


Research Article

Assessing Invasive Carp in the Neosho River-Grand Lake System of Kansas and Oklahoma

Ethan J. Rasset ¹, Hae H. Kim,² Ben C. Neely,³ Quinton E. Phelps,² and Greg W. Whitley⁴

¹Minnesota Department of Natural Resources, 1509 1st Ave North, Fergus Falls, MN 56537, USA

²Applied Fisheries Management Lab, Missouri State University, 901 S National Ave, Springfield, MO 65897, USA

³Kansas Department of Wildlife and Parks, 1830 Merchant St., Emporia, KS 66801, USA

⁴Southern Illinois University, 263 Lincoln Dr, Carbondale, IL 62901, USA

Correspondence should be addressed to Ethan J. Rasset; ejrasset@gmail.com

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Invasive carp populations have purported a negative influence on native biota at high densities. These invasive fishes (i.e., bighead carp *Hypophthalmichthys nobilis*, silver carp *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella*, and black carp *Mylopharyngodon piceus*) each exhibit similar life history characteristics. In the Neosho River-Grand Lake system (i.e., John Redmond Reservoir, Kansas, downstream to Grand Lake O' the Cherokees, Oklahoma), only bighead carp and grass carp have been documented to date. The distribution and status of bighead carp throughout this system were previously unknown due to limited historical data and low abundance. While few bighead carp are encountered within this system, grass carp exhibited relatively higher abundance and were used to provide insights into bighead carp. Captures of both species were used to inform management and suppression efforts. Sampling locations ($n = 18$) were established for environmental DNA analyses throughout the Neosho River-Grand Lake system. We sampled 13 sites using a suite of gears for standardized targeted fish sampling. All invasive carp were measured, sexed, and otoliths removed for ageing and microchemical analysis. Grass carp were processed for ploidy testing following the U.S. Fish and Wildlife Service protocol. Environmental DNA analyses generated positive results for the eDNA presence of bighead carp and silver carp. Otolith age estimates suggest fish are long lived and supported by multiple year classes. Additionally, a sampled two-year-old grass carp demonstrates spawning and recruitment potential. Otolith micro-chemistry suggests largescale broad movement patterns. Ploidy testing confirmed the first documented evidence of diploid grass carp in the Neosho River-Grand Lake system and revealed reproductive viability. Our results may provide future insights into locations for containment, removal, and/or eradication.

1. Introduction

Human activities are a vector of invasive species introductions in North America [1]. Specifically, fish species have been imported and introduced across the United States for various purposes, including food, aquaculture, angling, the aquarium trade, and aquatic vegetation control [1, 2]. Through intentional and unintentional introductions, various nonnative fish populations have been established in North America [2, 3]. These invasions have caused billions of dollars in annual expenditures relating to research, mitigating or preventing impacts, and removal efforts [1, 4–6]. For example, invasive fishes can cause ecological damages in

the form of reduced body condition of native competitors, alteration of aquatic habitats, and modification of trophic cascades [6–10]. Proper management of these invasive fish populations is imperative, given the potential impacts [11].

Invasive carp species (i.e., bighead carp *Hypophthalmichthys nobilis*, grass carp *Ctenopharyngodon idella*, black carp *Mylopharyngodon piceus*, and silver carp *Hypophthalmichthys molitrix*) were introduced in North America for various biological and ecological benefits (e.g., algae, vegetation, and gastropod control [6, 7, 12]). However, invasive carp escaped and subsequently established populations across North America [13]. These fish exhibit life history characteristics and behavior (e.g., extensive

movement and dispersal) of a successful invader [8, 11, 14]. Specifically, bighead carp and grass carp exhibit protracted spawning, high dispersal, movement, fecundity, and survive in a wide range of habitats [15, 16].

Management and monitoring of bighead carp and grass carp require a thorough understanding of abundance, distribution, population dynamics, and their biological and ecological response to a new environment [1, 17]. Consequently, various direct and indirect approaches have been utilized to monitor and study these invasive fish [12, 17]. Intensive sampling and monitoring provide the most accurate information and ecological insights that inform management decisions [18–20]. Such a practice has delivered insights into abiotic and biotic conditions of a system via the assessment of tolerant or intolerant species corresponding to pollution, demonstrated impacts from invasive fish establishment, and documented baseline fish assemblages for comparison following the potential future impacts or changes [6, 9, 18–20]. Riverine fish sampling methods vary across specific research and monitoring needs, but multigear approaches are commonly used to minimize sampling biases, obtain biologically representative samples, and increase the detection probability of rare or difficult-to-acquire species [21–24]. Additionally, newly emerging environmental DNA (eDNA) metabarcoding techniques have unique applications in detecting riverine species, particularly low-abundance species [25–30]. Otolith trace element analyses have also been used to provide an understanding of broadscale movement patterns (i.e., environmental life history) and natal origin, furthering ecological knowledge [31–33]. Ultimately, optimal management of invasive fishes requires a multitiered approach based on biological (e.g., ploidy, fecundity, and longevity) and ecological (e.g., movement) insights [9].

Numerous studies have proven that bighead carp can directly compete with paddlefish *Polyodon spathula*, gizzard shad *Dorosoma cepedianum*, bigmouth buffalo *Ictiobus cyprinellus*, and other native planktivores [6–9, 34, 35]. Specifically, the reduced body condition of native planktivorous fishes has been observed following bighead carp establishment [7–9, 34]. Similarly, studies on grass carp have documented their ability to impact aquatic ecosystems through the consumption of aquatic vegetation, and in the absence of such, invertebrates and various fishes [10, 17, 36]. This behavior modifies the native aquatic vegetation assemblage and impacts invertebrates and fishes via reduced food availability and direct consumption [10, 17, 36]. Subsequently, the removal of aquatic vegetation can negatively impact fishes that rely upon it [17, 36]. Both bighead carp and grass carp can alter fish community interactions and impact recreationally and ecologically important species through their deleterious behavior, ultimately leading to negative economic impacts [8–10, 13, 34]. The potential of bighead carp and grass carp to disrupt aquatic ecosystems further establishes the need to monitor populations through all stages of invasion (i.e., low abundance, hyperabundance, and stabilized abundance [17]).

Abundance and distribution of bighead carp and grass carp vary across the United States, with previous studies

having largely focused on areas of moderate to high abundances where impacts are more discernible [7, 8, 34, 37–40]. It is plausible that low-density populations of bigheaded carp do not generate detrimental effects on the surrounding aquatic ecosystem even though competition and impacts are occurring [6, 8, 9]. As such, few studies have evaluated lower-density populations. Grass carp, however, can generate discernible impacts even in low densities due to their habitat-altering consumptive behavior(s) [10, 17]. Baseline population demographics of bighead carp and grass carp should be documented while they persist in low densities to allow for future determination of impacts (e.g., fish community and habitat) and assessment for potential population growth, mitigation, or eradication [3, 6, 7, 9, 10, 35].

The Neosho River-Grand Lake system in Kansas and Oklahoma provides an opportunity to study a perceived low-density population of bighead carp and grass carp [35, 41]. Bighead carp were first discovered in the Neosho River-Grand Lake system in 1992 when specimens were snagged by anglers in two separate locations (i.e., Miami and Pensacola Dam) on the Neosho River in Oklahoma [42, 43]. Another specimen was subsequently snagged in Grand Lake O' the Cherokees in 1996 [43]. Bighead carp were brought into southeast Kansas in 1988 by a commercial fish farmer for aquaculture purposes and likely escaped from stocked farm ponds [42, 43] potentially due to inundation with the river or some other human-mediated actions. These original escapees were likely diploid and reproductively viable although ploidy of bighead carp from this system is undocumented. Additionally, grass carp are currently used as a biological control for aquatic vegetation in the state of Kansas [44, 45]. As such, they have been stocked throughout the surrounding watershed in both private ponds and public waterbodies [44, 45]. The Kansas Department of Wildlife and Parks (KDWP) currently requires all stocked grass carp to be triploid to prevent reproduction [45]. However, it was legal to stock or sell diploid grass carp in Kansas prior to 2006 [46]. These stocking practices coupled with longevity observed in other grass carp populations suggest that diploid fish may be present in the Neosho River-Grand Lake system [46].

The Neosho River-Grand Lake system supports a world-renowned fishery for paddlefish that draws anglers from throughout North America, supporting the local economy [33, 47–49]. Paddlefish and other abundant native planktivorous fishes in this system such as gizzard shad and bigmouth buffalo could experience deleterious effects due to diet overlap with bighead carp [34, 35, 41]. Little information exists about the present grass carp population although this species is likely more prevalent than bighead carp based on the stocking history and subsequent use for vegetation control throughout the Neosho River basin [44, 50]. Unlike bighead carp, grass carp have been previously documented in the Neosho River in Kansas [50]. However, the natural reproduction of bighead carp and grass carp in the Neosho River-Grand Lake system is undocumented. A unique opportunity exists to evaluate the status of these fishes in this system; documenting their distribution and abundance can inform future barrier placement or other control methods [12, 14, 17, 51].

Bighead carp captures are relatively infrequent throughout this system [43, 52]. However, grass carp in the Neosho River-Grand Lake system are more frequently encountered. Grass carp display similar reproductive patterns and may provide some valuable insights into bighead carp life history [53–58]. Considering the lack of knowledge on bighead carp and grass carp in the Neosho River-Grand Lake system, we evaluated their status by documenting the distribution (i.e., eDNA sampling and standardized or targeted sampling), ages of individuals, ploidy testing to test reproductive potential, fecundity analyses to display reproductive status, and otolith microchemistry to inform about potential broadscale movement or natal origination [9, 38, 39, 59–62]. Our objectives of this project were to document the abundance and spatial extent of bighead carp and grass carp, collect baseline population demographic information (i.e., status), examine for broadscale movement patterns, and determine locations for future containment, removal, and/or eradication efforts.

2. Methodology

2.1. Study Area. The Neosho River system is over 700 km in its entirety, with the stretch from the John Redmond Dam in Kansas to the Oklahoma border (approximately 338 river km). The Spring River joins with the Neosho River south of the Oklahoma border and flows into Grand Lake O' the Cherokees. We selected sampling sites (i.e., 19 locations) across the Neosho River-Grand Lake system in Kansas and Oklahoma to correspond to low head dams and reservoirs along a longitudinal gradient ([61, 63]; Figure 1). We separated the Neosho River into three geographically distinct regions (i.e., upper, middle, and lower) to compare invasive carp relative densities (via catch per unit effort) across broad geographic areas.

2.2. Environmental DNA. We conducted eDNA water sampling at all sites (as feasible) except for John Redmond Reservoir (i.e., 18 locations). Water samples were collected once per season for five consecutive seasons (i.e., winter 2020–2021 through winter 2021–2022) according to the USGS standardized protocol [61]. Seasons constituted winter (i.e., winter solstice to spring equinox), spring (i.e., spring equinox to summer solstice), summer (i.e., summer solstice to autumn equinox), and fall (i.e., autumn equinox to winter solstice). We recorded the GPS coordinates, position of the sample location relative to the low head dam (i.e., above or below), time of collection, and river position of the sample (right descending or left descending) for each sample. All 90 samples were collected in 500 mL or 1,000 mL plastic sterile containers, kept on ice, and shipped to the Whitney Genetics Lab of the U.S. Fish and Wildlife Service for processing [61].

2.3. Sampling. We randomly allocated sampling gears at the designated sampling sites above or below fish barriers (i.e., low head dams) and utilized them according to the Long Term Resource Monitoring (LTRM) element (used on the Upper

Mississippi River system) for standardized targeted sampling [24]. Our sampling occurred seasonally to examine for potential seasonal variations from June 2021 through November 2022. The protocol used to determine the number and location of net sets, selection of alternative sites, and short-term versus overnight gill net and mini gill net sets was identical to the procedure established by Rasset et al. [64]. Passive or active fisheries sampling occurred at all sites except Chanute 1, Erie, Neosho Wildlife Area, Miami, Grand Lake O' the Cherokees, and Pensacola Dam according to the feasibility and landowner permissions (see Figure 1). All invasive carp were euthanized via cranial piting after capture (Missouri State University IACUC Protocol 2020–14 [65]).

Our effort of passive gears varied at some sites due to the size of the sampling area or river conditions; the overall effort of each gear ranged from one to four sets per site per season [24, 64]. We set American Fisheries Society (AFS) experimental gill nets, mini gill nets, hoop nets, modified fyke nets, and mini fyke nets; the dimensions of nets and deployment technique were identical to the dimensions and deployment described by Rasset et al. [64].

Electrofishing followed LTRM sampling protocols and settings (60 Hz; 25% duty cycle) with a boat-mounted ETS electrofishing control box (ETS Electrofishing Systems, LLC; Madison, WI [24, 64]). Runs were 900 seconds, and power was standardized across observed water temperatures and conductivities [24, 64]. A uniform minimum power goal target of 3,000 W was generally exceeded, given the water conditions (3,895–5,181 W). Fish community sampling occurred simultaneously while targeting bighead carp and grass carp across a standardized number of runs [64]. Additional targeted runs were conducted if time allowed, where only bighead carp or grass carp were netted. Electrofishing occurred within the scope of the LTRM protocol and according to the procedure described by Rasset et al. [64] during boat operation [24]. The guidelines we set for shocking the Neosho and Spring River(s) were also used in tributaries and lentic habitats of John Redmond Reservoir.

2.4. Invasive Carp Ageing. We embedded bighead carp and grass carp lapillus otoliths in a two-part epoxy and allowed that to harden. Subsequently, epoxied otoliths were thin-sectioned (i.e., 350 micrometer width) using a low-speed saw; samples were sanded with silicon carbide sandpaper and polished on lapping film [39, 59, 66]. Each section was viewed under a dissecting microscope, and annuli were enumerated by two independent readers; discrepancies were discussed until a consensus age was obtained [39, 59, 66].

2.5. Ploidy Assessments. One grass carp specimen from each region (i.e., four total) of the Neosho River system (including the Spring River) was assessed for ploidy [60, 67]. We made an incision along the ventral portion of the caudal fin and collected a minimum of 1 mL of blood in a heparinized tube [60]. We then mixed the blood sample with an acid citrate dextrose (ACD) solution, kept it on ice, and shipped it overnight to the La Crosse Fish Health Center (U.S. Fish and Wildlife Service) for ploidy analyses via flow cytometry [60].

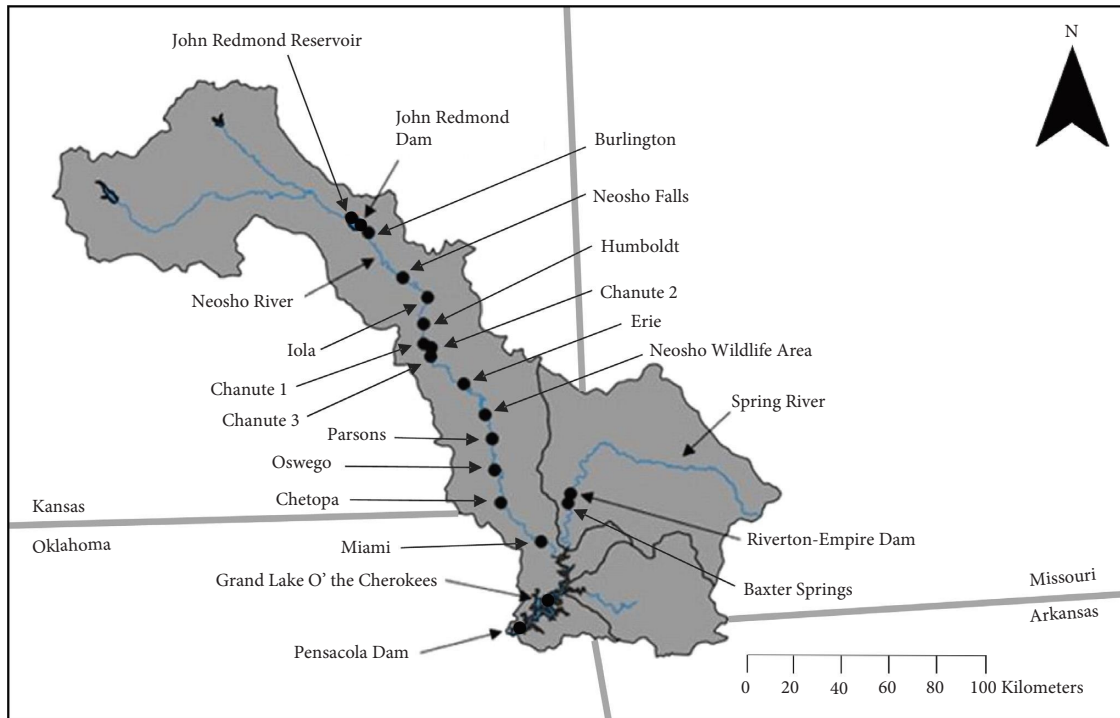


FIGURE 1: Map of the Neosho River-Grand Lake system study area (adapted from [33]; reproduced from [64]). Environmental DNA and/or fish sampling sites are represented by circles. Sites in “Upper Neosho” constituted the John Redmond Dam, Burlington, Neosho Falls, and Iola. Sites in “Middle Neosho” included Humboldt, Chanute 2, and Chanute 3. Sites in “Lower Neosho” included Parsons, Oswego, and Chetopa.

2.6. Fecundity Analyses. We analyzed ovary egg masses collected from female bighead carp and grass carp for fecundity similarly to methods outlined by Schrank and Guy [38]. Oocytes were counted from subsamples (i.e., nine per specimen) of ovary mass, and fecundity was determined by extrapolating the number of oocytes in the subsamples to the total ovary weight [38].

2.7. Otolith Microchemistry. We ablated otolith thin sections on a CETAC Technologies (Teledyne CETAC Technologies, Omaha, Nebraska) LSX-266 system; trace element concentrations were analyzed using a Thermo X-Series II inductively coupled plasma mass spectrometer (ICP-MS). Each transect encompassed the core and terminated at the outer edge (beam diameter = 25 μm , scan rate = 5 $\mu\text{m}/\text{sec}$, laser pulse rate = 20 Hz, and laser energy level = 35%). Additionally, reference materials were analyzed and corrected for potential machine drift. All otolith samples were preceded by microanalytical reference materials (MACS-3) replicated three times at approximately 300 μm distance. Furthermore, all standards were preceded by a 30-second gas blank, followed by a 30-second wash-out period. Raw elemental counts were obtained and corrected using MACS-3 standards as a reference; raw counts were converted to molar ratios using elementR [68]. Elements were normalized to calcium (Ca) concentrations based on considerations of Ca as a pseudointernal standard. As such, trace elemental ratios of strontium 86 and calcium 43 (Sr86:Ca43) were evaluated to assess the natal origin and broadscale movement patterns

[33]. Water samples obtained by Whitley and Schooley [33] from throughout the Neosho River-Grand Lake system yielded estimates of water Sr86:Ca43 signatures for comparison to Sr86:Ca43 signatures within otoliths [69–72]. These water signatures were transformed identically to the methodology used by Whitley et al. [70] to allow for direct comparison to both bighead carp and grass carp otoliths [69, 71, 72].

3. Results

Analyses conducted by the Whitney Genetics Lab on 89 water samples yielded two positive eDNA results, one for bighead carp and one for silver carp. Both positive results were from water samples collected in May of 2021. Bighead carp eDNA was confirmed in a water sample from the dam at Miami, Oklahoma. Furthermore, silver carp eDNA was confirmed in a water sample from above the most upstream Chanute dam (i.e., Chanute 1) in Kansas.

Passive sampling on the Neosho River for invasive carp across five seasons consisted of 321 net sets, of which eight were overnight gill nets, 65 were short-term gill nets, six were overnight mini gill nets, 42 were short-term mini gill nets, 50 were hoop nets, 47 were modified fyke nets, and 103 were mini fyke nets (Table 1). We set 26 total nets in spring sampling, 173 nets throughout summer sampling, and 122 nets in the fall. Effort also varied by the region of the Neosho River; we set 156 nets on Upper Neosho, 52 nets on Middle Neosho, and 117 nets on Lower Neosho (Table 2). This cumulative effort resulted in the capture of one grass carp

TABLE 1: Netting effort and catches of grass carp on the Neosho River, Kansas, from the summer of 2021 through the fall of 2022.

	Spring 2022	Summer 2021–2022	Fall 2021–2022	Total
Netting effort				
Overnight gill sets	6	2	—	8
Short-term gill sets	—	38	27	65
Overnight mini gill sets	4	2	—	6
Short-term mini gill sets	—	24	18	42
Hoop sets	4	28	18	50
Modified fyke sets	4	26	17	47
Mini fyke sets	8	53	42	103
Total samples (sets)	26	173	122	321
Netting catch				
Overnight gill	—	—	—	—
Short-term gill	—	1	—	1
Overnight mini gill	—	—	—	—
Short-term mini gill	—	—	—	—
Hoop	—	—	—	—
Modified fyke	—	—	—	—
Mini fyke	—	—	—	—
Total (N)	—	1	—	1
CPUE (fish/net)	0.00 (0.00)	0.02 (0.02)	0.00 (0.00)	<0.01 (0.01)

Standard error is in parentheses.

TABLE 2: Netting effort and catches of grass carp from the Neosho River, Kansas, by the geographic region.

	Upper	Middle	Lower
Netting effort			
Overnight gill sets	8	—	—
Short-term gill sets	26	12	27
Overnight mini gill sets	6	—	—
Short-term mini gill sets	16	8	18
Hoop sets	28	8	18
Modified fyke sets	21	8	18
Mini fyke sets	51	16	36
Total samples (sets)	156	52	117
Netting catch			
Overnight gill	—	—	—
Short-term gill	—	1	—
Overnight mini gill	—	—	—
Short-term mini gill	—	—	—
Hoop	—	—	—
Modified fyke	—	—	—
Mini fyke	—	—	—
Total (N)	—	—	—
CPUE (fish/net)	0.00 (0.00)	0.08 (0.08)	0.00 (0.00)

Standard error is in parentheses.

(standard error in parentheses; CPUE = 0.01 (0.01) fish/net) via a short-term gill net at Chanute 2 (i.e., Middle Neosho) in the summer of 2021. This lone specimen was an 854 mm female. Additionally, we set 95 nets on the Spring River, where effort varied by the season (i.e., 26 sets in spring, 43 sets in summer, and 26 sets in fall) and by the gear type (i.e., six overnight gill nets, 18 short-term gill nets, four overnight mini gill nets, 12 short-term mini gill nets, 13 hoop nets, 14 modified fyke nets, and 28 mini fyke nets). We obtained zero grass carp from the Spring River (Table 3).

Pulsed-DC boat electrofishing at John Redmond Reservoir resulted in the acquisition of two grass carp (standard error in parentheses; CPUE = 0.07 (0.07) fish/hr) in 13.5 hours of effort (54 runs; Table 4). Both fish were

obtained from spring sampling. Additionally, we collected seven grass carp across 57 hours (228 runs) of electrofishing on the Neosho River (CPUE = 0.12 (0.06) fish/hr; Table 5). Effort by season varied; we conducted electrofishing for 16.0 hours (64 runs) in the spring, 21.3 hours (85 runs) throughout the summer, and 19.8 hours (79 runs) in the fall. Fall electrofishing generated the greatest number (i.e., four) of grass carp (CPUE = 0.20 (0.10) fish/hr). We obtained four, two, and one grass carp respectively from Upper Neosho, Middle Neosho, and Lower Neosho (Table 6). Neosho River grass carp ranged from 840 to 983 mm and 6.52 to 10.36 kg. Similarly, we conducted 14.5 hours (58 runs) of electrofishing on the Spring River, resulting in a capture of 10 grass carp (CPUE = 0.69 (0.28) fish/hr; Table 7).

TABLE 3: Netting effort and catches of grass carp on the Spring River, Kansas, from the summer of 2021 through the fall of 2022.

	Spring 2022	Summer 2021–2022	Fall 2021–2022	Total
Netting effort				
Overnight gill sets	6	—	—	6
Short-term gill sets	—	12	6	18
Overnight mini gill sets	4	—	—	4
Short-term mini gill sets	—	8	4	12
Hoop sets	4	5	4	13
Modified fyke sets	4	6	4	14
Mini fyke sets	8	12	8	28
Total samples (sets)	26	43	26	95
Netting catch				
Overnight gill	—	—	—	—
Short-term gill	—	—	—	—
Overnight mini gill	—	—	—	—
Short-term mini gill	—	—	—	—
Hoop	—	—	—	—
Modified fyke	—	—	—	—
Mini fyke	—	—	—	—
Total (N)	—	—	—	—
CPUE (fish/net)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Standard error is in parentheses.

TABLE 4: Grass carp electrofishing effort and catches from the John Redmond Reservoir, Kansas, in the spring of 2022.

	Spring 2022
Electrofishing effort	
Samples (runs)	54
Electrofishing hours	13.5
Electrofishing catch	
Total (N)	2
CPUE (fish/hr)	0.07 (0.07)

Standard error is in parentheses.

Bighead carp were not captured via traditional sampling methods, and as such, we sought to obtain specimens via other means. We managed to acquire five total bighead carp from paddlefish anglers in Oklahoma and ODWC. These fish were obtained from Grand Lake O' the Cherokees (i.e., three) and the Neosho River (i.e., two) downstream from the Miami Dam and ranged in size from 1,025 to 1,300 mm and 31.8 to 36.1 kg (Table 8).

We removed lapillus otoliths from the five bighead carp specimens for ageing purposes. Subsequent age estimates ranged from 14 to 32 years of age (mean of 24). Similarly, we removed lapillus otoliths from 17 grass carp for ageing, obtaining age estimates ranging from 2 to 37 years of age with a mean age of 18 (Table 9). A single two-year-old fish was the only specimen under 11 years of age, and the second fish obtained from John Redmond Reservoir was aged at 19. Additionally, the Spring River produced eight fish for ageing purposes, yielding age estimates that ranged from 13 to 25 (mean age of 19). The Neosho River provided seven fish for ageing purposes; estimates ranged from 11 to 37 (mean age of 19).

Ploidy assessments via blood collection on a subsample of grass carp confirmed three diploid individuals (Table 10). A fourth grass carp from Upper Neosho was tested, but the blood sample went bad prior to analyses and was unusable.

Bighead carp specimens were not kept alive prior to our collection, rendering ploidy assessments noneffective [60].

We performed fecundity analyses on two female bighead carp specimens; fecundity ranged from 2,967,234 to 4,734,800 (mean of 3,851,017; Table 11). Pulsed-DC boat electrofishing provided us two grass carp with eggs suitable for fecundity analyses. The fecundity of these two fish was 580,593 (i.e., John Redmond Reservoir) and 944,632 (i.e., Oswego; Table 12).

Bighead carp ($N=5$) and grass carp ($N=3$) were analyzed via otolith microchemistry to examine for potential broadscale movement patterns (e.g., reconstructing life history). We used estimated and transformed Sr86:Ca43 signatures from water samples taken throughout Grand Lake O' the Cherokees (i.e., 378–2,028 $\mu\text{mol/mol}$) and the Neosho (i.e., 2,256–3,735 $\mu\text{mol/mol}$), Spring (i.e., 254–400 $\mu\text{mol/mol}$), and Elk River(s) (i.e., 193–268 $\mu\text{mol/mol}$) as a baseline for direct comparison with Sr86:Ca43 signatures of bighead carp and grass carp otoliths [33, 69–72]. Trace elemental ratios varied across both species and among individuals. The natal origin appears to be variable across sampled fishes. Across all sampled bighead carp, core signatures ranged from approximately 300–2,000 Sr86:Ca43 $\mu\text{mol/mol}$ (Figure 2). Grass carp core signatures appeared to be relatively higher in comparison to bighead carp and ranged approximately 1,500–4,000 Sr86:Ca43 $\mu\text{mol/mol}$ (Figure 3). Most sampled fishes exhibited movement between chemically distinct areas across the otolith transect. Some individuals appeared to stay within a river basin, while others exhibited interbasin movement.

4. Discussion

Bighead carp and grass carp are present in low densities throughout the Neosho River-Grand Lake system and are likely reproductively viable. Studies have shown that

TABLE 5: Grass carp electrofishing effort and catches from the Neosho River, Kansas, from the summer of 2021 through the fall of 2022.

	Spring 2022	Summer 2021–2022	Fall 2021–2022	Total
Electrofishing effort				
Samples (runs)	64	85	79	228
Electrofishing hours	16.0	21.3	19.8	57.0
Electrofishing catch				
Total (N)	1	2	4	7
CPUE (fish/hr)	0.06 (0.06)	0.09 (0.07)	0.20 (0.10)	0.12 (0.05)

Standard error is in parentheses.

TABLE 6: Grass carp electrofishing effort and catches on the Neosho River, Kansas, by the geographic region.

	Upper	Middle	Lower
Electrofishing effort			
Samples (runs)	111	38	79
Electrofishing hours	27.8	9.5	19.8
Electrofishing catch			
Total (N)	4	2	1
CPUE (fish/hr)	0.14 (0.07)	0.22 (0.15)	0.05 (0.05)

Standard error is in parentheses.

negative impacts can arise from the presence of these invasive fishes [6–10, 34]. As such, deleterious impacts on native fishes may occur if invasive carp population sizes increase in the future. We did not find conclusive evidence pointing toward a future increase in the abundance of bighead carp and grass carp although the presence of reproductively viable grass carp and fecund fishes of both species indicates a potential for population growth [17, 73]. Impacts from bighead carp and grass carp are unlikely to be readily discernible due to their low abundance, and subsequently, it remains unknown if the deleterious effects of these fish are occurring.

Grass carp can directly influence aquatic habitats through vegetation consumption [10, 17, 36]. Other cascading impacts have been observed and various indirect impacts (e.g., altering aquatic invertebrate communities) may negatively influence native fish populations [10, 17, 36]. While aquatic vegetation is not a main habitat component of the Neosho River-Grand Lake system, detritus is prevalent. Consumption of this detritus can reduce organic matter for other organisms and processes, subsequently decreasing the energy base availability within the aquatic ecosystem [10, 17, 36, 74]. Grass carp are currently low in abundance in this system, and their specific impacts are unknown. Should abundance increase in the future, both direct and trickle-down impacts on the aquatic ecosystem will likely become more discernible.

Confirmed diploid grass carp, a young capture, and fecund specimens from this research support their potential for episodic recruitment within the Neosho River-Grand Lake system. It is suspected that bighead carp present are diploid, but a lack of ploidy analyses on collected specimens leaves reproductive viability undocumented. Grass carp and bighead carp reproduction is triggered by a combination of water temperature (i.e., greater than 18°C) and increasing current velocity or discharge during spring and early summer [37, 53, 55–57, 73, 75, 76]. Grass carp reproduction has been documented in free-flowing river reaches less than 28 km in length; bighead carp requirements are similar to

that of grass carp [37, 53, 55–57, 73, 75, 76]. Currently, only triploid grass carp can legally be stocked in Kansas. Diploid fish ages represent recruitment following the regulation change and could suggest natural recruitment. Overall, it appears the Neosho River-Grand Lake system could support natural reproduction. Additionally, combining diploid grass carp captures with apparent broadscale movement patterns of both species, variable natal origin locations (inconclusive whether stocked or recruited), high longevity, and large body sizes displays the potential for an increase in the bighead carp and grass carp presence in the Neosho River-Grand Lake system. However, it is apparent due to the current low densities that ideal reproductive conditions either have not been or infrequently are met within this system. Grass carp and bighead carp reproduction remains undocumented due to the difficulties associated with obtaining these fish, a lack of ploidy analyses on bighead carp, and zero young-of-the-year captures.

Our eDNA results for bighead carp coincide with where they have been found previously. A lack of bighead carp eDNA presence upstream of their furthest north confirmed presence (Miami, Oklahoma) could suggest that dams are preventing the movement although it is likely that bighead carp can bypass present barriers during floods. Additionally, it could have been beneficial to test water samples for grass carp eDNA to compare the standardized targeted sampling results to the eDNA presence locations. Silver carp eDNA from a water sample at Chanute 1 requires further investigation. Silver carp are legal to use as dead bait in Kansas but are not known to exist within the study area [77]. Due to this, a false positive is plausible; identifications via eDNA analyses cannot be interpreted with absolute certainty [30]. Additional eDNA samples should be collected for all invasive carp species for monitoring the future distribution.

Otolith microchemistry results suggest that bighead carp and grass carp originate in chemically distinct environments and move between these environments throughout their life [33]. For example, some fish exhibited

TABLE 7: Grass carp electrofishing effort and catches from the Spring River, Kansas, from the summer of 2021 through the fall of 2022.

	Spring 2022	Summer 2021–2022	Fall 2021–2022	Total
Electrofishing effort				
Samples (runs)	22	23	13	58
Electrofishing hours	5.5	5.8	3.3	14.5
Electrofishing catch				
Total (N)	2	1	7	10
CPUE (fish/hr)	0.36 (0.25)	0.17 (0.17)	2.15 (1.07)	0.69 (0.28)

Standard error is in parentheses.

TABLE 8: Collection location, date acquired, method of acquisition, total length (mm), body mass (kg), sex, and age (years) of bighead carp specimens from Grand Lake O' the Cherokees and the Neosho River in Oklahoma from 2021 to 2022.

Location	Data obtained	Method	Total length (mm)	Body Mass (Kg)	Sex	Age
Grand Lake	12/8/2021	ODWC	1,218	33.6	F	26
Miami Fairgrounds (Neosho River)	5/4/2021	Angler	1,025	—	M	20
Grand Lake	3/10/2022	Angler	1,300	36.0	F	14
Connor Bridge (Neosho River)	4/15/2022	Angler	1,200	36.1	M	29
Grand Lake	4/16/2022	Angler	1,220	31.8	M	32

TABLE 9: Sampling location, season and method of acquisition, total length (mm), body mass (kg), sex, and age of grass carp specimens obtained via netting and electrofishing at John Redmond Reservoir, the Neosho River, and the Spring River, Kansas, from 2021 to 2022.

Site	Season	Method	Total length (mm)	Body mass (kg)	Sex	Age
John Redmond Reservoir	Spring 2022	Day electrofishing	923	10.2	F	19
John Redmond Reservoir	Spring 2022	Day electrofishing	213	0.1	I	2
Neosho River						
Burlington	Summer 2021	Day electrofishing	890	—	M	22
Iola	Fall 2021	Day electrofishing	895	—	F	14
Iola	Fall 2021	Day electrofishing	876	—	M	37
Iola	Fall 2021	Day electrofishing	852	—	—	—
Chanute 2	Summer 2021	Short-term gill netting	854	—	F	16
Chanute 2	Spring 2022	Day electrofishing	840	6.5	F	21
Chanute 2	Fall 2022	Day electrofishing	980	10.4	F	11
Oswego	Summer 2022	Day electrofishing	983	9.4	F	11
Spring River						
Baxter Springs	Fall 2021	Day electrofishing	1,049	—	M	—
Baxter Springs	Fall 2021	Day electrofishing	864	—	M	17
Baxter Springs	Fall 2021	Day electrofishing	960	—	F	24
Baxter Springs	Fall 2021	Day electrofishing	892	—	M	17
Baxter Springs	Fall 2021	Day electrofishing	930	—	M	25
Baxter Springs	Fall 2021	Day electrofishing	874	—	F	—
Baxter Springs	Spring 2022	Day electrofishing	921	9.4	M	13
Baxter Springs	Spring 2022	Day electrofishing	904	—	M	19
Baxter Springs	Summer 2022	Day electrofishing	847	6.1	M	18
Baxter Springs	Fall 2022	Day electrofishing	1,092	11.9	M	15

TABLE 10: Sampling location, total length (mm), body mass (kg), sex, and age of the confirmed diploid grass carp obtained from the Neosho River-Grand Lake system in 2022.

Site	Total length (mm)	Body mass (kg)	Sex	Age
Neosho River				
Chanute 2	980	10.4	F	11
Oswego	983	9.4	F	11
Spring River				
Baxter Springs	1,092	11.9	M	15

TABLE 11: Fecundity data for two bighead carp obtained from the Neosho River-Grand Lake system from 2021 to 2022.

Metric	Grand Lake O' the Cherokees	
Total length (mm)	1,300	1,218
Body mass (kg)	36.0	33.6
Ovary mass (kg)	6.2	5.0
Eggs/g of ovary	760	590
Fecundity	4,734,800	2,967,234
Eggs/kg of body mass	131,522	88,389
GSI (%)	17.3	15.0
Age	14	26

TABLE 12: Fecundity data obtained for two grass carp obtained from the Neosho River-Grand Lake system from 2021 to 2022.

Metric	John Redmond Reservoir	Oswego
Total length (mm)	923	983
Body mass (kg)	10.2	9.4
Ovary mass (kg)	0.7	0.7
Eggs/g of ovary	853	1,297
Fecundity	580,593	944,632
Eggs/kg of body mass	61,897	92,339
GSI (%)	7.3	7.1
Age	19	11

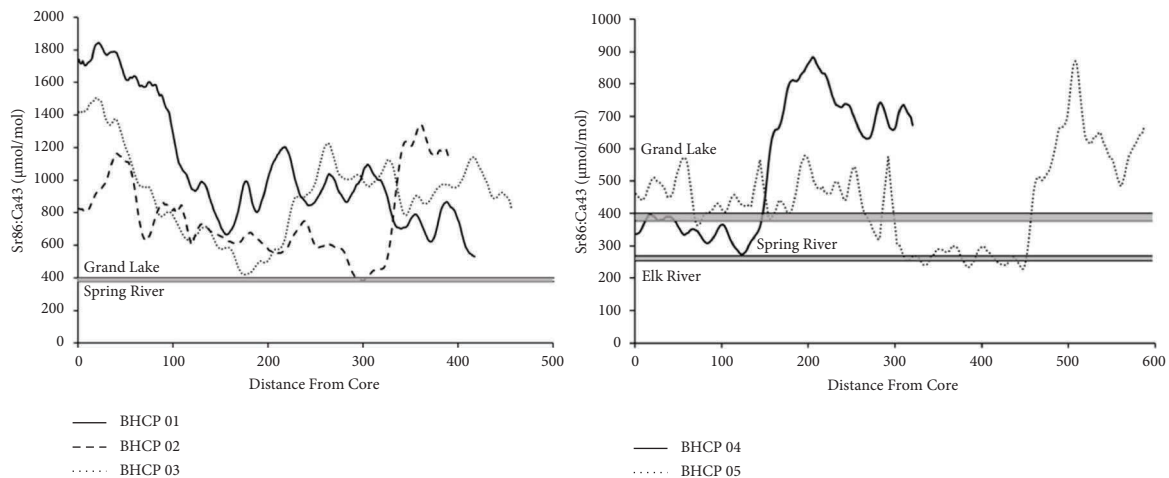


FIGURE 2: Microchemical results from bighead carp otoliths collected from 2021 to 2022. Bighead captures represent one scientific collection (i.e., ODWC Paddlefish Research Center Annual Standard Sampling) and four incidental angler captures. The shaded regions represent the overlap in Sr86:Ca43 signatures estimated from water samples converted for direct comparison to bighead carp otoliths [33, 69–72]. The shaded region in the upper figure with BHCP 01, BHCP 02, and BHCP 04 represents the overlap between the lower estimated Grand Lake Sr86:Ca43 signature (378 $\mu\text{mol/mol}$) and the upper estimated Spring River Sr86:Ca43 signature (400 $\mu\text{mol/mol}$). The shaded regions within the lower figure represent the same Grand Lake–Spring River overlap and the overlap between the lower estimated Spring River Sr86:Ca43 signature (254 $\mu\text{mol/mol}$) and the upper estimated Elk River Sr86:Ca43 signature (268 $\mu\text{mol/mol}$). Edge signatures across all sampled bighead carp reflect chemical signatures unique to capture locations. Movement appears to be high within chemically distinct regions and also occurs across chemically distinct regions. Movement across the Spring River, the Neosho River, the Elk River, and Grand Lake likely occurred across BHCP 03 and BHCP 05. BHCP 01 was a 1,300 mm specimen obtained from Grand Lake on 3/10/2022. BHCP 02 was a 1,220 mm specimen collected from Grand Lake on 4/16/2022. BHCP 03 was a 1,218 mm fish obtained on 12/8/2021 from Grand Lake. BHCP 04 was a 1,200 mm fish acquired on 4/15/2022 from the Neosho River. BHCP 05 was a 1,025 mm specimen collected from the Neosho River on 5/4/2021.

shifts in Sr86:Ca43 consistent with the movement between the Neosho River and Spring River [33]. While our sample size was small for microchemistry analyses, some specimens exhibited apparent broadscale movement patterns (i.e., moving between chemically distinct environments) within the system. Subsequently, these movement patterns

may be biologically (e.g., spawning) or ecologically (e.g., flow response) driven [57, 58, 76]. We cannot conclusively say fish originate within the system despite variable Sr86:Ca43 signatures between specimens; origination outside of the system (i.e., farm pond escapement or unauthorized stocking) remains a possibility.

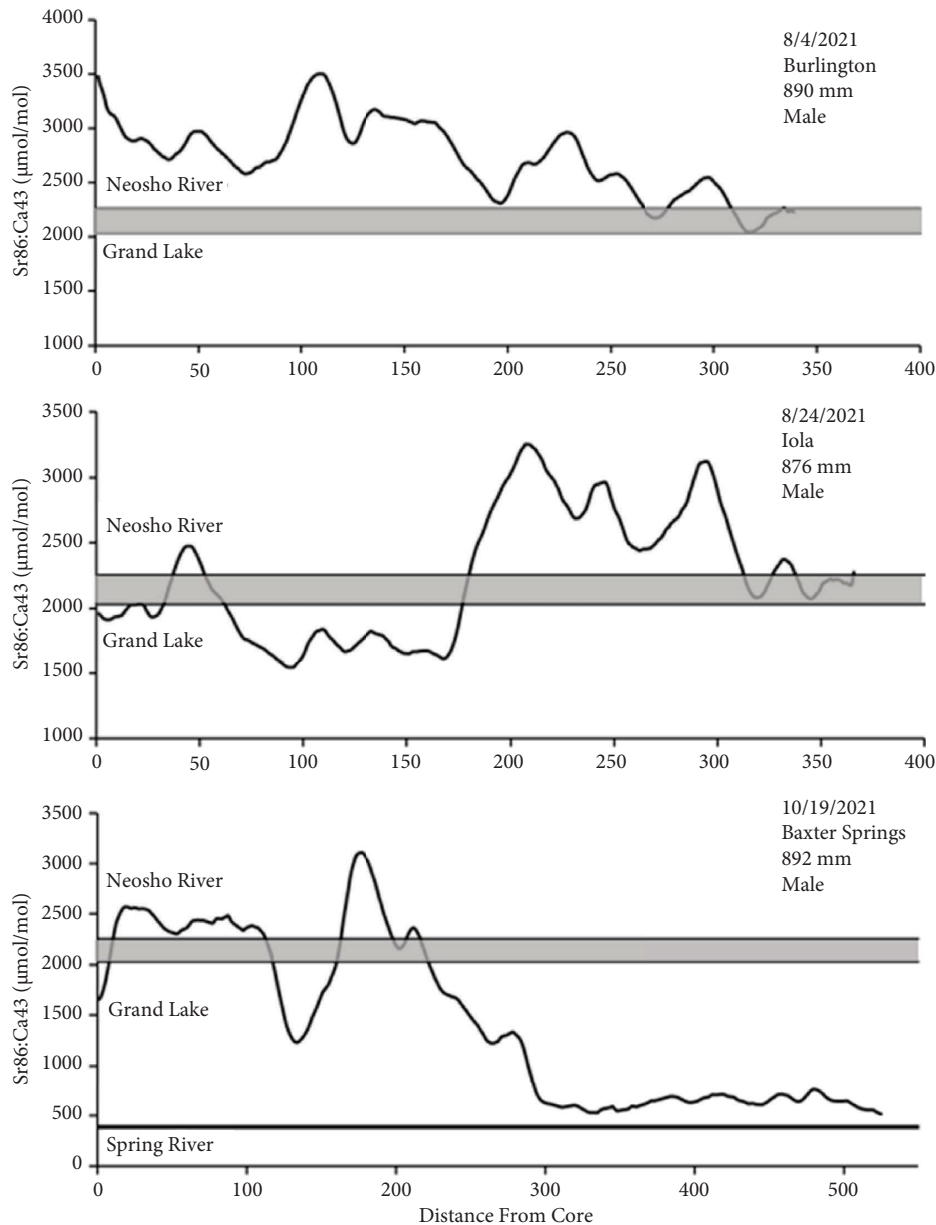


FIGURE 3: Grass carp microchemical results from three adult male fish collected in the Neosho River and Spring River. The shaded regions reflect overlaps in the estimated Sr86 : Ca43 signatures from water samples for each respective location [33]. Signatures from water samples were converted for direct comparison to grass carp otoliths [69–72]. Grass carp displayed movement across chemically distinct environments. Natal origins appeared to vary and could be within the Neosho River-Grand Lake system.

Given the low abundance of bighead carp and new insights garnered from grass carp in this study, grass carp can be targeted at locations (i.e., Chaunte 2, Iola, Baxter Springs) identified in this study for future studies. Developing a better understanding of their movement and habitat use can provide insights into areas and times for novel suppression methods [57, 58, 78]. A study utilizing grass carp as a surrogate species could inform about bighead carp seasonal migrations and corresponding habitat usage in the Neosho River-Grand Lake system [53–58]. Our study established sites where grass carp can be collected for tagging (i.e., telemetry) purposes. Additionally, ploidy assessments should be performed on future bighead carp captures to

investigate the recruitment potential. Similarly, eDNA water sampling should be continued to guide future targeted sampling efforts. Furthermore, this allows fisheries biologists early detection of new invasive species (e.g., silver carp). It is important to develop novel methods to target bighead carp, while in low abundance, and as such, we recommend the pursuit of nonconventional techniques such as targeted snagging (or simply counting individuals) with forward-facing sonar or angler reward programs to obtain these fish in the future.

Invasive species management requires multitiered approaches, especially when these species exhibit variable densities and deleterious impacts on native species

[1, 12, 17, 41, 51]. Ultimately, novel control and suppression methods can only be developed and refined through continuous ecological and biological monitoring [1, 12, 17]. We used the framework of direct (e.g., standardized targeted sampling) and indirect (e.g., eDNA water sampling) approaches to monitor and investigate bighead carp and grass carp in the Neosho River-Grand Lake system. Continual utilization and further refinement of these methods we outlined will lead to a better understanding and management of invasive carp in the system.

Data Availability

The data that support the findings of this study are available from the corresponding author, EJR, upon reasonable request.

Ethical Approval

The research was conducted under compliance with the following permits: Institutional Animal Care and Use Committee Approval (Missouri State University, IACUC protocol 2020-14), Scientific, Education, or Exhibition Wildlife Permit (Kansas Department of Wildlife and Parks, SC-003-2022), and Scientific, Education, or Exhibition Wildlife Permit (Kansas Department of Wildlife and Parks, SC-008-2021).

Conflicts of Interest

Ben C. Neely is the sole author with potential conflicts of interest as he is employed by the agency that administered funding for this research.

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