. 2006. The Relationship between Body and Scale Growth Proportions and Validation of Two Back-Calculation Methods Using Individually Tagged and Recaptured Wild Atlantic Salmon. Transactions of the American Fisheries Society 135(5):1156–1164.

Heppell, S. S., S. A. Heppell, F. C. Coleman, and C. C. Koenig. 2006. Models To Compare Management Options For A Protogynous Fish. Ecological Applications 16(1):238–249.

Hirsch, R. M., and L. A. D. Cicco. 2015. User guide to Exploration and Graphics for RivEr Trends (EGRET) and dataRetrieval: R packages for hydrologic data. Page Techniques and Methods. U.S. Geological Survey, 4-A10.

Hoff, G. R., D. J. Logan, and D. F. Markle. 1997. Notes: Otolith Morphology and Increment Validation in Young Lost River and Shortnose Suckers. Transactions of the American Fisheries Society 126(3):488–494.

Honsey, A. E., D. F. Staples, and P. A. Venturelli. 2017. Accurate estimates of age at maturity from the growth trajectories of fishes and other ectotherms. Ecological Applications 27(1):182–192.

Hoyt, R. D., G. J. Overmann, and G. A. Kindschi. 1979. Observations on the larval ecology of the smallmouth buffalo. Pages 1–16 Proceedings of the third symposium on larval fish. Western Kentucky University, Bowling Green, Kentucky, USA.

Irons, K. S., G. G. Sass, M. A. McClelland, and J. D. Stafford. 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? Journal of Fish Biology 71(sd):258–273.

Isermann, D. A., W. L. McKibbin, and D. W. Willis. 2002. An Analysis of Methods for Quantifying Crappie Recruitment Variability. North American Journal of Fisheries Management 22(4):1124–1135.

Jacquemin, S. J., J. C. Doll, M. Pyron, M. Allen, and D. A. S. Owen. 2015. Effects of flow regime on growth rate in freshwater drum, Aplodinotus grunniens. Environmental Biology of Fishes 98(4):993–1003.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren Jr. 2008. Conservation Status of Imperiled North American Freshwater and Diadromous Fishes. Fisheries 33(8):372–407.

Jezierska, B., K. Ługowska, and M. Witeska. 2009. The effects of heavy metals on embryonic development of fish (a review). Fish Physiology and Biochemistry 35(4):625–640.

Johnson, D. W., and W. Minckley. 1972. Variability in Arizona buffalofishes. Copeia:12–17.

Johnson, J. E., and B. D. McAleer. 1987. Protected fishes of the United States and Canada.

Johnson, R. P. 1963. Studies on the Life History and Ecology of the Bigmouth Buffalo, Ictiobus cyprinellus (Valenciennes). Journal of the Fisheries Research Board of Canada 20(6):1397–1429.

Junk, W. J., P. B. Bayley, R. E. Sparks, and others. 1989. The flood pulse concept in river-floodplain systems. Canadian special publication of fisheries and aquatic sciences 106(1):110–127.

Junk, W. J., and K. M. Wantzen. 2004. The flood pulse concept: new aspects, approaches and applications-an update. Pages 117–149 Second international symposium on the management of large rivers for fisheries. Food and Agriculture Organization and Mekong River Commission, FAO Regional ….

Katsanevakis, S., and C. D. Maravelias. 2008. Modelling fish growth: multi-model inference as a better alternative to a priori using von Bertalanffy equation. Fish and Fisheries 9(2):178–187.

Keeley, E. R. 2001. Demographic Responses to Food and Space Competition by Juvenile Steelhead Trout. Ecology 82(5):1247–1259.

Kjesbu, O. S. 1994. Time of start of spawning in Atlantic cod (Gadus morhua) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. Journal of Fish Biology 45(5):719–735.

Klein, Z. B., M. C. Quist, L. E. Miranda, M. M. Marron, M. J. Steuck, and K. A. Hansen. 2018. Commercial Fisheries of the Upper Mississippi River: A Century of Sustained Harvest. Fisheries 43(12):563–574.

Kramer, D. L. 1978. Reproductive Seasonality in the Fishes of a Tropical Stream. Ecology 59(5):976–985.

Krueger, C. C., W. C. Shepherd, and A. M. Muir. 2014. Predation by alewife on lake trout fry emerging from laboratory reefs: Estimation of fry survival and assessment of predation potential. Journal of Great Lakes Research 40(2):429–434.

Lackmann, A. R., A. H. Andrews, M. G. Butler, E. S. Bielak-Lackmann, and M. E. Clark. 2019. Bigmouth Buffalo Ictiobus cyprinellus sets freshwater teleost record as improved age analysis reveals centenarian longevity. Communications Biology 2(1):1–14.

Lackmann, A. R., E. S. Bielak-Lackmann, R. I. Jacobson, M. G. Butler, and M. E. Clark. 2022a. Otolith allometry informs age and growth of long-lived Quillback Carpiodes cyprinus. Environmental Biology of Fishes 105(8):1051–1064.

Lackmann, A. R., S. A. Black, E. S. Bielak-Lackmann, and J. A. Lackmann. 2023. Centenarian lifespans of three freshwater fish species in Arizona reveal the exceptional longevity of the buffalofishes (Ictiobus). Scientific Reports 13(1):17401.

Lackmann, A. R., B. J. Kratz, E. S. Bielak-Lackmann, R. I. Jacobson, D. J. Sauer, A. H. Andrews, M. G. Butler, and M. E. Clark. 2021. Long-lived population demographics in a declining, vulnerable fishery-bigmouth buffalo (Ictiobus cyprinellus) of Jamestown Reservoir, North Dakota. Canadian Journal of Fisheries and Aquatic Sciences 78(10):1486.

Lackmann, A. R., J. Sereda, M. Pollock, R. Bryshun, M. Chupik, K. McCallum, J. Villeneuve, E. S. Bielak-Lackmann, and M. E. Clark. 2022b. Bet-hedging bigmouth buffalo (Ictiobus cyprinellus) recruit episodically over a 127-year timeframe in Saskatchewan. Canadian Journal of Fisheries and Aquatic Sciences.

Lackmann, A. R., S. Seybold, E. S. Bielak-Lackmann, W. Ford, M. G. Butler, and M. E. Clark. 2024. Analysis of bigmouth buffalo Ictiobus cyprinellus spawning phenology in

Minnesota reveals 50-year recruitment failure and conservation concern. Scientific Reports 14(1):19444.

Lambert, D. 1992. Zero-Inflated Poisson Regression, With an Application to Defects in Manufacturing. Technometrics 34(1):1–14.

Leaman, B., and R. J. Beamish. 1984. Ecological and management implications of longevity in some Northeast Pacific groundfishes. Page International North Pacific Fisheries Commission Bulletin.

Li, T., K. Mo, J. Wang, Q. Chen, J. Zhang, C. Zeng, H. Zhang, and P. Yang. 2021. Mismatch between critical and accumulated temperature following river damming impacts fish spawning. Science of The Total Environment 756:144052.

Litvak, M. K., and W. C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Marine ecology progress series. Oldendorf 81(1):13–24.

Lombardi, P. M., F. L. Rodrigues, and J. P. Vieira. 2014. Longer is not always better: The influence of beach seine net haul distance on fish catchability. Zoologia (Curitiba) 31:35–41.

Long, J. M., R. A. Snow, D. E. Shoup, and J. B. Bartnicki. 2023. Validation and Comparison of Age Estimates for Smallmouth Buffalo in Oklahoma Based on Otoliths, Pectoral Fin Rays, and Opercula. North American Journal of Fisheries Management 43(3):618–627.

Lorenzen, K. 2022. Size- and age-dependent natural mortality in fish populations: Biology, models, implications, and a generalized length-inverse mortality paradigm. Fisheries Research 255:106454.

Lorenzen, K., I. G. Cowx, R. E. M. Entsua-Mensah, N. P. Lester, J. D. Koehn, R. G. Randall, N. So, S. A. Bonar, D. B. Bunnell, P. Venturelli, S. D. Bower, and S. J. Cooke. 2016. Stock assessment in inland fisheries: a foundation for sustainable use and conservation. Reviews in Fish Biology and Fisheries 26(3):405–440.

Love, S. A., S. J. Tripp, and Q. E. Phelps. 2019. Age and Growth of Middle Mississippi River Smallmouth Buffalo. The American Midland Naturalist 182(1):118–123.

Lowerre-Barbieri, S. K., K. Ganias, F. Saborido-Rey, H. Murua, and J. R. Hunter. 2011. Reproductive Timing in Marine Fishes: Variability, Temporal Scales, and Methods. Marine and Coastal Fisheries 3(1):71–91.

Maceina, M. J. 1997. Simple application of using residuals from catch-curve regressions to assess year-class strength in fish. Fisheries Research 32(2):115–121.

Maceina, M. J., and S. M. Sammons. 2016. Assessing the Accuracy of Published Natural Mortality Estimators Using Rates Determined from Five Unexploited Freshwater Fish Populations. North American Journal of Fisheries Management 36(2):433–446.

Martell, S. J. D., W. E. Pine, and C. J. Walters. 2008. Parameterizing age-structured models from a fisheries management perspective. Canadian Journal of Fisheries and Aquatic Sciences 65(8):1586–1600.

Martin, R. E., S. I. Auerbach, and D. J. Nelson. 1964. GROWTH AND MOVEMENT OF SMALLMOUTH BUFFALO, ICTIOBUS BUBALUS (RAFINESQUE), IN WATTS BAR RESERVOIR, TENNESSEE. Oak Ridge National Lab. (ORNL), Oak Ridge, TN (United States), ORNL-3530.

Martin, T. G., B. A. Wintle, J. R. Rhodes, P. M. Kuhnert, S. A. Field, S. J. Low-Choy, A. J. Tyre, and H. P. Possingham. 2005. Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecology Letters 8(11):1235–1246.

Maunder, M. N., O. S. Hamel, H.-H. Lee, K. R. Piner, J. M. Cope, A. E. Punt, J. N. Ianelli, C. Castillo-Jordán, M. S. Kapur, and R. D. Methot. 2023. A review of estimation methods for natural mortality and their performance in the context of fishery stock assessment. Fisheries Research 257:106489.

Maunder, M. N., and J. T. Thorson. 2019. Modeling temporal variation in recruitment in fisheries stock assessment: A review of theory and practice. Fisheries Research 217:71–86.

Maxson, K. A., L. E. Solomon, T. A. Bookout, S. A. DeLain, A. D. Bartels, M. C. Bowler, E. J. Gittinger, E. N. Ratcliff, J. L. West, S. A. Love, J. A. DeBoer, A. L. Whitten-Harris, M. J. Spear, B. S. Ickes, A. F. Casper, and J. T. Lamer. 2024. Smallmouth buffalo (Ictiobus bubalus Rafinesque) population trends and demographics in the Upper Mississippi River System. Environmental Biology of Fishes.

Mazerolle, M. J. 2017. Package 'AICcmodavg.' R package 281.

McCorkle, T. A., S. S. Williams, T. A. Pfeiffer, and J. B. Basara. 2016. Atmospheric Contributors to Heavy Rainfall Events in the Arkansas-Red River Basin. Advances in Meteorology 2016:e4597912.

McPherson, L. R., K. Ganias, and C. T. Marshall. 2011. Inaccuracies in routinely collected Atlantic herring (Clupea harengus) maturity data and correction using a gonadosomatic index model. Journal of the Marine Biological Association of the United Kingdom 91(7):1477–1487.

Michie, L. E., J. D. Thiem, C. A. Boys, and S. M. Mitrovic. 2020. The effects of cold shock on freshwater fish larvae and early-stage juveniles: implications for river management. Conservation Physiology 8(1):coaa092.

Mignien, L., and S. Stoll. 2024. Reproductive success of stream fish species in relation to high and low flow patterns: The role of life history strategies and species traits. Science of The Total Environment 946:174366.

Miller, R. J., and H. W. Robison. 2004. Fishes of Oklahoma. University of Oklahoma Press.

Minckley, W. L., J. E. Johnson, J. N. Rinne, and S. E. Willoughby. 1970. Foods of Buffalofishes, Genus Ictiobus, in Central Arizona Reservoirs. Transactions of the American Fisheries Society 99(2):333–342.

Miranda, L. E., and W. D. Hubbard. 1994. Winter Survival of Age-0 Largemouth Bass Relative to Size, Predators, and Shelter. North American Journal of Fisheries Management 14(4):790–796.

Mollenhauer, R., S. K. Brewer, J. S. Perkin, D. Swedberg, M. Wedgeworth, and Z. D. Steffensmeier. 2021. Connectivity and flow regime direct conservation priorities for pelagophil fishes. Aquatic Conservation: Marine and Freshwater Ecosystems 31(11):3215–3227.

Mollenhauer, R., J. B. Mouser, V. L. Roland, and S. K. Brewer. 2022. Increased landscape disturbance and streamflow variability threaten fish biodiversity in the Red River catchment, USA. Diversity and Distributions 28(9):1934–1950.

Morrill, J. C., R. C. Bales, and M. H. Conklin. 2005. Estimating Stream Temperature from Air Temperature: Implications for Future Water Quality. Journal of Environmental Engineering 131(1):139–146.

Morrongiello, J. R., D. A. Crook, A. J. King, D. S. L. Ramsey, and P. Brown. 2011. Impacts of drought and predicted effects of climate change on fish growth in temperate Australian lakes. Global Change Biology 17(2):745–755.

Mumford, S. L. 2004. Histology of finfish. USFWS, Olympia Fish Health Center. Olympia. Washington.

Myers, J. N., N. G. Chatakondi, R. A. Dunham, and I. A. E. Butts. 2020. Genetic architecture of early life history traits for channel catfish, *Ictalurus punctatus* ♀ × blue catfish, *I. furcatus* ♂ hybrid production. Aquaculture 514:734436.

Myers, R. A., J. A. Hutchings, and N. J. Barrowman. 1997. Why Do Fish Stocks Collapse? The Example of Cod in Atlantic Canada. Ecological Applications 7(1):91–106.

Nack, C. C., D. P. Swaney, and K. E. Limburg. 2019. Historical and Projected Changes in Spawning Phenologies of American Shad and Striped Bass in the Hudson River Estuary. Marine and Coastal Fisheries 11(3):271–284.

Nelson, W. R. 1974. Age, growth, and maturity of thirteen species of fish from Lake Oahe during the early years of impoundment, 1963-68. US Department of the Interior, Fish and Wildlife Service.

Nitychoruk, J. M., L. F. G. Gutowsky, P. M. Harrison, T. J. Hossie, M. Power, and S. J. Cooke. 2013. Sexual and seasonal dimorphism in adult adfluvial bull trout (Salvelinus confluentus). Canadian Journal of Zoology 91(7):480–488.

Olden, J. D., and M. J. Kennard. 2010. Intercontinental comparison of fish life history strategies along a gradient of hydrologic variability. Pages 83–107 American Fisheries Society Symposium.

Osborn, R. S. 1966. Observations on the spawning ecology of buffalos (Ictiobus bubalus and I. cyprinellus) in relation to parasitism. Pages 54–57 Proceedings of the Oklahoma Academy of Science.

Ouellet, V., A. H. Fullerton, M. Kaylor, S. Naman, R. Bellmore, J. Rosenfeld, G. Rossi, S. White, S. Rhoades, D. A. Beauchamp, and others. 2024. Food for fish: Challenges and opportunities for quantifying foodscapes in river networks. Wiley Interdisciplinary Reviews: Water:e1752.

Padilla, R. 1972. Reproduction of carp, smallmouth buffalo and river carpsucker in Elephant Butte Lake. PhD Thesis, New Mexico State University.

Parker, G. A. 1992. The evolution of sexual size dimorphism in fish*. Journal of Fish Biology 41(sB):1–20.

Pauly, D., R. Froese, C. Liang, J. Müller, and P. Sorensen. 2023. Post-spawning growth acceleration in fish as a result of reduced live weight and thus, increased food conversion efficiency. Environmental Biology of Fishes 106(11):2031–2043.

Pearson, K. N., W. L. Kendall, D. L. Winkelman, and W. R. Persons. 2015. Evidence for skipped spawning in a potamodromous cyprinid, humpback chub (Gila cypha), with implications for demographic parameter estimates. Fisheries Research 170:50–59.

Perkin, J. S., T. A. Starks, C. A. Pennock, K. B. Gido, G. W. Hopper, and S. C. Hedden. 2019. Extreme drought causes fish recruitment failure in a fragmented Great Plains riverscape. Ecohydrology 12(6):e2120.

Peterson, R. C., and C. A. Jennings. 2007. Effects of river discharge on abundance and instantaneous growth of age-0 carpsuckers in the Oconee River, Georgia, USA. River Research and Applications 23(9):1016–1025.

Pine, W. E., K. H. Pollock, J. E. Hightower, T. J. Kwak, and J. A. Rice. 2003. A Review of Tagging Methods for Estimating Fish Population Size and Components of Mortality. Fisheries 28(10):10–23.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The Natural Flow Regime. BioScience 47(11):769–784.

Poff, N. L., and J. K. H. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55(1):194–205.

van Poorten, B. T., S. p. Cox, and A. B. Cooper. 2013. Efficacy of harvest and minimum size limit regulations for controlling short-term harvest in recreational fisheries. Fisheries Management and Ecology 20(2–3):258–267.

Prull, M., M. A. Simonson, and M. J. Weber. 2023. Precision and Bias of Three Bigmouth Buffalo Aging Structures. North American Journal of Fisheries Management 43(3):646–655.

Quinn, J. W. 2010. A Survey of Bowfishing Tournaments in Arkansas. North American Journal of Fisheries Management 30(6):1376–1384.

Quist, M. C., and D. A. Isermann. 2017. Age and growth of fishes: principles and techniques. American Fisheries Society.

Quist, M. C., Z. J. Jackson, M. R. Bower, and W. A. Hubert. 2007. Precision of Hard Structures Used to Estimate Age of Riverine Catostomids and Cyprinids in the Upper Colorado River Basin. North American Journal of Fisheries Management 27(2):643–649.

Quist, M. C., and J. R. Spiegel. 2012. Population Demographics of Catostomids in Large River Ecosystems: Effects of Discharge and Temperature on Recruitment Dynamics and Growth. River Research and Applications 28(9):1567–1586.

Radford, D. S., A. R. Lackmann, C. J. Moody-Carpenter, and R. E. Colombo. 2021. Comparison of Four Hard Structures Including Otoliths for Estimating Age in Blue Suckers. Transactions of the American Fisheries Society 150(4):514–527.

Ramsey, P. Q. 2023. Nursery Habitat and Hatch Dates of Large River Fishes of the Lower Red River Catchment. M.Sc., Auburn University, United States -- Alabama.

Ramsey, P. Q., J. Dattilo, D. R. DeVries, and S. K. Brewer. 2024. Evaluating the context dependency of large river nursery habitats for fishes in the lower Red River catchment. River Research and Applications 40(5):761–779.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries and Marine Service.

Rideout, R. M., G. A. Rose, and M. P. M. Burton. 2005. Skipped spawning in female iteroparous fishes. Fish and Fisheries 6(1):50–72.

Rideout, R. M., and J. Tomkiewicz. 2011. Skipped Spawning in Fishes: More Common than You Might Think. Marine and Coastal Fisheries 3(1):176–189.

Riggs, C. D., and E. W. Bonn. 1959. An Annotated List of the Fishes of Lake Texoma, Oklahoma and Texas. The Southwestern Naturalist 4(4):157–168.

Rissik, D., E. H. Shon, B. Newell, M. E. Baird, and I. M. Suthers. 2009. Plankton dynamics due to rainfall, eutrophication, dilution, grazing and assimilation in an urbanized coastal lagoon. Estuarine, Coastal and Shelf Science 84(1):99–107.

Roberts, D. T., L. J. Duivenvoorden, and I. G. Stuart. 2008. Factors influencing recruitment patterns of Golden Perch (Macquaria ambigua oriens) within a hydrologically variable and regulated Australian tropical river system. Ecology of Freshwater Fish 17(4):577–589.

Roever, C. L., H. Beyer, M. J. Chase, and R. J. Van Aarde. 2014. The pitfalls of ignoring behaviour when quantifying habitat selection. Diversity and Distributions 20(3):322–333.

de Roos, A. M., D. S. Boukal, and L. Persson. 2006. Evolutionary regime shifts in age and size at maturation of exploited fish stocks. Proceedings of the Royal Society B: Biological Sciences 273(1596):1873–1880.

Röpke, C., A. Cella-Ribeiro, F. C. Ferreira, T. R. de Araújo, C. R. da Costa Dória, F. Gusmão, A. Lonardoni, and G. Torrente-Vilara. 2024. The seasonal rate of discharge change as the primary trigger synchronizing freshwater fish reproduction in an Amazonian River. Reviews in Fish Biology and Fisheries 34(4):1619–1635.

Ross, S. T., W. J. Matthews, and A. A. Echelle. 1985. Persistence of Stream Fish Assemblages: Effects of Environmental Change. The American Naturalist 126(1):24–40.

Rothschild, B. J. 2000. “Fish stocks and recruitment”: the past thirty years. ICES Journal of Marine Science 57(2):191–201.

Rypel, A. L. 2012. Meta-analysis of growth rates for a circumpolar fish, the northern pike (sox lucius), with emphasis on effects of continent, climate and latitude. Ecology of Freshwater Fish 21(4):521–532.

Rypel, A. L. 2014. The Cold-Water Connection: Bergmann’s Rule in North American Freshwater Fishes. The American Naturalist 183(1):147–156.

Rypel, A. L., P. Saffarinia, C. C. Vaughn, L. Nesper, K. O'Reilly, C. A. Parisek, M. L. Miller, P. B. Moyle, N. A. Fangue, M. Bell-Tilcock, D. Ayers, and S. R. David. 2021. Goodbye to "Rough Fish": Paradigm Shift in the Conservation of Native Fishes. Fisheries 46(12):605–616.

Sammons, S. M., L. A. Earley, and M. R. Goclowski. 2021. Effect of Discharge on Hatching and Growth of Age-0 Black Bass in Two Southeastern U.S. Rivers. Journal of Fish and Wildlife Management 12(2):434–452.

Samy-Kamal, M., A. Forcada, and J. L. S. Lizaso. 2015. Effects of seasonal closures in a multi-specific fishery. Fisheries Research 172:303–317.

Sauer, D. J., B. J. Heidinger, J. D. Kittilson, A. R. Lackmann, and M. E. Clark. 2021. No evidence of physiological declines with age in an extremely long-lived fish. Scientific Reports 11(1):9065.

Scarnecchia, D. L., and J. D. Schooley. 2020. Bowfishing in the United States: History, Status, Ecological Impact, and a Need for Management. Transactions of the Kansas Academy of Science 123(3–4):285–338.

Scarnecchia, D. L., J. D. Schooley, A. R. Lackmann, S. J. Rider, D. K. Riecke, J. McMullen, J. E. Ganus, K. D. Steffensen, N. W. Kramer, and Z. R. Shattuck. 2021. The Sport Fish Restoration Program as a Funding Source to Manage and Monitor Bowfishing and Monitor Inland Commercial Fisheries. Fisheries 46(12):595–604.

Scharbert, A., and J. Borcherding. 2013. Relationships of hydrology and life-history strategies on the spatio-temporal habitat utilisation of fish in European temperate river floodplains. Ecological Indicators 29:348–360.

Simpfendorfer, C. A. 2000. Predicting Population Recovery Rates for Endangered Western Atlantic Sawfishes Using Demographic Analysis. Environmental Biology of Fishes 58(4):371–377.

Sitar, S. P., A. J. Jasonowicz, C. A. Murphy, and F. W. Goetz. 2014. Estimates of Skipped Spawning in Lean and Siscowet Lake Trout in Southern Lake Superior: Implications for Stock Assessment. Transactions of the American Fisheries Society 143(3):660–672.

Skjæraasen, J. E., K. Korsbrekke, G. E. Dingsør, Ø. Langangen, A. F. Opdal, and C. Jørgensen. 2020. Large annual variation in the amount of skipped spawning for female Northeast Arctic haddock Melanogrammus aeglefinus. Fisheries Research 230:105670.

Skjæraasen, J. E., R. D. M. Nash, K. Korsbrekke, M. Fonn, T. Nilsen, J. Kennedy, K. H. Nedreaas, A. Thorsen, P. R. Witthames, A. J. Geffen, H. Høie, and O. S. Kjesbu. 2012. Frequent skipped spawning in the world's largest cod population. Proceedings of the National Academy of Sciences 109(23):8995–8999.

van der Sleen, P., C. Stransky, J. R. Morrongiello, H. Haslob, M. Peharda, and B. A. Black. 2018. Otolith increments in European plaice (Pleuronectes platessa) reveal temperature and density-dependent effects on growth. ICES Journal of Marine Science 75(5):1655–1663.

Smith, H., A. Garcia Lozano, D. Baker, H. Blondin, J. Hamilton, J. Choi, X. Basurto, and B. Silliman. 2021. Ecology and the science of small-scale fisheries: A synthetic review of research effort for the Anthropocene. Biological Conservation 254:108895.

Smith, M. W., A. Y. Then, C. Wor, G. Ralph, K. H. Pollock, and J. M. Hoenig. 2012. Recommendations for Catch-Curve Analysis. North American Journal of Fisheries Management 32(5):956–967.

Snow, R. A., M. J. Porta, and D. M. Bogner. 2020. Examination of the Current Oklahoma State Record Smallmouth Buffalo. Proceedings of the Oklahoma Academy of Science 100.

Song, Z., Z. Fu, J. Li, and B. Yue. 2008. Validation of daily otolith increments in larval and juvenile Chinese sucker, Myxocyprinus asiaticus. Environmental Biology of Fishes 82(2):165–171.

Steinhart, G. B., M. E. Sandrene, S. Weaver, R. A. Stein, and E. A. Marschall. 2005. Increased parental care cost for nest-guarding fish in a lake with hyperabundant nest predators. Behavioral Ecology 16(2):427–434.

Stevenson, D. K., and S. E. Campana. 1992. Otolith Microstructure Examination and Analysis.

Stoffels, R. J., K. E. Weatherman, N. R. Bond, J. R. Morrongiello, J. D. Thiem, G. Butler, W. Koster, R. K. Kopf, N. McCasker, Q. Ye, B. Zampatti, and B. Broadhurst. 2020. Stage-dependent effects of river flow and temperature regimes on the growth dynamics of an apex predator. Global Change Biology 26(12):6880–6894.

Thompson, K. R., and D. W. Beckman. 1995. Validation of Age Estimates from White Sucker Otoliths. Transactions of the American Fisheries Society 124(4):637–639.

Tockner, K., F. Malard, and J. V. Ward. 2000. An extension of the flood pulse concept. Hydrological Processes 14(16–17):2861–2883.

Tonkin, Z. D., A. J. King, A. I. Robertson, and D. S. L. Ramsey. 2011. Early fish growth varies in response to components of the flow regime in a temperate floodplain river. Freshwater Biology 56(9):1769–1782.

Tonkin, Z., A. Kitchingman, J. Lyon, J. Kearns, G. Hackett, J. O'Mahony, P. D. Moloney, K. Krusic-Golub, and T. Bird. 2017. Flow magnitude and variability influence growth of two freshwater fish species in a large regulated floodplain river. Hydrobiologia 797(1):289–301.

Tonkin, Z., J. Yen, J. Lyon, A. Kitchingman, J. D. Koehn, W. M. Koster, J. Lieschke, S. Raymond, J. Sharley, I. Stuart, and C. Todd. 2021. Linking flow attributes to recruitment to inform water management for an Australian freshwater fish with an equilibrium life-history strategy. Science of The Total Environment 752:141863.

Tornabene, B. J., T. W. Smith, A. E. Tews, R. P. Beattie, W. M. Gardner, and L. A. Eby. 2020. Trends in River Discharge and Water Temperature Cue Spawning Movements of Blue Sucker, Cycleptus elongatus, in an Impounded Great Plains River. Copeia 108(1):151–162.

Torres, N., and D. W. Harrelson. 2012. The Great Red River Raft and its Sedimentological Implications. Pages 35–55 *in* B. T. McLaurin, A. C. Elliott, and N. Torres, editors. Reconstructing Human-Landscape Interactions - Volume 1: Interpreting Desert and Fluvial Environments. Springer, Berlin, Heidelberg.

Tripp, S. J., Q. E. Phelps, R. E. Colombo, J. E. Garvey, B. M. Burr, D. P. Herzog, and R. A. Hrabik. 2009. Maturation and Reproduction of Shovelnose Sturgeon in the Middle Mississippi River. North American Journal of Fisheries Management 29(3):730–738.

Trippel, E. A. 1995. Age at Maturity as a Stress Indicator in Fisheries. BioScience 45(11):759–771.

Trippel, E. A., and H. H. Harvey. 1989. Missing opportunities to reproduce: an energy dependent or fecundity gaining strategy in white sucker (Catostomus commersoni)? Canadian Journal of Zoology 67(9):2180–2188.

Trippel, E. A., and H. H. Harvey. 1991. Comparison of Methods Used to Estimate Age and Length of Fishes at Sexual Maturity Using Populations of White Sucker (*Catostomus commersoni*). Canadian Journal of Fisheries and Aquatic Sciences 48(8):1446–1459.

Trotter, A. A., D. A. Blewett, R. G. Taylor, and P. W. Stevens. 2012. Migrations of Common Snook from a Tidal River with Implications for Skipped Spawning. Transactions of the American Fisheries Society 141(4):1016–1025.

Valdez, R. A., G. M. Haggerty, K. Richard, and D. Klobucar. 2019. Managed spring runoff to improve nursery floodplain habitat for endangered Rio Grande silvery minnow. Ecohydrology 12(7):e2134.

Verhelst, P., R. Brys, S. J. Cooke, I. Pauwels, M. Rohtla, and J. Reubens. 2023. Enhancing our understanding of fish movement ecology through interdisciplinary and cross-boundary research. Reviews in Fish Biology and Fisheries 33(1):111–135.

Villamizar, N., A. García-Alcazar, and F. J. Sánchez-Vázquez. 2009. Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax)* larvae. Aquaculture 292(1):80–86.

Vitale, F., H. Svedäng, and M. Cardinale. 2006. Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (Gadus morhua) and suggests new proxies for estimating maturity status of individual fish. ICES Journal of Marine Science 63(3):485–492.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467(7315):555–561.

Walburg, C. H., and W. R. Nelson. 1966. Carp, river carpsucker, smallmouth buffalo, and bigmouth buffalo in Lewis and Clark Lake, Missouri River. Bureau of Sport Fisheries and Wildlife.

Ward, P. E. 1963. Geology and ground-water features of salt springs, seeps, and plains in the Arkansas and Red River basins of western Oklahoma and adjacent parts of Kansas and Texas. Open-File Report.

Watkins, C. J., T. J. Ross, M. C. Quist, and R. S. Hardy. 2017. Response of Fish Population Dynamics to Mitigation Activities in a Large Regulated River. Transactions of the American Fisheries Society 146(4):703–715.

Wedekind, C., and C. Küng. 2010. Shift of Spawning Season and Effects of Climate Warming on Developmental Stages of a Grayling (Salmonidae). Conservation Biology 24(5):1418–1423.

Wedgeworth, M., R. Mollenhauer, and S. K. Brewer. 2023. Variation in Prairie Chub hatch relationships across wet and dry years in the upper Red River basin. North American Journal of Fisheries Management 43(5):1246–1259.

Weisberg, S., G. Spangler, and L. S. Richmond. 2010. Mixed effects models for fish growth. Canadian Journal of Fisheries and Aquatic Sciences 67(2):269–277.

Westrheim, S. J., and W. E. Ricker. 1978. Bias in Using an Age–Length Key to Estimate Age-Frequency Distributions. Journal of the Fisheries Research Board of Canada 35(2):184–189.

Whittemore, D. O. 1995. Geochemical Differentiation of Oil and Gas Brine from Other Saltwater Sources Contaminating Water Resources: Case Studies from Kansas and Oklahoma. Environmental Geosciences 2(1):15–31.

Winemiller, K. O. 2005. Life history strategies, population regulation, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 62(4):872–885.

Winemiller, K. O. 2011. Life history strategies, population regulation, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences.

Winemiller, K. O., J. S. Perkin, J. F. Trungale, D. J. Hoeinghaus, G. W. Moore, A. N. Schwalb, Z. A. Mitchell, A. Trimble, C. Reeves, T. B. Hardy, and D. Buzan. 2024. Advancing Environmental Flows Science: Hindcasting and Forecasting Flow–Ecology Relationships. Fisheries 49(8):353–368.

Winemiller, K. O., and K. A. Rose. 1992. Patterns of Life-History Diversification in North American Fishes: implications for Population Regulation. Canadian Journal of Fisheries and Aquatic Sciences 49(10):2196–2218.

Woodling, J. D., E. M. Lopez, T. A. Maldonado, D. O. Norris, and A. M. Vajda. 2006. Intersex and other reproductive disruption of fish in wastewater effluent dominated Colorado streams. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 144(1):10–15.

Worthington, T. A., A. A. Echelle, J. S. Perkin, R. Mollenhauer, N. Farless, J. J. Dyer, D. Logue, and S. K. Brewer. 2018. The emblematic minnows of the North American Great Plains: A synthesis of threats and conservation opportunities. Fish and Fisheries 19(2):271–307.

Wright, P. J. 2013. Methodological challenges to examining the causes of variation in stock reproductive potential. Fisheries Research 138:14–22.

Wright, P. J., and E. A. Trippel. 2009. Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. Fish and Fisheries 10(3):283–304.

York, B., J. D. Schooley, and D. L. Scarnecchia. 2022. Oklahoma Bowfishing Values and Perspectives toward Nongame Fishes and Their Management. North American Journal of Fisheries Management 42(4):1020–1040.

Zemeckis, D. R., M. J. Dean, and S. X. Cadrin. 2014. Spawning Dynamics and Associated Management Implications for Atlantic Cod. North American Journal of Fisheries Management 34(2):424–442.

Zeug, S. C., and K. O. Winemiller. 2008. Relationships between hydrology, spatial heterogeneity, and fish recruitment dynamics in a temperate floodplain river. River Research and Applications 24(1):90–102.