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

by M.A. Petty, P.L. Rakes, C.L. Ruble, J.R. Shute, and R.A. Xiques
Conservation Fisheries, Inc.
October 24, 2013

FINAL REPORT TO:
NC DIVISION OF WATER RESOURCES, CHEOAH FUND
RALEIGH, NORTH CAROLINA
SUMMARY OF SERVICES PERFORMED
January 1, 2013—October 31, 2013
Contract No. DENR 3481

Wounded darters observed/captured in Cheoah River, 2 August 2013.

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. While some fauna can likely be restored via translocations of wild individuals, numerous fish species would be too difficult or costly to translocate in significant numbers. These include the stonecat (*Noturus flavus*), the sicklefin redhorse (*Moxostoma sp.*), the olive darter (*Percina squamata*), and the wounded darter (*Etheostoma vulneratum*). Conservation Fisheries, Inc. (CFI) was contracted in 2007 to develop captive propagation techniques for the wounded darter and attempt reintroductions and restoration of the species to the Cheoah River. This report summarizes propagation and restoration efforts for the project through 24 October 2013.

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 and/or were photographed in situ with a compact underwater camera. 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 ppt) to reduce stress and fight parasitic infections. Beginning in late fall 2012, 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 June, 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.

2013 Results

Propagation efforts in spring 2013 commenced with 31 wounded darter breeders (10 were 2010 captive propagates; 21 were 2011 wild collected). The total number of eggs spawned was estimated to be ~230. Hatching success was an extremely low ~10% with only 25 larvae transferred from the catch tub to the feeding tub. Approximately 16 juveniles were transferred out into tanks, yielding a ~64% survival rate from pelagic larvae to benthic juveniles.

The 61 young produced in 2012 were released 3 April 2013 into Cheoah River at mile 7.4 (release site) adjacent to the Santeetlah Baptist Church (Table 1; Figure 1). Snorkel surveys were conducted in 2013 at five different localities in the Cheoah River. Three wounded darters were observed on 2 August at the release site (CRM 7.4) and all

were propagates released in April (Table 2). No evidence of wild reproduction was observed.

Discussion

Wounded darter propagation protocols in 2013 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), and successfully employed in earlier years of this project. However, the wounded darters spawned for only approximately 11 weeks, from mid-April through June. This was significantly shorter than the ~20 weeks of spawning noted in the early years of the project (2008—2011). Early termination of spawning in 2012 (~14 weeks) initially appeared to be linked to the young age of the females collected in 2011. Sexual maturity in most wounded darters is not attained until age 2 when individuals exceed average sizes of ~44 (females) – 48 (males) mm SL; life span is an estimated 4-5 years (Stiles 1972; Etnier and Starnes 1993). It therefore appeared that the females had not reached full sexual maturity as they were less than 44 mm SL in 2012. By 2013 the broodstock females had reached reproductive maturity and ages/sizes of relatively high fecundity, however, production declined even further. These observations suggested that the age and reproductive maturity of the females was not the issue for poor spawning production in 2013, and perhaps in 2012 as well. We suspect that the breeders may have had tapeworm (*Bothiocephalus sp.*) infestations, given prior observations of this parasite in spotfin chubs (*Erimonax monachus*) collected from the Little Tennessee River system. Medication (Praziquantel) to "de-worm" the broodstock was administered in August and December 2011 and again in mid-June 2013 but the latter was unfortunately too late in the breeding season to be effective in improving reproductive condition of the breeders. We suspect that a reproductive life cycle of the parasite may be resident in the breeding system, with resulting reoccurrence following (only temporary) elimination of adult worms. All the broodstock and their 2013 young will be sacrificed for disease diagnosis later this year to hopefully resolve the question.

Monitoring results over the lifetime of this project (13 surveys; 36.25 p-hrs) have repeatedly provided documentation of survival of stocked fish over periods of months, including over winter, but have failed to detect evidence of reproduction and recruitment (Table 2). All wounded darters observed in monitoring surveys were spawned in captivity (based on tags or age/size class). Finding evidence of reproduction and recruitment was not really expected before 2012 or 2013 based on our experience with the closely related boulder darter, but might have been expected during this year's monitoring. Stiles (1972) noted that the wounded darter inhabits moderate to large rivers in areas of gentle to moderate current and is typically associated with boulder or fine cobble with coarse rubble substrates. Although substrate habitats in the Cheoah River are in the process of improving with ongoing gravel and substrate additions, the relatively high gradients and paucity of pool mesohabitats may be permanently unsuitable for survival of the weak-swimming pelagic larvae of wounded darters. With all these observations and issues considered, we recommend suspending efforts to propagate and restore wounded darters to the Cheoah River.

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
4/3/13	CFI13-010	Adjacent to Santeetlah Baptist Church	7.4	61	N35.3895805	W83.8650722
		TOTAL RELEASED:		**1,566**		

Table 1. Wounded darters released to Cheoah River 2008-2013.

Date	Field #	Locality	R Mi	# Observed	Person-hrs
4/28/09	CFI09-016	Mouth of Yellow Crk	4.4	0	1.5
7/23/09	CF09-051	Lowest bridge above TAPOCO lodge	1.6	1	2.5
10/1/09	CF09-089	Just below dam, 50 m above flume crossing	8.75	0	3
10/1/09	CF09-090	Mouth of Yellow Crk	4.4	1	2.25
7/22/10	CFI0-050	Adjacent to Santeetlah Baptist Church	7.4	2	5
7/22/10	CFI0-051	Mouth of Yellow Crk	4.4	3	3.75
8/10/11	CFI11-080	Lowest bridge above TAPOCO lodge	1.6	3	3.5
10/17/11	CFI11-125	1400m above lowest bridge above TAPOCO lodge	2.5	0	2.5
6/4/13	CFI13-034	Mouth of Yellow Crk	4.4	0	3
6/4/13	CFI13-035	Uppermost bridge NC1134-Santeetlah Rd	7.95	0	2
6/4/13	CFI13-036	Above bridge @ TAPOCO lodge	0.3	0	1
8/2/13	CFI13-068	Adjacent to Santeetlah Baptist Church	7.4	3	5.25
8/2/13	CFI13-069	Lowest bridge above TAPOCO lodge	1.6	0	1

Table 2. Surveys of Cheoah River 2009-2013; no evidence of wild reproduction.

Acknowledgements

Funding provided by North Carolina Division of Water Resources, Cheoah Fund. Field assistance thanks to Mark Cantrell (USFWS), as well as Steve Fraley and T. R Russ (NCWRC).

Literature Cited

Etnier, D.A. and W.C. Starnes. 1993. The Fishes of Tennessee. The Univ. of Tenn. Press. Knoxville.

Rakes, P.L., J.R. Shute and P.W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. Environmental Biology of Fishes. 55:31-42.

Stiles, R.A. 1972. The comparative ecology of three species of *Nothonotus* (Percidae-*Etheostoma*) in Tennessee's Little River. Ph.D. Dissert., Univ. Tenn., 97 p.

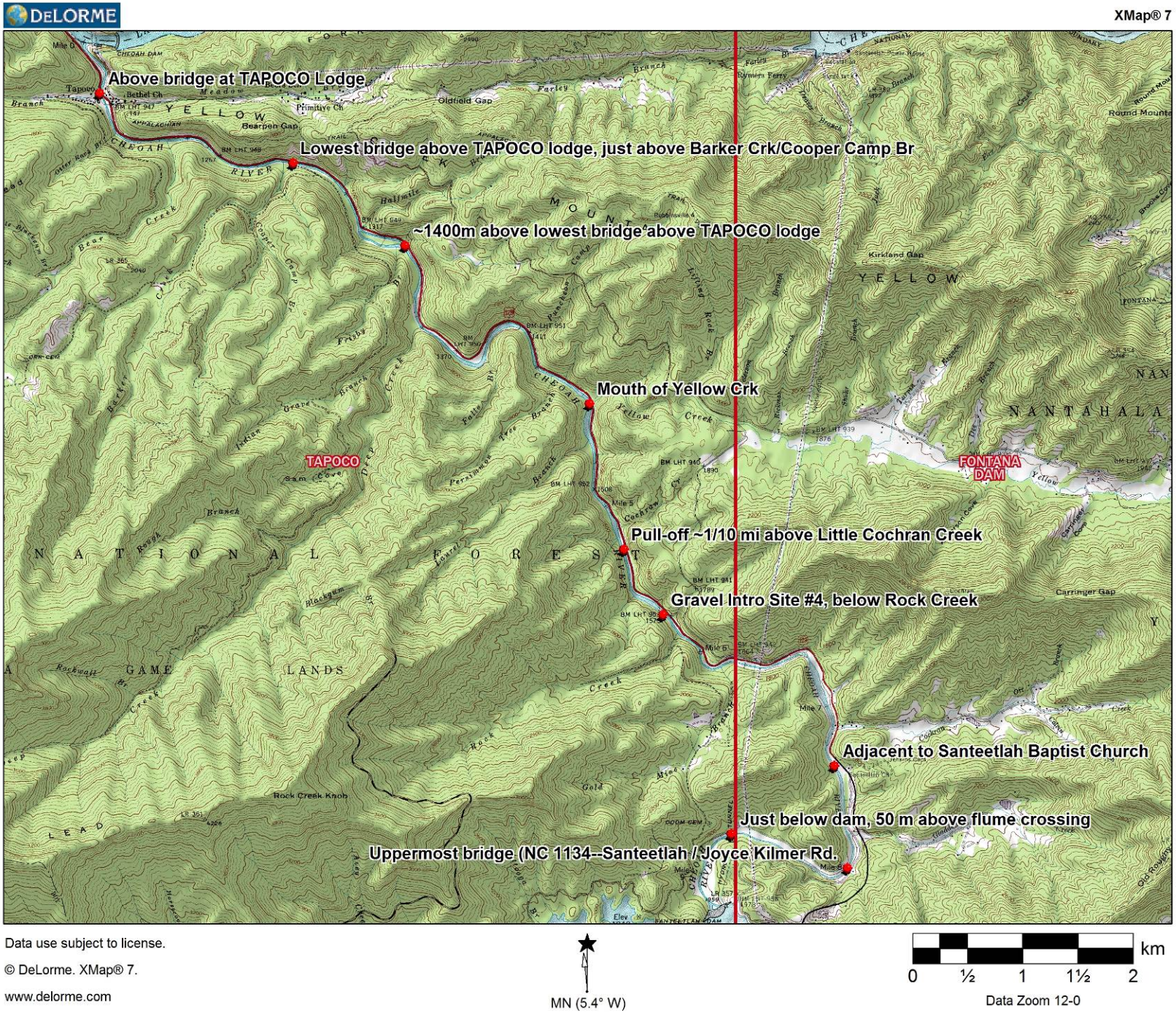

Figure 1. Wounded darter release & survey sites on the Cheoah River 2008-2013.