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ORIGINAL PAPER

Establishment of invasive Black Carp (*Mylopharyngodon piceus*) in the Mississippi River basin: identifying sources and year classes contributing to recruitment

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Abstract Black Carp (*Mylopharyngodon piceus*) was imported to the USA to control aquaculture pond snails. This species has escaped captivity and occurs in parts of the Mississippi River, several tributaries, and floodplain lakes, which is concerning due to potential competition with native fishes and predation on native mussels, many of which are imperiled. However, Black Carp captures have primarily been incidental by commercial fishers, and evidence of reproduction in the wild is limited. The objectives of this study were to assess relative abundance of aquaculture-origin and wild Black Carp using ploidy and otolith stable isotope analysis, identify spatial extent of natural reproduction using otolith microchemistry, assess age distributions of wild and aquaculture-source Black Carp to infer years in which natural reproduction occurred and timing of aquaculture escapement or introductions, and estimate size and age at maturation to assess whether recruitment to adulthood has occurred. Results revealed that Black Carp are established in parts of the Mississippi River basin based on findings that: (1) non-captive Black Carp primarily consist of fertile, naturally-reproduced fish, (2) reproduction has occurred in several rivers, (3) multiple year classes of wild fish are present, and (4) wild fish have recruited to adulthood. Multiple introductions or escapements of aquaculture-source fish into the wild, including both fertile and functionally sterile individuals, were also inferred. Individual growth appears to be rapid, although considerable variation was observed among fish. Additional study is suggested to refine understanding of where and when Black Carp reproduction is occurring in the Mississippi River basin.

Keywords Black carp · Invasion history · Age and growth · Stable isotopes · Otolith microchemistry · Ploidy

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Introduction

Development of successful control strategies for invasive species often requires knowledge of the relative importance of multiple invasion pathways to curtail further introductions (Hulme et al. 2008). Assessment of the invasion stage that a non-native species has reached (Blackburn et al. 2011) can also inform development of control measures and goals, because eradication becomes difficult following establishment (Simberloff 2003). For species that are reproducing in their invaded range, knowledge of life history and demographics are useful to direct control efforts to times, locations, and life stages that will enhance effectiveness of management actions intended to limit the invader's abundance and geographic range (Sakai et al. 2001; Dana et al. 2019). However, identifying sources, timing, and pathways of non-native species introductions is often challenging (Hebert and Cristescu 2002; Munro et al. 2005). Determining whether invasive fishes are reproducing and where and when reproduction has occurred can be particularly problematic due to the inherent challenges of sampling in aquatic environments, especially when targeting early life stages (Kelso et al. 2012) or sampling during the early stages of an invasion when abundance is typically low (Trebitz et al. 2017).

The Black Carp (*Mylopharyngodon piceus*) is a large (maximum total length > 1000 mm) fish species native to eastern Asia. There is considerable uncertainty regarding recruitment sources and year classes that have contributed to their invasion and expansion in the Mississippi River basin, USA (Nico et al. 2005). Internationally, Black Carp introductions in Asian, African, European, and Central, and North American countries have been facilitated by their uses as a food fish and biological control of snails (Nico et al. 2005). Black Carp are benthic invertivores (Poulton et al. 2019) and listed as injurious in the USA under the Lacey Act (USFWS 2007) due to their potential predation on imperiled, native freshwater mussels (Nico and Jelks 2011). Stable isotope and diet studies have documented trophic overlap between Black Carp and native benthic fishes in the Mississippi River basin, suggesting the potential for interspecific competition for benthic invertebrate prey (Nico et al. 2011; Poulton et al. 2019; Evans 2020). Black Carp were first imported into the USA during the 1970's and have commonly been used for biological control of snails (Nico et al. 2005). Snails targeted for biological control using Black Carp are intermediate hosts of parasitic trematodes which can infest Channel Catfish (*Ictalurus punctatus*) and other food fish raised in aquaculture ponds (Nico et al. 2005; Nico and Jelks 2011). Some commercial aquaculture facilities in the south-central USA began producing and rearing Black Carp in the 1980's, including diploid (fertile) and triploid (functionally sterile) fish, for sale and distribution to food-fish aquaculture farms for snail control (Nico et al. 2005; Papoulias et al. 2011). The triploid Black Carp certification program provided by the U.S. Fish and Wildlife Service was discontinued in 2000, limiting the capacity to track distribution of triploid and diploid fish (Nico et al. 2005). Some states ban possession of Black Carp, and importation of Black Carp into the USA has been prohibited since their 2007 listing as an injurious species under the Lacey Act. Use of Black Carp in aquaculture is permitted in Arkansas and Mississippi, where most of the known captive Black Carp in the USA are located (Hunter and Nico 2015). The total number of Black Carp that have been used in aquaculture facilities in the USA is unknown (Nico and Jelks 2011; Hunter and Nico 2015). However, between 2000 and 2007 more than 170,000 Black Carp were stocked into aquaculture ponds in Mississippi alone, and at least 10,000 of those fish were diploid (Dennis Riecke, Mississippi Department of Wildlife, Fisheries and Parks, written communication, 3 August 2018). Moreover, Black Carp presumably have escaped from aquaculture ponds into the wild; mitochondrial and microsatellite DNA markers suggest that multiple introductions or escapements have occurred in the Mississippi River basin (Hunter and Nico 2015).

The first documented non-captive Black Carp in the Mississippi River basin was a 783-mm triploid fish caught in 2003 from an oxbow lake adjacent to the Mississippi River in southern Illinois (Chick et al. 2003). Later reports indicated that commercial fishers had been catching Black Carp in the Red and Atchafalaya rivers in Louisiana on an annual or near-annual basis since the early 1990's (Nico and Jelks 2011). Reported captures of Black Carp in the Mississippi River and tributaries have substantially increased between 2003 and 2019, especially since 2015 (Kroboth et al. 2019b). Most reported Black Carp captures in the USA are incidental captures by commercial fishers (Kroboth et al. 2019b) due to limited agency and university sampling targeting Black Carp and low capture probability in the large rivers where they occur (Nico and Jelks 2011; Whitledge 2020). The documented range of Black Carp in the Mississippi River basin has expanded and now encompasses the main-stem Mississippi River between New Orleans, Louisiana and Pool 20 near Keokuk, Iowa, the lower Red-Atchafalaya River system, and several major Mississippi River tributaries, including parts of the Cumberland, Illinois, Kaskaskia, Missouri, Ohio, Tennessee, Wabash, and White rivers (Kroboth et al. 2019b; USGS 2020).

Both escapement or release of fish (either diploid or triploid) from aquaculture facilities and natural reproduction by diploid fish may contribute to non-captive Black Carp in the Mississippi River basin, although evidence of reproduction in the wild is limited. Thus, relative contributions of aquaculture-origin individuals and natural reproduction to the apparently increasing abundance and range of Black Carp in the Mississippi River basin are unknown. Both triploid and diploid Black Carp have been reported in the Mississippi River and tributaries (Hunter and Nico 2015; USGS 2020), although diploid individuals may be escapees or releases from aquaculture or the progeny of non-captive, diploid fish (Nico and Jelks 2011). Nico et al. (2005) suggested that Black Carp may have been reproducing and were possibly established in the lower portion of the Mississippi River basin based on multiple reported captures by commercial fishers over several years, presence of diploid fish, and habitats deemed suitable for all life stages. Larval and small juvenile Black Carp (< 150 mm total length) have only been collected from three locations in the USA, including 120 fish caught in Dutchtown Ditch and one in the Headwater Diversion Channel, Missouri between 2015 and 2018 and one fish caught in Gar Creek, a small tributary of the lower Ohio River in Kentucky in 2018 (USGS 2020). Thus, the spatial and temporal extents of natural reproduction by Black Carp in the Mississippi River basin are unclear. Additionally, whether any naturally-reproduced fish have recruited to adulthood is unknown.

Stable isotope and trace element compositions of otoliths have been shown to be useful to identify sources of invasive fishes when relative importance of different invasion pathways or areas where reproduction has occurred are unclear. Concentrations or stable isotope ratios of some chemical elements in otoliths are strongly correlated with corresponding elemental concentrations or stable isotope ratios of waters in which a fish resides (Pracheil et al. 2014). Otoliths are metabolically inert and contain a permanent record of environmental chemistry experienced by individual fish (Campana and Thorrold 2001). Identification of locations previously occupied by an individual fish using otolith chemistry is possible if persistent, location-specific differences in water and corresponding otolith chemistry are present and well-characterized, and an individual fish was present in a location long enough to acquire the chemical "signature" of that site (Pracheil et al. 2014). Otolith chemistry has proven useful for distinguishing introduced fish from wild fish in recently invaded locations (Munro et al. 2005; Whitledge et al. 2021a), identifying sources of introduced fish (Munro et al. 2005; Love et al. 2019), and inferring natal environments supporting natural recruitment of non-native fishes in invaded locations (Whitledge et al. 2007, 2019, 2021a; Norman and Whitledge 2015). For fish species in which triploid individuals are produced in aquaculture facilities (e.g., Grass Carp *Ctenopharyngodon idella* and Black Carp), combining data on otolith chemistry and somatic cell ploidy is advantageous for identifying fertile (diploid) and functionally sterile (triploid) individuals of aquaculture origin captured in the wild (Whitledge et al. 2021a, b). Assessing individual age and maturity status in combination with inferences regarding sources of non-native fishes can be useful for determining where and when natural reproduction or introductions happened (particularly if early life stages are rare or difficult to sample) and whether recruitment to adulthood has occurred (Whitledge et al. 2021a).

Otolith and fin ray chemistry have been used to identify natal river of native and non-native fishes within the invaded range of Black Carp in the USA, including the Illinois (Zeigler and Whitledge 2010; Norman and Whitledge 2015), lower Ohio (Abell et al. 2018), and lower Missouri (Phelps et al. 2012) rivers and the impounded and unimpounded reaches of the Mississippi River (Zeigler and Whitledge 2011; Laughlin et al. 2016; Whitledge et al. 2019). Thus, otolith chemistry has potential applicability for identifying sources of Black Carp captured in the Mississippi River and tributaries. However, no studies have applied otolith chemistry to investigate contributions of escapement or releases of aquaculture-origin fish and natural recruitment to non-captive Black Carp in the Mississippi River basin or to identify natal river for wild fish.

The goal of this study was to identify sources and assess spatial and temporal patterns of natural reproduction and recruitment of invasive Black Carp in the Mississippi River and tributaries. Study objectives were to: (1) assess relative abundance of aquaculture-origin and wild Black Carp using data from ploidy and otolith stable isotope analyses, (2) identify the spatial extent of natural reproduction by Black Carp using otolith microchemistry, (3) assess age distributions of wild and aquaculture-source Black Carp to infer years in which natural reproduction occurred and general timing of aquaculture escapement or introductions, and (4) estimate size and age at maturation for Black Carp in their invaded range to assess whether recruitment to adulthood has occurred. Knowledge of the relative contributions of aquaculture-origin and wild fish and fish from different year classes (whether spawned in captivity or the wild) to the non-captive Black Carp stock in the Mississippi River basin could be useful for informing whether actions to limit additional introductions of aquaculture-source fish or efforts to curtail natural reproduction and recruitment should be prioritized or if both require further emphasis. Greater insight regarding locations where natural reproduction has occurred may assist in guiding targeted sampling and potential control measures directed at early life stages of Black Carp. Additionally, fish source, age, growth, maturity, and ploidy data may aid in development of population models to evaluate potential control actions and quantify risks of further expansions in Black Carp range and abundance.

Materials and methods

Study area

The Mississippi River drains an area of 2,981,000 km^2, encompassing all or part of 32 states in the USA and small parts of two Canadian provinces. The river originates in northern Minnesota and flows generally south for 3730 km to Louisiana, where it drains into the Gulf of Mexico. The section of the upper Mississippi River between Minneapolis, Minnesota and the Mississippi-Missouri River confluence contains a series of impounded pools formed by 27 navigation locks and dams. The middle Mississippi River (between the mouths of the Missouri and Ohio rivers; Fig. 1) and the lower Mississippi River (downstream of the confluence of the Ohio and Mississippi rivers) are free flowing. Several of the Mississippi River's major tributaries are impounded by dams, including the Illinois, Kaskaskia, Ohio, and Arkansas rivers; large dams are also present on the Tennessee and Cumberland rivers, two major Ohio River tributaries. The lowermost 1305 km of the Missouri River are unimpounded.

Fish collection, processing of carcasses, and ploidy determination

Black Carp were obtained from captures reported by commercial fishers or state and federal agency biologists between May 2011 and September 2018 and identified using characteristics described in Kroboth et al. (2019a). Collection date and location information were obtained from personal communication with fishers or agency biologists (Whitledge et al. 2021b). Most fish were captured using hoop nets or gill nets (Kroboth et al. 2019b). Thirty-one age-0 Black Carp (24.5–116 mm total length) collected from Dutchtown Ditch (connected to the Headwater Diversion Channel, a tributary of the Mississippi River near Cape Girardeau, MO) by Missouri Department of Conservation personnel using seines during 2015–2016 were also included in this study. Ten Black Carp were obtained from an aquaculture facility in Arkansas (one of the three Black Carp production facilities in the USA) to characterize otolith stable isotope ratios of aquaculture-origin fish.

Most Black Carp (except for age-0 fish) captured in the wild were processed by a standard protocol distributed to biologists throughout the Mississippi River Basin. This protocol established the methods for collection and preservation of eyeballs for testing ploidy, gonad tissue for measuring reproductive development, fish dimensions, and capture data. Following the reported capture, most Black Carp were shipped overnight on ice to Southern Illinois University (SIU) or the U.S. Geological Survey (USGS) Columbia Environmental Research Center (CERC). A few fish were frozen by commercial fishers after collection and were shipped frozen. Location (description and GPS coordinates) and date of capture, total

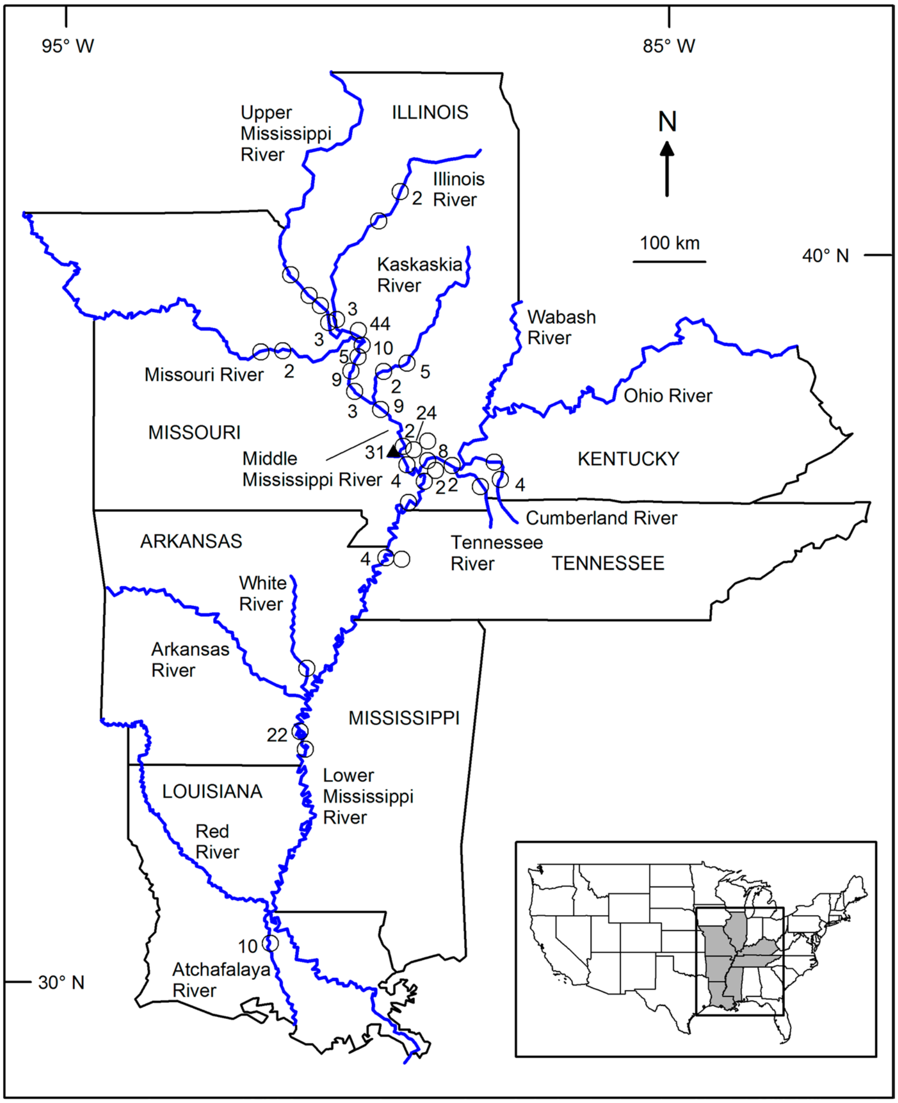

Fig. 1 Map of collection locations for Black Carp caught between May 2011 and September 2018 that were used in this study. Blue lines depict mainstem rivers, tributaries, and adjacent floodplain habitats. Circles are collection locations of fish > 400 mm total length (n = 190); filled triangle is collection location of age-0 Black Carp (n = 31). Numbers next to collection locations indicate number of individuals collected at each location when > 1 fish was caught at that site

length (TL), and weight were recorded for each fish in the lab as validation of initial biologists' measurements or for precise measurements of commercially caught fish that were directly shipped without initial state biologist contact. Eyes were removed from fish > 400 mm total length, stored in saline solution at 4–8 °C, and ploidy was assessed from vitreous humor cells by flow cytometry using procedures described in Jenkins and Thomas (2007), although ploidy determination was not possible for fish that were frozen for more than approximately one week following capture. Ploidy from age-0 fish and a few live adult captures was determined from blood collected in acid citrate dextrose and analyzed by flow cytometry immediately or within 36 h of capture (Jenkins and Thomas 2007). Collection and ploidy information for each fish was submitted to the USGS Nonindigenous Aquatic Species database (USGS 2020).

Otolith preparation and analyses

Both lapilli otoliths were extracted from each fish, rinsed with distilled water, and stored dry in microcentrifuge tubes until preparation for stable isotope and elemental analyses. One lapillus from each fish was prepared for stable oxygen and carbon isotope analysis. Otoliths used for stable isotope analysis were embedded in Epo-fix epoxy (Electron

Microscopy Sciences Inc., Hatfield, PA), sectioned in the transverse plane using an ISOMET low-speed saw, sanded using silicon carbide sandpaper (800 and 1000 grit in sequence) to achieve a 0.8-mm section centered on the otolith primordium, and polished with lapping film. Sectioned otoliths were affixed to glass microscope slides using cyanoacrylate glue. A 50-μg subsample of $CaCO_3$ powder was drilled from core of each otolith (centered on the primordium) using a New Wave Research micromill (New Wave Research, Inc., Fremont, CA). Another 50-μg subsample was drilled from the otolith edge (the most recently accreted material) for 10 fish from the aquaculture facility in Arkansas for comparison with otolith core stable isotope data from aquaculture-origin fish (including captive fish and triploid fish caught in the wild). A 50-μg subsample was also drilled from the otolith edge for each of 50 fish captured from across the Mississippi River basin (including fish from the upper Mississippi, middle Mississippi, lower Mississippi, Illinois, Ohio, Missouri, Cumberland, and Kaskaskia rivers) to characterize otolith stable oxygen and carbon isotopic compositions of fish residing in rivers.

Stable oxygen and carbon isotope analysis of otolith subsamples was conducted using a ThermoFinnigan MAT-252® isotope ratio mass spectrometer interfaced with a Kiel III® carbonate preparation device. Powdered samples from otoliths were reacted with dehydrated phosphoric acid under vacuum at 70 °C (Hoefs 2004). Isotope ratio measurements were calibrated based on repeated measurements of NBS-19 and NBS-18 standards (Friedman et al. 1982). All measurements are reported in standard delta notation as $\delta^{18}O$ or $\delta^{13}C$ (‰) = [(Rsample/Rstandard) − 1) × 1000 where R represents $^{18}O/^{16}O$ or $^{13}C/^{12}C$; data were referenced to the Vienna Pee Dee Belemnite standard. Analytical precision estimated from analysis of NBS-18 and NBS-19 standards was 0.08‰ for $\delta^{13}C$ and 0.10‰ for $\delta^{18}O$.

The second lapillus from each Black Carp was used for analysis of strontium:calcium (Sr:Ca) and magnesium:calcium (Mg:Ca) ratios. Otolith embedding, sectioning, and polishing were conducted using methods described for stable isotope analysis to expose the otolith core. Otolith thin sections were prepared for analysis under a class 100 laminar flow hood and handled only with nonmetallic forceps. Thin sections were cleaned with Milli-Q water and dried for 24 h under the laminar flow hood. Polished otolith thin sections were mounted on glass microscope slides using double-sided tape and stored in polypropylene Petri dishes in a sealed container until analysis. Sectioned otoliths were analyzed for strontium, magnesium, and calcium concentrations using a Thermo X-Series2 inductively coupled plasma mass spectrometer (ICPMS) coupled with a CETAC Technologies LSX-266 laser ablation system. The laser ablated a transect along the longest axis of the sectioned otolith, beginning approximately 100 μm from the otolith primordium, traveling through the primordium, and ending at the otolith edge on the opposite side of the otolith from the starting location (beam diameter = 25 μm, scan rate = 5 μm/s, laser pulse rate = 20 Hz, laser energy level = 35%). Each sample analysis was preceded by a 30 s argon gas blank measurement and followed by a 30 s washout period. A standard developed by the USGS (MACS-3; $CaCO_3$ matrix) was analyzed every 12–15 samples to enable quantification and correction of possible instrumental drift. Isotopes assayed included ^{25}Mg, ^{43}Ca, and ^{86}Sr. Correction for gas blank and drift effects and conversion of raw isotopic counts to elemental concentrations (μg/g) were performed using a Microsoft Excel macro (GeoPro) developed by CETAC Technologies (Honig et al. 2020). Mean limits of detection for ^{25}Mg and ^{86}Sr were 6 and 10 μg/g, respectively; concentrations of these elements in all otoliths were well above detection limits. Concentrations of strontium and magnesium were converted to molar element:calcium ratios (Sr:Ca, Mg:Ca; μmol/mol) using calcium as an internal standard and the stoichiometric concentration of calcium in aragonite (Pracheil et al. 2014). The relative standard deviations for Sr:Ca and Mg:Ca measurements in the MACS-3 reference standard were 4.6 and 2.7, respectively. Otolith Mg:Ca was used in conjunction with Sr:Ca as an indicator of vaterite (a crystalline form of $CaCO_3$ that exhibits inherently higher Mg and lower Sr concentrations than the more common aragonite form of $CaCO_3$ typically found in teleost otoliths; Melancon et al. 2005). Otolith Mg:Ca > 1000 μmol/mol in combination with Sr:Ca < 100 μmol/mol was considered indicative of vaterite (Whitledge et al. 2019). Data from vateritic otoliths (or portions thereof) were excluded from analyses to avoid potential misinterpretation of fish environmental history, because data from vateritic and aragonitic otoliths are not directly comparable (Pracheil et al. 2019a).

Distinguishing aquaculture-origin and wild Black Carp

Use of otolith stable isotope data to identify aquaculture-origin and wild Black Carp caught in the Mississippi River basin required characterization of otolith $\delta^{18}O$ and $\delta^{13}C$ of both aquaculture-source and wild fish. Otolith core and edge $\delta^{18}O$ and $\delta^{13}C$ of fish obtained from the aquaculture facility and otolith core $\delta^{18}O$ and $\delta^{13}C$ of triploid Black Carp (considered aquaculture-origin because spontaneously wild-produced triploidy is extremely rare in most fishes) captured in Mississippi River basin rivers and floodplain lakes ($n = 8$) were used to characterize otolith $\delta^{18}O$ and $\delta^{13}C$ of aquaculture-origin Black Carp. Otolith edge $\delta^{18}O$ and $\delta^{13}C$ data from Black Carp sampled from the Mississippi River basin ($n = 50$) were used to characterize otolith $\delta^{18}O$ and $\delta^{13}C$ of wild fish. To depict isotopic signatures representing aquaculture-origin and wild Black Carp in stable isotope biplots and facilitate comparison with otolith core $\delta^{18}O$ and $\delta^{13}C$ data from fish of unknown origin, 99% credible interval ellipses were fit to stable isotope data from known aquaculture-source fish and otolith edge $\delta^{18}O$ and $\delta^{13}C$ data from Black Carp caught in the wild using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011) in R (v. 3.4.0) with uninformed priors (R Core Team 2017). Otolith core $\delta^{18}O$ and $\delta^{13}C$ data for diploid or unknown ploidy, wild-caught Black Carp were compared with ranges of otolith $\delta^{18}O$ and $\delta^{13}C$ considered to be indicative of aquaculture-origin and wild Black Carp to infer origin of each fish. Diploid or unknown ploidy individuals with otolith core $\delta^{18}O$ and $\delta^{13}C$ within the range of known aquaculture-source fish were identified as fish that either may have escaped from aquaculture facilities or may have been released into the wild; diploid or unknown ploidy fish with otolith core $\delta^{18}O$ and $\delta^{13}C$ within the range of otolith edge $\delta^{18}O$ and $\delta^{13}C$ for Black Carp sampled from the Mississippi River basin were inferred to be wild fish.

Identifying natal river of wild Black Carp

Otolith core Sr:Ca was used to infer potential natal rivers for wild fish. Other natural markers commonly used in studies of fish hard-part microchemistry (e.g., Ba:Ca, Mg:Ca, Mn:Ca, $^{87}Sr/^{86}Sr$) were not used in this study due to no differences among rivers within or near the known range of Black Carp in the USA or no additional capability of distinguishing locations beyond that enabled by use of otolith Sr:Ca (Zeigler and Whitledge 2011; Whitledge, unpublished data). Scant water Ba:Ca data for some lower Mississippi River tributaries also limited the ability to interpret otolith Ba:Ca data. Application of otolith core Sr:Ca to infer natal river required knowledge of spatial and temporal variability in water Sr:Ca among potential Black Carp natal rivers and river-specific ranges of otolith Sr:Ca for Black Carp to characterize the chemical 'signature' representative of each river. Known-origin, age-0 fish are commonly used to define otolith chemical 'signatures' of natal locations, but this approach could not be used in this study because age-0 Black Carp otoliths were only available from one location (Dutchtown Ditch), and fish may have moved there from another location. The limited number and geographic locations of commercial aquaculture facilities that spawn Black Carp (three, all in eastern Arkansas) also precluded use of fish residing in aquaculture facilities for characterizing the relation between water and Black Carp otolith Sr:Ca across the broad range of water Sr:Ca documented in the Mississippi River and tributaries (Zeigler and Whitledge 2010, 2011; Laughlin et al. 2016; Abell et al. 2018; Whitledge et al. 2019). Therefore, otolith edge Sr:Ca (mean of last 25 µm of laser ablation transects) for wild-caught Black Carp in the Mississippi River basin were used to estimate river-specific ranges of otolith Sr:Ca that were assumed to be characteristic of resident Black Carp in the river in which the fish were captured. Otolith edge Sr:Ca and water Sr:Ca data from sampling during June through October 2006–2018 for rivers where fish were collected (Zeigler and Whitledge 2010, 2011; Laughlin et al. 2016; Abell et al. 2018; Whitledge et al. 2019) were used to assess whether differences in water Sr:Ca among rivers corresponded with differences in otolith edge Sr:Ca among rivers. This approach may result in inclusion of otolith chemistry data from recent immigrants that have not resided in their collection location long enough to acquire that site's chemical signature. However, the absence of Black Carp of known environmental history in their invaded range prevented use of known-history individuals for characterizing river-specific otolith Sr:Ca values or assessing the relation between water and Black Carp otolith Sr:Ca.

Differences in mean water Sr:Ca among rivers across years were assessed using mixed model analysis of variance and least squares means with Tukey's adjustment for multiple comparisons. Otolith core Sr:Ca (mean for a 25 µm section of the laser ablation transect centered on the primordium) for each wild, diploid and unknown ploidy Black Carp collected from the Mississippi River and tributaries was compared with river-specific ranges of otolith edge Sr:Ca to infer potential natal rivers for each of these individuals.

Estimating age, year of hatch, growth, and age at maturity

Black Carp ages were estimated using fin rays and vertebrae due to allocation of otoliths for stable isotope and microchemical analyses, thus requiring the use of alternative structures for age estimation. Fin rays and vertebrae have both been applied for the age estimation of Grass Carp (Whitledge et al. 2021a), the closest relative of Black Carp. Nico et al. (2005) indicated that scales are the common traditional method for aging Black Carp, but they found that in past studies not all authors reported complete methods. We did not use scales for aging due to the common occurrence of regenerated scales observed while sampling. Prior to 2015, postcleithra were also collected, but this was discontinued because postcleithra were found to be inferior aging structures for Black Carp. Pectoral fin rays, dorsal fin rays, and ossified vertebrae were removed from fish > 400 mm TL and frozen until batch cleaning. Black Carp < 400 mm TL are not fully recruited to the commercial capture methods from which most of the study fish were sourced. Thus, there were few available individuals < 400 mm TL and those fish were from a single collection site and may not have been representative of fish across a broader geographic area. Excess flesh and fats were boiled and manually removed from structures. Structures were then soaked in a 2% sodium hypochlorite solution and desiccated at 55-65℃ for 24 h. Cleaned structures were sectioned and mounted on microscope slides. The dorsal ray consists of two filaments which divide upon cleaning. One filament was mounted and sectioned closest to the point of articulation. The leading pectoral fin ray consists of two filaments; each of these were sectioned close to the point of articulation and mounted for aging. Vertebrae were sectioned diagonally and mounted. Digital images were recorded with a Nikon SMZ25 microscope using transmitted light to enhance annuli. Ages were estimated independently by two readers based on images, with disputes settled by a third. If multiple structures from the same fish produced conflicting age estimates, the reported age is the observed range of ages, and these fish were excluded from any further analyses.

Annuli were assigned as translucent bands in the sectioned images of each structure. An annulus is a translucent band that persists throughout all measured axes of a structure. For sectioned vertebrae, presence of corresponding annuli on the four axes identified that an annulus was not a false or pseudo-annulus. False annuli are often incomplete or irregular zones, found only in one region of a structure that are not associated with growth zones formed during annual growth reductions (VanderKooy 2009). False annuli were identified based on proximity and equivalent morphology to adjacent annuli, and commonly represented double bands with less than the anticipated annual growth observed in the structure. For use as reference fish for aging, one-year old Black Carp (n = 10) from hatchery stock (Keo Farms, Keo, Arkansas) were obtained to assess size and shape of the first annulus.

Age for each structure was assigned as the number of fully developed annuli based on an assigned January 1st hatch date. Black carp are assumed to spawn in the summer in North America, similar to their Chinese counterparts (Nico et al. 2005; Song et al. 2018). The final age for each fish was designated from a minimum of two structures, except for one 2011 and two 2014 samples from which only vertebrae or pectoral fin rays were available. Year of hatch was estimated for individual fish by subtracting estimated age from year of capture.

Due to latitudinal and range-wide variation in growth and assumed hatching date, time of year of capture, and growth rate among lotic and lentic environments, a standard von Bertalanffy growth model was not fit to length-at-age data. Instead, mean length-at-age data were fit with a local polynomial regression and 95% confidence interval for samples collected from 2011–2017. This regression is presented as a reference for future population level age and growth analyses.

To assess size at maturity for male and female Black Carp, sex was determined for individual fish > 400 mm TL by visual examination of the gonads. Gonadosomatic index (GSI) was calculated as gonad weight/total body weight *100. Gonadosomatic index can be applied as a relative measurement of reproductive condition throughout the year and estimation of spawning period (Papoulias et al. 2006). Black Carp were collected throughout the year for this study but did not approach the required sample sizes for this type of analysis. Thus, results represent only relative gonadal development in relation to TL of wild Black Carp. Local polynomial regressions were used to assess trends in GSI with TL by sex and to estimate size at maturity for males and females from shifts in GSI distribution with fish TL. Most gonad samples were obtained from commercial captures of Black Carp between 2011 and 2017, either processed in the field or shipped with the whole carcass overnight on ice for extraction. Only one triploid Black Carp was collected during this period. We excluded this sample from analysis because of the direct effect of triploidy on reproductive development (Papoulias et al. 2011). Gonads deteriorate rapidly, and thus, 16 samples that were not properly preserved after capture were excluded from analysis. Additionally, male specimens < 600 mm TL possessed a greater volume of fat and connective tissue relative to body mass of fish, inflating estimated GSI values. This result is anticipated based on the histological observations of Papoulias et al. (2011) for triploid Black Carp. Female samples were not available for fish < 600 mm TL, and thus, GSI values are reported only for female and male Black Carp > 600 mm TL.

Results

The 221 Black Carp used in this study included fish collected in the upper, middle, and lower Mississippi rivers, the Atchafalaya River, Mississippi River floodplain lakes, sloughs and connected ditches, and several tributaries, including the Illinois, Missouri, Kaskaskia, lower Ohio, Tennessee, Cumberland, and White rivers (Fig. 1). Fish ranged from 24.5 to 1607 mm TL, with most individuals between 600 and 1000 mm TL (Fig. 2). Of the 165 Black Carp > 400 mm TL captured between May 2011 and September 2018 that were tested for ploidy, 137 were diploid and 8 (5.5%) were triploid; ploidy testing was inconclusive for 20 individuals due to eye cell degradation (carcasses frozen too long or eyes warmed excessively during shipment). All age-0 Black Carp included in this study (n = 31) were confirmed diploid. The data set for this project is available in Whitledge et al. (2021b).

Otolith chemistry

Otolith stable isotope data indicated a distinction between aquaculture-source Black Carp and non-captive Black Carp in the Mississippi River basin (Whitledge et al. 2021b). Otolith core $\delta^{18}O$ of aquaculture-source Black Carp ranged from − 0.56‰ to − 4.35‰ and otolith edge $\delta^{18}O$ of aquaculture-source fish ranged from − 2.63 to − 3.97‰. Neither otolith core nor edge $\delta^{18}O$ values for aquaculture-source fish overlapped with the range of otolith edge $\delta^{18}O$ for wild-caught Black Carp in the Mississippi River basin (− 4.96 to − 9.92‰; Fig. 3a). Otolith core $\delta^{13}C$ of aquaculture-source Black Carp ranged from − 5.66 to − 10.89‰, and otolith edge $\delta^{13}C$ of aquaculture-source Black Carp ranged from − 5.50 to − 11.57‰,

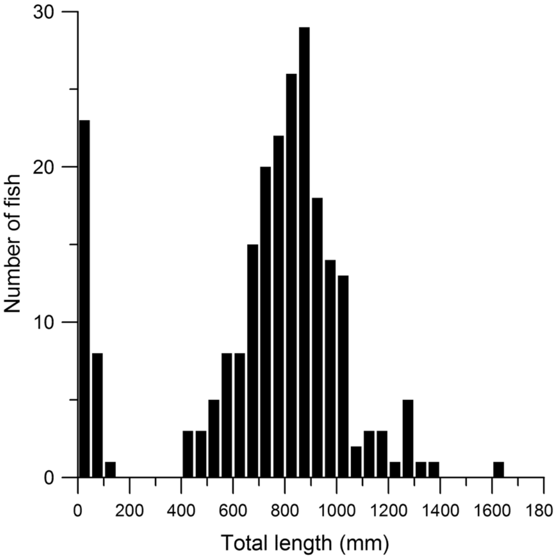

Fig. 2 Length-frequency distribution of Black Carp collected in the Mississippi River basin during May 2011–September 2018 used in this study

whereas otolith edge δ^{13}C of Black Carp caught in the Mississippi River and tributaries ranged from – 10.01 to – 15.43‰ (Fig. 3a). Combined measurements of otolith δ^{18}O and δ^{13}C resulted in non-overlapping ranges of otolith stable isotope signatures for Black Carp that resided in aquaculture facilities and Black Carp inhabiting the Mississippi River and tributaries (Fig. 3a).

Among the 180 diploid and unknown ploidy Black Carp captured in the Mississippi River basin that were analyzed for otolith core δ^{18}O and δ^{13}C, 18 had otolith core δ^{18}O and δ^{13}C values that fell within the ranges of otolith δ^{18}O and δ^{13}C for known aquaculture-source fish and 161 (89%) had otolith core δ^{18}O and δ^{13}C values that were within or just outside the periphery of otolith edge δ^{18}O and δ^{13}C values measured in wild-caught Black Carp (Fig. 3b). One diploid fish had otolith core δ^{18}O and δ^{13}C that plotted outside of the combined ranges of δ^{18}O and δ^{13}C measured in aquaculture-origin and river-resident Black Carp, although its otolith core δ^{18}O (– 2.53‰) was within the range of otolith δ^{18}O for aquaculture-source fish.

Persistent differences in water Sr:Ca among several rivers in or near the known range of Black Carp in the Mississippi River basin and corresponding differences in Black Carp otolith edge Sr:Ca among rivers provide evidence of the utility of otolith core Sr:Ca to identify the set of potential natal rivers for individual fish identified as wild by otolith core δ^{18}O and δ^{13}C. Available data indicated that mean water Sr:Ca differed among multiple rivers within and near the known range of Black Carp ($F_{11,76} = 63.98$, $P < 0.0001$), although ranges of water Sr:Ca for several rivers partially overlapped (Fig. 4). The highest water Sr:Ca occurred in the Missouri and Arkansas rivers and Dutchtown Ditch (Fig. 4). Mean water Sr:Ca did not differ among the middle and lower Mississippi, Ohio, and Atchafalaya rivers ($P > 0.05$). Water Sr:Ca values < 1.65 mmol/mol only occurred in the upper Mississippi, Illinois, Kaskaskia, Tennessee, Cumberland, and White rivers (Fig. 4).

Distributions of otolith edge Sr:Ca among water bodies where Black Carp were collected were consistent with the pattern of observed differences in water Sr:Ca among locations (Figs. 4 and 5; Whitledge et al. 2021b). All but three Black Carp with otolith edge Sr:Ca > 1000 µmol/mol were collected in the Missouri River or Dutchtown Ditch. Black Carp

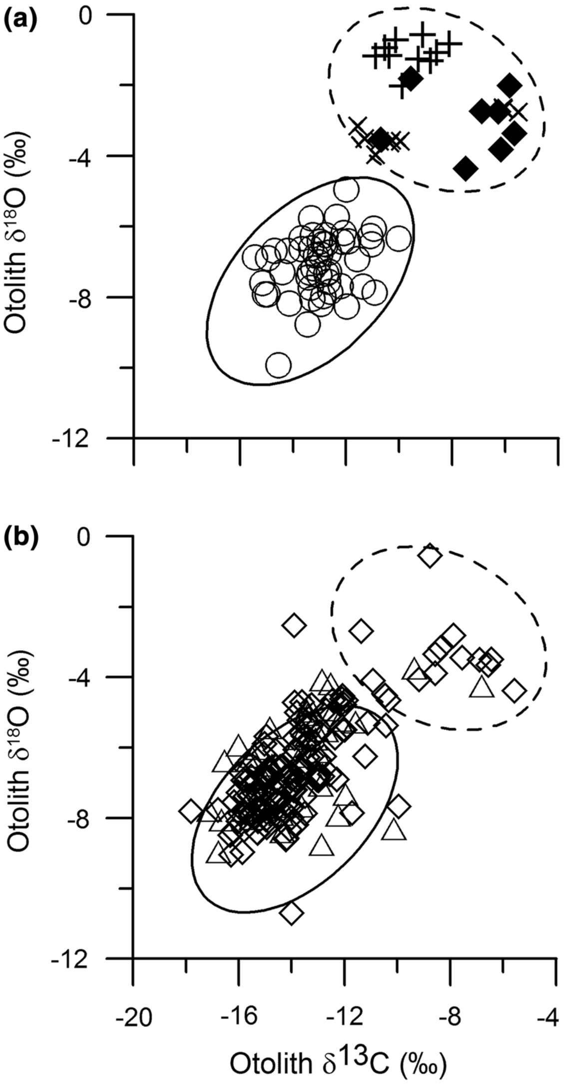

Fig. 3 **a** Otolith core δ^{18}O and δ^{13}C of Black Carp obtained from an aquaculture facility (n = 10; plus symbols), otolith edge δ^{18}O and δ^{13}C of aquaculture fish (n = 10; 'x' symbols), otolith core δ^{18}O and δ^{13}C of triploid Black Carp caught in the Mississippi River basin (n = 8; filled diamonds), and otolith edge δ^{18}O and δ^{13}C of Black Carp captured in the Mississippi River basin (n = 50; open circles). Ellipses were fit to otolith δ^{18}O and δ^{13}C data using Stable Isotope Bayesian Ellipses in R (SIBER) package (99% credible intervals, uninformed priors) for aquaculture-sourced fish (dashed) and otolith edge data from non-captive fish (solid). **b** Otolith core δ^{18}O and δ^{13}C of diploid (open circles) and unknown ploidy (open triangles) Black Carp collected from the Mississippi River basin during 2011–2018 (n = 180). Ellipses are identical to those shown in Fig. 3a and depict distributions of otolith δ^{18}O and δ^{13}C of aquaculture-origin fish (dashed) and otolith edge δ^{18}O and δ^{13}C of wild fish (solid) for comparison with otolith core δ^{18}O and δ^{13}C data from non-captive, diploid and unknown ploidy Black Carp collected in the Mississippi River basin

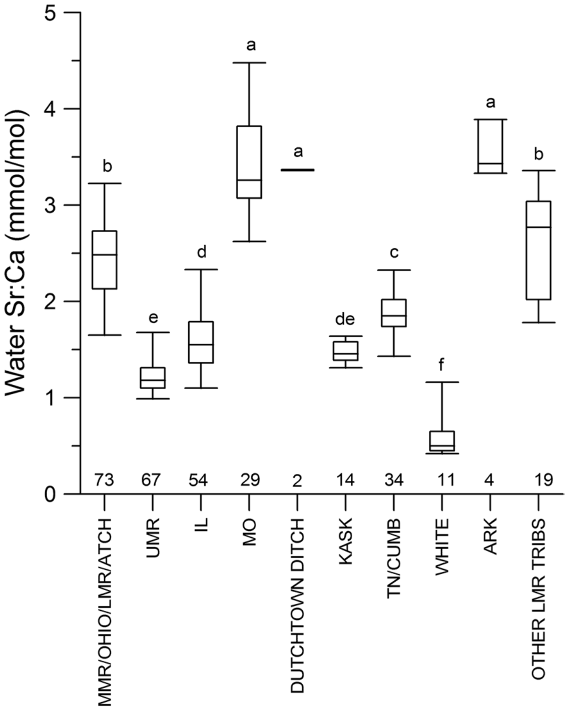

Fig. 4 Boxplot showing medians, inter-quartile ranges, and ranges of water Sr:Ca for water samples collected during 2006–2018 from the Middle Mississippi (MMR), Ohio (OHIO), Lower Mississippi (LMR), and Atchafalaya (ATCH) rivers; Upper Mississippi River (UMR; Illinois River (IL); Missouri River (MO); Dutchtown Ditch; Kaskaskia River (KASK); Tennessee (TN) and Cumberland (CUMB) rivers; White River (WHITE); Arkansas River (ARK); and other tributaries of the Lower Mississippi River. Numbers above site names are sample sizes. Mean water Sr:Ca differs among locations that do not bear the same letter above boxplots (mixed model ANOVA followed by least squares means (with Tukey's adjustment) test for multiple comparisons, $P < 0.05$)

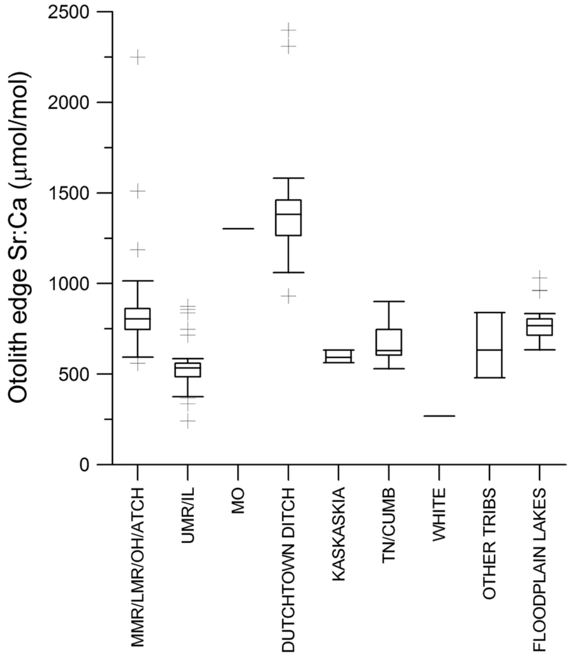

Fig. 5 Boxplot showing medians, inter-quartile ranges, and ranges of otolith edge Sr:Ca for Black Carp collected in the Middle Mississippi (MMR), Lower Mississippi (LMR), lower Ohio (OH) and Atchafalaya (ATCH) rivers ($n = 86$); Upper Mississippi (UMR) and Illinois (IL) rivers ($n = 46$); Missouri River (MO; $n = 3$); Dutchtown Ditch ($n = 30$); Kaskaskia River ($n = 3$); Tennessee (TN) and Cumberland (CUMB) rivers ($n = 6$); White River, (WHITE; $n = 1$); other Mississippi River tributaries (OTHER; $n = 3$), and Mississippi River floodplain lakes ($n = 23$). Outliers (data points > 1.5 × interquartile range above the 3rd quartile or 1.5 × interquartile range below the 1st quartile) are plotted as '+' symbols

caught in Mississippi River floodplain lakes had otolith edge Sr:Ca within the range of otolith edge Sr:Ca for fish caught in the middle and lower Mississippi rivers. Fish that had otolith edge Sr:Ca < 600 µmol/mol (the minimum value observed among Black Carp captured in the middle and lower Mississippi, Ohio, and Atchafalaya rivers) were only collected in the set of rivers (upper Mississippi, Illinois, Kaskaskia, Tennessee, Cumberland, and White) in which water Sr:Ca values < 1.65 mmol/mol were observed and mean water Sr:Ca was lower compared to the middle and lower Mississippi, Ohio, and Atchafalaya rivers. Five Black Carp (including one age-0 fish) with entirely vateritic lapilli and two fish with vaterite at the otolith edge were excluded from analyses of otolith edge Sr:Ca data.

Otolith core Sr:Ca of wild Black Carp > 400 mm TL ranged from 218 to 1475 µmol/mol, with most fish having otolith core Sr:Ca values between 600 and 1000 µmol/mol (Fig. 6; Whitledge et al. 2021b). Thirty-one fish had otolith core Sr:Ca < 600 µmol/mol; one of these fish was caught in the White River, Arkansas and had otolith Sr:Ca < 300 µmol/mol along the entire laser ablation transect (Fig. 7). Twenty-nine of the other 30 Black Carp with otolith core Sr:Ca < 600 µmol/mol were caught in locations upriver from the confluence of the Mississippi and Ohio Rivers; three of these fish had otolith Sr:Ca < 600 µmol/mol throughout the entire laser traverse and were captured in the upper Mississippi or Illinois rivers (Fig. 7). No inferences regarding natal

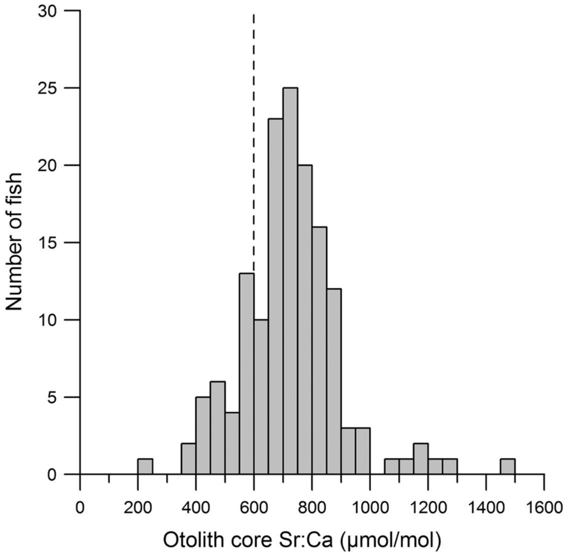

Fig. 6 Frequency distribution of otolith core Sr:Ca for wild Black Carp > 400 mm total length collected in the Mississippi River basin during 2011–2018. Vertical dashed line is lower limit of otolith edge Sr:Ca for Black Carp collected in the Middle Mississippi, Lower Mississippi, Ohio and Atchafalaya rivers (600 µmol/mol)

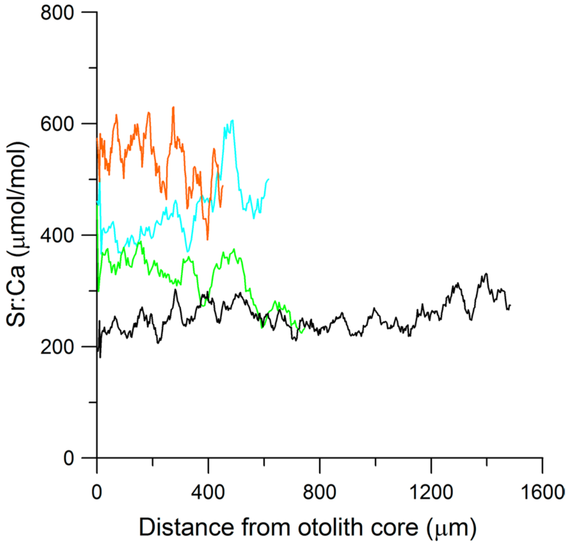

Fig. 7 Otolith Sr:Ca along laser ablation transects from otolith core to edge for four Black Carp captured in the Illinois River (two fish; light blue and green lines), Upper Mississippi River (orange line), and White River (black line)

river could be made for the four fish > 400 mm total length that had entirely vateritic lapilli and one fish that had a vateritic otolith core.

Age distributions of wild and aquaculture-origin fish

Age data suggest that captured individuals > 400 mm TL ranged from one to nine years old. The strongest year classes present among fish captured at > 400 mm TL were 2010, 2014, and 2015 (Fig. 8). Relative frequencies of year classes among rivers in our sample were undoubtedly affected by collection efforts, as equivalent sample sizes were not available from each river or river reach where Black Carp were captured. The upper Mississippi River (n = 43), lower Mississippi River (n = 38), middle Mississippi River (n = 22), Atchafalaya River (n = 12), and Kaskaskia River (n = 6) possessed the largest sample sizes for identifying year classes of captured Black Carp. The Illinois, Ohio, Tennessee and Cumberland, and White rivers had three or fewer reported captures within the time frame of the study.

Of the 27 Black Carp identified as hatchery origin by otolith microchemistry and/or ploidy analyses, 17 Black Carp were captured in a southern Illinois oxbow lake in 2018, including seven triploid fish, six diploid individuals, and four fish of undetermined ploidy (Whitledge et al. 2021b). Age was not clear for many structures but ranged from 2–4 years with hatch years in 2014 and 2015. The remaining ten aquaculture-origin

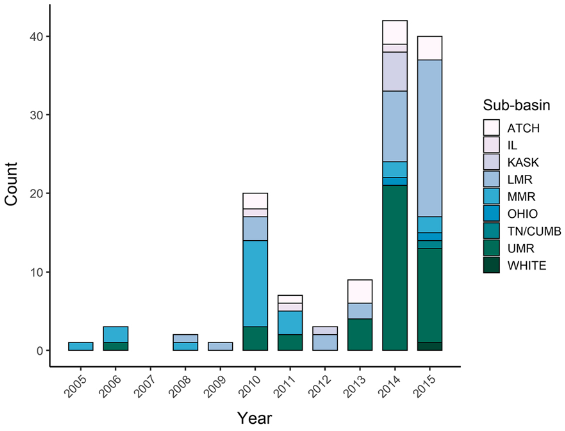

Fig. 8 Frequency distribution of year classes represented among Black Carp > 400 mm total length collected from the Mississippi River basin during 2011–2017. Sub-basin labels represent the final capture location of individual fish, as the location of reproduction may differ from capture location

fish were spread across the range of reporting locations in the upper Mississippi, middle Mississippi, lower Mississippi, Illinois, and Ohio rivers from 2011–2018. Three of these 10 fish had undetermined ages. The remaining six fish ranged from 2–4 years old. Of the ten aquaculture-origin fish caught in the Mississippi River or tributaries, only one individual (captured from the middle Mississippi River near St. Louis, MO) was a triploid; six fish were diploid, and the remaining three fish were of undetermined ploidy.

Size and age at maturation and growth

Gonadosomatic index varied by gender and length. Males appear to begin maturation at around 800 mm TL, whereas females appear to mature at around 1000 mm TL based on the asymptotes in relation between GSI and total length by gender (Fig. 9a; Whitledge et al. 2021b). Male GSI values were less than those of female fish due to low gonadal body mass variation.

Length-at-age data for fish collected during 2011–2017 suggest that growth is variable within the known range of Black Carp (Fig. 9b). Of the 132 Black Carp aged, four samples were excluded due to lack of agreement between readers or indistinct annuli. Pooling length-at-age data for individuals collected from several rivers and habitats across the known range of Black Carp in the Mississippi River basin and variability in time of year in which fish were collected resulted in broad ranges in total length of fish at estimated ages. For example, variability in total length of age-2 fish exceeded 500 mm (Fig. 9b).

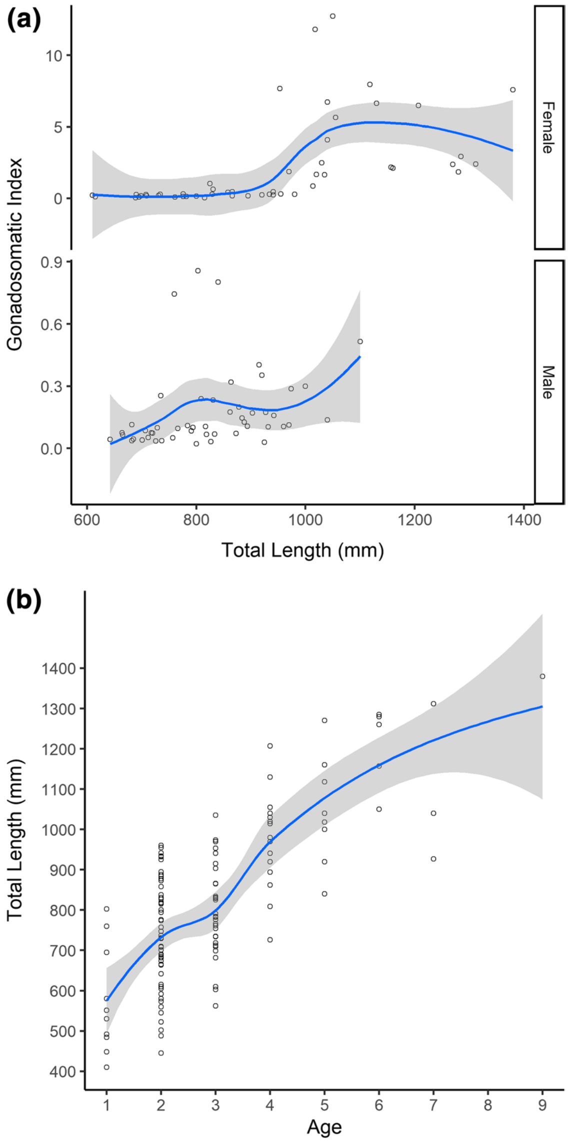

Fig. 9 Relation between gonadosomatic index (**a**) and length-at-age (**b**) of Black Carp > 400 mm total length collected from the Mississippi River basin during 2011–2017. Blue curves are polynomial regressions fit with grey shading depicting 95% confidence intervals around regression curves. Gonadosomatic index is presented for both female and male Black Carp with plots truncated for fish > 600 mm total length (n = 100). Length-at-age is plotted for 128 Black Carp to depict variability in growth among individuals collected from across the current species range during the collection timeframe

Discussion

Evidence of Black Carp establishment in the Mississippi River basin

Black Carp otolith core $\delta^{18}O$ and $\delta^{13}C$, ploidy, age, and GSI data, coupled with prior collections of age-0 fish from a few locations, collectively provide strong evidence that Black Carp are established in the Mississippi River basin due to multiple year classes of wild fish that have recruited to adulthood. Ninety-four percent of Black Carp for which ploidy was determined were diploid and 89% of diploid and unknown ploidy individuals had otolith core $\delta^{18}O$ and $\delta^{13}C$ characteristic of wild fish. Thus, non-captive Black Carp in the Mississippi River basin appear to primarily consist of fertile, naturally-reproduced fish. If the increase in reported Black Carp captures in the Mississippi River basin during the time frame of this study (Kroboth et al. 2019b) is at least partly due to increasing fish abundance in the wild rather than simply an increase in reporting rate, our results indicate that increasing abundance would be primarily attributable to natural reproduction rather than escapement or release of captive fish. Age estimates of wild Black Carp indicated that multiple year classes are present in the Mississippi River and tributaries, and examination of gonads revealed that mature fish appear to be common. Although collections of age-0 Black Carp have been limited in terms of locations and number of individuals captured, age-0 fish collections confirm that reproduction occurred during at least three years (USGS 2020). Identification of several mature, diploid individuals of wild origin using otolith core δ^{18}O and δ^{13}C provides the first evidence of recruitment to adulthood for naturally-reproduced Black Carp in the Mississippi River basin. Nico et al. (2005) suggested that Black Carp may have been reproducing and were possibly established in the lower portion of the Mississippi River basin based on multiple reported captures by commercial fishers over several years, presence of diploid fish, and habitats deemed suitable for all life stages. Our results indicate that Black Carp are indeed established in the lower Mississippi and Atchafalaya rivers, as well as in the middle Mississippi River, part of the upper Mississippi River basin, and potentially in some tributaries based on collection of predominantly naturally-reproduced fish, including adults, representing multiple year classes in each of these areas during the time frame of this study.

Identifying wild and aquaculture-origin Black Carp

Results of this study illustrate the utility of otolith core δ^{18}O and δ^{13}C for determining whether non-captive, diploid Black Carp caught in the Mississippi River basin represent escapees or releases from aquaculture or are naturally reproduced (wild) fish. Non-overlapping stable isotope signatures (δ^{18}O and δ^{13}C in combination) of otolith core and edge samples from known aquaculture-origin fish compared to otolith edge samples from Black Carp captured in multiple rivers across the species' invaded range demonstrate a distinction between fish residing in aquaculture facilities and fish living in the wild; this distinction is required to identify the source (aquaculture or wild) of non-captive, diploid individuals. Use of this approach to identify aquaculture-source Black Carp captured in the wild requires that fish were in captivity long enough to accrue otolith core stable isotope ratios characteristic of the aquaculture facility from which they originated. Overlapping ranges of otolith core and edge δ^{18}O and δ^{13}C for fish obtained from an aquaculture facility and otolith core δ^{18}O and δ^{13}C of triploid Black Carp captured in the wild corroborates the applicability of otolith core δ^{18}O and δ^{13}C to identify aquaculture-origin Black Carp caught in the Mississippi River basin.

Differences in δ^{13}C and δ^{18}O between otolith samples reflecting Black Carp held in captivity and fish living in the wild likely resulted from differences in δ^{13}C of dissolved inorganic carbon (DIC) and water δ^{18}O in aquaculture ponds compared to the Mississippi River and tributaries. Otolith δ^{13}C is strongly influenced by δ^{13}C of DIC (Solomon et al. 2006) and δ^{13}C values of DIC in lakes are often higher (less negative) compared to rivers due to the predominance of isotopically light, respired carbon in the latter (Hoefs 2004). Otolith and water δ^{18}O are highly correlated (Zeigler and Whitledge 2010; Norman and Whitledge 2015), suggesting that the Mississippi River and tributaries likely have lower (more negative) water δ^{18}O than aquaculture ponds where Black Carp are raised or used for snail control. Lower water δ^{18}O in the Mississippi River and tributaries compared to aquaculture ponds in the south-central USA might be expected due to decreasing δ^{18}O of precipitation and surface waters with increasing latitude, altitude, and distance from the ocean and conveyance of isotopically light water in river flow from areas of the Mississippi River basin where mean precipitation δ^{18}O values are more negative (Kendall and Coplen 2001; Hoefs 2004); the middle and lower Mississippi rivers have lower δ^{18}O than nearby rivers and lakes supplied by local precipitation (Coplen and Kendall 2000; Zeigler and Whitledge 2011). Higher (less negative) water δ^{18}O might also occur in aquaculture ponds than in rivers due to a higher rate of loss of isotopically light water from evaporation, particularly if water residence time is longer in ponds (Hoefs 2004). Higher otolith δ^{18}O has been observed for Bighead Carp (*Hypophthalmichthys nobilis*) from aquaculture ponds compared to wild Bighead Carp from the

Illinois River (Love et al. 2019) and for aquaculture-source Grass Carp compared to wild Grass Carp captured in the Illinois River and the Great Lakes basin (Whitledge et al. 2021a), consistent with the pattern observed for Black Carp in this study. Bighead Carp and Grass Carp obtained from aquaculture facilities for analysis of otolith $\delta^{18}O$ by Love et al. (2019) and Whitledge et al. (2021a) included fish from three farms that currently produce or formerly raised Black Carp, only one of which was the source of farm-resident fish used in this study. Thus, although Black Carp were obtained from only one of the three current commercial suppliers of Black Carp in the USA (all located in Arkansas), Black Carp from other aquaculture facilities would also be expected to differ in otolith $\delta^{18}O$ from wild fish. However, our data do not enable identification of specific aquaculture facilities that represent sources (either sites where fish were hatched or sites from which fish escaped or were released) of aquaculture-origin Black Carp captured in the wild. Therefore, our results do not implicate any individual aquaculture facility (production source or pond where Black Carp were used for snail control) as the source or escapement location of these fish.

Although most diploid Black Carp captured in the Mississippi River, its tributaries, and floodplain lakes were wild, some aquaculture-origin fish (including both diploid and triploid individuals) were identified, indicating that escapement to the wild occurred during the time frame encompassed by the lifetimes of fish identified as aquaculture-origin individuals in this study. Identification of diploid, aquaculture-origin individuals in the wild is consistent with aquaculture escapement and/or release as the presumed mechanism by which non-captive Black Carp initiated a breeding population in the Mississippi River basin. Evidence of multiple hatch years for aquaculture-source fish is consistent with diverse haplotypes and microsatellite DNA groupings in a small sample of non-captive Black Carp, which suggested multiple introductions and/or escapements occurred in the Mississippi River basin (Hunter and Nico 2015). Some aquaculture-source fish identified in our study were captured in the middle Mississippi River and an adjacent floodplain lake, and in the upper Mississippi and Illinois rivers, far from areas where use of Black Carp in aquaculture is permitted, suggesting the potential for long-distance dispersal or human-mediated transport of escaped or released fish. Evidence of aquaculture-origin Black Carp in the wild is of particular concern for areas where Black Carp are not present but could potentially become established.

Spatial and temporal patterns of natural reproduction and recruitment

Age estimates of Black Carp identified as wild fish using otolith core $\delta^{18}O$ and $\delta^{13}C$ indicated that this species has been reproducing in the Mississippi River basin since at least 2005. This confirms a previous suggestion that Black Carp may have been reproducing in the lower portion of the Mississippi River basin for more than a decade prior to collection of the first age-0 fish in the wild (Nico et al. 2005). The substantial time lag between the onset of natural reproduction and collection of age-0 Black Carp in the wild is likely due to the near absence of sampling efforts targeting Black Carp prior to 2015 (Hunter and Nico 2015) and the relatively low probability of capturing age-0 fish in a large river system during the early stages of an invasion when abundance is low (Trebitz et al. 2017). Similarity of the larvae and young of year to those of the related Grass Carp (Yi et al. 2006), coupled with unfamiliarity with this species in North America, may also have contributed to low probability of detection. Our results highlight the utility of otolith chemistry to distinguish natural recruitment from introduction or escapement of fish early in an invasion. Application of otolith chemistry for this purpose is possible provided that elemental or isotopic compositions of otoliths from exogenous fish and individuals residing in the invaded locations are distinct and well-characterized, otolith size provides sufficient sample for analysis, and that fish resided in their source location long enough to acquire the chemical signature of that site (Munro et al. 2005; Whitledge et al. 2021a).

Considering documented differences in water Sr:Ca and corresponding Black Carp otolith edge Sr:Ca among some rivers within and near the invaded range of Black Carp in the Mississippi River basin, the broad range of otolith core Sr:Ca among wild, large juvenile and adult Black Carp suggests that reproduction has likely occurred in multiple locations. However, inferences regarding natal river of individual fish in this study required making the assumption that otolith edge Sr:Ca accurately represented the range of otolith Sr:Ca for resident Black Carp in each river. The absence of Black Carp of known environmental history in the study area did not permit testing of this assumption or use of known-origin fish to characterize Sr:Ca signatures of potential natal rivers. Otolith edge Sr:Ca data were obtained from fish collected in different seasons over a period of several years (May 2011 through September 2018), and thus incorporated temporal variability in river-specific otolith Sr:Ca signatures. Use of otolith edge chemistry data to characterize river-specific otolith Sr:Ca signatures likely resulted in inclusion of data from recent immigrants that did not reside in their collection location long enough to acquire that site's chemical signature; this would have resulted in overestimation of the range of otolith Sr:Ca for a given location. A second assumption for inferring natal river of wild fish is that individuals remained in their natal river long enough to acquire its Sr:Ca signature in their otoliths. Thus, rapid downstream drift or movement of early life stages out of their natal river could potentially result in misidentification of a fish's origin. However, our sample of Black Carp included individuals that exhibited little to no change in Sr:Ca across the otolith (indicative of fish remaining in their natal river) and fish with otolith core Sr:Ca reflective of a tributary upstream of their collection location. Therefore, at least some individuals showed evidence of not having drifted or moved downstream out of a tributary prior to accruing otolith core growth reflective of their natal location.

Most Black Carp > 400 mm TL had otolith core Sr:Ca between 600–1000 µmol/mol, consistent with the observed range of otolith edge Sr:Ca for fish captured in the middle and lower Mississippi, lower Ohio, and Atchafalaya rivers. Thus, Black Carp may be reproducing in one or more of these rivers, although fish originating in a tributary with water Sr:Ca within the combined ranges for these rivers (1.65–3.25 mmol/mol) cannot be ruled out. Large juvenile and adult fish with otolith core Sr:Ca > 1150 µmol/mol were rare in our sample, suggesting that locations that would be expected to yield such high otolith Sr:Ca (where water Sr:Ca is higher than approximately 3 mmol/mol) have probably not played a substantial role as recruitment sources for wild Black Carp across their invaded range; this includes Dutchtown Ditch, where most of the age-0 fish have been caught to date. The 31 fish with otolith core Sr:Ca lower than the minimum otolith edge Sr:Ca among fish caught in the middle and lower Mississippi, lower Ohio, and Atchafalaya rivers (600 µmol/mol) are strongly suggestive of reproduction in locations where water Sr:Ca is less than the observed lower limit of water Sr:Ca in these rivers (1.65 mmol/mol) during 2006–2018. Rivers where water Sr:Ca < 1.65 mmol/mol has been observed are rare downstream of the confluence of the Ohio and Mississippi rivers, but are common upstream of this location (Fig. 4; Whitledge et al. 2019; Zeigler and Whitledge 2010, 2011). The fact that 29 of the 31 Black Carp with otolith core Sr:Ca < 600 µmol/mol were captured upstream of the Ohio-Mississippi confluence suggests that reproduction may have occurred in one or more locations in the upper or middle Mississippi River basins where water Sr:Ca is lower than in the mainstem middle and lower Mississippi rivers. There are several potential natal rivers for these fish based on this criterion, including five where large juvenile or adult Black Carp have been captured (Cumberland, Illinois, Kaskaskia, Tennessee, and upper Mississippi rivers), although the specific sources of these individuals cannot be identified due to overlapping ranges of water and otolith edge Sr:Ca for these locations. However, the three Black Carp caught in the upper Mississippi and Illinois rivers that had otolith Sr:Ca < 600 µmol/mol along the entire laser ablation transect apparently originated upstream of the Missouri River-Mississippi River confluence. Such otolith Sr:Ca values indicate that these fish were either never downstream of this location where water Sr:Ca abruptly increases due to influx of Missouri River water, or at least did not spend sufficient time downstream of this point to accrue otolith growth with higher Sr:Ca reflective of occupancy of the middle Mississippi or Missouri rivers. Thus, reproduction appears to have occurred in the upper Mississippi or Illinois rivers. Evidence of reproduction in the White River, Arkansas was inferred from the Black Carp captured there that had Sr:Ca < 300 µmol/mol along the entire laser traverse of the otolith section. This likely reflects lifetime residency in the White River, which is unique among rivers in the lower Mississippi River basin in having water Sr:Ca < 1 mmol/mol. We were often unable to identify particular rivers as the source of individual Black Carp in our sample due to the inherent limitation of having one informative natural chemical marker (Sr:Ca) and many potential natal rivers, some of which have partially or completely overlapping ranges of water Sr:Ca. However, our results nonetheless provide evidence that Black Carp have likely reproduced in several rivers and suggest that broadening the geographic extent of sampling for early life stages may need to be considered to refine our knowledge of where and when reproduction is occurring.

Age, growth, and maturation

Black Carp ages determined from the suite of ossified structures indicated that multiple year classes are present for fish collected among the sub-basins, tributaries, and mainstem of the Mississippi River (Fig. 8). Within their native range, Black Carp growth and maturation vary latitudinally (Nico et al. 2005). This variation is divided among the large river systems in east Asia where Black Carp are native, that follow predominately west to east paths. Black Carp range from the Red River, Vietnam (22°N) to the Amur River (51°N) in northeastern China and Russia. Within our samples, the latitudinal extent is Simmesport, LA (31°N) to Meyer, Illinois (40°N), representing only a portion of the variability known from the species native range. In general, northern populations of Black Carp grow slower and mature later than in southern populations, but difficulty with translations and poorly defined methodologies limit comparisons (Nico et al. 2005). In the Amur River, Gorbach (1961) noted maturation at 8–9 years and 750–800 mm, whereas in the Yangtze River maturation varies with reports from as early as 3 years to as late as 7 years, and roughly 900 mm TL (Nico et al. 2005). Our GSI data indicate that males mature at smaller sizes (800 mm TL) than females (1000 mm TL), but histological examination of these tissues is required to confirm that gonad mass coincides with reproductive development. Of the population growth rates examined by Nico et al. (2005), the introduced population in the Kara Kum Canal, Turkmenistan appears to have the most similar characteristics to our samples, which indicate a somewhat rapid growth with age 1+Black Carp at least 400 mm TL. Our results indicate variability in growth that is assumed to be influenced by differences in climate across the invaded range, as well as individual variation in diet, habitat, and development. Because our reported length and age data were determined from several structures, lengths were not adjusted for additional growth after last annulus formation and should be considered as the defined age with a range of annual growth. Collections of Black Carp throughout the year were combined, leading to a greater variation in length-at-age among fish with recently formed annuli and those near the extent of annual growth.

Implications for Black Carp monitoring and control

Successful control of invasive species is often dependent on life history information to inform control strategies (Simberloff 2003; Dana et al., 2019), although obtaining such information is challenging when an invasion occurs over a large geographic area or where capture of the species is difficult. Our findings highlight the utility of obtaining a variety of data (otolith chemistry, ploidy, age and growth, maturity status) from reported captures of an invasive fish species when sources and year classes that have contributed to apparent increases in abundance and range are unknown. Such data are also particularly useful when it is unclear whether recruitment to adulthood or establishment in the wild have occurred due to limited collections, particularly for early life stages. Most reported Black Carp were incidental captures by commercial fishers (Kroboth et al. 2019b) due to limited agency and university sampling targeting Black Carp and low capture probability in their invaded range (Nico and Jelks 2011; Whitledge 2020). Reporting of Black Carp captures by commercial fishers was facilitated by payments to fishers from the Illinois Department of Natural Resources. Additional commercially caught Black Carp from the lower Mississippi and Atchafalaya rivers were purchased to provide samples beyond locations within or near Illinois. These payments likely resulted in substantially increased availability of wild-caught Black Carp for acquisition of data to infer sources, fertility, maturity, and age and year class distributions of this species in the Mississippi River basin. However, using fish primarily captured and reported by commercial fishers may have influenced the geographic distribution and sizes of fish included in this study, because commercial fishing is permitted only in parts of the Mississippi River basin and because commercial fishing gears are size-selective (Kroboth et al. 2019b). Nonetheless, gathering a variety of data from reported captures of individuals can offer insight into sources and status of invasive fishes when low capture probability limits the number of individuals that can be obtained from targeted sampling.

Results also suggest several areas for potential future research on Black Carp life history and assessment of their invasion status and impacts in the Mississippi River basin. Expansion of targeted sampling programs and refinement of sampling methods will likely be required to better assess distribution and abundance of early life stages of Black Carp, characterize spatial and temporal patterns of reproduction and recruitment, and potentially develop control strategies that incorporate targeting of early life stages based on knowledge of their habitat use patterns. Multiple sources of wild fish inferred from otolith microchemistry suggest that substantial geographic expansion of sampling for early life stages of Black Carp in the Mississippi River basin may be warranted. Laboratory studies to define environmental requirements for survival and development of egg and larval stages and enable application of the FluEgg model (Garcia et al. 2015) may also aid in refining understanding of locations and hydrologic conditions conducive to Black Carp reproduction. We anticipate that otolith chemistry will continue to prove useful for identifying additional areas where Black Carp reproduction may occur in the Mississippi River basin based on documented spatial differences in water chemistry among several locations that have persisted across years (Zeigler and Whitledge 2010, 2011; Phelps et al. 2012; Abell et al. 2018; Spurgeon et al. 2018; Pracheil et al. 2019b; Whitledge et al. 2019). Our results also suggest the potential for long-distance movement by Black Carp in the Mississippi River basin and identify a need for more precise definitions of movement and habitat use patterns by older juvenile and adult fish, potentially through telemetry. Evidence of Black Carp establishment in parts of the Mississippi River basin, coupled with documented range expansion and increasing reported captures (Kroboth et al. 2019b), highlight the need to assess the ecological effects of Black Carp and for development and evaluation of potential containment and control techniques and strategies.

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Data availability statement The data set for this project is available in Whitledge et al (2021b) (https://doi.org/10.5066/P90DD6NT).

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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References

Abell NJ, Oliver DC, Whitledge GW (2018) Recruitment sources and spatial patterns of population demographics of Spotted Bass *Micropterus punctulatus* in a large river-tributary network. Fisheries Manag Ecol 25:339–349

Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339

Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58:30–38

Chick JH, Maher RJ, Burr BM, Thomas MR (2003) First black carp captured in the U.S. Science 300:1876–1877

Coplen T, Kendall C (2000) Stable hydrogen and oxygen isotope ratios for selected sites of the U.S. Geological Survey's NASQAN and BENCHMARK surface water networks. U.S. Geological Survey Open-file Report 00–160, Reston, VA. doi: https://doi.org/10.3133/ofr00160

Dana ED, Garcia-de-Lomas J, Verloove F, Vila M (2019) Common deficiencies of actions for managing invasive alien species: a decision-support checklist. NeoBiota 48:97–112

Evans HS Jr (2020) Comparison of resource use by invasive Black Carp and native fish using isotopic niche and diet analyses. MS Thesis, Southern Illinois University, Carbondale, IL

Friedman I, O'Neil J, Cebula G (1982) Two new carbonate stable isotope standards. Geostand Newsl 6:11–12

Garcia T, Murphy EA, Jackson PR, Garcia MH (2015) Application of the FluEgg model to predict transport of Asian carp eggs in the Saint Joseph River (Great Lakes tributary). J Great Lakes Res 41:374–386

Gorbach EI (1961) Age composition, growth and age at puberty of grass carp *Ctenopharyngodon idella* (Val.) and black carp *Mylopharyngodon piceus* (Rich.) in the Amur River basin. J Ichthyol 1:119–126

Hebert PDN, Cristescu MEA (2002) Genetic perspectives on invasions: the case of the Cladocera. Can J Fish Aquat Sci 59:1229–1234

Hoefs J (2004) Stable isotope geochemistry. Springer, Berlin

Honig A, Etter R, Pepperman K, Morello S, Hannigan R (2020) Site and age discrimination using trace element fingerprints in the blue mussel, *Mytilus edulis*. J Exp Mar Biol Ecol 522:151249

Hulme PE, Bacher S, Kenis M, Klotz S, Kuhn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, Pyšek P, Roques A, Sol D, Solarz W, Vilà M (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. J Appl Ecol 45:403–414

Hunter ME, Nico LG (2015) Genetic analysis of invasive Asian black carp (*Mylopharyngodon piceus*) in the Mississippi River Basin: evidence for multiple introductions. Biol Invasions 17:99–114

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Animal Ecol 80:595–602

Jenkins JA, Thomas RG (2007) Use of eyeballs for establishing ploidy of Asian carp. N Am J Fish Manag 27:1195–1202

Kelso WE, Kaller MD, Rutherford DA (2012) Collection, processing, and identification of fish eggs and larvae and zooplankton. In: Zale AV, Parrish DL, Sutton TM (eds) Fisheries techniques, 3rd edn. American Fisheries Society, Bethesda, Maryland, pp 363–452

Kendall C, Coplen TB (2001) Distribution of oxygen-18 and deuterium in river waters across the United States. Hydrol Process 15:1363–1393

Kroboth PT, Chapman DC, Hrabik RA, Neely DA (2019a) Characteristics for the external identification of Black Carp *Mylopharyngodon piceus* from Grass Carp *Ctenopharyngodon idella*. J Fish Wildlife Manag 10:304–313

Kroboth PT, Cox CL, Chapman DC, Whitledge GW (2019b) Black Carp in North America: a description of range, habitats, time of year, and methods of reported captures. N Am J Fish Manag 39:1046–1055

Laughlin TW, Whitledge GW, Oliver DC, Rude NP (2016) Recruitment sources of channel and blue catfishes inhabiting the middle Mississippi River. River Res Appl 32:1808–1818

Love SA, Lederman NJ, Widloe T, Whitledge GW (2019) Sources of bigheaded carps found in Chicago urban fishing program ponds. Trans Am Fish Soc 148:417–425

Melancon S, Fryer BJ, Ludsin SA, Gagnon JA, Yang Z (2005) Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can J Fish Aquat Sci 62:2609–2619

Munro AR, McMahon TE, Ruzycki JR (2005) Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Can J Fish Aquat Sci 62:79–87

Nico LG, Williams JD, Jelks HL (2005) Black carp: biological synopsis and risk assessment of an introduced fish. American Fisheries Society, Bethesda, Maryland

Nico LG, Jelks HL (2011) The black carp in North America: an update. In: Chapman DC, Hoff MH (eds) Invasive Asian Carps in North America. American Fisheries Society Symposium 74, Bethesda, Maryland, pp 89–104.

Nico LG, Demopoulos A, Gualtieri M, Wieser C (2011) Use of stable isotopes and mercury to assess trophic positions of black carp and other large fishes in the Red-Atchafalaya River System, Louisiana, USA. In: Chapman DC, Hoff MH (eds) Invasive Asian Carps in North America. American Fisheries Society Symposium 74, Bethesda, Maryland, pp 105–119.

Norman JD, Whitledge GW (2015) Recruitment sources of invasive bighead carp (*Hypopthalmichthys nobilis*) and silver carp (*H. molitrix*) inhabiting the Illinois River. Biol Invasions 17:2999–3014

Papoulias DM, Chapman DC, Tillitt DE (2006) Reproductive condition and occurrence of intersex in bighead carp and silver carp in the Missouri River. Hydrobiologia 571:355–360

Papoulias DM, Candrl J, Jenkins JA, Tillitt DE (2011) Verification of ploidy and reproductive potential in triploid black carp and grass carp. In: Chapman DC, Hoff MH (eds) Invasive Asian Carps in North America. American Fisheries Society Symposium 74, Bethesda, Maryland, pp. 251–266.

Phelps QE, Whitledge GW, Tripp SJ, Smith KT, Garvey JE, Herzog DP, Ostendorf DE, Ridings JW, Crites JW, Hrabik RA, Doyle WJ, Hill TD (2012) Identifying river of origin for age-0 *Scaphirhynchus* sturgeons in the Missouri and Mississippi rivers using fin ray microchemistry. Can J Fish Aquat Sci 69:930–941

Poulton BC, Kroboth PT, Bailey J, George AE, McMurray SE, Faiman JS, Chapman DC (2019) First examination of diet items consumed by wild-caught Black Carp (*Mylopharyngodon piceus*) in the U.S. Am Midland Nat 182:89–108

Pracheil BM, Hogan JD, Lyons J, McIntyre PB (2014) Using hard-part microchemistry to advance conservation and management of North American freshwater fishes. Fisheries 39:451–465

Pracheil BM, George R, Chakoumakos BC (2019a) Significance of otolith calcium carbonate crystal structure diversity to microchemistry studies. Rev Fish Biol Fisheries 29:569–588

Pracheil BM, Lyons J, Hamann EJ, Short PH, McIntyre PB (2019b) Lifelong population connectivity between large rivers and their tributaries: a case study of shovelnose sturgeon from the Mississippi and Wisconsin rivers. Ecol Freshwater Fish 28:20–32

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Simberloff D (2003) Eradication - preventing invasion at the outset. Weed Sci 51:247–253

Solomon CT, Weber PK, Cech JJ Jr, Ingram BL, Conrad ME, Machavaram MV, Pogodina AR, Franklin RL (2006) Experimental determination of the sources of otolith carbon and associated isotopic fractionation. Can J Fish Aquat Sci 63:79–89

Song Y, Cheng F, Murphy BR, Xie S (2018) Downstream effects of the Three Gorges Dam on larval dispersal, spatial distribution, and growth of the four major Chinese carps call for reprioritizing conservation measures. Can J Fish Aquat Sci 75:141–151

Spurgeon JJ, Pegg MA, Halden NM (2018) Mixed origins of channel catfish in a large-river tributary. Fish Res 198:195–202

Trebitz AS, Hoffman JC, Darling JA, Pilgrim EM, Kelly JR, Brown EA, Chadderton WL, Egan SP, Grey EK, Hashsham SA, Klymus KE, Mahon AR, Ram JL, Schultz MT, Stepien CA, Schardt JC (2017) Early detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating advanced technologies, and identifying research needs. J Environ Manag 202:299–310

USFWS (U.S. Fish and Wildlife Service) (2007) Rules and regulations: injurious wildlife species: black carp (*Mylopharyngodon piceus*). Federal Register 72:201(18 October 2007): 59019–59035.

USGS (U.S. Geological Survey) (2020) Nonindigenous aquatic species database https://nas.er.usgs.gov. Accessed 28 July 2020

VanderKooy S (2009) A practical handbook for determining the ages of Gulf of Mexico fishes, 2nd edn. Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi.

Whitledge GW, Johnson BM, Martinez PJ, Martinez AM (2007) Sources of nonnative centrarchids in the upper Colorado River revealed by stable isotope and microchemical analysis of otoliths. Trans Am Fish Soc 136:1263–1275

Whitledge GW, Knights B, Vallazza J, Larson J, Weber MJ, Lamer JT, Phelps QE, Norman JD (2019) Identification of Bighead Carp and Silver Carp early life environments and inferring Lock and Dam 19 passage in the Upper Mississippi River: insights from otolith chemistry. Biol Invasions 21:1007–1020

Whitledge GW, Chapman DC, Farver JR, Herbst SJ, Mandrak NE, Miner JG, Pangle KL, Kocovsky PM (2021a) Identifying sources and year classes contributing to invasive Grass Carp in the Laurentian Great Lakes. J Great Lakes Res 47:14–28

Whitledge, GW, Kroboth PT, Chapman DC, Phelps QE, Sleeper W, Bailey J, and Jenkins JA (2021b) Microchemistry demographics and development data from wild caught black carp in the Mississippi River basin, 2011–18: U.S. Geological Survey data release, https://doi.org/10.5066/P90DD6NT

Whitledge GW (2020) Development of sampling techniques for Black Carp to aid assessment of natural Reproduction, distribution, and ecological impacts. Project F17AP00159, final report to the U.S. Fish and Wildlife Service.

Yi, B, Liang Z, Yu Z, Lin R, He M (2006) A study of the early development of grass carp, black carp, silver carp, and bighead carp in the Yangtze River, China. In: Chapman DC, Wang N (eds) Early development of four cyprinids native to the Yangtze River, China. U.S. Geological Survey, Data Series 239.

Zeigler JM, Whitledge GW (2010) Assessment of otolith chemistry for identifying source environment of fishes in the lower Illinois River, Illinois. Hydrobiologia 638:109–119

Zeigler JM, Whitledge GW (2011) Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes. Hydrobiologia 66:289–302