FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-155-R

F13AP00668

Endangered and Threatened Species Conservation

Propagation and repatriation of native prairie stream minnows in the Middle Brazos River

Prepared by:

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16 November 2017

FINAL PERFORMANCE REPORT

STATE: Texas **GRANT NUMBER:** TX E-155-R

GRANT TITLE: Propagation and repatriation of native prairie stream minnows in the Middle Brazos River

REPORTING PERIOD: 1 September 2013 to 30 31 August 2017

OBJECTIVE(S): To propagate and raise sharpnose shiner, smalleye shiner, and other species of pelagic-spawning minnows, for release into the middle Brazos River to assess the feasibility of repatriating these species, determine river-length fragments and flow regimes necessary for maintenance of Brazos River pelagic-spawning species, and provide an ecological buffer against establishment of invasive species.

Segment Objectives:

Task 1: Propagate, Raise, and Repatriate Native Prairie Stream Minnows

Task 2: Relocate and Repatriated Native Prairie Stream Minnows

Significant Deviations: None.

Summary Of Progress: See Attachment A.

Location: Brazos River, between Possum Kingdom Reservoir and Lake Granbury, Palo Pinto and Parker counties, Texas.

Cost: Costs were not available at time of this report.

Prepared by: Craig Farquhar **Date:** 16 November 2017

Approved by: _____ **Date:** 16 November 2017
C. Craig Farquhar

ATTACHMENT A

Texas Parks and Wildlife Department

Final Section 6 Project Report

TITLE: Propagation and Repatriation of Native Prairie Stream Minnows in the Middle Brazos River

DATE: 15 November 2017

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The Sharpnose Shiner *Notropis oxyrhynchus* and Smalleye Shiner *N. buccula* are endemic to the Brazos River drainage, Texas. Historically, both species ranged widely in the Brazos River main-stem, and its upper tributaries, from just off the escarpment of the Llano Estacado, downstream to within 100 km of the Gulf Coast. The Sharpnose Shiner also occurred in the North and South Wichita rivers (Cross et al. 1986; Moss and Mayes 1993), but it is now generally believed to be extirpated from the Wichita River drainage.

Both the Brazos and Wichita River drainages have been extensively modified by impoundments. The Brazos River is the most fragmented river basin in Texas (Wilde and Urbanczyk 2013). There are three main-stem impoundments, and nearly forty tributary impoundments, in the Brazos River drainage and an additional eight impoundments are permitted or are under construction. Although the Wichita River drainage is smaller than the Brazos River drainage, it is fragmented by two main-stem impoundments and at least eight tributary impoundments. As a result of modifications of the Brazos and Wichita rivers, the distributions of both Sharpnose Shiner and Smalleye Shiner have decreased substantially (Moss and Mayes 1993): both Sharpnose Shiner and Smalleye Shiner occur in the upper Brazos River, upstream from Possum Kingdom Reservoir, and a small population of Sharpnose Shiner occurs in the lower Brazos River, downstream from Lake Whitney. Since initiation of this project both species have been listed as Endangered Species (USFWS 2014).

Sharpnose Shiner and Smalleye Shiner are members of a reproductive guild of pelagic-spawning cyprinids common in Great Plains rivers that broadcast spawn semi-buoyant ova into the current (Platania and Altenbach 1998; Durham 2007; Durham and Wilde 2008, 2009a, 2009b)). Ova and newly hatched swim-up fry are kept afloat by the current, which prevents them from settling to the river bottom where they may be covered and suffocated by silt and sand sediments (e.g., Moore 1944). Platania and Altenbach (1998) calculated that the developing ova could be displaced as far as 72 to 144 km downstream before hatching and that fry could be displaced an additional 216 km downstream before they could move out of the current into backwater areas. Known members of this reproductive guild, in addition to Sharpnose Shiner and Smalleye Shiner, include Arkansas River Shiner *Notropis girardi*, Red River Shiner *N. bairdi*, Rio Grande Silver Minnow *Hybognathus amarus*, Plains Minnow *H. placitus*, Speckled Chub *Macrhybopsis aestivalis*, Peppered Chub *M. tetranema*, and Shoal Chub *M. hyostoma*.

Perkin and Gido (2011) found that stream fragment length was a reliable predictor of the conservation status of Great Plains pelagic-spawning cyprinids. Although there analysis did not include Sharpnose Shiner and Smalleye Shiner, a similar analysis for these species suggested the importance of river fragment length in maintenance of these species (Wilde and Urbanczyk 2013). Because of the extensive fragmentation of the Brazos River drainage basin, and the threat of continued fragmentation as proposed and ongoing water development projects are completed, conservation of the Sharpnose Shiner and Smalleye Shiner will require efforts to repatriate the species to other portions of their historic distributions. The Species Status and Assessment for Sharpnose Shiner and Smalleye Shiner notes the lack or redundancy for these species, which will repatriation of these species into former portions of their historic ranges (ATESFO 2014).

Objectives

Our original objective was to propagate and raise Sharpnose Shiner and Smalleye Shiner for release into the middle Brazos River to (1) assess the feasibility of repatriating these species, (2) determine river-length fragments and flow regimes necessary for maintenance of Brazos River pelagic-spawning species, and (3) provide an ecological buffer against establishment of invasive species. However, the listing of these two species in 2014 required us to substitute Plains Minnow and Shoal Chub, as surrogate species, to assess these objectives. The middle Brazos River was chosen as a study site because Sharpnose Shiner and Smalleye Shiner, as well as the surrogate species Plains Minnow and Shoal Chub, historically occurred in this reach, until impoundment of Possum Kingdom Reservoir (upstream) and Lake Granbury (downstream).

Methods

Broodstock Collection and Maintenance

Adult male and female Plains Minnow and Shoal Chub were collected from the Double Mountain Fork of the Brazos River and the upper Brazos River main-stem upstream from Possum Kingdom Reservoir. Fish were collected before and during their natural spawning season, March through August, in 2014, 2015, and 2016. Fish were collected by seining. Captured fish were transported in aerated 94-l ice chests to the Prairie Stream Fish Conservation and Propagation Laboratory (Figure 1) located at The Institute of Environmental and Human Health (TIEHH) Texas Tech University.

Plains Minnow and Shoal Chub were held in separate 380-l recirculating holding aquaria in a shaded outdoor facility at TIEHH. Each recirculating aquarium contained de-chlorinated tap

Figure 1. Prairie Stream Fish Conservation and Propagation Laboratory located at The Institute of Environmental and Human Health (TIEHH), Reese Center, Texas Tech University.

water from the City of Lubbock, Texas. The shaded outdoor facility allowed the fish to be held at ambient temperatures with a natural photoperiod. Water quality was maintained at temperatures between 20 and 32°C, dissolved oxygen 5 to 13 mg/l, conductivity 4,000 to 5,500 µS/cm, salinity 2 to 3 ppt, and pH 8.2 to 9.0. Fish were fed daily with a combination of commercially available Purina AquaMax Fry Starter fish pellets, freeze-dried brine shrimp, and freeze-dried bloodworms. Fish were fed a daily ration of 3% body weight per day. Holding aquaria were treated with air driven biological filters for water detoxification. Fish were acclimated to holding conditions for at least two weeks before spawning was attempted.

Hormonal Injections

The morning before spawning, males and females of either Plains Minnow or Shoal Chub were removed from their holding aquarium and were anesthetized in a buffered 100-ppm Tricaine methanesulfonate (MS-222) (Sigma-Aldrich) aerated bath. While the fish were anesthetized, they were sexed by applying gentle pressure to the abdominal cavity. Males of expressed a small amount of sperm from their urogenital opening, whereas in females the urogenital pore would swell but would expel gametes. Males were given an intraperitoneal injection of approximately 0.1-ml of a 5-ppm aqueous suspension of acetone dried carp pituitary extract (CPE). After males were injected with CPE, they were held in aerated 75-l static aquaria for approximately 30 to 32 hr. Females that were anesthetized along with males, for sexing, were placed into 75-l static aquaria to recover.

Approximately 24-hr after males were injected with CPE, females of that species were removed from their holding aquaria, between 0600 and 0900 hrs, and were re-anesthetized using a buffered 100-ppm MS-222 aerated bath. Each female received an intraperitoneal injection of aqueous CPE. After being injected with CPE, females were placed into aerated 75-l static aquaria.

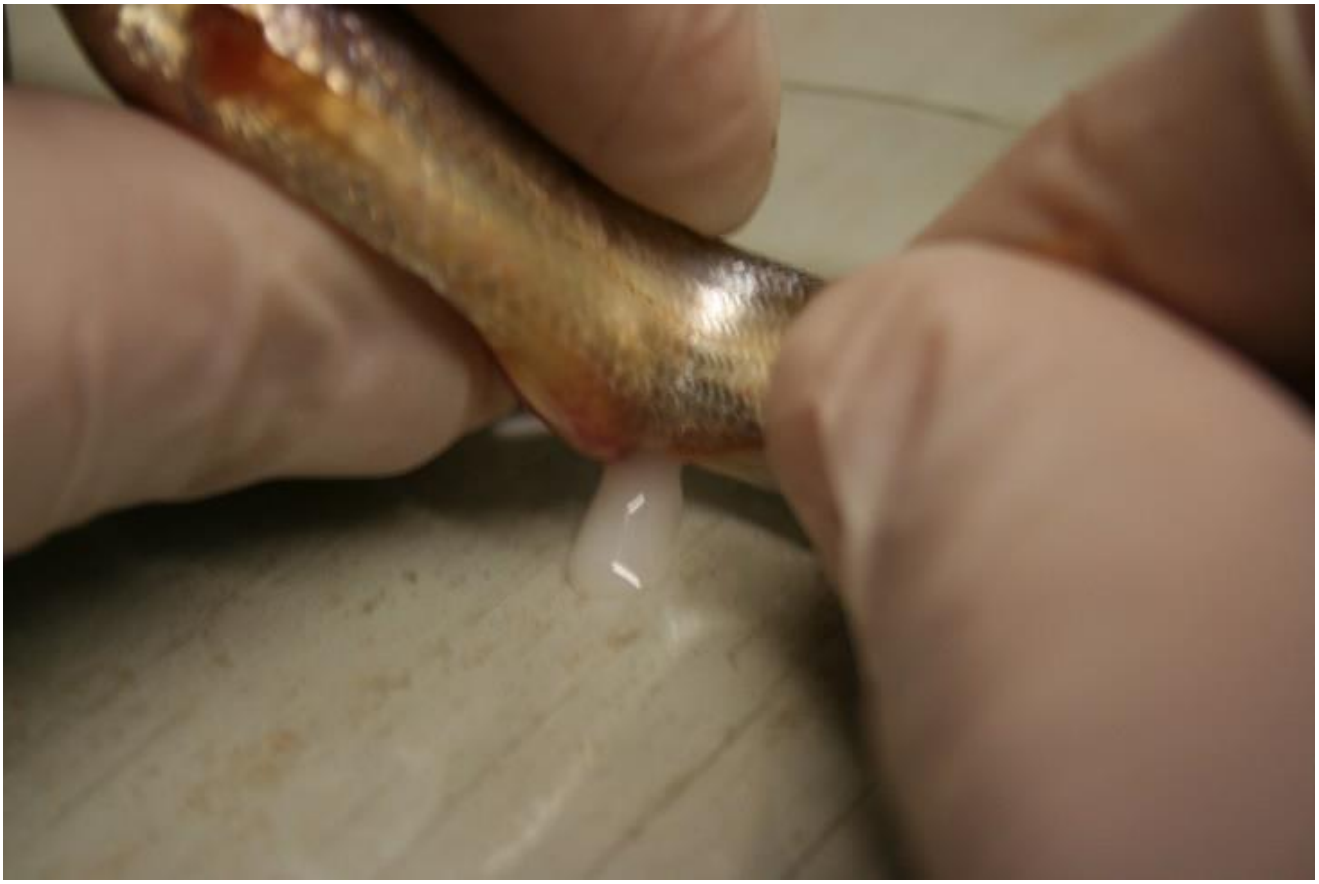
Hatching Aquaria

Twelve 38-l hatching aquaria were filled with conditioned de-chlorinated tap water similar in composition to that in holding aquaria of post-injected female. This procedure was necessary to ensure that water temperatures in the hatching aquaria were similar to those at which females were held because ova survival, after fertilization, was adversely affected by temperature fluctuations.

Stripping Procedure

Six to seven hr after females were injected, male fish were removed from their holding aquaria and anesthetized using an aerated buffered 100-ppm MS-222 bath. Males were removed from the bath one at a time and stripped of sperm utilizing the dry technique (Rothbard 1981). Each fish was rolled in a paper towel to remove excess water from the skin and the urogenital pore was wiped to remove water. Once the fish was dry, it was held over a petri-dish and gentle pressure was applied to the abdomen utilizing a stripping motion starting at the anterior end of the abdomen and gentling moving toward the urogenital pore (Figure 2). Expelled sperm was was collected using an insulin syringe and was kept on ice until female ovulation began. Sperm could be stored on ice and utilized for up to seven hr post-collection without the use of extenders. Males were returned to the aerated 380-L recirculating holding aquaria for future spawning.

Figure 2. Sperm being expressed (stripped) from a male Plains Minnow.



Depending on the ambient temperature, ovulation occurred between seven and 12 hr post-CPE injection in females. Approximately seven hr post-injection, females were tested for ovulation by applying gentle pressure to the abdomen. If the female was ovulating, a small

number of ova would be expelled from the urogenital pore. Females that were not ovulating were placed back into their holding aquaria and were then tested hourly for ovulation.

Once ovulation occurred, females were removed from their 75-l holding aquaria and dried using a paper towel (dry technique Rothbard 1981). After the body and urogenital pore were dried, gentle pressure was applied to the abdomen to expel ova into a petri-dish (Figure 3.

Three to five females were stripped into a single petri dish and the combined ova were fertilized utilizing sperm that had been collected earlier in the day. Sperm, held on ice in an insulin syringe, was warmed ambient temperatures to avoid temperature shocking the ova. Quantity of sperm used to fertilize ova was variable, depending on the number of spawned females. Once sperm was added to the ova, in a petri-dish, a feather was used to mix the sperm and ova for approximately 30 seconds until sperm was evenly distributed among the ova. Two ml of conditioned water from the hatching aquarium was added to the petri-dish with sperm and ova to activate them. Once activation of the sperm and ova occurred, mixing of the sperm and ova with a feather for 90 seconds was necessary for optimal fertilization. After the ova and sperm were mixed, fertilized ova were allowed to rest for 2 minutes, during which time the ova began to absorb water and swell. After this two minute period, the fertilized ova were mixed once more with a feather and placed into a 38-l static aerated aquaria.

Figure 3. Ova being stripped from a female Plains Minnow.

Ova and larval fish husbandry

Between 17 and 24-hr post-fertilization, larval fish hatched and began swim-up fry. After hatching, the fry, are not as buoyant as the fertilized ova and would sink to the bottom of the holding aquaria if the air stone was removed (Coleman 2015). During this period, fry were not capable of swimming against the current generated by an air stone. These fish were sensitive to handling and changes in water quality. Therefore, as fish began to hatch each 38-l aquaria was treated with 1 ml of commercially available Seachem Prime daily to detoxify ammonia and nitrites. Feeding of juvenile fish began four days post-hatch. Juveniles were fed commercially available Larval AP100 (microparticle size <100μm).

Five days post hatch; the juvenile fish were transferred to 11-l flow-through systems (Figure 4, Figure 5). These flow-through systems allowed us to simulate natural river conditions and assist in achieving exogenous feeding. Approximately 15 days after the fry had been moved into the flow-through systems, powdered Rio Grande Silvery Minnow Flakes (Dexter National Fish Hatchery and Technology Center) were added to the diet at a ration of approximately 3% body weight per day.

Thirty days post hatch, juvenile fish were transferred into outdoor 4,542-l grow-out tanks (Figure 6). The grow-out tanks were not shaded allowing for natural ambient temperatures and natural photoperiod. Grow-out tanks contained de-chlorinated tap water from the City of Lubbock. Water quality in the grow-out tanks was maintained at temperatures between 20 and 32°C, dissolved oxygen 5 to 13 mg/l, conductivity 4,000 to 5,500 μS/cm, salinity 2 to 3 ppt, pH 8.2 to 9.5. Fish were fed a daily ration of 3% body weight with a combination of Rio Grande Silvery Minnow Flakes, freeze dried brine shrimp, and freeze dried bloodworms. Grow-out tanks were treated with air driven biological filters for water detoxification.

Figure 4. Two air driven flow-through systems, under construction, that were used to simulate flowing river conditions for juvenile Plains Minnow and Shoal Chub.

Figure 5. Close up of flow-through system with feeding Sharpnose Shiner fry.

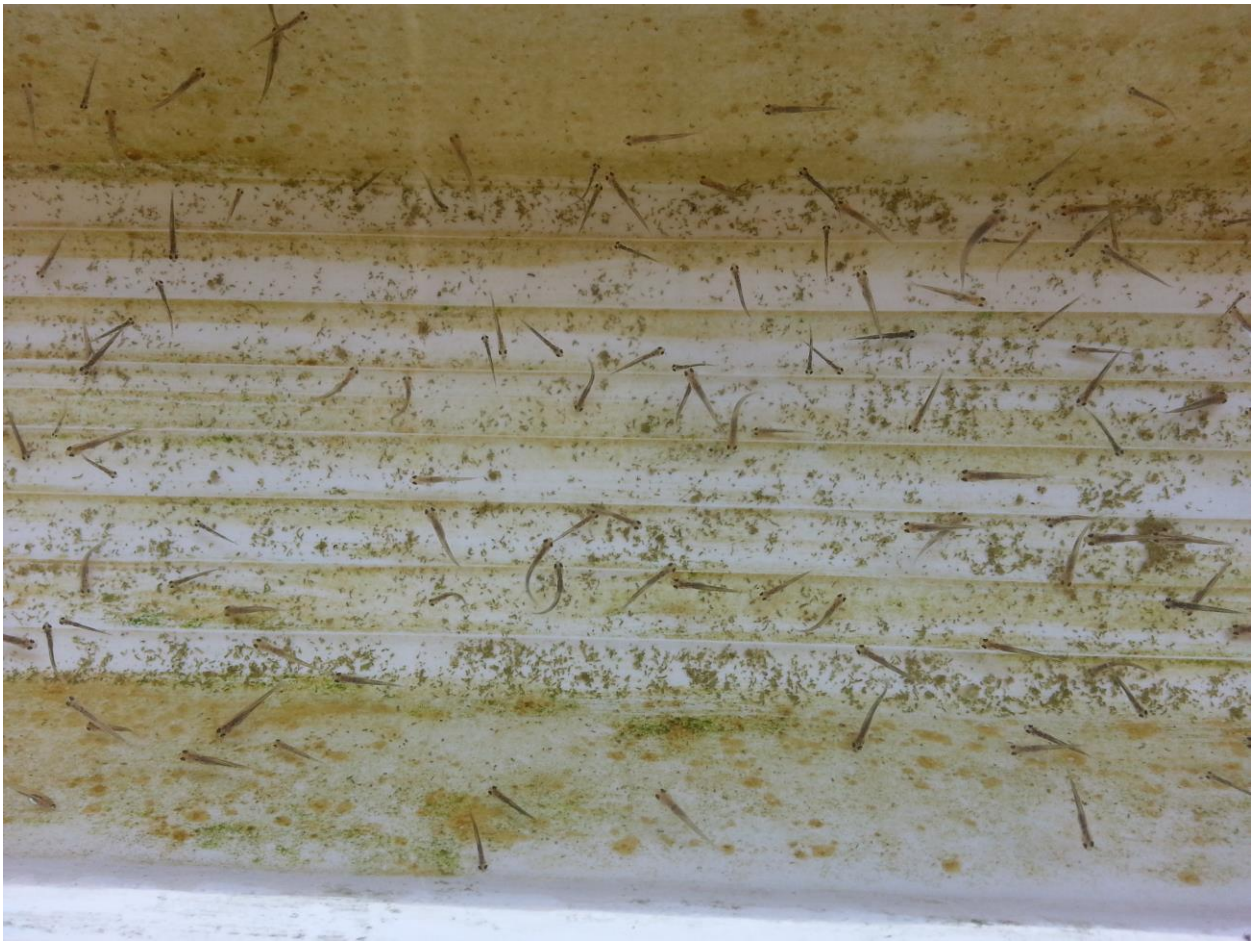
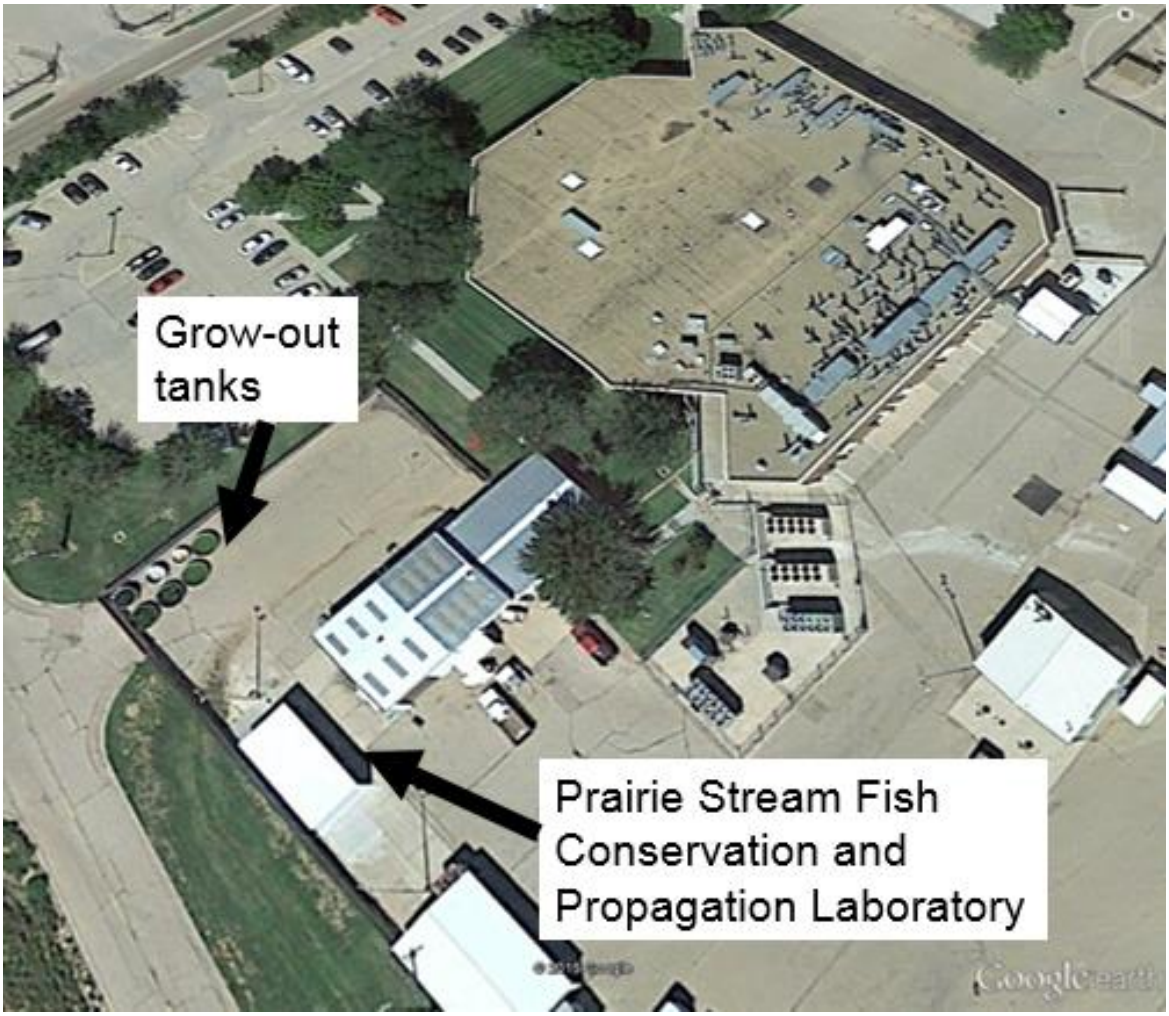


Figure 5. Aerial (Google Earth) view of the Prairie Stream Fish Conservation and Propagation Laboratory (Figure 1) showing the laboratory building and grow-out tanks.



Release of Fish

We released propagated Plains Minnow and Shoal Chub in October in 2014, 2015, and 2016. We chose October to maximize growth before release and to reduce handing and transport stress which would have been exacerbated by high summer temperatures. Prior to transport, fish were anaesthesized in an aerated buffered 100-ppm MS-222 bath to reduce handling stress while fish were loaded in an aerated stocking tank. During 2014 and 2015, fish were chosen for measurement of total length (TL, mm) at haphazard to provide a general estimate of the range of sizes released. In 2015, fish were marked with Visable Elastomer Tags (VIE) to allow us to differiate fish releaase in that year from possible progeny of the 2014 release. In 2016, released fish were exposed to oxytetracycline for 8 hr to allow us to differentiate these fish from other releases or their progeny.

After fish were loaded into the stocking tank, they were transported in the evening to Mineral Wells, where they were held overnight. In the morning, fish were transported to the stocking site (Hillbilly Haven RV Park, Millsap, Parker County, Texas; Table 1) and water temperature and salinity in the stocking tank were tempered to within 1^{o}C and 2 ppt salinity of Brazos River conditions. Once tempering was completed, the fish were released.

Recapture Sampling

We attempted to locate and capture released Plains Minnow and Shoal Chub three times in 2014-2015 and 2015-2016. Due to river conditions, recapture sampling was limited in 2015-2016. Sampling was conducted by seining at all river sites with public access (Table 1). Seining was conducted in all available habitats over a distance of 1+ km at each site. In fall 2017 (31 October - 1 November 2017) we attempted to relocate released fish or their progeny using

Table 1. Middle Brazos River sample sites listed from downstream to upstream. Plains Minnow and Shoal Chub were released at Hillbilly Haven RV Park, Millsap, Texas (second from the top).

Sampling Location	Distance from release site (km)	Latitude	Longitude
Brazos River, Dennis, Parker County, Texas	-7.94	32.6161	-97.9255
Brazos River, Hillbilly Haven RV Park, Millsap, Parker County, Texas	0	32.6664	-99.1049
Brazos River, at Hwy 281, SE of Brazos, Palo Pinto County, Texas	7.62	32.6413	-98.1005
Brazos River, Water Plant Rd, Brazos, Palo Pinto County, Texas	8.87	32.6666	-98.1137
Brazos River, Oaks Crossing Rd, SW of Mineral Wells, Palo Pinto County, Texas	25.50	32.7560	-98.1635
Brazos River, at Hwy 180, E of Mineral Wells, Palo Pinto County, Texas	30.69	32.7980	-98.1861
Brazos River, at Hwy 4 NW of Mineral Wells, Palo Pinto County, Texas	42.44	32.8631	-98.3022
Brazos River, at Hwy 16, NW of Mineral Wells, Palo Pinto County, Texas	54.31	32.8579	-98.4117

electrofishing. Due to river conditions the boat could only be launched at two sites. Pedal time at the two sites was 351 seconds at Hwy 4 and 2328 seconds at Highway 16 (see Table 1). All available habitats at these two sites were extensively sampled.

Results

We released a total of 20,080 Plains Minnow and 2840 Shoal Chub during 2014 through 2016 (Table 2). After release, we watched fish until they dispersed, usually upstream, from the release site. Released fish ranged from 17- to 41-mm TL for Plains Minnow and 10- to 45-mm TL for Shoal Chub. In general, individuals of both species were 25- to 30-mm TL.

Our attempts to recapture fish yielded modest results. We recaptured a total of seven fish (Table 3). Plains Minnow accounted for four of the recaptures. All of these individuals were recaptured within 1 km upstream of the release site and within 50 to 115 days of release. We recaptured two Shoal Chub. One individual was recaptured with 1 km of the release site and the other was recaptured 62 km upstream. These fish were recaptured, respectively, 151 and 389 days after release. Our attempt to recapture fish using electrofishing in fall 2017 yielded no Plains Minnow or Shoal Chub.

Discussion

Despite the small number of recaptures, our results show that Plains Minnow and Shoal Chub survived transport and release into the Middle Brazos River. Further, our results show that,

Table 2. Numbers of Plains Minnow and Shoal Chub released into the Middle Brazos River, 2014 to 2016.

	Plains Minnow		Shoal Chub	
Date of release	Number released	TL (mm) range	Number released	TL (mm) range
3 October 2014	5576	17 - 41	949	10 - 23
17 October 2015	9115	22 - 53	1391	14 - 45
3 October 2016	5389		500	

Table 3. Recaptures of Plains Minnow and Shoal Chub released into the Middle Brazos River.

Species	Length (TL, mm)	Recapture date	Days since release	Distance upstream (km)	Recapture location	Recapture latitude	Recapture longitude
Plains Minnow	48	11/22/2014	50	0.77	Hillbilly Haven	32.6735	-98.0344
Plains Minnow	45	11/22/2014	50	0.12	Hillbilly Haven	32.6678	-98.0330
Plains Minnow	39	1/25/2015	114	0.96	Hillbilly Haven	32.6747	-98.0358
Plains Minnow	42	1/26/2015	115	0.10	Hillbilly Haven	32.6676	-98.0331
Shoal Chub	40	3/20/2015	151	0.27	Hillbilly Haven	32.6692	-98.0329
Shoal Chub	71	10/27/2015	389	62.44	Hwy 4	32.7552	-98.1641

based on recaptures, fish survived for 2 to 13 months. Therefore, released Shoal Chub, at least, did survive into the following reproductive season. In addition to our results an ongoing study, conducted by Chris Taylor, University of Texas Rio Grande Valley, documented capture of a Shoal Chub (42-mm TL) at Dennis, Texas (see Table 1) in July 2017. This fish may have been a member of the cohort released in 2016, or it may derive from natural reproduction by previously released fish (the presence of an oxytetracline mark was not assessed).

Lengths of recaptured fish were consistently at, or beyond, the upper range of those released into the Brazos River. This indicates that beyond surviving, released fish were able to acclimate to local conditions and begin feeding and growing.

At this time, it is difficult to definitively evaluate the success of our reintroduction effort because of the small number of recaptures. The study area is approximately 200 km in length and we were only able to sample in the vicinity of a small number of access points. These access points are heavily utilized by vehicles, boats, and recreationists and habitat characteristics- shallow with gravel and cobble- may not be representative of more remote areas. Given the ability of Plains Minnow and Peppered Chub, related to Shoal Chub, to move an average of 0.34 km per day over the course of a year (Wilde 2016), we would expect Plains Minnow and Shoal Chub to become widely dispersed in the Middle Brazos River.

Including the Shoal Chub captured in 2017 (see above), the recapture rate of Shoal Chub was much greater than that of Plains Minnow. There are two possible explanations for this. First, as noted above, access sites generally are shallow with firm substrates, a habitat type preferred by Shoal Chub. Therefore, these results may represent a sampling bias because deeper, lower waters preferred by Plains Minnow were poorly sampled. Alternatively, habitat quality and length of the

study are may not be suitable for Plains Minnow. Platania and Altenbach (1998) noted that Speckled Chub was able to persist in smaller river fragments than were other broadcast spawning species. This observation is in agreement with model results presented by Perkin and Gido (2011). Therefore, our releases might be much more likely to establish Shoal Chub than Plains Minnow.

Wilde and Urbanczyk (2013) found that Sharpnose Shiner and Smalleye Shiner were absent from river fragments less than 187 km in length, so the study area is presumably of adequate length to support both species. Our releases and recaptures of Plains Minnow and Shoal Chub suggest Sharpnose Shiner and Smalleye Shiner could survive introduction into the middle Brazos River and would likely feed and grow. Given this, we would expect both species to reproduce (although we have no way to evaluate the potential success of that effort). We conclude that re-introduction of large numbers of both species into the Middle Brazos River could be successful in establishment of both species.

A growing number of Great Plains pelagic-spawning cyprinids (e.g., Cross et al. 1985; Luttrell et al. 1999, Wilde 2002, Gido et al. 2010; Perkin and Gido 2011) are undergoing declines in distribution and abundance and many are already listed as of conservation concern. This is reflected in a growing number of state and federal facilities dedicated to propagation of these fish. Ideally, males and females (with or without hormone injections) would be placed together and successfully spawn. Platania and Altenbach (1998) describe results of such matings. However, successful spawns were the exception, rather than the rule. Captive propagation of fishes, especially minnows, has proven to be difficult and is not always effective. This is in part due to an apparent dopamine inhibition during different parts of oocyte development as well as during the ovulation and spawning process (Zohar 1988, 1989a, b; Sokolowska-Mikolajczyk and Mikolajczyk 1991; Peter et al. 1993; Yaron 1995; Mananos et al. 2009). As a result, it usually

takes years to develop successful protocols for spawning. Herein, we have described our spawning methods in great detail so they can be adopted where necessary and until other, less labor intensive protocols can be developed. In addition to Plains Minnow and Shoal Chub, we have successfully used this method to spawn Arkansas River Shiner, Red River Shiner, Sharpnose Shiner, Smalleye Shiner, Peppered Chub, Prairie Chub *Macrhybopsis australis* among other species.

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