Relict Darter
(Etheostoma chienense)

5-Year Review:
Summary and Evaluation

USFWS Photo (Brent Harrel)

U.S. Fish and Wildlife Service
Southeast Region
Kentucky Ecological Services Field Office
Frankfort, Kentucky

5-YEAR REVIEW
Relict Darter (*Etheostoma chienense*)

1.0. GENERAL INFORMATION

1.1. Reviewers:

Lead Regional Office: Southeast Region: Kelly Bibb, 404-679-7132

Lead Field Office: Kentucky Ecological Services Field Office: Dr. Michael Floyd, 502-695-0468, x102

Peer Reviewers:

Dr. David Eisenhour, Morehead State University, Morehead, Kentucky
Dr. Sherry Harrel, Eastern Kentucky University, Richmond, Kentucky
Dr. Rebecca Johansen, Austin Peay State University, Clarksville, Tennessee
Dr. Melvin L. Warren, Jr., U.S. Forest Service, Oxford, Mississippi

1.2. Methods Used to Complete the Review:

We provided public notice of this 5-Year Review in the *Federal Register* on August 6, 2018 (83 FR 38320) and opened a 60-day comment period. During this comment period, we obtained information on the status of this species from several experts; additional data was obtained from the draft recovery plan, peer-reviewed scientific literature, and our state partners. Once all known literature and information was collected for this species, the review was completed by Michael Compton, Aquatic Zoologist with the Office of Kentucky Nature Preserves (KNP), and Dr. Michael A. Floyd, Fish and Wildlife Biologist with the Kentucky Ecological Services Field Office (KFO), U.S. Fish and Wildlife Service (Service). The draft document was peer-reviewed by Dr. David Eisenhour, Morehead State University, Morehead, Kentucky; Dr. Sherry Harrel, Eastern Kentucky University, Richmond, Kentucky; Dr. Rebecca Johansen, Austin Peay State University, Clarksville, Tennessee; and Dr. Melvin L. Warren, Jr., U.S. Forest Service, Oxford, Mississippi. Peer review comments were incorporated as appropriate (see Appendix A).

1.3. Background

1.3.1 Federal Register Notice citation announcing initiation of this review: 83 FR 38320, August 6, 2018

1.3.2 Species Status: Improving. Threats continue to impact the species, but based on continued conservation efforts and repeated observations of the species by the Service and its partners, the species' status has improved, and habitat threats have decreased in magnitude in some portions of the species' range. Recent surveys (2017-2018) indicate that estimates of Relict Darter abundance, mean density, and population size are greatest in Jackson Creek and middle reaches of Bayou de Chien. There is also ample evidence of reproduction and recruitment

in these streams, and these trends have remained relatively constant or have improved during surveys completed during the past nine years. The Service continues to work with its partners to implement stream and riparian habitat restoration projects in middle and headwater reaches of the Bayou de Chien system (especially Jackson Creek), and these projects have helped reduce the magnitude of habitat threats in these reaches. The species' limited range continues to make it vulnerable to stochastic disturbance, and its low genetic diversity may reduce its ability to adapt to environmental change.

1.3.3. Listing history:

Final Rule

FR notice: 58 FR 68480
Date listed: December 27, 1993
Entity listed: Species
Classification: Endangered

1.3.3 Review History:

Technical/Agency Draft Recovery Plan for the Relict Darter (*Etheostoma chienense*), July 31, 1994

Each year, the Service reviews and updates listed species information for inclusion in the required Recovery Report to Congress. Through 2013, we conducted a recovery data call that included status recommendations like "Stable" for this species. We continue to show that status recommendation as part of our 5-Year Review (note the Improving call under 1.3.2). The most recent evaluation for this fish for the Recovery Report to Congress was completed in 2018.

Previous 5-Year Reviews: 2013

1.3.5 Species' Recovery Priority Number at start of 5-Year Review (48 FR 43098):

5 – indicating that the Relict Darter is taxonomically categorized as a species, has a high degree of threat, and has a low recovery potential.

1.3.6 Recovery plan:

Name of plan: Technical/Agency Draft Recovery Plan for the Relict Darter (*Etheostoma chienense*). (The final plan is not yet complete; this is the title for our draft plan.)
Date issued: July 31, 1994

2.0 REVIEW ANALYSIS

2.1. Application of the 1996 Distinct Population Segment (DPS) policy

2.1.1. Is the species under review listed as a DPS? No

2.1.2. Is there relevant new information that would lead you to consider listing this species as a DPS in accordance with the 1996 policy? No

2.2. Recovery Criteria

2.2.1 Does the species have a final, approved recovery plan containing objective, measurable criteria? No. The Service announced a technical/agency draft recovery plan in 1994, but we have gained substantial new scientific information since that time and intend to release a new technical/agency draft recovery plan for public review and comment in 2019.

2.3. Updated Information and Current Species Status

2.3.1. Biology and Habitat

2.3.1.1. Abundance, population trends (e.g. increasing, decreasing, stable), demographic features, or demographic trends:

Abundance / Population Trends

The Relict Darter is endemic to the Bayou de Chien system in western Kentucky (Figure 1) (Burr and Warren 1986, Piller and Burr 1998, Service unpublished data – see Appendix B). The first comprehensive fish surveys in the Bayou de Chien system were completed by Webb and Sisk (1975) and Warren et al. (1994), who determined that that the Relict Darter was restricted to about 28 stream kilometers (km) (17.4 miles (mi.)) of the Bayou de Chien system in Graves and Hickman counties, Kentucky. Evidence of spawning was observed within portions of the Bayou de Chien mainstem and Jackson Creek, a small northeastern tributary of Bayou de Chien near Water Valley, Graves County, Kentucky (Warren et al. 1994). Warren *et al.* (1994) estimated the extent (in meters (m)) of suitable habitat at the two sites where Relict Darters were most abundant, Jackson Creek at Lawrence Road and Bayou de Chien at KY 1283. Their calculations determined that approximately 160 m (525 feet (ft)) of suitable habitat were present at these sites, with a mean of 1.25 individuals per meter (3.28 ft) of suitable habitat and an estimated population size of 200 individuals. Warren *et al.* (1994) also estimated the presence of about 35 m (115 ft) of suitable habitat at the remaining sites where the species was observed.

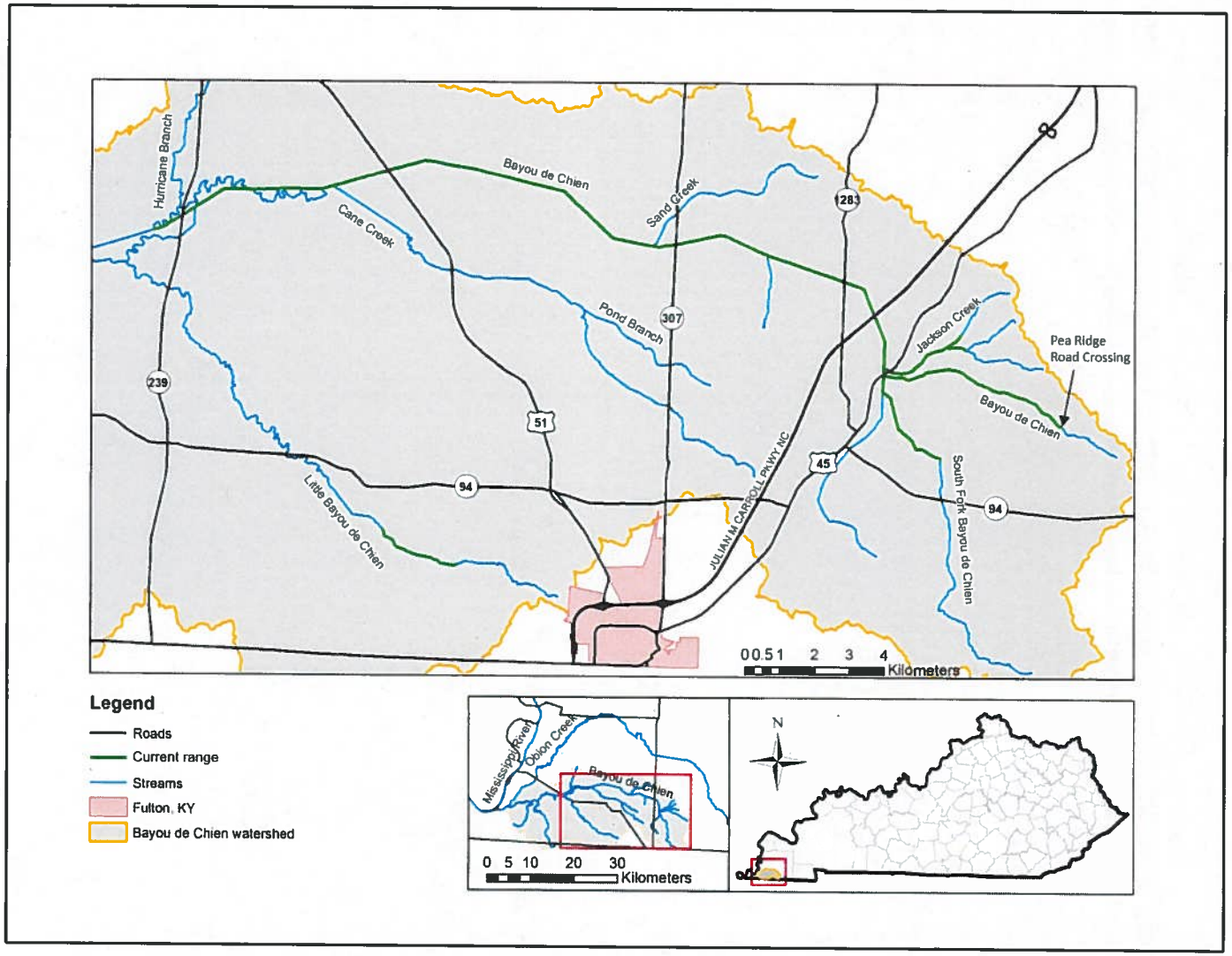

Figure 1. Current distribution of the Relict Darter in the Bayou de Chien system, Fulton, Graves, and Hickman counties, Kentucky. The extent of occupied habitat is based on surveys completed by the Service, KDFWR, and KNP during 2010-2012 and 2017-2018 (see Appendix B; map prepared by the Office of Kentucky Nature Preserves).

From 1995-1996, Piller and Burr (1998) surveyed all known historical sites and several new sites within the Bayou de Chien system. Relict Darters were observed at 16 of 28 sites and inhabited approximately 47.1 linear stream km (29.3 mi) based on spring collections (March – May). Their population estimate was between 9,553 and 31,293 individuals, with the greatest abundances observed in areas having gently flowing water, good undercut bank habitat, low silt load, and suitable quantities of spawning substrates and instream cover. Piller and Burr (1998) asserted that the Relict Darter was maintaining an effective population size sufficient for continued persistence.

During 2006, KDFWR sampled two sites where Relict Darters had been reported previously (Thomas 2008, pers. comm.). On April 27, 2006, 35 individuals (28 females, several in gravid condition, and 7 males, all in nuptial condition) were captured at the KY 1283 bridge crossing of Bayou de Chien in Graves County. Previous sampling efforts at this site by Piller and Burr (1998) estimated a population size of 60 individuals. On May 25, 2006, KDFWR and the Kentucky Division of Water (KDOW) observed 28 individuals at the US Highway 51 (US 51) bridge crossing of Bayou de Chien in Hickman County. Previous collections at this locality by Piller and Burr (1998) and others yielded lower numbers (3-5 individuals). Piller and Burr (1998) estimated a population size of 46 individuals at this site.

During October 2010, KNP, KFO, The Nature Conservancy, and the Kentucky Transportation Cabinet conducted qualitative surveys (seine hauls) at 13 of 16 historical sites in the Bayou de Chien system. Three historical sites, Cane Creek (upstream site), Sand Creek, and an unnamed tributary of Bayou de Chien (at Rose Road) were not surveyed because of minimal flow, resulting in dry or pooled channels. Relict Darters were captured at all of the historical sites surveyed, and Relict Darter abundance was similar to that reported by Piller and Burr (1998). In addition to these historical sites, six Relict Darters were observed at a new site on Jackson Creek, approximately 100 meters upstream of its confluence with Bayou de Chien and 1 km (0.60 mi) downstream of Lawrence Road. The discovery of a new Jackson Creek site was not surprising, given its proximity to the Bayou de Chien confluence, the abundance of Relict Darters observed in other reaches of Jackson Creek, and the large number of individuals in reproductive condition regularly observed in upstream portions of Jackson Creek.

In 2011, KNP, KDFWR, and KFO initiated a long-term, quantitative monitoring effort for the Relict Darter (methods and results summarized in Appendix B). The goal was to establish a consistent and repeatable monitoring strategy that would document Relict Darter occurrence and density over time and estimate the species' population size within the Bayou de Chien system. Surveys were completed in 2011-2012 and 2017-2018 at reaches on Bayou de Chien and Jackson Creek (Appendix B, Figure B-1). Two reaches on Little Bayou de Chien were added to the study in 2017 based on KDOW's discovery of the species at the State Route (SR) 1125 bridge crossing in Fulton County. Reach locations were selected randomly on each stream based on the species' known

range, its habitat affinities, and the extent of available habitat (the number of reaches was proportional to the total stream length in each stream).

Total Relict Darter abundance in Jackson Creek was essentially the same during 2011 and 2017, while total darter abundance in Bayou de Chien was 2.3 times greater in 2018 than in 2012 (Table 1). Relict Darter abundance at two reaches of Little Bayou de Chien was low compared to Jackson Creek, a stream of similar size. For Jackson Creek, overall mean density (number of darters/m^2) in 2011 and 2017 was 0.23 and 0.26, respectively. In Bayou de Chien, overall mean density in 2012 and 2018 was 0.04 and 0.10, respectively. Values in 2018 were significantly higher than those observed in 2012 (T value, -3.29; $p < 0.001$). The 2018 increase in abundance and density in Bayou de Chien can be attributed to increased recruitment (Appendix B, Figure B-5). The 0+ age class (< 50 mm TL) increased from 35.8% of the population in 2012 to 67.3% in 2018. Overall mean density at two Little Bayou de Chien reaches (0.06) was comparable to mean density values observed in Bayou de Chien.

The estimated mean population size for Jackson Creek was 1,791 and 1,888 in 2011 and 2017, respectively (95% confidence intervals of 277 – 3,304 and 682 – 3,093 individuals, respectively) (Table 1; Appendix B, Figure B-6). The estimated mean population size for Bayou de Chien was 14,581 and 22,798 in 2012 and 2018, respectively (95% confidence intervals of 4,919 – 24,243 and 4,172 – 41,424 individuals, respectively). Range-wide mean population estimates for 2011 – 2012 (16,372 individuals) and 2017 – 2018 (24,686) were relatively similar to earlier estimates for the entire system (21,740; Piller and Burr 1998). We did not estimate population size for Little Bayou de Chien due to a limited survey effort (i.e., small sample size) that included only two 100-m (328-ft) survey reaches within the 2.3-km (1.4-mi) study reach.

Life History

The only information on reproductive habits of the species was provided by Piller and Burr (1999) during their investigation in 1995 and 1996. Spawning occurred from mid-March to early June at water temperatures ranging from 11 to 22 °C (52 to 72 °F). A total of 166 nests were observed on 16 different substrate types. Most nests were located on natural materials such as small rocks, woody debris, and live tree roots, but 37 percent of nests were found on anthropogenic materials such as rubber tires, plastic, roof shingles, glass, concrete blocks, metal road signs, and concrete slabs. Nests were found at a mean depth of 16.9 cm (6.6 inches (in)) (range: 4.5 to 38 cm (1.8 to 15 in)), and the cavity between the stream bottom and the spawning substrate averaged 2.9 cm (1.1 in) (range: 2 – 5 cm (0.79 to 1.9 in)). Nests with clutches of eggs attached to naturally occurring materials contained a range of 12 to 789 eggs (mean = 255) in 1995 and from 12 to 1,275 eggs (mean = 343) in 1996.

Table 1. Summary of quantitative survey results for the Relict Darter in the Bayou de Chien system, 2011-2012 and 2017–2018 (see Appendix B).

	Jackson Creek		Bayou de Chien		Little Bayou de Chien
	2011	2017	2012	2018	2017
# Survey Reaches	4	4	12	12	2
# Reaches w/ Darters	4	4	8	10	2
# Darters Observed	91	89	89	200	9
Mean Density (# Darters/m^2)	0.23	0.26	0.04	0.10	0.06
Mean Population Estimate*	1,791 (278 - 3,304)	1,888 (683 - 3,093)	14,581 (4,919 - 24,243)	22,798 (4,172 - 41,424)	N/A

* 95% confidence intervals shown in parentheses; we did not calculate a population estimate for Little Bayou de Chien because of our limited survey effort – only 2 100-m (328-ft) reaches were surveyed within a 2.3-km (1.4-mi) study reach (from SR 1125 bridge crossing upstream to a point approximately 300 m (984 ft) upstream of SR 1706).

Due to an apparent lack of suitable spawning substrates in the Bayou de Chien system, Piller and Burr (1999) seeded several reaches with ceramic tiles to increase Relict Darter reproductive success. During the two-year study (1995 – 1996) at sites on Bayou de Chien and Jackson Creek, 25 to 88 percent of tiles were used for spawning at least once during the study, and several tiles were used multiple times. The number of eggs deposited on introduced tiles was significantly greater than the number deposited on natural substrates. Piller and Burr (1999) also performed two laboratory experiments to attempt to determine nest preferences of the species. Female Relict Darters were provided spawning substrates of different sizes. Six of eight laboratory spawnings occurred on the larger substrates, but these differences were not statistically different. In the second experiment, females were added to aquaria with a large male (standard length (SL, from the tip of the snout to the base of the tail fin) of 68 to 72 mm (2.7 to 2.8 in)) and a small male (60 to 64 mm (2.4 to 2.5 in) SL). Seven of eight spawning events occurred with the larger male - a statistically significant result. Several spawnings were videotaped in the laboratory (Piller and Burr 1999), and spawning occurred between 20 and 21°C (68 to 70°F). Males and females were positioned in a head-to-head, inverted position for 1.5 to 3 seconds, during which time eggs were released and fertilized.

In 2016, Fangman and Clausen (2016) initiated a second study of artificial substrate use by Relict Darters in the Bayou de Chien system. Artificial

structures (half-cylindrical ceramic tiles and concrete culvert pieces) were installed at seven sites and monitored every two weeks during the spawning season (March 18-June 24). Use of artificial structures was observed at six of seven sites, with the most observed use at the Bard Road crossing of Bayou de Chien (Site 4). The most widespread use of spawning structures was observed on April 18-19, when 27 percent of all available structures exhibited newly deposited, viable eggs. Male Relict Darter total lengths ranged from 68-93 mm (2.7-3.7 in), with an average length of 81 mm (3.2 in). The vertical cavity height of utilized structures ranged from 1-7 cm (0.4-2.8 in), with an average of 3.4 cm (1.3 in). Utilized structures were located in water depths ranging from 7.6-45.7 cm (3.0-18.0 in), with an average of 19.6 cm (7.7 in).

Non-breeding Relict Darters limit their activities to undercut banks or other near bank areas (Piller and Burr 1999). During spawning, some adult males leave undercut banks in spring to guard territories beneath instream objects, but seining indicated that many nuptial males and gravid females remained beneath undercut banks, where females may attach eggs to the ceilings of these habitats. The overall body color of breeding males was darkened, and the dorsal fins were knobbed. When captured, breeding females would release eggs with only slight pressure on their abdomens.

Length frequency estimates by Piller and Burr (1998) appeared to indicate three to four age classes and an estimated life expectancy of 3+ years. Males were approximately 40 mm (1.6 in) SL by age 1, 52 to 62 mm (2.0 to 2.4 in) SL at age 2, and 63 to 76 mm (2.5 to 3.0 in) SL at age 3. Females were slightly smaller at each class. Age 1 females were almost 35 mm (1.4 in) SL, 47 to 54 mm (1.8 to 2.1 in) SL at age 2, and 55 to 68 mm (2.2 to 2.7 in) SL at age 3. Piller and Burr (1998) observed no larval Relict Darters during their study, and only a few juveniles were captured in late spring and early summer.

Relict Darter length measurements (total length, mm) from 2011 – 2018 (Appendix B, Figure B-5) appeared to indicate three to four age classes and a life span of 3+ years, similar to that reported by Piller and Burr (1998). These measurements also indicated that recruitment was strong in 2018, with approximately two-thirds of all captured individuals belonging to the 0+ age class. Nearly all of the 0+ age class members were observed in Jackson Creek and in Bayou de Chien upstream of the US 51 bridge crossing, suggesting that the most successful spawning occurs within these reaches.

2.3.1.2. Genetics, genetic variation, or trends in genetic variation:

Kattawar (2019) utilized a comparative approach to investigate the genetic structure, gene flow, genetic variation, and effective population sizes of the Relict Darter and a closely related species, the Guardian Darter (*E. cf. oophylax*). Tissue samples from the Bayou de Chien (Relict Darter) and Clarks River (Guardian Darter) systems were collected in 2017 and analyzed using mitochondrial DNA sequence data and Single Nucleotide Polymorphisms (SNP). The study determined that the Relict Darter has low levels of genetic variation throughout its range and a low effective population size (mean of

138.9, with a 95% confidence interval of 73.4 – 530.3). Kattawar (2019) suggested a population bottleneck and subsequent genetic drift as a possible scenario to explain the low level of observed genetic variation. Genetic clustering analyses for the Relict Darter indicated a single panmictic population, where random mating occurs among all individuals (i.e., individuals can interbreed without restrictions). Based on the lack of genetic structure, Kattawar (2019) concluded that the species could be managed as a single conservation unit.

2.3.1.3. Taxonomic classification or changes in nomenclature:

The Relict Darter is 1 of 10 recognized species in the *Etheostoma squamiceps* complex (subgenus *Catonotus*, family Percidae) (Page *et al.* 1992). It was first discovered in Bayou de Chien by Webb and Sisk (1975, reported as *Etheostoma squamiceps*), but it was not recognized as a distinct species and described until 1992 (Page *et al.* 1992). The species can be differentiated from the other members of the *E. squamiceps* complex with certainty only by the color and morphology of the dorsal fins of nuptial males (Page *et al.* 1992). The Relict Darter is unique in that the second dorsal fin of each breeding male has two equal branches per ray that are tipped with small white knobs. The Relict Darter's taxonomy and common name have been accepted by the scientific community, as evidenced by the species' inclusion in Page *et al.* (2013, p. 136), which is a list of common and scientific names of fishes from the United States, Canada, and Mexico published by the American Fisheries Society (7th edition).

2.3.1.4. Spatial distribution, trends in spatial distribution, or historical range:

The Relict Darter is endemic to the Bayou de Chien system in Fulton, Graves, and Hickman counties, Kentucky (Figure 1) (Burr and Warren 1986, Piller and Burr 1998). At the time of listing in 1993, the species was known only from the Bayou de Chien mainstem and Jackson Creek. The species was considered to be most abundant in Jackson Creek and a limited reach of the headwaters of Bayou de Chien near the town of Water Valley in Graves County (Webb and Sisk 1975, Warren and Burr 1991, Warren *et al.* 1994). Piller and Burr (1998) documented the species' presence at 16 of 28 sites surveyed, including 6 new sites in Graves and Hickman counties. The species was most commonly collected in the middle and headwater reaches of the system, where it was described as "abundant" in Jackson Creek and "common" at four Bayou de Chien sites. Relict Darters or nests were also observed at sites on South Fork Bayou de Chien, Cane Creek, Sand Creek, and two unnamed tributaries; however, the species' summer and fall distribution was limited to the Bayou de Chien mainstem, Jackson Creek, and South Fork Bayou de Chien. Tributaries such as Cane Creek and Sand Creek were dry or consisted of isolated pools by late summer or fall. Piller and Burr (1998) concluded that these intermittent reaches likely contributed little to recruitment. Based on these intermittent and/or ephemeral conditions and a 20-year lack of collection records, we do not consider Cane Creek or Sand Creek to be part of the species' current range.

In July 2017, a second population of the species was discovered in Little Bayou de Chien in Fulton County (Figure 1). KDOW personnel collected two Relict Darters during routine biological investigations just upstream of the SR 1125 crossing (Culp 2017, pers. comm.). The species has not been observed in downstream reaches of Little Bayou de Chien, which are more lowland in character, with deep pools and low current velocities.

Based on surveys completed since 2010, the Relict Darter continues to be most abundant in Jackson Creek and in middle and headwater reaches of Bayou de Chien (from the US 51 bridge crossing in Hickman County upstream to the Pea Ridge Road bridge crossing in Graves County) (Appendix B, Figure B-1) (Remley and Olsen 2012, Fangman and Clausen 2013, Fangman and Clausen 2016, Bishop and Clausen 2018, Service unpublished data – see Appendix B). The species is less common and occurs in lower densities in Little Bayou de Chien, South Fork Bayou de Chien, and portions of Bayou de Chien downstream of the US 51 bridge crossing. Only one individual has been observed in Bayou de Chien downstream of the State Route (SR) 239 bridge crossing since quantitative surveys began in 2011. The species currently occupies a 30.4-km (18.9-mi) reach of the Bayou de Chien mainstem, a 3.6-km (2.3-mi) reach of Jackson Creek and its tributaries, a 3.2-km (2.0-mi) reach of South Fork Bayou de Chien, and a 2.6-km (1.6-mi) reach of Little Bayou de Chien (between SR 1125 and SR 1706) (Figure 1). The greatest mean densities have been observed in Jackson Creek (the type locality) and in middle and headwater reaches of Bayou de Chien (from US 51 bridge crossing upstream to Pea Ridge Road bridge crossing).

The recent discovery of the species in Little Bayou de Chien is encouraging for the species' continued persistence and conservation; however, the length of the occupied reach is short (only 2.6 km (1.6 mi)), and Relict Darter abundance and mean density were low relative to Jackson Creek. Sampling efforts to encounter the species further downstream in Little Bayou de Chien have been unsuccessful to date. It is unlikely a viable population occurs in the lower reaches of Little Bayou de Chien because habitats in these reaches are unsuitable for the species (e.g., deep pools, low current velocities, and soft sediments). It is anticipated that only transient individuals would occur in this portion of Little Bayou de Chien; however, we have identified surveys of downstream reaches of Little Bayou de Chien as a future recovery task (see Section 4.0 of this document) because we want to investigate the species' occupancy in these reaches.

The endemism of the Relict Darter in Bayou de Chien is unique (Warren *et al.* 1994) as no other fish species shares a similarly restricted distribution anywhere on the northern Gulf Coastal Plain of Arkansas, Kentucky, Missouri, or Tennessee (Pflieger 1975, Burr and Warren 1986, Robison and Buchanan 1988, Etnier and Starnes 1993). Other species restricted to the northern Gulf Coastal Plain, such as the Least Madtom (*Noturus hildebrandi*) and Firebelly Darter (*Etheostoma pyrrhogaster*) are not known from Bayou de Chien and are distributed in at least two other Mississippi River tributaries (Warren *et al.* 1994).

Warren *et al.* (1994) speculated that the Relict Darter was more widespread in the Bayou de Chien system prior to human settlement, but it was likely restricted to reaches of the system lying upstream of the Mississippi River floodplain (generally east of the KY 239 bridge crossings of Bayou de Chien and Little Bayou de Chien). Based on historical and current collection records, Warren et al. (1994) reported that there was no documented evidence that the Relict Darter ever occurred outside the Bayou de Chien system (Warren *et al.* 1994). In fact, they asserted that it was extremely unlikely that additional populations would be found outside the Bayou de Chien system for several reasons: (1) the habitat affinities of the species, (2) the complete allopatry (separation) between the Relict Darter and its closest relatives, (3) the absence of any other species in the *E. squamiceps* complex in Mississippi River tributaries in Kentucky and Tennessee, and (4) the availability of summaries of species composition in these drainages that did not record the Relict Darter (Etnier and Starnes 1993; Burr and Warren 1986).

2.3.1.5. Habitat:

The Relict Darter occupies the same general habitat as most members of the *E. squamiceps* complex (Service 1994). Adults are concentrated in headwaters of streams in slow flowing pools (0.2 – 0.6 m/sec (0.7 – 2.0 ft/sec)), usually over gravel mixed with sand and under or near cover such as fallen tree branches, undercut banks, or overhanging riparian vegetation (Warren *et al.* 1994; Piller and Burr 1998). At sites along the Bayou de Chien mainstem, the species has shown an affinity for undercut banks and adjacent narrow side channels (2 to 3 m (6.6 to 9.8 ft) wide) underlain by gravel mixed with sand (Warren *et al.* 1994). Individuals were occasionally collected by Piller and Burr (1998) in mid-stream areas with slow flowing water, but rarely was the species collected in riffle habitats. Piller and Burr (1998) observed the most Relict Darters in areas having gently flowing water, good undercut bank habitat, low silt load, and a suitable quantity of spawning substrata or instream cover. Adults occurred almost exclusively in reaches with appropriate cover and spawning substrates and were absent or in low abundance at sites that lacked these features.

Historically, Bayou de Chien was presumably a free-flowing stream with alternating areas of riffles, runs, and pools; however, only a few of these reaches remain because much of the stream has been channelized and converted to a deep ditch with uniform depth, velocity, and substrate (Piller and Burr 1998). Based on previous surveys by Warren *et al.* (1994) and Piller and Burr (1998) and recent surveys by the Service and its partners (2010 – 2018), Jackson Creek is the least modified stream in the system and appears to support the greatest darter densities. An abundance of woody debris in Jackson Creek provides a sufficient supply of spawning substrates, and consequently, surveys in Jackson Creek since 2011 have produced the greatest densities of the species. Piller and Burr (1998) considered the species to be "abundant" (commonly collected in large numbers, one of the dominant species) in Jackson Creek. Piller and Burr (1998) considered the species to be "common" (collected regularly and usually found in moderate to large numbers) at four Bayou de Chien sites that had been

only moderately modified in the past. These areas still had adequate quantities of spawning materials and instream cover, and the species was doing well at these sites. At the 11 remaining sites where the Relict Darter was detected by Piller and Burr (1998), the species was reported as "uncommon" (captured semi-regularly but usually only in small numbers) or "rare" (species captured or vouchered only once or very infrequently). These stream reaches had been radically modified and lacked suitable riparian zones and instream cover. Consequently, these sites supported low numbers of Relict Darters.

2.3.1.6. Other:

Within middle and headwater reaches of the Bayou de Chien system, the Relict Darter is most commonly associated with Creek Chub (*Semotilus atromaculatus*) and Blackspotted Topminnow (*Fundulus olivaceus*) (Piller and Burr 1996, Warren *et al.* 1994). Creek Chubs have been reported as the most common fish species in Jackson Creek (Piller and Burr 1996) and could represent a potential predator of the Relict Darter. Creek Chubs greater than 81 mm (3.2 in) SL are predominately piscivores (Barber and Minckley 1971, Newsome and Gee 1978, Keast 1985), with as much as 70 percent of the diet consisting of fishes (Piller and Burr 1996). Creek Chubs of this size were observed by Piller and Burr (1996) and have also been observed during recent surveys (2017 – 2018). Piller and Burr (1996) provided direct evidence of predation by Largemouth Bass (*Micropterus salmoides*) and observed the caudal region of a female Relict Darter protruding from a bass' mouth. They determined that the ingested female was partially digested and gravid. Additional frequent associates reported by Warren *et al.* (1994) and observed in recent surveys in the Bayou de Chien mainstem include the Saddleback Darter (*Percina vigil*), Suckermouth Minnow (*Phenacobius mirabilis*), and Freckled Madtom (*Noturus nocturnus*).

The Relict Darter's food habits are unknown, but it is assumed that their diet is similar to other members of the *E. squamiceps* complex (Service 1994). The diet of related darters consists mainly of aquatic insects and small crustaceans (Page 1980). Juveniles feed on copepods, cladocerans, ostracods, and chironomids, and adults feed mainly on amphipods, isopods, chironomids, and caddisflies.

To guard against a potential catastrophic extirpation event in the Bayou de Chien system, a captive breeding population was established (via Service funding) in the late 1990s by Conservation Fisheries, Inc. (CFI), a non-profit fish propagation facility in Knoxville, Tennessee (Rakes and Shute 2003). Relict Darters (19 individuals) were first collected in September 1999 at two sites on Bayou de Chien, Graves County, Kentucky and transported to CFI's facility. The species proved to be relatively easy to propagate and maintain in captivity. With the exception of an apparent sensitivity to declines in pH, the species was hardy in aquaria, easily spawned, and the eggs and young amenable to already well developed incubation and rearing protocols. The species also appeared to live far longer in captivity than in the wild (>4 years). Excess adults (a total of 190 individuals) were transferred to Wolf Creek National Fish

Hatchery (Wolf Creek NFH), Russell County, Kentucky in February 2001 and April 2002. The project was concluded at CFI in April 2008, and all remaining darters (74 individuals) were released by KDFWR at three sites in the Bayou de Chien system (Thomas 2018, pers. comm.). The decision to release captive individuals was made collectively by Wolf Creek NFH, KDFWR, and KFO.

2.3.2 Five-Factor Analysis

In the following discussion, we present a summary of threats affecting the Relict Darter and its habitat. Our summary is organized according to the Service's five listing factors or threat categories: (A) the present or threatened destruction, modification, or curtailment of its habitat or range; (B) overutilization for commercial, recreational, scientific, or educational purposes; (C) disease or predation; (D) the inadequacy of existing regulatory mechanisms; and (E) other natural or manmade factors affecting its continued existence. As shared in our 2013 5-Year Review and based on information gathered since that review, the most significant threat to the species continues to be Factor A (destruction, modification, curtailment of habitat or range) caused by a variety of human-induced impacts such as stream channelization, disturbance/loss of riparian corridors, siltation, and water pollution (Waters 1995, KDOW 2018). The species is also threatened by the inadequacy of existing regulatory mechanisms in protecting against habitat alteration or destruction (Factor D) and its restricted range and small population size (Factor E).

2.3.2.1. Present or threatened destruction, modification or curtailment of its habitat or range:

The primary threats to the Relict Darter and its habitat are (1) channelization of the Bayou de Chien mainstem and its tributaries; (2) the removal and lack of shade-producing and habitat-producing (e.g., instream small wood) riparian vegetation; (3) increased sediment loads associated with poor land-use practices; (4) deforestation and drainage of riparian wetlands; and (5) pollutants originating from municipal wastewater plants or agricultural livestock operations (Warren et al. 1994; Piller and Burr 1998; Jackson Purchase RCD Foundation 2009; KDOW 2018). These habitat changes have limited the distribution of Relict Darters within portions of the Bayou de Chien mainstem and its tributaries. In general, higher numbers of Relict Darters are observed in areas with the least amount of disturbance (e.g., Jackson Creek), while more altered or disturbed portions of the system tend to have fewer darters or the species is absent entirely.

Channelization / riparian vegetation removal

Historically, Bayou de Chien was presumably a free-flowing stream with alternating areas of riffles, runs, and pools. Since that time, many stream channels within the system have been channelized and converted to deep ditches with uniform depth, velocity, and substrate. Stream channelization is a common land practice used to control flooding, increase the drainage rate of agricultural land, and maximize the amount of tillable land (Piller and Burr 1998). Channelization has impacted the Bayou de Chien system by changing stream

flow patterns, reducing instream base flows (especially during drier periods), decreasing aquatic habitat complexity, and reducing stream bank and floodplain (riparian) vegetation (Piller and Burr 1998). Channelized reaches have higher stream velocities during high flow periods (which leads to channel instability and bank erosion), less instream cover and habitat for aquatic organisms (decreased habitat complexity), less riparian vegetation and correspondingly reduced canopies (reduced shade), and below normal flows during drier periods (Warren *et al.* 1994; Piller and Burr 1998). The Relict Darter is susceptible to reductions in riparian vegetation because these losses reduce the amount of woody material that is available for shelter and reproduction.

In 2017, KNP and KFO staff observed and briefly discussed channelization and impacts to stream health with a resident who was actively modifying an unnamed tributary of Bayou de Chien, near the SR 1283 bridge crossing (Compton 2017, pers. comm.). Approximately 300 meters of the tributary was in the process of being channelized, and vegetation within and along the riparian zone was being removed. This incident and numerous other undocumented incidences over the past century have extensively altered habitats within the Bayou de Chien system. These alterations can reduce Relict Darter numbers and the amount of suitable habitat for feeding and reproduction. Excessive sediment from the 2017 tributary channelization was obvious for approximately 0.33 km (0.2 mi) downstream and turbid conditions were observed at least 14.4 km (9 mi) downstream within the Bayou de Chien mainstem. A variable layer of sediment coated all substrates immediately downstream of SR 1283, with occasional excessive mounds of sediment present in depositional zones.

Siltation

The Bayou de Chien system is extensively farmed (e.g., row crops and livestock), and most of the system has been deforested. These land use practices result in a fairly high silt load within the Bayou de Chien system that continues to degrade habitat and impact the species. Sediment (siltation) has been listed repeatedly by KDOW as one of the most common stressors of aquatic communities in the Bayou de Chien system (KDOW 2018). The primary sources of sediment were identified as agriculture (crop production) and habitat impacts (channel erosion/incision from upstream hydromodifications, dredging, and loss of riparian habitat). Several streams within the Bayou de Chien system have been identified as impaired due to siltation and have been included on Kentucky's 303(d) list of impaired waters (KDOW 2018). These streams include Cane Creek (stream km 0 to 8.5 (stream mi 0 to 5.3) in Hickman County; Little Bayou de Chien (stream km 1.8 to 3.8 and 18.8 to 22.5 (stream mi 1.1 to 2.4 and 11.7 to 14.0)) in Fulton and Hickman counties; and South Fork Bayou de Chien (stream km 0 to 12.6 (stream mi 0 to 7.8)) in Graves County. Sediment abrades and/or suffocates bottom dwelling algae and other organisms, reduces aquatic insect diversity and abundance (the Relict Darter's prey), and ultimately, negatively impacts fish growth, survival, and reproduction (Waters 1995). Wood and Armitage (1997) identified at least five impacts of sedimentation on fishes, including (1) reduction of growth rate, disease tolerance, and gill function; (2) reduction of spawning habitat and egg, larvae, and juvenile development; (3) modification of migration patterns; (4) reduction

of food availability through the blockage of primary production; and (5) reduction of foraging efficiency. All of these potential impacts would apply to the Relict Darter.

Drainage of riparian wetlands

With increased agricultural activity in the Bayou de Chien system over the last century, much of the system has been cleared, and many riparian wetlands have been drained to make additional lands available for farming. This has caused an overall reduction in the groundwater level and base flows within the Bayou de Chien mainstem and its tributaries. Warren *et al.* (1994) observed that many small streams in the system were completely dry or consisted of isolated pools by the early fall months. These conditions serve to isolate populations and subject both the adults and juveniles to increased pressure from predators (Service 1994). Warren et al. (1991) asserted that dispersal of the species upstream of the Jackson Creek area or into many downstream tributaries may be limited by instream flow conditions.

Other Pollutants

The Kentucky Natural Resources and Environmental Protection Cabinet (Division of Water) has identified several point-source and nonpoint-source pollutants to aquatic life in Bayou de Chien (KDOW 2018). These stressors included copper, iron, lead, excess nutrients, and eutrophication originating from two suspected sources – municipal point source discharges and agriculture. Three streams, Bayou de Chien (stream km 14.2 to 23.0 (stream mi 8.8 to 14.3)), Cane Creek (stream km 0 to 8.5 (stream mi 0 to 5.3)), and South Fork Bayou de Chien (stream km 0 to 3.2 (stream mi 0 to 2.0)), were identified as impaired due to these stressors (KDOW 2018). The impacts of copper, lead, and iron inputs are unknown, but nutrient inputs and eutrophication can lead to excessive algal growths and instream oxygen deficiencies that can seriously impact aquatic species, including the Relict Darter.

Currently, 13 National Pollutant Discharge Elimination System (NPDES) permitted discharges impact portions of the Bayou de Chien system. Two sewage treatment plants, City of Fulton Treatment Works (Kentucky Pollutant Discharge Elimination System (KPDES) #KY0026913) and Hickman East Sewage Treatment Plant (KPDES #KY0028436), discharge treated wastewater directly into the Bayou de Chien mainstem. The Hickman East Sewage Treatment Plant discharges approximately 12.8 km (8 mi) downstream of areas known to support Relict Darters and has little expected impact on the species. The City of Fulton Treatment Works discharges into the Bayou de Chien mainstem near the Illinois Central Gulf Railroad crossing at stream km 24.6 (stream mi 15.3). While conducting fish surveys near this location in September 2018, KFO and KDFWR staff encountered a pungent, sewage smell coming from the discharge. The KDOW was notified by KFO and later investigated the outfall and treatment facility for any potential water quality violations. KDOW discovered some ongoing operation and maintenance issues with the treatment facility, and water quality samples taken on the same day of the reported smell showed elevated levels for two parameters – biological oxygen demand (BOD) and total suspended solids (TSS). A notice of violation was issued by KDOW,

and the facility is now under an agreed order to fix any problems associated with operation of their treatment facility and the quality of their discharge. Inputs of untreated wastewater could harm Relict Darter populations, by introducing bacteria and disease, and well as decreasing available oxygen levels.

Other permitted discharges within the Bayou de Chien system include stormwater permits for SGL Carbon LLC (KPDES #KY0000094), a manufacturer of cathodes in Hickman, Kentucky; Stella-Jones Corporation (KPDES #KY0107298), a manufacturer of treated lumber products; two sand and gravel operations, Harold Coffey Construction Company, Inc. in Crutchfield (KPDES #KYG840167) and Ford Construction Company in Water Valley (KPDES #KYG840160); Heritage Distribution (KPDES #KY0107336), a petroleum transport and services company; Fulton County Highway Maintenance Garage (KPDES #KYG500071), and Hickman Motors (KPDES #KYR003478), a motor vehicle parts company. Parameters listed on each of these permits include pH, total suspended solids, oil and grease, and flow. All of the discharges pose some risk to the species, but the Stella-Jones Corporation and Ford Construction Company discharges have the greatest potential to impact the species because of their close proximity to the Bayou du Chien mainstem and Jackson Creek.

2.3.2.2. Overutilization for commercial, recreational, scientific, or educational purposes:

The Relict Darter is not believed to be utilized for commercial, recreational, scientific, or educational purposes. When the species was described and listed in the early 1990s, it was suggested that the species' rareness would make it desirable to private and institutional collectors; however, since that time, over-collecting has not become a threat.

2.3.2.3. Disease or predation:

The Relict Darter is undoubtedly consumed by predators (Piller and Burr 1996); however, there is no evidence that predation is a significant threat to the species. The species has evolved with various predators over thousands of years and has continued to persist within the system. There are no known diseases that currently pose a threat to the species.

2.3.2.4. Inadequacy of existing regulatory mechanisms:

The Relict Darter and its habitats are afforded some protection from water quality and habitat degradation under the Clean Water Act of 1977 (33 U.S.C. 1251 et seq.), Kentucky's Forest Conservation Act of 1998 (KRS 149.330-355), Kentucky's Agriculture Water Quality Act of 1994 (KRS 224.71-140), and additional Kentucky laws and regulations regarding natural resources and environmental protection (KRS 146.200-360; KRS 224; 401 KAR 5:026, 5:031). The species is also afforded protection by the Endangered Species Act (ESA) of 1973, as amended (16 U.S.C. 1531 *et seq*), which requires federal agencies to consult with the Service when activities they fund, authorize, or

carry out may affect a listed species. The ESA requires federal permits for any activity that may result in "take" of a listed species.

States maintain water-use classifications through issuance of National Pollutant Discharge Elimination System (NPDES) permits to industries, municipalities, and others that set maximum limits on certain pollutants or pollutant parameters. For water bodies on the 303(d) list, States are required under the Clean Water Act to establish a total maximum daily load (TMDL) for the pollutants of concern that will bring water quality into the applicable standard. Segments of four streams within the Bayou de Chien system, Bayou de Chien, Cane Creek, Little Bayou de Chien, and South Fork Bayou de Chien, have been identified as impaired by KDOW and placed on the State's 303(d) list (KDOW 2018). Causes of impairment were listed as siltation/sedimentation, nutrients, eutrophication, and elevated levels of copper, iron, and lead (see Factor A discussion above). Contaminant sources were listed as agriculture, channel erosion, channel dredging, riparian habitat loss, and municipal point-source discharges. TMDLs have not yet been developed for these pollutants.

The Relict Darter has been designated as an endangered species in Kentucky (KSNPC 2005), but the designation conveys no legal protection. Kentucky law prohibits the collection of the species for scientific purposes without a valid state-issued collecting permit (KRS 150.183), but this regulation provides no protection to the species' habitat. Within Kentucky, persons who hold a valid fishing license KDFWR are prohibited from using listed fish species, including the Relict Darter, as bait (KDFWR 2019).

Despite the limited protection afforded by the laws and corresponding regulations cited above, the Relict Darter continues to be impacted by poor water quality and habitat degradation resulting from stream channelization, reductions in riparian cover, siltation caused by poor land use practices, and by other nonpoint-source pollutants (see discussion under Factor A above). Existing regulatory mechanisms have been inadequate to protect the species and its habitat from these impacts.

2.3.2.5. Other natural or manmade factors affecting its continued existence:

Restricted Range

The Relict Darter has a limited geographic range, consisting of about 39.8 stream km (24.7 stream mi) within a single stream system in western Kentucky (Bayou de Chien system). The occurrence of Relict Darters within the Bayou de Chien system is restricted to Bayou de Chien, Jackson Creek, South Fork Bayou de Chien, and Little Bayou de Chien. Piller and Burr (1996) characterized the species as uncommon or rare at most collection sites, generally consisting of 1 to 23 individuals per site. Recent surveys indicate the species continues to be rare in some reaches but is more common in others. The greatest number of darters per sampling reach have been observed in Jackson Creek and an approximate 22.6-km (14.1-mi) reach of Bayou de Chien, extending from just downstream of the US 51 bridge crossing in Hickman County upstream to the Pea Ridge Road bridge crossing in Graves County.

The species' restricted range and low abundance in some reaches makes it more vulnerable to extirpation from toxic chemical spills, habitat modifications, progressive degradation from land surface runoff (nonpoint-source pollutions), and natural catastrophic changes to their habitat (*e.g.*, flood scour, drought). In particular, the weight of the evidence indicates the Relict Darter's largest and most significant breeding populations occur in Jackson Creek and middle and headwater reaches of Bayou de Chien, which are vulnerable to stochastic events, such as a single toxic chemical spill or an extreme weather event such as a drought or flash flood. These events would have devastating effects on darters in these reaches (Piller and Burr 1996) and could threaten the long-term viability of the species.

The Bayou de Chien/Jackson Creek and Little Bayou de Chien populations appear to be geographically separated (disjunct) from each other based on recent surveys (2011- present) completed by the Service and its partners. This may reduce or limit the potential for natural interchange of genetic material between the two watersheds. The apparent small population size in Little Bayou de Chien reduces the reservoir of genetic diversity within that population. This can lead to inbreeding depression, a reduced ability to adapt to environmental change, and reduced fitness of individuals (Soule 1980; Hunter 2002; Allendorf and Luikart 2007). As mentioned previously, genetic analyses indicate that the genetic diversity of the Relict Darter is low, potentially reducing its ability to adapt to changing environmental conditions over time and making it more susceptible to local extirpations.

Climate Change

In its Fifth Assessment Report, the Intergovernmental Panel on Climate Change (IPCC) concluded that warming of the climate system is unequivocal (IPCC 2014). Numerous long-term climate changes have been observed including changes in arctic temperatures and ice, widespread changes in precipitation amounts, ocean salinity, wind patterns and aspects of extreme weather including droughts, heavy precipitation, heat waves, and the intensity of tropical cyclones (IPCC 2014). Species that are dependent on specialized habitat types, limited in distribution, or at the extreme periphery of their range may be most susceptible to the impacts of climate change (see 75 FR 48911, August 12, 2010); however, while continued change is certain, the magnitude and rate of change is unknown in many cases.

Climate change has the potential to increase the vulnerability of the Relict Darter to random catastrophic events (McLaughlin *et al.* 2002; Thomas *et al.* 2004). An increase in both severity and variation in climate patterns is expected; extreme floods, strong storms, and droughts will become more common (Cook *et al.* 2004, Ford *et al.* 2011, IPCC 2014). Thomas *et al.* (2004) report that frequency, duration, and intensity of droughts are likely to increase in the Southeast as a result of global climate change. Predicted impacts of climate change on fishes include disruption to their physiology (such as temperature tolerance, dissolved oxygen needs, and metabolic rates), life history (such as timing of reproduction, growth rate), and distribution (range shifts, migration of

new predators) (Jackson and Mandrak 2002, Heino *et al.* 2009, Strayer and Dudgeon 2010, Comte *et al.* 2013). According to Kaushal *et al.* (2010), stream temperatures in the Southeast have increased roughly 0.2 – 0.4°C (0.4 – 0.7°F) per decade over the past 30 years, and as air temperature is a strong predictor of water temperature, stream temperatures are expected to continue to rise.

Estimates of the effects of climate change using available climate models typically lack the geographic precision needed to predict the magnitude of effects at a scale small enough to discretely apply to the range of a given species. However, data on recent trends and predicted changes for Kentucky (Girvetz *et al.* 2009), and, more specifically, the Bayou de Chien/Mayfield Creek drainages (Alder and Hostetler 2017), provide some insight for evaluating the potential threat of climate change to the Relict Darter. Alder and Hostetler (2017) use different emission scenarios to calculate estimates of average annual increases in maximum and minimum temperature, precipitation, snowfall, and other variables. These scenarios, called "representative concentration pathways" (RCPs) are plausible pathways toward reaching a target radiative forcing (the change in energy in the atmosphere due to greenhouse gases) by the year 2100 (Moss *et al.* 2010). Depending on the chosen model and emission scenario (RCP 8.5 (high) vs. 4.5 (moderate)), annual mean maximum temperatures for the Bayou de Chien system are expected to increase by 2.3 to 3.4°C (4.1 to 6.1°F) by 2074, while precipitation models predict that the Bayou de Chien system will experience a slight increase in annual mean precipitation (0.5 cm/month (0.2 in/month)) through 2074 (Girvetz *et al.* 2009, Alder and Hostetler 2017).

There is uncertainty about the specific effects of climate change (and their magnitude) on the Relict Darter; however, climate change is almost certain to affect aquatic habitats in the Bayou de Chien system of western Kentucky through increased water temperatures and more frequent droughts (Alder and Hostetler 2017), and species with limited ranges, fragmented distributions, and small population size are thought to be especially vulnerable to the effects of climate change (Byers and Norris 2011). Thus, we consider climate change to be a potential threat to the Relict Darter.

2.4 Synthesis

The Relict Darter is endemic to and has a limited range within the Bayou de Chien system in Fulton, Graves, and Hickman counties, Kentucky. Recent surveys indicate the species occupies a 30.4-km (18.9-mi) reach of Bayou de Chien, a 3.6-km (2.3-mi) reach of Jackson Creek, a 3.2-km (2.0-mi) reach of South Fork Bayou de Chien, and a 2.6-km (1.6-mi) reach of Little Bayou de Chien. During field surveys completed since 2011, the greatest abundance and greatest mean densities of Relict Darters have been observed within Jackson Creek and middle and headwater reaches of Bayou du Chien, with ample evidence of recruitment and mean population estimates ranging from 1,791-1,888 individuals in Jackson Creek (95% confidence intervals of 278 - 3,000) and from 14,581-22,798 individuals in Bayou de Chien (95% confidence intervals of 4,172-41,424). The species continues to occupy lower reaches of

South Fork Bayou de Chien, where it occurs in low densities. All recent survey methods in South Fork Bayou de Chien have been qualitative, so no estimate of population size has been possible. A reliable population estimate has not been obtained for Little Bayou de Chien; however, mean density values were similar to those observed in some Bayou de Chien reaches, and observed age-class structure provided evidence of recruitment. The species may be present in the lower reaches of several other Bayou de Chien tributaries (e.g., Cane Creek, Sand Creek) during spring (March – May); however, these streams are typically dry or consist of isolated pools by late summer or fall. Because of these conditions, these intermittent reaches likely contribute little to recruitment.

The Relict Darter continues to be threatened by three of the Service's five listing factors: the present or threatened destruction, modification, or curtailment of its habitat or range (Factor A), the inadequacy of existing regulatory mechanisms in protecting against habitat alteration or destruction (Factor D), and other natural or manmade factors affecting its continued existence (Factor E). While habitat threats remain and current regulatory mechanisms have been inadequate to prevent all of these impacts, we conclude that habitat threats in Jackson Creek and Bayou de Chien have decreased from a magnitude level of high to a magnitude level of moderate. We base our conclusion on observed trends of abundance and mean density in Jackson Creek and Bayou de Chien, our estimates of the species' population size in both streams, the ample evidence of reproduction and recruitment in both streams, and our repeated observations of these conditions during major survey efforts in 2011-2012 and 2017-2018 (see Appendix B). In addition to these factors, threats to the species' habitat have been reduced, and in some cases eliminated, by multiple habitat protection projects (e.g., cattle exclusion, riparian plantings) in Jackson Creek and Bayou de Chien. The Service continues to work with its partners to implement additional projects in these watersheds.

With respect to Factor E, the species' linear distribution and limited range within the Bayou de Chien watershed continue to make it vulnerable to stochastic events (e.g., drought or toxic chemical spills) that could cause the extirpation of the species from portions of Bayou de Chien, Jackson Creek, or Little Bayou de Chien. The species' discovery in Little Bayou de Chien offers some protection against catastrophic events that could lead to the species' extinction (e.g., improved redundancy); however, the Little Bayou de Chien population appears to be small relative to Bayou de Chien and Jackson Creek (i.e., lower resiliency), and habitat conditions are not as favorable for the species. In addition to the species' limited range, genetic analyses indicate low genetic diversity for the species, suggesting a reduced ability to adapt to changing environmental conditions and greater vulnerability to local extirpations.

The ESA defines an endangered species as any species, which is in danger of extinction throughout all or a significant portion of its range. Based on our status review, threat analysis, and evaluation of conservation measures, we believe that the Relict Darter no longer meets the definition of endangered and should be reclassified as a threatened species. We recommend a recovery

priority number of 8, corresponding to a moderate degree of threat, a low recovery potential, and species level taxonomy.

3.0 RESULTS

3.1 Recommended Classification: Downlist to Threatened.

Recent surveys (2017-2018) indicate that estimates of Relict Darter abundance, mean density, and population size continue to be greatest in Jackson Creek and middle to headwater reaches of Bayou de Chien. There is also ample evidence of reproduction and recruitment in these streams, and these trends have remained relatively constant or have improved based on surveys completed during the past nine years. Habitat improvement and protection projects have reduced habitat threats in portions of Bayou de Chien and Jackson Creek, and the Service continues to work with its partners on these projects. In 2017, a new population was discovered in Little Bayou de Chien, providing increased redundancy and offering some protection against catastrophic events that could lead to the species' extinction. The species' linear distribution and limited range within the Bayou de Chien watershed continue to make it vulnerable to stochastic events (e.g., drought or toxic chemical spills), and the species' low genetic diversity suggests a reduced ability to adapt to changing environmental conditions. Based on these observations, the 5-factor analysis shows threats associated with Factors A and D have decreased in magnitude (from high to moderate) in Bayou de Chien and Jackson Creek, while threats associated with Factor E have remained at a high magnitude level across the species' range.

Therefore, based on our status review, threats analysis, and evaluation of conservation measures, we believe that the Relict Darter no longer meets the ESA definition of endangered and should be reclassified to threatened. The ESA defines a threatened species as any species, which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.

3.2 Recommended Recovery Priority Number: 8

A Recovery Priority Number of 8 indicates that the Relict Darter has a moderate degree of threat, a low recovery potential, and is taxonomically categorized as a species.

3.3 Listing and Reclassification Priority Number: 6

The Service has not been petitioned to reclassify the Relict Darter to threatened status, and this action will have a low management impact because this fish occupies a sparsely populated watershed, little additional watershed manipulation is expected, and there are few human activities that occur in the watershed that would require consultation with the Service.

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

- Initiate efforts to propose reclassifying this fish from endangered to threatened status under the ESA.

- Complete new draft and final recovery plans.

- Determine habitat preferences of juvenile and larval Relict Darters. The biology of larvae is unknown.

- Continue research examining the level of genetic exchange, effective population size, and overall genetic diversity. This will provide important information on the long-term viability of the species and help focus habitat recovery efforts in stream reaches that would improve population connectivity.

- Continue periodic quantitative survey efforts (fall surveys) on Bayou de Chien, Jackson Creek, and Little Bayou de Chien. Consider inclusion of survey reaches on South Fork Bayou de Chien and/or other tributaries and conduct a power analysis to determine if quantitative survey methods should be modified.

- Search for unknown occupied reaches in other Bayou de Chien tributaries (e.g., Cane Creek) and downstream reaches of Bayou de Chien and Little Bayou de Chien.

- Continue to protect, restore, and enhance habitat quality throughout the drainage. Federal, state, and private parties should continue to work cooperatively (through Farm Bill programs, Partners for Fish and Wildlife projects, etc.) to restore and protect habitats, especially those areas where reproduction and recruitment has been documented (Jackson Creek). The number of permits granted to snag, channelize, or modify the existing stream system should be limited.

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U.S. FISH AND WILDLIFE SERVICE

5-YEAR REVIEW of Relict Darter (*Etheostoma chienense*)

Current classification: Endangered

Recommendation resulting from the 5-Year Review: Downlist to Threatened

Review conducted by: Dr. Michael A. Floyd, Kentucky ES Field Office, Frankfort, Kentucky

Appropriate Listing/Reclassification Priority Number: 6

FIELD OFFICE APPROVAL:

Lead Field Supervisor, Fish and Wildlife Service

Approve VIRGIL ANDREWS Digitally signed by VIRGIL ANDREWS Date: 2019.08.16 10:49:58 -04'00' Date August 16, 2019

REGIONAL OFFICE APPROVAL

Lead Regional Director, Fish and Wildlife Service

for

Approve Date 8/30/2019

APPENDIX A: Summary of peer review for the 5-Year Review of the Relict Darter (*Etheostoma chienense*)

A. Peer Review Method: The draft document was peer-reviewed by Dr. David Eisenhour, Morehead State University, Morehead, Kentucky; Dr. Sherry Harrel, Eastern Kentucky University, Richmond, Kentucky; Dr. Rebecca Johansen, Austin Peay State University, Clarksville, Tennessee; and Dr. Melvin L. Warren, Jr., U.S. Forest Service, Oxford, Mississippi.

B. Peer Review Charge: Peer reviewers were asked to read the 5-Year Review and provide any comments, both editorial and content related. They were not asked to comment on the recommendation regarding listing status.

C. Summary of Peer Review Comments/Report: The peer reviewers considered the revised 5-Year Review to be biologically sound and generally agreed with the species' status information and proposed conservation actions. They agreed that the review was based on the best available scientific information. Most comments were editorial in nature. Substantive comments included (1) a recommendation that the Service revise its summary and graphical representation of survey methods and results, including its characterization of abundance, mean density, and population size (M Warren, Jr. and R. Johansen); (2) a recommendation to improve and/or expand future quantitative survey methods (and reduce sample variance) though completion of a power analysis (M. Warren); (3) a request that the Service complete a thorough study of the species' population genetics (M. Warren, Jr. and R. Johansen); (4) a recommendation that Cane Creek be considered as part of the species' current range (D. Eisenhour); (5) a suggestion that Figure 1 be modified to show the location of Pea Ridge Road (M. Warren, Jr.); and (6) a request that the Service explain why captive individuals from Wolf Creek NFH were released back into the wild (M. Warren, Jr.).

D. Response to Peer Review

General edits and minor substantive changes were added to the review as appropriate. Substantive comments summarized above were addressed as follows: (1) the Service's description of survey methods (Appendix B) and our summary and analysis of field data were modified through revisions to Tables 1 and B-1 and inclusion of revised text that more accurately describes survey results and acknowledges the high sample variance; (2) a statistical power analysis was added as a future conservation action; (3) a study of the species' population genetics is underway (see pages 9-10), with an expected completion date of Fall 2019 (4) intensive surveys of Cane Creek were added as a future conservation action; however, recent surveys indicate that habitat conditions in Cane Creek are not suitable for the species; (5) Figure 1 was modified to include the location of the Pea Ridge Road crossing; and (6) text was added explaining the Service's decision to release captive individuals from Wolf Creek NFH.

Appendix B

Quantitative Surveys for the Relict Darter in the Bayou de Chien System (2011-2018)
Kentucky Department of Fish and Wildlife Resources
Office of Kentucky Nature Preserves
Kentucky Ecological Services Field Office

In 2011, KNP, KDFWR, and KFO initiated a long-term, quantitative monitoring effort for the Relict Darter. The goal was to establish a consistent and repeatable monitoring strategy that would document Relict Darter occurrence and density over time and estimate the species' population size within the Bayou de Chien system. A total of 16 sampling reaches were selected randomly on Bayou de Chien (12 reaches) and Jackson Creek (4) based on the species' known range, its habitat affinities, and the extent of available habitat on each stream (the number of reaches was proportional to the total stream length in each stream) (Figure B-1; Table B-1). Surveys on Jackson Creek were completed in October 2011 and October 2017, and surveys on Bayou de Chien were completed in October 2012 and September 2018. Two survey reaches on Little Bayou de Chien were added to the monitoring effort in 2017.

Within each sampling reach, surveys were completed in 10, randomly placed sub-units (plots). Reach length and plot dimensions varied based on stream size. Sampling reaches on Jackson Creek and Little Bayou de Chien were approximately 100 meters in length, and plot dimensions were 2 x 5 meters. The length of sampling reaches on the Bayou de Chien mainstem was 150 m, and plot dimensions were 2 x 10 meters. For each plot, wetted stream width (m) was measured, and fishes were surveyed using a backpack electrofishing unit (Smith-Root LR-24). All fishes were identified, enumerated, and released approximately 5-10 m downstream of the sampling plot (to avoid recapture). Total length (mm) was recorded for all Relict Darters.

Relict Darter density in each plot (#darters/m^2) was determined using plot area (m^2) and Relict Darter abundance (# darters/plot). Values of Relict Darter density from each plot were averaged to produce a mean density value for the reach. A population estimate for each reach was calculated using total reach abundance and an extrapolation coefficient (total reach area/total area surveyed). Population estimates for Bayou de Chien and Jackson Creek were calculated using an average population estimate (average of all reach estimates) and the number of 100-m reaches in each stream.

Relict Darters were observed at least once in each of the survey reaches. Total Relict Darter abundance in Jackson Creek was about the same during 2011 and 2017, but total darter abundance and overall mean density in Bayou de Chien was statistically higher in 2018 than in 2012 (T value, -3.29; $p < 0.001$) (Table 1). The 2018 increase in darter abundance and density within the Bayou de Chien mainstem can be attributed to greater recruitment in 2018 (Figure B-5). The 0+ age class (< 50 mm TL) increased from 35.8% of the population in 2012 to 67.3% in 2018. Relict Darter abundance in two Little Bayou de Chien reaches was low compared to Jackson Creek, a stream of similar size.

Overall mean density in Jackson Creek was 0.23 and 0.26 in 2011 and 2017, respectively (Figure B-2). Mean density values per reach were greatest in middle reaches of Jackson Creek, near and upstream of the Lawrence Road bridge crossing (Figure B-2). For Bayou de Chien, overall mean density in 2012 and 2018 was 0.04 and 0.10, respectively (Table 1). Mean density values per reach were generally greatest upstream of the US 51 bridge crossing (Reaches 6-12) (Figures B-1, B-3, and B-4). Mean

Relict Darter density for Little Bayou de Chien (2 reaches) was comparable to mean density for the Bayou de Chien mainstem (12 reaches).

The estimated population size for Jackson Creek was 1,791 individuals in 2011 (95% confidence interval of 278 – 3,304) and 1,888 individuals in 2017 (95% confidence interval of 683 – 3,093 individuals) (Table 1, Figure B-6). The estimated population size for the Bayou de Chien mainstem was 14,581 individuals in 2012 (95% confidence interval of 4,919 – 24,243) and 22,798 individuals in 2018 (95% confidence interval of 4172 – 41,424 individuals). Population estimates for Jackson Creek (2011 and 2017) and Bayou de Chien (2012 and 2018) were not statistically different (Figure B-6), but range-wide population estimates for 2011 – 2012 (16,372 individuals) and 2017 – 2018 (24,686) were relatively similar to a system-wide estimate (21,740) completed by Piller and Burr (1998).

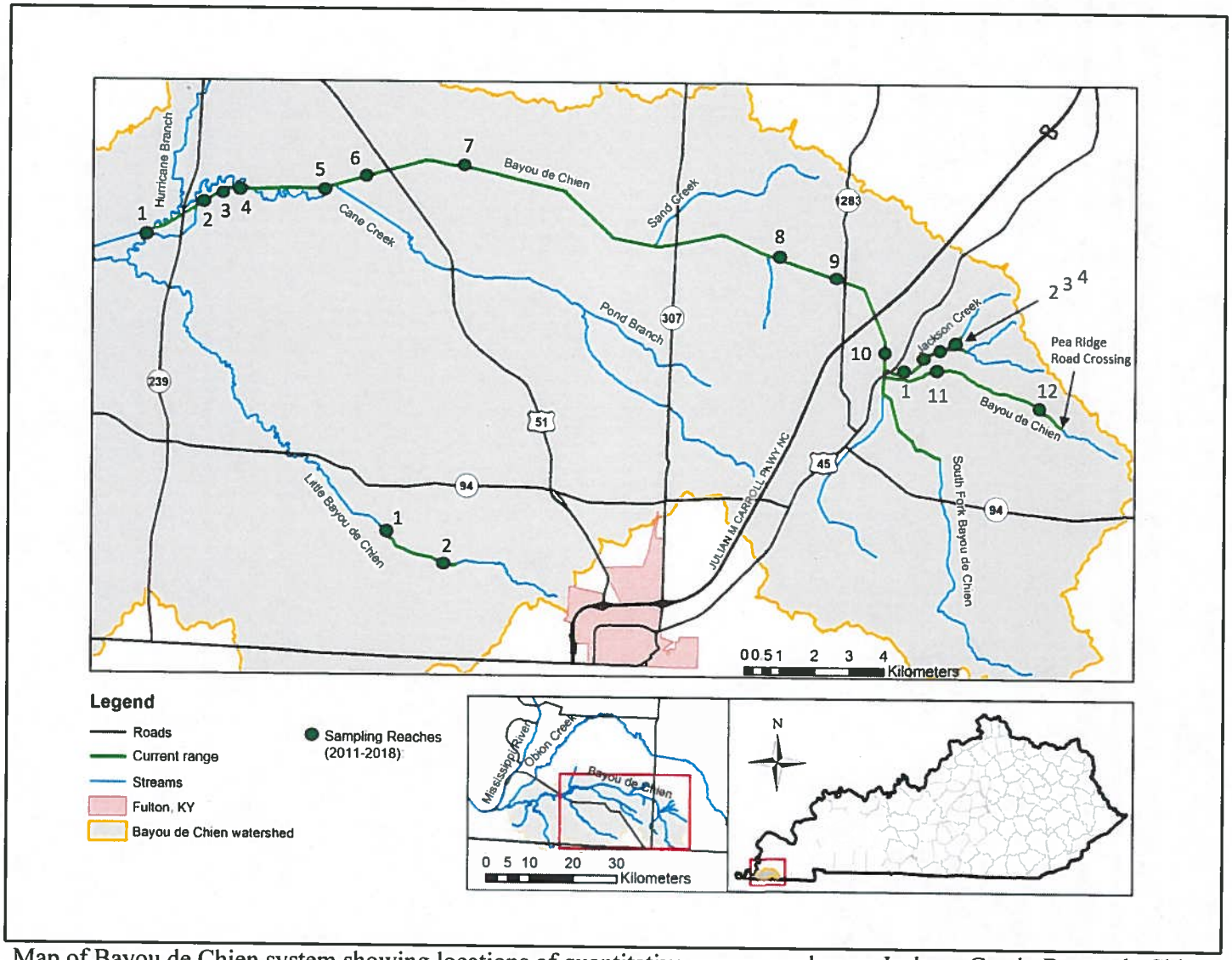

Figure B-1. Map of Bayou de Chien system showing locations of quantitative survey reaches on Jackson Creek, Bayou de Chien, and Little Bayou de Chien (2011-2018).

Table B-1. Summary of quantitative survey reach (site) locations visited by the Service, KDFWR, and KNP (2011-2012, 2017-2018).

Stream	Reach (Site)	Location	Latitude	Longitude	River Mile (km)	County
Bayou de Chien	1	0.65 mi downstream SR 239, near Deweese Road	36.61084	-89.04049	10.8 (17.4)	Hickman
Bayou de Chien	2	0.33 mi upstream SR 239, off dirt road	36.61817	-89.02511	11.8 (19.0)	Hickman
Bayou de Chien	3	0.54 mi upstream SR 239, off dirt road	36.61980	-89.02214	12.0 (19.3)	Hickman
Bayou de Chien	4	0.84 mi upstream SR 239, off dirt road	36.62227	-89.01770	12.3 (19.8)	Hickman
Bayou de Chien	5	0.30 miles downstream Illinois Central Railroad Line Bridge	36.62299	-88.98721	14.0 (22.5)	Hickman
Bayou de Chien	6	0.52 mi downstream US Hwy 51 Bridge	36.62625	-88.97347	14.8 (23.8)	Hickman
Bayou de Chien	7	0.50 mi downstream Howell Road crossing	36.62980	-88.93691	16.9 (27.2)	Hickman
Bayou de Chien	8	Off Stephens Road; 0.6 mi upstream Illinois Central Railroad Line	36.60986	-88.83887	22.8 (36.7)	Hickman
Bayou de Chien	9	0.2 mi downstream SR 1283 bridge	36.60514	-88.81994	23.9 (38.5)	Hickman
Bayou de Chien	10	0.3 mi downstream Jackson Creek confluence, off US HWY 45	36.58601	-88.80291	25.7 (41.4)	Graves
Bayou de Chien	11	0.2 mi upstream Lawrence Road; NW of Bayou de Chien Church	36.58209	-88.78561	27.1 (43.6)	Graves
Bayou de Chien	12	0.75 mi downstream Pea Ridge Road	36.57475	-88.75556	28.9 (46.5)	Graves
Jackson Creek	1	0.15 mi upstream US Hwy 45 bridge	36.58109	-88.79846	0.25 (0.4)	Graves
Jackson Creek	2	0.05 mi upstream Lawrence Road Bridge	36.58494	-88.79025	0.85 (1.4)	Graves
Jackson Creek	3	0.40 mi upstream Lawrence Road Bridge; 0.10 mi downstream of UT confluence	36.58741	-88.78492	1.2 (1.9)	Graves
Jackson Creek (UT)	4	0.10 mi upstream Jackson Creek confluence	36.58804	-88.78206	0.1 (0.2)	Graves
Little Bayou de Chien	1	0.10 mi upstream SR 1125 bridge crossing	36.53435	-88.96173	11.7 (18.8)	Fulton
Little Bayou de Chien	2	0.10 mi upstream SR 1706 bridge crossing	36.57593	-89.01412	13.0 (30.9)	Fulton

UT – Unnamed tributary

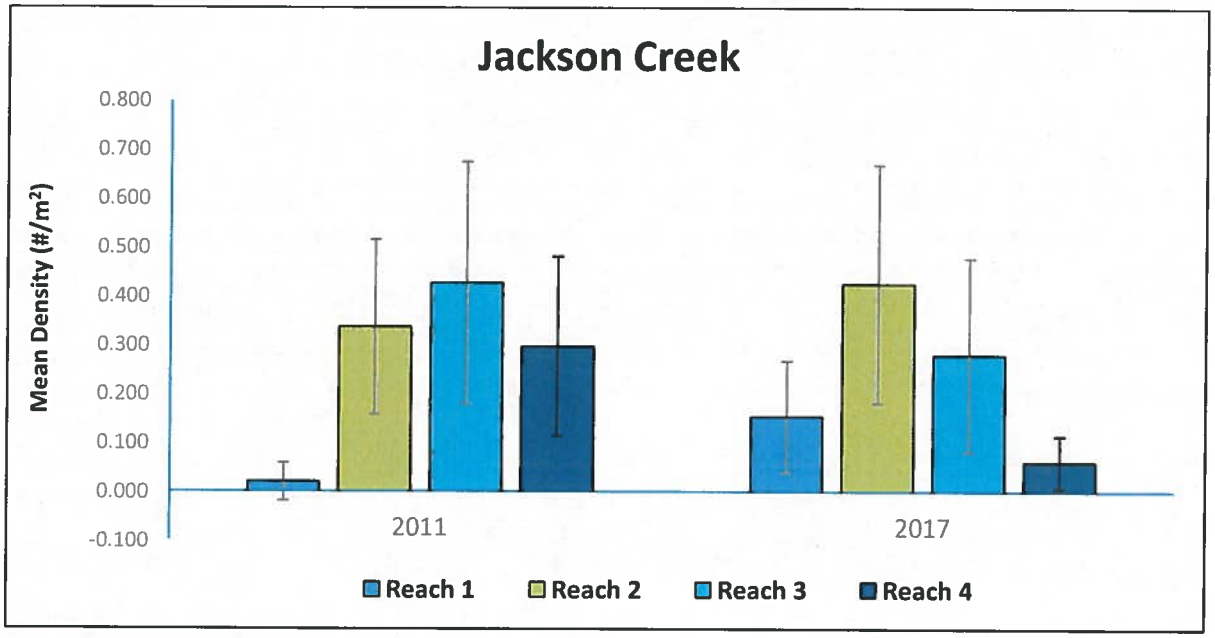

Figure B-2. Mean density (#/m^2) of Relict Darters at four reaches (sites) in Jackson Creek during fall surveys in 2011 and 2017, with 95% confidence intervals.

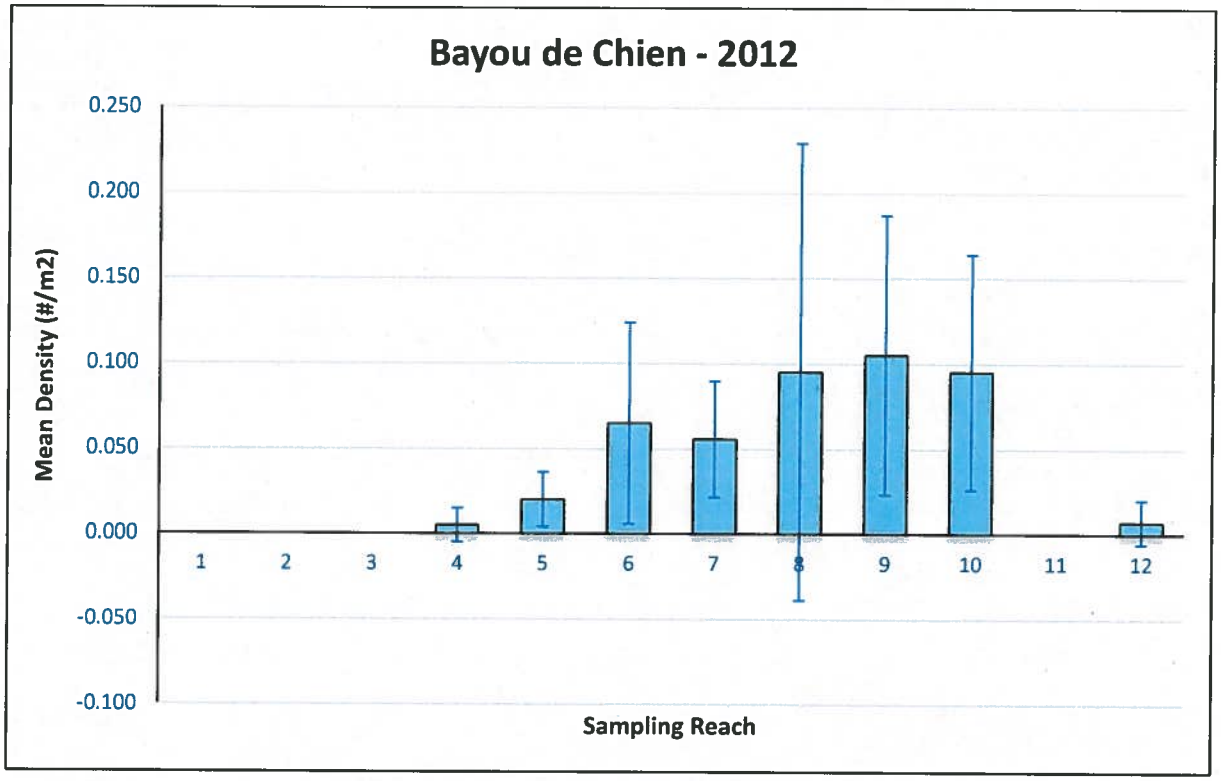

Figure B-3. Mean density (#/m^2) of Relict Darters at 12 reaches of Bayou de Chien during fall surveys in 2012, with 95% confidence intervals.

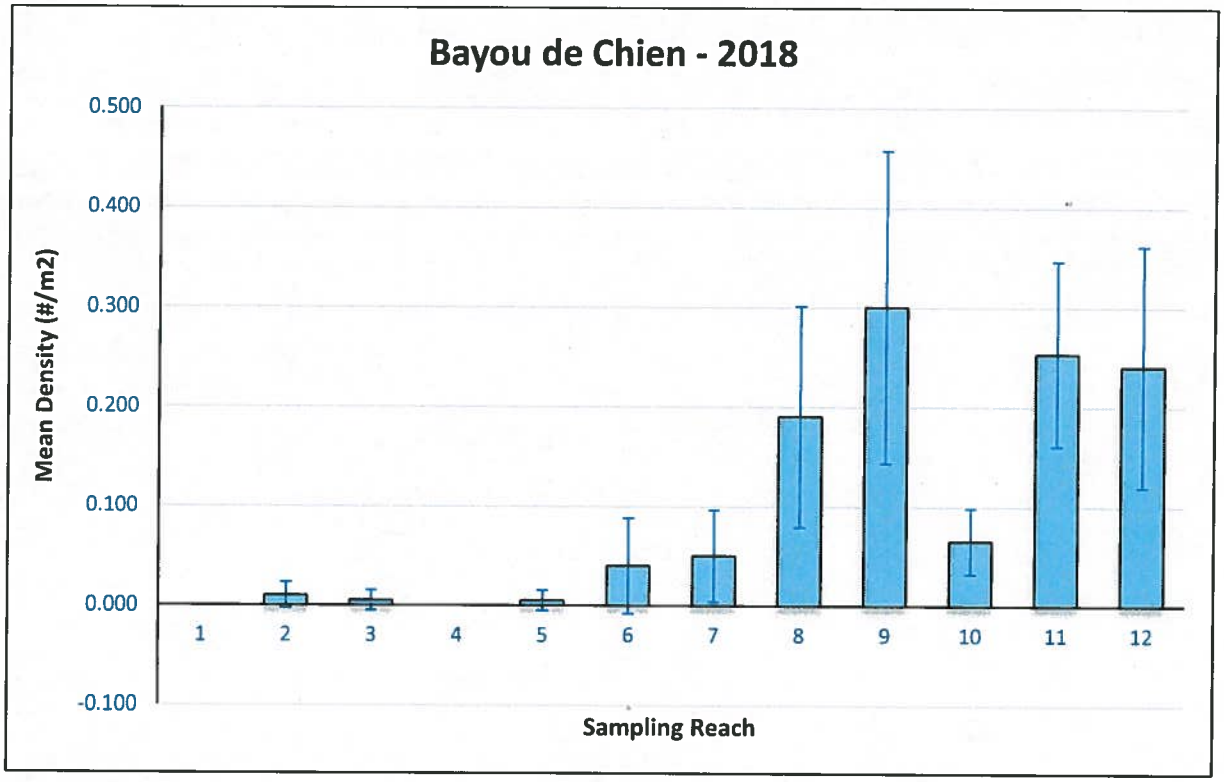

Figure B-4. Mean density (#/m^2) of Relict Darters at 12 reaches of Bayou de Chien during fall surveys in 2018, with 95% confidence intervals.

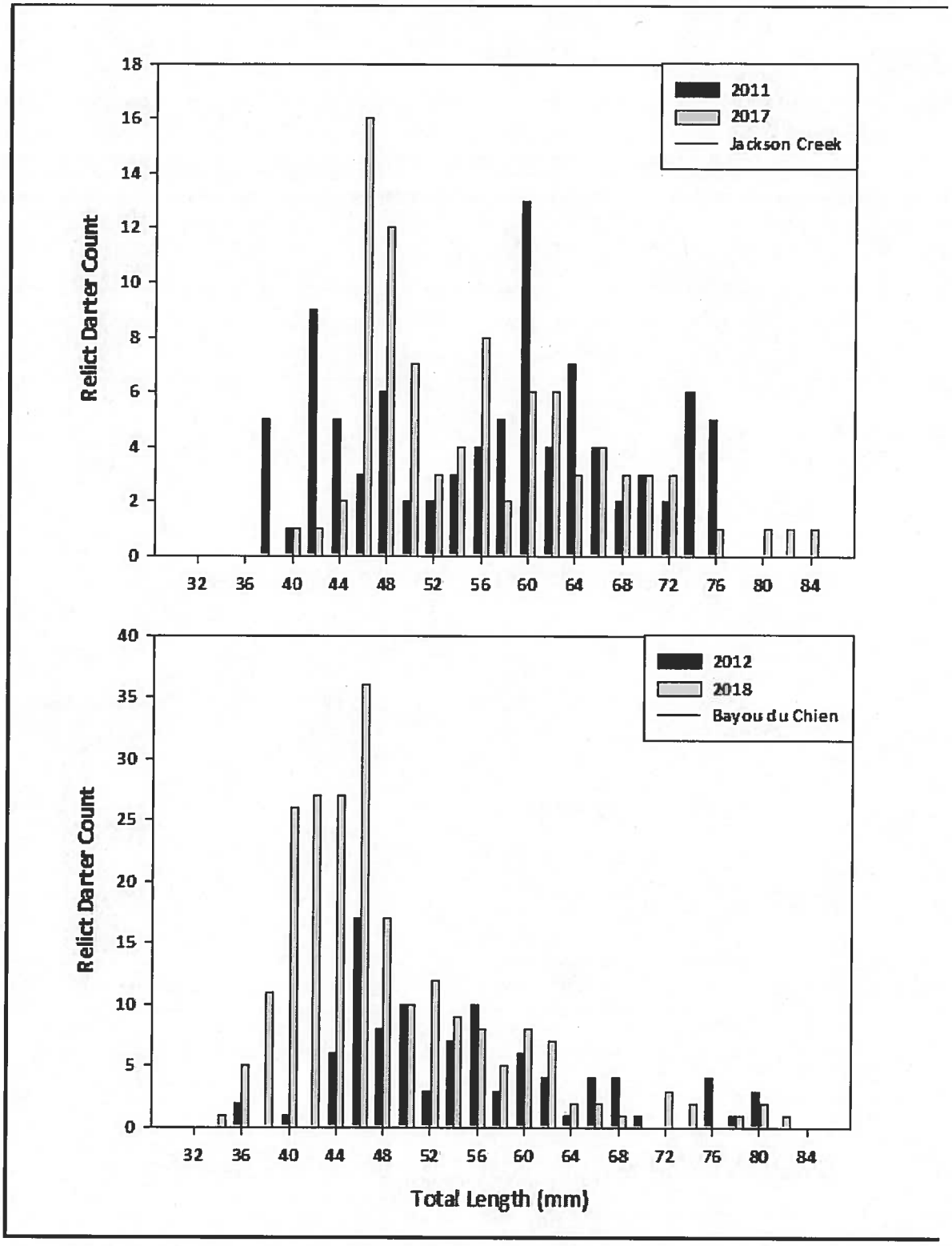

Figure B-5. Length-frequency histograms of Relict Darters captured during fall quantitative surveys in Jackson Creek (top) and Bayou de Chien (bottom).

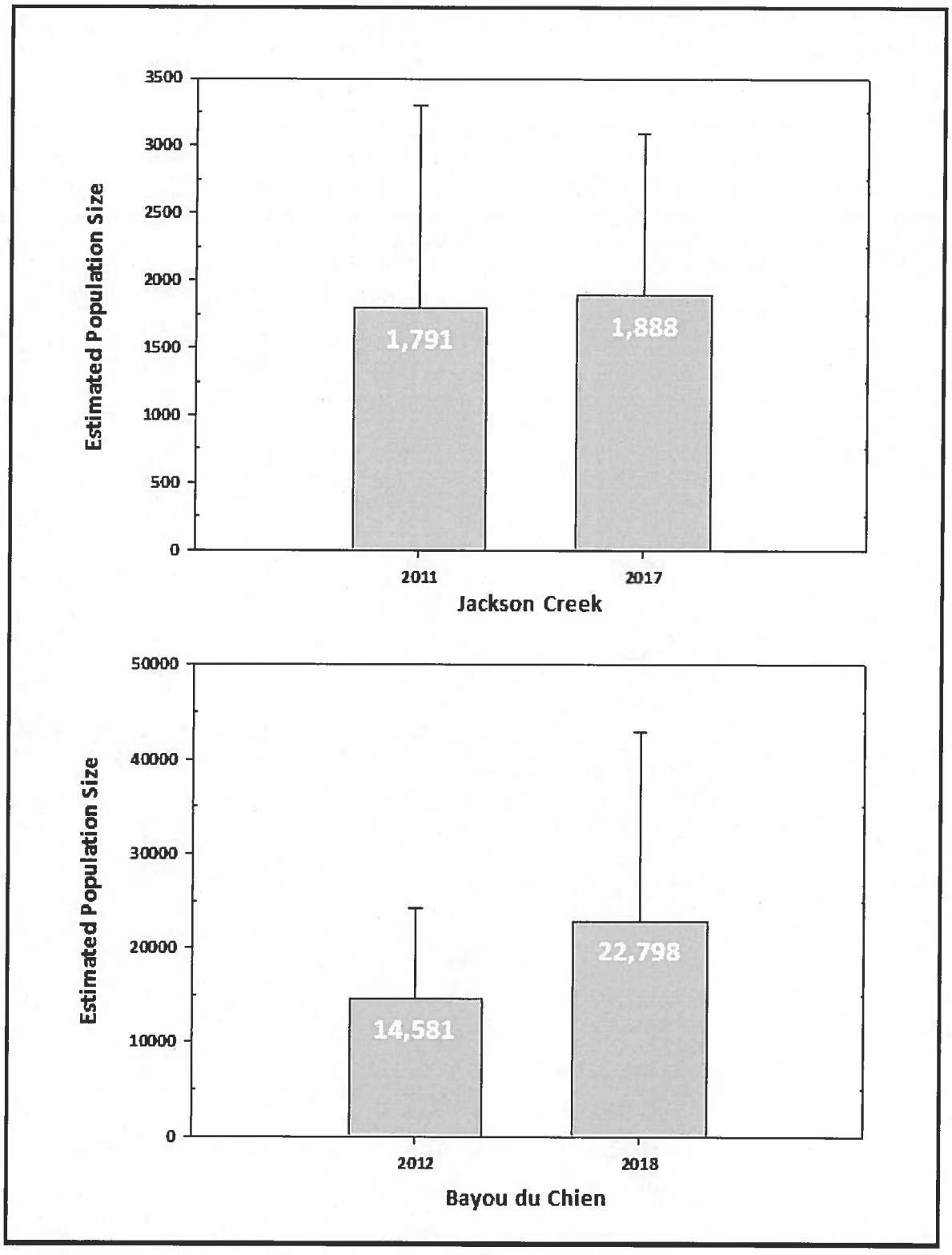

Figure B-6. Estimated mean population size of Relict Darters within Jackson Creek and Bayou de Chien during fall surveys in 2011-2012 and 2017-2018, with upper 95 % confidence bars.