Captive Propagation and Reintroduction of the Kentucky Arrow Darter, *Etheostoma sagitta spilotum*, in the Upper Kentucky River Drainage of Kentucky

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Introduction from Project Grant Agreement by Matt Thomas
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Kentucky arrow darter (*Etheostoma sagitta spilotum*). Photo by J.R. Shute.

INTRODUCTION

The Kentucky Arrow Darter, *Etheostoma sagitta spilotum*, has a limited range in the upper Cumberland River drainage, most of which is in Kentucky. Recent analyses of morphological and genetic data have shown that *E. s. spilotum* and *E. s. sagitta* (Cumberland River drainage) represent distinct evolutionary lineages and should be treated as separate management units for conservation management purposes. A status survey of *E. s. spilotum* in the Kentucky River basin has shown that populations have declined considerably during the past two decades. Kentucky Arrow Darters were detected in only 29 of 50 historic streams sampled in 2007 and 2008. This has led the U.S. Fish and Wildlife service to consider this taxon as a candidate for listing as threatened or endangered. Conservation Fisheries, Inc. (CFI), in cooperation with Kentucky Department of Fish & Wildlife, proposes to develop captive propagation protocols for reintroduction of the Kentucky Arrow Darter into streams within its native range to restore populations that have been extirpated. 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.

Details of the reproductive biology (e.g., spawning behavior) of the Kentucky Arrow Darter and other environmental conditions necessary for spawning to occur are poorly known. Captive propagation and reintroduction is considered warranted to prevent the Kentucky Arrow Darter from being added to the federal list of Endangered and Threatened Wildlife.

METHODS

All brood stock were collected with a fine mesh seine. The fish were transported to CFI in oxygen-filled bags of water in coolers and acclimated to aquaria. During the winter months, like all the fish at CFI, the Kentucky arrow darters were maintained and conditioned through water temperature and photoperiod manipulation in preparation for attempted captive spawning. Photoperiod was controlled with an astronomic timer to mimic natural winter lighting conditions with slowly decreasing, then increasing day length on a schedule comparable to the actual season. Temperatures were similarly manipulated. By using ventilation of outdoor air water temperatures were maintained as low as 35°F to "winter condition" the fish. Fish were housed in a 150 L tank within a larger 500 L recirculating system. Food quantities were provided dependent upon water temperature and the accompanying activity levels and willingness of the fish to feed. All males were separated from females due to aggression, and only introduced singly into the breeding tank for one day at a time. Three males were rotated for breeding to maximize genetic diversity. After breeding was observed, most eggs were allowed to remain in situ to continue development undisturbed. A gravel siphon was used to collect some eggs in order to observe egg development and estimate hatch time after spawning events. These eggs were incubated in a Petri dish in a 4 L plastic flow-through tray nested over a 76 L aquarium.

The 150 L breeding tank drained from the overflow of the adult tank to an oval catch tub measuring 63.5 cm X 53 cm and 18 cm deep with a 2 cm diameter PVC central standpipe drain (Figure 1). Hatching larvae were monitored by checking the overflow collection tub daily from April through June. Once the larvae hatched and swam up to be collected by the overflow into the catch tub, they were then removed with a baster and transferred to a larger rearing tub measuring 70 cm wide and 33 cm deep. This tub also had a PVC standpipe widened at the top with a 250-500 µ screen around it. The standpipe was positioned in the center of the tub with a flexible air wand around it to prevent larval drift into the overflow screen.

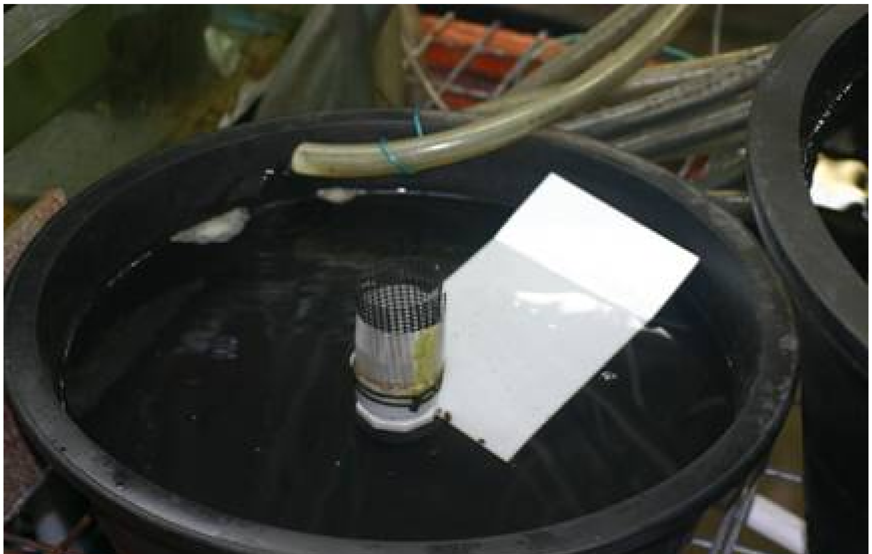

Figure 1. Typical catch tub for collecting larvae from adult tank

The rearing of the 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 consisted of an 11 L opaque plastic tub with a faucet installed at the bottom. A solenoid controlled releases of food, turned on and off by a timer that was set up to dose for 8-10 seconds every 2 minutes during daylight hours. 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 ReedMarineculture Inc.), and *Ceriodaphnia dubia* neonates.

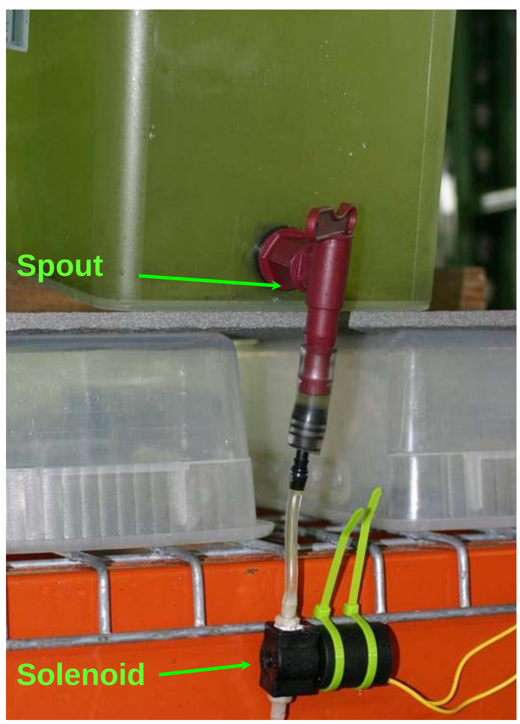

Figure 2. Feeding reservoir and solenoid.

Newly hatched brine shrimp *Artemia* nauplii 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. The powder consisted of equal parts A.P.R. (Artificial Plankton – Rotifer) by Ocean Star International, Larval AP 100 (<100 µ and 100-150 µ), by Zeigler Bros., Inc., and spirulina by Salt Creek, Inc. 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. This was done with either a large baster, or a small flexible tube siphoning into a bucket, so any larvae that were captured in the process were visible and could be returned to the rearing tub. Snails were also added to help clean up excess food and waste. As larvae transformed into juveniles they were moved to 76 liter tanks for further grow out. With this transformation to juvenile stage new foods were also added, including chopped blackworms, bloodworms, and adult *Ceriodaphnia*.

RESULTS AND DISCUSSION

Two females, two males, and one juvenile Kentucky arrow darter were collected on December 18th, 2008 from Big Double Creek, Clay County, Kentucky (N 37.0904666 W 83.6022722). These were initially housed together in the 150 L tank, but the males soon proved too aggressive towards each other and were separated. Even after separation, the one male left in the tank was still very aggressive towards the females. On March 11th, 2009, two gravid females, a breeding condition male, and 6 juveniles were collected from also collected from Big Double Creek, near where the original group was taken. The two females were added to the breeding tank with the one male (to be referred to as male #1) and two females collected in December. Immediately the male followed the most gravid new female in a nonaggressive "solicitous" manner and behaved almost gently towards both the new females. This contrasted markedly with the earlier aggressive behavior towards the females that he had been housed with since December (which did not show signs of being gravid). The next day, March 12th, active spawns were observed—and some videotaped—between the male and the newly introduced gravid female. The female would select an area of fine sand and bury herself in it. The male would mount beside her and a brief vibration would take place where they would bury together deeper into the substrate and then exit. This happened multiple times throughout the day. By the end of the day this female appeared far less gravid and started avoiding the male. On March 13th, she was moved to an empty tank to recover. The male now pursued the second gravid female brought in on the

Figure 3. Kentucky arrow darters spawning.

11th and spawned throughout the day. After this spawning the male was removed to allow the females time to recover.

On March 16th the substrate in the area of spawning was sifted through with a fork to see if eggs were deposited in clumps. Nothing was found with this method, so the substrate was then siphoned to collect individual eggs. A total of 71 eggs were collected, all but one fertile and developing. Eggs were about 1.6 -1.8mm in size. Eggs exhibited two distinct stages corresponding to the two days of spawning. Both ages were already eyed, but the older eggs were also heavily pigmented (Figure 4). This was unusually quick development for such relatively low temperatures (<60°F).

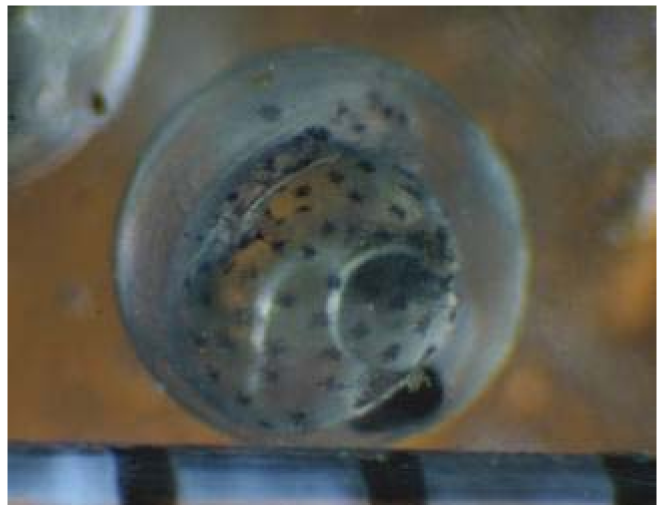

Figure 4. Four-day-old Kentucky arrow darter egg.

Additional fine substrate was added to the tank, along with a new male (#2) and the female separated out on the 12th. The new male and one of the females collected in December immediately began spawning. The March collected females also seemed receptive and were also observed spawning with the male. By the next day, March 17th, none of the females seemed receptive to spawning and the male was removed to allow the females to recover condition. On March 18th a small portion of the tank was vacuumed for eggs, recovering 37. The eggs' location revealed that Male #2 had been spawning in different locations in the tank compared to #1, in many cases unobserved. This might have been due to the new sand additions.

The female fish quickly recovered condition, so male #3 was added to the tank for two days. The switching of males in and out of the breeding tank continued with all three males in this manner until the females no longer seemed receptive. March 20th marked the first day of larval swim up from both the vacuumed eggs as well as the appearance of larvae in the capture tub. Table 1 summarizes larval transfers into the feeding and rearing tub from both the capture tub and egg incubation tray. By the beginning of April the males no longer seemed interested in breeding even though females seemed to be receptive still, as indicated by a presumed 'courtship solicitation' behavior consisting of shaking the head at a male. The last larvae were captured on April 19th, indicating the last spawns occurred around the 12th. Spawning appeared to peak about the 3rd week of March.

Development of eggs to hatch was surprisingly rapid at the incubation water temperatures in the upper 50s and low 60s°F (Figure 5). Only 7-8 days elapsed between initial spawning to swim up of the first larvae. Many other species of darters propagated at CFI have required two weeks or more to develop at the same temperatures. Larvae were small, but grew quickly. Measurements were not taken at this time as the potential number of larvae to be produced was still unknown and unnecessary mortality was avoided. If production is sufficient in the subsequent 2010 effort, larval development may be documented closely. Larvae began to settle and transform to juvenile stage at approximately 27-28 days after larval swim up.

Date	Tray	Catch	Notes
3/20/09	1	2	
3/21/09		1	some larvae in tray failing to develop/dying
3/23/09	4	4	
3/24/09		4	-1 dead on screen of feed tub
3/25/09	2	6	-1 egg in tray
3/26/09	51	23	
3/27/09	38	52	
3/28/09	3	48	
3/29/09		12	
3/30/09		27	-1 from tray, -8 from tub, bloated
3/31/09	3	25	-5 from tub 1; density too high now…
4/1/09		71	-4 from tub 1, still looking odd – Started tub 2
4/2/09		59	already in catch a.m. before lights were on
4/3/09		15	-~13 from tubs 1 & 2, start formalin
4/4/09		20	-2 from tub 1, starting to look better
4/5/09		11	-2 from tub 1, system needs water change
4/6/09		6	-3 from tub 2
4/7/09		4	
4/8/09		1	-3 from tub 2
4/10/09			-4 from tub 2
4/13/09			-4 from tub 2, -1 from tub 1
4/14/09		1	vacuumed tank, 58 bad eggs, 13 good, 2 larvae
larvae sizes different enough now for competition/cannibalism concerns			
4/15/09		2	
4/17/09		3	One larva benthic in tub 1, transferred out to tanks
112 larvae out of tub 1; unobserved losses = competition? cannibalism?			
4/18/09		3	
4/19/09		3	
4/21/09			-7 in tub 2 after quick warm up!
5/27/09			41 out of tub 2; must transfer sooner in future
Totals	102	403	~59 observed losses – 110 released 8/25/09

Table 1. Summary of Kentucky arrow darter larval transfers to the rearing tub.

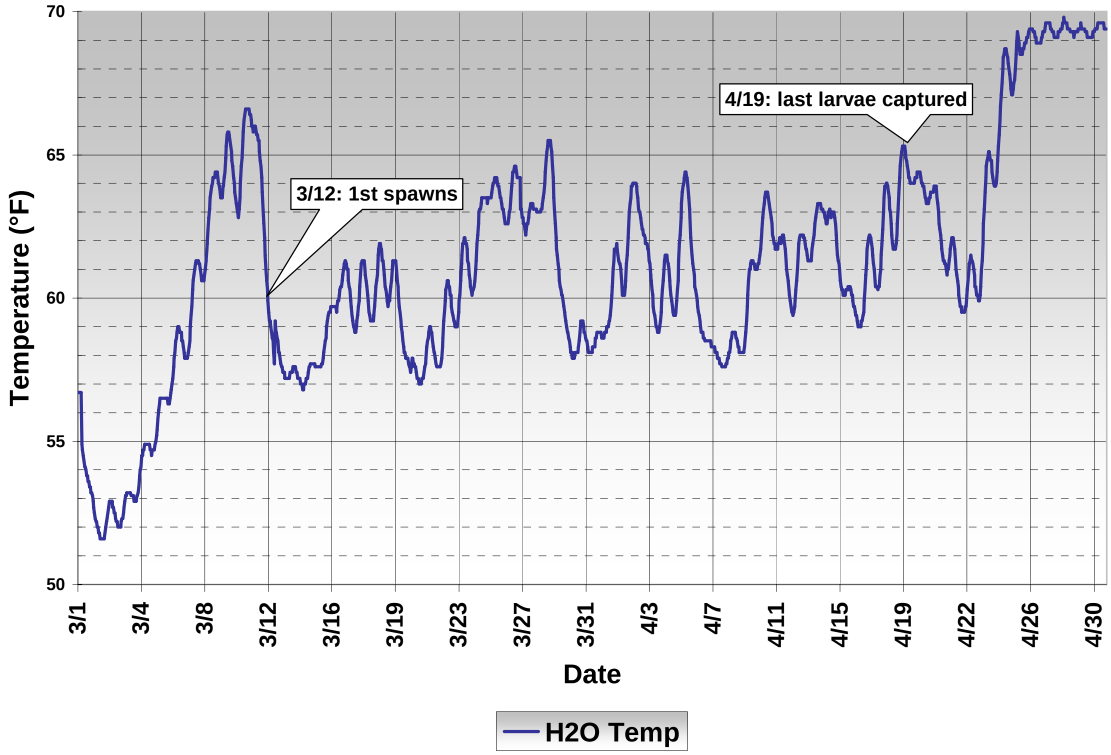

Figure 5. Temperature data at Conservation Fisheries, Inc. March-April 2009.

Survivorship of Kentucky arrow darter larvae was relatively low compared to some other pelagic larvae regularly produced at CFI. Only 30% of larvae that were transferred into feeding tubs survived to juvenile stage (N=153). Of these juvenile fish 72% survived to be released (N=110), yielding an overall survivorship of 21.8%. There are several possible explanations for these somewhat poor results. The system that these fish occupied was about 720 liters, which is about ½ the size of other systems we typically use for breeding darters with pelagic larvae. The smaller system may have been more adversely affected by enrichment that results from the automatic plankton feeding, leading to a build up in organics in the system and possibly leading to an observed disease issue. Excessive crowding of larvae in the feeding tubs also could have caused stress and losses due to disease. These larvae and juveniles may be very aggressively competitive, like the adults, and housing high numbers together may have been detrimental. When losses were observed over several days a treatment of formalin at a dose of 1 drop per gallon was administered. After the system was treated with formalin observed losses seemed to decrease, but many losses were unaccounted for and their timing unknown. Space and density issues for the larvae may have been a more limiting factor with rearing this species in comparison to other darter species reared at CFI. For efforts in 2010 a new system of twice the capacity of this year's will be utilized with additional rearing tubs. The expected result will be at least a doubling of production to 300-400 individuals from a comparable number of brood stock.

The 110 juvenile Kentucky arrow darters that were produced were released on July 15th to Sugar Creek (N 37.12706, W 83.53771) Leslie County, Kentucky in an effort to restore the species to a stream (near the source population) where the species had apparently been extirpated, but which exhibited currently suitable habitat. Prior to release (June 22nd) all the fish were marked with a Northwest Marine Technologies elastomer tag (pink, dorso-lateral left side of dorsal fin) at CFI. A small number of the fish were observed to have lost their tags prior to release, but given that none were currently thought to inhabit Sugar Creek, they were released untagged. The darters averaged approximately 30-35 mm TL at release.

On August 25th, a seining survey of the release area was conducted by CFI, KDFWR, and USFWS personnel. Eight fish species, including four darter species were collected. A lone Kentucky arrow darter was taken. Because the fish was untagged and measured about 70 mm TL, it was presumed to be a 1+ age individual that had probably recently immigrated into the creek. Consideration of future stocking in this creek should probably be dependent upon additional surveys to determine whether a population is naturally recolonizing.

ACKNOWLEDGEMENTS

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