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

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Large (96 mm TL) male Kentucky Arrow Darter in Long Fork, Clay County, Kentucky, 30 September 2014; no VIE tag visible, but probably stocked in 2012.

INTRODUCTION

The Arrow Darter, *Etheostoma sagitta*, has a limited range in only two river drainages, mostly in Kentucky. Recent analyses of morphological and genetic data provided evidence that the Cumberland Arrow Darter, *E. s. sagitta* (Cumberland River drainage) and the Kentucky Arrow Darter, *E. s. spilotum*, (Kentucky River drainage) represent distinct evolutionary lineages and should be treated as separate management units for conservation management purposes. The Kentucky Department of Fish and Wildlife Resources (KDFWR) identified 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 status survey of *E. s. spilotum* in the Kentucky River basin ascertained that populations have declined considerably during the past two decades. Kentucky Arrow Darters were detected in only 33 of 68 historic streams sampled in 2007-2009 (Thomas 2008; Floyd and Thomas 2010). This and the recent elevation of the subspecies to species status recently led the U.S. Fish and Wildlife Service to increase the candidate listing priority number (LPN) from 2 to 3, the highest priority for non-monotypic genus species (USFWS 2014).

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. Details of the reproductive biology (e.g., spawning behavior) of the Kentucky Arrow Darter and other environmental conditions necessary for spawning to occur were poorly known. Since 2008 Conservation Fisheries, Inc. (CFI), in cooperation with KDFWR, has developed successful captive propagation protocols to produce the offspring needed to restore extirpated populations into streams within the species' native range. This report summarizes propagation efforts to determine the feasibility of re-establishing viable populations of this species within its former range. The sixth year's efforts (2014) with the Arrow Darter are described herein.

METHODS

Brood Source and Recipient Stream(s)

Adults were collected for brood stock from Big Double Creek, Clay County, Kentucky, a third-order tributary of the Red Bird River (South Fork Kentucky River drainage) that lies entirely within the Daniel Boone National Forest (DBNF) and contains a healthy population of Kentucky Arrow Darters. Kentucky Arrow Darters were collected with a fine mesh seine, mainly by downstream hauls. 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 and acclimated to aquaria.

Reintroduction sites were chosen where habitat conditions are suitable and there is some level of protection (e.g., within wildlife management area or national forest boundaries). In 2012, 2013 and 2014 Long Fork was chosen as the recipient stream for captive-spawned Kentucky Arrow Darters because: 1) it was within the historic range of the species and within close proximity to (but isolated from) the brood source; 2) it has good water quality, suitable habitat, and is within the DBNF; 3) available survey data indicated no pre-existing population; and 4) it has been disrupted by a barrier (e.g., impassable culvert or section of degraded habitat), effectively isolating an upper reach with suitable habitat that is depauperate of Kentucky Arrow Darters. 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; Thomas and Brandt 2012).

Limited numbers of captive spawned individuals (157) were released into Sugar Creek (a second-order tributary to Red Bird River upstream of Big Double Creek) in 2009 and 2011 (Table 1). Failure to detect any propagated and tagged Kentucky Arrow Darters following their release into Sugar Creek and the increasing trend in numbers of untagged individuals (31) caught since 2009 suggested dispersal of wild fish into the stream followed by successful reproduction and recruitment (Thomas and Brandt 2012; Table 2) after the extreme drought of 2007; therefore, continued stocking of propagated fish for the purpose of reestablishing a population was deemed inappropriate for Sugar Creek. Reintroduction efforts were relocated to a new stream, Long Fork, a second-order tributary to Hector Branch, a fourth-order tributary to Red Bird River downstream of Big Double Creek.

Periodic surveys were conducted from 2009-2012 in Sugar Creek and 2012 in Long Fork using backpack electrofishing within the release section by Thomas and Brandt (2012). Monitoring of Long Fork since 2013 was conducted by CFI biologists and KDFWR by performing a combination of visual surveys and seine hauls.

Propagation Methods

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. For efforts in 2014 fish were housed in two breeding tanks (2 x 200 L) within a larger 1400 L recirculating system. Tanks were divided up into groups of breeders with sex ratios of 1 male: 2 females per 200 L tank. In addition, abundant cover was provided in the form of slab rocks, tiles, and black plastic slabs on a mixed sand substrate added to the tanks for the spawning season.

Filtration included individual tank sponge filters and airstones as well as system filters (for multiple redundancy back-up); internal tank circulation was enhanced with "underwater fans" (Hydor Koralia evolution©). Both 200 L breeding tanks were modified with reverse flow undergravel filters to provide interstitial water and gas movement within the spawning substrates in the hopes of enhancing egg survivorship. Implemented in 2012, a water chiller was also added to the propagation system in order to prevent the excessively warm water temperatures experienced in 2009 and 2011 which may have prematurely terminated breeding condition for many of the Arrow Darters, especially older individuals. Buffered reverse osmosis (RO) water (pH ~ 7.0) was utilized to maintain softer water than the municipal water source utilized at CFI. A 15kW generator insured back-up power for essential life support functions for the entire facility in the event of an electrical outage.

Winter conditioning included reduction of water temperatures to as low as 3°C and photoperiod shortened to 9 hours of light. Reproductive condition was induced by gradually increasing water temperatures, photoperiod, and food quantity offered, to mimic natural seasonal changes. As well, in the late winter—early spring (spawning season) temperatures were held below hatchery ambient with moderate daily fluctuations of 2-3°C. Fish were fed frozen bloodworms (chironomids) and brine shrimp, and live blackworms (*Lumbriculus variegatus*, a small aquatic annelid) and *Daphnia* provided ad libitum with quantities and frequency seasonally adjusted. After breeding was observed, eggs were allowed to remain in situ to continue development undisturbed.

Each of the 200 L breeding tanks drained from an overflow to a dedicated white plastic ~20 L catch tub with a 2 cm diameter PVC central standpipe drain. Hatching larvae were monitored by checking the two overflow collection tubs daily from March through early May. Once the larvae hatched and swam up to be collected by the overflow into a catch tub, they were then removed with a baster and transferred to a larger (~35-40 L) common black plastic feeding and rearing tub. 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. A total of two of these rearing tubs were set up and used through the breeding season.

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. Each rearing tub was set up with a reservoir, timer, and solenoid for constant food dispersal during the day. 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 Reed Mariculture Inc.), and *Ceriodaphnia dubia* neonates.

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. Snails were also added to help clean up excess food and waste. As larvae transformed into juveniles they were moved to 75 liter tanks for further grow out. With this transformation to juvenile stage new foods were also added, including grindal worms (*Enchytraeus buchholzi*, a tiny annelid white worm), chopped blackworms, bloodworms, and adult *Ceriodaphnia*.

Prior to any reintroductions, a sample of the appropriate captive population, representing each system occupied, if applicable, was sent to the Warm Springs National Fish Hatchery to screen for any detectable disease pathogens. Disease detection would initiate actions necessary to prevent the transfer of any pathogens to wild populations of fish. A week or so 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. Fish were transported for releases as described above for brood stock acquisition except that backpacks were used where necessary to hike to remote release sites. Survivability and movement patterns of released fish were assessed through mark-recapture methods and through periodic monitoring using non-invasive methods, such as visual census techniques.

RESULTS

One male and 7 adult female Kentucky Arrow Darters were collected on 20 February 2014 from Big Double Creek (N 37.0904666 W 83.6022722), near where other brood stock were collected from 2008—2012. The new wild-caught females and 1 male were used in this year's effort in addition to 3 captively conditioned (2012) wild males and 6 captively conditioned (2012) wild females. The new females were quarantined in one of the 200 L breeding tanks adjacent to the other housing the captively overwintered females. All males were separated from females due to aggression, held individually in 75 L tanks, and only introduced singly into the separate breeding tanks for a few days at a time.

Breeders were tracked individually in two groups each of whose egg/larval production was recorded:

- **I2-1** (200 L): Reverse undergravel; 2 males conditioned 2012 (wild); a mix of 6 new wild females collected February 2014 and 4 females conditioned 2012 (wild); drained into dedicated larval catch tub; 1 male: 2 females. ***Larval production = 170***.
- **I2-2** (200 L): Reverse undergravel; 1 new wild male collected February 2014 and 1 male conditioned 2012 (wild); a mix of 5 new wild females collected February 2014 and 5 females conditioned 2012 (wild); drained into dedicated larval catch tub; 1 male: 2 females. ***Larval production = 278***.

Breeding groups were first introduced to the two tanks on 17 March. On 18 March spawning was observed in both tanks after temperatures briefly exceeded 13°C (see Figure 1). Spawning activity quickly declined in late April and on 5 May the chiller was removed and water temperatures allowed to rise above 19°C.

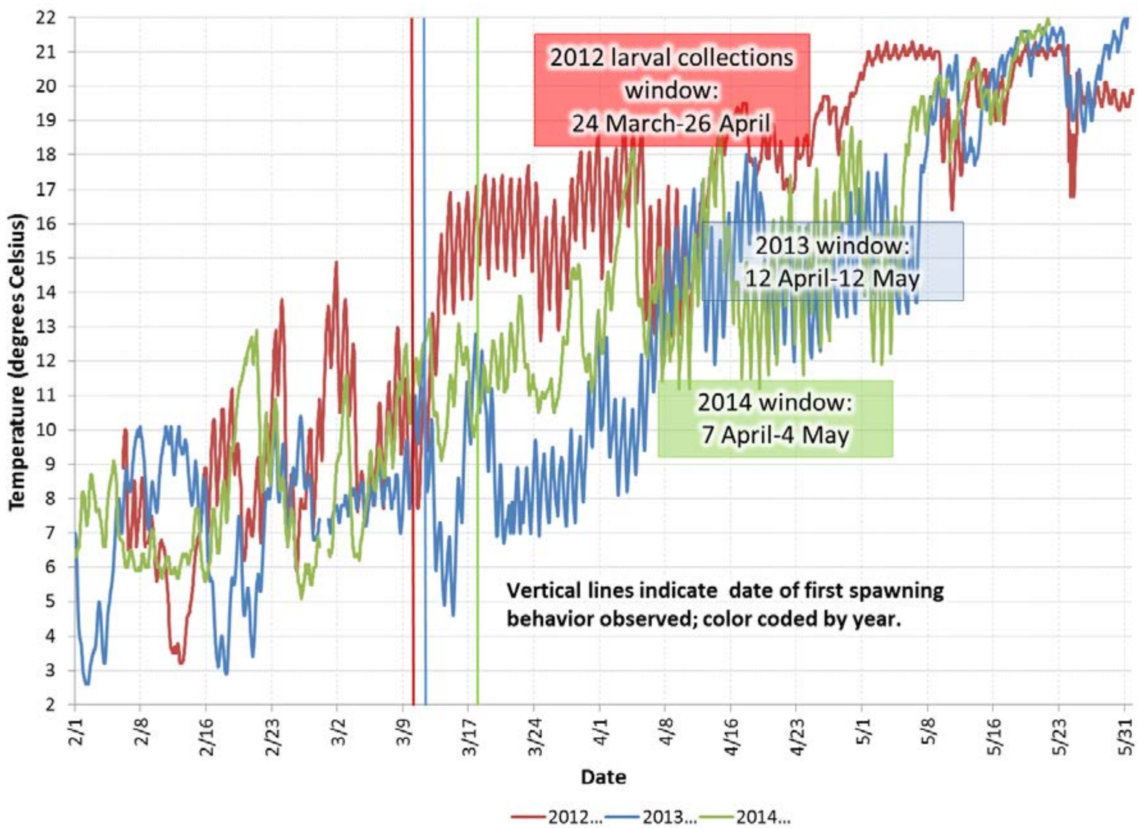

Figure 1. Kentucky Arrow Darter system water temperatures and reproductive observations in 2012, 2013, and 2014 at CFI.

April 7 marked the first day of the appearance of larvae in a passive collection tub, and by 4 May, the date of the last larval collection, ~448 larvae had been captured by the set-up, with 408 eventually surviving to the early juvenile stage (~91% survivorship). Total passive collection of Kentucky Arrow Darter larvae was higher than 2013 (2012 was the highest to date).

On 30 July the young (~400) were released at multiple sites in Long Fork, a tributary of the Red Bird River, Clay County. These sites spanned Long Fork from the mouth to ~1.5 km upstream at the Long Fork Road crossing (Table 1). At release, the size of the 2014 propagates ranged from 35-45 mm total length (TL). All the fish were marked with a Northwest Marine Technologies elastomer tag (red, right first dorsal) in preparation for release.

During pre-release surveys on 10 March and 30 July 37 tagged Kentucky Arrow Darters released in 2012 and 2013 were observed as well as 14 Arrow Darters without tags, representing at least two age classes (Table 2). Two were young of the year (2014 age class) and most of the remainder were clearly 2013 age-class, but at least one individual (92 mm TL) was either a very large fast-growing 2013 or else a 2012 age class fish that had lost a tag or else was of wild origin. Post-release surveys on 27 August, 30 September, and 4 November collected 68 tagged and 10 untagged darters (two 2014 YOYs, six 2013s, and two 96 mm TL fish of unknown status like the large individual described above).

A total of 1447 Kentucky Arrow Darters have been stocked in Long Fork since 2012 (Table 1). Monitoring results for all surveys are summarized in Table 2. A total of 404 tagged (propagated) and 44 untagged (wild-spawned) Kentucky Arrow Darters have been observed through 2014. Additional wild broodstock will be collected from Big Double Creek prior to wild spawning to be used in 2015 captive production.

DISCUSSION

Monitoring efforts so far have confirmed the survival of tagged Kentucky Arrow Darters released into Long Fork for periods exceeding two years. Surveys have detected tagged individuals during every survey in Long Fork and instances of significant upstream dispersal from stocking points. The increasing trend in numbers of untagged individuals in 2013-2014 is evidence of successful reproduction and recruitment, but it would be premature at this point to suggest that the project has been successful/unsuccessful (Thomas and Brandt 2012). Non-game fish restoration attempts may take many years to establish viable populations when stocking relatively limited numbers of individuals, particularly small species that are short-lived and cryptic (Shute et al. 2005).

Spawning Kentucky Arrow Darters in captivity has been variably successful, with attempts made to address key production limiting issues each successive year (Ruble et al., 2010; Petty et al., 2011, 2012, 2013, and 2014). Keeping males segregated from each other and nonreceptive females in order to limit territorial aggression and permit female survivorship and conditioning has been critical, but necessarily limits broodstock numbers (and therefore production) due to resulting space requirements. Reverse flow undergravel filters have enhanced egg survivorship by increasing interstitial water circulation. The addition of a chiller for better regulation of water temperatures has allowed maintenance of significantly lower daily maximum temperatures for much improved and prolonged maintenance of reproductive condition by both sexes, although determining the ideal temperature ranges for pre-spawn, spawning, egg incubation, and larval development remains a work in progress. Primary production-limiting factors that persist include the size and condition of available broodstock each year, which varies dependent upon collection conditions and success, as well as the physiological condition of the breeders and the eggs and larvae produced. These factors were likely the primary reason(s) for lower production in 2013 and 2014 relative to 2012. The 90% survivorship of larvae in 2014 was exceptional relative to any darter species produced at CFI and a significant improvement over the ~60% the previous two years.

Although it is admittedly speculative to make any hard conclusions based on the limited data to date, water temperatures and reproductive/developmental observations in the hatchery over the past three years (Figure 1) suggest at least some interesting discussion. Water temperatures in 2014 immediately prior to larval captures were more or less intermediate between temperatures that were significantly warmer in 2012 and mostly cooler in 2013. The relative warmth in 2012 appeared to both initiate and terminate captures earlier, while the relatively cool temperatures in 2013 appeared to delay both. In 2014 both initiation and termination were intermediate to the two prior years. This is perhaps not surprising given the known correlation between temperature and development rates of eggs and larvae. The long delay between the onset of spawning and the first larval captures in 2013 suggested that temperatures below 10°C might negatively affect either egg viability and/or larval survivorship and/or simply increase development times of both more than might be predicted; in any case, temperatures that low should probably be avoided in the future in the breeding system after spawning commences.

As noted above and in prior reports water temperature is likely a significant determinant of when spawning begins and how long it continues. The “start dates” observed the last three years should be considered with caution in that each followed within 24 hours of moving females into breeding tanks with males; however, the moves were always driven by close observation of the gravid condition of the females, which in turn is likely driven by some combination of photoperiod, water temperature, and female age/size and condition. Spawning began March 10 in 2012, March 12 in 2013, and March 18 in 2014. Note that spawning began on nearly the same date in 2012 and 2013 even though temperatures were significantly different in late February and early March, suggesting one or more of the other factors were offsetting the effect of temperature. However, note that in every year in the few days immediately prior to spawning water temperatures reached 11-13°C. Finally, we have always assumed that warmer water temperatures shorten the breeding season by negatively affecting breeding condition; however, the duration between spawning onset and larval capture termination in 2012 and 2014 were both 47 days, despite a much warmer 2012. The relatively longer duration of 61 days in the very cool 2013 season was more like “expected”. It seems highly likely that age and size of breeding darters creates additional variation beyond the effects of photoperiod and water temperature, with larger, older individuals spawning earlier and probably terminating earlier, with smaller, younger individuals maturing and spawning later. Hatchery observations are necessarily biased by the selection and use of mostly larger individuals in attempts to maximize production and may not reflect natural variation in wild populations with greater demographic (and environmental) diversity.

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Field Note #	Date	Location	*Tag Color & Position	Age-Class	# Released
CFI09-048	15-Jul-09	Sugar Crk above old 2nd road crossing	Pink L D1	2009	110
KDFWR	21-Oct-11	Sugar Crk: KDFWR released	Red L D1	2011	35
CFI12-134	14-Aug-12	Long Fork lower section to upper release section	Red L D1	2012	751
CFI12-195	9-Oct-12	Long Fork lower to upstream above midpoint	Red L D1	2012	78
CFI13-051	15-Jul-13	Long Fork lower section to upper release section	Green R D1	2013	218
CFI14-083	30-Jul-14	Long Fork lower upstream to midpoint	Red R D1	2014	103
CFI14-084	30-Jul-14	Long Fork midpoint upstream to road crossing	Red R D1	2014	297
				Total:	**1592**
				2009 age class:	110
				2010 age class:	0
				2011 age class:	35
				Total Sugar Crk:	**145**
				2012 age class:	829
				2013 age class:	218
				2014 age class:	400
				Total Long Fk:	**1447**

* Tag codes: L D1 = left, 1st dorsal; R D1 = right, 1st dorsal (colors green, red or pink)

Table 1. Numbers of Kentucky Arrow Darter released into Sugar Creek and Long Fork from 2009-2014.

Date	Lat/Long	Locality	# Obs	Age Class	Total Length mm	Tag color & position *P/W
25-Aug-09	37.1249/-83.54117	SC: release section	1		69	W no tag
13-Oct-09	37.11973/-83.54765	SC: 900 m below release section	4		44-47	W no tag
13-Oct-09	37.1249/-83.54117	SC: release section	0			
31-Mar-10	37.1249/-83.54117	SC: release section	0			
31-Mar-10	37.12376/-83.5243	SC: 3.2 km above release section	4		63-88	W no tag
9-Jan-12	37.1249/-83.54117	SC: release section	3		72-87	W no tag
9-Jan-12	37.11973/-83.54765	SC: 900 m below release section	6		38-75	W no tag
15-Feb-12	37.1249/-83.54117	SC: release section	4		59-87	W no tag
15-Feb-12	37.127766/-83.51067	SC: 3 km above release section	9		60-102	W no tag
		Sugar Creek Total Observations:	**30**			**SC all wild-spawned**
9-Oct-12	37.17728/-83.63516	LF: entire stocked section	18	2012		P Red L D1
29-Jan-13	37.17728/-83.63516	LF: entire stocked section	47	2012	50+	P Red L D1
13-Mar-13	37.172281/-83.649174	LF: road xing to 250m above stocked reach	10	2012	50+	P Red L D1
13-Mar-13	37.175735/-83.640079	LF: mid-point parking spot to road xing	37	2012	50+	P Red L D1
15-Jul-13	37.17728/-83.63516	LF: entire stocked section	46	2012	55-80	P Red L D1
15-Jul-13	37.17728/-83.63516	LF: entire stocked section	9	2013	42-45	W no tag
29-Aug-13	37.17728/-83.63516	LF: entire stocked section	55	2012	55-80	P Red L D1
29-Aug-13	37.17728/-83.63516	LF: entire stocked section	28	2013	44-51	P Green R D1
29-Aug-13	37.17728/-83.63516	LF: entire stocked section	3	2013	42-45	W no tag
29-Oct-13	37.17728/-83.63516	LF: entire stocked section + Hector Br	13	2012		P Red L D1
29-Oct-13	37.17728/-83.63516	LF: entire stocked section + Hector Br	1	2013		P Green R D1
29-Oct-13	37.17728/-83.63516	LF: entire stocked section + Hector Br	8	2013	46-54	W no tag
10-Mar-14	37.17728/-83.63516	LF: entire stocked section + Hector Br	9	2012	65-84	P Red L D1
10-Mar-14	37.17728/-83.63516	LF: entire stocked section + Hector Br	4	2013	43-55	P Green R D1
10-Mar-14	37.17728/-83.63516	LF: entire stocked section + Hector Br	1	2013	50	P Green L D1
10-Mar-14	37.17728/-83.63516	LF: entire stocked section + Hector Br	5	2013	49-58	W no tag
10-Mar-14	37.17071/-83.6508	LF: ~200m above stocked section	5	2012	69-75	P Red L D1
10-Mar-14	37.17071/-83.6508	LF: ~200m above stocked section	2	2013	52-55	P Green R D1
30-Jul-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	5	Adults	60-92	W no tag
30-Jul-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	2	2014	37/40	W no tag
30-Jul-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	2	2013	62/66	P Green R D1
30-Jul-14	37.175735/-83.640079	LF: mid-point parking spot to road xing	2	Adults	64/67	W no tag
30-Jul-14	37.175735/-83.640079	LF: mid-point parking spot to road xing	6	2012	70-90	P Red L D1
30-Jul-14	37.175735/-83.640079	LF: mid-point parking spot to road xing	5	2013	62-74	P Green R D1
30-Jul-14	37.175735/-83.640079	LF: mid-point parking spot to road xing	3	2013	65-70	P Green L D1
27-Aug-14	37.17756/-83.6345	Hector Br ~100m below LF up to bridge	1	2014	45	W no tag
27-Aug-14	37.17756/-83.6345	Hector Br ~100m below LF up to bridge	1	Adult	81	W no tag
27-Aug-14	37.17756/-83.6345	Hector Br ~100m below LF up to bridge	1	2012	70+	P Red L D1
27-Aug-14	37.17756/-83.6345	Hector Br ~100m below LF up to bridge	2	2014	45/45	P Red R D1
27-Aug-14	37.17071/-83.6508	LF: ~200m above stocked section	1	2012	96	W no tag
27-Aug-14	37.17071/-83.6508	LF: ~200m above stocked section	8	2012	67-96	P Red L D1
27-Aug-14	37.17071/-83.6508	LF: ~200m above stocked section	4	2013	65-91	P Green R D1
27-Aug-14	37.17071/-83.6508	LF: ~200m above stocked section	5	2014	45-47	P Red R D1
30-Sep-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	6	2-3 yr classes	47-96	W no tag
30-Sep-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	7	2012	70-91	P Red L D1
30-Sep-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	5	2013	64-73	P Green R D1
30-Sep-14	37.17728/-83.63516	LF: mouth up to mid-point parking spot	10	2014	48-70	P Red R D1
4-Nov-14	37.17756/-83.6345	Hector Br ~100m below LF up to bridge	1	2014	51	W no tag
4-Nov-14	37.17728/-83.63516	LF: entire stocked section	10	2012	67-91	P Red L D1
4-Nov-14	37.17728/-83.63516	LF: entire stocked section	5	2013	67-85	P Green R D1
4-Nov-14	37.17728/-83.63516	LF: entire stocked section	11	2014	42-58	P Red R D1
		Long Fork Total Observations:	**404**			**LF Wild-spawned: 44**

*P = propagated fish; W = wild spawned fish

Table 2. Kentucky Arrow Darter observations in Sugar Creek (SC) and Long Fork (LF), 2009-2014. Monitoring in 2009-2012 was conducted by Thomas and Brandt (2012) using backpack electrofishing and their results are briefly summarized in this table. Monitoring in 2012-2014 was conducted by CFI biologists and KDFWR using visuals and seine hauls.