

Project Title: Abundance and distribution of early life stages of Asian carp in Red River Basin.

Geographic Location: Red River Basin

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Participating Agencies: Louisiana Department of Wildlife and Fisheries (LDWF) Nicholls State University (Quenton Fontenot, NSU); U.S. Fish and Wildlife Service (Oklahoma Fish and Wildlife Conservation Office)

Statement of Need:

Successful management of invasive carp is dependent on a thorough understanding of life histories and interactions with local environments. Reproduction, a key component of life history, can be used to guide control efforts by identifying where large spawning aggregations occur, and by identifying source and sink populations.

The purpose of this project is to determine where successful reproduction of invasive carp is occurring through the identification and cataloging of larval fish samples in Louisiana and Oklahoma waters. The documentation of the presence/absence of invasive carp larvae will assist in determining the leading edge of expansion throughout the Red River Basin and will help document where self-sustaining populations have established. This project will inform the direct management activities to contain the spread of invasive carp such as placement of deterrents and possible locations for other control activities.

In Louisiana, the Red, Atchafalaya, and Mississippi Rivers are connected at the Old River complex, which has allowed invasive carp to spread to all three rivers, and further spread to smaller rivers in LA through natural and man-made connections such as flood relief or freshwater diversions used for wetland restoration. Commercial navigation channels have allowed migration between river basins, thereby allowing invasive carp and other fish to move

freely between basins. The LDWF began monitoring ichthyoplankton in 2013 to better understand the extent of invasive carp reproduction and to quantify larval invasive carp in Louisiana. In 2013, the samples were taken in April, May, and June. This project was modified to have the samples taken in May, June, and July in 2014 to try to define a temporal pattern of the presence of invasive carp early life stages. Sample sites were revisited in 2019 to see if invasive carp reproduction has remained constant over time or has spread to other areas where carp reproduction was not detected in 2013-14. The 2019 samples were taken during a historic flood on the Mississippi and Red Rivers and the data does not reflect what we would expect of a “typical” year’s reproduction. Future studies will either confirm changes in reproductive patterns as reflected by the 2019 results or that the 2013 and 2014 years are more typical.

Previous studies were funded by the state Aquatic Nuisance Species (ANS) grants, which must support all ANS activities in Louisiana. In order to conduct additional research on temporal and spatial variation in invasive carp breeding, other funding sources must be utilized. The long-term goal for the research conducted through this early life stage study, in conjunction with telemetry-based movement studies, is to determine if it would be possible to direct harvest or place barriers or deterrents to further restrict invasive carp movement and reproduction.

In Oklahoma, efforts to evaluate recruitment by invasive carp species in the Red River have not yet been conducted. The OKFWCO began monitoring for the presence of larval Bighead Carp (*Hypophthalmichthys nobilis*), Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idella*), and Black Carp (*Mylopharyngodon piceus*) in 2021 to determine the distribution and extent of successful reproduction by these species in the Red River and its tributaries. Initiating these assessments for understudied systems will inform long-term control strategies and provide a baseline for monitoring the extent of invasion in Oklahoma and surrounding states.

The Red River flows east in Oklahoma to form its border with Texas. This section of the Red River is characterized by numerous southern-flowing tributaries, which typically possess an impoundment and an associated reservoir above. While these impoundments are considered to be impassable by invasive carp species, the connectivity of the Red River with the lower reaches

of its tributaries foster the appropriate conditions for spawning below the dams, as well as the potential for bait fish transfer to nearby reservoirs. This region supports a thriving recreational fishery that could potentially be impacted by invasive carp invasion.

This study supports the objectives of the Lower Mississippi River Basin invasive carp control plan, by helping to determine what aquatic habitats in Louisiana and Oklahoma are suitable for invasive carp spawning. When combined with other studies it may help determine what populations are source populations and help in directing efforts for harvest or exclusion.

Project Objectives:

1. Determine the extent of invasive carp spawning activity in the Red River basin.

Project Highlights:

- Invasive carp are reproducing in Red River in LA.
- Invasive carp have not been detected in Oklahoma; however less than 25% of the total samples have been fully processed.
- The carp were not a high percentage of the ichthyoplankton in the 2021 samples.
- The 2021 samples indicate that there may have been environmental conditions that hindered reproduction in the Red River.

Methods:**Louisiana:**

Ichthyoplankton samples were collected at 14 sites throughout Louisiana by LDWF personnel in April, May, June, and July of 2021. At each sample station, Ichthyoplankton samples were collected by towing a 0.5m diameter 500µm mesh ichthyoplankton net just below the water surface for a duration of 10 minutes per tow. A separate tow was made on the left, middle, and right portions of the channel. Samples were preserved in 70% ethanol or isopropyl alcohol and delivered to NSU.

Upon arrival at NSU, larval and juvenile fish were separated from debris for each sample and stored in plastic containers or scintillating vials containing 70% ethanol or isopropyl alcohol. Each fish was identified to at least the family taxonomic level. Cyprinids were further identified as either an invasive carp species or not an invasive carp species. All fish were identified fishes to family based on Auer (1982). Identification of cyprinids such as invasive carp larvae were based on Chapman (2006), Chapman and George (2011) and George and Chapman (2013). Taxonomic classification beyond family for non-cyprinids was based on Auer (1982).

Oklahoma:*Ichthyoplankton Tows.*—

OKFWCO methodologies were analogous to those implemented LDWF with the exception that tandem nets were used by OKFWCO. Water temperature (°C; YSI Professional Plus Multiparameter Meter), water velocity (m/s; Marsh McBirney, Flo-Mate), and turbidity (NTU; Apera Instruments, Model TN400) was recorded approximately 0.25-m below the surface.

Light Trapping.—During the ichthyoplankton sampling season, we concurrently deployed Quadrafoil Light Traps (WaterMark, Jackson, MS) in the upper portions of the Red River and its tributaries between the Denison Dam and the Kiamichi River (Fig.2). These cylindrical traps are characterized by their series of polycarbonate tubes arranged in a cloverleaf shape and lower a collection chamber where contents are funneled. In the center of the tubes, a green LED light (FishXtrada/Lumica USA, Inc., Anaheim, CA) was suspended to attract phototactic aquatic organisms, such as macroinvertebrates, and numerous species of juvenile fishes, including invasive carp (Roth 2018; Brandenburg et al. 2019). Traps were anchored in place and floated just below the water's surface with an agency-labeled buoy attached. The majority of our light trapping was done in eddies, side channels, floodplains, and small tributaries (especially during dam discharge events), which often serve as habitats for drifting larvae to reside and develop into free-swimming mesolarvae. After approximately 24 hours, traps were retrieved, water was drained through 500 µm mesh, and contents were rinsed and preserved in 95% non-denatured ethanol.

Sorting and identification.—To reduce dilution of our ethanol solutions during specimen dehydration, we replaced the ethanol in each sample 24 to 48 hours after initial preservation by filtering the solution through a 500- μ m sieve (Nagy 2010; U.S. Fish and Wildlife Service n.d.). The sample contents were retained and non-denatured ethanol replenished. After removing debris from the samples, fishes were identified to family based on Auer (1982). Further identification of Cyprinids to genus was completed using Holland-Bartels et al. (1990), Taber (1969), May and Gasaway (1967), and Snyder (1979). Potential invasive carp genera were identified using Chapman (2006), Chapman and George (2011) and George and Chapman (2013). We will examine fishes with two stereo microscopes: an Amscope SM-4TPZZ (3.5x–180x total magnification) and a Nikon SMZ800N stereo microscope (5x–480x total magnification).

Genetic Confirmation.—Larval fish and eggs often suffer from deformation, loss of pigmentation, and significant shrinkage, particularly in highly concentrated alcohol fixatives and towed nets (Kelso and Rutherford, 1996; Smith and Walker, 2003; Frimpong and Henebry 2012; König and Borchering, 2012; Larson et al. 2016). Consequently, individual eggs and larvae identified as potential invasive carp, or specimens deemed unidentifiable, were separated for genetic analysis by the U.S. Fish and Wildlife Service’s Southwest Native Aquatic Resource and Recovery Center (SNARRC) in Dexter, New Mexico to confirm species identity.

Results and Discussion:

Louisiana:

Sample site location data is listed in Appendix 1. Table 1 shows the results of the 105 samples collected. Out of the 105 samples that have been received, 98 have had 100% of their contents identified (Table 1). The majority of fish that have been sorted but not identified include cyprinids that have either not been confirmed as an invasive carp or not an invasive carp, or are a species waiting on a second opinion to confirm identification.

The identified larval fish consisted of 4034 individuals from 10 families (Table 2). Clupeidae was the most abundant, occurring in the most samples, and Poeciliidae was the least abundant, occurring in the fewest samples (Table 2). Invasive carp were the 7th most abundant species

represented. However, we expect their abundance to increase once we complete all identifications. There are also a few yolk-sac larvae (N=5) that have yet to be identified. There were also some larvae (N=7) that were too degraded to confirm identification and were labeled as unidentified degraded. The total number of fish to date (including unidentified yolk-sac and degraded) was 4046 (Table 2).

Although we have not confirmed the identification of larvae from all samples, invasive carp were only identified in samples from June (Table 3). In June, invasive carp were identified in only 1 tow sample for the 2 stations where they were found (Figure 1). The low numbers make it difficult to determine if large scale reproduction is occurring in the Red River or if the reproduction was missed by sampling.

Table 1. Status of the samples we have received (N=105) from the Red River as of January 2022. Sorted samples have had the larvae separated from the sample debris and all fish have been identified to family in the 100% Identified samples.

Sample Status	Total Number	Percent Total
Received	105	100
Sorted	7	7
100% Identified	98	93

Table 2. Total number of larvae identified within each family and larvae that could not be identified because they were too degraded or at the yolk-sac stage as of January 2022. Frequency represents the number of samples that contained at least one individual in that family. Invasive carp were the seventh most abundant larvae and occurred in 2 samples. There were 17 samples that had no larvae.

Family	Frequency	Number
Clupeidae	67	2,970
Centrarchidae	47	382
Percichthyidae	29	233
Atherinidae	43	191
Catostomidae	34	136
Cyprinidae	18	61
Invasive Carp	2	28
Percidae	9	13
Lepisosteidae	8	11

Poeciliidae	8	9
Subtotal		4,034
Unidentified Yolk-sac		
Larvae	3	5
Unidentified Degraded	6	7
Subtotal		12
Total Fish		4,046

Table 3. Total number of invasive carp larvae collected each month at each station as of January 2022. The frequency indicates the number of net tows that collected an invasive carp from that station. For example, if the frequency is one, then only one all tow at that station had an invasive carp in it. No invasive carp have been identified in April, May, or July samples, but some samples from 2021 have not been completely processed.

Month	River	Station	Frequency	Number
April	None	None	0	0
May	None	None	0	0
June	Red	Pool 3 – Station 1	1	21
June	Red	Pool 3 – Station 2	1	7
		Subtotal		28
July	None	None	0	0

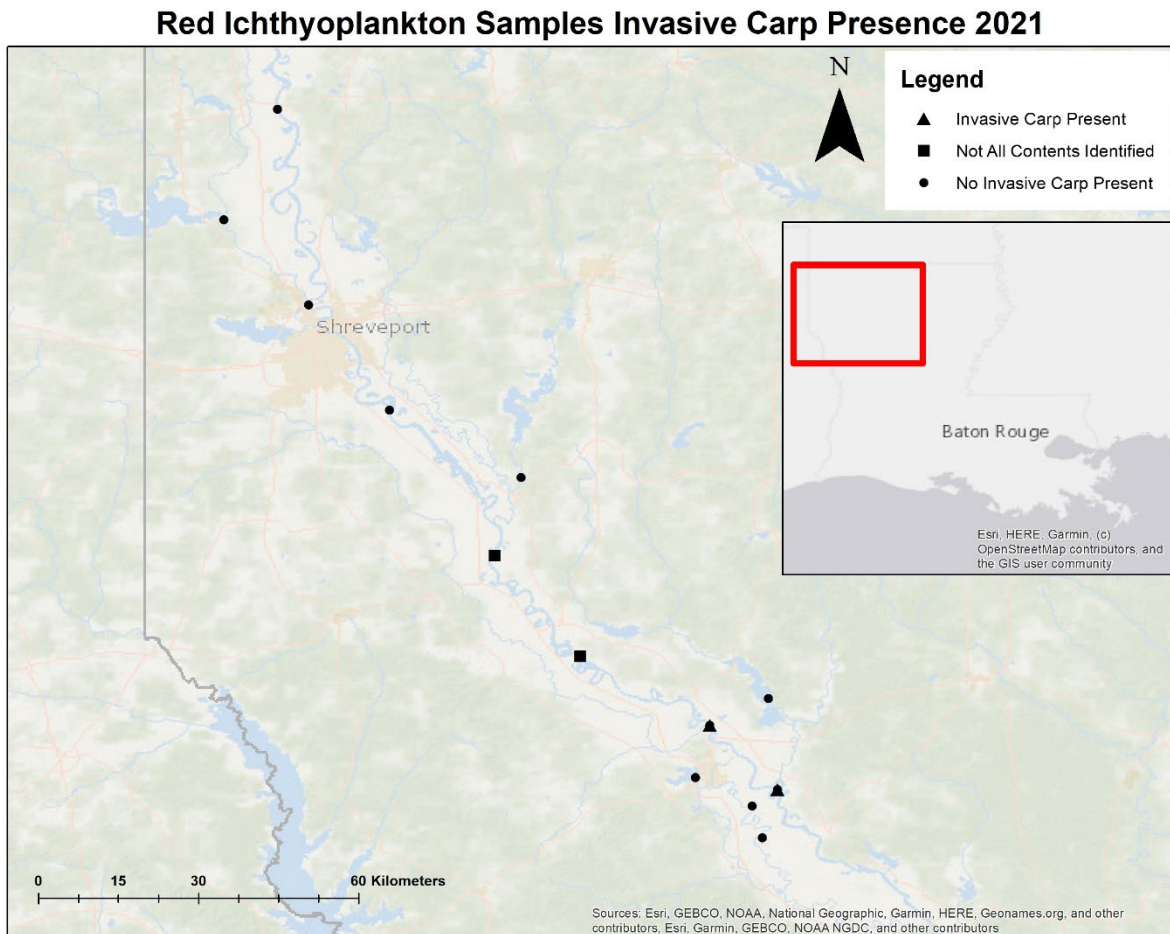


Figure 1. Sample locations where invasive carp have been identified (triangle), not all contents have been identified (squares), and where no invasive carp were identified (circles) as of January 2022.

Oklahoma:

Ichthyoplankton Tows.—A combined total of 52 ichthyoplankton tows were completed on the Red River and its smaller tributaries ($N = 30$; 12 transects; Figure 2), and the Kiamichi River ($N = 22$; 8 transects; Figure 3) during the summer of 2021. Dates and location data are listed in Appendix 2.

Light Trapping.—Nine quadrafoil light trapping events resulted in a combined total of 69 trap-nights on the Red River (including Webb Creek and Choctaw Creek) ($N = 52$; Figure 2), and the Kiamichi River ($N = 17$; Figure 3). Dates and location data are listed in Appendix 3.

Sorting and identification.—As of January 2022, a total of 332 fishes have been identified across 14 light trap samples and four ichthyoplankton tow samples, and contained fishes belonging to nine different families (Table 4). No suspect invasive carp have been identified; however, identification is still in progress for 93 samples (Table 5). An additional six fish were deemed “unidentifiable” and have been isolated for future genetic analyses.

Genetic Confirmation.—DNA from confirmed Grass Carp, Silver Carp, Bighead Carp, and Black Carp fin clips (50+ samples per species) was sequenced by SNARRC in preparation for comparison with field samples.

Table 4. Fish families identified by the OKFWCO in light trap and ichthyoplankton tow samples as of January 2022. Asterisk denotes individuals that were not visually identifiable by our staff, and require further observation and/or genetic confirmation.

Family	Ichthyoplankton Tows	Light Traps	% of Total
Atherinidae	-	189	56.9
Catastomidae	1	-	0.3
Centrarchidae	-	4	1.2
Clupeidae	18	80	29.5
Cyprinidae	1	20	6.3
Hiodontidae	1	3	1.2
Moronidae	5	-	1.5
Poeciliidae	-	1	0.3
Sciaenidae	3	-	0.9
Unidentified*	4	2	1.8
Total	33	299	

Table 5. Current status of larval fish identification by the OKFWCO in samples collected in light traps and ichthyoplankton tows in summer 2021 as of January 2022. Processing of samples is ongoing. Asterisk indicates samples that are currently unidentifiable and will undergo genetic analyses and/or second opinion.

	Samples		
	Completed	In Progress	# Fish
Light Traps	14	55	344
Ichthyoplankton Tows	4	38	33
Total	18	93	377

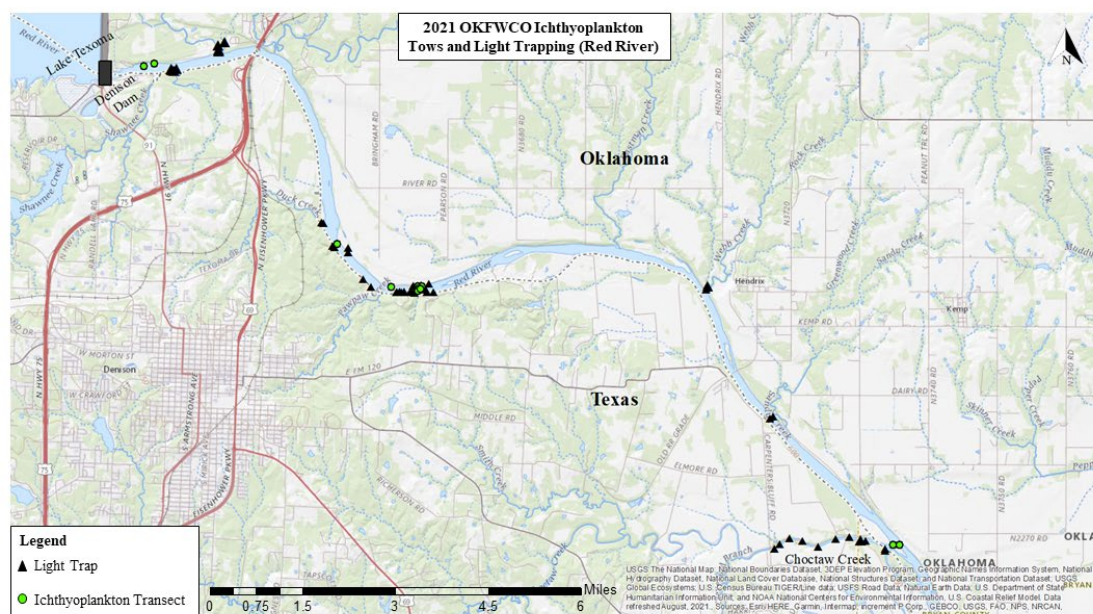


Figure 2. Locations of larval fish sampling completed by the OKFWCO on the Red River and its tributaries between the Denison Dam and the Kiamichi River. Ichthyoplankton tow transects are denoted by green circles and light trap locations by black triangles.

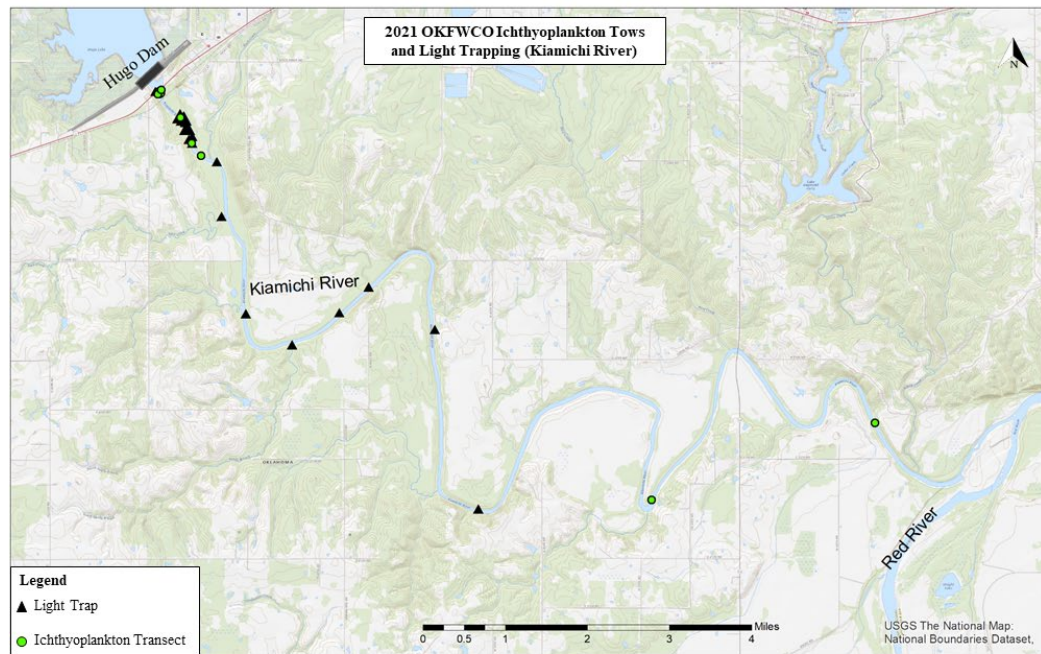


Figure 3. Locations of larval fish sampling completed by the OKFWCO on the Kiamichi River below the Hugo Dam. Ichthyoplankton tow transects are denoted by green circles and light trap locations by black triangles.

Recommendations:

Successful reproduction of invasive carps was not detected in large numbers on the Red River and its tributaries; however, sampling efforts for early detection of larval invasive carp should be robust and consistent, especially in flashy systems with sexually mature adult fish. After consulting with other biologists, expanding ichthyoplankton sampling to areas lower in the water column may capture semi-buoyant drifting larvae and eggs.

Hydrological and chemical analysis as well as analysis of barrier possibility of the Red River may help determine if certain reaches are less favorable for invasive carp spawning.

More sites need to be sampled upstream and downstream of sites where invasive carp were located in order to better define the breeding areas in Louisiana.

Increased throughput of ichthyoplankton processing will help with more timely information which may be used for immediate management actions. This may entail eDNA tests on the samples to determine the possible presence of invasive carp prior to completely processing the samples.

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- morphology alone cannot distinguish Asian carp eggs from those of other cyprinid species. *North American Journal of Fisheries Management*, 36:1053–1058.
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APPENDIX 1. LDWF ichthyoplankton sample site locations that were sampled during the summer of 2021. Each month consisted of three tows per site.

River	Station	Latitude	Longitude
Cane	2 Shell Beach Ramp	31.657577	-93.001269
Cane	1	31.711349	-93.018668
Red	Pool 3-Station 1	31.738706	-92.975817
Sibley Lake	1	31.759069	-93.114349
Red	Pool 3-Station 2	31.847154	-93.089743
Black Lake	Sandy Point	31.891978	-92.990851
Red	Pool 4-Station 1	31.963696	-93.308669
Red	Pool 4-Station 2	32.133624	-93.453075
Loggy Bayou	2 Loggy Bayou Boat Ramp	32.264933	-93.407883
Red	3 South	32.36304	-93.6259
Red	4 Teague	32.5556	-93.767
Twleve Mile Bayou Caddo	1 Soda Lake	32.699391	-93.909546
Red	5 Hwy 2	32.88526	-93.8191

APPENDIX 2. Ichthyoplankton tow transects completed by the OKFWCO during summer 2021 on the Kiamichi River below Hugo Dam, and the Red River and its tributaries below Denison Dam. Each site consisted of three tows.

River	Date	Latitude	Longitude
Kiamichi	5/12/2021	34.0073699	-95.38049
Kiamichi	5/12/2021	33.94804	-95.29375
Red	5/13/2021	33.8200345	-96.35604368
Red	5/13/2021	33.77366	-96.4912
Red	5/19/2021	33.77372	-96.49119
Kiamichi	5/21/2021	33.99827	-95.37305
Kiamichi	5/21/2021	34.00723	-95.38066

Red	6/3/2021	33.82045	-96.55389
Red	6/4/2021	33.77366	-96.49101
Kiamichi	6/15/2021	33.959323	-95.25442
Kiamichi	6/16/2021	34.000098	-95.37476
Red	6/22/2021	33.77415	-96.49128
Kiamichi	6/29/2021	33.77349	-96.49213
Red	7/6/2021	33.78314	-96.51149
Red	7/6/2021	33.77363	-96.49075
Kiamichi	7/13/2021	34.00382	-95.37668
Red	7/20/2021	33.77402	-96.4979
Red	7/22/2021	33.71975	-96.36915
Choctaw Creek	7/22/2021	33.721286	-96.382245

APPENDIX 2. Quadrafoil light traps deployed by the OKFWCO during summer 2021 on the Kiamichi River below Hugo Dam, and the Red River and its tributaries below Denison Dam.

River	Date	Latitude	Longitude
Red	5/13/2021	33.8199683	-96.5536059
Red	5/13/2021	33.8196048	-96.5533689
Red	5/13/2021	33.8195683	-96.5528348
Red	5/13/2021	33.8199393	-96.5521686
Red	6/3/2021	33.7812500	-96.5086400
Red	6/3/2021	33.7822600	-96.5086700
Red	6/3/2021	33.7874600	-96.5149900
Red	6/3/2021	33.7876100	-96.5154000
Red	6/3/2021	33.8236400	-96.5415500
Red	6/3/2021	33.8239800	-96.5415200
Red	6/3/2021	33.8249000	-96.5416200
Red	6/3/2021	33.8256100	-96.5400000
Kiamichi	6/15/2021	34.0023100	-95.3755300
Kiamichi	6/15/2021	33.9974100	-95.3702800

Kiamichi	6/15/2021	33.9894000	-95.3694400
Kiamichi	6/15/2021	33.9886200	-95.3679000
Kiamichi	6/15/2021	33.9752100	-95.3651600
Kiamichi	6/15/2021	33.9707000	-95.3570200
Kiamichi	6/15/2021	33.9791300	-95.3435200
Kiamichi	6/15/2021	33.9729100	-95.3318800
Kiamichi	6/15/2021	33.9467200	-95.3242400
Red	6/23/2021	33.7729200	-96.4887000
Red	6/23/2021	33.7729800	-96.4871500
Red	6/23/2021	33.7747300	-96.4883200
Red	6/23/2021	33.7744400	-96.4902500
Red	6/23/2021	33.7743800	-96.4922400
Red	6/23/2021	33.7729300	-96.4963300
Red	6/23/2021	33.7730100	-96.4951600
Red	6/23/2021	33.7729600	-96.4925500
Red	6/30/2021	34.0076400	-95.3811000
Red	6/30/2021	33.7730000	-96.4965100
Red	6/30/2021	33.7729300	-96.4963200
Red	6/30/2021	33.7730300	-96.4956100
Red	6/30/2021	33.7730900	-96.4946000
Red	6/30/2021	33.7729700	-96.4930100
Red	6/30/2021	33.7729900	-96.4920380
Red	6/30/2021	33.7729900	-96.4920000
Red	7/6/2021	33.7734500	-96.4891100
Red	7/6/2021	33.7734500	-96.4891100
Red	7/6/2021	33.7827300	-96.5126500
Red	7/6/2021	33.7823500	-96.5121100
Red	7/6/2021	33.7757500	-96.5049000
Red	7/6/2021	33.7740900	-96.5029800
Kiamichi	7/13/2021	34.0036500	-95.3769100
Kiamichi	7/13/2021	34.0031200	-95.3758900

Kiamichi	7/13/2021	34.0006400	-95.3751800
Kiamichi	7/13/2021	34.0001000	-95.3746200
Kiamichi	7/13/2021	34.0010900	-95.3746300
Kiamichi	7/13/2021	34.0036900	-95.3760800
Kiamichi	7/13/2021	34.0036900	-95.3760800
Kiamichi	7/13/2021	34.0039900	-95.3767400
Choctaw Creek	7/21/2021	33.7189800	-96.4008100
Choctaw Creek	7/21/2021	33.7199000	-96.3994200
Choctaw Creek	7/21/2021	33.7211000	-96.3969400
Choctaw Creek	7/21/2021	33.7206500	-96.3935900
Choctaw Creek	7/21/2021	33.7193500	-96.3896100
Choctaw Creek	7/21/2021	33.7210700	-96.3852200
Choctaw Creek	7/21/2021	33.7214700	-96.3818300
Choctaw Creek	7/21/2021	33.7190100	-96.3726800
Choctaw Creek	7/21/2021	33.7185700	-96.3727200
Choctaw Creek	7/21/2021	33.7205900	-96.3775900
Webb Creek	8/3/2021	33.7207100	-96.3789900
Webb Creek	8/3/2021	33.7738500	-96.4173600
Webb Creek	8/3/2021	33.7742400	-96.4174700
Webb Creek	8/3/2021	33.7743500	-96.4178300
Webb Creek	8/3/2021	33.7740900	-96.4179000
Webb Creek	8/3/2021	33.7736800	-96.4181900
Red	8/3/2021	33.7474900	-96.4012400
Red	8/3/2021	33.7473900	-96.4011400
