

Propagation and Reintroduction of Wounded Darters, *Etheostoma vulneratum*, in the Cheoah River, North Carolina

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Conservation Fisheries, Inc.
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NC DIVISION OF WATER RESOURCES, CHEOAH FUND
RALEIGH, NORTH CAROLINA
SUMMARY OF SERVICES PERFORMED
January 1, 2012—December 31, 2012
Contract No. DENR 3481



Wounded darters observed in Cheoah River 10 August 2011. All with red tags right side of dorsum near first dorsal fin, released 3 November 2010.

Introduction

On January 25, 2005, the Federal Energy Regulatory Commission (FERC) issued an Order Approving Settlement and Issuing a New License for the Tapoco Hydroelectric Project (FERC No. 2169). The Tapoco Project Relicensing Settlement Agreement (RSA), filed with FERC in May 2004, established two funds, the Tallassee Fund and the North Carolina Resource Management and Enhancement Fund (now Cheoah Fund) to support restoration, recovery, and conservation efforts in Tennessee and North Carolina, respectively. In accordance with the RSA, for the next 40 years seasonal and base flow regimens are being regulated in the Cheoah River, along with gravel augmentations and efforts to restore indigenous fish and other fauna that were extirpated when the river was bypassed by a hydroelectric diversion flume. Conservation Fisheries, Inc. (CFI) was contracted to develop captive propagation techniques for several species which are too difficult to translocate in significant numbers. These include the stonecat (*Noturus flavus*), the sicklefin redhorse (*Moxostoma sp.*), and the wounded darter (*Etheostoma vulneratum*). This report summarizes ongoing propagation and restoration efforts in the project through 23 October 2012.

Materials and Methods

All monitoring and collections of fish were performed by snorkeling. Fish were captured with 10 inch hand nets or by setting a fine mesh seine. Fish were transported inside plastic bags with water from the river and topped off with oxygen, then placed in insulated coolers. All were transported back to the CFI facility in Knoxville where they were slowly acclimated into 76-liter aquaria, part of a ~760-liter multi-aquarium recirculating system (similar to other systems at CFI, see Rakes et al. 1999). Each tank was set up with appropriate cover items consisting of ceramic slates, PVC pipes, and natural stone slabs. Filtration included individual tank sponge filters as well as system filters (for multiple redundancy back-up). A 15kW generator insured back-up power for essential life support functions for the entire facility in the event of an electrical outage.

Adult fish were fed live blackworms, mosquito larvae and *Daphnia* when they were first collected. They were later supplemented with frozen bloodworms after they had properly acclimated to captivity. The recirculating system was treated with salt (~2%) to reduce stress and fight parasitic infections. Beginning in late fall 2011, a portion of the adults kept as breeders were winter-conditioned from November through February through water temperature and photoperiod manipulation in preparation for spawning. 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. An astronomic timer controlled artificial lighting inside the facility with automated daily adjustments to closely mimic seasonally changing daylength. Food quantities were provided dependent upon water temperature and the accompanying activity levels and willingness of the fish to feed.

The darters were distributed into 76 liter 'breeder' tanks and each tank was ultimately set up with 2 -3 males and 2-3 females each. Spawning sites for each tank were set up, each consisting of two tile slates positioned to form a wedge. The males

were periodically shuffled to new tanks to increase genetic diversity of the propagates. From mid April through mid-July, nests were collected from the breeding tanks weekly. Tiles with eggs were removed and placed in an empty egg incubation tank (76 liter) for passive capture of the larvae as they hatched and swam up. An overflow situated at the back of the tank drained into a black oval rubber tub (~50 x 70 cm / ~15 cm depth) designed to capture pelagic darter larvae. Hatching larvae were monitored by checking the overflow collection tub daily. Once they were fully pelagic in the collection tub they were transferred to a larger 'rearing tub' measuring 69 cm diameter by 33 cm deep.

The rearing of tiny pelagic larvae required a balance between providing adequate zooplanktonic food densities while simultaneously maintaining adequate water quality and avoiding excessive larval densities. The rearing tub was set up with a reservoir, timer, and solenoid for constant food dispersal during the day. The feeding reservoir was filled with water from the system, then with a portion of *Brachionus* rotifers, Nanno 3600™ *Nannochloropsis* sp. (Instant Algae® produced by Reed Marineculture Inc.), and *Ceriodaphnia dubia* neonates. Newly hatched brine shrimp *Artemia* nauplii and grindal worms were added to the mix when larvae were large enough eat them. To supplement the reservoir feeding, commercial larval feed/powder was lightly dusted on top of the rearing tub several times daily. Routine cleaning of the feeding reservoir and rearing tub was necessary to maintain water quality and prevent unwanted bacterial and/or fungal growths on uneaten food and waste. The use of aquatic snails also aided in maintaining a clean environment for the larvae by eating excess powders.

As larvae grew and transformed to benthic juveniles they were separated from younger larvae and transferred to empty tanks for further grow out. Feeding of these juveniles was supplemented with larger food items such as chopped blackworms, and later frozen chopped bloodworms. Brine shrimp nauplii and *Ceriodaphnia dubia* adults were also offered for smaller individuals. Prior to release all of the young wounded darters were tagged using Visible Implant Elastomer (VIE) tags from Northwest Marine Technologies. A few sample individuals were screened for disease organisms at the U.S. Fish and Wildlife Service Warm Springs (GA) Fish Health Center prior to release.

Results

All of the oldest (2007/2009 wild collected) wounded darter broodstock were released prior to the 2012 spawning season. Consequently, propagation efforts in spring 2012 commenced with 31 "new" wounded darter breeders (10 were 2010 captive propagates; 21 were 2011 wild collected). This breeding group included fish propagated in 2010 that were approaching two yrs of age and fish collected in 2011 that included females that were mostly young adults or subadults (1⁺ yr or less) when collected and ultimately proved too young to breed during 2012 production. Since sexual maturity does not occur until age 2, only a few of the females became gravid and egg production was reduced by half in comparison to previous years.

The total number of eggs spawned was estimated to be ~540. A total of ~123 larvae were transferred from the catch tub to the feeding tub. Approximately 75 juveniles were transferred out into tanks, yielding a ~61% survival rate from pelagic larvae to benthic juveniles to be tagged and released to the Cheoah River this spring 2013. The less than 130 young collected seemed to indicate participation by either only a few females or

else very few eggs were produced by each individual. No snorkel surveys were conducted in 2012 in the Cheoah River.

Discussion

Monitoring results in 2011 provided documentation of survival of stocked fish over winter but failed to detect evidence of reproduction and recruitment. Such observations are not really expected yet (before 2012 or 2013 based on our experience with the closely related boulder darter), but will certainly be watched for during this year's monitoring.

Wounded darter propagation protocols in 2012 were little changed from those we have developed and refined since 1995 for a close relative, the boulder darter, *Etheostoma wapiti* (Rakes et al. 1999). The wounded darters spawned for approximately 14 weeks, from mid-April through mid-July. This was significantly shorter than the ~20 weeks of spawning noted in years past (2009—2011). Termination of spawning appeared to be linked to the age of the females collected in 2011. Sexual maturity does not occur until age 2 with average size being ~44 (females) – 48 (males) mm SL at age 2 with a life span of 4-5 yrs (Stiles 1972; Etnier and Starnes 1993).

It would appear that the females had not reached sexual maturity for this spawning season as they were likely less than 2 yrs old and less than 44 mm SL. Hopefully by 2013 more females will have reached reproductive maturity and an age of relatively high fecundity. These observations suggest that the most productive females are those at least 2 yrs of age. Hence, sufficiently large, older females are key to successful captive spawning.

Future efforts will include refining all protocols to increase egg and early larval survivorship, experimenting with breeding ratios and breeding population sizes, and determining maximum reproductive age and age/size-related egg production. We will continue to acquire additional broodstock to further diversify genetic variation of propagated darters and in the reintroduced population. We will greatly expand snorkel monitoring efforts in 2013 to determine survivorship and status of the reintroduced population, with an emphasis later in the year to detect evidence of wild reproduction.

Date	Field #	Locality	R Mi	# released	Lat	Long
8/19/08	CFI08-073	Adjacent to Santeetlah Baptist Church	7.4	120	N35.3895805	W83.8650722
8/19/08	CFI08-074	Just below dam, 50 m above flume crossing	8.75	123	N35.3840444	W83.8753111
8/19/08	CFI08-075	Pull-off ~1/10 mi above Little Cochran Creek	5.2	99	N35.4071305	W83.8860194
4/28/09	CFI09-015	Lowest bridge above TAPOCO lodge	1.6	73	N35.4384693	W83.9190314
4/28/09	CFI09-016	Mouth of Yellow Crk	4.4	79	N35.4189563	W83.8894662
10/1/09	CFI09-088	Adjacent to Santeetlah Baptist Church	7.4	138	N35.3895805	W83.8650722
10/1/09	CFI09-090	Mouth of Yellow Crk	4.4	250	N35.4189563	W83.8894662
11/3/10	CFI10-132	Lowest bridge above TAPOCO lodge	1.6	99	N35.4384693	W83.9190314
11/3/10	CFI10-133	Mouth of Yellow Crk	4.4	100	N35.4189563	W83.8894662
11/3/10	CFI10-134	Adjacent to Santeetlah Baptist Church	7.4	101	N35.3895805	W83.8650722
4/10/12	CFI12-022	Lowest bridge above TAPOCO lodge	1.6	96	N35.4384693	W83.9190314
4/10/12	CFI12-023	Mouth of Yellow Crk	4.4	96	N35.4189563	W83.8894662
4/10/12	CFI12-024	Gravel intro site #4 below Rock Creek	5.7	131	N35.4018355	W83.8821713

TOTAL RELEASED: 1,505

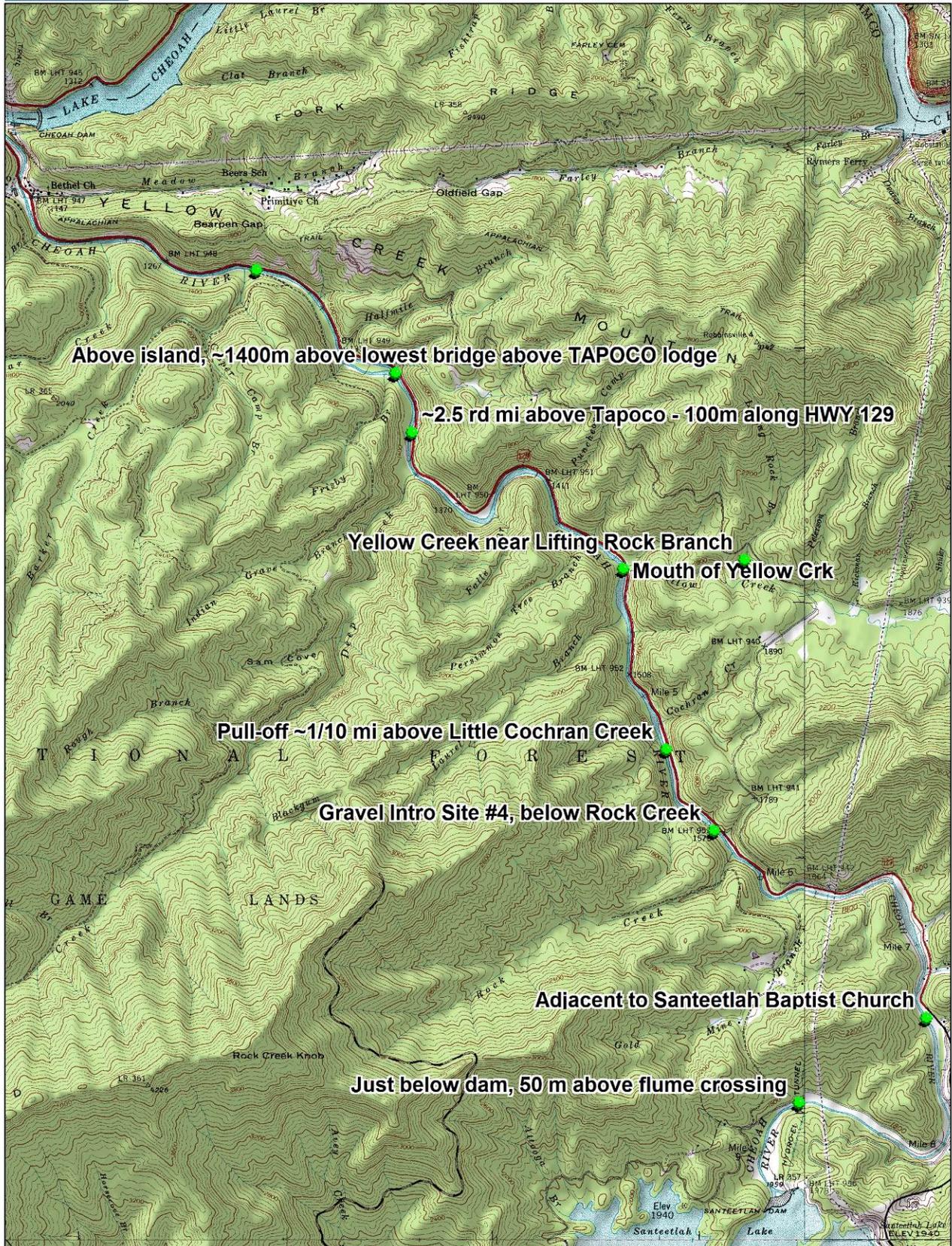
Table 1. Wounded darters released to Cheoah River 2008-2012.

Acknowledgements

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Literature Cited

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MN (5.3° W)



0 1/4 1/2 3/4 1 1 1/4 1 1/2 1 3/4 km

Data Zoom 12-0

Figure 1. Wounded darter release & survey sites on the Cheoah River 2008-2012.