Identifying the Status of Invasive Carp Recruitment in the Red River and its Tributaries below Denison Dam in Oklahoma and Texas

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Introduction

Like many invasive species, Bighead (*Hypophthalmichthys nobilis*) and Silver Carp (*H. molitrix*)—collectively nicknamed 'bigheaded carp'—are known for their hardiness, high fecundity, dispersal abilities, and competitive advantage against native aquatic species in the United States. While our understanding of their ecology, life history, and population dynamics in the upper Mississippi River basin has grown substantially over the last two decades, bigheaded carp continue to overturn the limiting factors we have ascribed to their reproduction and movement (Sampson et al. 2009; Deters et al. 2013; Coulter et al. 2013). As a result, the development of effective management and control strategies in these inundated regions has been slow and arduous. In recent years, an influx of resources was allocated to the understudied sub-basins of the lower Mississippi River, where bigheaded carp currently persist in relatively lower densities. This opportunity has resulted in the formation of extensive partnerships between government agencies, conservation groups, universities, commercial and recreational anglers, business owners, and landowners—all with the common goal of developing proactive strategies for managing populations and preventing further translocation of bigheaded carp.

There are numerous native, state-listed species vulnerable to the growth of bigheaded carp populations in the Red River, including the planktivorous American Paddlefish (*Polyodon spathula*), Blue Sucker (*Cycleptus elongates*), Plains Minnow (*Hybognathus placitus*), American Eel (*Anguilla rostrate*), and Blackspot Shiner (*Notropis atrocaudalis*). Also facing significant risk is the recreational value of Lake Texoma and its renowned recreational Striped Bass (*Morone saxatilis*) fishery. Each year, the reservoir attracts approximately 965,000 visitors, generating a regional total of $159 million (U.S. Army Corps of Engineers 2012). While the Denison Dam currently serves as an effective barrier to all fish movement from the Red River upstream into Lake Texoma, the translocation of juvenile bigheaded carp (closely resembling native Clupeids) via angler bait buckets is a viable concern. The threat of Silver and Bighead Carp introduction to Lake Texoma is compounded by the potential for translocated individuals to find suitable spawning habitat on the Washita and Red River arms of the lake. Grass Carp (*Ctenopharyngodon idella*), which share similar spawning cues and conditions with bigheaded carp, have demonstrated successful recruitment here already (Hargrave and Gido 2004; Shelton and Snow 2017).

To date, there has been no evidence to suggest that bigheaded carp have recruited within our Red River study area; however, Patton and Tackett (2012) speculated that the river conditions appeared to be conducive to their spawning, with the extreme hydrologic fluctuations from the Denison Dam potentially contributing to a flood pulse spawning cue. Since the inception of the Arkansas-Red-White partnership for invasive carp in 2020, hundreds of fecund bigheaded carp have been collected from the Red River drainage in Texas and Oklahoma. As the partnership continues evaluating the status of these species to inform future management strategies, the Oklahoma Fish and Wildlife Conservation Office (OKFWCO) will assist efforts by monitoring the status of bigheaded carp recruitment in the Red River and its tributaries below the Denison Dam in Oklahoma and Texas (Figure 1).

The OKFWCO's Monitoring for bigheaded carp recruitment in the form of eggs and larvae was conducted using larval fish tows and light trapping. Using traditional methods, samples collected from these efforts would need to be physically sorted and visually inspected to identify potential bigheaded carp. This process is incredibly time consuming, and species ID based on physical characteristics of these samples is not possible in many cases (Camacho et al. 2019). Recently, Fritts et al. (2019) demonstrated that aliquots of the ethanol used to preserve these samples could be screened using quantitative PCR (qPCR) to identify collections which contained invasive carp eggs and or larvae. Although this method was not 100% accurate, this information could then be used to select those tow and trap samples which should be further screened for the presence of larval fish and eggs. The objective of this study was to use qPCR screening protocols developed by Fritts et al. (2019) to screen ethanol preserved samples collected by the OKFWCO for the presence of Silver and Bighead carp DNA. Any samples that do test positive for bigheaded carp DNA can then be further screened using visual ID and additional genetic methods (e.g., species ID sequencing).

Study Location

The Red River is considered a major tributary in the Lower Mississippi River sub-basin and has a drainage area of approximately 240,000 km^2. The Red River flows east from its headwaters in the Great Plains of eastern New Mexico, forms the Texas-Oklahoma border, and curves southward through Louisiana until terminating at its swampy confluence with the Atchafalaya and Mississippi Rivers. The Red River is characterized by its sandy/silty bottoms, low gradient, and areas of high salinity. Substantial hydrologic fluctuations from the Denison Dam result in an incredibly dynamic system in Texas and Oklahoma.

Field Methods

*Ichthyoplankton tow*s

During May–August 2023, we monitored for drifting bigheaded carp eggs and larvae using ichthyoplankton tows conducted 1–2 times per week; river conditions permitting. Sampling locations each week were often dictated by unpredictable dam water release schedules, which significantly impacted our already constrained boat launch options and river navigability. When possible, FWCO staff preferentially chose locations based on distance from potential spawning habitat (primarily dams and confluences), time since last significant hydrological pulse, and channel width. Samples were collected using ichthyoplankton nets (0.5-m diameter; 2-m length; 500 µm mesh) deployed from each side of the boat—one sampling at the water's surface and the other sampling at approximately mid-column depth. The net contained a calibrated mechanical flowmeter (General Oceanics, Inc.; Model 2030R) mounted across the mouth to quantify the volume of water sampled (m^3). The boat was oriented facing upstream and its position was maintained stationary relative to the shore. To reduce debris load and damage to specimens, the duration of tows was kept to three minutes but was shortened in the presence of excess debris. After removing nets from the water, the contents were rinsed into the collection chambers at the cod ends and water was drained through the 500-µm mesh vents. We used 95% non-denatured ethanol solution to rinse and preserve ichthyoplankton into separate 1-Liter high-density polyethylene bottles (Nalgene Labware, Rochester, NY). We conducted each sampling

event in replicates of three at each location—one in the thalweg and one in proximity to each bank. To reduce DNA cross-contamination, nets and associated sampling tools were soaked in 2% Virkon solution for 20 minutes before hanging in the sunlight to be dried.

Quadrafoil light traps

We also concurrently deployed Quadrafoil Light Traps (Watermark) during the sampling season. The traps contained a green LED light (FishXtrada. Anaheim, CA) surrounded by polycarbonate tubes arranged in a cloverleaf-shaped arrangement and a lower collection chamber where contents were funneled. The light attracts phototactic species of macroinvertebrates and larval fishes, including invasive carp (Roth 2018; Brandenburg et al. 2019). Traps were set during morning/early afternoon in slack water areas such as eddies, side channels, floodplains, oxbows, and small tributaries, where drifting protolarvae often reside until they develop into free-swimming flexion larvae. Floating and sinking traps sampled the upper and lower water columns, respectively. Each were anchored in place and had an agency-labeled buoy attached. Light traps were then picked up the following day. We applied the same preservation and sanitation protocols to light trap samples as those described for ichthyoplankton tows in this proposal.

Sample Processing

Each preserved sample's ethanol solution was replaced within 48 hours of collection to remove DNA not directly associated with its specimens and to reduce dilution for better preservation (Kelso et al. 2012; Nagy 2010; Fritts et al. 2019). This consisted of pouring contents of each sample through a sanitized 500-µm stainless steel sieve, placing contents back into their bottle, and adding fresh 95% ethanol solution. Dense/debris-heavy samples had the ethanol replaced two or three times. qPCR screening only requires subsamples of the ethanol solution from each sample. Prior to subsampling, each sample was gently agitated and left to rest until contents had settled. We then used a sanitized 2-mL pipette to extract three aliquots of ethanol solution and deposit 1-mL into leak-proof microcentrifuge tubes. The aliquots were sent to the Whitney Genetics Lab for subsequent qPCR screening. The ethanol-preserved sample contents remained available for alternative analyses and processing (i.e., visual inspection) if any bigheaded carp DNA was detected.

Laboratory Methods

DNA Extraction

The samples which were collected and subsampled in triplicate as outlined above were stored in 1.5 mL microcentrifuge tubes (MCTs) for DNA extraction at Whitney Genetics Lab. During extraction, we kept each of the three tubes that constitute one sample separate (i.e., each sample was analyzed three separate times). Samples were centrifuged at max speed (~18,000 rpm) to concentrate any cells and/or eDNA at the bottom of the MCT, the supernatant was discarded, and the samples were set to dry in a 60°C bead bath for 15 minutes to remove any excess ethanol. Ethanol is a well-documented PCR inhibitor, so care was taken to ensure each sample was dry before continuing with subsequent steps. Once the samples were dry, they were frozen in -20°C storage to preserve the eDNA until we were ready to extract. DNA was extracted using the commercially available gMax mini genomic DNA extraction kit (IBI scientific) and

final extracts contained a volume of 200µL elution buffer. Samples were extracted in batches of up to 45 samples and each DNA extraction batch included a negative control (water only) and a positive control (Brook Trout cells) to ensure that our extraction procedure was successful and that there was no laboratory contamination. All DNA extractions were conducted in rooms dedicated to invasive carp eDNA extractions where no other laboratory procedures (e.g., qPCR) were performed.

Quantitative PCR (qPCR)

DNA extracts were analyzed using qPCR to determine the presence of Silver or Bighead carp DNA. We used a multiplex qPCR assay that contains six total loci. The qPCR multiplex includes two general invasive carp markers (i.e., can detect Silver and Bighead carp, but cannot differentiate between the two species) located on the cytochrome oxidase I gene (ACTM 1/3), two markers specific to Silver Carp located on the ND6 and ND2 regions of the mtDNA genome (SCTM 4/5), and two markers specific to Bighead Carp located on the ND4 and ND6 regions of the mtDNA genome (BHTM1/2) (Farrington et. al 2016). qPCR master mix concentrations and thermal profiles followed standard USFWS lab protocols for these markers developed for eDNA monitoring (USFWS 2023). qPCR master mixes were made in a room dedicated to reagent prep. Master mix was added to samples using an automated liquid handling robot (Eppendorf EP motion 5075) and each sample was analyzed in replicates of eight. qPCR replicates were considered positive if they crossed the threshold fluorescence detection limit between 15 and 40 cycles. Any sample that had at least one detection for any of the eight replicates was considered a positive detection. All qPCR plates included both positive (invasive carp DNA extracted from cells) and negative (water only) controls and two, five-point standard curves consisting of 10 ng/µl, 50 ng/µl, 250 ng/µl, 1250 ng/µl, and 6250 ng/µl standards.

Results and Discussion

We collected and analyzed a total of 88 samples that were extracted in triplicate for a total of 276 samples including lab-generated positive and negative controls. We were unable to detect the presence of any Bighead or Silver carp eDNA. To ensure that field collection and DNA extractions were successful, we ran the samples again for a Bluegill (*Lepomis macrochirus*) qPCR assay (Takahara et al. 2013) to ensure that we could detect a species we knew was present in both the sampling environment and in the preserved larval fish samples. Twenty-eight of 273 samples analyzed for the Bluegill qPCR assay were positive indicating that our DNA extraction procedure was successful and that there were likely no invasive carp in any of the preserved samples.

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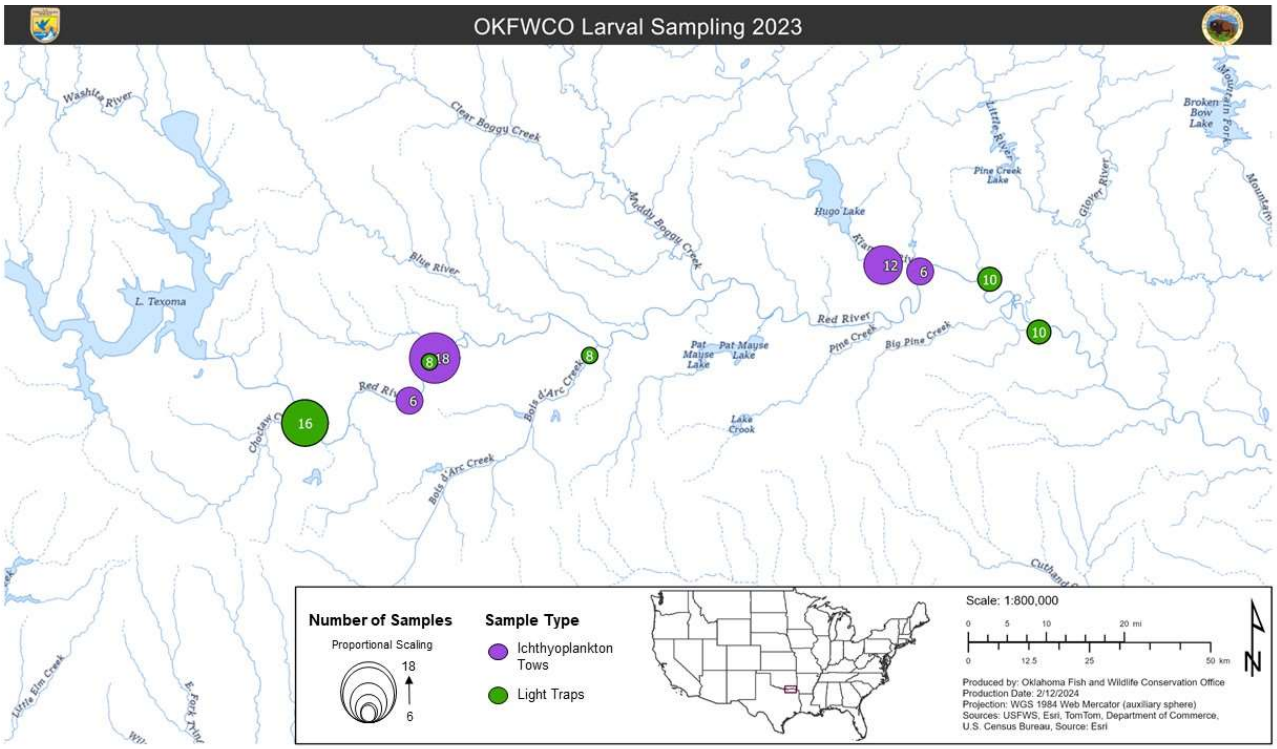

Figure 1. Ichthyoplankton tow (Purple) and light trap (green) sample locations on the Red River and its tributaries between the Denison Dam and the Arkansas border. The size of the dot corresponds to the number of samples collected at that location.