PROJECT TITLE: Distribution and Population Demographics of Invasive Carp in the Lower Red River Basin

GEOGRAPHIC LOCATION: Lower Red River Basin (OK, TX, AR)

LEAD AGENCY: Texas Parks and Wildlife Department (TPWD)

PARTICIPATING AGENCIES: Oklahoma Department of Wildlife Conservation (ODWC), Arkansas Game and Fish Commission (AGFC), USFWS Oklahoma Fish and Wildlife Conservation Office (OKFWCO); contracted researchers - Auburn Cooperative Fish and Wildlife Research Unit (ACFWRU), Texas Tech University (TTU)

STATEMENT OF NEED:
Invasive bigheaded carp pose significant ecological and economic threats to freshwaters around the globe, including in the Mississippi River Basin. Bigheaded carp research activities have primarily focused on large floodplain rivers of the Upper Mississippi River Basin (e.g., Illinois, Mississippi, and Missouri rivers) where substantial advances in understanding their ecology have been achieved. However, substantially less is known about bigheaded carp populations in tributaries of the Lower Mississippi River Basin where they have been studied less frequently (Chapman and Hoff 2011; Ochs et al. 2019). Their presence has been noted across the Lower Mississippi River Basin for a while (Thomas et al. 2011; Rodgers 2019) and sampling and landings data suggest their prevalence is increasing in the Lower Red River Basin (TPWD, ODWC, AGFC, unpublished data). However, there is a general lack of information regarding the population dynamics of bigheaded carps and their effects on native fish communities of the Lower Mississippi River Basin. Understanding occupancy by bigheaded carps in the basin, both spatially and temporally, is vital for directing the management actions. Furthermore, there exists a great need to understand the trajectory of the bigheaded carps' invasion to predict their influences on native fish assemblages within these large tributary basins and associated reservoirs.

The objectives of this project, as outlined in this document, are aligned with and support the goals and objectives of the Lower Mississippi River Asian Carp Control Strategy Framework. This project will initiate a surveillance effort for bigheaded carp at a broad geographic scale across the Lower Red River Basin as well as establish baseline data for native fish assemblages potentially impacted by bigheaded carp needed for future evaluations of deleterious impacts. This project will address the general lack of knowledge of bigheaded carp population dynamics and native fish assemblages in this sub-basin. The intentions of this effort are to aid in early detection, assess population distribution and status, and facilitate future evaluations of deleterious impacts to native fishes. Data on bigheaded carp populations would also inform potential removal efforts. Collaborative efforts of multiple partners and agencies (state, federal, and university) will be implemented to accomplish the project goals and objectives. This project will provide an ongoing, coordinated effort to evaluate bigheaded carp distribution and status in the Lower Red River Basin that will contribute to a better understanding of the status of this species in the Mississippi River Basin as a whole.

PROJECT OBJECTIVES:

1) Determine the spatial and temporal distribution and adult population demographics of invasive bigheaded carp (Silver Carp and Bighead Carp)
2) Establish baseline native and non-native fish assemblage and habitat association data

PROJECT HIGHLIGHTS:

- Frequent, often dramatic shifts in flow regime (i.e., fluctuation between flood and baseflow conditions) limited gear types that could be used effectively and, at times, limited access to sampling sites. Electrofishing, including in combination with gill nets and herding, was found to be the a very effective sampling method in terms of catch rates, catch precision, and fish species collected.
- A total of 42 bigheaded carp were captured in the Red River and tributaries or donated by bowfishermen over the course of this project to date—23 Silver Carp and 19 Bighead Carp. Six male and 13 female Bighead Carp were collected. For Silver Carp, 15 males and 8 females were collected. Additional bigheaded carp were observed jumping or during electrofishing but were not able to be captured.
- No bigheaded carp have been captured to date in the Sulphur River, although conversations with anglers suggest they are sporadically observed in the tailwaters of Wright Patman Dam and two Silver Carp were observed jumping in the Red River confluence but not captured.
- Most captures of bigheaded carp were from connected oxbows, backwater locations, and tributaries; only one Silver Carp was collected from the main channel.
- All bigheaded carp collected were adults, including gravid females; however, no fish younger than age 3 were collected. We were not able to successfully detect age-0 carp either due to detection, lack of spawning in 2021, or other influence such as extensive high flow events. Our 2021 sampling season may be emblematic of an extremely low capture year where adults have chosen not to reproduce.
- The Silver Carp collected ranged in length from 708 mm to 1020 mm (±1- mm, TL), whereas the Bighead Carp ranged from 990 mm to 1245 mm (±1- mm, TL) (Appendix I - Table S1). The Bighead Carp ranged from 3 to 10 years of age, with the most numerous (40%) being 5 years old. The Silver Carp ranged from 3 to 8 years of age with the majority (65%) from 3-4 years old.
- These captured, adult bigheaded carp may not have recruited within the Red River and could originate in a different basin (i.e., Mississippi River) expanding the invasion front. A telemetry effort would be helpful to determine the source of these fishes.
- Native fish assemblage assessment indicated a functionally diverse group of fishes including large river planktivores, benthic invertivores, generalists, and predators. A total of 67 species was collected in the Red River and Tributaries and 43 species were

collected in the Sulphur River. In the Red River, species diversity was greatest in the Arkansas section of the river. Sulphur River fish assemblages exhibited a pronounced shift between flood and baseflow conditions.

METHODS:

Red River and Tributaries (ACFWRU, OKFWCO)

Objective 1. Invasive Carp Population Assessment

Juvenile Carp sampling
We sampled stream reaches approximately 300m in length in the Lower Red River Basin for juvenile carp. Our sites were distributed across tributaries and within the mainstem Red River (Figure 1). Sites were selected based on river access, proximity to USGS stream gauges, and the likelihood of detection of the target species. Our sites were selected approximately 25-100 km downriver of major dams and confluences because this is the suggested length of river needed to allow Carp eggs to develop and hatch while in suspension (Kolar et al. 2007; Garcia et al. 2015). Our sample sites included slackwater habitats such as forewaters, backwaters, side channels, sandbars, and pool complexes. These slackwater habitats are thought to be important nursery areas for a variety of age-0 fish including Bighead Carp and Silver Carp (Jurajda 1999; Love et al. 2017; George et al. 2018). Lastly, discharge and temperature conditions are relatively homogenous across our sites, and the sites are large enough to be considered closed to species immigrations during sampling.

We sampled age-0 Carp using three different gear types during daylight hours. Using a combination of gears diminishes some of the sample bias associated with a single gear approach (Clark et al. 2007). For example, passive gears tend to target more active individuals (Fago 1998). At each site, we set mini-fyke nets, sampled using beach seines, and conducted larval tows. First, we set 3 mini-fyke nets in <2 m of water at locations adjacent to the shoreline to target small-bodied fishes (Eggleton et al. 2010). Mini-fyke nets are commonly used to sample age-0 carp (Wanner and Klumb 2009; Gibson-Reinemer et al. 2017; Williams 2020) and sometimes capture high numbers compared to other gears (Collins et al. 2017). Next, a beach seine was used to sample wadeable habitat across the site using a modified version of the encirclement technique (Bayley and Herendeen 2000). Transects were established throughout wadeable habitat at each site and seine hauls were completed across each transect. Seine hauls were limited to 25m to maintain the efficiency of the gear because longer hauls are less efficient (Lombardi et al. 2014). We quantified total seine distance, seine width, and maximum depth for each haul to calculate the area sampled. We completed a sub-surface larval tow at a representative location of deeper water (i.e., where we could not seine or place fyke nets). Each tow was executed for 10 minutes and the volume of water sampled was quantified using a flow meter (General Oceanics Mechanical Flowmeter Model 2030R) attached to the mouth of the net. We standardized larval tows based on the volume of water filtered by the net. Any samples that could not be identified in the field were preserved in 70% ethanol and brought back to the lab for processing.

Juvenile Carp Habitat

We quantified the physicochemical factors that may be related to bigheaded carp or native fish distributions across multiple spatial scales (i.e., reach, segment, and catchment). The physicochemical factors are divided into detection (i.e., temperature, dissolved oxygen, turbidity, discharge) and occupancy (i.e., salinity, average depth, width:depth, zooplankton biomass, large woody debris, % backwater, % pools, discharge) covariates and identified as those quantified in the field or via existing geospatial data (i.e., distance from dam, distance from confluence, sinuosity, slope, drainage area, lithology). Stream habitat use by fishes is hierarchical where finer levels of organization are nested within coarser landscape constraints (Frissell et al. 1986; Imhof et al. 1996). Coarse scale (e.g., segment and catchment) habitat factors are applied to multiple reaches that occur within the same stream segment or catchment (i.e., nested). For example, finer-scale channel unit conditions (i.e., pH and substrate) used by fish are often influenced by coarse factors (i.e., drainage area and geology) of the surrounding watershed (Mollenhauer et al. 2019). Including coarse-scale habitat factors helps explain fish distributions and account for pseudoreplication caused by sampling multiple sites in the same stream segment or river system (i.e., nested).

We measured several factors across each sample site that described the general water-quality conditions. First, we collected temperature and dissolved oxygen samples at 0.5 m below the water's surface for each site using a multi-parameter water-quality meter (YSI ProDSS). We collected salinity from a well-mixed location of each site approximately 0.5m below the surface. We also measured water clarity using a secchi disk, because turbidity can influence resource use, foraging success, and even provide shelter from predators (Zamor and Grossman 2007; Reichert et al. 2010). To characterize the general conditions of each site, we measured all water-quality parameters three times at each site and averaged these values.

We also quantified the proportion of select channel unit features in each site. Because forewater and backwater habitat are often important nursery habitat for many large river fishes (Galat et al. 2004), we quantified the area of each using a meter tape or rangefinder (Simmons Volt 600 Laser Rangefinder) to measure length and average width. Other slackwater areas such as pools offer low-velocity areas in the main channel (Schwartz and Herricks 2005); therefore, we measured pool area using side-scan sonar (Humminbird Helix 12). The proportion of each of the slackwater channel units will be expressed as a proportion of the available habitat in each site. Because age-0 Carp are associated with large woody debris in some systems (George et al. 2018), we also used side-scan sonar to quantify the percentage of large woody debris following the methods of Gordon et al. (1992).

We quantified several hydraulic variables to describe the fluvial dynamics of our sampling sites. Species often use specific depths within a water column (Lamouroux et al. 1998); therefore, we quantified the average thalweg depth by measuring depth at 10-m increments along the thalweg of the site using side-scan sonar. Further, because the shape of the channel dictates habitat availability (Thomson et al. 2001), we quantified width to depth ratios in each site. We measured three representative wetted width measurements using a rangefinder. Average thalweg depth of the site was then divided by the average widths. We will also obtain discharge data from the nearest USGS stream gauges to apply to sampling sites within the same stream segments to examine both detection and occupancy.

Some habitat metrics will be quantified using existing geospatial data. At the reach-scale, we will quantify distance to the nearest dam by measuring the distance from the most downstream point of our sites to the nearest upstream using National Hydrology Dataset (NHDplus) flowlines and ArcMap spatial analyst. We will also measure distance from our sites and the nearest upstream 5th order tributary. Areas below dams and major tributary confluences are potential spawning locations for carp species (Kolar et al. 2007; George et al. 2018; Camacho et al. 2020). At the stream segment scale, we will use the NHDplus flowlines and ArcMap spatial analyst to calculate stream sinuosity and slope. Sinuosity (i.e., channel migration of meandering rivers) affects fish habitat use including choice of spawning location (Fukushima 2001; Lazarus and Constantine 2013) and will be calculated by dividing the thalweg length by the straight line distance of the segment. (Camana et al. 2016). We will calculate river slope using ArcMap spatial analysis to determine the change in elevation between the upstream and downstream points of each stream segment and divide by the thalweg length (i.e., channel distance measured down the middle of the channel, Bain and Stevenson 1999).

We will also measure several habitat variables that may affect fish distributions at the catchment scale. We will measure drainage area (km^2) upstream of each site (i.e., catchment draining to each site) using NHDplus flow lines to determine the size and relative position of sites within the network. Because catchment lithology controls many local physicochemical conditions (Frissell et al. 1986; Stevenson 1997), we will quantify the dominant lithology that drains to each site. We will use United States Geological Survey's (USGS) National Geologic Map Database and the identify tool in ArcMap to determine the percentage of dominant lithology.

Juvenile Carp Collection

We will collect age-0 Bighead and Silver Carp before they reach 60-mm total length (TL). It is difficult to enumerate daily bands in fish >100 days old (Long and Grabowski 2017). Therefore, we will collect age-0 Bighead and Silver Carp until they reach approximately 60-mm TL as they are estimated to reach the juvenile transition at 36 and 34-mm TL in China, respectively (Chapman 2006). By using a more liberal cutoff, we can determine band counts at the Red River latitude based on our daily ages. Each site will be sampled 1-3 times using seines, mini-fyke nets, and larval tows.

All captured carp will be enumerated and measured; however, for catches with more than 50 individuals, we will take five randomly selected individuals from 5-mm length bins up to 60-mm (e.g., 0-5-mm, 5-10-mm, and 10-15-mm). If catches are less than 50 individuals per site, all fish will be kept for aging. All captured carp will be euthanized using an overdose of tricane methanesulphonate (MS-222) (300 mg/L; Neiffer and Stamper 2009), then preserved in 1-L bottles containing 70% ethanol for future laboratory processing.

Juvenile Carp Otolith Extraction, Processing, and Ageing

If we capture juvenile carp, we will remove and mount lapilli otoliths to estimate hatch dates. Daily band deposition on lapilli otoliths has been validated to estimate spawning dates in age-0 carp (Lohmeyer and Garvey 2009; Williams 2020) and other cyprinid species (e.g., Sharpnose Shiners *Notropis oxyrhynchus*, Smalleye Shiners *Notropis buccula*, and Plains Minnow *Hybognathus placitus*; Durham and Wilde 2008). We will remove lapilli otoliths under a stereo dissection microscope using a fine-tipped probing needle and forceps to remove the otoliths from the top of the skull. Once the otoliths have been removed, we will place them in a petri dish. We will then mount the otoliths to slides using thermoplastic (Lakeside No. 70C, Monee, IL). We will melt the cement on the slide until it is pooled. The otoliths will then be placed convex side down in the cement and allowed to cool at ambient temperature.

The mounted otoliths will be polished in a circular pattern to allow band enumeration. We will polish the otoliths using diamond lapping paper (Diamond Lapping Film, 8" diameter, plain backing, Electron Microscopy Sciences, Hatfield, PA). Polishing will be complete once all the bands become visible at the nucleus (Campana and Neilson 1985).

We will quantify daily bands of age-0 Carp to estimate spawning dates and average daily incremental growth rates. We will enumerate daily bands using a compound microscope. Daily bands will be counted from the outer edge toward the center to enhance accuracy (Campana and Moksness 1991). Two independent readers will count bands and record estimates. Band counts within 10% difference between readers will be averaged, if >10% differences exist, then readers will attempt to reach a consensus. If a consensus can still not be reached, the otolith will be removed from the dataset. We will also measure otolith radii (1-µm TL) from the central point of the otolith nucleus to the outer edge to estimate growth (Infinity analyze-7 software, Lumenera infinity 2 camera). We will also measure radii from the nucleus to the edge of otolith bands at 10-day increments to calculate average daily incremental growth (e.g., 0-10, 10-20, 30-40 days). We will estimate hatch dates by subtracting the daily band counts from the date of capture. We will calculate spawn dates by subtracting an additional day from estimated hatch dates (i.e. ~27-29 hour incubation, Chapman and George 2011).

Adult Fish Sampling

We sampled for adult Silver Carp and Bighead Carp at 48 sites (Figure 2) throughout the Lower Red River Basin of Oklahoma, Texas, and Arkansas. Each site was approximately 1.5 to 2 river km (rkm) and was sampled 1-3 times using gillnets, electrofishing, and hoop nets. Access can be problematic in the Lower Red River Basin and thus, sites were selected based on accessibility (i.e., access to private lands and conditions conducive to boat launching) (Figure 2). We also sampled Bois D'Arc Lake in June 2021. For reservoir sampling, we set 3 gillnets 180 feet long and used two electrofishing boats. Gill nets were soaked for 6 hours, and we (in conjunction with TPWD) sampled 10 electrofishing transects for 15 min each (300 sampling minutes).

We sampled adult fishes using a combination of gillnets, hoop nets, and electrofishing because they have been shown useful for sampling both carp species in perceived low-density environments (Butler et al. 2019; Norman and Whitledge 2015). Three experimental sinking gillnets (54.8-m long for mainstem and 30.5-m long for tributary sampling with 8.9, 10.16, and 10.8-cm bar-length mesh panels) and three hoop nets (4.88-m long with a 1.2-m diameter opening) were placed throughout each site. Gillnets were either deployed perpendicular to the shoreline or parallel if large amounts of woody debris were present near the shoreline. One gillnet was placed near each end of the site and the third net placed in the middle of the site at the narrowest portion of the channel to restrict carp movement. Hoop nets were placed parallel to the shoreline with the opening facing downstream in locations that included channel edges and channel crossovers but lacking extensive woody debris. Both gillnets and hoopnets were soaked for approximately 6 h. After net placement, we electrofished using an 80-amp Midwest Lakes Electrofishing Systems shocking unit (Polo, Michigan). We used standard AFS electrofishing settings based on conductivity (i.e., though we tried several others- see below). Water conductivity in the tributaries was much lower than the mainstem Red River. As such, voltage was typically set to high range (pulsed DC current, >300 volts, 60Hz) for tributaries and low range (pulsed DC current, <300 volts, 60Hz) for the mainstem sites. Beginning at the upriver end of the site, the boat traversed downstream in a cloverleaf pattern with electrical current applied for 10-sec with 5-sec “off peddle” intervals to increase the effectiveness of capturing Silver Carp and to attempt to drive fish into the nets and shoreline (Bouska et al. 2017). Electrofishing continued until the entirety of the site was sampled. Invasive carp collected by the OKFWCO (1; jumped into boat) and bowfisherman were also obtained.

Before we established our electrofishing protocol, we used several electrofishing settings at sites where carp were observed on previous occasions or during that trip. During experimental electrofishing trials, we used pulsed DC current at both low and high frequencies, with Hz ranging from 15 to 60 and a target amperage of 4 and 20, respectively. Boat electrofishing was also used in an attempt to drive carp into set nets. All carp collected during our sampling events were euthanized. Total length (mm, +/- 1 mm), and weight (g, +/- 10 g), were recorded for captured carp, except for a few captured while our scale was malfunctioning.

Adult Fish Habitat

We quantified the physicochemical factors that may be related to Carp distributions across multiple spatial scales. We quantified habitat factors at the catchment, segment, and site scales. The habitat factors were either collected in the field or obtained using existing geospatial data.

The catchment scale habitat factors we will consider are drainage area, disturbance, and lithology. Drainage area (km^2) is a coarse scale habitat factor that influences fish distributions, community structure, and species richness (Osborne and Wiley 1992; Newall and Magnuson 1999; Griffiths 2018) and will be measured using National Hydrography Database Plus (NHDplus) (https://apps.nationalmap.gov/downloader/#/) flow lines in ArcMap 10.6. Disturbance can affect fish community structure and distribution by altering nutrient flow and habitat availability and lead to decreased diversity throughout trophic levels (Scrimgeour et al. 2008; Wang et al. 2008; Johnson and Angeler 2014). We will use ArcMap 10.6 to create a buffer of the floodplain and classify land use type using the National Land Cover Database (NLCD). Each land type will receive a disturbance value using the Landscape Development Index (LDI) (Brown and Vivas 2005). Lithology constitutes the predominant bedrock of a riverscape and can alter sedimentation, pH, and control the macro and micronutrient cycling load within a catchment (Sarkar et al. 2007; Zeng et al. 2007; McDowell et al. 2013; Glaus et al. 2019). We will analyze lithology by classifying the dominant rock type in the catchment using United States Geological Survey’s (USGS) National Geologic Map Database (https://mrdata.usgs.gov/geology/state/) and the identify tool in ArcMap 10.6.

The segment scale habitat factors we will quantify are sinuosity, slope, elevation, temperature, and discharge. Stream sinuosity, the ratio of the straight-line segment of river to the channel distance (Rowe et al. 2009), is associated with habitat complexity (e.g., woody debris, canopy cover), backwater connection, and overall habitat variability (Nagayama and Nakamura 2018). Sinuous reaches in a river system can be areas of particular importance for specific species or life-history strategies (e.g., *Hucho perryi*; Fukushima et al. 2001). Sinuosity will be calculated by dividing the river kilometer (rkm) distance by the straight-line distance using the distance tool in ArcMap 10.6. Slope can affect the species richness of a river by influencing water velocity, channel morphology, and substrate which are often correlated with the stream gradient (Camana et al. 2016). We will quantify slope using spatial analysis in ArcMap 10.6 by dividing the change in elevation from the upstream to downstream end of the segment by the segment length. Elevation is commonly used to describe changes in climate (Bozek and Hubert 1992) and influences channel gradient, water temperature, and species diversity and distributions

(Quist et al. 2004a; Quist et al. 2004b). We will calculate elevation using ArcMap 10.6 spatial analysis. Temperature controls fish metabolism and can cause fish to alter distributions to meet the requirements for growth, forage, spawning, and social interactions (Sloat et al. 2005; Sloat and Osterback 2013; Kuparinen et al. 2011). We will collect temperature across stream segments using Onset HOBO MX2201 Pendant Wireless Temperature Data Loggers (Bourne, Massachusetts) (Figure 1). Discharge affects fish density and occurrence, habitat associations, recruitment success, and can be altered for mitigation purposes (Valdez et al. 2001; Gillette et al. 2006; Work et al. 2017; Love et al. 2017; Bašić et al. 2018). We will obtain discharge data from the nearest USGS stream gauges and calculate the median discharge for the sampling periods.

At the reach scale (i.e., site), we will calculate the distance to the nearest upstream dam, percent slackwater, width to depth ratios, salinity, and chlorophyl-a. Dam construction changes both biotic and abiotic riverine attributes (Catalano et al. 2007). Nutrients important for biological function, such as phosphorus, are sequestered by dams (Maavara et al. 2015). Effects of dams on fish assemblages and habitat include altered flows, nutrient availability, habitat degradation, reduced spawning potential (Catalano et al, 2007; Wang et al. 2011; Rolls et al. 2013), and alter fish assemblage structure (Wang et al. 2011). We will use NHDplus flowlines and ArcMap 10.6 spatial analyst to quantify the distance from the site to the nearest upstream dam. Slackwaters, areas of decreased velocity and depth (Vietz et al. 2013), are used as a refuge by juvenile fishes for increased growth and forage potential (Humphries et al. 2006). These habitats help facilitate the growth of macrophytes that are intolerant of highly fluctuating discharge (Nielsen et al. 2009) and increase habitat complexity within sites. We calculated the percent slackwater by taking width and length measurement in the field using a handheld rangefinder. Width to depth ratios describe the structural variation of a stream channel where increasing ratios are emblematic of wider and shallower stream channels (Gordon et al. 1992; Dunham et al. 2002).

Fishes have specific conductivity tolerances and will use habitat within their salinity range over preferred dissolved oxygen and temperature ranges (e.g., *Acipenser brevirostrum*; Farrae et al. 2014). Abnormal salinity environments can lead to poor osmoregulation and eventual death (Oto et al. 2017). We collected three salinity measurements (ppt) at the upper, middle, and bottom portions of the site using a Yellow Springs Instrument (YSI) (Yellow Springs, Ohio). Chlorophyll-a (chl-a) is widely used as a surrogate for productivity and algal biomass (Pinder et. al 1997). Carp are omnivores, consuming both zooplankton and phytoplankton (Calkins et al. 2012), and may be associated with varying chl-a densities in the basin. A water sample was collected using an integrating tube sampler to sample the top 2 m of the water column at the most downstream end of each site (Raikow et. al 2004). Within 24 h of water collection, three 500-mL subsamples were then placed into a 47-mm diameter filter tower (PALL, Port Washington, New York) and filtered through a 1-µm glass fiber filter. The filter was then placed into a light-proof container and frozen for later laboratory analysis. In the laboratory, we will soak the filters in 95% ethanol and then filter a second time. Chl-a will then be estimated using a fluorometer.

Egg Estimates from Female Carp

We estimated the total eggs contained within the ovary of each female collected via sampling. We began by taking the total weight (g, +/- 1 g) of the ovary. We then took subsamples (0.3 – 0.5 g) from the anterior, middle, and posterior of the ovary and enumerated the eggs for each subsample. From these enumerated subsamples, we then estimated the average eggs per gram and extrapolated that to the respective ovary weight.

Adult Otolith Extraction, Processing, Ageing, and Growth

Lapilli otoliths were removed for age and growth analysis following Seibert and Phelps (2013). The lapilli otoliths, located at the posterior of the skull, were accessed using a hacksaw. A cut was made through the top of skull at the juncture of the preopercle and opercula. Otoliths were then removed using forceps and placed into coin envelopes marked with an individual fish number for later laboratory analysis.

In the laboratory, otoliths were sectioned and prepared for age estimation. First, the nucleus was marked on the exterior of the otolith with a ballpoint pen. The otolith was then placed in epoxy resin and allowed to harden for 24 h. After hardening, the otolith was sectioned with an isomet saw (Buehler IsoMet Low Speed Precision Cutter, Lake Bluff, Illinois) and a single 0.5 to 0.6-mm section was removed from the center of the otolith ensuring the inclusion of the nucleus. The sectioned otolith was then polished on each side with diamond lapping paper (Diamond Lapping Film, 8" diameter, plain backing, Electron Microscopy Sciences, Hatfield, PA). Subsequently, the sectioned otolith was mounted onto a slide with thermoplastic. The slide was then placed under a dissecting microscope equipped with a light source and imaged with a Luminera Infinity 2 (Tyledyne Luminera, Ontario). The images were saved for later age and growth analysis.

Two readers separately enumerated the annuli of the imaged otolith to age each fish. An annulus is a pair of translucent and opaque bands that continue uninterrupted around the nucleus (Dzul et al. 2012). The edge was counted as an annulus for fish captured prior to August 31st because an annulus will be created during the spawning season (Minard 1998; Ericksen 1999). There was no prior knowledge of the length, weight, or age estimation of either reader to avoid reader bias. If there was no consensus on the age of a fish, the readers discussed how they derived the age until a consensus was reached. We analyzed the between-reader agreement and mean-CV of otoliths compared to other structures to ensure that lapilli otoliths were the proper structure to use.

We will quantify the proportional growth of carp to determine how growth relates to their spatial distribution. The annuli and edge will be analyzed for proportional growth using Infinity Analyze 7 software 2 (Tyledyne Luminera, Ontario; Quist and Isermann 2017). Otoliths will be measured for incremental growth along the same axis. The focus will be identified, and then individual radii distances will be recorded from the focus longitudinally to the outside edge of each opaque band to determine individual year growth (Weisberg et al. 2010). The distance from the focus to the edge will be used to relate incremental growth to fish length. Back calculation for age-at-length will be conducted using the Fraser-Lea method if the body-scale relationship is strongly correlated (R2>0.80, Quist and Guy 2001). If the body-scale relationship is weak, then we will use the Dahl-Lea method (Quist and Isermann 2017). A von Bertalanffy growth model (vBGM) will be fit to both Silver Carp and Bighead Carp to assess growth using the previously collected back-calculated age-at-length data. The vBGM will be implemented in a hierarchical model using maximum likelihood to compare the growth parameters L and k spatially across ecoregions of the Lower Red River Basin. The ecoregions will include the USEPA-defined level III ecoregions: Central Great Plains, Cross Timber, and the South-Central Plains (Longing and Haggard 2010).

Objective 2. Native fish Assemblage Assessment

Native Fish Sampling

At each juvenile and adult site, we sampled native fishes using multiple gears as described for Objective 1. Briefly, sites targeting juvenile and smaller-bodied fishes was conducted using three gear types: mini-fyke nets, beach seines, and larval tows. Mini-fyke nets were set in 1-2 m of water for approximately 6 h during daylight. Beach seining was conducted within areas of the site that allowed for seining (i.e., depths <1m). Larval tows were conducted by towing an ichthyoplankton net upstream for approximately 10 min at each site. Identifiable species were enumerated and recorded for each gear used. All larval individuals and unknown species were preserved in a 70% ethanol solution for later identification in the lab. At sites targeting larger-bodied fishes, we conducted electrofishing and net surveys. Three gill nets and three hoop nets were placed throughout each site to soak for approximately 6 h. Following net placement, the site was sampled via boat electrofishing. All sampled fish were identified to species, and the sampling method associated with each catch was recorded.

Native Fish Habitat

At each site, we quantified physicochemical factors that may also be related to native fish distributions as described for Objective 1. Briefly, we collected factors that are divided into detection and occupancy covariates. For juvenile and smaller-bodied fishes we collected: temperature (°C), dissolved oxygen (mg/L), turbidity (cm), discharge (m^3/s), salinity (ppt), average depth (m), width-to-depth ratio (m), zooplankton biomass (µg), large woody debris (%), forewater/backwater (%), pools (%). We will also collect geospatial covariates, which include distance from dam, distance from confluence, sinuosity, slope, drainage area, and lithology. For adult and larger-bodied fishes we collected: chlorophyll-a (mg/L), conductivity (µ/S), salinity (ppt), temperature (°C), dissolved oxygen (mg/L), turbidity (cm), discharge (m^3/s), max depth (m), and width-to-depth ratio (m). We will also calculate distance from dam, distance from confluence, sinuosity, slope, elevation, drainage area, disturbance, and lithology using existing geospatial data and tools.

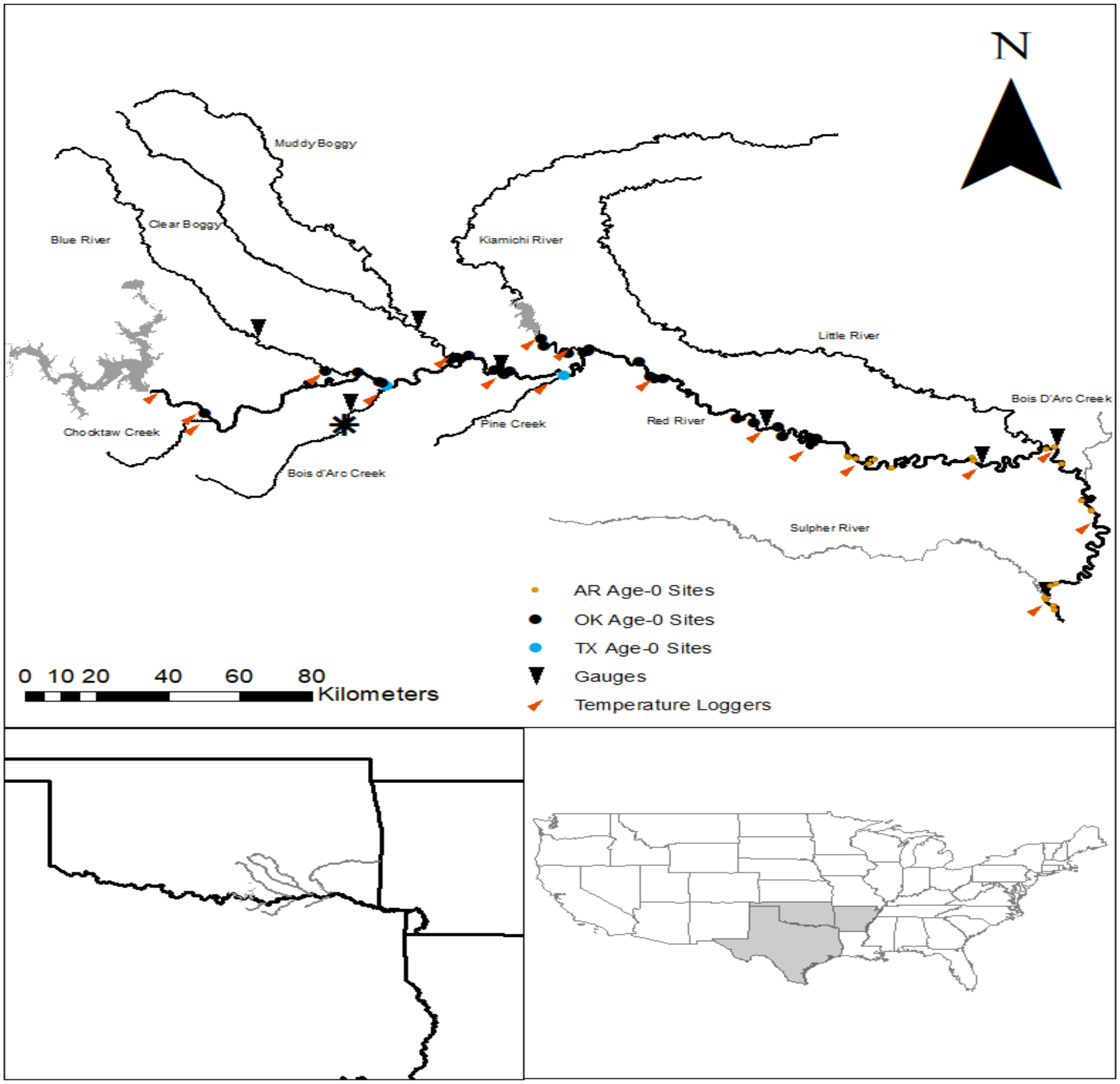

Figure 1. Age-0 fish sampling locations (circles) in the Lower Red River Basin. The circle colors reflect the state where the sample site was located (blue = TX, black = OK, orange = AR). The gray lines represent major rivers with black arrows denoting U.S. Geological Survey stream gages and the red arrow denoting temperature loggers. Black asterisk denotes Bois d'Arc Lake location. Each site was sampled 1-3 times using seines, mini-fyke nets, and larval tows.

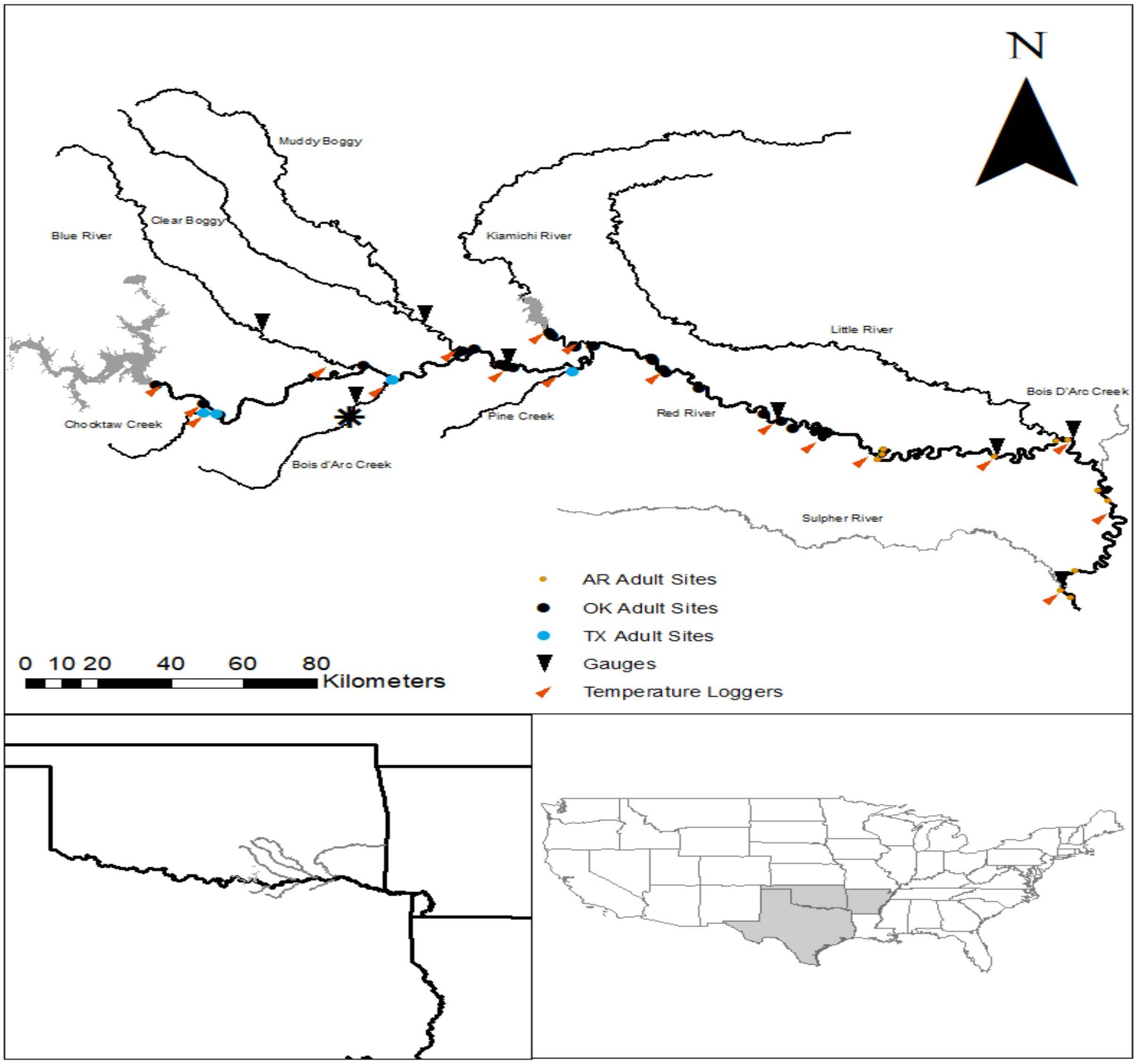

Figure 2. Adult fish sampling locations (circles) in the Lower Red River Basin. The circle colors reflect the state where the sample site is located. The circle colors reflect the state where the sample site was located (blue = TX, black = OK, orange = AR). The gray lines represent major rivers with black arrows denoting U.S. Geological Survey stream gauges and the red arrow denoting temperature loggers. Each site was sampled 1-3 times using gillnets, electrofishing, and hoop nets.

Sulphur River (TTU)

Objectives 1 & 2. Invasive Carp Population and Native Fish Assemblage Assessment

Methods did not differ between objectives with the exception that invasive carp would not be released, and thus these methods are presented together in a single section.

River Hydrology

River hydrology was examined in relation to fish data. Hydrographs of the Sulphur River and mainstem Red River were constructed for all of 2021. Flow conditions were examined to better understand how the regulated flows impact invasive species management and the native fish community. River stage heights were visually assessed from USGS gauging stations at the tailwater of the Wright Patman Dam (USGS station: 07344210) and at the confluence of the Sulphur and Red Rivers (USGS station: 07344370). Visual examination of data was sufficient to characterize periods of flooding and baseflow conditions, which were incorporated into data analyses. Although characterization of Sulphur River hydrology was not an explicit objective, it is integral component of each objective.

Fish Sampling

Unlike the Mississippi and its large tributaries, the Sulphur River is a mid-sized river, and thus, comparatively smaller in channel width, discharge, and other features. Fish were sampled at a total of six sites. Five sites were located on the river and the sixth site was located within Wright Patman Lake (Figure 3). For river sites, reaches were approximately 3.5 - 4 rkm in length. For the lake site, we selected habitat approximately 20-50 meters offshore so depths were similar to the river and to ensure boat electrofishing was effective. Sampling was distributed over a similar 3-4 km distance across the lake for consistency. In large rivers with complex river-floodplain configurations, Asian carp rapidly move amongst habitats, making their capture more difficult. In smaller systems like the Sulphur River, the lateral movements of bigheaded carp are more constrained by the width of the channel. Consequently, gears can fish a greater proportion of cross-sectional area of the river channel. Tailwater and confluence habitats were prioritized to increase the likelihood of capturing bigheaded carp in the Sulphur River. We also identified an oxbow lake that provided suitable habitat for sampling. To sample these habitat types, a combination of gill nets, pulsed-DC electrofishing, herding methods, and hoop nets were used to capture bigheaded carp and other fishes in the Sulphur River.

Pulsed-DC boat electrofishing (Smith-Root Inc, Apex) was conducted at each sampling location (minimum 3 transects per site; 10 mins. pedal time per transect) to capture fishes. Pulsed-DC electrofishing has been an effective means of sampling juvenile and adult bigheaded carp across the Mississippi River Basin (Collins et al. 2015; Culver and Chick 2015; Collins et al. 2017). For each transect, the boat traversed the river channel to cover dominant habitats to capture fishes within the river. In doing so, riverbank, channel margin, and thalweg habitats were sampled in an integrative manner. Electrofishing settings ranged from 100-375 volts, 25% duty cycle, 25% frequency, 60 pulses/s, with amps ranging from 4-10. Voltages were adjusted based on river conductivity.

Entanglement gears are effective at catching carp. However, gill and trammel nets are not always suitable for sampling during flood conditions because drifting logs can quickly destroy them. Indeed, several nets were damaged or destroyed during early sampling during the project. Recent methodological developments have shown that hybrid herding approaches can be effective at catching and detecting bigheaded carps (Butler et al. 2019; Ridgway et al. 2021). Herding approaches use sound or electricity to drive fish into a gill net. The gill nets are deployed for 10 mins during herding sampling whereas traditional gill net sets can sit for >2 hours (4-8 hours is common). Because of the shorter duration in the water, there is the reduced chance that the net will be destroyed by drifting logs. However, the shortened duration may limit gill net effectiveness. Thus, TTU will assess differences in catch rates, species captured, and sampling precision. When flow and habitat conditions permitted, experimental AFS gill nets (monofilament; multi-panel, varied meshes; Duluth Nets) were deployed and the boat herded fish into nets following procedures described by Butler et al. (2019).

Hoop nets were set parallel to the shoreline to capture fish moving upriver. Hoop nets were 1.07-m in diameter and 4.27-m in length when fully extended, with square mesh sizes of 6.4 cm (Memphis Net & Twine). All hoop nets remained deployed in the water for 4-hour periods before removal to avoid damage and minimize by-catch mortality of certain reptiles. Sites with relatively shallow depths (1-2 m) were selected for all hoop net sets. Hoop nets were spaced approximately 250-m apart to avoid potential gear interference.

Juvenile fish sampling was conducted through a combination of seining, mini-fyke netting, and pulsed-DC boat electrofishing. Seining and mini-fyke netting was only possible during low flow conditions due risk of damage to gears and because of safety concerns. Because of river channel form (e.g., steep banks) and habitat conditions (e.g., dense logs/branches) at our sample sites, seining yielded only a few individual fish. Notably, our large bag-seine yielded no fish because it was frequently obstructed by woody debris. A shorter seine (10-ft length) was subsequently selected and still performed poorly in the deeper scour pools because depths were 2-5 meters, which hindered sampling efficiency. Because of supply chain issues, delivery of mini-fykes was substantially delayed (netting, frame materials), limiting our usage in 2021. Given so few fish were collected, we do not report values because such comparisons are biased because of extenuating factors. Despite these setbacks, pulsed-DC electrofishing was effective and captured many small-bodied fishes the Sulphur River because we could effectively shock in woody debris piles and deeper scour pools. For example, approximately 50% of all captured fishes were <150 mm, which is a size range characteristic of juvenile bigheaded carp in the Upper Mississippi River (Culver and Chick 2015; Collins et al. 2017). Our integrative sampling of nearshore and thalweg microhabitats collected large numbers of small-bodied fishes including juvenile Gizzard Shad, Lepomis species, minnows, and shiners.

Habitat assessments were conducted concurrently with fish sampling at each site during each sampling event. Surface waters were collected (100 ml; 4 subsamples per site, per date) to determine the concentration of phytoplankton at sampling locations. Additionally, water temperature (°C), dissolved oxygen (mg/L), and conductivity (µS) were recorded at each site. Plankton samples were preserved for future analysis to determine spatial patterns of plankton in relation to the dam and examine data in relation to relative abundance of bigheaded carp (Williamson & Garvey 2005). Data may be needed as explanatory covariates to analyze catch rates of bigheaded carp in gears along the Sulphur River to assess whether catch rates or community composition are influenced by food availability. Habitat data were included in fish community analyses to identify potential drivers/environmental gradients of community change in the Sulphur River (see Data Analysis).

All individual fish were identified and measured for total length (mm) and weight (g or kg). Length-weight data were recorded for all fish to determine average body size and to construct baseline length-mass relationships to track changes in body condition of important native species (Bigmouth Buffalo, Gizzard Shad; Irons et al. 2007). All native fish were released after processing. If captured, bigheaded carps would be retained for collection of other information.

If bigheaded carps are collected, individuals will be dissected to determine an individual's sex and overall population sex ratio. Gonads will be removed to determine weight and GSI values. Gonads will be weighed (g), and GSI (GSI = 100 ∗ gonad weight [g]/body weight [g]) will be calculated for males and females (Crim & Glebe 1990). Developmental stages of both genders will be assessed for collected individuals to determine reproduction potential within the Sulphur River. Such assessments can be used to identify whether females spawned once, spawned multiple times, or reabsorbed their eggs (Papoulias et al. 2006; Camacho 2016). Information can be examined relative to hydrographs and water temperatures to assess correlations between potential spawning events and environmental cues. Total numbers of eggs will be determined based on density-weight relationships for each female captured. The ova from a ~1 g sample of the ovaries will be enumerated and multiplied by the weight of both ovaries to estimate fecundity (Crim & Glebe 1990). Findings will be scaled to the population to determine the potential reproductive output by the population.

Otoliths will be extracted to determine annual growth rates, associated age-length relationships of the population, and relationships with gonad weights. Potential hybrids (Silver carp x Bighead carp) will be visually identified by inspecting the gills rakers for prominent "twisting" deformations (Lamer et al. 2010). Fin clips or liver samples from each bigheaded carp would be collected and archived for potential future DNA analysis to assess the degree of hybridization within the sampled population.

Data Analysis

Catch data were summarized over the 2021 season. Catch rates (CPUE, # per 10 mins) of bigheaded carp and native fishes were calculated for the entire Sulphur River for each gear type using boat electrofishing data. Catch rates were aggregated into 'flooding' and 'baseflow' conditions for easy of comparison. Additionally, average lengths were summarized to represent the range of sizes encountered. Additionally, catch rates were contrasted among gears to determine the most effective gear for sampling the Sulphur River. Due to high flow conditions, hoop nets could not reliably be deployed. Thus, gear comparisons were made only during low flow conditions when all gears could be deployed concurrently. Catch rates were log10 transformed and tested for differences using ANOVA ($\alpha = 0.05$). Seasonal and annual changes in the fish assemblage will be monitored to establish an ecological baseline.

The Sulphur River fish assemblage was also analyzed. Electrofishing was the most reliable means of fish sampling (see below). Therefore, electrofishing data (CPUE) were used to analyze the structure and dynamics of the Sulphur River fish assemblage. First, the capture of new fish species through time was examined. Afterwards, non-metric multidimensional scaling was used to examine compositional differences in the fish community across sample sites and months (PC-ORD 7; Bray-Curtis distance). Habitat variables from each site/date (temperature (°C), chlorophyll-a (mg/L), conductivity (µS), dissolved oxygen (mg/L), and transect depth (m)) were examined to determine if gradients of habitat conditions explained changes in fish assemblages. In addition, river hydrology conditions (categorical: flooding, baseflow) were included in the analysis to account for potential drivers of fish community changes. To determine whether the structure of the fish assemblage differed between flooding and baseflow conditions, we applied the multi-response permutation procedure (MRPP; bootstrapped 5000 iterations) to test if groups differed statistically ($\alpha = 0.05$). Finally, we used Indicator Species Analysis (ISA) to identify certain taxa that are characteristic of each flow condition. Indicator species analysis is an analytic approach for identifying species found mostly in a single category, location, or time. ISA was used to evaluate fish species indicative of flood or baseflow conditions within the Sulphur River.

Body condition indices were created from measurements of Bigmouth Buffalo and Gizzard Shad lengths and weights. Fish body condition is a useful and simple indicator for tracking changes in fish populations. For example, changes in body condition of Bigmouth Buffalo and Gizzard Shad have been used to document the negative effects of bigheaded carps in the La Grange pool of the Illinois River (Upper Mississippi River; Irons et al. 2007). Using data from 2021, we first developed separate linear models of length-weight relationships for Gizzard Shad (size range: 95 - 414 mm) and Bigmouth Buffalo (size range: 287 - 870 mm). Length (mm) and weight (g) measurements were log10 transformed prior to analysis. Linear models were then created. Each model serves as an ecological indicator and provide a baseline representation of body condition in the Sulphur River system. If bigheaded carp populations increase in the Sulphur River, future weight-length measurements can be used to track potential shifts in body condition. If few or no bigheaded carp are captured, additional length and weight measurements will be included to make the models robust.

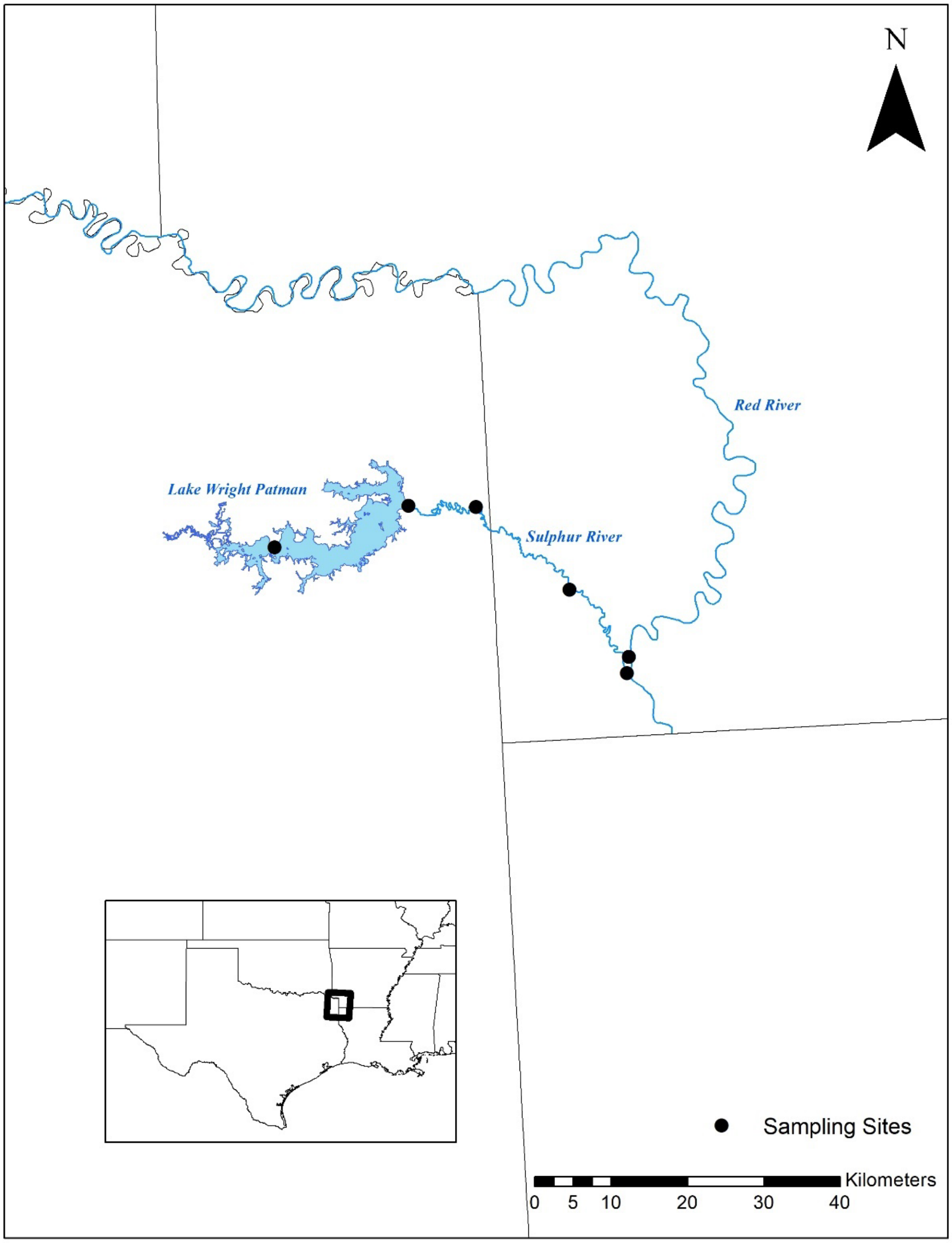

Figure 3. Sulphur River invasive carp sampling sites in Texas and Arkansas.

RESULTS:

Red River and Tributaries (ACFWRU, OKFWCO)

Objective 1. Invasive Carp Population Assessment

We captured, or obtained from bowfishermen, 42 bigheaded carp throughout the Lower Red River Basin, 19 in the mainstem Red River and 23 in tributaries. Most carp captured in the mainstem Red River were sampled from connected oxbows and backwater locations (Appendix I - Table S1). Thus far, only three Silver Carp have been collected in the main channel of the Red River as opposed to these oxbows and backwaters. Carp from the mainstem Red River comprised 11 Silver Carp and 8 Bighead Carp. Of the 23 Carp collected from tributaries, the majority (i.e., 18) were captured in Choctaw Creek where detection appears higher than the other deeper-water tributaries. The carp that were collected from the Red River tributaries comprised 11 Bighead Carp and 12 Silver Carp. Both species were observed during surveys in which neither species were captured (Appendix I - Table S2). Silver Carp were observed at each site located in the Muddy Boggy. Additionally, numerous other Silver Carp (>10) were observed jumping throughout each site in Choctaw Creek. One Bighead Carp was observed during electrofishing at Pine Creek, though the individual was not successfully netted (Appendix I - Table S2). Bois d'Arc Lake was sampled for carp via electrofishing and gillnets in June 2021; no carp were observed or captured during sampling.

All Silver Carp and Bighead Carp collected were adults. No age-0 Silver Carp or Bighead Carp were observed. The Silver Carp collected ranged in length from 708 mm to 1020 mm (±1- mm, TL), whereas the Bighead Carp ranged from 925 mm to 1245 mm (±1- mm, TL) (Appendix I - Table S1). Six male and 13 female Bighead Carp were collected. For Silver Carp, 15 males and 8 females were collected.

The ovaries of female carp species occupied much of the body cavity and were full of well-developed eggs. We estimated egg totals for 4 Bighead Carp and 8 Silver Carp (Appendix I - Table S1). Bighead Carp egg estimates ranged from 256,313 eggs to 722,638 eggs. Silver Carp egg estimates ranged from 233,739 eggs to 1,110,147 eggs. Although Bighead Carp were larger than Silver Carp, the Silver Carp had a higher average total egg estimate (713,587) compared to Bighead Carp (486,897).

The lapilli otolith had the highest between-reader agreement of 0.81 for Silver Carp and 0.72 for Bighead Carp and lowest mean-CV of 2.82 for Silver Carp and 3.47 for Bighead Carp. We aged all Bighead Carp and Silver Carp (Appendix I - Table S1). The Bighead Carp ranged from 3 to 14 years of age, with the most numerous being 5 years old. The Silver Carp ranged from 3 to 14 years of age with the majority from 3-4 years old.

Objective 2. Native Fish Assemblage Assessment

Habitat metrics are currently being compiled to relate to detection and occupancy of native fishes. We placed temperature loggers at sites in the tributaries and the mainstem Red River (Figure 1). Several conductivity loggers have been placed at sites, and we are in the process of getting the remaining loggers set. One temperature logger and one conductivity logger from below Lake Texoma on the mainstem Red River were stolen from their mounted location within the river. We will find a new location near that site to place new loggers.

Habitat differences were evident when comparing the Red River of Oklahoma and Arkansas and tributary sites. On average, Red River sites in Arkansas were deeper and more narrow (smaller wetted widths) compared to mainstem Red River sites in Oklahoma. Additionally, the Arkansas portion of the Red River contained more available backwater habitats due to the presence of dikes. As such, species collection data were divided into three groups based on habitat differences: the Arkansas portion of the Red River, the Oklahoma portion of the Red River, and major tributaries of the Red River sampled. We did not have evidence to suggest many of these riverine populations differed based on the changes in river morphology (i.e., between AR and OK). Arkansas allows regulated commercial fishing, whereas Oklahoma does not and Texas currently has no permitted commercial nongame fishing occurring in the study area, thereby possibly affecting population numbers. However, no differences in length-weight relationships were observed when plotted separately indicating these are likely populations that mix or regulations are not affecting length distributions.

A total of 59,955 fishes, comprising 67 species and 39 genera, were identified during sampling of the Lower Red River Basin. Vouchered fishes have been reviewed in the laboratory. Of the three river sections sampled, species diversity was highest in the Arkansas section of the Red River (63 species, Appendix I – Table S3), followed by the Oklahoma section of the Red River (54 species, Appendix I – Table S4), and finally the major tributaries sampled (49 species, Appendix I – Table S5). The most abundant species was Red Shiner (32,790), followed by Bullhead Minnow (6,341), Western Mosquitofish (3,845), and Inland Silverside (2,713). Of the 67 fish species, four were non-native including Bighead Carp, Silver Carp, Common Carp, and Grass Carp. The genus that contained the most species collected was Lepomis (7 species). As the weather got cooler and water levels dropped, we observed an increase in capture of several more species that are considered relatively rare in the basin including Blue Sucker and Shovelnose Sturgeon.

Sulphur River (TTU)

Sulphur River Hydrology

The flow regime of the Sulphur River existed primarily in flood or baseflow conditions during 2021 (Figure 4). Flow regulation resulting from dam operations at the Lake Wright Patman impoundment rapidly altered river flow conditions during the year. The transition from spring flood to summer baseflow conditions entailed a four-fold reduction in stage height, dropping from ~30 ft to ~7 ft over several days. Such pronounced changes in water levels can affect fish community composition and influence effectiveness of sampling (see Objective 2).

A key component of the sampling regime was to identify habitats that would increase the likelihood of capturing bigheaded carps and other fishes. We identified and sampled locations that had side channel and oxbows habitats. Unfortunately, the drop in water levels eliminated connectivity to the oxbow lake and emergency spillway side channel. During low flows, we could no longer access the oxbow lake habitat. In addition, both the northern side channel and the emergency spillway side channel at the dam (south of main channel) were inaccessible at low flows.

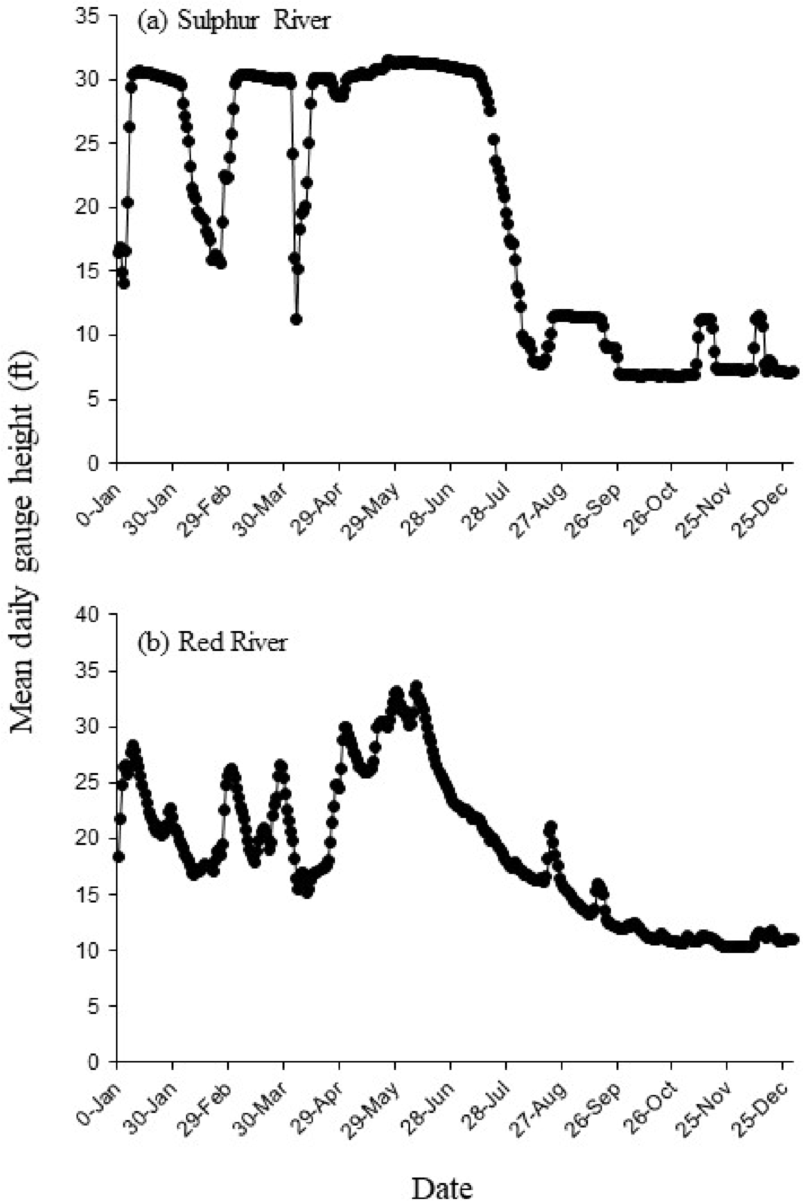

Figure 1. Hydrograph of the (a) Sulphur River (USGS station: 07344210) and (b) Red River (USGS station: 07344370) through 2021. Note the abrupt transitions on the Sulphur River flow regime resulting from Army Corps dam operations.

Objective 1. Invasive Carp Population Assessment

The Sulphur River was sampled extensively during 2021, with a total of seven sampling events. A total of 22.3 hours of electrofishing pedal time was spent capturing fishes along the river. Additionally, 4.6 hours of pedal time was spent herding fishes into nets with the electrofishing boat. Hoop net sampling started during periods of low flow. A total of 284 hours of set time was accumulated from August through November. Flood conditions and associated drifting logs damaged or destroyed several gill nets during sampling. We determined that extended gill net sets (>4 hrs.) were not appropriate for a mid-sized river.

Despite the intensive sampling effort, no bigheaded carp were captured along the 72 km segment of the Sulphur River. Consequently, comparison of catch rates among gears was not possible. Moreover, evaluation of other population characteristics of bigheaded carps (sex ratios, ages, etc.) was not possible. Conversations with recreational anglers, including bowfishing anglers, revealed that fishes resembling bigheaded carps are sporadically observed in the tailwaters of Wright Patman Dam. TTU has been communicating with local angling groups face-to-face and through social media to encourage reporting of catches of potential bigheaded carps so that we can verify the species.

Non-native Common Carp were frequently encountered along the Sulphur River. DC boat electrofishing was the most effective means of capturing Common Carp in the Sulphur River system. No Common Carp were captured in gill nets or hoop nets. In contrast, one Common Carp was collected via herding and 29 via electrofishing. Body sizes were relatively consistent over 2021, ranging from an average of 560 to 747 mm (total length). No small bodied, juvenile Common Carp were encountered during sampling.

Objective 2. Native Fish Assemblage Assessment

Adaptive sampling approaches/methods were necessary because of the river flow conditions. During flood conditions, multiple nets were lost or destroyed due to the prevalence of drifting logs and other materials. During such conditions, deployment of gill nets and hoop nets for >1 hour was infeasible. We adapted our sampling approach and used herding techniques to drive fishes into deployed gill nets; gill nets were deployed and TTU used the electric current (10 mins pedal time) from the electrofishing unit to drive fish into the net. This approach minimized the time nets are exposed to drifting debris. Consequently, we experienced less gill net damage during this form of sampling. During flooding, traditional boat electrofishing and herding were determined to be the only effective means of sampling during these river conditions. At low flows, boat electrofishing, herding, and hoop netting were feasible.

Juvenile fish sampling was hindered flooding and other extenuating issues. Seining captured <20 total fish during multiple attempts. Mini-fyke were set in deeper pools (2-4 meters) and captured 50 individuals total. In contrast, pulsed-DC boat electrofishing was effective at capturing small fishes in flood and baseflow conditions. Approximately 50% of all captured fishes were <150 mm, which is a size range characteristic of juvenile bigheaded carp in the Upper Mississippi River (e.g., Culver and Chick 2015; Collins et al. 2017). Integrative sampling of nearshore and thalweg microhabitats within transects collected large numbers of small-bodied fishes including juvenile Gizzard Shad, Lepomis species, minnows, and shiners.

Based on our sampling scheme, we observed substantial differences among gears in terms of catch rate and precision (Figure 5). Overall, pulsed-DC boat electrofishing was the most consistent sampling approach in the mid-sized river. Electrofishing captured the most species (43 species), compared to herding (10 species) and hoop netting (6 species). Notably, any species found by herding or hoop netting were also represented by electrofishing. Additionally, catch rates were approximately 3 times greater than herding and 17 times greater than hoop nets when averaged across all captured species (Figure 5a; ANOVA, $p = 0.015$). In addition to capturing more fishes, electrofishing and herding were comparatively more precise (i.e., how close your replicate values of the sample statistic are to each other) than hoop netting in terms of catch variability (Figure 5b; ANOVA, $p = 0.034$).

The Sulphur River has a functionally diverse group of fishes including large river planktivores, benthic invertivores, generalists, and predators (Appendix I - Table S6). A total of 43 fish species were encountered during 2021. Across sampling periods, between 3-6 new species were typically encountered on each trip. As the river shifted from high to low flow conditions, we continued to document new and often smaller bodied fishes. Catch rates of fishes were typically higher during low flow conditions (35 of 43 species). However, several native species including Bigmouth Buffalo, Gizzard Shad, and Longnose Gar were, on average, higher during periods of high flows (Appendix I - Table S6).

The Sulphur River fish assemblage exhibited a pronounced shift between flood and baseflow conditions (Figure 6). Shifts appear to be a combination of increased catch rates and species composition. Post-hoc analysis revealed that the structure and composition of the fish assemblage differed between flooding and baseflow conditions (MRPP, $p < 0.001$). Visual inspection of individual data points (Site_Month) further indicate a gradual shift in the fish community across the sampling season. Analyses of fine scale habitat factors including water temperature, phytoplankton, conductivity, and transect depth yielded no detectable response gradients in the multivariate analysis and appear to be superseded by flood and baseflow conditions.

Indicator species analysis was conducted to identify which taxa were present in most sampling sites during either flooding or baseflow conditions (Table 1). Overall, significant indicator species were only observed during low flow conditions. During flooding, River Carpsucker, Bigmouth Buffalo, and Longnose Gar each had high (>25) indicator values, but these taxa were not significant indicators (Table 1). These taxa are common in warmwater river systems. Although they exhibited higher catch rates during flooding, they were still captured during low flows, rendering them weak indicators. During baseflow, 13 species were significantly indicative of low flow conditions in the Sulphur River. Of the 13 species, most were typically associated with benthic environments (e.g., Freshwater Drum, catfishes) or small bodied taxa (minnows, shiners; Table 1). Additionally, many taxa (e.g., Largemouth Bass, Common Carp) were readily captured in high and low flow conditions and thus were poor indicators of either flow condition.

Fish body condition is a useful and simple response metric for tracking changes in fish populations. For example, changes in body condition of Bigmouth Buffalo and Gizzard Shad have been used to document the negative effects of bigheaded carps in the La Grange pool of the Illinois River (Upper Mississippi River; Irons et al. 2007). Using data from 2021, we first developed separate linear models of length-weight relationships for Gizzard Shad (size range: 95 - 414 mm) and Bigmouth Buffalo (size range: 287 - 870 mm). Length (mm) and weight (g) measurements were log10 transformed prior to analysis. Linear models were then created. Strong relationships were detected for both Bigmouth Buffalo ($N = 82$, $R2 = 0.97$, $p < 0.001$; $M = 3.1313(\log10L) + 5.128$) and Gizzard Shad ($N = 458$, $R2 = 0.95$, $p<0.001$; $M = 2.8127(\log10L) + 4.604$). Each model serves as an ecological indicator and provides a baseline representation of body condition in the Sulphur River system. If bigheaded carp populations increase in the Sulphur River, future weight-length measurements can be used to track potential shifts in body condition. We validated the model by comparing models of body condition to models reported in Irons et al. (2007). We entered the Sulphur River weight-length data into the Irons et al. (2007) models to estimate body condition. We then compared our estimates of condition to those predicted by the Irons et al. (2007) model. Ideally, the relationship of both models should be close to 1. We observed that predictions from the Bigmouth Buffalo model were very similar, deviating by only 0.25%, on average. The relationship between models was highly significant, with a slope of 1.0028, indicating strong congruence between models. We observed similar findings for Gizzard Shad, however predictions from each model were slightly more variable, differing by about 1.19% in body condition.

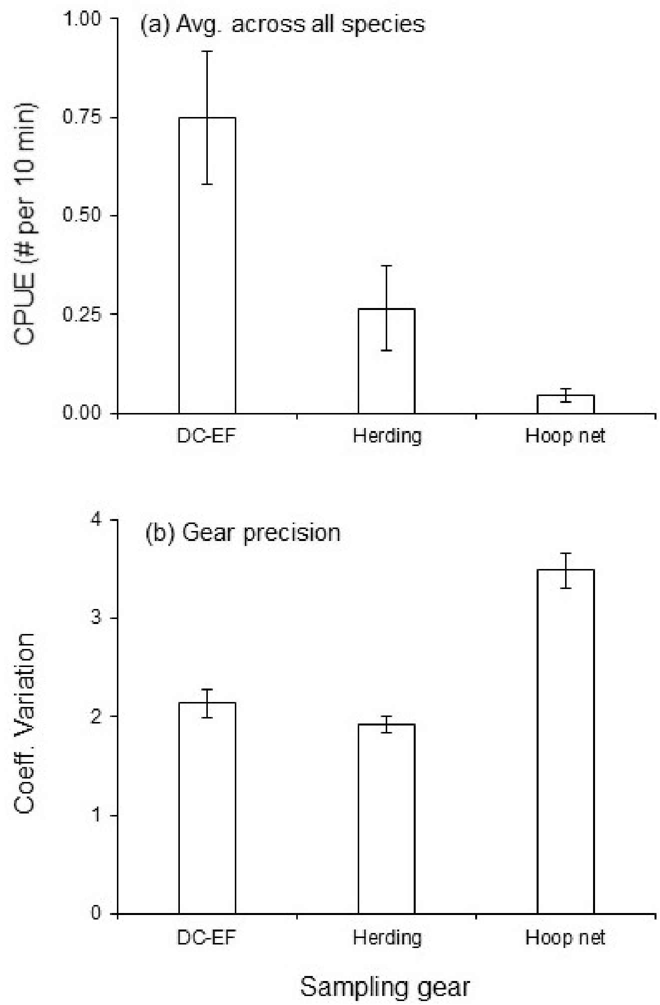

Figure 5. Examination of gear performance in the Sulphur River, a mid-sized tributary of the Red River. (a) Average fish CPUE among DC electrofishing (DC-EF), fish herding, and hoop nets. (b) Gear sampling precision, as determined by the coefficient of variation of catch data (Std. Dev / Mean). Values reflect average C.V.'s of all fish species captured by the gear type; note only native fishes were captured in the Sulphur River.

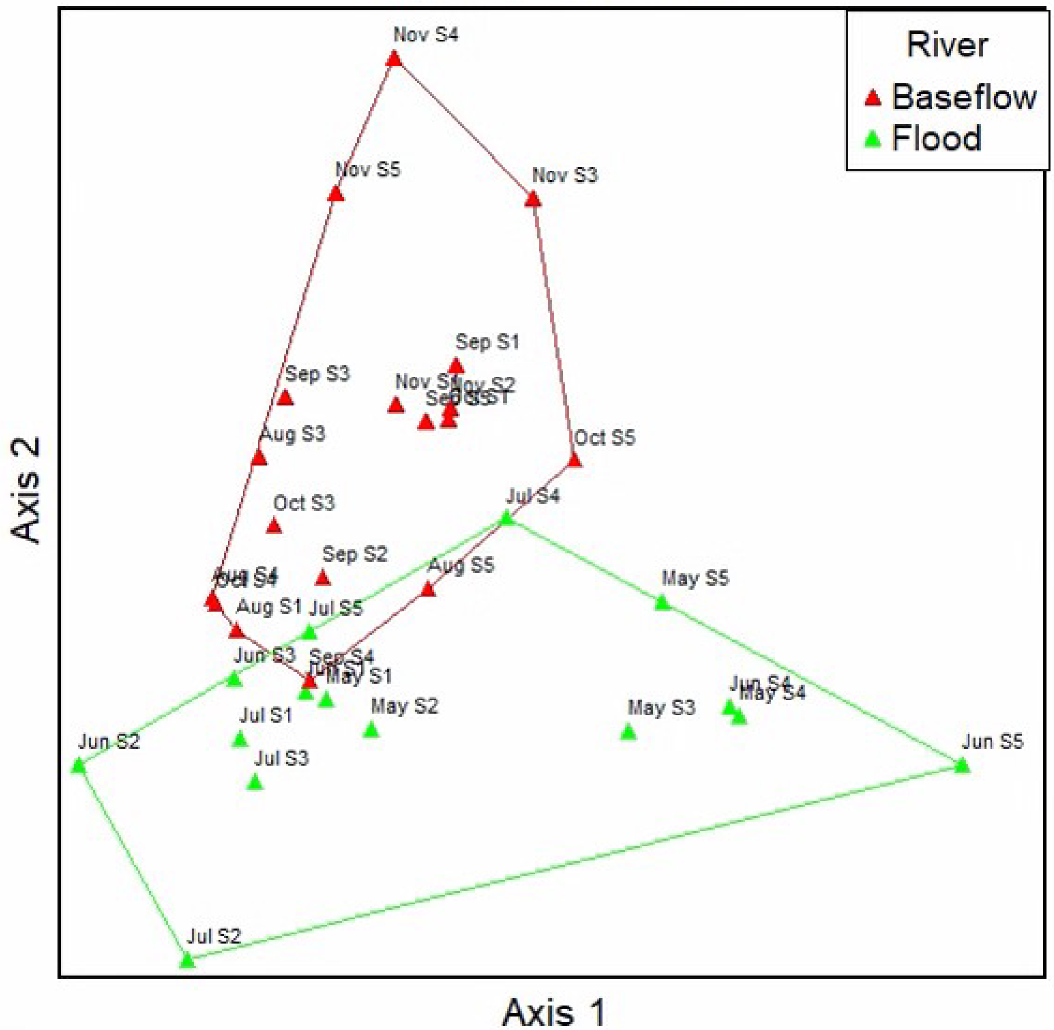

Figure 6. Changes in the Sulphur River fish assemblage from May through November 2021 in relation to the altered flow regime. Composition and structure of the fish assemblage differed between the spring flood (green triangles, lines) and summer baseflow (red triangle, line) conditions (analysis: non-metric multidimensional scaling (NMDS); PC-ORD 7).

Table 1. Indicator species analysis (ISA) is an analytic approach for identifying species found mostly in a single category, location, or time. ISA was used to evaluate fish species indicative of flood or baseflow conditions within the Sulphur River. Significance between flooding and baseflow was assessed using Monte Carlo bootstrapping (4,999 permutations). Species were included if either the indicator value exceeded 25 or p-values were <0.05 (in bold). Habitat traits: B = benthic; WC = water column.

Species	Trait	Flow condition	Ind. Value	Mean	SD	P-value
River Carpsucker	B	Flood	37.4	42.2	7.01	0.70
Bigmouth Buffalo	B, WC	Flood	30.4	40.9	8.35	0.9
Longnose Gar	WC	Flood	25	28.2	7.02	0.58
Spotted Bass	B, WC	Baseflow	87	44.7	6.57	**<0.001**

Freshwater Drum	B, WC	Baseflow	81.1	43.9	7.28	**<0.001**
Blue Catfish	B, WC	Baseflow	79	37.2	8.11	**<0.001**
Flathead Catfish	B, WC	Baseflow	78.4	43.2	9.16	**<0.001**
Longear Sunfish	B, WC	Baseflow	70.1	42	6.89	**0.001**
Black Buffalo	B	Baseflow	66.7	27	7.35	**<0.001**
Bluegill	B, WC	Baseflow	66.6	46.4	8.5	**0.02**
Smallmouth Buffalo	B	Baseflow	59.4	47.5	7.5	0.08
Spotted Gar	WC	Baseflow	58.2	53.3	7.41	0.25
Inland Silverside	WC	Baseflow	50	22.3	7.34	**0.004**
Gizzard Shad	B, WC	Baseflow	49.2	54.6	6.84	0.74
Shortnose Gar	WC	Baseflow	46.8	38.6	6.8	0.12
Blacktail Shiner	B, WC	Baseflow	44.4	19.7	6.41	**0.005**
Orangespotted Sunfish	B, WC	Baseflow	44.4	20.6	7.06	**0.008**
Ribbon Shiner	B, WC	Baseflow	42.5	22	6.93	**0.01**
Channel Catfish	B, WC	Baseflow	36.9	26.6	6.99	0.08
Black Crappie	B, WC	Baseflow	31.4	20.1	6.72	0.06
White Crappie	B, WC	Baseflow	31.1	25	7.6	0.21
White Bass	WC	Baseflow	29	25.8	7.56	0.30
Threadfin Shad	B, WC	Baseflow	27.8	14.5	5.84	**0.04**
Flier	B, WC	Baseflow	27.8	14.1	5.4	**0.04**
Yellow Bass	WC	Baseflow	27.7	23.9	7.09	0.28
Common Carp	B, WC	Baseflow	27.4	30.4	7.63	0.57

DISCUSSION:

Objective 1. Invasive Carp Population Assessment

Red River and Tributaries

Many age-0 fishes are difficult to detect in large river systems (Brewer and Ellersieck 2011), including Bighead and Silver Carp (Roth et al. 2020). Carp are extremely difficult to sample (Wanner and Klumb 2009; Bouska et al. 2017; Roth et al. 2020) and have detection rates as low as 38% in the presumably highly populated Illinois River Basin (Coulter et al. 2018b). We selected sampling gears following the suggestions of Collins et al. (2017), who found both mini-fyke nets and beach seines to be the most efficient for capturing age-0 carp. However, we were not able to successfully detect age-0 carp either due to detection, lack of spawning in 2021, or other influence. Camacho (2016), Collins et al. (2017), and Chick et al. (2020) have reported stark differences in the successful collection of larval and juvenile carp in successive years. For example, Collins et al. (2017) collected 39,398 Silver Carp in 2014; however, they collected only 116 in 2015. During the same years, Camacho (2016) captured a higher density of eggs and larval fish in 2014 than in 2015. Our 2021 sampling season may be emblematic of an extremely low capture year where adults have chosen not to—or were unable to—reproduce. Because carp in the Lower Red River Basin have not been documented in densities as high as the Upper Mississippi River Basin, the effect may be exacerbated.

Sandbed streams of the Central Great Plains, including the Red River are extremely dynamic and continuously shift over time (e.g., a backwater may be present during the wet months and absent during the dry months). Due to the constant shifts and extreme conditions emblematic of sandbed streams, detection of fishes is quite variable and often imperfect (Mollenhauer et al. 2018). The extensive high flow events observed in 2021 may have influenced our ability to successfully detect both age-0 species of carp (Figure 7). In June 2021, Red River discharge reached near 2,549 m^3/s (90,000 ft^3/s), roughly 1,982 m^3/s (70,000 ft^3/s) higher than the 78-year median (USGS gage 07337000). Discharge is assumed to be a spawning cue for carp and both our seining efficiency and mini-fyke net effort may have been affected by high flows. However, the abnormally high flows may have also led to unfavorable spawning conditions.

Most of the adult Bighead Carp and Silver Carp were captured in either the tributaries or backwater habitats on the mainstem Red River. The only carp captured in the mainstem Red River during sampling occurred directly below the confluence of a major tributary, the Little River. Carp exhibit very strong gear avoidance behaviors, and this may be a function of poor detection in the Red River. In fact, we sampled very few native fishes and no carp with hoop nets suggesting we should discontinue use of that gear and focus use on the other gears. However previous research by Coulter et al. (2016) through acoustic telemetry, demonstrated that outside of large migratory movements, Silver Carp were highly related to backwater environments and remained in those locations throughout the summer months. The low quantity of connected backwater in the Red River Basin from Denison Dam to the Arkansas-Louisiana border may limit the availability of suitable habitat for both carp species, but additional years of data are needed given the variations in flow.

We did not sample individuals of either species that were younger than age 3. Previous research by Coulter et. al (2018a) showed that the larger individuals are more likely to be located on the fringe of the species distribution as they are primarily responsible for expanding the species range. These fish may not have recruited within the Red River and could originate in a different basin (i.e., Mississippi River) expanding the invasion front. A telemetry effort would be helpful to determine the source of these fishes. Moreover, a wetter or drier year may produce much different patterns than observed in 2021.

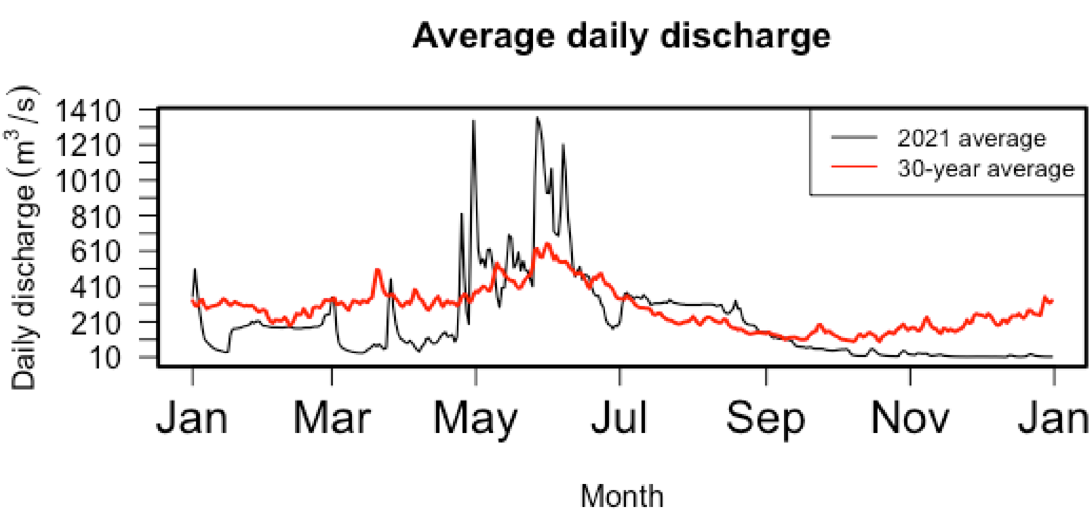

Figure 7. Average Lower Red River daily discharge over the 2021 sampling period. The red line indicates the 30-year average discharge conditions. Data are from U.S. Geological Survey, stream gauge 07335500 at Author City, TX.

Sulphur River

The detection, capture, and removal of bigheaded carp has been a management priority over the last decade in the Mississippi River Basin (Collins et al. 2015, 2017; ACRCC 2016; Rodgers 2019). Assessing invasive species at their 'invasion front' is challenging but necessary because such information is needed to inform management decisions regarding monitoring and targeted removal efforts. At the fringes of the invasion front, bigheaded carps are less abundant but larger in size (Coulter et al. 2018c). Fortunately, no bigheaded carp were captured in 2021 in the Sulphur River system. Given the heavy sampling effort, vulnerability of large-bodied fishes to electrofishing, and wide array of species collected, we are reasonably confident that bigheaded carp are likely at low numbers or were not present in the Sulphur River during 2021. At the "leading edge" of their geographical range (Coulter et al. 2018c), there are noticeably lower numbers when compared to adjacent downstream locations. Such a pattern appears to be the case between the Sulphur River and the mainstem Red River. A recent study detected bigheaded carp eDNA suggested both Bighead Carp and Silver Carp were present in the Sulphur River near our sampling locations but did not capture individuals via electrofishing (Barnes 2017). As part of the current project, TTU researchers visually observed two Silver Carp jump out of the water below the confluence of the Sulphur River and the Red River. Attempts to capture the fish were unsuccessful. Conversations with Arkansas Game and Fish Commission fisheries scientists have noted prior observations of bigheaded carps near the Red River-Sulphur River confluence and there have been previous documentations of Bighead Carp below the dam at Wright Patman Lake. Based on sporadic reports by anglers, detection of eDNA by others, and our own visual observations and catch data, evidence indicates that bigheaded carps from the mainstem Red River do enter the Sulphur River sporadically. The reasons for these movements remain uncertain. From a management perspective, understanding how environmental and flow conditions mediate movements of invasive planktivores from mainstem to tributary environments is important because the Wright Patman Dam is currently blocking the expansion of their range.

Although we anticipated using several sampling approaches, flow conditions (Figure 1) rendered many ineffective in the Sulphur River. This was due, in part, because the gears are better suited to large river environments and because their usage in the Upper Mississippi River Basin usually follows spring flooding. Usually, gill and trammel nets are deployed in the river channel margins, with relatively consistent depths adjacent to the river thalweg (to avoid barges). The Sulphur River generally lacks these types of habitats. The Sulphur River system largely existed in flood or baseflow conditions, with a brief transition phase. We observed that gill nets performed poorly at both hydrologic extremes because of floating debris during flooding or shallow water levels (gill nets were deeper than water, causing them to bunch up). Seining was not conducted during flooding because of safety concerns. Overall, we determined that pulsed-DC electrofishing was the best means of sampling fishes in the Sulphur River as it yielded the highest catch rates, collected the most fish species, collected a broad range of sizes (40 – 1460 mm) that span juvenile and adult life stages, and had the highest sampling precision. Local habitat conditions yielded no detectable patterns in the fish community, indicating river flow conditions (flooding, baseflow) superseded any local effect. For most fish species, catch rates were higher during low flow conditions. Such a pattern is likely because reduced water volumes can increase capture probability and also because reduced flow/velocity reduces the energetic costs of occupying the river segment. Future analyses will try to parse out what factor has the greatest influence. Given the success of electrofishing, we adapted our sampling by using herding methods, which allowed us to use a combination of gill nets and the electrofishing boat. Overall, the combination of boat electrofishing plus herding appears to be the best sampling combination for the Sulphur River.

The hydrology of the Sulphur River is regulated by the U.S. Army Corps of Engineers and their requirement to store or release water from Wright Patman Lake. Unlike unregulated rivers whose hydrographs typically ascend and descend gradually over days to weeks, the Sulphur River exhibits comparatively drastic shifts in discharge over a matter of days to weeks. The hydrology of the Sulphur River appears to alter the fish assemblage. Such stark changes will likely affect the management of bigheaded carps, as flow conditions may influence whether these large bodied planktivores enter or leave the Sulphur River system. During 2021, the Sulphur River existed largely in two conditions, at flood stage and at baseflows with minimal water. Visual inspection of historic flow data in the Sulphur River suggests this dynamic is common across years. In the Upper Mississippi River system, most large river sampling occurs after spring floods to avoid damage to nets; however, monitoring for bigheaded carp egg and larvae does occur during flooding (ACCRC 2016). The protracted flood regime, which can extend into July, creates challenges for monitoring programs because only a certain gears or approaches are effective during flood conditions. Whereas larger rivers like the Red River exhibit a more gradual decline in water levels, comparatively smaller systems like the Sulphur River are greatly and abruptly altered by dam operations.

The regulated flow regime of the Sulphur River presents challenges for invasive species management. Decisions to release or store water within the reservoir directly affect river flows, fish assemblages, habitat availability, and possibly movements of fishes between tributary and mainstem environments. High flows make sampling more challenging, as it limits gear effectiveness, the types of gears that can be used, and likely detection probabilities. At the sustained flood stage, the river is non-wadable. However, when water levels drop, the system transitions to a wadable stream environment. In a few places, the river channel was blocked by the accumulation of large woody debris piles. Such impediments would likely present a challenge for the upriver and/or downriver movements of fishes. In other locations, pools of water were connected by a narrow and shallow channel of water. This stark transition has strong implications for large river species like bigheaded carps.

Objective 2. Native Fish Assemblage Assessment

Red River and Tributaries

Throughout the sampling period, we documented 67 different fish species throughout the Lower Red River Basin. Relatively few sampling efforts covering this spatial extent have been devoted to collecting data on the native fish assemblage within the Lower Red River Basin. From 1995 to 2001, Buchanan et al. (2003) sampled the Arkansas portion of the Red River and reported the collection of 72 fish species. Of the 72 species they collected from 1995 to 2001, we collected 60 from all sample sites. In addition to the 60 species caught by Buchanan et al. (2003), we collected seven unique species including: American Eel, Bluntnose Minnow, Flier, Mooneye, Quillback, Sand Shiner, and Smallmouth Bass.

Fish diversity was highest in the Arkansas portion of the Red River, where the Red River is typified by both pools within the thalweg throughout the year as well as sections of shallow braided channels during low flow. There are abundant wing dikes and rip-rap lined banks throughout the Arkansas portion, directing flow to established channels. The river in the Oklahoma portion has little to no artificial channelization, allowing for a more dynamic, though shallower, channel. However, the wing dikes and levees in Arkansas create unique habitat that may effectively “attract” species like other forms of cover. We know that Pirate Perch has been sampled from Oklahoma waters (Brewer, Unpublished data), so we suspect we may find additional species as we continue to sample. However, the shallow braided stretches of the Red River in Oklahoma provide habitat niches that are favorable for some small-bodied fishes such as the Western Sand Darter, where 35 individuals were captured compared to the 8 captured in Arkansas. Only one unique species was observed in the tributaries; however, its capture was a historically significant one. One American Eel was collected in a tributary to the Red River, and to our knowledge it is the first documented capture within our study area since 1973 (Buchanan et al. 2003).

Two species that may be in decline in some areas, Blue Suckers and Shovelnose Sturgeon, were seldom captured prior to November. However, we began to capture more individuals in the mainstem Red River as temperature and flow decreased. Abnormally high spring and summer flows occurred in 2021 in the Lower Red River Basin. Sampling full species assemblages is increasingly difficult as river size, flow, and turbidity increase (Flotemersch et al. 2006), and the high spring flows could have limited capture efficiency of these and other species. It is thought that adult Blue Suckers move into tributaries to spawn in late winter or early spring and migrate back into the mainstem of large rivers afterwards (Neely et al. 2009; Dyer and Brewer 2020), but little is published on their preferred over-wintering habitats (with the following exceptions). Shovelnose Sturgeon use shallow (1.0 - 2.0 m) water depths, over sand substrate, and relatively low velocities (Quist et al. 1999) when they overwinter in the Kansas River, which is consistent with the habitat where we observed them. Although it is likely that lower water levels, decreased turbidity, and cooler water temperatures contributed to our increased catch rate, our results suggest these species use shallow water over sand substrate and slower flowing habitats for winter refugia. Our increased catch of these species serves as a good reminder that sampling seasonally is important to document information on species considered to be of conservation concern. These results also indicate that our sampling for these species was less efficient at other times of the year.

Sulphur River

Progress towards establishing an ecological baseline in the Sulphur River advanced in multiple ways. First, we documented a diverse fish assemblage and anticipate detecting more species during the 2022 field season. The collection of 43 fish species suggests that our sampling has been effective. For perspective, the Lower Red River Basin, including small tributaries, supports about 135 native and non-native fish species (Douglas and Hoover 2008). Others focused on a broader geographical area of the Lower Red River Basin than the Sulphur River detected 65 species in the mainstem Red River (Buchanan et al. 2003) and 67 species in the mainstem Red River and major tributaries (Brewer, current study) and should be representative of the general species pool. Our sampling effectively captured a large representative sample of fishes that occupy mid-sized and large river environments. Comparisons with these other fish assessments suggest more species are likely to be encountered during future sampling. In the event of rapid population growth by bigheaded carp, our baseline data will be used to describe the proportions of native and invasive fishes in terms of relative abundance and biomass (Coulter et al. 2018c). Although populations fluctuate, we also have a baseline assessment of common carp relative abundance and size. Common carp were consistently encountered along the river. As part of the ecological baseline, we summarized their catch rates and sizes to track how their populations may change in response to bigheaded carp populations through time.

We also developed body condition indices for Bigmouth Buffalo and Gizzard Shad. These simple length-mass models provide a convenient means of tracking potential effects of invasive carp. Interestingly, our Sulphur River models were very similar to those developed for the La Grange pool of the Illinois River (Irons et al. 2007). Predictions of body condition from each study were accurate to within 1.25 percent or less in most cases, depending on the species. The Sulphur River data was surprisingly similar to the "pre-invasion" models developed and used to quantify reductions in body condition of Bigmouth Buffalo and Gizzard Shad. Our analyses indicate that our Sulphur River models should detect changes in native fish body condition if bigheaded carp drastically reduce resource availability for buffalo and shad. If bigheaded carps are not encountered in future years, Gizzard Shad and Bigmouth Buffalo data (weight and length measurements) will be added to the existing Sulphur River models to increase sample size and to make them more robust.

RECOMMENDATIONS:

Management of invasive bigheaded carps in the Upper Mississippi River benefitted immensely from multiple long-term monitoring programs on the Mississippi and Illinois Rivers because data spanned 'pre' and 'post' invasion phases. Several long-term river monitoring programs exist within the Upper Mississippi River Basin (e.g., LTRMP, state agency datasets, commercial harvest records). These programs have tracked biotic and abiotic changes for varying durations—some of which started in the 1990's prior to the rapid expansion and growth of bigheaded carp populations (Gutreuter, 1993). It is rather fortunate that the rapid population growth of Silver Carp and Bighead Carp overlapped with these sampling programs because it allowed agencies and researchers to track the many effects of these invasive fish across major tributaries of the Upper Mississippi River. Complementary programs in the Lower Mississippi River Basin are less common, to our knowledge, and non-existent for the Red River Basin; this is the first bigheaded carp population assessment in this basin.

Continued monitoring is needed to fully assess the population and identify the biological, hydrological, and environmental factors that influence bigheaded carp occupancy and abundance in the Lower Red River Basin. Additional information will benefit the management of bigheaded carps by providing a more comprehensive assessment of the population and identifying conditions best suited for capturing these invasive fishes. Carp are certainly present in the Lower Red River Basin; however, they are very difficult to sample. The spring was particularly wet and thus, setting nets was difficult to impossible at times (resulting in loss of several nets and nets full of leaves and woody debris), and a wetter or drier year may produce much different patterns than observed in 2021. Alternatively, and emblematic of the basin (i.e., extremes), the summer and autumn have been much drier than normal thereby making access and navigation difficult (i.e., boat ramps disconnected from the river). Given the sporadic reports of bigheaded carp in the Sulphur River over the years and lack of captures to date during this study, continued monitoring is needed to better understand the flow conditions that may promote movements of carp from the Red River into the Sulphur River (e.g., ascending limbs from dam operations). Furthermore, continuation of the study would allow researchers to take advantage of a planned Sulphur River dewatering event below Wright Patman Dam to potentially capture bigheaded carp. Improved understanding may translate to other mid-sized rivers, as these systems are not typically the focus of bigheaded carp management. Moreover, such an understanding will help direct the timing and effort of sampling within the study areas and similar mid-sized river systems.

A telemetry study is also recommended to assess potential recruitment. We did not sample individuals of either bigheaded carp species that were younger than age 3. Previous research by Coulter et. al (2018a) showed that the larger individuals are more likely to be located on the fringe of the species distribution as they are primarily responsible for expanding the species range. These fish may not have recruited within the Red River and could originate in a different basin (i.e., Mississippi River) expanding the invasion front. A telemetry effort would be helpful to determine the source of these fishes.

State and federal agencies and other partners should continue to engage and educate members of the recreational angling community about the threats of bigheaded carp. Informal conversations with multiple anglers revealed that many anglers possessed general knowledge about bigheaded carps. Verbal descriptions about carp morphology and their distinguishing features (e.g., position of eye) suggested that some anglers were knowledgeable and have observed these fishes in the Sulphur River and several invasive carp were provided to researchers by an angler from other tributaries of the Red River. An informed angling community could be used to help document bigheaded carp in the Lower Red River Basin, as many anglers fish the river far more frequently than it is sampled scientifically. Information about documenting the catch, preserving the fish (i.e., freezing) and whom to contact to donate study specimens should be provided.

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APPENDIX I: SUPPLEMENTARY TABLES

Table S1**.** Demographic information of most Bighead Carp and Silver Carp collected during sampling events. The sample date, site, and gears used are provided. Total length (TL, mm), weight (W, g), and sex (male [M] or female [F]) of each fish are provided. The preliminary age estimates (Age) using otoliths are provided. These carp were sampled using gillnets (GN), electrofishing (EF), bow-fisherman (BF) or jumped in the boat during a survey (JM). Lastly, estimated egg counts for some female fish is provided.

River	Date	Latitue	Longitude	Species	TL	TW	Gear	Sex	Age	Eggs
Bois d'Arc Creek	7/7/2021	33.83864	-95.84481	BC	1048	12840	GN	Female	3	561,374
Bois d'Arc Creek	7/23/2021	33.83864	-95.84481	BC	1090	-	GN	Female	9	-
Bois d'Arc Creek	7/23/2021	33.83864	-95.84481	BC	1245	-	GN	Male	10	-
Choctaw Creek	8/10/2021	33.72021	-96.37333	BC	990	9260	GN	Male	5	-
Choctaw Creek	8/10/2021	33.72021	-96.37333	BC	1097	14220	GN	Female	3	722,638
Choctaw Creek	8/10/2021	33.72021	-96.37333	BC	1100	13480	GN	Male	5	-
Choctaw Creek	8/10/2021	33.72021	-96.37333	BC	1140	15180	GN	Male	5	-
Choctaw Creek	8/11/2021	33.72223	-96.41024	BC	1069	1200	GN	Male	5	-
Choctaw Creek	11/16/2021	33.72021	-96.37333	BC	1033	10025	EF	Female	7	407,264
Choctaw Creek	11/16/2021	33.72021	-96.37333	BC	1205	1800	GN	Male	6	-
Choctaw Creek	12/15/2021	33.72021	-96.37333	BC	1225	23000	EF	Female	8	-
Choctaw Creek	6/23/2021	33.77369	-96.41828	SC	745	4900	BF	Male	3	-
Choctaw Creek	7/19/2021	33.72074	-96.3769	SC	910	9500	BF	Male	7	-
Choctaw Creek	7/21/2021	33.72004	-96.39877	SC	850	8160	JM	Male	5	-
Choctaw Creek	8/10/2021	33.72021	-96.37333	SC	850	7600	GN	Male	6	-
Choctaw Creek	8/11/2021	33.72223	-96.41024	SC	851	8100	EF	Male	7	-
Choctaw Creek	8/11/2021	33.72223	-96.41024	SC	882	8350	EF	Female	3	1,217,828
Choctaw Creek	11/16/2021	33.72021	-96.37333	SC	765	6000	EF	Male	4	-
Choctaw Creek	11/16/2021	33.72021	-96.37333	SC	932	10750	GN	Female	3	381,742
Choctaw Creek	11/16/2021	33.72021	-96.37333	SC	1020	12050	EF	Female	8	1,022,782
Choctaw Creek	12/15/2021	33.72021	-96.37333	SC	902	8000	GN	Male	7	-
Kiamichi	7/15/2021	33.94832	-95.29562	SC	708	3850	GN	Male	3	-

Red River	7/16/2021	33.65393	-94.56868	BC	1240	-	GN	Female	4	256,314
Red River	8/4/2021	33.56842	-94.38122	BC	1108	13670	GN	Male	4	-
Red River	8/10/2021	33.77693	-96.47264	BC	9250	6350	BF	Male	6	-
Red River	8/23/2021	33.80257	-94.9285	BC	1230	21500	GN	Male	5	-
Red River	8/24/2021	33.56842	-94.38122	BC	960	17500	GN	Male	5	-
Red River	9/5/2021	33.79629	-96.51526	BC	1130	15600	BF	Female	9	-
Red River	9/6/2021	33.79629	-96.51526	BC	1090	14600	BF	Female	5	-
Red River	9/6/2021	33.79629	-96.51526	BC	1130	19700	BF	Female	7	-
Red River	7/5/2021	33.60915	-93.8242	SC	710	3880	EF	Female	4	233,740
Red River	7/9/2021	33.56842	-94.38122	SC	897	7260	GN	Male	6	-
Red River	7/12/2021	33.58881	-94.37804	SC	912	7460	GN	Male	4	-
Red River	8/4/2021	33.56842	-94.38122	SC	808	6460	EF	Male	6	-
Red River	8/24/2021	33.56842	-94.38122	SC	752	5020	EF	Female	4	720,804
Red River	8/24/2021	33.56842	-94.38122	SC	783	6300	GN	Male	3	-
Red River	8/24/2021	33.56842	-94.38122	SC	850	9000	EF	Male	4	-
Red River	9/21/2021	33.58881	-94.37804	SC	752	4800	GN	Female	3	308,066
Red River	9/21/2021	33.58881	-94.37804	SC	876	8500	JM	Female	3	1,110,148
Red River	12/1/2021	33.7742	-96.421	SC	883	7900	BF	Male	9	-
Red River	12/1/2021	33.7729	-96.418	SC	864	8300	BF	Male	14	-
Webb Creek	7/25/2021	33.77366	-96.41828	SC	720	4620	BF	Male	4	-

Table S2**.** Carp visually confirmed but not collected during sampling in the Lower Red River Basin and tributaries (other than the Sulphur River). The observations indicate the date, location, and species observed.

River	Date	Latitude	Longitude	Species
Bois d'Arc Creek	7/23/2021	33.8386	-95.8448	Silver Carp
Kiamichi	11/29/2021	33.9483	-95.2956	Silver Carp
Muddy Boggy	7/2/2021	33.9434	-95.6017	Silver Carp
Muddy Boggy	7/27/2021	33.9356	-95.6349	Silver Carp
Muddy Boggy	7/28/2021	33.9284	-95.651	Silver Carp
Pine Creek	8/3/2021	33.8648	-95.3079	Bighead Carp
Red River	7/9/2021	33.5684	-94.3812	Silver Carp
Red River	7/16/2021	33.6539	-94.5687	Silver Carp
Red River	7/29/2021	33.6539	-94.5687	Silver Carp
Red River	8/31/2021	33.397	-93.7117	Silver Carp
Red River	10/8/2021	33.397	-93.7117	Silver Carp
Red River	10/14/2021	33.6485	-94.5432	Silver Carp
Red River	11/11/2021	33.6092	-93.8242	Silver Carp

Table S3. The number of individuals, by species and by sampling gear (EF=electrofishing, FN= mini-fyke net, GN = gillnet, HN=hoopnet, LT=larval tow, SE=seine) collected from the Arkansas portion of the Red River (Bighead Carp and Silver Carp collections/observations are not included in the table).

Species	EF	FN	GN	HN	LT	SE	Total
Alligator Gar	-	-	13	1	-	-	14
Black Buffalo	12	-	77	1	-	-	90
Blue Catfish	7	-	3	-	-	-	10
Bullhead Minnow	3	469	-	-	-	2464	2936
Black Crappie	2	87	-	-	-	5	94
Bluegill	4	390	-	-	-	440	834
Bigmouth Buffalo	35	-	129	-	-	1	165
Bluntnose Darter	-	3	-	-	-	-	6
Bluntnose Minnow	-	2	-	-	-	-	2
Blue Sucker	83	-	17	1	-	-	101
Brook Silverside	-	3	-	-	-	96	99
Blackstripe Topminnow	-	9	-	-	-	18	27
Blacktail Shiner	1	-	-	-	-	46	47
Catostomidae spp.	-	-	-	-	1	-	1
Chub Shiner	-	130	-	-	-	1140	1270
Channel Catfish	3	5	2	-	-	13	23
Common Carp	-	-	2	-	-	-	2
Dusky Darter	-	2	-	-	-	1	3
Emerald Shiner	6	543	-	-	5	769	1323
Flathead Catfish	69	1	1	-	-	2	73
Flier	-	1	-	-	-	-	1
Freshwater Drum	22	14	2	1	-	24	63
Golden Shiner	-	-	-	-	-	5	5
Grass Carp	-	-	11	-	-	-	11

Green Sunfish	14	3	-	-	-	7	24
Golden Topminnow	-	6	-	-	-	10	16
Gizzard Shad	233	43	3	-	1	336	616
Inland Silverside	5	201	-	-	-	1098	1304
Lepomis spp.	-	55	-	-	1	83	139
Longear Sunfish	10	18	-	-	-	4	32
Logperch	-	4	-	-	-	1	5
Largemouth Bass	2	-	-	-	-	-	2
Longnose Gar	39	3	27	3	-	5	77
Mississippi Silvery Minnow	-	-	-	-	-	1	1
Mosquitofish	-	344	-	-	-	1035	1379
Orangespotted Sunfish	5	973	-	-	-	645	1623
Paddlefish	-	-	17	-	-	-	17
Pallid Shiner	-	-	-	-	-	2	2
Pirate Perch	-	-	-	-	-	1	1
Pomoxis spp.	-	-	-	-	-	22	22
Quillback	-	1	-	-	-	3	4
River Carpsucker	111	4	-	-	-	63	178
Redear Sunfish	-	-	-	-	-	1	1
Red Shiner	106	4504	-	-	9	12288	16907
River Darter	-	3	-	-	-	2	5
Sand Shiner	-	20	-	-	-	-	20
Smallmouth Buffalo	41	1	101	7	-	-	150
Silverband Shiner	-	-	-	-	-	11	11
Shoal Chub	-	5	-	-	-	79	84
Silver Chub	14	1	-	-	-	20	35
Skipjack Herring	-	2	-	-	-	2	4
Slough Darter	-	2	-	-	-	3	5

Smallmouth Bass	1	-	2	-	-	-	3
Shortnose Gar	21	28	2	-	-	-	51
Shovelnose Sturgeon	4	-	-	-	-	-	4
Spotted Bass	18	13	-	-	-	172	203
Spotted Gar	10	2	-	-	-	1	13
Silver Chub	4	-	4	-	-	-	9
Threadfin Shad	113	852	-	-	3	307	1275
Tadpole Madtom	-	2	-	-	-	-	2
White Bass	4	-	-	-	-	63	67
White Crappie	6	521	-	2	-	111	640
Warmouth	1	23	-	-	-	17	41
Western Sand Darter	-	3	-	-	-	5	8
Yellow Bullhead	-	1	-	-	-	-	1

Table S4. The number of individuals, by species and by sampling gear (EF=electrofishing, FN= mini-fyke net, GN = gillnet, HN=hoopnet, LT=larval tow, SE=seine) collected from the Oklahoma portion of the Red River (Bighead Carp and Silver Carp collections/observations are not included in the table).

Species	EF	FN	GN	HN	LT	SE	Total
Alligator Gar	-	-	8	-	-	-	8
Black Buffalo	12	-	51	1	-	-	64
Blue Catfish	4	-	13	1	-	1	19
Bullhead Minnow	15	176	-	-	-	1785	1976
Black Crappie	-	6	-	-	1	5	12
Bluegill	14	19	-	-	-	61	94
Bigmouth Buffalo	27	-	37	-	-	-	64
Bluntnose Minnow	3	-	-	-	-	-	3
Blackspotted Topminnow	-	-	-	-	-	1	1
Blue Sucker	67	-	2	-	-	-	69
Blackstripe Topminnow	-	-	-	-	-	1	1
Blacktail Shiner	-	4	-	-	-	28	32
Catostomidae spp.	-	-	-	-	-	4	4
Chub Shiner	-	49	-	-	-	619	668
Channel Catfish	19	3	3	-	-	4	29
Common Carp	2	-	-	-	-	-	2
Dusky Darter	-	-	-	-	-	1	1
Emerald Shiner	9	25	-	-	-	160	194
Flathead Catfish	29	-	1	-	-	-	30
Freshwater Drum	25	8	1	-	-	12	46
Goldeye	1	-	-	-	-	-	1
Grass Carp	1	-	4	-	-	-	5

Green Sunfish	3	5	-	-	-	8	16
Golden Topminnow	-	3	-	-	-	2	5
Gizzard Shad	123	9	-	-	-	149	281
Inland Silverside	3	62	-	-	-	1233	1298
Lepomis spp.	-	-	-	-	-	21	21
Longear Sunfish	4	26	-	-	-	4	34
Logperch	-	-	-	-	-	1	1
Longnose Gar	46	5	42	5	-	3	101
Mosquitofish	-	9	-	-	-	806	815
Orangespotted Sunfish	72	5	-	-	-	12	89
Paddlefish	-	-	4	-	-	-	4
Pallid shiner	-	-	-	-	-	2	2
Pomoxis spp.	-	-	-	-	-	2	2
Quillback	4	-	-	-	17	-	21
River Carpsucker	103	7	2	-	-	145	257
Redear Sunfish	1	-	-	-	-	-	1
Red Shiner	91	2043	1	-	3	12412	14550
River Darter	-	-	-	-	-	1	1
Sand Shiner	-	1	-	-	-	4	5
Smallmouth Buffalo	85	-	99	14	-	-	198
Shoal Chub	-	9	-	-	-	352	361
Silver Chub	-	-	-	-	-	4	4
Suckermouth Minnow	-	1	-	-	-	10	11
Slough Darter	-	1	-	-	-	-	1
Smallmouth Bass	-	-	1	-	-	-	1
Shortnose Gar	16	6	3	1	-	4	30
Shovelnose Sturgeon	9	-	1	-	-	-	10

Spotted Bass	7	5	-	-	-	37	49
Spotted Gar	15	-	-	1	-	-	16
Striped Bass	2	-	-	-	-	-	2
Threadfin Shad	101	3	-	-	-	314	418
White Bass	3	3	-	-	-	72	78
White Crappie	5	58	-	-	1	134	198
Warmouth	2	-	-	-	-	-	2
Western Sand Darter	-	-	-	-	-	35	35

Table S5. The number of individuals, by species and by sampling gear (EF=electrofishing, FN= mini-fyke net, GN = gillnet, HN=hoopnet, LT=larval tow, SE=seine) collected from the tributaries of the Red River (Bighead Carp and Silver Carp collections/observations are not included in the table).

Species	EF	FN	GN	HN	LT	SE	Total
Alligator Gar	-	-	7	-	-	-	7
American Eel	1	-	-	-	-	-	1
Black Buffalo	8	-	40	-	-	-	48
Blue Catfish	6	-	24	-	-	-	30
Bullhead Minnow	42	78	-	-	-	1357	1477
Black Crappie	-	1	-	-	-	9	10
Bluegill	21	100	-	-	-	65	186
Bigmouth Buffalo	60	-	72	2	-	-	134
Bluntnose Minnow	2	-	-	-	-	-	2
Brook Silverside	-	-	-	-	-	10	10
Blackstripe Topminnow	1	4	-	-	-	11	16
Blacktail Shiner	27	105	-	-	-	542	674
Chub Shiner	-	2	-	-	-	1	3
Channel Catfish	2	4	9	-	-	13	28
Common Carp	3	-	7	-	-	-	10
Dusky Darter	-	2	-	-	-	6	8
Emerald Shiner	44	2	-	-	-	14	60
Flathead Catfish	4	-	2	1	-	-	7
Freshwater Drum	28	-	20	-	-	1	49
Grass Carp	3	-	13	1	-	-	17
Green Sunfish	4	-	-	-	-	1	5
Gizzard Shad	264	-	58	-	-	63	385
Inland Silverside	17	5	-	-	-	108	130
Lepomis spp.	-	-	-	-	-	133	133

Longear Sunfish	21	31	1	-	-	59	112
Logperch	-	1	-	-	-	1	2
Largemouth Bass	5	1	-	-	-	-	6
Longnose Gar	60	-	13	2	-	2	77
Mooneye	1	-	-	-	-	-	1
Mosquitofish	1	-	-	-	-	1750	1751
Orangespotted Sunfish	4	16	-	-	-	14	34
Paddlefish	2	-	53	-	-	-	55
Pallid Shiner	-	4	-	-	-	-	4
Pomoxis spp.	-	-	-	-	-	1	1
River Carpsucker	230	-	3	-	-	62	295
Red Shiner	206	136	2	-	9	732	1085
River Darter	-	1	-	-	-	1	2
Sand Shiner	-	-	-	-	-	1	1
Smallmouth Buffalo	95	-	79	5	-	1	180
Silver Chub	-	-	-	-	-	3	3
Suckermouth Minnow	-	-	-	-	-	1	1
Slough Darter	-	-	-	-	-	1	1
Smallmouth Bass	2	-	-	1	-	-	3
Shortnose Gar	14	3	4	-	-	-	21
Spotted Bass	10	2	-	-	-	83	95
Spotted Gar	29	-	1	-	-	-	30
Threadfin Shad	122	-	-	-	-	4	126
Tadpole Madtom	-	-	-	-	-	2	2
White Bass	3	-	-	-	-	-	3
White Crappie	8	25	-	1	-	62	96
Warmouth	1	5	-	-	-	2	8

Table S6. Summary of fish species collected in the Sulphur River during the 2021 sampling season. Attributes including the total numbers captured, average body lengths, and relative abundances during flooding and baseflow conditions are reported.

Species	Captured	Avg. length	±	SD	Flooding CPUE	Baseflow CPUE
Blue catfish	97	350	±	220	0.272	1.100
Black buffalo	39	442	±	88	0.069	0.594
Black crappie	52	233	±	44	0.047	0.863
Bluegill	178	105	±	42	0.663	2.320
Blue sucker	7	559	±	78		0.137
Bigmouth buffalo	84	593	±	141	0.917	0.539
Bowfin	16	563	±	103		0.288
Blacktail shiner	39	48	±	8		0.595
Channel catfish	50	299	±	112	0.308	0.488
Common logperch	6	83	±	11	0.036	0.038
Common carp	33	573	±	144	0.149	0.429
Dollar sunfish	1	126	±			0.015
Emerald shiner	2	88	±	11		0.039
Flathead catfish	71	255	±	119	0.134	1.095
Fathead minnow	5	66	±	6	0.043	0.015
Flier	8	93	±	19		0.166
Freshwater drum	173	282	±	107	0.409	2.289
Golden Redhorse	5	104	±	23		0.118
Golden shiner	2	88	±	40		0.026
Green sunfish	13	76	±	21	0.033	0.167
Gizzard shad	938	119	±	63	6.967	6.090
Inland silverside	141	67	±	11	0.036	2.679
Largemouth bass	125	206	±	120	0.967	0.847
Longnose gar	23	891	±	344	0.228	0.130
Longear sunfish	137	100	±	19	0.543	1.427
Orangespotted sunfish	34	64	±	11	0.098	0.389
Paddlefish	1	720	±		0.014	
Pirate perch	2	92	±	22		0.029

Redbreast sunfish	2	128	±	4		0.034
River carpsucker	84	292	±	75	0.558	0.765
Red shiner	20	58	±	13	0.174	0.071
Redear sunfish	3	163	±	18		0.041
Ribbon shiner	50	55	±	7	0.047	1.105
Redspotted sunfish	1	86	±			0.020
Smallmouth buffalo	232	434	±	92	1.221	2.174
Shortnose gar	56	630	±	54	0.373	0.438
Spotted bass	97	185	±	110	0.351	1.010
Spotted gar	146	579	±	76	0.732	1.380
Threadfin shad	19	79	±	30		0.345
White bass	33	231	±	193	0.225	0.310
White crappie	37	129	±	86	0.105	0.507
Warmouth	20	113	±	39	0.272	0.100
Yellow bass	25	124	±	64	0.178	0.203