

## ANNUAL PERFORMANCE REPORT

State: Kentucky  
Grant: T-60-R  
Project Type: Research and Survey  
Project Title: Fish Surveys in Kentucky  
Grant Period: November 1, 2010 to December 31, 2015  
Period Covered: January 1, 2012 to December 31, 2012

Project Objective: To collect and compile distributional records, obtain population trend information, and develop procedures to attempt to preserve, enhance, or restore Kentucky's important ichthyofauna.

### A. ACTIVITY

- I. Ichthyofaunal Assessment of Buck Creek, Upper Cumberland River Drainage, Kentucky.—Fish community sampling in Buck Creek was completed in 2012. Overall faunal composition has changed slightly during the past 25 years, although shifts in relative abundances in several species and the longitudinal distribution of species have also occurred. Twelve species reported previously were not detected in our study; however, we documented new drainage records for seven species, including three fish SGCN. This discrepancy likely resulted in large part from differences in sampling gear used between the present and past surveys, but may also reflect changes in habitat and environmental fluctuations. The Buck Creek drainage supports seven fish SGCN. Among these, the Bloodfin Darter has the strongest population with the most occurrences and highest abundance levels; it was one of the most abundant species in the middle and lower sections of the mainstem and was also present in lower Brushy Creek. Mountain Brook Lamprey, Lake Sturgeon, and Southern Cavefish (undescribed form) are documented here for the first time in the Buck Creek drainage. These species appear to be rare and limited to specific habitat types in the lower portion of the drainage. The Popeye Shiner, Redlips Darter, and Striped Darter (undescribed form) were documented previously and continue to persist, but are rare (i.e., fewer than 20 total individuals at 1-3 sites) within the drainage. The undescribed form of the Striped Darter is of particular concern because it was detected at less than 10% of sites having historic presence and now appears to be restricted to Flat Lick Creek.
  
- II. Ichthyofaunal Assessment of the Red River, Lower Cumberland River Drainage, Kentucky.—Field sampling in the Red River began in 2011 to assess the status of SGCN based on historic occurrences and obtain baseline data for species lacking complete distributional information. As of 31 December 2012, fish community data were obtained from a total of 41 sites, including 12 with

baseline data for comparison. A total of 55 species have been recorded to date, including five of eight fish SGCN known from the drainage. In 2012, the Flame Chub was collected at two additional localities in the Spring Creek drainage. Historic occurrences for Spring Cavefish were substantiated and new occurrences in three streams were documented. The Stone Darter (*Etheostoma derivativum*) was present at an additional site in Whippoorwill Creek, representing a downstream expansion of its known distribution within this system. Sampling in 2012 detected the Blotched Chub at two sites in the South Fork Red River, one of which represents an upstream expansion of the known distribution of this species in the drainage. A new drainage record for Smallscale Darter was also documented in the South Fork Red River.

III. Kentucky Arrow Darter (*Etheostoma spilotum*) Reintroduction in Long Fork, Red Bird River Drainage, Kentucky: 2012 Monitoring Results.—In 2008 the KDFWR partnered with Conservation Fisheries, Inc. (CFI) to develop successful spawning protocols for the Kentucky Arrow Darter and produce the offspring needed to re-establish extirpated populations within the species' historic range. Beginning in 2012, annual stocking of propagated Kentucky Arrow Darters was relocated from Sugar Creek to Long Fork, a new recipient stream within the Red Bird River drainage having suitable habitat, but without a wild population. The objective is to establish a naturally reproducing population within this small watershed. A total of 829 captive-spawned juveniles were marked with VIE (Visible Implant Elastomer) tags and released into Long Fork in 2012. Monitoring results have resulted in capture rates of up to 5.7%. Recaptured individuals were in good condition and appeared to be making pre-spawning movements similar to those made by adults in other Red Bird River tributaries. Females appeared to be developing ova and males had red color on the body and fins typical of breeding condition.

IV. Cumberland Darter (*Etheostoma susanae*) Reintroduction in Cogur Fork, Upper Cumberland River Drainage, Kentucky: 2009-2012 Monitoring Results.—In 2008 the KDFWR partnered with Conservation Fisheries, Inc. (CFI) to develop successful spawning protocols for the Cumberland Darter and produce the offspring needed to re-establish extirpated populations within the species' historic range. A total of 2,579 captive-spawned juveniles were marked with VIE (Visible Implant Elastomer) tags and released into Cogur Fork from 2009 to 2012, for the purpose of restoring a naturally-reproducing population. Monitoring efforts have resulted in recapture of up to 15% of tagged fish and have confirmed the survival of propagated individuals released into Cogur Fork for periods exceeding one year; however, evidence of natural reproduction has not yet been detected.

#### B. TARGET DATES FOR ACCOMPLISHMENT

Planned Achievement Date – December 31, 2015  
Work Accomplished –



C. SIGNIFICANT DEVIATIONS

None.

D. REMARKS

See accompanying report.

E. RECOMMENDATIONS

Continue fish community sampling needed to complete basin-wide ichthyofaunal assessments for the Red River. This project will provide information necessary to facilitate appropriate conservation actions that would benefit fish SGCN within these watersheds.

Changes in fish species composition and community structure documented in Buck Creek demonstrate the need for periodic surveys to monitor the distribution and population status of rare species. We recommend periodic (every 5-10 years) fish sampling in Buck Creek at fixed locations having baseline data to assess changes to the fish community. A more intensive sampling approach should target the Striped Darter for a more robust assessment of its distributional status and abundance. Because our surveys during 2010-2012 involved only single visits to historic sites, there is an inherent amount of error in our ability to detect this species at a given location. Repeated sampling at historic sites as well as an additional array of randomly selected sites in tributaries of Buck Creek could be used to estimate occupancy and detection probability.

Increase monitoring efforts associated with annual propagation and reintroduction of Kentucky Arrow Darter and Cumberland Darter. Hatchery production should be scaled back for both species with smaller annual releases in their respective recipient streams (Long Fork and Cogur Fork); more effort should be dedicated to monitoring reintroductions in both streams. Monitoring using invasive methods (e.g., seining) should be conducted only during summer, fall, and winter to avoid impacts to spawning, nests/eggs, and early larval development. During the spring spawning period, non-invasive survey methods (e.g., snorkeling or visual observation) should be used to document spawning activity.

Additional surveys of targeted fish SGCN will be conducted to update their current distributional status, evaluate population densities, and habitat conditions. This will be accomplished through collaboration with the Kentucky State Nature Preserves Commission's monitoring of state heritage-listed species.

F. COST

\$382,417.38	(\$238,520.57 – Federal Share)	62.4%
	(\$143,896.81 – State Share)	37.6%

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### Section III

## Kentucky Arrow Darter (*Etheostoma spilotum*) Reintroduction in Long Fork, Red Bird River Drainage, Kentucky: 2012 Monitoring Results



Long Fork, Clay Co., Kentucky (above); adult male Kentucky Arrow Darter used for captive spawning at CFI (below), J. R. Shute photo.

## INTRODUCTION

The Kentucky Arrow Darter, *Etheostoma spilotum*, has a limited distribution in the upper Kentucky River drainage, where it inhabits headwater (mostly first- and second-order) streams. The Kentucky Department of Fish and Wildlife Resources (KDFWR) indentified the Kentucky Arrow Darter as a Species of Greatest Conservation Need (SGCN) in its State Wildlife Action Plan to address research and survey needs for the species (KDFWR, 2010). A comprehensive status survey conducted by KDFWR and USFWS during 2007-2009 indicated that the species was present at only 33 of 68 historic streams. A variety of human activities, including coal mining, logging, agriculture, gas/oil exploration, and land development have contributed to the species decline; however, coal mining activities represent the most imminent and significant source of these threats, especially within the North and Middle forks of the Kentucky River basin (Thomas, 2008; Floyd and Thomas, 2010). Based on its decline and the magnitude and imminence of its threats, the U.S. Fish and Wildlife Service (USFWS) determined that the Kentucky Arrow Darter warrants listing under the Endangered Species Act. It is currently a Candidate for Federal Listing based on its inclusion in the USFWS Candidate Notice of Review published in the Federal Register on November 10, 2010.

In 2008 the KDFWR partnered with Conservation Fisheries, Inc. (CFI) to develop successful spawning protocols and produce the offspring needed to re-establish extirpated populations within the species' historic range. Spawning Kentucky Arrow Darters in captivity has been met with varying degrees of success, but overall poor larval survivorship (Ruble et al., 2010; Petty et al., 2011 and 2012). Limited numbers of captive spawned individuals were released into Sugar Creek (South Fork Kentucky River – Red Bird River drainage) in 2009 and 2011. In 2012, CFI attempted to correct warm water temperatures and gas interstitial movement through spawning substrates for normal egg survivorship leading to a higher larval survivorship (Petty, 2013). The dramatic increase in production allowed more individuals to be released in Long Fork in 2012 than previously released in Sugar Creek. This report summarizes monitoring efforts following the releases to determine the feasibility of re-establishing viable populations of this species within its former range.

## METHODS AND MATERIALS

### Brood Source

Adult individuals were collected for brood stock from Big Double Creek, a third-order tributary of the Red Bird River (South Fork Kentucky River drainage) in the Central Appalachian Ecoregion of the Cumberland Plateau. The Big Double Creek watershed has an area of approximately 8.5 square miles and lies entirely within the proclamation boundaries of the Daniel Boone National Forest (DBNF). This stream was chosen as the source for brood stock because it is one of several streams within the Red Bird River drainage that supports the healthiest Kentucky Arrow Darter populations and it is on public land.



## **Recipient Stream**

Long Fork, a first-order tributary of Hector Branch approximately 9.8 river km upstream of Big Double Creek, was chosen as the recipient stream for captive-spawned Kentucky Arrow Darters (Figure 1). The watershed has an area of approximately 1.4 square miles and lies entirely within the DBNF. Like most streams in the Red Bird River Basin, Long Fork is characterized by moderate to steep gradients with predominantly bedrock, cobble, and boulder substrates. This stream was chosen to receive propagated individuals because: 1) it was within the historic range of the species and within close proximity to (but apparently isolated from) the brood source (Big Double Creek); 2) it has good water quality, suitable habitat, and is within the DBNF; and 3) available survey data indicated no pre-existing population. These are all important criteria that must be met in order to improve the likelihood of successful population establishment through reintroduction (George et al., 2009). Sugar Creek was considered unsuitable following the discovery of a pre-existing population after stocking began in 2009.

## **Monitoring Methods**

Prior to stocking, all propagated fish were injected with Northwest Marine Technologies' VIE (Visible Implant Elastomer) tags to allow for mark-recapture evaluation of survival and movements of the reintroduced population. Following each release of tagged juveniles, periodic surveys were conducted in Long Fork using a 10' X 6' (1/8" mesh) seine, visual surveys, and dip nets within the release section, as well as arbitrarily chosen distances upstream and downstream of that section (Figure 2). All fishes captured were identified, enumerated, and released on site. Each sampling effort was recorded with GPS coordinates at start and stop points. The following data were recorded for each Kentucky Arrow Darter captured: tag presence, color, and location; total length (TL); sex (if possible); capture location (GPS coordinates); capture depth; substrate composition and flow (descriptive).

## **PRELIMINARY RESULTS AND DISCUSSION**

### **Accomplishments as of 31 December 2012**

A total of 829 captive-spawned Kentucky Arrow Darters were released into Long Fork in 2012, for the purpose of restoring a naturally-reproducing population. Fish community composition in Long Fork based on 2012 sampling is summarized in Table 1.

### *Monitoring Results Following 2012 Stocking*

On 14 August 2012, 751 tagged juveniles were released within a 1.7 km section beginning approximately 100 stream km above the confluence with Hector Branch. The



size range of individuals released on 14 August 2012 was 35-72 mm total length (TL), with most in the 50-55 mm TL range (Figure 3) (Petty 2013).

At day 56 (9 October 2012), surveys were conducted within the stocking reach by kick-sets with a seine and dip nets around cobble and boulders in pools and runs. We worked from the mouth of Long Fork upstream to Long Fork Rd. crossing (1.5 km). Water levels were low and clear, but the stream was flowing. Although captive darters have been observed to reject elastomer tags at a low frequency shortly after implantation (C. Ruble, CFI, pers. comm.), 18 (2.4%) tagged Kentucky Arrow Darters were encountered with 100 % tag retention. A total of 78 tagged juveniles were released into Long Fork on the same day immediately following the survey effort. These individuals were marked with a red VIE tag on the left dorsal side of the body.

At day 168 (29 January 2013) surveys were conducted using a seine, dip nets, and visual inspection. We captured 47 (5.7%) of the tagged fish; most were found in pools (~ 8" to 24" deep) with mixed-sized rock substrate with exposed areas of bedrock and often with some marginal cover (e.g., undercut or tree roots). These individuals appeared to be in very good condition. We observed an abundance of aquatic insect larvae actively swimming over the substrate, suggesting food resources were plentiful. Interestingly, our capture rates increased as we progressed farther upstream. This pattern is consistent with pre-spawning movements of adults observed in other Red Bird River tributaries. No individuals were observed in the lowermost reach of Long Fork or its confluence with Hector Branch. Our catch rate was 2-3 individuals per pool below the mid-point of the release section, increasing to 4-6 per pool above the midpoint. Most individuals were still too small to spawn this spring; however, some of the larger females appeared to be developing ova (becoming gravid) and males had developed red color on the body and fins typical of breeding condition. Although these results are preliminary, they are encouraging.

## **Conclusions and Recommendations**

Monitoring efforts so far have confirmed the survival of tagged Kentucky Arrow Darters released into Long Fork. High capture rates suggest that Long Fork is an appropriate recipient stream for the Kentucky Arrow Darter. The appearance and condition of some individuals (e.g., females becoming gravid and males developing adult color) suggests the possibility of natural reproduction in 2013. Surveys will continue on a seasonal basis in Long Fork during 2013.

The following actions are recommended for the continuation of the Kentucky Arrow Darter reintroduction project in Long Fork:

1. Continue annual releases, but scale back hatchery production and dedicate more effort to monitoring individuals released into Long Fork.
2. Monitoring using invasive methods (e.g., seining) should be conducted only during summer, fall, and winter to avoid impacts to spawning, nests/eggs, and

early larval development. During the spring spawning period (mid-March through June), non-invasive survey methods (e.g., snorkeling or visual observation) should be used to document spawning activity.

3. Standardize the monitoring effort. Develop an abundance index from natural populations (range and average) to serve as a basis for comparison with the reintroduced population.
4. Commit to a long-term project. Other non-game fish restoration attempts have shown that it takes several years to document success when stocking relatively limited numbers of individuals, particularly small species that are short-lived and cryptic (Shute et al., 2005).
5. Once the presence or increase in numbers of Kentucky Arrow Darters is consistently documented in the study stream, further evaluation of success should include growth and condition, movement of tagged individuals, and genetic diversity of surviving individuals.

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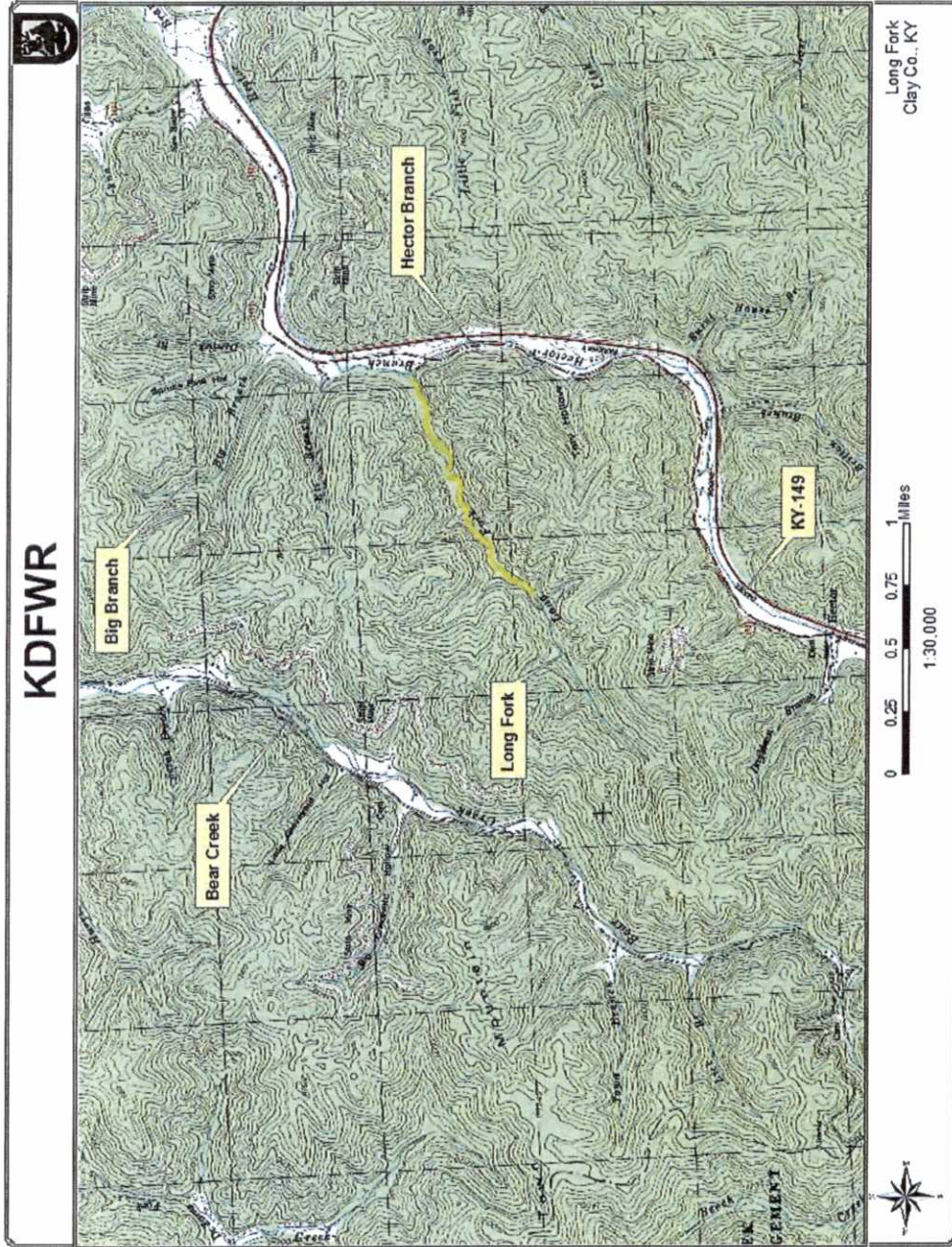


Figure 1. Study area for Kentucky Arrow Darter reintroduction effort showing the location of Long Fork, a tributary of Hector Branch in the lower Red Bird River drainage, Clay County. Propagated, VIE-tagged individuals were released in the lower half of Long Fork (yellow colored line).





Figure 2. CFI and KDFWR staff conducting mark-recapture surveys in Long Fork, Clay Co., KY. CFI photo.



Figure 3. Juvenile Kentucky Arrow Darter marked with a Visible Implant Elastomer (VIE) tag (red, left dorso-lateral side) to prior to stocking in Long Fork, P. L. Rakes photo.

Table 1. Fishes collected from Long Fork in 2012. Baseline data was collected in April and June. Surveys following the release of VIE-tagged Kentucky arrow darters was conducted in October 2012 and January 2013.

Family	2012		
Scientific name	April	June	October
<b>Cyprinidae</b>			
<i>Chrosomus erythrogaster</i>	X	X	X
<i>Luxilus chrysocephalus</i>			X
<i>Semotilus atromaculatus</i>	X	X	X
<b>Percidae</b>			
<i>Etheostoma caeruleum</i>			X
<i>Etheostoma flabellare</i>	X	X	X
<i>Etheostoma spilotum</i>			X