

**Captive Propagation and Reintroduction  
of the Cumberland Darter, *Etheostoma susanae*,  
in the Upper Cumberland River Drainage of Kentucky**

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Conservation Fisheries, Inc.  
Introduction from Project Grant Agreement by Matt Thomas  
Kentucky Department of Fish and Wildlife Resources**

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*Interim Performance Report to:*  
**Kentucky Department of Fish and Wildlife Resources  
Contract No. PON2 660 0900011426 1  
Summary of services performed from 12/15/08 – 12/31/09**



**Cumberland darter (*Etheostoma susanae*). Photo by J.R. Shute.**

## INTRODUCTION

The Cumberland darter, *Etheostoma susanae*, has a limited range in the upper Cumberland River drainage, most of which is in Kentucky. A proposed rule is currently in review to federally list this species as endangered, because of recent range curtailment and fragmentation resulting from habitat degradation. Conservation Fisheries, Inc. (CFI), in cooperation with Kentucky Department of Fish & Wildlife (KDFWR), proposes to develop captive propagation protocols for reintroduction of the Cumberland Darter into streams within its native range to restore populations that have been extirpated. Because of the apparent rarity of this species, captive propagation and reintroduction is considered an appropriate tool for its recovery and eventual delisting. Propagated individuals will be released within the watershed from which brood stock are taken, to avoid mixing potentially unique evolutionary lineages. Reintroduction sites will be chosen where habitat conditions are suitable and there is some level of protection (e.g., within wildlife management area or national forest boundaries). Survivability and movement patterns of released fish will be assessed through mark-recapture methods and through periodic monitoring using non-invasive methods, such as visual census techniques.

Captive propagation and reintroduction are considered appropriate tools for the recovery and eventual delisting of the Cumberland darter. Although the life history of the johnny darter (*Etheostoma nigrum*) has been relatively well studied, details of the reproductive biology (e.g., spawning behavior) of the Cumberland darter is unknown, although likely to be similar. Identification of critical habitat and other environmental conditions necessary for spawning to occur is essential to the recovery of this species. Restoration of extirpated populations through reintroduction is considered a necessary step to remove the Cumberland darter from the Endangered and Threatened Wildlife list.

## METHODS

Cumberland darters were collected with a fine mesh seine, mainly by downstream hauls over bedrock with sand patches. Fish were held in minnow buckets in the stream until packed with oxygen in plastic bags in coolers for transportation back to the CFI facility in Knoxville. The fish were housed in three separate 76 liter tanks, divided up into two groups of ten and one group of eleven. These tanks were part of a larger 750 liter multi-tank system similar to others currently used to house and propagate other fish species at CFI. Filtration included individual tank sponge filters and airstones as well as system filters (for multiple redundancy back-up); internal tank circulation was enhanced with small submersible powerhead pumps. A 15kW generator insured back-up power for essential life support functions for the entire facility in the event of an electrical outage. The system was salted to a 2.5 ppt solution to aid in acclimation and fight off parasites and disease. Abundant cover was provided in the form of slab rocks, tiles, and black plastic slabs on a fine gravel and sand substrate. Fish were provided live chopped blackworms and live *Ceriodaphnia* as a first food. Winter conditioning included reduction of water temperatures to as low as 4-5°C and photoperiod shortened to 9 hours of light. Reproductive condition was induced by gradually increasing water temperatures, photoperiod, and food quantity offered, in concert with natural seasonal changes.

Tiles and thin slab rocks were set up through the Cumberland darter aquaria with just enough space under them for a male to set up territory for breeding. Potential nesting cavities were checked weekly for eggs, and if found, the entire slab with eggs attached was removed to incubate in a tray so that development could be tracked. As larvae hatched they were fed *Brachionus* rotifers and early instars of *Ceriodaphnia dubia*. Newly hatched brine shrimp *Artemia* nauplii were added when larvae were large enough eat them. As larvae transformed into juveniles they were moved to a 76 liter tank and fed chopped blackworms and adult *Ceriodaphnia*.

## RESULTS AND DISCUSSION

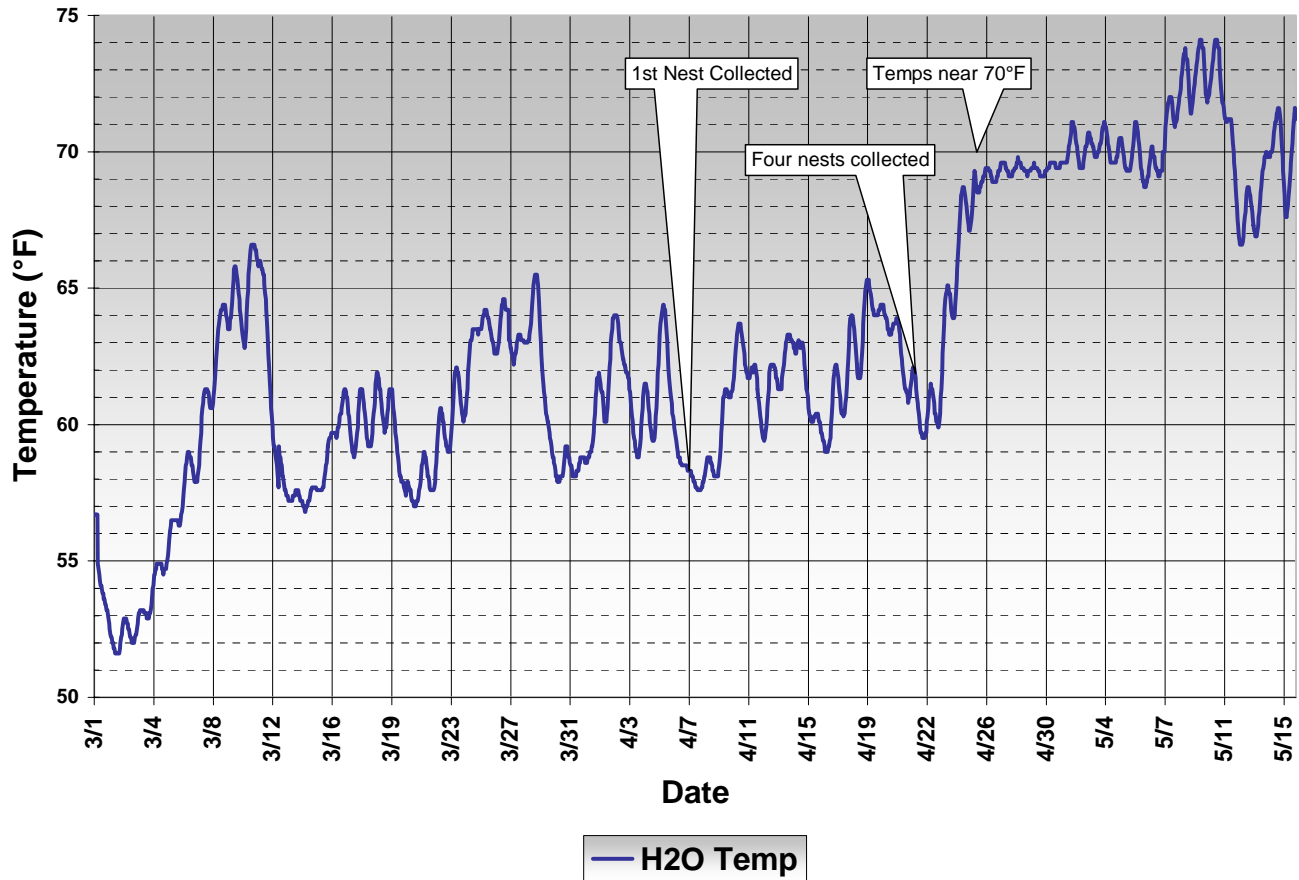
A total of 31 Cumberland darters were collected for broodstock on 18 December 2008 from Barren Fork, just above the Taylor Ridge Rd crossing, McCreary County, Kentucky (N36.77565 W84.4359388). Nearly all individuals were small (<40 mm TL) presumed young-of-the-year (YOYs). The fish acclimated quickly to captivity, immediately feeding on live *Ceriodaphnia* and *Artemia* nauplii. The larger, older darters tended to remain under cover most of the time, whereas smaller individuals tended to remain out in the open, often over sandy areas. As winter photoperiod and water temperatures progressed, nearly all began to stay under cover.

Following observations of darkly pigmented males (heads and fins) defending cavities under slabs, weekly checks for eggs were initiated on 7 April 2009, at which time the first nest was collected from tank #2. Table 1 summarizes all egg collections. Figure 1 illustrates water temperatures before and during the spawning season. Eggs were golden-amber with a large oil droplet and slightly orange yolk on eyed embryos. Size was 1.6-1.8mm diameter. Two eggs were removed from the tile to track development. The two separated eggs were at a very early developmental stage, late morulas to early blastulas. Given that there were also eyed eggs in the recovered clutch, there either were multiple female additions or multiple spawns from the same female spanning several days. On 8 April the separated eggs were at the hemispherical blastula stage. Development was slow in sub-60° F water. By 11 April the separated egg pair had fully developed somites, eyes, and brain divisions. Pigmentation was sparse with only a few melanocytes scattered on the yolk of the embryos. Both eggs still exhibited a flattened side with thickened outer ring where originally attached to the rock. By 20 April all eggs from this first clutch (ES09-01) had hatched. The older, more developed larvae were observed sitting upright, but still had heavy yolk sacs, and would not yet feed when offered rotifers and small *Ceriodaphnia*.

A second small clutch was removed from a different tank (#3) on 14 April, but none of these eggs were observed to develop and all were quickly lost. On 21 April four clutches were removed from tanks #2 and #3 containing over 140 eggs total. These developed normally until day four at which point many mortalities were observed which continued until all the eggs were lost by 1 May. No bacterial or fungal infections were evident and all the losses followed an unusual warm period outside that caused water temperatures to suddenly rise from 60°F to near 70°F in only two days. A final clutch that was recovered on 6 May developed normally and hatched with excellent survivorship to the juvenile stage (90%). Overall survivorship of the first two and last nests was an acceptable 69%.

Date	Tank 1	Tank 2	Tank 3	Notes
4/7/09	0	40	0	ES09-01
4/10/09				1 egg lost
4/13/09				1 egg lost (separated egg)
4/14/09	0	0	8	ES09-02 ; Eggs starting to hatch from ES09-01
4/15/09				Suspect ES09-02 infertile
4/16/09				All ES09-02 lost
4/17/09				-4 deformed larvae
4/20/09				All eggs hatch from ES09-01, started feeding
4/21/09		7, 37	80, 17	ES09-03, 04, 05, 06 eggs at various stages
4/25/09				Lots of losses, temps over 70!!
5/1/09				ES09-03, 04, 05, 06 completely lost – high temp
5/6/09		39		ES09-07 eyed
5/7/09				27 larvae dropped to tank from ES09-01
5/26/09				35 larvae dropped to tank from ES09-07
<b>Totals</b>	<b>0</b>	<b>123</b>	<b>105</b>	<b>60 juveniles released 8/25/09</b>

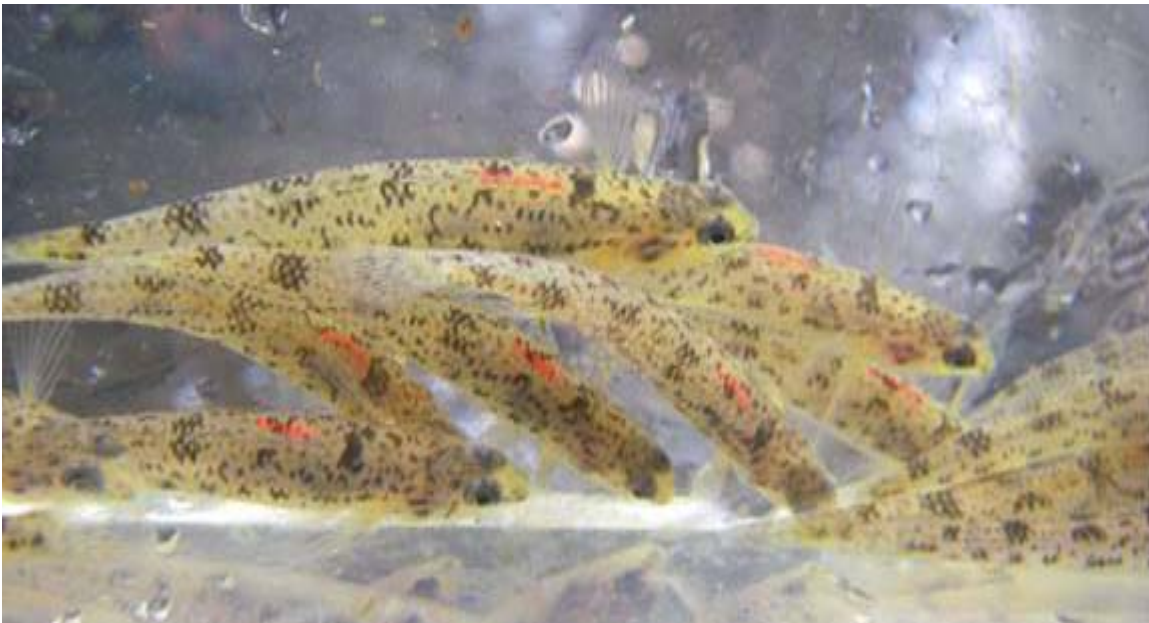
**Table 2. Cumberland darter egg collections and larval development summary.**



**Figure 1. Temperature data at Conservation Fisheries, Inc. March 1-May 15, 2009.**

From 228 eggs produced by the Cumberland darters, 60 larvae were successfully raised to the juvenile stage, yielding 26% overall survival. Most of the losses occurred among the developing eggs collected on 21 April and appeared to be due to the suddenly warm period in April that produced what was very likely an excessively stressful nearly 10°F rise in water temperature within 48 hours. Embryonic development was observed in the eggs in nests ES09-03, -04, -05, and -06, indicating they were indeed fertile (unlike ES09-02). The larvae that had hatched prior to the increase in temperature were not affected. The last clutch of eggs spawned, which was consistently subjected to warmer temperatures in early May, exhibited the highest survivorship of all. This suggested that 70+°F water temperatures were not problematic for egg incubation and survival; instead, either the rapidity of the increase or the particular temperature range spanned by the change was excessively stressful.

On 25 August 2009 the 60 juvenile propagated Cumberland darters were released to Cogur Creek, McCreary County Kentucky (N36.80136 W84.39967). The darters ranged approximately 30-50 mm TL at release. A week or so prior to release all the fish were marked with a Northwest Marine Technologies visual implant elastomer (VIE) tag (red, dorso-lateral left side of dorsal fin) at CFI (Figure 2). Cogur Creek (Figure 3) is the next significant tributary to Indian Creek downstream of Barren Fork, where the parental stock was collected. Although Cogur Creek apparently lacked Cumberland darters prior to stocking, habitat appeared suitable, and two other commonly associated darter species were present (rainbow and stripetail). The release of the low number of propagated fish was intended to test potential survivorship and dispersal. ~~No follow-up monitoring surveys were performed in 2009.~~



**Figure 2. Tagged propagated Cumberland darters in transport bag immediately prior to release, August 25, 2009.**





**Figure 3. Cogur Creek just upstream of KY 1045/Beulah Heights Rd, McCreary County, KY.**

### **ACKNOWLEDGEMENTS**

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